

**HEPATOPROTECTIVE POTENTIAL OF *OROXYLUM*
INDICUM (L.) BENTH. EX. KURZ AGAINST NON-STEROIDAL
ANTI-INFLAMMATORY DRUG (DICLOFENAC) - INDUCED
HEPATOTOXICITY IN RAT**

**A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF DOCTOR OF
PHILOSOPHY**

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**DEPARTMENT OF BOTANY
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BY

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DEPARTMENT OF BOTANY

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SUBMITTED

**IN PARTIAL FULFILLMENT OF THE REQUIREMENT OF THE DEGREE
OF DOCTOR OF PHILOSOPHY IN BOTANY OF MIZORAM
UNIVERSITY, AIZAWL**



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CERTIFICATE

This is to certify that **Mr. Ran Bahadur Pradhan**, Registration No. **MZU/Ph.D./1211 of 31.08.2018** has submitted the thesis entitled **“Hepatoprotective Potential of *Oroxylum indicum* (L.) Benth. Ex. Kurz Against Non-Steroidal Anti-Inflammatory Drug (Diclofenac) - Induced Hepatotoxicity in Rat”** under my supervision, to fulfill the requirements for award of the Degree of Doctor of Philosophy in the Department of Botany, Mizoram University, Aizawl. The work is authentic, content of the thesis is the original work of the Research Scholar, and the nature and the presentation of the work are the first of its kind.

It is further certified that no portion (s) or part (s) of the content of the thesis has been submitted for any degree in Mizoram University or any other University or Institute.

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Yours sincerely,

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DECLARATION

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March, 2025

I, Mr. Ran Bahadur Pradhan, hereby declare that the subject matter of this thesis is the record of work done by me, that the contents of this thesis did not form basis of the award of any previous degree to me or to the best of my knowledge to anybody else, and that the thesis has not been submitted by me for any research degree in any other University/Institute.

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Date:

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PREFACE

Drug-induced hepatotoxicity, particularly from nonsteroidal anti-inflammatory drugs (NSAIDs), remains a major concern, with diclofenac being a well-documented culprit. Its prolonged use leads to liver damage through oxidative stress, mitochondrial dysfunction, and inflammatory responses, generating reactive oxygen species (ROS) and toxic metabolites that compromise hepatocyte integrity. Amid growing concerns over diclofenac-induced hepatotoxicity, the search for safer alternatives is crucial. *Oroxylum indicum*, a traditionally used medicinal plant among North East Indian tribes, holds potential hepatoprotective properties, yet scientific validation remains limited. This study explores its efficacy in mitigating diclofenac-induced liver injury, addressing a critical gap in hepatoprotective research.

The thesis is structured systematically to facilitate clear and comprehensive understanding. The main body of the thesis is organized into four chapters, each serving a distinct purpose: Chapter I, “Introduction” lays the groundwork by outlining the problem of diclofenac inducing hepatotoxicity and *O. indicum*’s efficacy to mitigate hepatotoxicity. Chapter II, “Materials and Methods” this section function as a detailed experimental blueprint, systematically describing experimental design, methodologies employed, analytical techniques and data collection procedures to ensure reproducibility and scientific rigor. Chapter III, “Results” The findings of the research are presented in a clear and structured manner, supported by visual aids such as figures, tables, and graphs to enhance data interpretation. Finally, Chapter IV, “Discussion” interprets the results within the broader context of existing scientific literature. It highlights the most significant findings, discusses their implications for mitigating oxidative stress and hepatoprotective potential. Following Chapter IV, “Summary” recapitulates the major research questions, consolidate the key findings, and emphasize the broader significance of the study in advancing hepatoprotection.

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ABBREBIATION

FTIR	Fourier transform - infrared radiation
UV	Ultraviolet rays
OD	Optical density
ABTS	2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)
$O_2^{\bullet -}$	Superoxide radical
OIE	<i>Oroxylum indicum</i> ethanolic extract
OIA	<i>Oroxylum indicum</i> aqueous extract
TCM	Traditional Chinese medicine
AAE	Ascorbic acid equivalent
BHT	Butylated hydroxy toluene
EDTA	Ethylenediamine tetraacetic acid
FCR	Folin Ciocalteus reagent
DPPH	2,2-Diphenyl-1-picryl-hydrazyl-hydrate
$OH^{\bullet}$	Hydroxyl radical
LC-ESI-MS	Liquid chromatography-electrospray ionization-mass spectrometry
TAE	Tannic acid equivalent
QE	Quercetin equivalent
NSAID	Nonsteroidal anti-inflammatory drugs
OECD	Organization for economic cooperation and development
rpm	Rotation per minute
IAEC	Institutional animal ethical committee
DMSO	Dimethyl sulfoxide

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CHAPTER - 1

INTRODUCTION

1. Background

The liver plays a key role in detoxification (Robinson et al., 2016). However, liver is sensitive to various drugs, alcohol, and several environmental pollutants (Andrade et al., 2019; Gu & Manautou, 2012). Among different kinds of liver injuries, drug-induced hepatotoxicity presents a primary concern (Devarbhavi, 2012; Pandit, 2012). Prolonged use or overdose of nonsteroidal anti-inflammatory drugs (NSAIDs) often causes adverse hepatic effects (Panchal and Sabina, 2023). Diclofenac, a commonly prescribed NSAID, causes hepatotoxicity in various forms ranging from an increase in hepatic enzymes to severe liver failure (Boelsterli, 2003).

Oxidative stress, mitochondrial dysfunction, and inflammatory response are the primary causes of diclofenac-induced hepatotoxicity. Diclofenac metabolism in the liver is associated with the generation of reactive oxygen species (ROS) and other toxic substances, resulting in disruption of cellular integrity, hepatocyte apoptosis, and necrosis. The above limitations of diclofenac and other NSAID, natural bioactive compounds, have attracted attention among researchers and medical practitioners.

Oroxylum indicum (L.) Kurz is widely used in traditional medicine system. It has been shown to have a great pharmacological potential. *O. indicum* belongs to the Bignoniaceae family and is rich in phytochemicals including flavonoids, alkaloids, and polyphenols. These phytochemicals have hepatoprotective effects, such as antioxidant and anti-inflammatory effects. Studies have demonstrated that bioactive compounds such as baicalein and chrysin protect the liver by mitigating oxidative stress, reducing inflammation, and promoting cellular repair mechanisms.

Regardless of the overuse of *O. indicum* in traditional medicine, systematic evaluation of its hepatoprotective properties against NSAID-induced liver injury is still insufficient. Therefore, this study aimed to examine hepatoprotective potential of *O. indicum* against diclofenac-induced toxicity of liver in rat. This study aimed to

examine biochemical markers, histopathological conditions, and molecular mechanisms of diclofenac-induced liver injury.

1.1. Botanical Classification of *Oroxylum indicum*

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Lamiales

Family: Bignoniaceae

Genus: *Oroxylum*

Species: *indicum*

1.2. Synonyms

There are several synonyms for *Oroxylum indicum* (Rao and Kumar, 2024).

- *Arthrophyllum ceylanicum* Miq.
- *Arthrophyllum reticulatum* Blume ex Miq.
- *Bignonia indica* L.
- *Bignonia lugubris* Salisb.
- *Bignonia pentandra* Lour.
- *Bignonia quadripinnata* Blanco
- *Bignonia tripinnata* Noronha
- *Bignonia tuberculata* Roxb. ex DC.
- *Calosanthès indica* (L.) Blume
- *Hippoxylon indica* (L.) Raf.
- *Oroxylum flavum* Rehder
- *Spathodea indica* (L.) Pers.



Figure 1. *Oroxylum indicum* in its natural habitat

1.3. Common Names

English: Broken bones tree, Indian calosanthes, Midnight horror, Indian trumpet flower, Tree of damocles.

1.4. Vernacular Names (Rao and Kumar, 2024):

- **Hindi:** Sonapatha, Sagru, Sauna, Sona, Karkath.
- **Mizo:** Archangkawm
- **Kannada:** Thigade mara.
- **Telugu:** Godugu chettu, Akumanu, Nemali chettu, Pampini, Peddakatti, Sakana-nasamu, Pampini chettu, Konda pampina chettu.
- **Oria:** Pependi, Bhalu-sakti, Phemphana, Phampan.
- **Sanskrit:** Syonaka.
- **Malayalam:** Aralu, Palakapayyani, Payyazhantha, Valpathiri, Veluthapathiri.

1.5. Geographical Distribution

Oroxylum indicum is prevalent in tropical and subtropical forests. In India, it exists throughout northeastern, central, and southern India and commonly grows in mixed-deciduous forests and riparian habitats. It shows a higher occurrence in the Vindhyan range and southward. It occurs naturally in the wild near rivers and streams up to an altitude of 1000 meters. However, it is typically missing in the arid and dry regions of Western India. Outside South Asia, *O. indicum* has a wide spatial distribution.

O. indicum is reported from Andaman Islands, Bangladesh, Bhutan, Cambodia, China, Laos, Malaysia, Myanmar, Nepal, Philippines, Sri Lanka, Thailand, Vietnam (south and southeast Asia), Taiwan, Java, Sumatra, Borneo, Sulawesi, Sunda Islands, Java, Sumatra, Borneo, Sulawesi, Sunda Islands. The tree is resilient to tropical forests and woodlands, frequently recorded in regions with high humidity and moderate rainfall (Dev et al., 2010; Rao and Kumar, 2024).

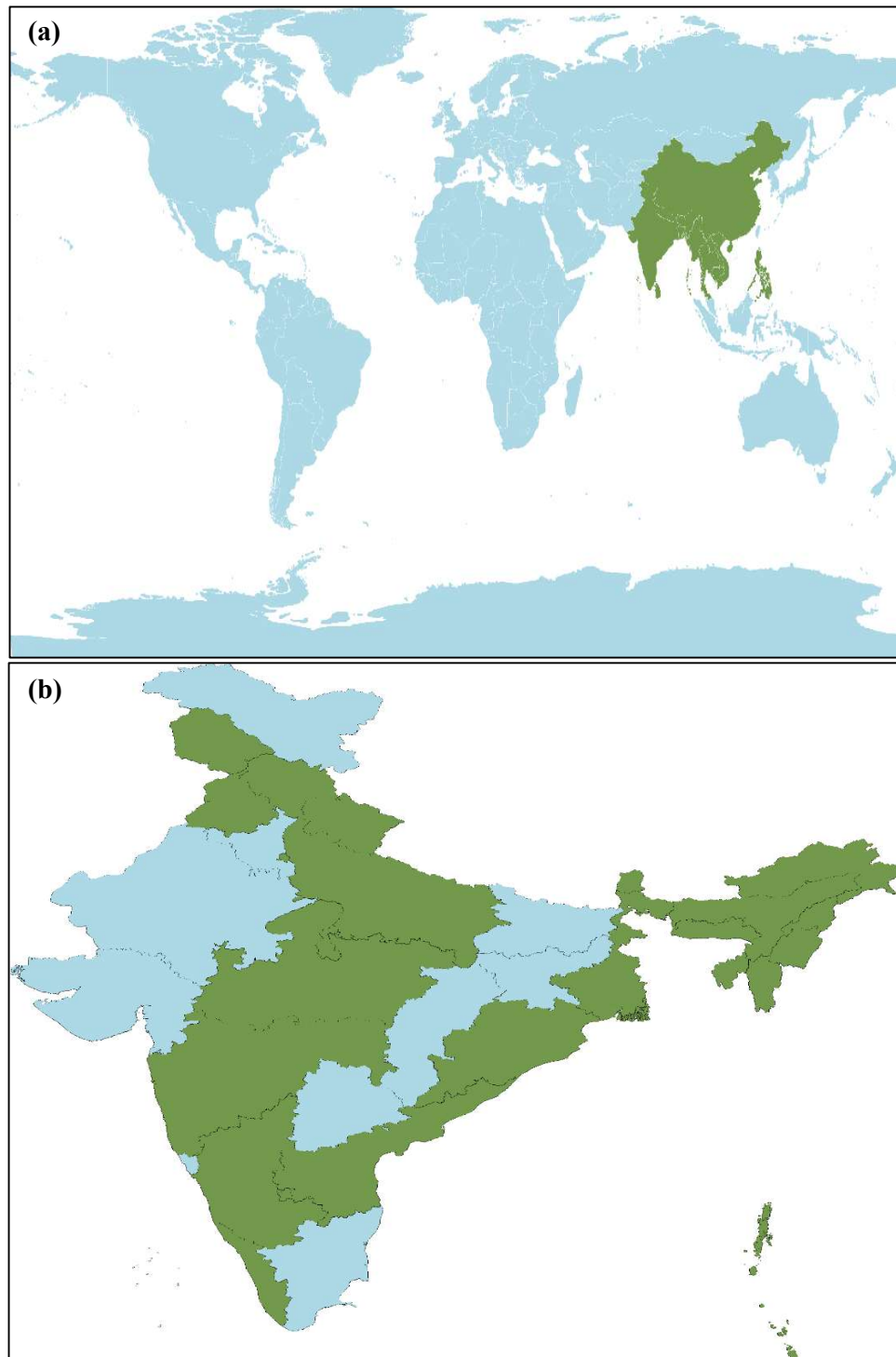


Figure 2. Distribution map of *Oroxylum indicum* indicated by green shaded regions: World (a) and India (b)

2. Phytochemical Composition and Medicinal Properties of *Oroxylum indicum*

2.1. Traditional Uses and Medicinal Properties

Indigenous medical systems depend on plants to cure various diseases. According to an estimate, approximately 80 % of the global population depends on plants for primary healthcare. Approximately 25 % of prescribed medicines are derived from plants. About 2,500 medicinal plant species have been documented worldwide (Mehta & Pradhan, 2024).

Oroxylum indicum holds a key place in traditional medicine and regional ethnobotanical food practices. The young pods, buds, and flowers of *O. indicum* are extensively adapted to Southeast Asian cuisines, either cooked in soups or stir-fries (Dinda et al., 2015). The plant is also of ethnocultural significance as it is recognized as a sacred entity in some tribal areas. Furthermore, this tree is cultivated for its noteworthy flowers and is frequently found in gardens and public spaces.

Across historical periods, *O. indicum* has been a well-documented medicinal plant among several tribal communities in Northeast India. Ethnobotanical studies have documented the historical uses of tribes such as the Rabha, Garo, Khasi, and Jaintia in Meghalaya to combat various diseases (Barchung et al., 2018). In Nagaland, the Naga tribe administers paste of *O. indicum* roots to cure dysentery, diarrhea, jaundice, and rheumatism (Jamir, 2010). The Halam tribe uses an aqueous extract of the bark along with sugar to cure jaundice (Das et al., 2012). Moreover, various tribal communities throughout India use the seeds and bark of *O. indicum* to manage pneumonia, respiratory diseases, and fever (Panghal et al., 2010; Patil et al., 2008).

The medicinal properties of *O. indicum* have been widely reported in Ayurveda, Siddha, and traditional Chinese medicines. Almost every part of the *O. indicum* plant is used to cure various diseases. *O. indicum* is widely used to treat several conditions, for example, inflammation, cancer, pain, bronchopulmonary conditions, hyperbilirubinemia, arthritis, autoimmune inflammatory disorders, gastric ulcers, diabetes, and gastrointestinal disorders (Ahmed et al., 2023; Dinda et al., 2017;

Sharma et al., 2023; Siriwatanametanon et al., 2010; Sun et al., 2018). In the Aurvedic system, *O. indicum* has been documented for its application as an adaptogenic remedy, renal stimulant, hepatoprotective, and astringent compound (Deka et al., 2013; Dinda et al., 2015).

Current literature has further endorsed the therapeutic properties of *O. indicum*. The antioxidant, cardiac function-preserving, anti-inflammatory, antidiabetic, antimicrobial, antiviral, and anticancer properties of *O. indicum* have been extensively demonstrated (Mairuae et al., 2019; Menon et al., 2022; Pondugula et al., 2022). Lalrinzuali et al. (2018) observed that ethanolic extracts of *O. indicum* wound closer via inhibition of molecular pathways of tissue regeneration. Parvin et al. (2023) examined the efficacy of *O. indicum* stem bark extract in inhibiting oral cancer. The above study reported that the stem bark extract suppresses cancer cell proliferation by interfering with signaling pathways. Furthermore, Reis et al. (2023) showed the antiviral potential of *O. indicum* against Chikungunya, Zika, and Mayaro viruses.

2.2. Phytochemical Constituents

Phytochemicals are mainly secondary metabolites and contribute to the medicinal properties of plants. These phytochemicals have been used extensively to treat several diseases and chronic conditions (Koche et al., 2016). Different plant parts, including leaves, roots, stems, bark, gums, flowers, fruits, and resins, have been examined for phytochemical extraction (Altemimi et al., 2017).

A diverse array of bioactive compounds (e.g., flavonoids, tannins, alkaloids, saponins, sterols, fats, oils, lignin, and phenolic acids) have been extracted from *Oroxylum indicum* (Rathod et al., 2022). *O. indicum* is widely recognized for its high amounts of flavonoids. Notable compounds identified in *O. indicum* include the following (Figure 3).

- **Flavonoids:** Oroxylin A, baicalin, chrysin, scutellarin, baicalein, daucosterol, baicalein-6-O-glucuronide, baicalein-7-O-glucoside, baicalein-7-O-glucuronide, chrysin-diglucoside, chrysin-7-O-glucuronide.

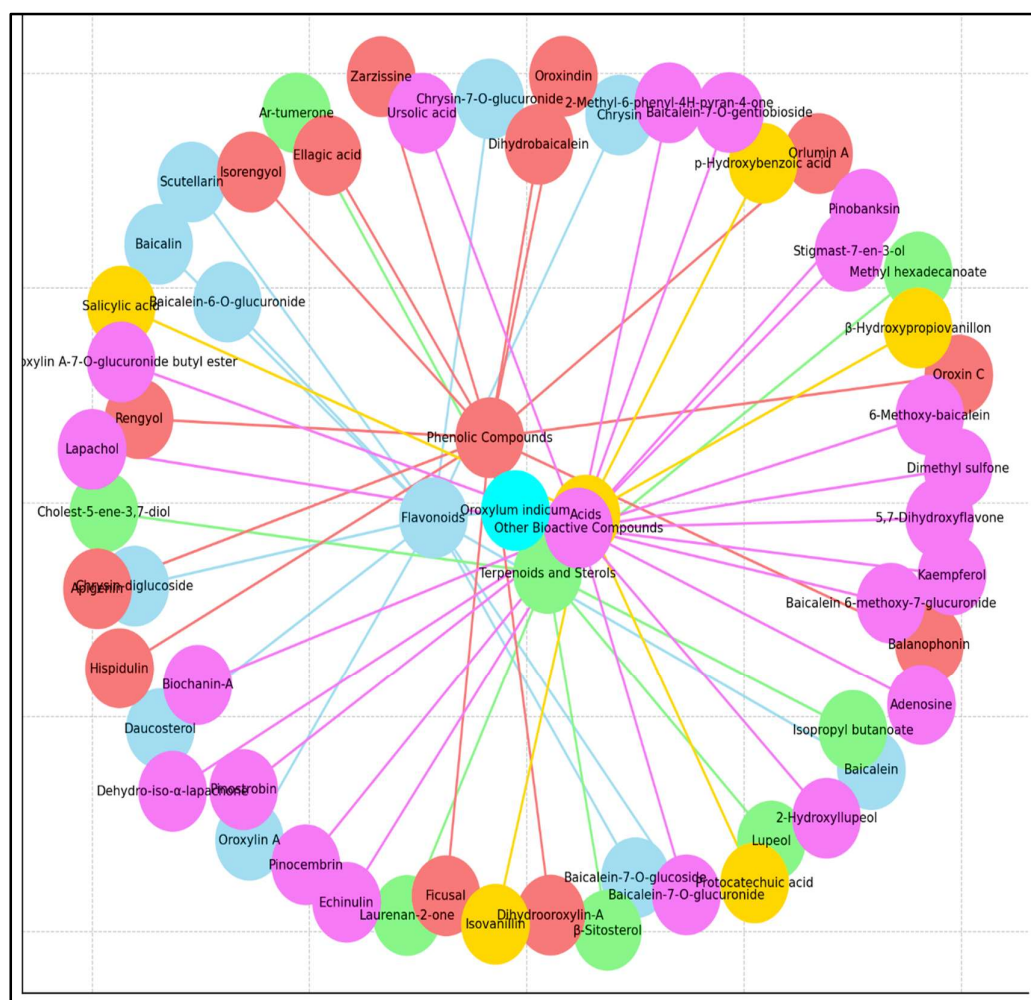


Figure 3. The phytochemical constituents of *Oroxyllum indicum* are categorized by chemical groups.

- **Terpenoids and sterols:** β -sitosterol, lupeol, ar-tumerone, methyl hexadecanoate, laurenan-2-one, isopropyl butanoate, cholest-5-ene-3,7-diol.
- **Phenolic compounds:** Ellagic acid, dihydrobaicalein, dihydrooroxylin-A, oroxin C, orlumin A, oroxindin, hispidulin, apigenin, ficalin, balanophonin, rengyol, isorengyol, and zarzissine.
- **Acids:** Salicylic acid, *p*-hydroxybenzoic acid, protocatechuic acid, isovanillin, and β -hydroxypropiovanillin.
- **Other bioactive compounds** included pinostrobin, stigmast-7-en-3-ol, 3,4',5,7-tetrahydroxy flavonol (kaempferol), 5,6,7-trimethoxyflavone-8-O- β -D-glucopyranoside, oroxylin A-7-O- β -D-glucuronide butyl ester, 6-methoxy-baicalein, 5,7-dihydroxyflavone, baicalein 6-methoxy-7-glucuronide, dehydro-iso- α -lapachone, lapachol, biochanin-A, adenosine, ursolic acid, echinulin, pinocembrin, pinobanksin, 2-methyl-6-phenyl-4H-pyran-4-one, 2-hydroxyllupeol, dimethyl sulfone, baicalein-7-O-gentiobioside, and baicalein-7-O-glucuronide.

2.3. Liver Diseases and Diclofenac-Induced Hepatotoxicity

2.3.1. Hepatotoxicity

The liver is an essential organ that performs several metabolic functions. The liver controls several biological functions, such as carbohydrate, protein, and fat metabolism; detoxification; secretion of bile; and storage of vitamins. However, the liver is at risk of hepatotoxicity induced by xenobiotics including several drugs (Campbell, 2006).

Hepatotoxicity describes the hepatic injury caused by chemicals, particularly drugs. Drug-induced hepatic injury (DILI) is a major contributor to hospitalization. Hepatic toxicity accounts for approximately 50% of cases of acute liver failure (Andrade et al. 2019). Oxidative stress is a core mechanism associated with DILI (Pandit, 2012). The summary of diclofenac-induced hepatotoxicity and various liver diseases is illustrated in Figure 4.

2.3.2. Common Liver Diseases

Hepatitis

Both infectious (parasites, bacteria, fungi, and viruses) and non-infectious factors (metabolic disorders, autoimmune diseases, alcohol consumption, and certain drugs) can induce hepatitis. Hepatitis A, B, C, D, and E, along with strains such as Hepatitis X and G, have been reported as primary hepatitis viruses (Pallavi et al., 2017). Hepatitis B and C mainly spread through the infected blood, unprotected sexual contact, and mother-to-child transmission. Common symptoms include fever, jaundice, dark urine, fatigue, nausea, loss of appetite, diarrhea, and abdominal pain (Gholizadeh et al., 2023; Pallavi et al., 2017).

Cirrhosis

Diseases such as alcoholism, hepatitis, and fatty liver disease can result in cirrhosis, an advanced stage of liver fibrosis. The common symptoms include fatigue, jaundice, and swelling of the abdomen and legs. The pathological signs of cirrhosis include portal hypertension, ascites, variceal bleeding, hepatic encephalopathy, and impaired liver function.

Liver cancer

Hepatocellular carcinoma (HCC) is a liver malignancy that has been reported worldwide (Aguayo and Patt, 2001; Yang et al., 2019). It commonly develops in patients with a history of cirrhosis or hepatitis. Symptoms commonly include weight loss, fatigue, abdominal pain, anorexia, constipation, ascites, and jaundice (Barber and Nelson, 2000; Chen et al., 2024). Liver transplantation, chemotherapy, radiation, and gene therapy are the common treatments for liver cancer (Mokdad et al., 2016).

Fatty liver disease

Fatty liver disease is classified into alcoholic fatty liver disease (AFLD) and nonalcoholic fatty liver disease (NAFLD). About 25% of the global population. NAFLD is associated with obesity, diabetes, and metabolic syndromes. NAFLD can



Figure 4. Summary of liver diseases and diclofenac-induced hepatotoxicity

lead to non-alcoholic steatohepatitis (NASH), cirrhosis, or hepatocellular carcinoma (Farrell and Larter, 2006; Zoller and Tilg, 2016).

Autoimmune liver diseases

Autoimmune liver diseases are triggered when the immune system wrongly attacks the liver cells, leading to chronic inflammation and damage. These diseases include primary biliary cholangitis (PBC), primary sclerosing cholangitis (PSC), and autoimmune hepatitis (AIH). Treatment typically involves immunosuppressive therapy to slow the disease progression (Liberal and Grant, 2016).

Genetic liver disorders

Hemochromatosis (excess iron accumulation) and Wilson disease (copper overload) may contribute to liver impairment. These diseases must be diagnosed early and managed to prevent liver damage (Pak et al., 2021).

2.3.3. Role of oxidative stress in liver injury

Oxidative stress plays a key role in liver damage. It is mainly caused by excessive generation of reactive oxygen species (ROS) during normal cellular metabolism as well as during stress conditions. In hepatic cells, ROS levels increase several-fold after alcohol consumption and viral infections (George et al., 2024). Furthermore, ROS (such as superoxide anions and hydroxyl radicals) can cause lipid peroxidation (Jomova et al., 2023; Ranneh et al., 2017). Prolonged oxidative stress can ultimately lead to hepatic cancer (Chen et al., 2020).

2.3.4. Diclofenac: hepatotoxicity and mechanisms of action

Diclofenac is a broadly applied nonsteroidal anti-inflammatory drug (NSAID). It is prescribed for arthritis, muscle pain, and menstrual cramps (Altman et al., 2015; Bindu et al., 2020; Zacher et al., 2008). Diclofenac mainly suppresses cyclooxygenase enzymes (COX-1 and COX-2) and decreases prostaglandin synthesis. Although COX-2 inhibition enhances anti-inflammatory properties, COX-1 suppression may cause gastrointestinal problems (Gan, 2010; Kaur and Singh, 2022; Peesa et al., 2016). As shown in Figure 5, cyclooxygenase enzymes regulate the conversion of arachidonic

acid into prostaglandins, which mediate inflammation and other physiological responses.

The hepatotoxic mechanism of diclofenac is primarily associated with Cytochrome P450 (CYP2C9). It participates in the oxidative stress pathway (Bort et al. 1999). Diclofenac triggers liver inflammation by initiating immune-mediated hypersensitivity (Selvaraj et al., 2017; Allameh et al., 2023; Banerjee et al., 2023). The mechanism of diclofenac toxicity also involves mitochondrial dysfunction (Jung et al., 2020; Syed et al., 2016). Furthermore, increased levels of hepatic enzymes such as ALT, AST, and ALP indicate diclofenac-induced hepatotoxicity (Boelsterli, 2003; Rani et al., 2024). Prolonged diclofenac intake increases the probability of gastrointestinal bleeding, renal impairment, and cardiovascular conditions (Shah and Sorathia, 2020; Hsu et al., 2015).

2.4. Need for Alternative Hepatoprotective Agents

The need for alternative hepatoprotective agents has become increasingly urgent owing to the increasing prevalence of liver diseases worldwide. While conventional treatments exist, they often have limitations, such as side effects, drug resistance, and limited efficacy in certain cases. Most of the available hepatoprotective therapies are disease-specific and have limited efficacy against broad-spectrum liver injuries. For example, medications effective for alcoholic liver disease may not be beneficial for viral hepatitis or NAFLD, necessitating condition-specific interventions (Gao and Bataller, 2011). Additionally, the complex nature of liver disorders and diverse mechanisms of hepatotoxicity call for a more comprehensive approach to liver protection. Certain hepatoprotective agents are expensive and inaccessible, particularly in low-resource settings. This economic burden restricts treatment availability, exacerbates health disparities, and prevents patients from receiving adequate liver disease management. Affordable and widely available alternatives are crucial for addressing this gap.

Natural compounds and herbal remedies have shown promise as potential alternatives, offering a wide range of bioactive components with antioxidant, anti-

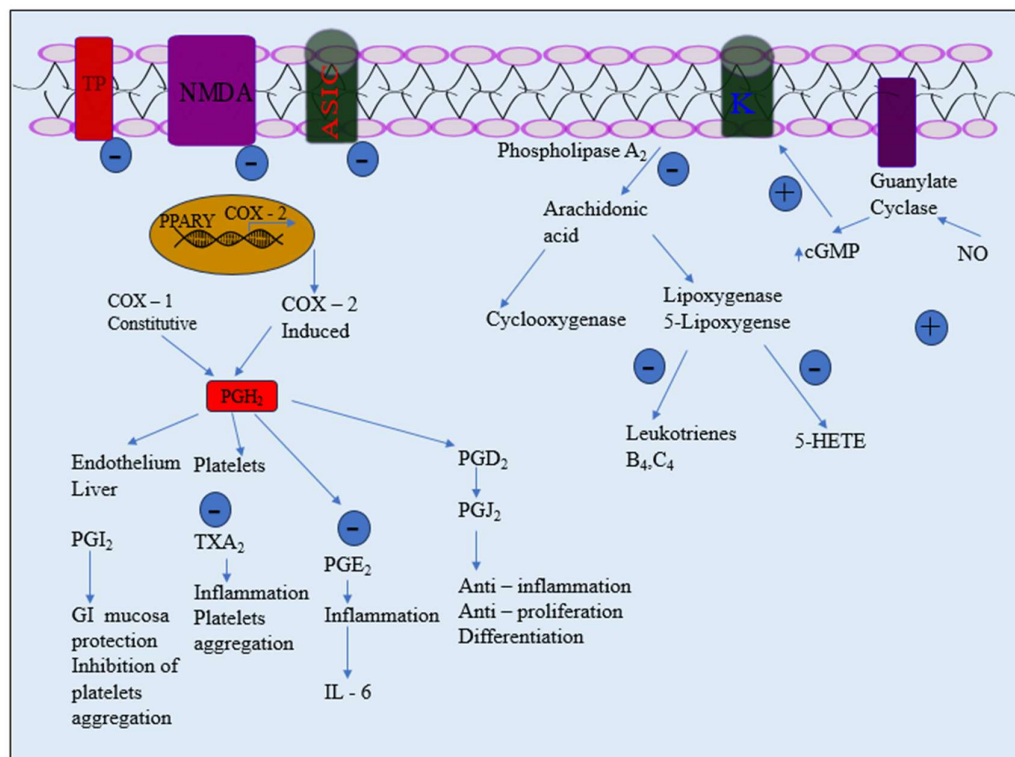


Figure 5. The diagram illustrates known, potential, and newly discovered mechanisms through which diclofenac operates. Circular markers denote areas where the drug is believed to exert either an inhibitory (-) or stimulatory (+) effect. ASIC, acid-sensing ion channel; cGMP, cyclic guanosine monophosphate; COX-1, cyclooxygenase-1; COX-2, cyclooxygenase-2; GI, gastrointestinal; 5-HETE, 5-hydroxyeicosatetraenoic acid; IL-6, interleukin-6; K, ATP-sensitive potassium channel; NMDA, N-methyl-D-aspartate receptor; NO, nitric oxide; PGD₂, prostaglandin D₂; PGE₂, prostaglandin E₂; PGH₂, prostaglandin H₂; PGI₂, prostaglandin I₂; PGJ₂, prostaglandin J₂; PPARγ, peroxisome proliferator activated receptor gamma; TP, thromboxane-prostanoid receptor; TXA₂, thromboxane A₂.

inflammatory, and regenerative properties (Parham et al., 2020). These alternative agents may provide safer, more accessible, and potentially more effective options for the prevention and treatment of liver damage. Furthermore, the development of novel hepatoprotective agents could address the growing need for personalized medicine in liver care, targeting specific pathways and addressing individual patient needs. As research in this field progresses, it is crucial to explore and validate alternative agents through rigorous scientific studies to ensure their safety and efficacy in clinical applications. Integrating traditional medicine with modern scientific approaches could lead to innovative hepatoprotective therapies that combine the best of both. Such integrative strategies may enhance the overall effectiveness of liver treatments, while potentially reducing the side effects associated with conventional medications. Additionally, investigating the synergistic effects of natural compounds and their interactions with pharmaceutical drugs could pave the way for a more comprehensive and tailored liver-protection protocol.

1.6. Bioactive Compounds Isolated from *Oroxylum indicum* and their Bioactivities: Literature Review

A comprehensive literature review of bioactive compounds isolated from *O. indicum* has revealed a diverse array of phytochemicals with significant potential therapeutic applications in various medical fields. Flavonoids are a major group of bioactive compounds in *O. indicum*. The plant is particularly abundant in baicalein, chrysin, and oroxylin A, which exhibit significant anti-inflammatory, antioxidant, and anticancer properties in numerous studies (Han et al., 2017; Zhu et al., 2013). Specifically, baicalein has demonstrated considerable potential for inhibiting tumor growth and metastasis in several cancer cell lines, including breast, prostate, and lung cancers (Park et al., 2017; Yan et al., 2018; Zheng et al., 2014). Chrysin has been reported to possess anxiolytic and anticonvulsant properties (Coleta et al., 2006), whereas oroxylin A exhibits neuroprotective effects and has the potential to treat neurodegenerative disorders (Jeon et al., 2011).

Anthraquinones represent another significant class of compounds isolated from *O. indicum*. Aloe-emodin and chrysophanol are the primary anthraquinones identified

in the plant. Aloe-emodin and chrysophanol, which are both found in *O. indicum*, exhibit significant antimicrobial and anticancer properties (Guo et al., 2008). Aloe-emodin exerts potent anticancer effects on gastric cancer cells by inhibiting cell proliferation, arresting the cell cycle at the G2/M phase, and suppressing c-Myc expression (Guo et al., 2008). Recent studies have shown that Aloe-emodin (AE) exerts significant antineoplastic properties in different cancer cell lines via triggering apoptosis and inhibiting angiogenesis. In colon cancer cells, AE inhibited cell proliferation by arresting the cell cycle at the G2/M phase and activating caspases 9 and 6, with caspase-6 playing a crucial role in apoptotic execution (Suboj et al., 2012).

The alkaloid content in *O. indicum* has received considerable attention. Several alkaloids, including oroxylin B, have been identified in plants (Chen et al., 2003) and have demonstrated potent antimicrobial and antifungal properties, suggesting their potential applications in the development of new antibiotics and antifungal agents. Some alkaloids isolated from *O. indicum* have also shown promise for the treatment of neurological disorders because of their ability to modulate neurotransmitter systems.

Phenolic compounds constitute an important group of bioactive substances in *O. indicum*. Ellagic acid and gallic acid were among the primary phenolic compounds identified in *O. indicum*. These substances are known for their strong antioxidant activities and potential to prevent chronic diseases. Ellagic acid, in particular, has shown promise for the prevention and treatment of various types of cancer, cardiovascular diseases, and neurodegenerative disorders (Zuccari et al., 2020). In contrast, gallic acid has hepatoprotective and antidiabetic properties in several studies. Studies have shown that GA protects the liver from damage caused by carbon tetrachloride (CCl₄) (Bhattacharyya et al., 2013), paracetamol (Rasool et al., 2010), N-nitrosodiethylamine (NDEA), and anti-tuberculosis agents (Karimi-Khouzani et al., 2017).

Terpenoids isolated from *O. indicum*, particularly β -sitosterol and stigmasterol, have been the subject of numerous studies. These compounds have demonstrated significant anti-inflammatory and cholesterol-lowering effects in various experimental models. Both compounds have been shown to ameliorate non-

alcoholic fatty liver disease (NAFLD) induced by a high-fat Western-style diet (HFWD) in mice, with stigmasterol being more effective than β -sitosterol (Feng et al., 2018). β -sitosterol has shown potential in treating benign prostatic hyperplasia (Berges et al., 1995) and may play a role in preventing colon cancer (Novotny et al., 2017). In contrast, stigmasterol has demonstrated potential as an antioxidant, hypoglycemic, and thyroid-inhibiting agent. Studies have shown that stigmasterol administration reduces serum triiodothyronine (T3), thyroxine (T4), and glucose concentrations, while increasing insulin levels, indicating its thyroid-inhibiting and hypoglycemic properties (Panda et al., 2009).

Naphthalene glycosides are a unique class of compounds found in *Oryza indicum*. Oroxindin and oroxin B are the primary naphthalene glycosides isolated from the plant. These compounds have shown potential in the treatment of respiratory disorders and possess anti-inflammatory properties. Recent studies have suggested that these compounds may have bronchodilatory effects and be beneficial in the management of asthma and other respiratory conditions (Liu et al., 2020). Oroxin B has been shown to be effective in treating metabolic-associated fatty liver disease (MAFLD) by modulating gut microbiota and strengthening the intestinal barrier (Huang et al., 2023).

Recent studies have increasingly focused on the synergistic effects of these compounds and their potential applications in the treatment of various diseases including cancer, diabetes, and inflammatory disorders (Zhang et al., 2017). The complex interplay between different phytochemicals in *O. indicum* may contribute to its overall therapeutic effects, highlighting the importance of studying the plant as a whole rather than isolating individual compounds.

Although the potential therapeutic applications of bioactive compounds from *O. indicum* are promising, it is important to note that many of these findings have been based on *in vitro* and animal studies. Further research, including properly structured clinical studies, are necessary to fully elucidate mechanisms of action, efficacy, and safety of these compounds in humans. Studies of the bioavailability, pharmacokinetics, and potential drug interactions of these compounds are crucial for their development as therapeutic agents.

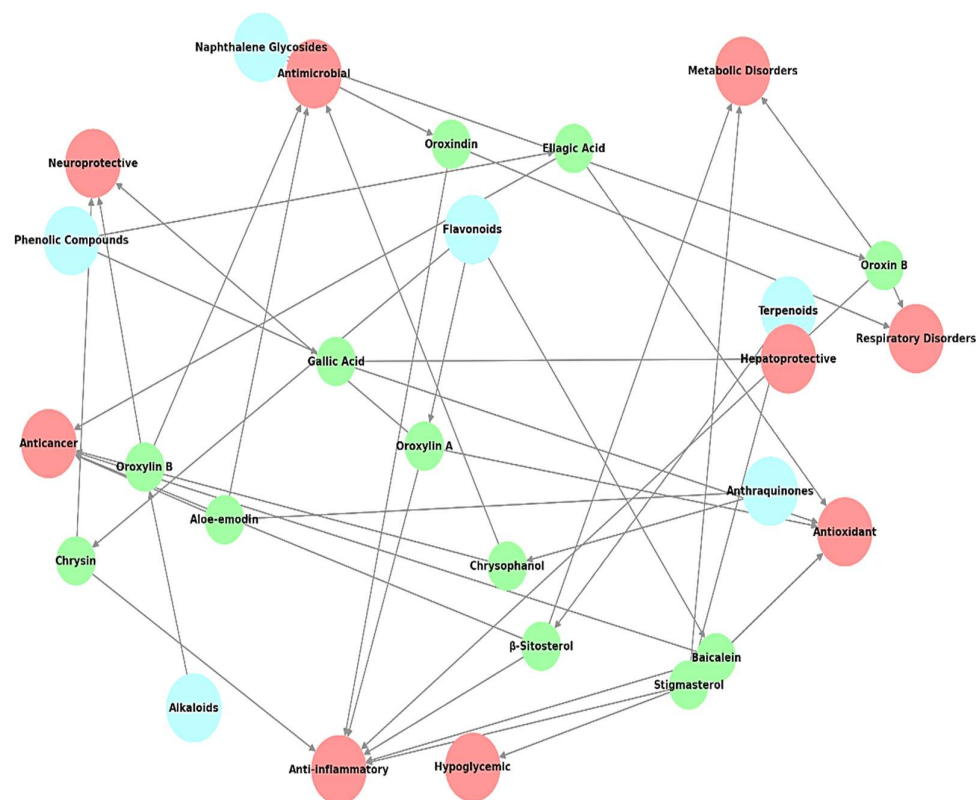


Figure 6. Bioactivities of *Oroxyllum indicum*. The diagram presents a network of bioactive compounds found in *O. indicum* and their associated therapeutic applications. The bioactive compounds are categorized into six major groups: 1. flavonoids (baicalein, chrysin, oroxylin A), 2. Anthraquinones (aloe-emodin, chrysophanol), 3. Alkaloids (oroxylin B), 4. Phenolic compounds (ellagic acid, gallic acid), 5. Terpenoids (β -sitosterol, stigmasterol) and 6. Naphthalene glycosides (oroxindin, oroxin B). Each category is linked to specific compounds, which in turn are associated with various therapeutic applications, such as anti-inflammatory, antioxidant, anticancer, neuroprotective, antimicrobial, hepatoprotective, hypoglycemic, and respiratory disorder treatments. Light blue nodes $\rightarrow$ Categories of bioactive compounds. Light green nodes $\rightarrow$ Specific bioactive compounds, Light coral nodes $\rightarrow$ Therapeutic applications.

1.7. *In Vivo* Studies on Bioactivities of *Oroxylum indicum*: Review

In vivo studies have demonstrated several bioactivities of *O. indicum* extracts and their constituents. Oroxylin A, a flavonoid found in *O. indicum*, demonstrated anti-allergic properties in a mouse model of ovalbumin-induced allergic asthma. This compound reduces the accumulation of inflammatory cells, especially eosinophils, lowers Th2 cytokine levels, and alleviates inflammatory indicators and mucin production in the lungs (Lee et al., 2016). Research has also revealed that Oroxylin A possesses anti-inflammatory effects beyond its impact on allergic asthma (Zhou et al., 2016; Li et al., 2016). Studies have shown that this compound can suppress the production of proinflammatory mediators in diverse cell types and animal models. Moreover, Oroxylin A shown promise in regulating immune responses, indicating its potential for broader therapeutic use in inflammatory conditions. Research has demonstrated that Oroxylin A, a naturally occurring flavonoid, can influence the immune response through various pathways. It triggers the release of apoptosis-related extracellular vesicles in hepatocellular carcinoma (HCC) cells, which in turn regulates the M1-like polarization of macrophages. Furthermore, Oroxylin A reduced the number of M2-like macrophages and promoted T-cell infiltration within the tumor microenvironment. These actions inhibit HCC progression and improve the effectiveness of immune checkpoint inhibitor treatments in mouse models (Wang et al., 2023).

Research has shown that *O. indicum* (OISE) boost the effectiveness of acarbose in lowering postprandial blood sugar levels in both normal and diabetic mice. OISE doses ranging from 50 to 200 mg kg⁻¹ were found to increase acarbose potency by as much as 5 times (Zhang et al., 2017).

Studies have revealed that raw extracts and compounds isolated from *O. indicum* have a broad range of pharmacological effects in living organisms. These include reducing inflammation, alleviating arthritis, fighting cancer, healing ulcers, protecting the liver, managing diabetes, and treating diarrhea (Dinda et al., 2014).

It is worth noting that while scientific studies have confirmed many traditional uses of *O. indicum*, some claimed benefits require further investigation. Additionally, safety assessments of water- and alcohol-based extracts from various parts of the plant,

including stem bark, root bark, and fruits, have shown them to be non-toxic (Dinda et al., 2014).

In conclusion, *O. indicum* and its components, especially flavonoids, such as baicalein, have shown promising biological activities in animal studies. However, additional clinical trials are needed to verify the effectiveness and safety of these substances as potential treatments in humans (Dinda et al., 2014; Salleh et al., 2020).

1.8. Research Gap and Rationale for the Study

Despite the widespread use of diclofenac as a non-steroidal anti-inflammatory drug (NSAID), its hepatotoxic effects are well-documented. However, research exploring alternative therapies, particularly herbal remedies, such as *Oroxylum indicum*, remains limited. While some studies have indicated that *O. indicum* possesses antioxidant and anti-inflammatory properties, comprehensive evidence supporting its hepatoprotective effects in diclofenac-induced liver injury is still lacking. There is a critical gap in understanding how bioactive compounds from *O. indicum* interact at the molecular level with the pathways involved in hepatotoxicity and liver regeneration. Elucidating these mechanisms may significantly enhance the therapeutic potential of this herbal remedy. Additionally, the comparative efficacy of *O. indicum* with conventional hepatoprotective agents has not been thoroughly investigated, leaving uncertainties regarding its clinical viability.

Given the widespread use of diclofenac and its associated hepatotoxic risks, identifying effective hepatoprotective agents such as *O. indicum* is of critical importance. The growing interest in natural therapeutic alternatives further underscores the relevance of exploring the hepatoprotective effects of this medicinal plant, aligning with the broader trend of integrating complementary and herbal medicines into modern healthcare. Validating the protective effects of *O. indicum* has substantial public health implications as it could contribute to reducing drug-induced liver injury, improving patient safety, and optimizing treatment outcomes. Scientific validation of traditional medicinal claims is essential to ensure the integration of herbal remedies into evidence-based medicine, necessitating robust experimental and clinical studies. Additionally, investigating the biochemical and molecular mechanisms

underlying the hepatoprotective effects of *O. indicum* will enhance the pharmacological understanding and facilitate its potential application in liver disease management. Thus, this study aimed to bridge these research gaps by providing comprehensive mechanistic insights and scientific validation of *O. indicum* as a potential hepatoprotective agent against diclofenac-induced liver toxicity.

1.9. Objectives

1. To evaluate safety profile of the *Oroxylum indicum* stem bark extracts in rat model.
2. To analyze the phytochemical profile using aqueous and ethanolic stem bark extracts of *Oroxylum indicum*.
3. To evaluate the effect of *Oroxylum indicum* extracts on oxidative status in the rats treated with diclofenac.

1.10. Hypothesis

The hypothesis of this study was that *Oroxylum indicum* possesses significant hepatoprotective effects against diclofenac-induced liver toxicity through its antioxidant, anti-inflammatory, and cytoprotective mechanisms. It has been proposed that bioactive compounds in *O. indicum*, particularly flavonoids and alkaloids, mitigate oxidative stress, reduce inflammatory cytokine levels, and enhance liver regeneration by modulating key molecular pathways. This study hypothesizes that treatment with *O. indicum* extract results in lower liver enzyme markers (ALT, AST, and ALP), reduced histopathological damage, and improved antioxidant defense compared to untreated hepatotoxic models, demonstrating its potential as a natural hepatoprotective agent.

1.11. Significance

The relevance of present research rests in its potential to establish *Oroxylum indicum* as a natural hepatoprotective agent against diclofenac-induced liver toxicity, thereby addressing the growing need for safer and more effective alternatives to conventional hepatoprotective drugs. By investigating the antioxidant, anti-inflammatory, and cytoprotective mechanisms of *O. indicum*, this study aimed to provide scientific

validation of its traditional medicinal use and contribute to the development of evidence-based herbal therapies. These findings could have important clinical and public health implications, offering insights into alternative strategies for managing drug-induced liver injury and promoting the integration of plant-based hepatoprotective agents into modern medical practices.

CHAPTER - 2

MATERIALS AND METHODS

2. Reagents and Chemicals

Chemicals employed in the current investigation were of research-grade and procured from Himedia, ensuring the highest quality and reliability in our experimental procedures. These substances encompass 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azinobis-3-ethylbenzothiozoline 6-sulfonic acid (ABTS), gallic acid (GA), quercetin, butylated hydroxy toluene (BHT), potassium ferricyanide, methanol, ethanol, trichloroacetic acid (TCA), ferric chloride, sodium carbonate (Na_2CO_3), dipotassium, Folin Ciocalteu reagent (FCR), hydrogen phosphate, acetyl acetone, aluminum chloride, potassium persulfate, sodium nitroprusside, naphthyl ethylene diamine dihydrochloride, sulfanilic acid, ammonium acetate, ferrous ammonium sulfate, EDTA, glacial acetic acid, DMSO, phosphoric acid, potassium dihydrogen phosphate (KH_2PO_4), phenazine methosulphate (PMS), hydrogen peroxide (H_2O_2), sodium hydroxide (NaOH), copper sulphate (CuSO_4), acetic acid, nitro blue tetrazolium chloride (NBT), disodium hydrogen phosphate (Na_2HPO_4), n-butanol, nicotinamide adenine dinucleotide reduced disodium salt (NADH), reduced glutathione (GSH), di-potassium phosphate (K_2HPO_4), thiobarbituric acid (TBA), cobalt (II) solution, sodium hexametaphosphate (NaPO_3)₆ and sodium bicarbonate (NaHCO_3). Absolute alcohol, xylene, wax, saturated picric acid, formalin, gallic acid, eosin, hematoxylin, ammonia water, n-butanol, sodium bicarbonate (NaHCO_3), reduced glutathione (GSH), cobalt (II) solution, sodium chloride, potassium phosphate (K_2HPO_4), potassium phosphate (KH_2PO_4), acetic acid, sodium hexametaphosphate (NaPO_3)₆, diclofenac and silymarin (Civil Hospital, Drug store, Mizoram).

3. Sample Collection

The non-infected stem bark of *O. indicum* (L.) Kurz (Sonapatha in Hindi, Archangkawm in Mizo) was collected from the Tanhril area of Aizawl, Mizoram, India, in February 2021. The Department of Botany at Mizoram University

authenticated the plant material catalogued under ACS No. MZUBOT:0088. The nomenclature was confirmed using 'World Flora Online' (<https://www.worldfloraonline.org/taxon/wfo-0000779181>).

3.1. Extract Preparation

The stem bark was washed thoroughly, chopped into small pieces, and air-dried. The bark was dried and ground into powder using an electric grinder. Powdered bark was kept in aseptic, airtight containers to maintain quality. Approximately 30 g of powdered bark was subjected to Soxhlet extraction at 40-50 °C temperatures (Rebaya et al., 2015). The ethanol and aqueous extracts were strained using Whatman filter paper no. 1. Extracts were dehydrated in a vacuum concentrator at 45 °C. Resulting concentrated extracts were stored in sterile containers in a refrigerator to maintain stability. The ethanol and aqueous extractions yielded 8 % and 9.7 % (w/w), respectively, indicating the success of the extraction process.

3.2. Qualitative Phytochemical Screening

The ethanolic and aqueous extracts of *O. indicum* stem bark were screened for the presence of various classes of phytochemicals, including phenols, flavonoids, tannins, saponins, glycosides, terpenoids, alkaloids, steroids, and phlobatannins, using standardized protocols (Deyab et al., 2016; Kale et al., 2018). The extracts were dissolved in corresponding solvents before analysis and sonicated.

3.2.1. Phenol test

A small amount of 15 % sodium carbonate and Folin's solution were introduced to 2 mL of each extract. Development of a dark green color suggested the occurrence phenolics.

3.2.2. Flavonoid test

Zinc hydrochloride test: Upon addition of hydrochloric acid and zinc dust to 2 mL of the plant extract, red color formation indicated the presence of flavonoids.

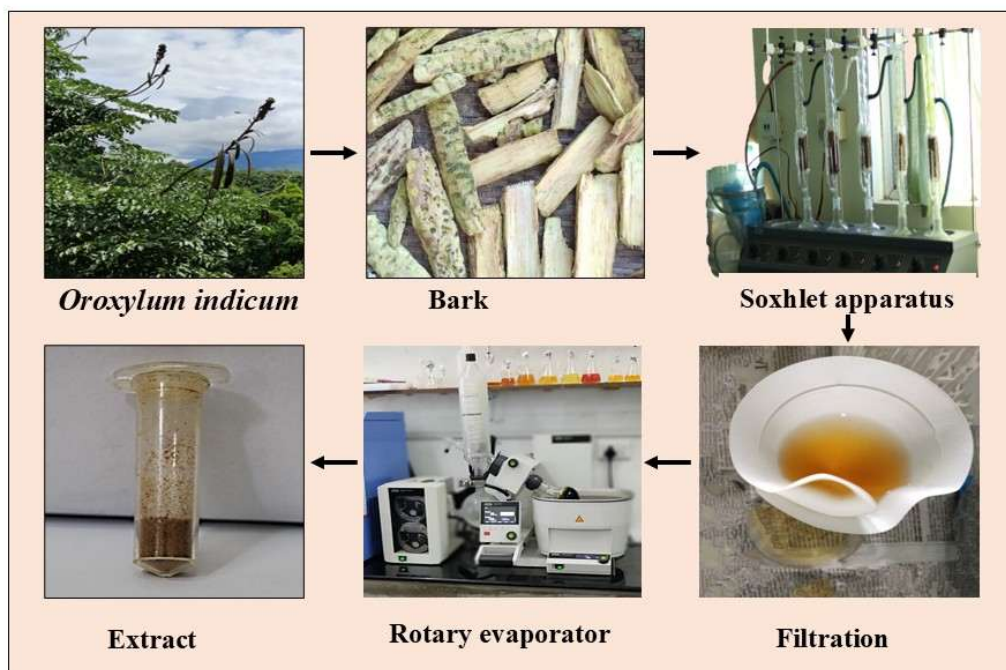


Figure 7. Phytochemicals were extracted from the shade-dried sterile small stem bark pieces of *Oroxyllum indicum* using ethanolic and aqueous solvents in a Soxhlet apparatus maintaining a temperature range of 40–50 °C. Extract were filtered using Whatman filter paper and dried using rotatory evaporator at 50 °C to obtain solid extract.

3.2.3. Tannin test

To detect the presence of tannins, approximately 2 mL of the filtrate was combined with an equal volume of Milli-Q water. Ferric chloride was added dropwise. Presence of green coloration confirmed existence of tannins in the sample.

3.2.4. Alkaloids test

Dragendroff's and Mayer's test was done by shaking a few drops of the respective reagent with 2 mL of every extract. Alkaloids were indicated by a reddish-brown precipitate in the presence of Dragendroff's reagent and a yellow or white precipitate when Mayer's reagent was present.

3.2.5. Saponin test

Approximately 3 mL of distilled water combined to 3 mL plant extract, and the solution was stirred for 10 minutes. Formation of a stable layer of foam indicates saponin.

3.2.6. Glycosides

Under the Lugal test, 2 mL of a mixture containing pyridine and sodium nitroprusside was added to 2 mL extract. Then, 2 mL of NaOH was introduced sequentially, with the development of a pink color to confirm the presence of glycoside.

3.2.7. Terpenoids

Approximately 2 mL of chloroform was combined with 5 mL of the plant extract and evaporated in a water bath. The mixture was then heated with 3 mL H_2SO_4 . The formation of a gray color suggests existence of terpenoids in extracts.

3.2.8. Phlobatannins

A volume of roughly 5 mL of 1 % HCl solution was introduced to each 10 mL extract. The resulting mixture was then heated in a boiling water bath. The production of a red precipitate suggested affirmative result.

3.3. Quantitative Phytochemical Screening

3.3.1. Total flavonoid content

Principle

Aluminum chloride (AlCl_3) forms acid-stable complexes with the C-4 keto groups and either the C-3 or C-5 hydroxyl groups of flavones and flavonols (Zou et al., 2004).

Reagents

5 % NaNO_3 , 10 % AlCl_3 , 1 M NaOH

Procedure

1. A stock solution of quercetin was created by dissolving 1 mg mL^{-1} in methanol. This solution was then diluted to create a range of concentrations from 10 to $100 \text{ } \mu\text{g mL}^{-1}$.
2. To create stock solutions, 5 mg dried extract was dispersed in 5 mL of methanol.
3. The experiment utilized 0.5 mL extract from the base solution. Every quercetin dilution was tested in triplicate in separate test tubes.
4. Then to all test tube added 1.5 mL CH_3OH , 0.3 mL AlCl_3 , 2 mL NaOH and 2.7 mL of Milli-Q water. The samples were kept at ambient temperature for 60 minutes.
5. Using a blank as a reference, the optical density was determined at 510 nm.
6. The standard curve was constructed using quercetin's absorbance, and the sample's unknown flavonoid concentration was determined through a linear equation.

3.3.2. Total phenol content

Principle

The Folin-Ciocalteu assay operates on the premise of FCR (Folin–Ciocalteu reagent) reduction when exposed to phenolic compounds, leading to the formation of molybdenum–tungsten blue. This product was measured spectrophotometrically at 765 nm and its intensity increased linearly with the concentration of phenolic compounds in the reaction medium (Alhakmani et al., 2013).

Reagents

Folin Ciocalteu reagent (FCR): 10-fold diluted, 0.7 M sodium carbonate.

Procedure

1. An initial solution of gallic acid was created by dissolving 1 mg mL⁻¹ in ethanol. This stock solution was then further diluted to produce a range of concentrations from 10 to 100 µg mL⁻¹.
2. To create stock solutions, 5 mg extract was dispersed in 5 mL of ethanol.
3. The experiments were conducted using 0.5 mL of each extract from the stock solution, along with all gallic acid dilutions, which were individually placed in test tubes in triplicate.
4. Following this, 5 mL of FCR and 4 mL of Na₂CO₃ were introduced into each test tube and kept for another 1 hour.
5. Using an appropriate control, OD was determined at a wavelength of 765 nm.
6. The calibration curve was established using gallic acid standards to quantify the total phenol content of the samples.

Total tannin contents

Principle

Tannins are polyphenolic compounds that react with vanillin (4-hydroxy-3-methoxybenzaldehyde) under acidic conditions to form colored complexes. The intensity of the resultant color was directly proportional to the tannin content of the sample (Islam et al., 2015).

Reagent

A mixture of Folin-Ciocalteu reagent (diluted 1:10) and Na₃CO₃ solution (20 % w/v).

Procedure

1. A range of tannic acid standard solutions with known concentrations from 10-100 µg mL⁻¹ were created using distilled water.
2. Individual test tubes were filled with 0.5 mL of each standard solution and the sample.
3. To each tube, 2.5 mL of diluted Folin-Ciocalteu reagent was introduced.
4. Subsequently, 2 mL of Na₂CO₃ was transferred to each tube.

5. The process continued for half an hour at room temperature in the absence of light.
6. Using a spectrophotometer, absorbance of standards was determined at 765 nm.
7. A standard curve was generated by plotting the absorbance values against the known concentrations.
8. The total tannin content was determined by comparing the sample's absorbance to the standard curve. Results are presented as tannic acid equivalents (TAE).

3.4. Antioxidant Activity Assessment: *In Vitro* Experiments

3.4.1. Estimation of activities of antioxidants by DPPH scavenging method

Principle

The antioxidant assay known as the DPPH free radical method utilizes 2, 2-diphenyl-1-picryl-hydrazyl-hydrate. DPPH produces a violet color in ethanol, which demonstrates its electron transfer capability. The DPPH assay is an efficient and expeditious method for evaluating antioxidants using spectrophotometry (Leong & Shui, 2002).

Reagents

DPPH reagent: 0.10 mM (3.94 mg in 100 mL of methanol), BHT (standard): 1 mg mL⁻¹ in ethanol.

Procedure

1. The stock solution 1 mg mL⁻¹ was diluted to concentrations ranging from 10 to 100 µg mL⁻¹ of either the standard or the extracts.
2. A volume of 2 mL of the DPPH radical solution was pipetted into all test tubes.
3. The samples were then kept in darkness for a duration of 30 minutes at ambient temperature.
4. Using a UV-VIS spectrophotometer, the absorbance was recorded at a wavelength of 517 nm.
5. Following equation was employed to compute radical scavenging activity:

$$\% \text{ Free radical scavenging activity} = \frac{A_c - A_s}{A_c} \times 100$$

In this equation, A_c denotes the optical density of the control, while A_s indicates the optical density of the sample.

6. The IC_{50} was derived by graphing % inhibition against the respective concentrations of the extract.

3.4.2. ABTS radical scavenging evaluation

Principle

The ABTS test is commonly employed to evaluate the antioxidant capacity of plant-derived extracts. This method relies on the capability of antioxidant compounds to neutralize the ABTS radical cation ($ABTS^+$). The antioxidant potential of plants leads to the rapid discoloration of the greenish-blue complex (Pellegrini et al., 1998).

Reagents

7 mM $ABTS^+$ solution, 2.45 mM ammonium persulfate, ascorbic acid

Procedure

1. An $ABTS^+$ solution with ammonium persulfate was combined to generate ABTS radicals.
2. Subsequently, mixture was set to incubate at ambient temperature in a dark environment for 12-16 hours. Following this, the solution underwent dilution with methanol to achieve an absorbance reading of 0.700 nm when measured at 734 nm.
4. Various stock dilutions ($10 \mu\text{g mL}^{-1}$ to $100 \mu\text{g mL}^{-1}$) of ascorbic acid standard were prepared in separate test tubes.
5. Approximately $50 \mu\text{g mL}^{-1}$ of each sample was placed in triplicate in test tubes.
6. To evaluate the radical scavenging activity, the aforementioned sample and standard concentrations were mixed with 1.5 mL of the $ABTS^+$ solution that had been diluted.
7. Using a spectrophotometer, the absorbance was recorded at 734 nm following a 30-minute incubation period. The equivalent concentration of ascorbic acid was then determined by referencing the ascorbic acid standard curve.

5. The inhibition percentage of the absorbance at 734 nm was computed using the following equation:

$$\text{Percent inhibition} = \frac{1-A_c}{A_s} \times 100$$

where A_c is the optical density of the control (without plant extract or standard) and A_s is the absorbance of the sample. Each assay included a solvent blank, and all measurements were performed in triplicates to ensure accuracy and reliability.

3.4.3. Hydroxyl radical scavenging activity (HRSA)

Principle

When combined with H_2O_2 in the presence of ascorbic acid, an iron-EDTA complex generates hydroxyl radicals. Upon reaction with deoxyribose, this mixture forms a product that, when heated with thiobarbituric acid at low pH, produces a pink chromogen. Substances that scavenge hydroxyl radicals compete with deoxyribose for these radicals, thereby reducing chromogen formation. By observing the inhibition of color formation, one can determine the rate constant for the interaction between the scavenger and hydroxyl radicals (Klein et al., 1981).

Reagents

Stock solutions of EDTA (1 mM), 17.5 % ice-cold TCA, 0.018 % EDTA, 0.85 % DMSO, 0.1 M phosphate buffer (pH 7.4), 0.22 % ascorbic acid.

Nash reagent: A solution was prepared by combining 7.5 g ammonium acetate, 300 μ L CH_3COOH , and 200 μ L acetyl acetone in 100 mL of Milli-Q water.

Procedure

1. The test tubes were filled with varying amounts of the extracts, ranging from 10 to 100 μ g mL^{-1} .
2. Following this, 1 mL of the iron-EDTA mixture, 0.018 % EDTA, and 1 mL of DMSO were combined with a 0.1 M phosphate buffer at pH 7.4.
3. Ascorbic acid, which serves as an initiator for hydroxyl radical generation, was then introduced. The tubes were sealed tightly and placed in an 85 °C water bath for a 15-minute incubation period.

4. After the incubation was complete, the tube caps were removed, and a cold solution of trichloroacetic acid (17.5 % w/v), used for protein precipitation, was promptly added.

5. Nash reagent, measuring about 3 mL, was introduced into each tube.

6. Following this, the samples were left to incubate at ambient temperature for a quarter of an hour. A spectrophotometer was then employed to determine the absorbance at a 412 nm.

The HRSA percentage was determined employing the following equation:

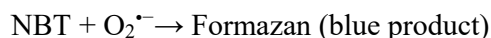
$$\text{HRSA (\%)} = \frac{A_c - A_s}{A_c} \times 100$$

In this equation, A_c represents the control's absorbance, while A_s denotes the extract's absorbance.

3.4.4. Superoxide radical scavenging activity

Principle

The principle involves the generation of superoxide radicals in an alkaline DMSO environment, which subsequently react with NBT to form a detectable formazan product. The reduction in the formation of this product in the presence of a test substance indicates its scavenging activity. Superoxide radicals reduce NBT to form a blue formazan product, which is quantified spectrophotometrically at 560 nm (Hyland et al., 1983).



Reagents:

Components required: NBT solution (1 mg mL⁻¹ dissolved in DMSO), DMSO with added alkali (1 mL DMSO combined with 0.1 mL distilled water containing 5 mM NaOH), 100 mM potassium phosphate buffer (at pH 7.4), ascorbic acid, and sample.

Procedure

1. Stock solution of standard ascorbic acid and plant extract: 2 mg ascorbic acid/plant extract was dissolved in 2 mL distilled water.
2. Triplicate test tubes were prepared using the stock solution, containing standard ascorbic acid and sample solution at concentrations ranging from 10 to 100 $\mu\text{g mL}^{-1}$. To all tubes MilliQ water was added to adjust final volume 1 mL.
3. Each tube received 0.2 mL NBT solution and 2 mL alkaline DMSO. Resulting mixture was incubated for 10 minutes.
4. Using a spectrophotometer, the solution's absorbance was determined at 560 nm, with a blank solution serving as the reference.

$$\text{Superoxide radical scavenging activity (\%)}: \frac{\text{Ac}-\text{As}}{\text{Ac}} \times 100$$

In this equation, Ac denotes the absorbance measured for the control sample (lacking the extract), while As indicates the absorbance observed for the sample containing the extract.

3.4.5. Determination of nitric oxide quenching activity

Principle

The nitric oxide (NO) quenching activity assay measures a substance's ability to neutralize NO free radicals. Sodium nitroprusside is used to generate nitric oxide, which subsequently reacts with oxygen to form nitrite ions (NO_2^-). These nitrite ions are detected using the Griess reagent. This assay was used to measure the reduction in nitrite formation in the presence of a potential NO scavenger (Bhakya et al., 2016).

Reagents

A 10 mM solution of sodium nitroprusside was prepared using phosphate buffer with a pH of 7.4. The Griess reagent has 1 % sulphanilamide, 2 % H_3PO_4 , and 0.1 % naphthylethylenediamine dihydrochloride.

Procedure

1. Both extracts and ascorbic acid (standard) were prepared at various concentrations (10-100 $\mu\text{g mL}^{-1}$) each in triplicate.

2. Next, 2 mL of sodium nitroprusside solution at a concentration of 10 mM was mixed with 0.5 mL of buffer solution containing phosphate, which had a pH of 7.4. Resulting mixture underwent incubation for 60 minutes at ambient temperature.
3. After adding Griess reagent (0.5 mL) the mixture was allowed to incubate for 30 minutes at 25 °C.
4. A control experiment was run similarly, but without the test extracts/standard.
5. The chromophores formed were measured at 550 nm using a spectrophotometer.
6. The equation below was utilized to determine the percentage of NO inhibition:

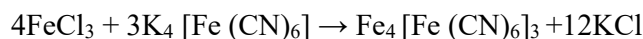
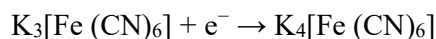
$$\text{Nitric oxide radical scavenging activity (\%)}: \frac{Ac-As}{Ac} \times 100$$

In this equation, Ac denotes the absorbance measured for the control sample (lacking the extract), while As indicates the absorbance observed for the sample containing the extract.

3.4.6. Reducing power assay

Principle

The reducing power analysis is grounded on the ability of antioxidants to donate electrons, thereby reducing oxidized intermediates. This assay specifically measures the reduction of ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions (Oyaizu 1986).



(Prussian blue)

Reagents

Potassium ferricyanide, Phosphate buffer (pH 6.6), Ferric chloride, Trichloroacetic acid

Procedure

1. Initially, varying concentrations of the extracts, ranging from 10 to 100 $\mu\text{g mL}^{-1}$, were prepared along with a standard solution of ascorbic acid.

2. The resulting mixtures were then amalgamated with phosphate buffer (pH 6.6) and potassium ferricyanide (2.5 mL each). Subsequently, the solution was heated at 50 °C.
3. The reaction was terminated after 20 minutes by introducing trichloroacetic acid (2.5 mL). Subsequently, the samples underwent centrifugation to isolate the supernatant.
4. The supernatant was combined with distilled water and treated with freshly prepared ferric chloride (0.5 mL).
5. The resulting solutions were then subjected to spectrophotometric analysis at 700 nm.

3.4.7. Determination of total antioxidant capacity

Principle

Total antioxidant capacity (TAC) assays generally measure the cumulative effect of all antioxidants present in a sample that reacts with a specific oxidant. The phosphomolybdenum method involves the reduction of Mo (VI) to Mo(V) by antioxidants, resulting in the formation of a green phosphate/Mo(V) complex spectrophotometrically measured at 695 nm (Prieto et al., 1999).

Reagents

28 mM Na₂HPO₄, ii. 0.6 M H₂SO₄, iii. 4 mM (NH₄)₆Mo₇O₂₄

Procedure

1. The stock solution of ascorbic acid 1 mg mL⁻¹ was diluted to concentrations ranging from 10-100 µg mL⁻¹.
2. Varying concentrations (0.01-0.1 mL) of the extract were combined to solution (H₂SO₄, Na₂HPO₄, and (NH₄)₆Mo₇O₂₄) and heated at 95 °C for 90 minutes.
3. Following incubation, solution was left to return to ambient temperature.
4. Using a UV-Vis spectrophotometer, the OD of all solution was determined at 695 nm. The overall antioxidant capacity (TAC) is presented in milligrams of ascorbic acid (AA) equivalent per gram of extract (mg AAE g⁻¹).

3.5. Liquid Chromatography-Mass Spectrometry (LC-MS)

Principle

The chemical structure, molecular structure, and quantitative analysis of separated components within a sample can be determined using LC-MS technology. The fundamental principle of LC-MS is that liquid chromatography (LC) separates the components of a sample dissolved in the mobile phase based on their affinity or retention time while passing through stationary phases, which can be solid or liquid. Subsequently, the separated components were ionized in the mass spectrometer and separated based on their mass-to-charge ratios (m/z). These ions are detected by a detector such as a photomultiplier tube or an electron multiplier, and their abundance is recorded as a mass spectrum.

Procedure

Phytochemicals in *O. indicum* extracts were identified using an LC-MS assay (LC-ESI-MS) conducted at Aakar Biotech, Lucknow, India. The extracts were centrifugation (4000 rpm for 10 min) to remove particulates prior to analysis. The separation of analytes was conducted using an Agilent Eclipse C18 column (5 μ m, 15 cm). A gradient mobile phase made up of formic acid (0.1%) in water and acetonitrile was utilized., The flow rate adjusted at 500 μ L min⁻¹. A waters UPLC-TQD mass spectrometer operated in the positive mode was utilized for data acquisition in both MS and MS/MS modes (a method adapted from Pai et al., 2015).

3.6. FTIR Analysis of Ethanolic and Aqueous Extracts of *Oroxylum indicum*

Principle

The fundamental principle underlying FTIR involves the interaction between infrared radiation and the components of a sample, resulting in the excitation of molecular rotations and vibrations.

Procedure

O. indicum extract spectra were analyzed using FTIR on a Bruker IFS66/S spectrometer (4000-400 cm^{-1}). Functional groups were identified by comparing the obtained spectra with those in existing literature.

4. Animal Model

For the present experiment, healthy Wistar albino rats within the weight range of 220-260 grams were utilized. The laboratory rats underwent an adaptation period to the controlled environment. The ambient conditions were maintained (22 ± 2 °C and a relative humidity 50 ± 10 %). The rats were placed under 12 hours light/dark cycle to facilitate the regulation of circadian rhythms. Rats were housed in well-ventilated, spacious polypropylene cages throughout the study. Standard rat pellets and fresh water were provided ad libitum to meet the nutritional and hydration requirements of animals. The experiments performed on these subjects rigorously followed the guidelines set forth by the Mizoram University Institutional Animal Ethical Committee (MZUIAEC). All procedures were carried out in compliance with the authorized protocol (Approval No. MZU/IAEC/2020/14). This adherence to ethical standards and consideration for animal welfare ensured humane treatment and ethical use of the rats employed in this study.

4.1. Acute Oral Toxicity Test

This study aimed to evaluate the possible harmful effects of administering a test substance in a single oral dose to a group of experimental subjects. The primary objective was to identify the dose that elicits adverse effects or mortality within a short duration, typically 14 days.

4.1.1. Acute toxicity experimental design

The research followed OECD protocol 423, with female rats ($n=3/\text{group}$) divided randomly into four groups, each containing three animals. The distribution was as follows:

Group 1: Rats in the control group (CG) was administered Saline water for one time on the dose day, exposure route orally by oral gavage

Group 2: Rats in the low dose group received 1000 mg kg⁻¹ *O. indicum* extract one time on the dose day, exposure route orally by oral gavage

Group 3: Rats in the medium-dose group received 3000 mg kg⁻¹ *O. indicum* extract once on the dose day, via oral exposure route by oral gavage, and observed for 14 days.

Group 4: Rats in the high dose group received 5000 mg kg⁻¹ *O. indicum* extract one time on the dose day, exposure route orally by oral gavage.

Procedure

1. For the acute toxicity test, a total of twelve healthy male Wistar albino rats were used. These rats, weighing between 220 and 260 g, were allocated to the previously mentioned groups, with each group containing three rats.

Before administering the extract, the rats were subjected to 12 hours fasting period overnight, during which they were allowed unrestricted access to water.

2. Prior to administering the test extracts, the weight of each rat was determined. The dosage of the extract was then calculated according to the individual body weight of each animal.

3. The suspensions were prepared by reconstituting the extracts in distilled water, which served as the vehicle. Using an oral gavage feeding needle, each rat was administered a 600 µL dose of the homogeneous mixture.

4. After administering the test substance, animals were deprived of food for 4 hours. During this period, they were monitored individually for the initial 30 minutes, then at 2-, 4-, and 6-hours post-administration, and subsequently once per day for two weeks. Observations focused on detecting clinical indicators of toxicity, including death, breathing patterns, alterations in overall behavior, and changes in skin, eyes, and fur condition.

5. We observed the animals' general characteristics, including eye color, activity level, and movement patterns before and after treatment, without employing any specific scoring system or measurement tools.

4.2. Experimental Design for Evaluating Hepatoprotective property

of *O. indicum* Extracts

OECD guideline 423 (OECD, 2002) served as the framework for this study.

Procedure

1. Twenty-one male Wistar albino rats (220-260 g) were allocated into seven groups (n=3/group):

Group 1: Normal control, followed by saline water for 10 days.

Group 2: Diclofenac ($100 \text{ mg kg}^{-1} \text{ day}^{-1}$) for 4 days, followed by distilled water for 10 days.

Groups 3-7: A daily dose of diclofenac (100 mg kg^{-1}) administered for 4 days, succeeded by a 10-day treatment period with:

Group 3: Silymarin ($50 \text{ mg kg}^{-1} \text{ day}^{-1}$)

Group 4: *O. indicum* ethanolic extract ($250 \text{ mg kg}^{-1} \text{ day}^{-1}$)

Group 5: *O. indicum* ethanolic extract ($500 \text{ mg kg}^{-1} \text{ day}^{-1}$)

Group 6: *O. indicum* aqueous extract ($250 \text{ mg kg}^{-1} \text{ day}^{-1}$)

Group 7: *O. indicum* aqueous extract ($500 \text{ mg kg}^{-1} \text{ day}^{-1}$)

2. On day 16, liver tissue was collected for the analysis of GSH, GPx, SOD, CAT, and MDA levels.

3. Hepatotoxicity was induced by intraperitoneal injection of DIC at 100 mg kg^{-1} for 4 days.

4. The suspensions were created by mixing the extracts with distilled water until homogeneous. Each rat received 600 μL of the suspension through oral gavage at 11:00 am.

5. Following extract administration, food was withheld for 4 hours.

6. On the day of sacrifice, the body masses of all rats were recorded prior to humane euthanasia under mild anesthesia induced by an injection of ketamine and xylazine.

7. Blood samples were collected and organs were extracted.

Hematological analysis

Hematological analysis involves examining the components of the blood to diagnose and monitor various medical conditions.

Procedure

1. At the end of the experiment on day 15, blood tests were conducted on all remaining subjects.
2. Blood samples were collected in tubes and used for hematological analysis.
3. The investigation assessed various hematological parameters, including erythrocyte (RBC) and leukocyte (WBC) counts. Furthermore, we quantified hemoglobin concentrations, % hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration to provide a comprehensive analysis of blood constituents.

4.2.1. Relative organs weight

Relative organ weight is used to compare the size and condition of certain vital organs with the total mass of the organism, and is often displayed as a percentage.

Procedure

11. Following euthanasia of the animals on the 16th day, the vital organs, including the liver, heart, lungs, spleen, kidneys, and testes, were systematically collected.
2. The adipose tissue adhering to the organs was removed, and the organs were carefully blotted with clean tissue paper before weighing.
3. The relative organ weight (ROW) was calculated to account for variations in animal size.
4. This value represents the ratio of the organ's weight to the entire body weight and was determined using the following equation:

$$\text{ROW} = \frac{\text{Absolute organ weight}}{\text{Body weight at sacrifice}} \times 100$$

4.2.2. Histopathological studies

Histopathology is the microscopic examination of tissues to elucidate disease mechanisms, their etiologies, and diagnostic modalities as well as to develop novel therapeutic interventions and compare them with existing ones during research and clinical practice. Furthermore, it facilitates the assessment of the safety and efficacy of the investigational drugs.

Procedure

1. Following the dissection, the target organ was placed in Bouin's solution for 24 hours to fix the tissue. It was then stored in 70 % ethanol until it was ready for additional processing steps.
2. Hepatic tissue samples were encased in paraffin wax using a block tray and sliced to a thickness of 7 μm with a Leica rotary microtome (RM2125 RTS).
3. Hepatic tissue ribbons were placed inside warm water to mount the ribbon with tissue on gelatin-coated glass slides.
4. The thin sections of hepatic tissues on glass slides were de-paraffinized with xylene and rehydrated with different grades of ethanol (100 %, 90 %, and 70 %) and distilled water involving two changes for 10 minutes each.
5. The samples were then gone for hematoxylin and eosin staining step.
6. Excess stain was removed by placing the slide with the tissue in a tray and kept in slow-running tap water.
5. The slides were then dehydrated using alcohol grades (70 %, 90 %, and 100 %), cleared with xylene, and mounted with DPX (Bancroft and Gamble, 2008).
6. The histopathology of the hepatic tissue was examined under a trinocular microscope (Olympus), and photographs were taken for analysis.

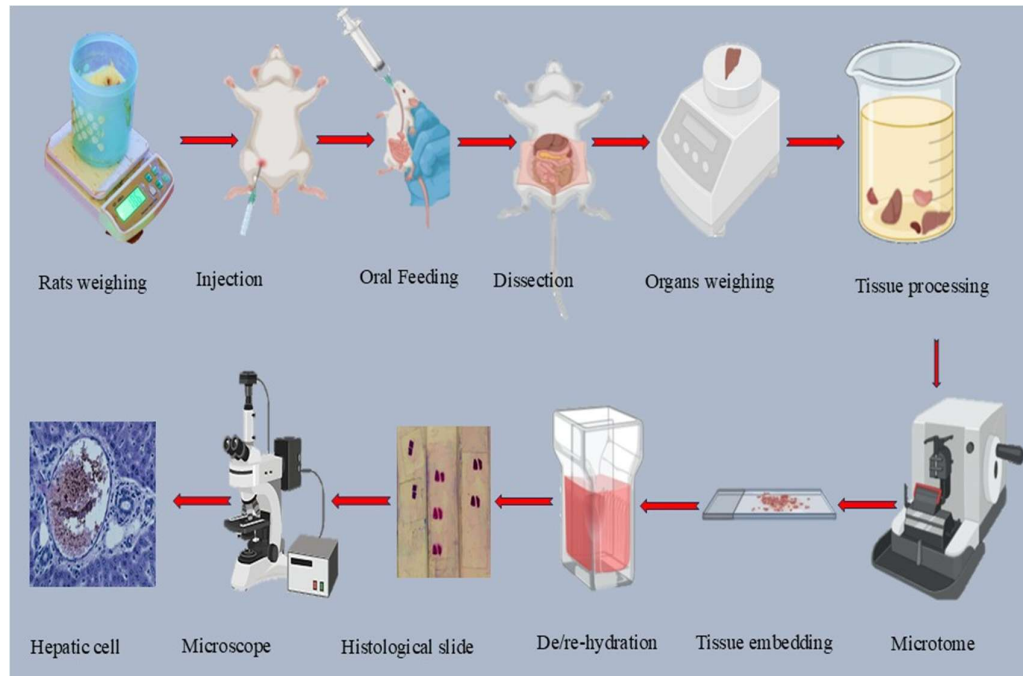


Figure 8. Histological methods for processing hepatic tissue for histopathological studies.

4.3. Biochemical Estimations

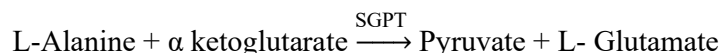
4.3.1. Alanine aminotransferase (ALT)

Serum ALT levels were estimated using the Modified IFCC method.

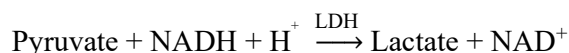
Principle

The enzyme SGPT (ALAT) facilitates the amino group transfer between l-alanine and α -ketoglutarate, resulting in the formation of pyruvate and glutamate. Following this, pyruvate undergoes a reaction with NADH, catalyzed by lactate dehydrogenase, resulting in NAD formation. The SGPT (ALAT) activity within the sample was quantified by observing the speed at which NADH converts to NAD. This was accomplished by quantifying the reduction in absorbance, which directly corresponded to the enzyme's activity.

Transamination reaction catalyzed by SGPT/ALT



Lactate Dehydrogenase Reaction:



Working Reagent:

The mixture consisted of enzyme reagent 1 and starter reagent 2 in a ratio of 4:1.

Assay Procedure

Test name	Working reagent (μL)	Sample (μL)	Incubation temperature (1 min) $^{\circ}\text{C}$	Wavelength (nm)
ALT	100	200	30	340

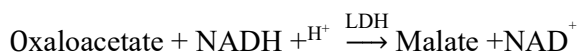
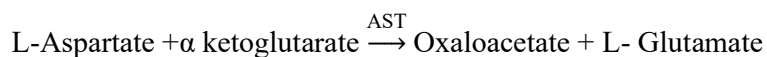
$$\text{SGPT (ALAT) activity in U L}^{-1} = \frac{\Delta A}{\text{min}} \times 952$$

4.3.2. Aspartate aminotransferase (AST)/SGOT ratio

Serum AST levels were estimated using the modified IFCC method.

Principle

The enzyme SGOT (AST) facilitates the amino group transfer between L-aspartate and alpha ketoglutarate, resulting in the formation of oxaloacetate and glutamate. In the presence of malate dehydrogenase, oxaloacetate interacts with NADH to generate NAD. The SGOT (AST) activity in the sample is determined by measuring the rate at which NADH oxidizes to NAD, which is reflected in the decrease of absorbance. This reduction in absorbance is directly proportional to the SGOT (AST) activity level in the given sample.



Working reagent:

The mixture consisted of enzyme reagent 1 and starter reagent 2 in a ratio of 4:1.

Assay procedure:

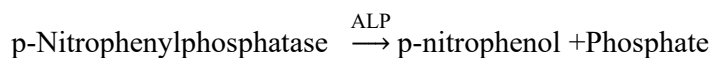
Test Name	Working reagent (μL)	Sample (μL)	Incubation temperature (1 min) °C	Wavelength (nm)
ALT	100	200	30	340

$$\text{SGOT (ALT) Activity in U L}^{-1} = \frac{\Delta A}{\text{min}} \times 952$$

4.3.3. Alkaline phosphatase (ALP)

Principle

At basic pH levels, ALP catalyzes the breakdown of p-nitrophenylphosphatase, yielding p-nitrophenol and phosphate. The ALP activity in the sample was quantified by measuring the increase in absorbance, which directly corresponds to the generation rate of p-nitrophenol.



Working reagent: Dissolved one substrate tablet in 3.2 mL of buffer reagent. The material was sensitive to both light and temperature. Proper precautions were taken, particularly during the reconstitution process.

Assay procedure

Test name	Working reagent (μL)	Sample (μL)	Incubation temp.(°C)	Wavelength (nm)
ALP	500	10	30	405

The mixture was mixed well, and the initial absorbance A₀ after 30 seconds. Absorbance readings were repeated every 1 min and 2 minutes. The mean absorbance change per minute was computed as per equation given below:

$$\text{ALP Activity in U L}^{-1} = \frac{\Delta A}{\text{min}} \times 2754$$

4.3.4. Bilirubin

The reaction between bilirubin and diazotized sulfanilic acid results in the formation of a colored azobilirubin. The process involves combining unconjugated bilirubin with sulfonic acid, facilitated by a caffeine-benzoate catalyst. The resulting color's strength correlates directly with the amount of bilirubin present in the sample.

Assay Procedure

Test name	Total bilirubin reagent (μL)	Direct nitrite reagent (μL)	Blank (μL)	Sample (μL)	Incubation temperature (°C)	Wavelength (nm)
Bilirubin	500	25	50	50	37	546

The solution was vigorously mixed and then allowed to settle at ambient temperature for 5 minutes. Immediately following this, the test samples' absorbance (Abs. T) was measured, using their corresponding blanks as a reference point.

$$\text{Total bilirubin in mg dL}^{-1} = \text{Abs.T} \times 13$$

4.3.5. Total protein

Principle

In an alkaline environment, cupric ions from the biuret reagent form a blue-violet complex when combined with proteins. The concentration of proteins in the sample is directly correlated with the intensity of this color. As the protein content increases, so does the vibrancy of the blue-violet hue.

Assay procedure

Test name	Biuret reagent (μL)	Distilled water (μL)	Sample (μL)	Incubation temperature (°C)	Wavelength (nm)
Total Protein	500	20	10	37	550

The solution was thoroughly combined and kept at 37 °C for a duration of 10 minutes. Absorbance of the standard (Abs. S.), and test samples (Abs. T.) against the blank within 60 minutes. Total protein content was calculated using the following formula:

$$\text{Total proteins in g dL}^{-1} = \frac{\text{Abs. T.}}{\text{Abs. S.}} \times 8$$

Here 8 is scaling factor

4.4. Oxidative Marker

Thiobarbituric acid reacting substances (TBARS)

(Balasubramanian et al., 1988)

Principle

Malondialdehyde (MDA) is generated through the degradation of polyunsaturated fatty acids, serving as an indicator for assessing the extent of peroxidation reactions. Lipid peroxidation produces malondialdehyde, which forms a pink complex when it interacts with thiobarbituric acid. This complex shows peak light absorption at 532 nm.

Reagents

0.375 % thiobarbituric acid (TBA), 15 % trichloroacetic acid (TCA), TRIS (hydroxy methyl)

Procedure

1. About 50 mg of liver tissue with 500 μ L PBS (1x) was sonicated to obtain a tissue homogenate.
2. Following centrifugation, equal volumes (500 μ L each) of the homogenate supernatant, trichloroacetic acid, and TBA were combined in a securely sealed tube.
3. Subsequently, the tubes underwent a 15 minutes heating process in water bath at its boiling point.
4. Following the cooling process, the sample was subjected to centrifugation at 2500 rpm for a duration of 10 minutes.
5. The supernatant's light absorption was determined by measuring its optical density at a wavelength of 532 nm, using a blank as a reference.
6. To determine the concentrations of the samples under analysis, we employed the molar extinction coefficient of MDA.

$$n \text{ moles (MDA)} = \left[\frac{2 \times V_s \times A_s}{1.56 \times 10^5 \times \text{mg of tissue}} \right] \times 10^9$$

V_s = Volume of sample taken, A_s = Absorbance of sample, 10^9 = Scaling factor

4.5. Antioxidant Enzymes

4.5.1. Reduced glutathione (GSH)

It is a tripeptide composed of glutamine, cysteine, and glycine. It acts as an important antioxidant in the body, protecting cells from oxidative stress and maintaining their redox state (Moron et al., 1979).

Reagents

4 mM reduced GSH, sodium phosphate buffer (0.2 M, pH = 7, 800 μ M EDTA, 0.01 M sodium azide, 250 μ M hydrogen peroxide, 10 % TCA, 0.2 M sodium phosphate, 0.6 mM DTNB, 1 % sodium citrate.

Procedure

1. The stock solution of standard GSH 1 mg mL^{-1} is divided into varying concentration of $10\text{-}100 \text{ }\mu\text{L mL}^{-1}$.
2. A mixture containing $200 \text{ }\mu\text{L}$ of homogenate and varying concentrations of the standard was combined with roughly $50 \text{ }\mu\text{L}$ of 10 % trichloroacetic acid. This solution was incubation at $37 \pm 2 \text{ }^{\circ}\text{C}$ for 10 minutes.
3. After centrifugation, $10 \text{ }\mu\text{L}$ of the supernatant was transferred to a test tube and $100 \text{ }\mu\text{L}$ of 0.5 mM DTNB, $500 \text{ }\mu\text{L}$ of sodium phosphate (pH 8), and $400 \text{ }\mu\text{L}$ of phosphate buffer were added, and the absorbance was read at 412 nm against a reagent blank.

$$\text{Activity} \rightarrow n \text{ moles of GSH } \text{mg}^{-1} \text{ protein}$$

4.5.2. Glutathione peroxidase (GPx)

Glutathione peroxidase (GPx) protect cells from oxidative damage by converting hydrogen peroxide and organic hydroperoxides to less toxic products. This assay relies on spectrophotometric quantification of the amount of hydrogen peroxide that was reduced by the enzyme in the presence of reduced glutathione (Paglia and Valentine, 1967).

Reagents

0.2 mM NADPH, 0.1 M phosphate buffer (pH 7.4), 50 mM of reduced glutathione, 0.25 Mm H_2O_2 .

Procedure

1. To $50 \text{ }\mu\text{L}$ of sample homogenate, approximately $100 \text{ }\mu\text{L}$ phosphate buffer, $50 \text{ }\mu\text{L}$, and $50 \text{ }\mu\text{L}$ were added.
2. The solution was kept at $37 \text{ }^{\circ}\text{C} \pm 2$ for 5 minutes and $50 \text{ }\mu\text{L}$ of NADPH was added. Following this, enzymatic activity was monitored at 60 seconds intervals for a duration of 5 minutes, using a wavelength of 320 nm .

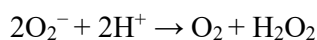
$$\text{GPx Activity} = \Delta A_{320} \times 3781 \text{ units.}$$

where, ΔA_{320} represents the rate of change in absorbance measured at a wavelength of 320 nm per minute, 3781- A constant that transforms the change in absorbance into units of enzyme activity.

4.5.3. Superoxide dismutase (SOD)

Principle

This technique quantifies superoxide generation by measuring the auto-oxidation of pyrogallol and subsequent suppression of SOD-mediated MTT reduction to its formazan derivative. The inhibition of MTT reduction is measured by recording the OD at 570 nm. SOD unit is defined as the amount in milligram of protein required to inhibit MTT reduction by 50 % (Madesh and Balasubramanian, 1998).



Reagents

MTT (100 μM Pyrogallol, 1.25 MTT, PBS. PBS was made by dispersing NaCl (8 g), potassium chloride (0.2 g), KH_2PO_4 (0.2 g), along with Na_2HPO_4 (0.94 g) in 800 mL of Milli-Q water. Acidity of solution was neutralized to pH 7.4. The solution is diluted with Milli-Q water to reach a final volume of 1 liter.

Procedure

1. Sample was prepared by grinding 10 % homogenate of liver tissue in PBS
2. The samples of different groups of rats and blank were treated with the appropriate reagents as indicated below.
3. Then vortex for 3 seconds and incubated for 10 minutes at room temperature.
4. After 10 minutes of incubation DMSO was added.
5. Centrifuged for 1 minute at 5000 rpm and using distilled water as the blank, absorbance measurements were taken at a wavelength of 570 nm.

	Reagents				
	PBS	MTT	Homogenate	Pyrogallol	DMSO
Blank	600 μL	30 μL	-	75 μL	0.75 μL
Sample	600 μL	30 μL	10 μL	75 μL	0.75 μL

Calculation:

The enzyme superoxide dismutase was quantified in terms of SOD units per milligram of protein.

$$\% \text{ inhibition} = \frac{\text{Absorbance of Blank} - \text{Absorbance of samples}}{\text{Absorbance of Blank}} \times 100$$

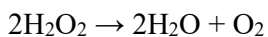
$$\text{SOD (U mg}^{-1} \text{ of protein)} = \frac{1 \times \% \text{ inhibition}}{50 \times \text{weight of protein in mg}} \times 100$$

Here, 1 unit SOD = 50 % inhibition, 1 % inhibition = 1/50

4.5.4. Estimation of catalase

Principle

Catalase activity was assessed by determining the rate at which the enzyme catalyzes the decomposition of hydrogen peroxide (H₂O₂). Catalase is an enzyme present in nearly all organisms (sample) that are subjected to oxidative stress, as it facilitates the breakdown of hydrogen peroxide into water and oxygen, thereby preventing oxygen-induced cellular damage (Sinha, 1972).



Procedure

1. To assay mixture containing 0.4 mL of 0.2 M H₂O₂ and 0.5 mL of 0.01 M phosphate buffer (pH.7) and 0.01 ml of liver tissue homogenate was vortex for 5 seconds
2. The dichromate acetic acid solution (2 mL) was blown exactly after 60 seconds to stop the reaction and was kept in a boiling water bath for 10 minutes.
3. Dichromate in acetic acid is reduced first to unstable blue colored perchromic acid and finally to stable green colored chromic acetate in the presence of H₂O₂, which is measured spectrophotometrically at 570 nm against blank containing 0.4 mL of 0.2 M H₂O₂ and 0.5 mL of 0.01 M phosphate buffer (pH.7).

Units of activity

One unit of catalase expressed as µg of H₂O₂ decomposed/minute/mg protein

4.6. Statistical Analysis

All tests were performed in triplicate and results are presented as mean $\pm$ SEM. Data were analyzed using SPSS and GraphPad Prism software. To compare the means of three or more independent groups, a one-way ANOVA was conducted in SPSS. This analysis was performed to identify any significant differences in group means, ensuring that the assumptions of normality and homogeneity of variances were met. After the one-way ANOVA, post hoc analysis was performed using Duncan's Multiple Range Test to identify which specific groups showed significant differences. SPSS's pairwise comparisons, which provided mean differences and p-values ($p < 0.05$), allowed me to identify statistically significant differences between groups. This approach enabled a comprehensive analysis of the data, ensuring the accuracy of the results.

CHAPTER - 3

RESULTS

3. Authentication of Plant Material

The identification of plant as *Oroxylum indicum* (L.) Kurz was conducted by the Department of Botany, Mizoram University, Aizawl, India. For the submitted specimens, accession numbers (ACS No. MZUBOT0088). Further, the identification of plant was confirmed online resource ('World Flora Online: <https://www.worldfloraonline.org/taxon/wfo-0000779181>).

3.1. Yield of Phytochemical Extraction

The ethanol extraction yield was found to be 8 %. The extraction yield of Milli-Q was 9.7 % (w/w). The higher yield of water as a solvent indicated that it was more effective for extracting polar compounds from plant material. The observed differences in the extraction efficiencies of ethanol and water can be attributed to their different polarities.

3.2. Qualitative Screening of Phytochemicals

Oroxylum indicum contains several secondary metabolites that have a range of pharmacological and biological activities. In the present study, a preliminary screening of the diversity of phytochemicals contained in *O. indium* was performed. The results of the phytochemical screening of the *O. indicum* extracts are shown in Table 1. The results of the primary screening showed the presence of flavonoids, alkaloids, saponins, phenols, and tannins in both the ethanolic (OIE) and aqueous (OIE) extracts (Table 1). The presence of these phytochemicals suggests the potential medicinal properties of the *O. indicum* extracts.

Table 1. Qualitative phytochemical screening of ethanolic and aqueous extract of *Oroxylum indicum*.

SI No.	Phytochemical	Ethanol extract	Aqueous extract
1	Glycoside	+++	+
2	Terpenoids	++	-
3	Steroids	-	-
4	Phlobatannins	-	-
5	Flavonoids	+++	++
6	Tannins	+++	++
7	Phenols	+++	++
8	Alkaloids	++	-
9	Saponins	-	++

Note: ++ present in moderate; +++ present in more quantity; + Present; - Absent

3.3. Quantitative Phytochemical Screening

3.3.1. Total phenolic, flavonoid and tannin content

The total phenolic content in the ethanolic extract was 557.21 ± 6.8 GAE mg g⁻¹, whereas the aqueous extract contained significantly lower amounts at 80.61 ± 0.64 GAE mg g⁻¹ (Figure 9a). This substantial difference suggests that ethanol is a more efficient solvent for extracting phenolic compounds, likely due to its ability to dissolve both polar and non-polar bioactive compounds. Similarly, the total flavonoid content was higher in the ethanolic extract, measuring 355.1 ± 1.6 QE mg g⁻¹, compared to 241 ± 1.3 QE mg g⁻¹ in the aqueous extract (Figure 9b). This indicates that flavonoids, like phenolics, exhibit greater solubility in ethanol, which enhances their extraction efficiency. In contrast, the tannin content showed minimal variation between the two solvents, with values of 108 ± 1.4 mg g⁻¹ TAE in the ethanolic extract and 106.2 ± 1.8 mg g⁻¹ TAE in the aqueous extract (Figure 9c). The relatively similar tannin concentrations suggest that these compounds may be more uniformly distributed between ethanol and water or that tannins have comparable solubility in both solvents. These results highlight the influence of solvent polarity on the extraction yield of bioactive compounds and emphasize the superior extraction capability of ethanol, particularly for phenolics and flavonoids.

3.4. *In Vitro* Antioxidant Activity of *Oroxylum indicum*

3.4.1. DPPH radical scavenging activities

The DPPH assay performed in this study revealed dose-dependent radical scavenging activity in the *O. indicum* extract. For the DPPH radical scavenging, the IC₅₀ value of 23.7 ± 1.8 µg mL⁻¹ and 30.4 ± 1.7 µg mL⁻¹ for ethanolic and aqueous extracts, respectively, were determined (Figure 10). The radical scavenging activity of the ethanolic extract was comparable to that of the standard BHT. BHT showed an IC₅₀ value of 22.9 ± 0.9 µg mL⁻¹. The percentage inhibition of DPPH at the highest dose (100 µg mL⁻¹) examined in this

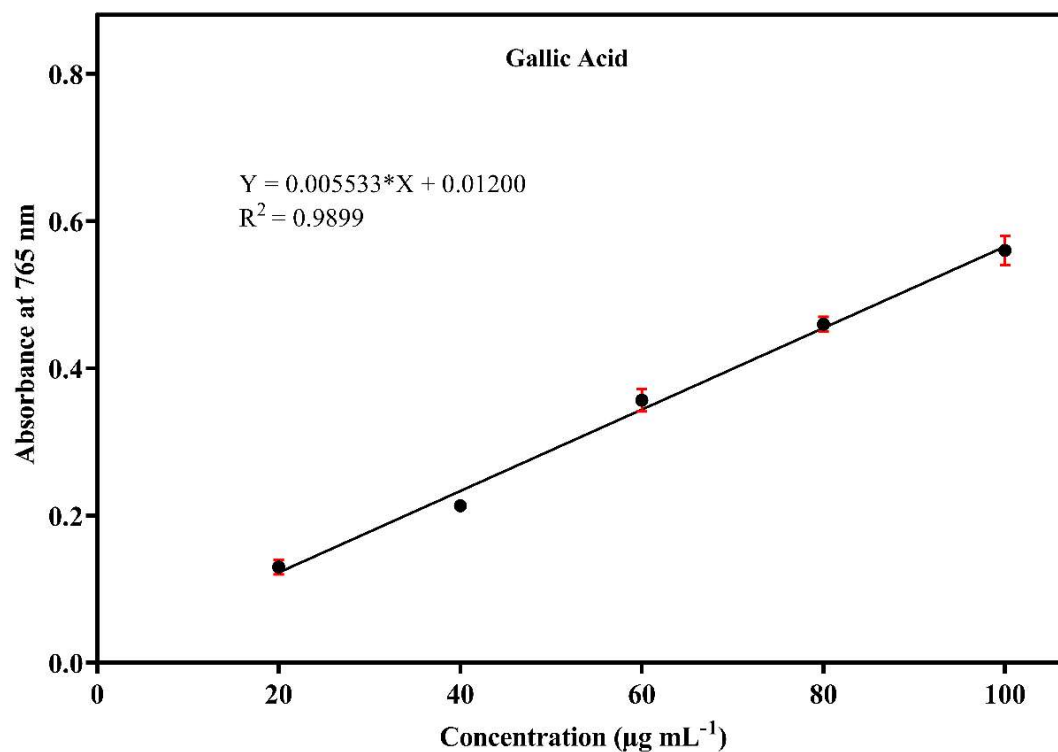


Figure 9a. Standard graph of gallic acid for the total phenol assay. The solid line represents the regression line. All values are in mean $\pm$ SEM (n=3).

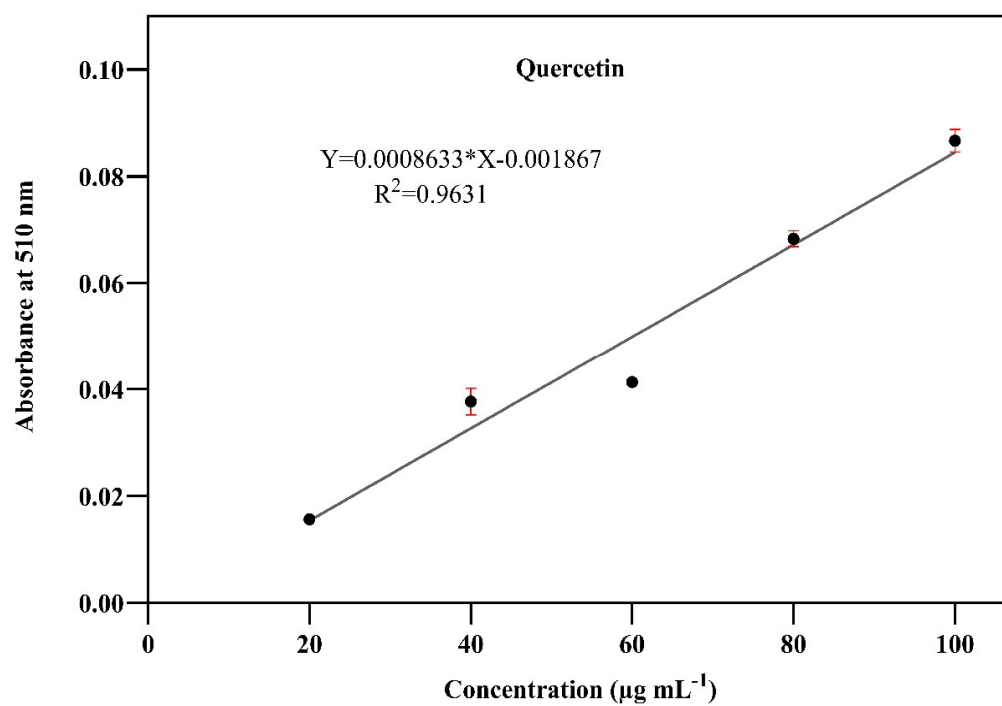


Figure 9b. Standard graph of quercetin for the total flavonoid assay. The solid line represents the regression line. All values are presented in triplicate ($n=3$) $\pm$ SEM.

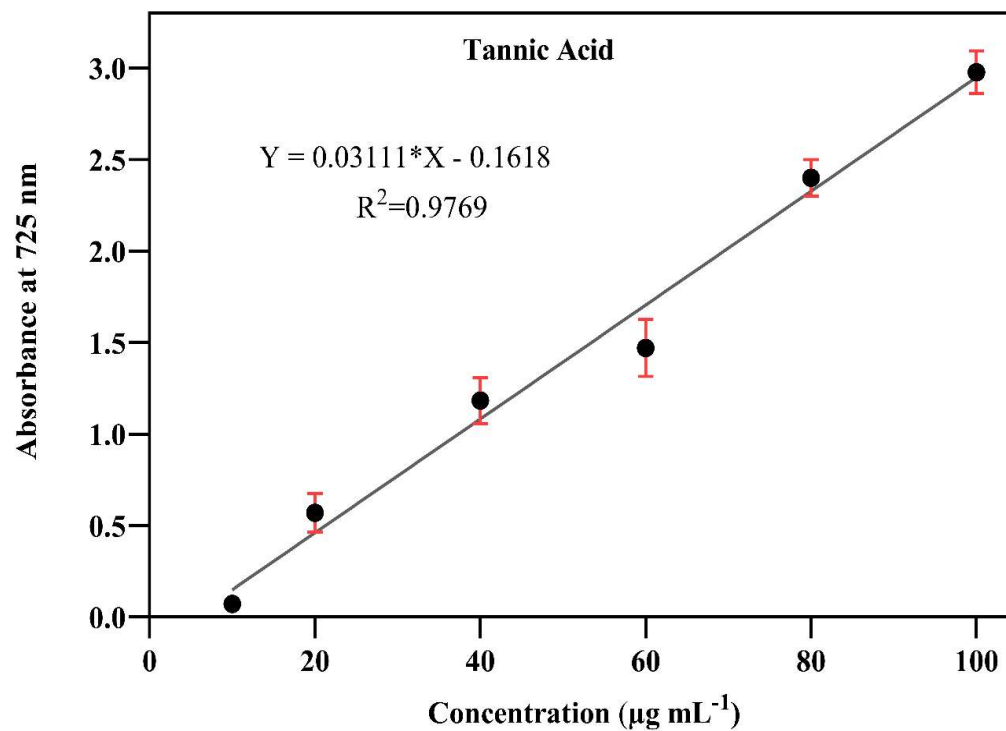


Figure 9c. Standard graph of tannic acid for total tannin assay. The solid line represents the linear graph. All values are presented in triplicate ($n=3$) $\pm$ SEM.

study was 94.68 %, 87.07 %, and 80.07 % for BHT, OIE, and OIA, respectively. These results suggest that the ethanolic extract of *O. indicum* possesses stronger antioxidant properties than those of the aqueous extract. The similarity in IC₅₀ values between the ethanolic extract and BHT indicates that the plant extract could potentially serve as a natural alternative to synthetic antioxidants.

3.4.2. ABTS scavenging activity

Oroxylum indicum extracts displayed concentration-dependent ABTS⁺ radical scavenging activity (Figure 11) with IC₅₀ value of $42.5 \pm 1.9 \mu\text{g mL}^{-1}$ and $75.1 \pm 3.7 \mu\text{g mL}^{-1}$ for the ethanolic and aqueous extracts, respectively (Table 2). The standard BHT had an IC₅₀ value of $21.1 \pm 0.4 \mu\text{g mL}^{-1}$. As evidenced by its lower IC₅₀ value, the ethanolic extract demonstrated stronger ABTS scavenging activity than the aqueous extract did. These findings suggest that ethanol is a potent solvent for the extraction of antioxidant compounds from *O. indicum*. However, both extracts demonstrated notable ABTS⁺ radical scavenging potential, but not effective as was BHT.

3.4.3. Hydroxyl radical scavenging

Both extracts showed considerable hydroxyl radical scavenging activity (Figure 12). It increased with increasing concentrations of both the extracts. The IC₅₀ were 24.4 ± 1.3 , 27.8 ± 0.8 , and $38.9 \pm 1.6 \mu\text{g mL}^{-1}$ for ascorbic acid, OIE and OIA, respectively (Table 2). These results imply that both OIE and OIA exhibit considerable antioxidant activity. OIE showed a slightly higher potency than OIA. The lower IC₅₀ value of OIE suggests that a lower concentration is required to inhibit hydroxyl radicals by 50 %, which makes it more effective than OIA. However, both extracts showed lower hydroxyl radical scavenging activity than ascorbic acid. Ascorbic acid had the lowest IC₅₀ value among all the tested samples.

3.4.4. Superoxide scavenging activities

Both extracts exhibited concentration-dependent superoxide radical scavenging activity (Figure 13). Ascorbic acid demonstrated the highest activity with an IC₅₀ of $30.1 \pm 0.06 \mu\text{g mL}^{-1}$, followed by OIE at $36.2 \pm 1.5 \mu\text{g mL}^{-1}$, followed by OIE at $36.2 \pm 1.5 \mu\text{g mL}^{-1}$ and OIA at $40.8 \pm 1 \mu\text{g mL}^{-1}$ (Table 2). These results indicate

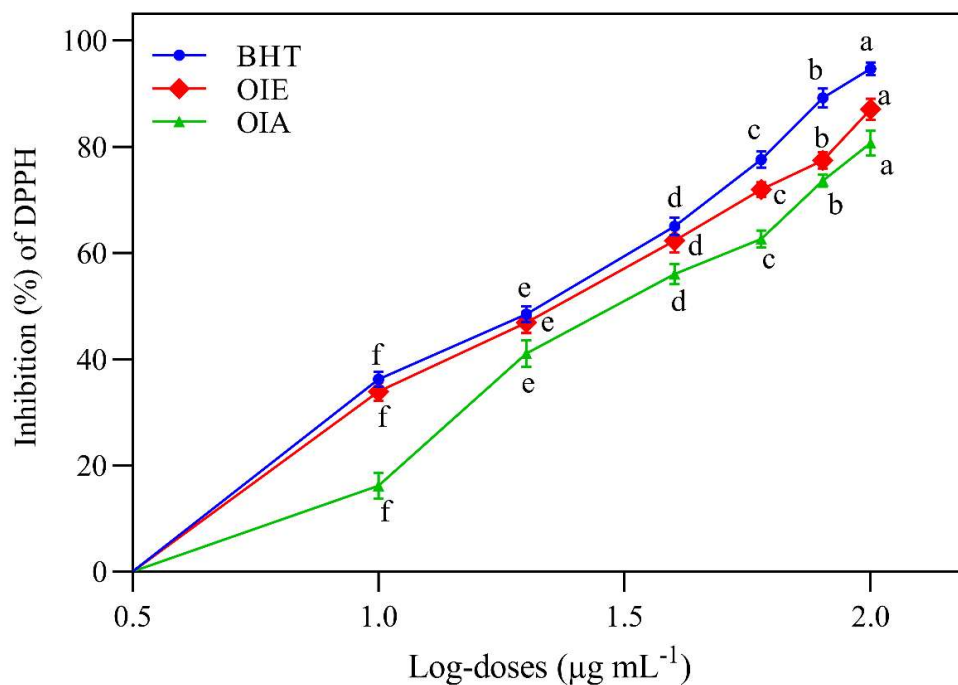


Figure 10. Log doses of *Oroxylum indicum* extract and standard BHT against DPPH. Data are presented as mean $\pm$ standard error of the mean (SEM), with $n = 3$ replicates. In a curve, data points indicated by the same letters are not significantly different ($p < 0.05$) according to one-way ANOVA, followed by Duncan's multiple range test.

that OIE possesses greater superoxide radical scavenging activity. This disparity in activities between OIE and OIA may be ascribed to the varying compositions and concentrations of the bioactive compounds extracted from the two extracts.

3.4.5. Nitric oxide inhibition

The extracts displayed concentration-dependent nitric oxide (NO) radical scavenging activity (Figure 14). The IC₅₀ value of Ascorbic acid, OIE and OIA was $40.8 \pm 1.2 \mu\text{g mL}^{-1}$, $43.0 \pm 5.4 \mu\text{g mL}^{-1}$ and $48.8 \pm 4.6 \mu\text{g mL}^{-1}$ respectively (Table 2). The results indicated that the OIE extract demonstrated marginally superior NO-scavenging activity compared with OIA. However, both the extracts exhibited potential as natural antioxidants. These findings suggest that the extracts contain compounds capable of neutralizing NO radicals.

3.4.6. Reducing power

The stem bark of *Oroxylum indicum* exhibited a dose-dependent response to the reducing power. The reducing power increased consistently with increasing concentrations of the extracts from 10 to 100 $\mu\text{g mL}^{-1}$ (Figure 15). The results showed that OIE demonstrated a comparatively higher reducing power than OIA and was comparable to that of ascorbic acid. The observed trend indicates that higher concentrations of *O. indicum* stem bark extract confer a greater antioxidant potential. This reducing power can be attributed to the presence of bioactive compounds such as electron-donating flavonoids and phenolic acids.

3.4.7. Total antioxidant capacity

The total antioxidant capacity of the ethanolic (OIE) and aqueous (OIA) extracts of *O. indicum* stem bark was studied with reference to ascorbic acid (Figure 16). The ethanolic extract exhibited an activity of $100.9 \pm 1.6 \text{ AAE mg g}^{-1}$, whereas the aqueous extract demonstrated an activity of $77.24 \pm 1.98 \text{ AAE mg g}^{-1}$ (Figure 17).

Table 2. IC₅₀ (µg mL⁻¹) of *Oroxylum indicum* extracts and standards for DPPH, ABTS, Hydroxyl, Superoxide and Nitric Oxide radical-scavenging activities. All values are presented as mean ± standard error of the mean (SEM) with n = 3 replicates. Values in columns with different letters denote significant variations (p < 0.05) based on Duncan's multiple range test.

Sample Name	IC ₅₀ (µg mL ⁻¹)				
	DPPH	ABTS	Hydroxyl	Superoxide	Nitric oxide
BHT	22.9 ± 0.9 ^b	21.1 ± 0.4 ^c	-	-	-
Ascorbic Acid	-	-	24.4 ± 1.3 ^b	30.1 ± 0.05 ^c	40.8 ± 1.2 ^a
OIE	23.7 ± 1.8 ^b	42.5 ± 1.9 ^b	27.8 ± 0.8 ^b	36.2 ± 1.5 ^b	43.0 ± 5.4 ^a
OIA	30.4 ± 1.7 ^a	75.1 ± 3.7 ^a	38.9 ± 1.6 ^a	40.8 ± 1 ^a	48.8 ± 4.6 ^a

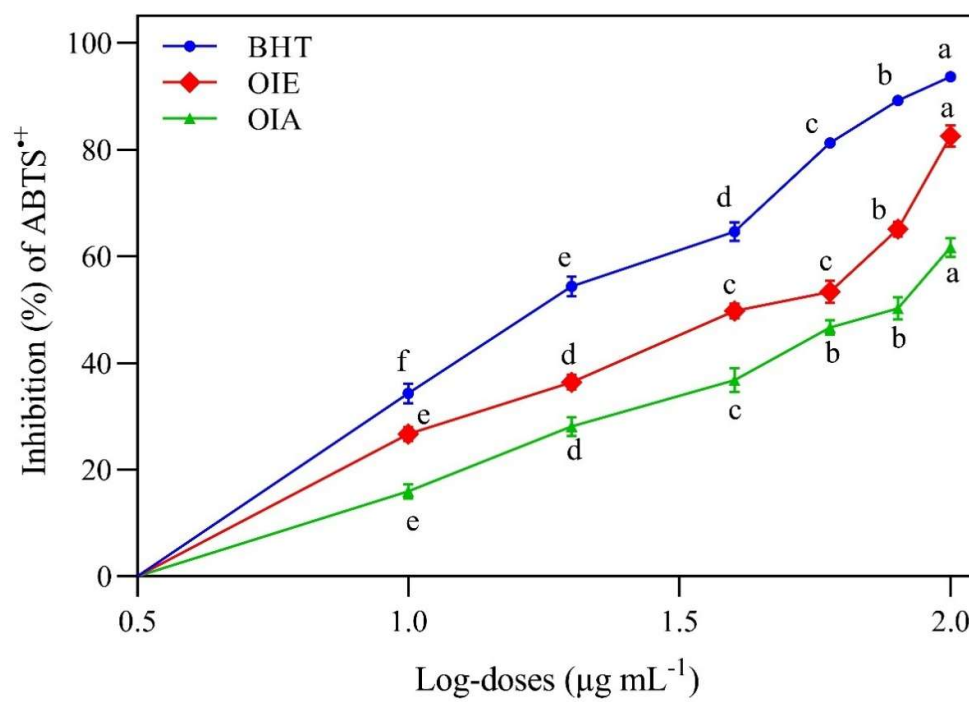


Figure 11. Log doses of *Oroxylum indicum* extract and BHT against ABTS radicals. Data are presented as mean $\pm$ standard error of the mean (SEM), with $n = 3$ replicates. In a curve, data points indicated by the same letters are not significantly different ($p < 0.05$) according to one-way ANOVA, followed by Duncan's multiple range test.

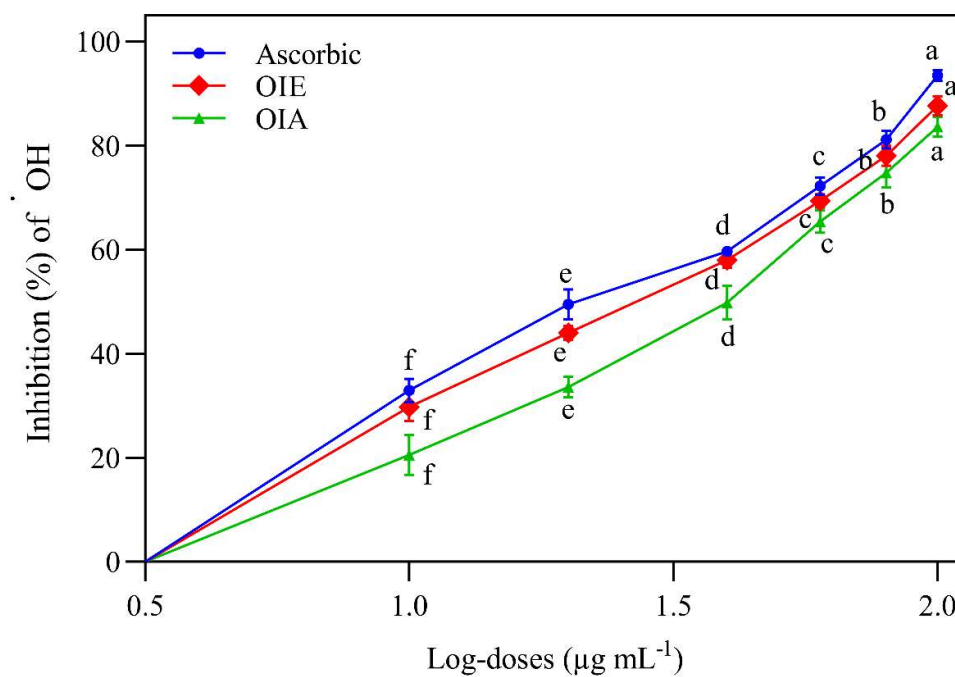


Figure 12. Hydroxyl scavenging activities of ethanolic and aqueous extracts of *Oroxylum indicum* and standard ascorbic acid. Data are presented as mean $\pm$ standard error of the mean (SEM), with $n = 3$ replicates. In a curve, data points indicated by the same letters are not significantly different ($p < 0.05$) according to one-way ANOVA, followed by Duncan's multiple range test.

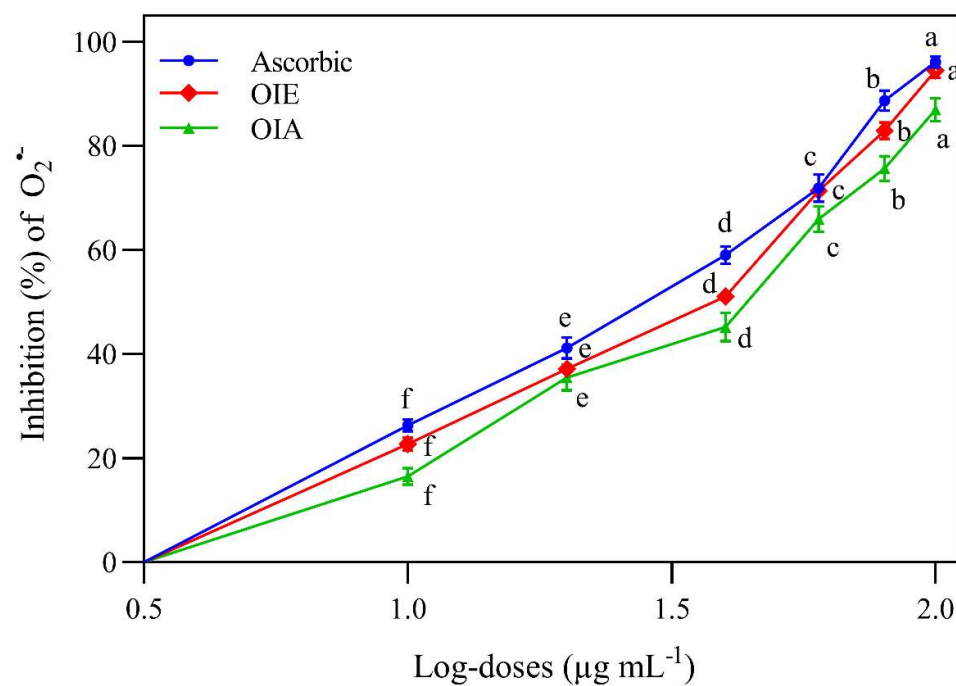


Figure 13. Superoxide scavenging ability of ethanolic and aqueous extracts of *Oroxylum indicum* and standard ascorbic acid. Data are presented as mean $\pm$ standard error of the mean (SEM), with $n = 3$ replicates. In a curve data point indicated by same alphabets are not significantly different ($p < 0.05$) according to one-way ANOVA followed by Duncan's multiple range test.

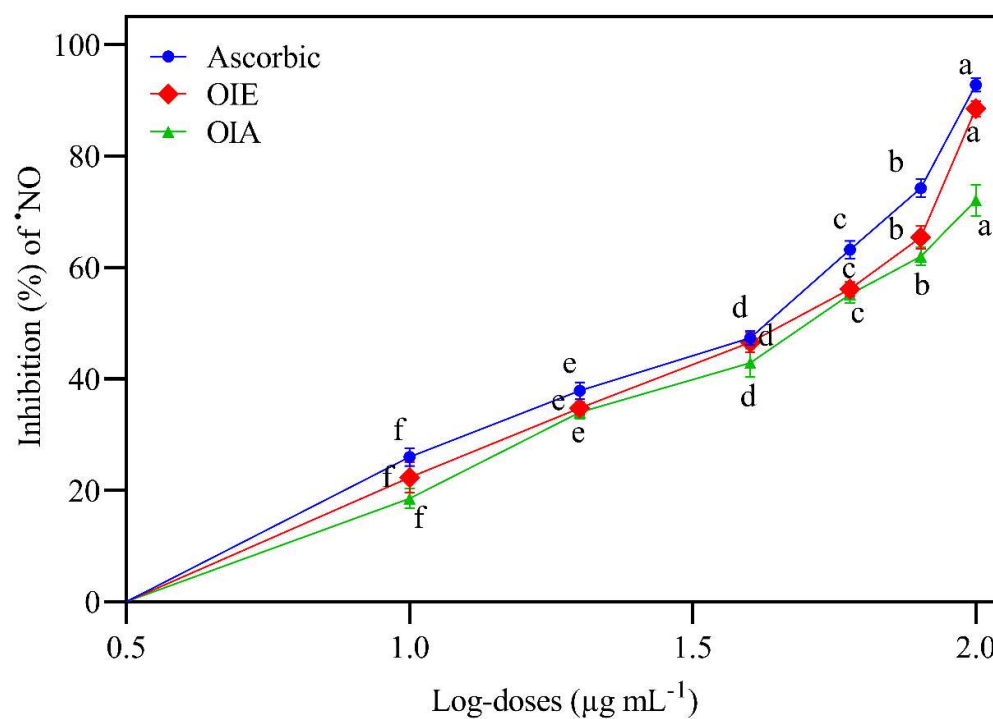


Figure 14. Nitric oxide inhibition (%) of ethanolic and aqueous extracts of *Oroxylum indicum* and standard ascorbic acid. Data are presented as mean $\pm$ standard error of the mean (SEM), with $n = 3$ replicates. In a curve, data points indicated by the same letters are not significantly different ($p < 0.05$) according to one-way ANOVA, followed by Duncan's multiple range test.

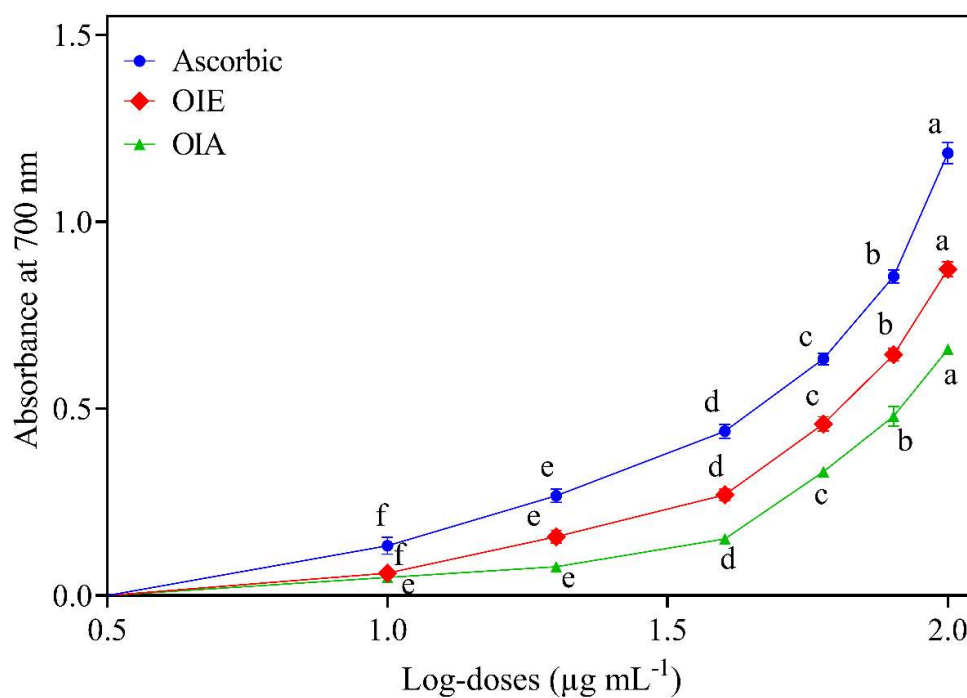


Figure 15. Reducing power of ethanolic and aqueous extracts of *Oroxylum indicum* and standard ascorbic acid. Data are presented as mean $\pm$ standard error of the mean (SEM), with $n = 3$ replicates. In a curve, data points indicated by the same letters are not significantly different ($p < 0.05$) according to one-way ANOVA, followed by Duncan's multiple range test.

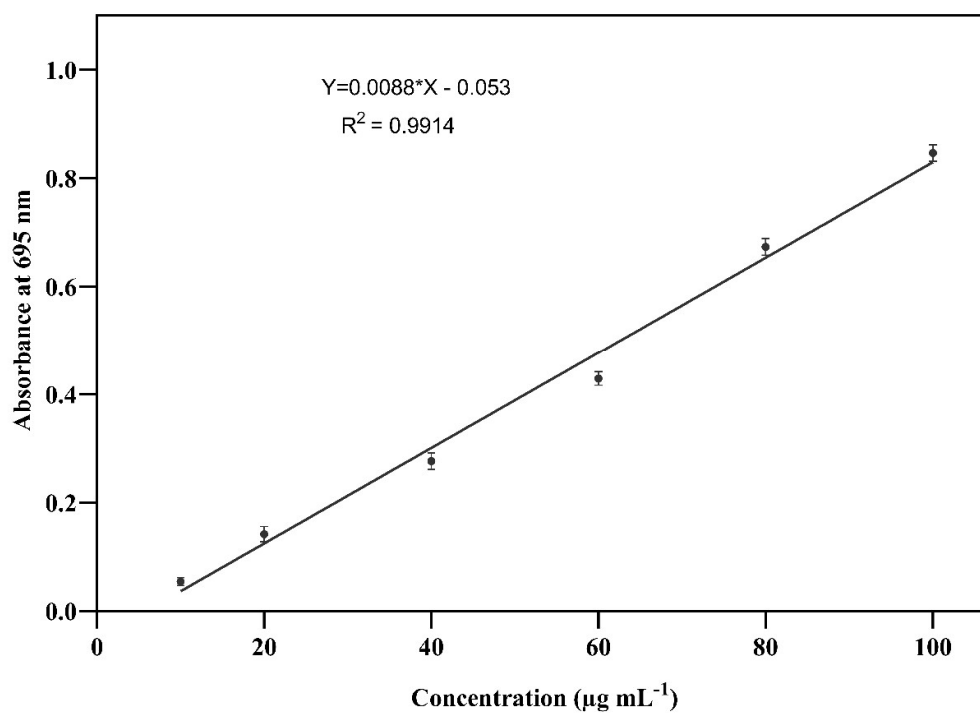


Figure 16. Standard graph for determination of total antioxidant capacity in ethanolic and aqueous stem bark extracts of *Oroxylum indicum*. All values are presented in triplicates (n=3).

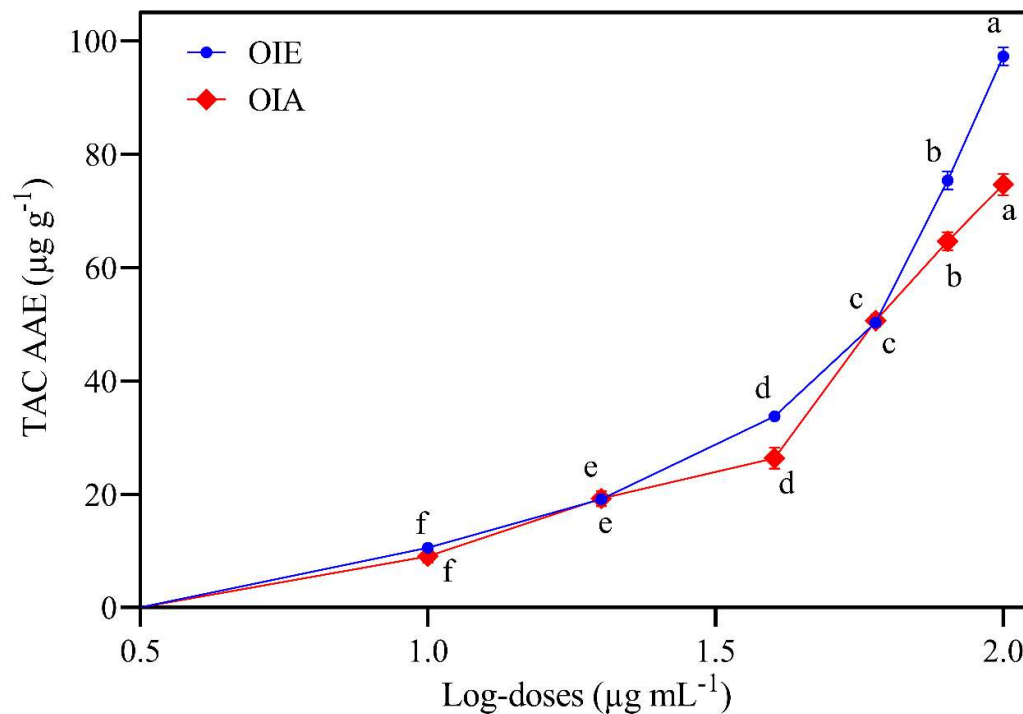


Figure 17. Total antioxidant capacity of the ethanolic and aqueous extracts of *Oroxylum indicum*. Data are presented as mean $\pm$ standard error of the mean (SEM), with $n = 3$ replicates. In a curve data point indicated by same alphabets are not significantly different ($p < 0.05$) according to one-way ANOVA followed by Duncan's multiple range test.

3.5. FTIR Analysis of Ethanolic and Aqueous Extract of *Oroxylum indicum*

FTIR analysis of the *O. indicum* bark extracts (OIE and OIA) revealed several functional groups (Figure 18 and 19; Table 3). FTIR analysis of OIE revealed the presence of diverse phytochemicals in the extracts. The broad O-H stretching vibration at 3343 cm^{-1} indicates the presence of hydrogen-bonded alcohols or phenols, which is likely attributed to the polyphenolic constituents. Aliphatic C-H stretching at 2974 cm^{-1} (asymmetric) and 2893 cm^{-1} (symmetric) correspond to saturated hydrocarbons, while a C=O stretching band at 2893 cm^{-1} suggest conjugation or electron-withdrawing effects perturbing the carbonyl group. The 1662 cm^{-1} absorption, characteristic of C=C stretching, confirms isolated alkenes, supported by out-of-plane C-H bending at 880 cm^{-1} , indicative of monosubstituted aromatic systems. Ether linkages are indicated by a C-O stretch at 1276 cm^{-1} , while amines are implicated via C-N stretches at 1088 and 1045 cm^{-1} , likely primary or secondary. The CH_3 bending mode at 1381 cm^{-1} further supports the occurrence of alkanes. Notably, halogenated organics were apparent through the C-Cl (642 cm^{-1}) and C-I (417 cm^{-1}) stretches. This observation indicated the presence of iodinated and chlorinated compounds in the extracts, potentially of terpenoid or alkaloid origin.

The aqueous extract exhibited distinct spectral features, including a broad O-H stretch at 3339 cm^{-1} , consistent with hydroxylated compounds. A sharp C $\equiv$ C stretch at 2158 cm^{-1} denotes terminal alkynes or nitriles, while conjugated dienes are identified via C=C stretches at 1647 and 1508 cm^{-1} , the latter's bathochromic shift implicating extended π -conjugation. Aliphatic C-H bending at 1458 cm^{-1} and a C-N stretch at 1342 cm^{-1} suggest alkylamines or heterocyclic bases. The C-O stretch at 1223 cm^{-1} aligns with ethers or phenolic hydroxyl groups. Halogenated species are again present, with C-Cl (775 cm^{-1}) and C-I (426 cm^{-1}) stretches, though at shifted wavenumbers relative to the ethanol extract, possibly due to differing electronegativities or molecular environments. The absence of carbonyl signals in the aqueous phase contrasts with that in the ethanol extract, suggesting polarity-driven

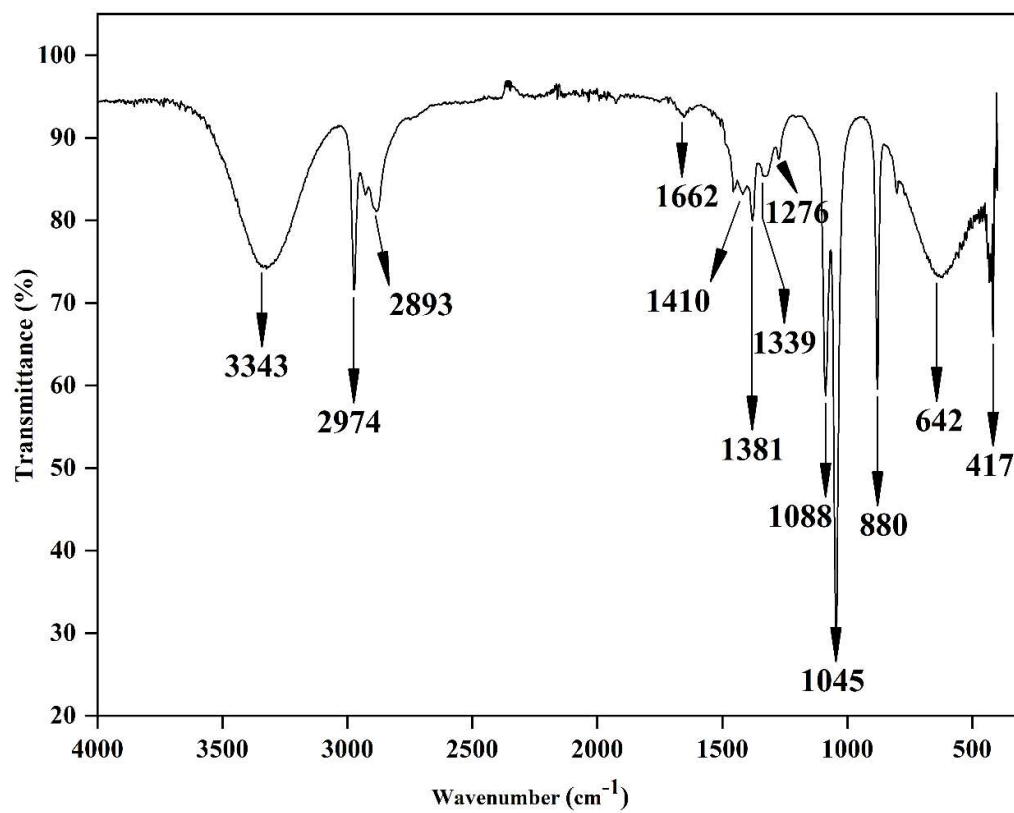


Figure 18. FTIR spectra of ethanolic stem bark extract of *Oroxylum indicum*.

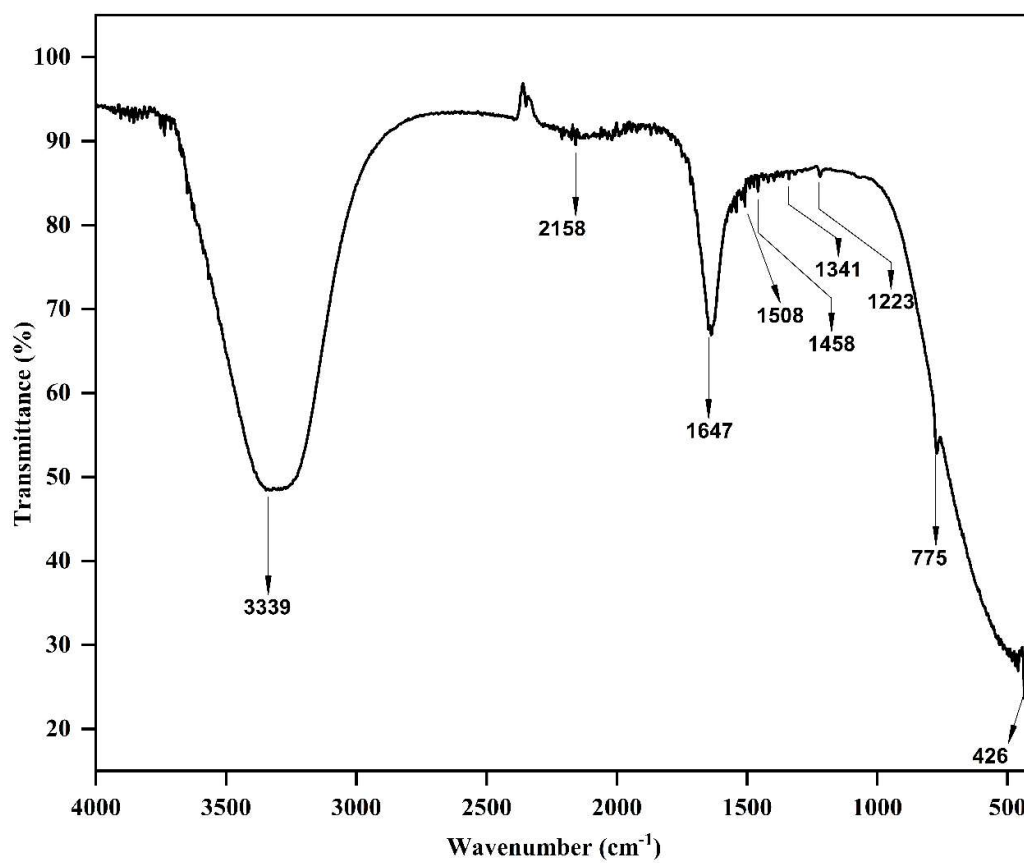


Figure 19. FTIR spectra of aqueous stem bark extract of *Oroxylum indicum*.

partitioning of nonpolar aldehydes or ketones into the organic phase. Collectively, these data underscore the chemical diversity of the extract, which is rich in halogenated alkanes, unsaturated hydrocarbons, and nitrogenous species, with implications for the isolation of bioactive compounds.

3.6. LC-MS Profiling of *Oroxylum indicum* Extracts

Liquid chromatography-mass spectrometry (LCMS) analysis revealed distinct phytochemical profiles of the *O. indicum* extracts (Figure 20 and 21; Tables 5). OIE exhibited a high concentration of scutellarein, hispidulin, oroxylin B, acacetin, quercetin, and a chrysin derivative (Table 5). Organic acids, sugars, and fatty acids were also identified. The primary phytochemicals detected in the ethanolic and aqueous extracts of *O. indicum* bark are displayed in Table 4, along with their reported hepatoprotective mechanisms and corresponding references in the literature. The ethanolic extract contains scutellarein and hispidulin, flavonoid aglycones that demonstrate anti-inflammatory, antifibrotic, and antioxidant activities, mitigating oxidative stress-induced liver injury and fibrosis (Miao et al., 2020; Ferrándiz et al., 1994). The aqueous extract was enriched in polyphenolic and phenolic acid derivatives. Quercetin and luteolin exhibit antioxidative and anti-inflammatory effects, attenuate non-alcoholic fatty liver disease (NAFLD), and enhance hepatocyte survival (Sotiropoulou et al., 2021; Pu et al., 2021). Chlorogenic acid and sinapic acid further contribute to hepatic protection via antioxidant activity, suppression of fibrotic markers (e.g., α -SMA), and modulation of stress-response proteins (e.g., HSP70), with efficacy in NAFLD and fibrosis models (Shi et al., 2016; Jabbar et al., 2023; Pandi and Kalappan, 2021). Biochanin A, an isoflavone, exerts hepatoprotective effects via immunomodulatory and anti-inflammatory pathways, although its extract specificity is not specified in the provided data (Breikaa et al., 2013; Sarfraz et al., 2020). Collectively, these compounds suggest the extract's therapeutic potential, driven by synergistic antioxidative and anti-inflammatory mechanisms, aligning with ethnopharmacological claims for *O. indicum* in managing hepatic disorders.

Table 3. FTIR peaks and functional groups detected in ethanolic and aqueous stem bark extracts of *Oroxylum indicum*.

Extract	IRvmax (cm ⁻¹)	Vibration mode	Functional groups
Ethanol	3343	O-H stretching	Alcohol, Phenols
	2974	C-H stretching	Alkanes
	2893	C=O stretching	Aldehydes
	1662	C=C Stretching	Alkenes
	1381	CH ₃ bending	Alkane
	1276	C-O stretching	Ethers
	1088	C-N stretching	Amine
	1045	C-N stretching	Amine
	880	C-H bending	Aromatic compound
	642	C-Cl stretching	Alkyl halide
	417	C-I stretching	Halo compound
Aqueous	3339	O-H stretching;	Alcohol
	2158	C≡C stretching	Alkynes
	1647	C=C stretching	Alkenes
	1508	C=C stretching	Conjugated alkenes
	1458	C-H bending	Alkanes

1342	C-N stretching	Amines
1223	C-O stretching	Alcohols, ethers
775	C-Cl stretching	Alkyl halide
426	C-I stretching	Halo compound

Table 4. Hepatoprotective compounds identified in *Oroxylum indicum* extract.

Compound	Extract	Putative Hepatoprotective Effects	References
Scutellarein	Ethanollic	Anti-inflammatory, antifibrotic, protects against oxidative stress-induced liver injury.	Miao et al., 2020
Hispidulin	Ethanollic	Antioxidant, anti-inflammatory, protects against liver fibrosis.	Ferrández et al., 1994
Quercetin	Aqueous	Antioxidant, anti-inflammatory, protects against non-alcoholic fatty liver disease (NAFLD)	Sotiropoulou et al., 2021
Luteolin	Aqueous	Antioxidant, anti-inflammatory, promotes liver cell survival	Pu et al., 2021
Chlorogenic acid	Aqueous	Antioxidant, anti-inflammatory, improves liver function in NAFLD models	Shi et al., 2016
Sinapic acid	Aqueous	Antioxidant, hepatoprotective, attenuate the liver fibrosis, liver cell proliferation, up-regulation of HSP 70, and downregulation of α -SMA expression	Jabbar et al., 2023; Pandi and Kalappan, 2021
Biochanin A		Antioxidant, hepatoprotective, attributed to its antioxidant, anti-inflammatory, and immunomodulatory actions	Breikaa et al., 2013; Sarfraz et al., 2020

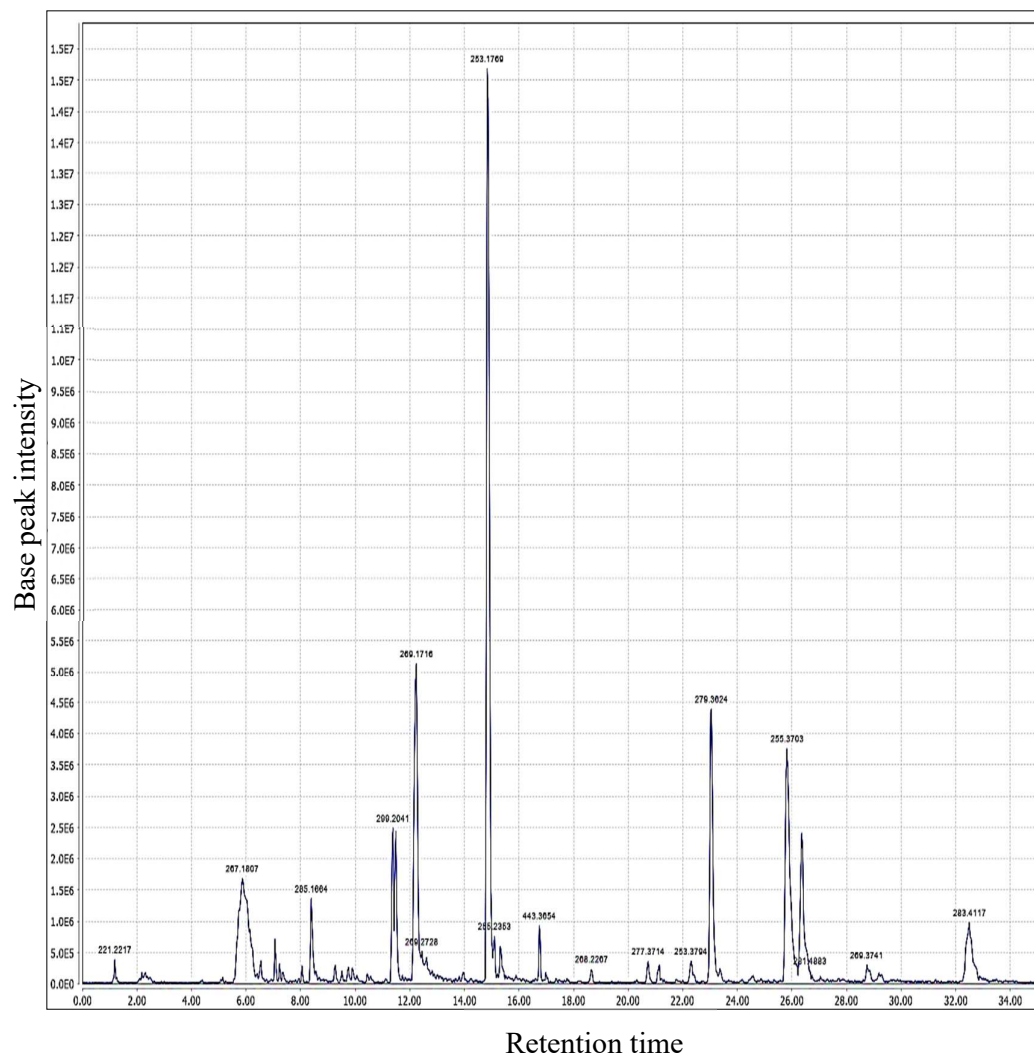


Figure 20a. The LC-MS chromatogram of the ethanolic extract of *Oroxylum indicum* was generated using a SUNFIRE C18 column with dimensions of 250 X 2.1 mm and 2.6 μ m particle size. Spectra were recorded in negative (Figure 20a) ionization mode across the m/z range of 150–2000.

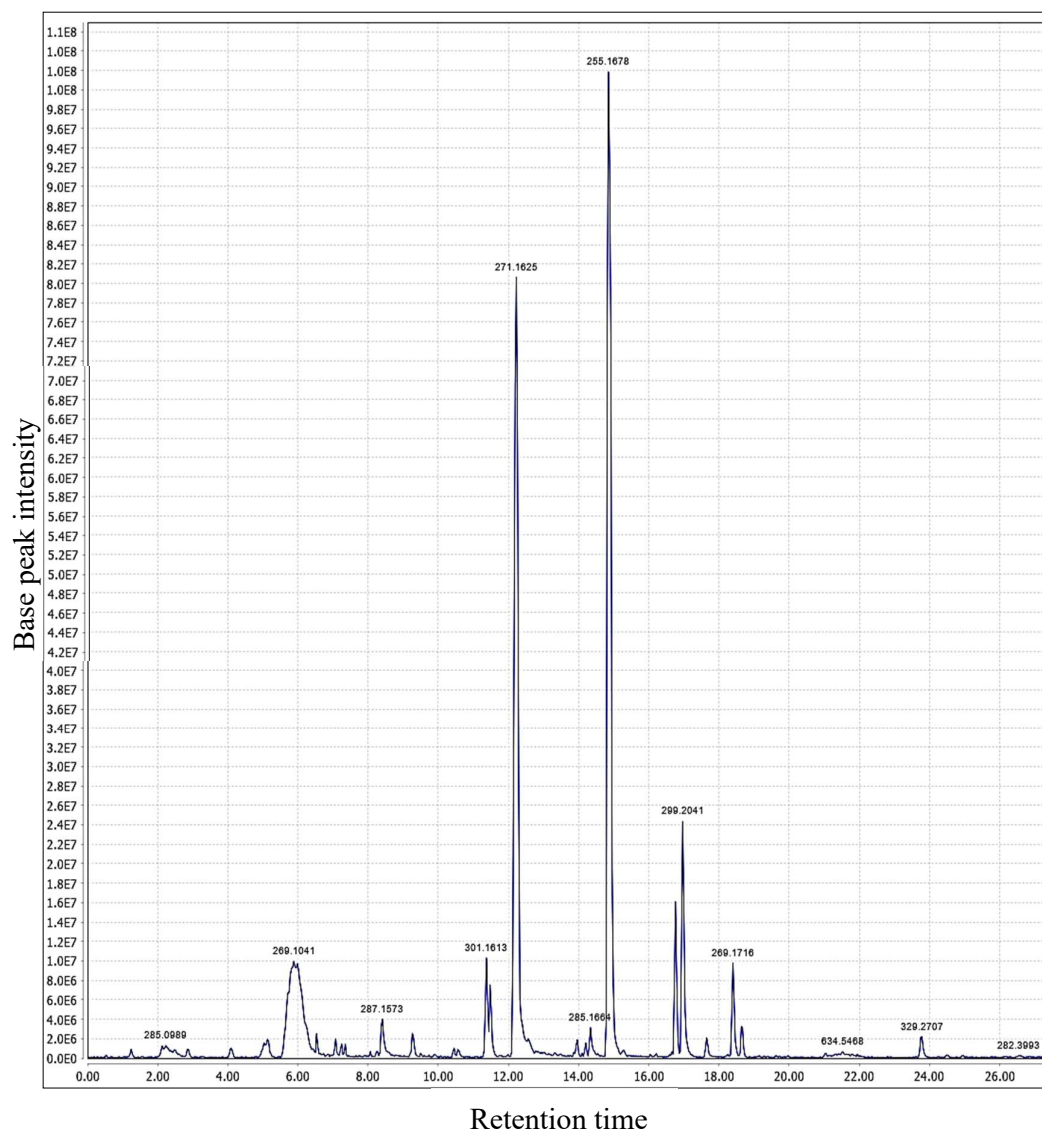


Figure 20b. Spectra of positive ionization modes across the m/z range 150–2000.

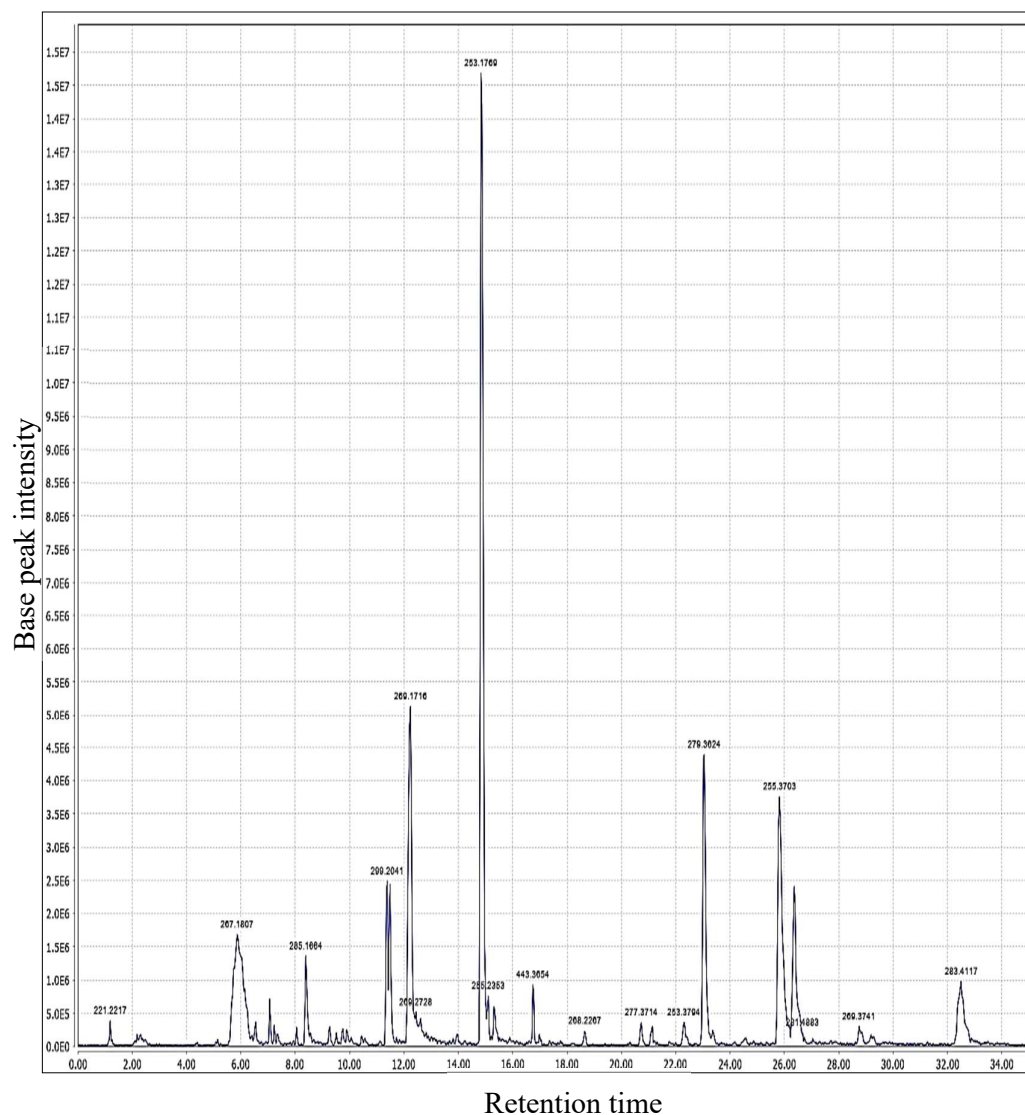


Figure 21a. The LC-MS chromatogram of the aqueous extract of *Oroxylum indicum* obtained using a SUNFIRE C18 column with dimensions of 250 X 2.1 mm and a particle size of 2.6 μm . Spectra were recorded in negative ionization mode across the mass-to-charge ratio (m/z) range of 150–2000 (Figure 21a).

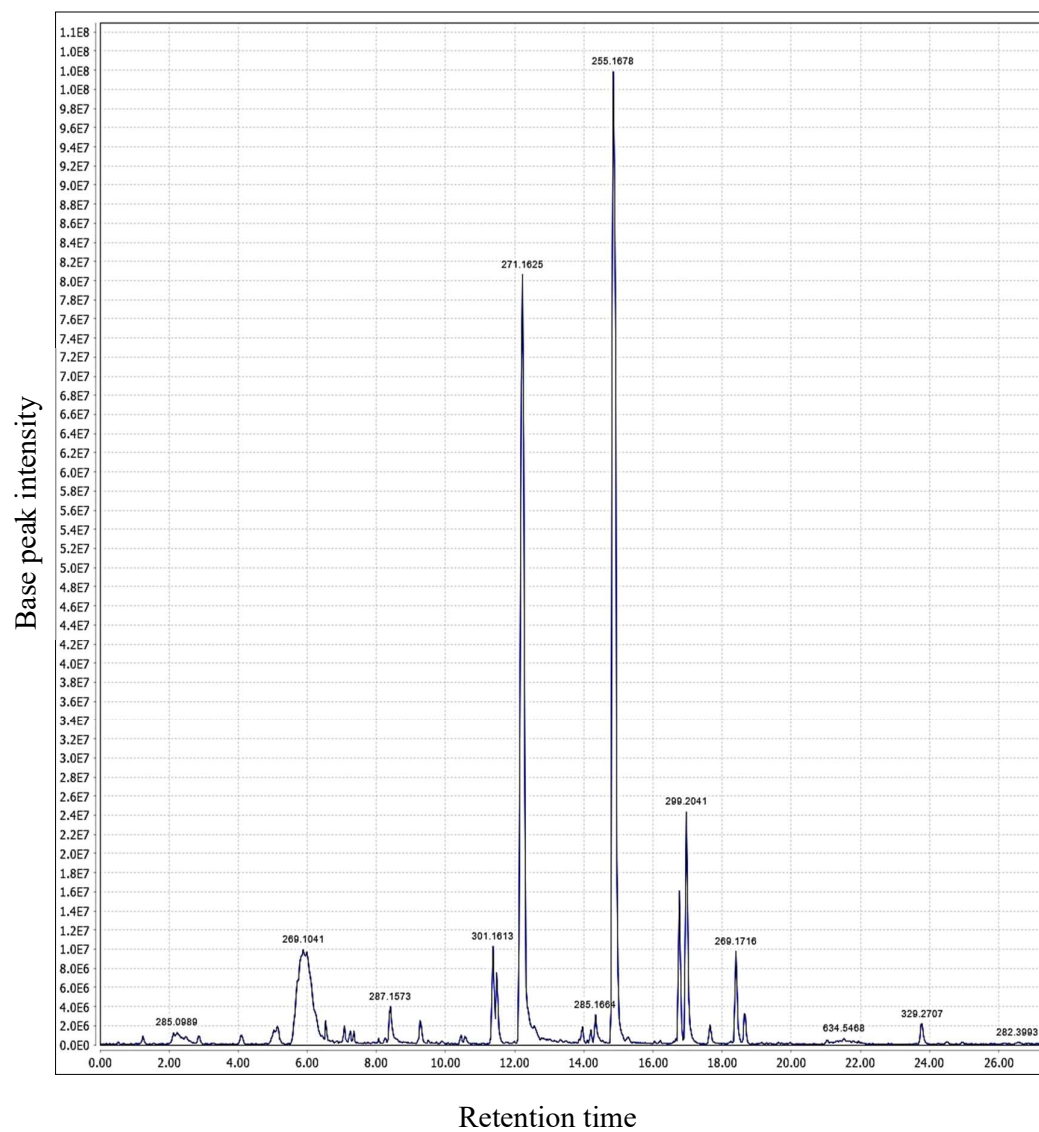


Figure 21b. Spectra were recorded in positive ionization mode across the mass-to-charge ratio (m/z) range of 150–2000.

Table 5. Metabolites detected by LC-MS in the ethanolic and aqueous extract of *Oroxylum indicum*.

Retention Time	Compound Name	Formula	Mass
5.87	2'-Deoxyguanosine monohydrate	C ₁₀ H ₁₃ N ₅ O ₄	267.1807
8.39	Scutellarein	C ₁₅ H ₁₀ O ₆	285.1664
11.39	Hispidulin	C ₁₆ H ₁₂ O ₆	299.2041
11.49	Oroxylin B	C ₁₆ H ₁₂ O ₄	269.2054
15.11	D-Glucosamine-6-phosphate sodium salt	C ₆ H ₁₄ NO ₈ P	255.2353
23.05	Gamma-linolenic acid	C ₁₈ H ₃₀ O ₂	279.3624
22.37	Palmitoleic acid	C ₁₆ H ₃₀ O ₂	253.1769
32.75	Acacetin	C ₁₆ H ₁₂ O ₅	283.4117
20.56	6-Phosphogluconic acid barium salt hydrate	C ₆ H ₁₃ O ₁₀ P	277.3714
8.41	Baicalein	C ₁₆ H ₁₄ O ₅	287.1573
11.47	Pelargonidin chloride	C ₁₅ H ₁₁ O ₅	271.1288
14.85	Chrysin derivative	C ₁₆ H ₃₀ O ₂	255.1678
11.37	Quercetin	C ₁₅ H ₁₀ O ₇	301.1631
2.43	Oroxylin A	C ₁₆ H ₁₂ O ₅	285.0989
5.95	Formononetin	C ₁₆ H ₁₂ O ₄	269.1041
17.25	All-trans retinoic acid	C ₂₀ H ₂₈ O ₂	299.2041
1.21	m-Hydroxycinnamic acid	C ₉ H ₈ O ₃	163.0152
1.55	Quercetin	C ₁₅ H ₁₀ O ₇	301.0938

4.18	Sinapic acid	C ₁₁ H ₁₂ O ₅	224.1236
6.50	UDP-beta-L-rhamnose	C ₁₅ H ₂₄ N ₂ O ₁₆ P ₂	549.1970
8.78	Trans-3,5-dimethoxy-4-hydroxycinnamaldehyde	C ₁₁ H ₁₂ O ₄	207.2520
8.81	Guanine	C ₅ H ₅ N ₅ O	151.2054
12.91	Alpha-D-glucose-1-phosphate dipotassium salt dihydrate	C ₆ H ₁₃ O ₉ P	260.1619
14.99	Thymidine-5'-diphosphate sodium salt	C ₁₀ H ₁₆ N ₂ O ₁₁ P ₂	403.2410
16.08	S-Lactoylglutathione	C ₁₃ H ₂₁ N ₃ O ₈ S	383.2072
16.76	Ferulic acid	C ₁₀ H ₁₀ O ₄	194.0922
16.93	Daidzin	C ₂₁ H ₂₀ O ₉	415.2884
19.15	Cis-Zeatin	C ₁₀ H ₁₃ N ₅ O	221.1205
19.97	Petunidin	C ₁₆ H ₁₃ O ₇	317.2238
23.75	Adenosine-3',5'-cyclicmonophosphate	C ₁₀ H ₁₂ N ₅ O ₆ P	329.2707
25.15	Isorhamnetin-3-galactoside-6"-rhamnoside	C ₂₈ H ₃₂ O ₁₆	621.4523
25.97	Chlorogenic acid Hemihydrate	C ₁₆ H ₁₈ O ₉	354.2084
26.85	Isorhamnetin	C ₁₆ H ₁₂ O ₇	313.5118
29.31	Sodium deoxycholate	C ₂₄ H ₄₀ O ₄	391.4299
1.16	D- (-)-Quinic acid	C ₇ H ₁₂ O ₆	193.1474

6.55	Quercetin-3-(6-malonyl) Glucoside	C ₂₄ H ₂₂₀ O ₁₅	547.3746
7.30	Linarin	C ₂₈ H ₃₂ O ₁₄	589.3574
7.40	Lupeol	C ₃₀ H ₅₀ O	427.3358
14.87	Chrysin	C ₁₅ H ₁₀ O ₄	253.1094
15.28	2'-Deoxyguanosine monohydrate	C ₁₀ H ₁₃ N ₅ O ₄	265.2910
16.16	N-acetylneuraminic acid	C ₁₁ H ₁₉ NO ₉	309.2600
16.61	Uridine-5'-monophosphate	C ₉ H ₁₃ N ₂ O ₉ P	325.2888
16.91	(+)-Catechin hydrate	C ₁₅ H ₁₄ O ₆	293.2988
17.36	Alpha-D-glucose-1,6- diphosphate potassium salt hydrate	C ₆ H ₁₄ O ₁₂ P ₂	339.3943
21.11	2'-Deoxycytidine	C ₉ H ₁₃ N ₃ O ₄	227.3293
21.52	6- (Gamma, gamma-dimethyl allylamino) purine	C ₁₀ H ₁₃ N ₅	205.2949
23.09	Gamma-linolenic acid	C ₁₈ H ₃₀ O ₂	279.3286
23.39	L-homocarnosine	C ₁₀ H ₁₆ N ₄ O ₃	241.3666
25.85	D-glucosamine-6-phosphate sodium salt	C ₆ H ₁₄ NO ₈ P	255.3703
32.67	Luteolin	C ₁₅ H ₁₀ O ₆	283.4117
32.77	Xanthosine	C ₁₀ H ₁₂ N ₄ O ₆	283.5129
32.84	Acacetin	C ₁₆ H ₁₂ O ₅	283.4117

3.7. Acute Oral Toxicity Test of *Oroxylum indicum* Extracts in Rat

Table 6 presents the clinical observations of female albino Wistar rats administered ethanolic and aqueous *O. indicum* extracts. Acute toxicity evaluation of *O. indicum* extract in female rats revealed no mortality or severe clinical manifestations across all examined doses (1000–5000 mg kg⁻¹). Control and 1000 mg kg⁻¹ groups exhibited no alterations in clinical parameters, including fur condition, ocular/tegumentary coloration, neurological activity (tremor, paralysis), or gastrointestinal function (diarrhea, loose stool). Transient hypoactivity (+) was observed at 3000 mg kg⁻¹, characterized by reduced exploratory behavior without progression to sedation or coma.

The 5000 mg kg⁻¹ cohort exhibited similar hypoactivity (+) alongside intermittent loose stool (+), indicative of mild gastrointestinal irritation. Notably, no dose elicited systemic toxicity markers, such as salivation, dermal inflammation (swollen paws), hemorrhagic events (ear bleeding, red tear), or autonomic dysregulation (altered respiration, thermoregulatory failure). The absence of histopathological correlates (coma, paralysis) suggests limited neurotoxic potential. These findings indicate a high acute safety threshold for *O. indicum* extract, with adverse effects limited to reversible, non-life-threatening endpoints at supratherapeutic doses.

3.8. Histopathological Assessment of Liver Tissue Following *Oroxylum indicum* Extract Administration

In the present study, histological examination of liver tissues was conducted to determine the probable toxicological effects of ethanolic (OIE) and aqueous (OIA) *Oroxylum indicum* stem bark extracts at varying doses (1000, 3000, and 5000 mg kg⁻¹). Liver sections were stained and observed under a light microscope at 40× magnification to evaluate structural alterations (Figure 22).

Table 6. Clinical observations of female albino Wistar rats treated with ethanolic and aqueous *Oroxylum indicum* extracts

Clinical signs	Control group	1000 mg kg ⁻¹	3000 mg kg ⁻¹	5000 mg kg ⁻¹
		group	group	group
	Female	Female	Female	Female
Mortality	-	-	-	-
sedation	-	-	-	-
Salivation	-	-	-	-
Fur	-	-	-	-
Tremor	-	-	-	-
Diarrhoea	-	-	-	-
Drowsiness	-	-	-	-
Skin colour	-	-	-	-
Eye colour	-	-	-	-
Coma	-	-	-	-
Ear bleeding	-	-	-	-
Movement	-	-	+	+
Swollen paws	-	-	-	-
Red tear	-	-	-	-
Paralysis	-	-	-	-
Loose stool	-	-	-	+

-: Not observed, +: Observed

Control group (A-B)

Liver sections from the control group showed typical liver histology, characterized by well-organized hepatocytes with centrally located nuclei, distinct hepatic veins, intact sinusoids, and a well-preserved portal triad. We did not find evidence of cellular degeneration, necrosis, or inflammatory infiltration.

Ethanollic and aqueous extract-treated groups (C-H)

Histological sections from the experimental groups receiving ethanollic and aqueous extracts at 1000 mg kg⁻¹ (C-D), 3000 mg kg⁻¹ (E-F), and 5000 mg kg⁻¹ (G-H) exhibited nonsignificant deviations from the usual hepatic histology. The hepatocytes maintained their polygonal shape with prominent nuclei and the sinusoids were clearly delineated without congestion. No evidence of hepatocellular necrosis, inflammatory infiltrates, bile duct hyperplasia, or vacuolar degeneration was observed in any of the treatment groups.

The absence of pathological abnormalities in liver sections across all extract-treated groups suggests that administration of ethanollic and aqueous *Oroxylum indicum* extracts up to 5000 mg kg⁻¹ did not elicit significant hepatotoxicity. The structure of the liver tissues remained preserved, supporting the safety profile of *O. indicum* extracts at high dosages. These findings support the non-toxic nature of plant extracts in hepatic tissues.

3.9. Diclofenac Exposure and Survival Test

Table 7 shows the survival outcomes of rats subjected to varying diclofenac doses over a 96 hours period. The results obtained in the present study revealed a dose-dependent mortality pattern across the administered range (50–500 mg kg⁻¹). At the highest dose (500 mg kg⁻¹ body weight) of diclofenac, total mortality was observed by the second day, with no subjects surviving beyond 48 hours. The 250 mg kg⁻¹ group exhibited a delayed lethal effect, with survival extending to Day 2 but ceasing by Day 3. A more pronounced latency was noted in the 100 mg kg⁻¹ cohort, where mortality onset occurred only on Day 4.

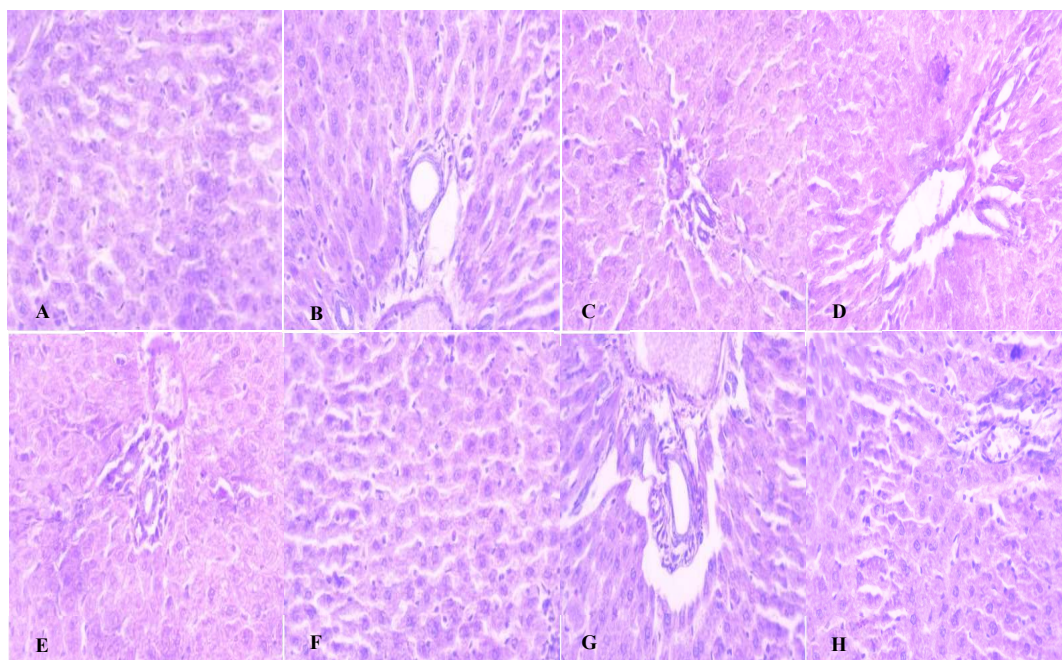


Figure 22. Photomicrographs depicting the histology of liver tissues treated with ethanolic and aqueous extracts of *Oroxylum indicum*. The control group, A-B, shows the normal histological architecture of the liver. In the experimental groups, C-D, E-F, and G-H correspond to the ethanolic and aqueous extract-treated groups at dosages of 1000, 3000, and 5000 mg kg⁻¹, respectively. No discernible alterations were apparent in the histological characteristics of the liver tissues within these experimental groups, reaffirming the maintenance of normal liver structure throughout the study.

Table 7. The table shows the survival outcomes of subjects administered different doses of diclofenac over four days. Each row represents a specific dosage (mg kg^{-1}), and each column indicates survival status on a given day. "Live" denotes survival, and "Death" marks the day of mortality. These data suggest a dose-dependent effect, with higher doses leading to earlier mortality.

Diclofenac	Diclofenac Treated Days			
	1	2	3	4
500 mg kg^{-1}	Live	Death		
250 mg kg^{-1}	Live	Live	Death	
100 mg kg^{-1}	Live	Live	Live	Death
50 mg kg^{-1}	Live	Live	Live	Live

In contrast, the lowest dose group (50 mg kg⁻¹) examined in the present study sustained 100 % survival throughout the four-day period, suggesting that this dose was non-lethal under acute exposure conditions. The results shown in Table 8 revealed a negative relationship between diclofenac dosage and survival duration. The absence of delayed mortality in the 50 mg kg⁻¹ group suggests a threshold below which acute toxicity does not appear. The results presented in the present study indicate a limited therapeutic index for diclofenac and imply dose-dependent hepatorenal risks in rodent models.

3.10 Effect of *Oroxylum indicum* Extracts on DIC-Induced Body Weight

Protective effects of ethanolic (OIE) and aqueous (OIA) of *O. indicum* stem bark extracts on body weight loss of subjects after exposure to diclofenac (DIC) was evaluated in the present study. The results are presented in Table 8. The initial body weights on day 0 were comparable across all the groups ($p > 0.05$). However, by Day 5, the DIC-treated group exhibited a marked decline in body weight (200 ± 4.6 g) compared to the control (230.3 ± 5.2 g). This reduction was also observed in the extract-treated groups, although the magnitude varied with the dose and extract type.

Among the treatment groups, silymarin (50 mg kg⁻¹) co-administration resulted in modest weight recovery (201.3 ± 1.2 g) but remained significantly lower than that of the control ($P < 0.05$). Subjects receiving ethanolic extract (OIE) at 250 mg kg⁻¹ and 500 mg kg⁻¹ exhibited dose-dependent protection, with the higher dose (500 mg kg⁻¹) showing better weight retention (211 ± 2.1 g) compared to the lower dose (185.3 ± 3.3 g). The aqueous extract (OIA) followed a similar trend, with the 500 mg kg⁻¹ dose (188.7 ± 2.2 g) offering better weight maintenance than the 250 mg kg⁻¹ group (208.7 ± 0.9 g).

By Day 16, control rats demonstrated a significant weight gain (262.7 ± 3.2 g). In contrast, DIC-treated rats maintained significantly lower body weight (183 ± 4.1 g), reflecting sustained hepatotoxic effects. Silymarin-treated rats showed substantial improvement (223.3 ± 1.45 g), with normal weight restoration. Among the extract-treated groups, OIE (500 mg kg⁻¹) resulted in the most pronounced recovery ($225.7 \pm$

0.7 g), which was comparable to that of silymarin. The aqueous extract (OIA) also promoted weight gain, with the highest dose (500 mg kg⁻¹) reaching 218.7 ± 1.5 g.

Overall, the diclofenac-induced weight loss was evident, confirming its hepatotoxic effects. Both extracts exhibited dose-dependent protective effects, with OIE demonstrating superior efficacy at equivalent doses. However, none of the treatments fully restored the body weight to control levels within the study period.

3.11. Effects of *Oroxylum indicum* Extracts on Organ Weights

The effects of ethanolic (OIE) and aqueous (OIA) stem bark extracts of *Oroxylum indicum* on the organ weights of Wistar albino male rats were evaluated following diclofenac (DIC)-induced hepatotoxicity. Significant weight reduction was observed in the vital organs of the DIC-treated group. This result confirmed the toxicity of diclofenac (Table 9).

Heart and kidney weights

The control group exhibited a mean heart weight of 0.58 ± 0.021 g g⁻¹ body weight, while diclofenac administration (50 mg kg⁻¹) resulted in a substantial decrease (0.25 ± 0.002 g g⁻¹; $p < 0.05$). Co-treatment with silymarin (0.35 ± 0.02 g g⁻¹) and OIE (500 mg kg⁻¹) (0.34 ± 0.009 g g⁻¹) significantly improved heart weight, though still lower than control values. Aqueous extract at 500 mg kg⁻¹ (0.31 ± 0.008 g g⁻¹) also restored heart weight to some extent, but was less effective than the ethanolic extract (Table 10).

Similarly, kidney weight declined significantly in DIC-treated rats (0.40 ± 0.01 g g⁻¹) compared to controls (0.66 ± 0.04 g g⁻¹). Silymarin-treated rats showed near-normal kidney weight (0.63 ± 0.02 g g⁻¹), comparable to the higher dose of OIE (0.60 ± 0.03 g g⁻¹). The aqueous extract at 500 mg kg⁻¹ restored kidney weight to 0.55 ± 0.03 g g⁻¹, showing moderate efficacy.

Table 8. Effects of ethanolic and aqueous stem bark extracts of *Oroxylum indicum* on body weight (g) of male Wistar albino rats. All data are expressed in triplicate $\pm$ SEM (n = 3). Different superscript letters within a column indicate significant differences at $p < 0.05$, according to Duncan's multiple comparison test.

Days	Control	DIC (100 mg kg ⁻¹)	DIC + Silymarin (50 mg kg ⁻¹)	DIC + OIE (250 mg kg ⁻¹)	DIC + OIE (500 mg kg ⁻¹)	DIC + OIA (250 mg kg ⁻¹)	DIC + OIA (500 mg kg ⁻¹)
Day 0	219 $\pm$ 6.4 ^b	228.7 $\pm$ 2.3 ^a	227.7 $\pm$ 1.8 ^a	224.7 $\pm$ 1.8 ^a	240 $\pm$ 2.1 ^a	235.3 $\pm$ 0.9 ^a	225 $\pm$ 1.2 ^a
Day 5	230.3 $\pm$ 5.2 ^b	200 $\pm$ 4.6 ^b	201.3 $\pm$ 1.2 ^b	185.3 $\pm$ 3.3 ^c	211 $\pm$ 2.1 ^c	208.7 $\pm$ 0.9 ^c	188.7 $\pm$ 2.2 ^c
Day 16	262.7 $\pm$ 3.2 ^a	183 $\pm$ 4.1 ^c	223.3 $\pm$ 1.45 ^a	209.7 $\pm$ 0.9 ^b	225.7 $\pm$ 0.7 ^b	213 $\pm$ 1.2 ^b	218.7 $\pm$ 1.5 ^b

Lungs and spleen weights

Lung weight was significantly reduced in the DIC group ($0.46 \pm 0.04 \text{ g g}^{-1}$), indicating possible pulmonary involvement in diclofenac toxicity. Among the treatments, silymarin ($0.67 \pm 0.02 \text{ g g}^{-1}$) and OIE (500 mg kg^{-1}) ($0.64 \pm 0.03 \text{ g g}^{-1}$) were most effective in reversing this reduction (Table 10).

The spleen followed a similar trend, with the DIC group showing a marked decrease ($0.15 \pm 0.01 \text{ g/g}$) compared to the control ($0.22 \pm 0.01 \text{ g g}^{-1}$). Silymarin ($0.18 \pm 0.01 \text{ g g}^{-1}$) and OIE (500 mg kg^{-1}) ($0.19 \pm 0.01 \text{ g g}^{-1}$) improved spleen weight, suggesting partial recovery of immune function (Table 10).

Liver and testis weights

Liver weight was significantly lower in DIC-treated rats ($1.8 \pm 0.05 \text{ g g}^{-1}$) than in controls ($2.57 \pm 0.07 \text{ g g}^{-1}$), reflecting hepatic atrophy (Table 10). Treatment with silymarin ($2.4 \pm 0.05 \text{ g g}^{-1}$) and OIE (500 mg kg^{-1}) ($2.4 \pm 0.05 \text{ g g}^{-1}$) restored liver weight close to normal levels. Interestingly, the aqueous extract at 500 mg kg^{-1} showed complete recovery ($2.6 \pm 0.05 \text{ g/g}$), indicating potential hepatoprotective superiority over OIE.

Testicular weight was also notably reduced in the DIC group ($0.52 \pm 0.02 \text{ g g}^{-1}$ vs. $0.93 \pm 0.08 \text{ g g}^{-1}$ in controls), showing possible gonadotoxic effects of diclofenac. Both the silymarin- and extract-treated groups showed similar recovery patterns. It was observed that OIE (500 mg kg^{-1}) ($0.6 \pm 0.03 \text{ g g}^{-1}$) and OIA (500 mg kg^{-1}) ($0.59 \pm 0.05 \text{ g g}^{-1}$) showed the most significant improvement in recovery of testicular weight (Table 10).

The above results showed that diclofenac administration caused significant organ weight reduction, particularly in the heart, liver, kidneys, and testes. Treatment with the *Oroxylum indicum* extract showed dose-dependent protection. OIE (500 mg kg^{-1}) and OIA (500 mg kg^{-1}) demonstrated protein levels that were comparable to those of silymarin. Notably, in the present study, 500 mg kg^{-1} OIA restored liver weight more effectively than did OIE. This trend can be attributed to marked differences in the phytochemical profiles of OIE and OIA.

Table 9. Effects of ethanolic and aqueous stem bark extracts of *Oroxylum indicum* on relative organ weight (g g⁻¹ body weight) of Wistar albino male rats. All data are expressed in triplicate $\pm$ SEM (n = 3). Values in rows with different superscript letters denote significant variations at p < 0.05, as per Duncan's multiple comparison test.

Organs	Control	DIC (100 mg kg ⁻¹)	DIC + Silymarin (50 mg kg ⁻¹)	DIC + OIE (250 mg kg ⁻¹)	DIC + OIE (500 mg kg ⁻¹)	DIC + OIA (250 mg kg ⁻¹)	DIC + OIA (500 mg kg ⁻¹)
Heart	0.58 $\pm$ 0.021 ^a	0.25 $\pm$ 0.002 ^d	0.35 $\pm$ 0.02 ^b	0.25 $\pm$ 0.007 ^d	0.34 $\pm$ 0.009 ^b	0.26 $\pm$ 0.007 ^d	0.31 $\pm$ 0.008 ^c
Kidneys	0.66 $\pm$ 0.04 ^a	0.40 $\pm$ 0.01 ^c	0.63 $\pm$ 0.02 ^{ab}	0.41 $\pm$ 0.021 ^c	0.60 $\pm$ 0.03 ^{ab}	0.37 $\pm$ 0.021 ^c	0.55 $\pm$ 0.03 ^b
Lungs	0.67 $\pm$ 0.03 ^{ab}	0.46 $\pm$ 0.04 ^d	0.67 $\pm$ 0.02 ^a	0.55 $\pm$ 0.01 ^{cd}	0.64 $\pm$ 0.03 ^{abc}	0.53 $\pm$ 0.05 ^d	0.56 $\pm$ 0.04 ^{bcd}
Spleen	0.22 $\pm$ 0.01 ^a	0.15 $\pm$ 0.01 ^d	0.18 $\pm$ 0.01 ^b	0.17 $\pm$ 0.01 ^{bcd}	0.19 $\pm$ 0.01 ^b	0.15 $\pm$ 0.004 ^{cd}	0.17 $\pm$ 0.01 ^{bc}
Liver	2.57 $\pm$ 0.07 ^a	1.8 $\pm$ 0.05 ^d	2.4 $\pm$ 0.05 ^b	2.0 $\pm$ 0.03 ^c	2.4 $\pm$ 0.05 ^b	1.9 $\pm$ 0.04 ^{cd}	2.6 $\pm$ 0.05 ^a
Testis	0.93 $\pm$ 0.08 ^a	0.52 $\pm$ 0.02 ^b	0.58 $\pm$ 0.01 ^b	0.56 $\pm$ 0.03 ^b	0.6 $\pm$ 0.03 ^b	0.49 $\pm$ 0.01 ^b	0.59 $\pm$ 0.05 ^b

3.12. Effects of *Oroxylum indicum* Stem Bark Extracts on Hematological Parameters

The hematological profile of Wistar albino male rats was assessed on day 5 and 16 after diclofenac-induced toxicity. The results indicated significant alterations in hematological parameters, with varying degrees of recovery upon pretreatment with ethanolic (OIE) and aqueous (OIA) extracts of *Oroxylum indicum* (Table 10).

White blood cell (WBC) count

On Day 5, the WBC count increased significantly in the DIC-treated group ($10.7 \pm 0.1 \times 10^3 \text{ mL}^{-1}$) compared to the control ($7.8 \pm 0.4 \times 10^3 \text{ mL}^{-1}$), indicating an inflammatory response. All treatment groups showed elevated WBC counts, without significant differences from those in the DIC group. By Day 16, the DIC group showed further WBC elevation ($11.6 \pm 0.17 \times 10^3 \text{ mL}^{-1}$), whereas silymarin ($8.5 \pm 0.2 \times 10^3 \text{ mL}^{-1}$) and OIE (500 mg kg^{-1}) ($8.5 \pm 0.6 \times 10^3 \text{ mL}^{-1}$) significantly reduced WBC levels close to control values ($8.3 \pm 0.4 \times 10^3 \text{ mL}^{-1}$).

Red blood cell (RBC) count

On Day 5, a marked reduction was observed in the DIC-treated rats ($5.3 \pm 0.1 \times 10^6 \text{ mL}^{-1}$), significantly lower than the control ($8.9 \pm 0.32 \times 10^6 \text{ mL}^{-1}$). All extract-treated groups showed slight improvements, but the RBC values remained lower than normal. By Day 16, there was partial recovery, with RBC counts in silymarin ($7.96 \pm 0.44 \times 10^6 \text{ mL}^{-1}$) and OIE (500 mg kg^{-1}) ($8 \pm 0.62 \times 10^6 \text{ mL}^{-1}$) approaching control levels ($8.3 \pm 1 \times 10^6 \text{ mL}^{-1}$).

Hemoglobin (Hb) levels

Diclofenac significantly lowered hemoglobin levels at Day 5 ($9.6 \pm 0.44 \text{ g dL}^{-1}$) compared to the control ($13.9 \pm 0.23 \text{ g dL}^{-1}$). All the treated groups exhibited minor improvements, but none showed full restoration. By Day 16, silymarin ($13.4 \pm 0.17 \text{ g dL}^{-1}$) and OIE (500 mg kg^{-1}) ($13.2 \pm 0.18 \text{ g dL}^{-1}$) were able to reverse diclofenac-induced hemoglobin reduction, nearly matching control values.

Hematocrit (HCT) levels

A significant reduction in hematocrit was observed in DIC-treated rats on day 5 (28.9 ± 1.3 %) compared to the control (41.7 ± 0.7 %). At this stage, recovery was minimal in all treated groups. By Day 16, silymarin (40.2 ± 0.52 %) and OIE (500 mg kg^{-1}) (39.5 ± 0.53 %) showed substantial recovery, demonstrating a protective effect on hematocrit levels.

Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC)

At Day 5, a significant increase in MCV and MCH was noted in the DIC-treated group ($54.6 \pm 2.6 \text{ fL}$ and $18.2 \pm 1 \text{ pg}$) compared to the control ($46.8 \pm 2.4 \text{ fL}$ and $15.6 \pm 0.8 \text{ pg}$, respectively), indicating macrocytosis. These values remained elevated across all the treatment groups. By Day 16, MCV and MCH normalized in the silymarin and OIE (500 mg kg^{-1}) groups, suggesting reversal of diclofenac-induced hematological alterations. MCHC levels followed a similar trend, with the DIC group showing a marked decline at Day 5 ($28.9 \pm 1.3 \text{ g dL}^{-1}$) compared to the control ($41.7 \pm 0.7 \text{ g dL}^{-1}$). While recovery was incomplete in most groups, silymarin ($40.2 \pm 0.52 \text{ g dL}^{-1}$) and OIE (500 mg kg^{-1}) ($39.5 \pm 0.53 \text{ g dL}^{-1}$) exhibited significant improvements by Day 16.

Therefore, the above results show that diclofenac administration severely alters hematological parameters, leading to leukocytosis, anemia, and reduced hematocrit levels. Treatment with *O. indicum* extracts attenuated these effects, with OIE (500 mg kg^{-1}) showing superior recovery, comparable to that of silymarin. While the WBC count remained elevated in most treatment groups at day 5, it normalized by day 16 in the silymarin and high-dose OIE groups. Similarly, hemoglobin and hematocrit levels were restored in these groups, indicating their potential role in mitigating diclofenac-induced hematotoxicity.

Table 10. Effects of ethanolic and aqueous *Oroxylum indicum* stem bark extracts on biochemical parameters of Wistar albino male rats. All data are expressed in triplicate $\pm$ SEM (n = 3). Values in rows with different superscript letters denote significant variations at $p < 0.05$, as per Duncan's multiple comparison test.

Days	Parameters	Control	DIC (100 mg kg ⁻¹)	DIC + Silymarin (50 mg kg ⁻¹)	DIC + OIE (250 mg kg ⁻¹)	DIC + OIE (500 mg kg ⁻¹)	DIC + OIA (250 mg kg ⁻¹)	DIC + OIA (500 mg kg ⁻¹)
5	WBC (10 ³ mL ⁻¹)	7.8 $\pm$ 0.4 ^b	10.7 $\pm$ 0.1 ^a	10.7 $\pm$ 0.2 ^a	10.9 $\pm$ 0.2 ^a	10.8 $\pm$ 0.3 ^a	10.9 $\pm$ 0.3 ^a	10.7 $\pm$ 0.3 ^a
16	WBC (10 ³ mL ⁻¹)	8.3 $\pm$ 0.4 ^d	11.6 $\pm$ 0.2 ^a	8.5 $\pm$ 0.2 ^{cd}	10.3 $\pm$ 0.2 ^b	8.5 $\pm$ 0.6 ^{cd}	10.5 $\pm$ 0.3 ^b	9.3 $\pm$ 0.4 ^c
5	RBC (10 ⁶ mL ⁻¹)	8.9 $\pm$ 0.3 ^a	5.3 $\pm$ 0.1 ^b	5.7 $\pm$ 0.2 ^b	5.8 $\pm$ 0.2 ^b	5.7 $\pm$ 0.2 ^b	5.5 $\pm$ 0.2 ^b	5.53 $\pm$ 0.3 ^b
16	RBC (10 ⁶ mL ⁻¹)	8.3 $\pm$ 1 ^a	7.1 $\pm$ 1 ^a	7.96 $\pm$ 0.4 ^a	7.5 $\pm$ 0.5 ^a	8 $\pm$ 0.6 ^a	7.3 $\pm$ 0.1 ^a	7.1 $\pm$ 0.2 ^a
5	Hb (g dL ⁻¹)	13.9 $\pm$ 0.2 ^a	9.6 $\pm$ 0.4 ^b	10 $\pm$ 0.1 ^b	9.6 $\pm$ 0.4 ^b	9.6 $\pm$ 0.4 ^b	9.8 $\pm$ 0.3 ^b	9.6 $\pm$ 0.4 ^b
16	Hb (g dL ⁻¹)	13.7 $\pm$ 0.2 ^a	9.7 $\pm$ 0.3 ^d	13.4 $\pm$ 0.2 ^a	10.9 $\pm$ 0.2 ^c	13.2 $\pm$ 0.2 ^a	10.6 $\pm$ 0.2 ^c	11.8 $\pm$ 0.1 ^b
5	HCT (%)	41.7 $\pm$ 0.7 ^a	28.9 $\pm$ 1.3 ^b	30.1 $\pm$ 0.4 ^b	28.9 $\pm$ 1.2 ^b	28.9 $\pm$ 1.3 ^b	29.4 $\pm$ 1 ^b	28.8 $\pm$ 1.1 ^b
16	HCT (%)	41.2 $\pm$ 0.6 ^a	29.1 $\pm$ 0.8 ^d	40.2 $\pm$ 0.5 ^a	32.7 $\pm$ 0.7 ^c	39.5 $\pm$ 0.5 ^a	31.9 $\pm$ 0.6 ^c	35.4 $\pm$ 0.4 ^b
5	MCV (fL)	46.8 $\pm$ 2.4 ^a	54.6 $\pm$ 2.6 ^a	53.3 $\pm$ 2.8 ^a	50.3 $\pm$ 0.1 ^a	51 $\pm$ 0.1a ^a	53 $\pm$ 2.6 ^a	52.3 $\pm$ 3.5 ^a
16	MCV (fL)	43.1 $\pm$ 1 ^c	47.2 $\pm$ 0.7 ^b	46.5 $\pm$ 1.4 ^{bc}	46.1 $\pm$ 1.5 ^{bc}	46.1 $\pm$ 0.6 ^{bc}	44.3 $\pm$ 1 ^{bc}	50.4 $\pm$ 0.6 ^a

5	MCH (pg)	15.6 ± 0.8^a	18.2 ± 1^a	17.8 ± 0.9^a	16.8 ± 1.2^a	17 ± 0.6^a	17.8 ± 0.9^a	17.2 ± 0.3^a
16	MCH (pg)	14.4 ± 0.3^c	15.7 ± 0.23^b	15.5 ± 0.46^b	15.4 ± 0.48^{bc}	15.4 ± 0.19^{bc}	14.8 ± 0.3^{bc}	16.8 ± 0.2^a
5	MCHC (g dL ⁻¹)	41.7 ± 0.7^a	28.9 ± 1.3^b	30.1 ± 0.4^b	28.9 ± 1.2^b	28.9 ± 1.3^b	29.4 ± 1^b	28.8 ± 1.1^b
16	MCHC (g dL ⁻¹)	41.2 ± 0.6^a	29.1 ± 0.8^d	40.2 ± 0.52^a	32.7 ± 0.7^c	39.5 ± 0.5^a	31.9 ± 0.6^c	35.4 ± 0.4^b

3.13. Ameliorative Effect of *Oroxylum indicum* Extract Against DIC-Induced Hepatotoxicity

The effects of ethanolic (OIE) and aqueous (OIA) stem bark extracts of *O. indicum* on serum enzyme activities in diclofenac (DIC)-treated and untreated Wistar albino male rats were assessed on days 5 and 16 (Table 11). Diclofenac administration led to significant alterations in the liver function markers, reflecting hepatocellular damage. The administration of OIE and OIA extracts demonstrated a dose-dependent hepatoprotective effect with variations in recovery rates.

Total protein (TP) levels

On Day 5, total protein levels declined significantly in the DIC group ($4.97 \pm 0.3 \text{ g dL}^{-1}$) compared to the control ($7.8 \pm 0.3 \text{ g dL}^{-1}$), indicating impaired hepatic synthetic function. None of the treatment groups showed significant improvement at this stage. By Day 16, silymarin ($6.8 \pm 0.04 \text{ g dL}^{-1}$) and OIE (500 mg kg^{-1}) ($7.4 \pm 0.2 \text{ g dL}^{-1}$) resulted in substantial recovery, approaching control levels ($8.1 \pm 0.08 \text{ g dL}^{-1}$). The aqueous extract (500 mg kg^{-1}) also improved TP levels ($6.8 \pm 0.4 \text{ g dL}^{-1}$) but was slightly less effective than the ethanolic extract.

Total bilirubin (TB) levels

At Day 5, total bilirubin levels increased significantly in DIC-treated rats ($2.1 \pm 0.3 \text{ mg dL}^{-1}$) compared to the control ($0.8 \pm 0.05 \text{ mg dL}^{-1}$), indicating hepatic dysfunction. None of the treatment groups showed a significant bilirubin reduction at this stage. By Day 16, silymarin ($0.91 \pm 0.06 \text{ mg dL}^{-1}$) and OIE (500 mg kg^{-1}) ($0.93 \pm 0.03 \text{ mg dL}^{-1}$) normalized bilirubin levels, whereas OIA (500 mg kg^{-1}) showed a moderate reduction ($0.98 \pm 0.02 \text{ mg dL}^{-1}$).

Serum glutamate-pyruvate transaminase (SGPT/ALT) levels

SGPT levels were markedly elevated in the DIC group at Day 5 ($87.2 \pm 0.9 \text{ U L}^{-1}$) compared to the control ($38.5 \pm 0.5 \text{ U L}^{-1}$). Silymarin and all treatment groups exhibited

elevated SGPT levels, indicating that hepatic injury persisted. However, by Day 16, SGPT levels decreased significantly in the silymarin ($48.2 \pm 0.8 \text{ U L}^{-1}$) and OIE (500 mg kg^{-1}) ($52.7 \pm 0.9 \text{ U L}^{-1}$) groups, nearing control values ($36.4 \pm 0.6 \text{ U L}^{-1}$). The OIA (500 mg kg^{-1}) group ($59 \pm 1.4 \text{ U L}^{-1}$) also showed improvement, but the reduction was less pronounced.

Serum glutamate-oxaloacetate transaminase (SGOT/AST) levels

SGOT levels at Day 5 were significantly increased in the DIC group ($337 \pm 9.3 \text{ U L}^{-1}$) compared to the control ($102.9 \pm 1.6 \text{ U L}^{-1}$), reflecting hepatic cellular damage. Although slight reductions were observed in the treatment groups, SGOT levels remained high. By Day 16, silymarin ($145.2 \pm 1.4 \text{ U L}^{-1}$) and OIE (500 mg kg^{-1}) ($148.8 \pm 1.4 \text{ U L}^{-1}$) exhibited a notable decrease, whereas OIA (500 mg kg^{-1}) ($154.6 \pm 1.9 \text{ U L}^{-1}$) showed moderate improvement.

Alkaline phosphatase (ALP) levels

ALP levels followed a similar pattern, with a marked increase in the DIC group ($307 \pm 3.9 \text{ U L}^{-1}$ at Day 5) compared to the control ($48.8 \pm 1.4 \text{ U L}^{-1}$). No significant changes were observed any in of the treatment groups at this stage. However, by Day 16, ALP levels normalized in the silymarin ($57.3 \pm 1.6 \text{ U L}^{-1}$) and OIE (500 mg kg^{-1}) ($58.5 \pm 1.3 \text{ U L}^{-1}$) groups, while OIA (500 mg kg^{-1}) ($62.8 \pm 1.9 \text{ U L}^{-1}$) also demonstrated hepatoprotective effects.

In conclusion, diclofenac administration caused significant hepatotoxicity, as indicated by the reduced total protein, increased bilirubin, and elevated liver enzymes (SGPT, SGOT, and ALP). Treatment with *Oroxylum indicum* extracts provided dose-dependent protection, with OIE (500 mg kg^{-1}) showing the highest efficacy, comparable to that of silymarin. SGPT, SGOT, and ALP levels were significantly restored by day 16, suggesting the hepatoprotective potential of the extract. However, OIA (500 mg kg^{-1}) showed moderate protection, particularly in bilirubin and total protein restoration, but was less effective than OIE in terms of enzyme recovery.

Table 11. Effects of ethanolic and aqueous *Oroxylum indicum* stem bark extracts on serum enzyme activities in Wistar albino male rats. All data are expressed in triplicate $\pm$ SEM (n = 3). Values in rows with different superscript letters denote significant variations at $p < 0.05$, as per Duncan's multiple comparison test.

Days	Serum Markers	Control	DIC (100 mg kg ⁻¹)	DIC + Silymarin (50 mg kg ⁻¹)	DIC + OIE (250 mg kg ⁻¹)	DIC + OIE (500 mg kg ⁻¹)	DIC + OIA (250 mg kg ⁻¹)	DIC + OIA (500 mg kg ⁻¹)
5	TP (g dL ⁻¹)	7.8 $\pm$ 0.3 ^a	4.97 $\pm$ 0.3 ^b	4.98 $\pm$ 0.2 ^b	4.9 $\pm$ 0.3 ^b	4.8 $\pm$ 0.14 ^b	4.6 $\pm$ 0.2 ^b	5 $\pm$ 0.3 ^b
16	TP (g dL ⁻¹)	8.1 $\pm$ 0.08 ^a	5.1 $\pm$ 0.4 ^d	6.8 $\pm$ 0.04 ^c	5.7 $\pm$ 0.24 ^d	7.4 $\pm$ 0.2 ^b	5.3 $\pm$ 0.08 ^d	6.8 $\pm$ 0.4 ^c
5	TB (mg dL ⁻¹)	0.8 $\pm$ 0.05 ^a	2.1 $\pm$ 0.3 ^b	2.06 $\pm$ 0.1 ^b	2.2 $\pm$ 0.1 ^b	2 $\pm$ 0.07 ^b	2.07 $\pm$ 0.08 ^b	2.09 $\pm$ 0.12 ^b
16	TB (mg dL ⁻¹)	0.84 $\pm$ 0.03 ^d	2.03 $\pm$ 0.2 ^a	0.91 $\pm$ 0.06 ^{cd}	1.2 $\pm$ 0.1 ^{bc}	0.93 $\pm$ 0.03 ^{cd}	1.3 $\pm$ 0.1 ^b	0.98 $\pm$ 0.02 ^{cd}
5	SGPT (U L ⁻¹)	38.5 $\pm$ 0.5 ^d	87.2 $\pm$ 0.9 ^c	97.5 $\pm$ 1.2 ^a	95.5 $\pm$ 0.6 ^{ab}	91 $\pm$ 2.6 ^{bc}	91.8 $\pm$ 2 ^{bc}	90.3 $\pm$ 1.8 ^c
16	SGPT (U L ⁻¹)	36.4 $\pm$ 0.6 ^g	88.6 $\pm$ 0.6 ^a	48.2 $\pm$ 0.8 ^f	65.7 $\pm$ 1.4 ^c	52.7 $\pm$ 0.9 ^e	69 $\pm$ 0.8 ^b	59 $\pm$ 1.4 ^d
5	SGOT (U L ⁻¹)	102.9 $\pm$ 1.6 ^c	337 $\pm$ 9.3 ^a	321.5 $\pm$ 1.2 ^b	320 $\pm$ 1.1 ^b	312 $\pm$ 3.3 ^b	318.2 $\pm$ 2 ^b	314.1 $\pm$ 1.4 ^b
16	SGOT (U L ⁻¹)	103.3 $\pm$ 1.5 ^d	334.7 $\pm$ 8.9 ^a	145.2 $\pm$ 1.4 ^c	171.5 $\pm$ 3.6 ^b	148.8 $\pm$ 1.4 ^c	177.4 $\pm$ 1.2 ^b	154.6 $\pm$ 1.9 ^c
5	ALP (U L ⁻¹)	48.8 $\pm$ 1.4 ^b	307 $\pm$ 3.9 ^a	307.9 $\pm$ 5.2 ^a	301 $\pm$ 7 ^a	298.4 $\pm$ 11 ^a	308.3 $\pm$ 4.1 ^a	312 $\pm$ 2.9 ^a
16	ALP (U L ⁻¹)	50 $\pm$ 1.1 ^d	308.4 $\pm$ 3.1 ^a	57.3 $\pm$ 1.6 ^c	79.3 $\pm$ 1.4 ^b	58.5 $\pm$ 1.3 ^c	83.1 $\pm$ 1.8 ^b	62.8 $\pm$ 1.9 ^c

3.14. Effect of *O. indicum* Extract on Diclofenac-Induced Oxidative Stress in Rat

The effects of ethanolic (OIE) and aqueous (OIA) stem bark extracts of *O. indicum* on diclofenac-induced oxidative stress were assessed by measuring lipid peroxidation and antioxidant enzyme activities in hepatic tissue (Table 12). Diclofenac administration led to increased lipid peroxidation and a marked decline in antioxidant enzyme levels, indicating hepatic oxidative damage. Treatment with OIE and OIA extracts provided varying degrees of antioxidant protection, with OIE at 500 mg kg⁻¹ demonstrating the most significant effect, comparable to that of silymarin.

Lipid peroxidation (TBARS levels)

Thiobarbituric acid reactive substances (TBARS) were significantly elevated in diclofenac-treated rats (0.584 ± 0.02 nM MDA mg⁻¹ protein) compared to the control (0.342 ± 0.007 nM MDA mg⁻¹ protein), indicating enhanced lipid peroxidation. Co-administration of silymarin (0.392 ± 0.005 nM) and OIE (500 mg kg⁻¹) (0.384 ± 0.005 nM) significantly reduced TBARS levels, approaching the control values. OIA (500 mg kg⁻¹) (0.464 ± 0.004 nM MDA mg⁻¹ protein) also demonstrated a protective effect, but the reduction was less pronounced than that of OIE.

Superoxide dismutase (SOD) activity

SOD activity was significantly reduced in the diclofenac group (5.95 ± 0.29 U mg⁻¹ protein) compared to the control (9.40 ± 0.21 U mg⁻¹ protein). Treatment with silymarin (8.80 ± 0.06 U mg⁻¹) and OIE (500 mg kg⁻¹) (8.47 ± 0.09 U mg⁻¹) restored SOD levels significantly. The OIA (500 mg kg⁻¹) group (7.44 ± 0.29 U mg⁻¹) also showed moderate recovery, though it remained lower than the OIE-treated groups.

Glutathione (GSH) levels

GSH, a key intracellular antioxidant, was significantly depleted in the diclofenac group (3.08 ± 0.06 nM mg⁻¹ protein) compared to the control (5.97 ± 0.5 nM mg⁻¹ protein).

Silymarin ($4.63 \pm 0.04 \text{ nM mg}^{-1}$) and OIE (500 mg kg^{-1}) ($4.45 \pm 0.6 \text{ nM mg}^{-1}$) significantly restored GSH levels. The OIA (500 mg kg^{-1}) group ($4.33 \pm 0.05 \text{ nM mg}^{-1}$) also showed protective effects but was slightly less effective than OIE.

Glutathione peroxidase (GPx) activity

GPx activity was significantly reduced in the diclofenac group ($21.49 \pm 0.46 \text{ U mg}^{-1} \text{ protein}$) compared to the control ($36.25 \pm 0.32 \text{ U mg}^{-1} \text{ protein}$), reflecting severe oxidative stress. Silymarin ($33.57 \pm 0.45 \text{ U mg}^{-1}$) and OIE (500 mg kg^{-1}) ($31.27 \pm 0.49 \text{ U mg}^{-1}$) markedly improved GPx levels. The OIA (500 mg kg^{-1}) ($28.5 \pm 0.41 \text{ U mg}^{-1}$) also enhanced GPx activity but remained lower than OIE.

Catalase activity

Compared to the control, diclofenac significantly lowered catalase activity ($3.53 \pm 0.08 \text{ U mg}^{-1} \text{ protein}$) compared to the control ($5.2 \pm 0.17 \text{ U mg}^{-1} \text{ protein}$). Treatment with silymarin ($4.39 \pm 0.10 \text{ U mg}^{-1}$) and OIE (500 mg kg^{-1}) ($4.43 \pm 0.04 \text{ U mg}^{-1}$) significantly improved catalase levels. OIA (500 mg kg^{-1}) ($4.03 \pm 0.12 \text{ U mg}^{-1} \text{ protein}$) also enhanced catalase activity but to a lesser extent than OIE.

These results suggest that diclofenac administration leads to severe oxidative stress, as indicated by increased TBARS levels and reduced antioxidant enzyme activity (SOD, GSH, GPx, and catalase). Treatment with *Oroxylum indicum* extracts provided dose-dependent antioxidant protection, with OIE (500 mg kg^{-1}) demonstrating the most significant improvement, comparable to that of silymarin. OIA (500 mg kg^{-1}) also exhibited protective effects, particularly in restoring GSH and catalase activities, but was slightly less effective than OIE.

3.15. Hepatoprotective Effect of *Oroxylum indicum* Extracts Against Diclofenac-induced Hepatotoxicity

Figure 23 summarizes the hepatoprotective effects of *O. indicum* extract against diclofenac-induced hepatotoxicity. The chart compares key hepatic and oxidative stress

Table 12. Effect of *Oroxylum indicum* stem bark extracts on lipid peroxidation and antioxidant status. All values are presented as mean $\pm$ standard error of the mean (SEM) with n = 3 replicates. Values in rows with different letters denote significant variations ($p < 0.05$) based on Duncan's multiple range test.

Parameters	Control	Diclofenac (100 mg kg ⁻¹ for 4 days)	Diclofenac + silymarin (50 mg kg ⁻¹)	Diclofenac + OIE (250 mg kg ⁻¹)	Diclofenac + OIE (500 mg kg ⁻¹)	Diclofenac + OIA (250 mg kg ⁻¹)	Diclofenac + OIA (500 mg kg ⁻¹)
TBARS (n moles of MDA mg ⁻¹ protein)	0.342 $\pm$ 0.007 ^e	0.584 $\pm$ 0.02 ^a	0.392 $\pm$ 0.005 ^d	0.451 $\pm$ 0.009 ^c	0.384 $\pm$ 0.005 ^d	0.506 $\pm$ 0.002 ^b	0.464 $\pm$ 0.004 ^c
SOD (U mg ⁻¹ protein)	9.40 $\pm$ 0.21 ^a	5.95 $\pm$ 0.29 ^e	8.80 $\pm$ 0.06 ^b	7.0 $\pm$ 0.10 ^{cd}	8.47 $\pm$ 0.09 ^b	6.57 $\pm$ 0.15 ^d	7.44 $\pm$ 0.29 ^c
GSH (n moles mg ⁻¹ protein)	5.97 $\pm$ 0.5 ^a	3.08 $\pm$ 0.06 ^f	4.63 $\pm$ 0.04 ^b	4.03 $\pm$ 0.01 ^d	4.45 $\pm$ 0.6 ^c	3.79 $\pm$ 0.07 ^e	4.33 $\pm$ 0.05 ^c
GPX (U mg ⁻¹ protein)	36.25 $\pm$ 0.32 ^a	21.49 $\pm$ 0.46 ^f	33.57 $\pm$ 0.45 ^b	28.60 $\pm$ 0.38 ^d	31.27 $\pm$ 0.49 ^c	25.37 $\pm$ 0.35 ^e	28.5 $\pm$ 0.41 ^d
Catalase (U mg ⁻¹ protein)	5.2 $\pm$ 0.17 ^a	3.53 $\pm$ 0.08 ^e	4.39 $\pm$ 0.10 ^b	3.83 $\pm$ 0.08 ^{cd}	4.43 $\pm$ 0.04 ^b	3.65 $\pm$ 0.05 ^{de}	4.03 $\pm$ 0.12 ^c

markers, showing the effect of diclofenac (DIC) treatment and the degree of amelioration by different treatments, including silymarin, OIE (500 mg kg⁻¹), and OIA (500 mg kg⁻¹). Diclofenac (red) showed severe hepatotoxicity, indicated by increased TBARS, SGPT, SGOT, and ALP, and reduced SOD, GSH, GPx, Catalase, and Total Protein. Silymarin (green) and OIE (blue) at 500 mg kg⁻¹ significantly reversed toxicity, bringing values close to control levels. OIA (purple) at a dose of 500 mg kg⁻¹ also exhibited hepatoprotection, but its effect was slightly lower than that of OIE.

In summary, *O. indicum* extracts, particularly OIE (500 mg kg⁻¹), demonstrated strong antioxidant and hepatoprotective properties comparable to silymarin. OIA (500 mg kg⁻¹) also had protective effects but was slightly less effective in restoring hepatic markers.

3.16. *Oroxylum indicum* Extract Protects Liver from DIC-Induced Damage: Histological Evidence

Histopathological examination of hepatic tissue in Wistar albino rats was conducted to evaluate the protective effects of *O. indicum* stem bark extracts against diclofenac-induced hepatotoxicity. Representative liver sections were analyzed under 40× magnification to assess morphological alterations across the experimental groups (Figure 24).

Control group (A-B)

Liver sections from the control group exhibited normal hepatic histoarchitecture, characterized by well-preserved hepatocytes with centrally located nuclei, distinct hepatic cords, intact sinusoids, and a normal Kupffer cell distribution. No evidence of necrosis, congestion, or inflammatory infiltration was observed.

Diclofenac-treated group (C-D)

In contrast, the diclofenac (100 mg kg⁻¹)-treated group demonstrated severe hepatocellular damage, including marked hepatocyte degeneration, cytoplasmic vacuolization, sinusoidal dilation, pyknosis and periportal inflammation. Additionally, cellular necrosis and disrupted hepatic cords was evident, indicating severe hepatotoxicity.

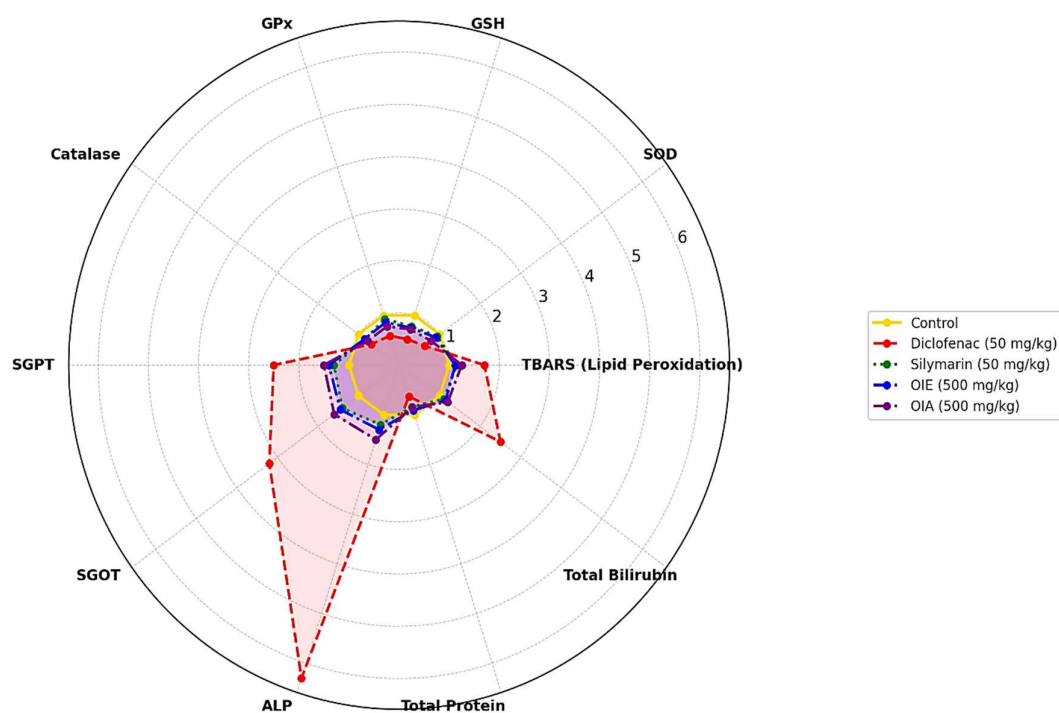


Figure 23. Radar chart showing the hepatotoxicity of diclofenac and its amelioration by stem bark extracts (ethanolic - OIE), aqueous -OIA) of *Oroxylum indicum*. [Diclofenac (red) showed severe hepatotoxicity, indicated by increased TBARS, SGPT, SGOT, and ALP, and reduced SOD, GSH, GPx, Catalase, and Total Protein. Silymarin (green) and OIE (blue) at 500 mg kg⁻¹ significantly reversed toxicity, bringing values close to control levels. OIA (purple) at a dose of 500 mg kg⁻¹] also exhibited hepatoprotection, but its effect was slightly lower than that of OIE.

Silymarin-treated group (E-F)

The silymarin (50 mg kg⁻¹) co-treated group exhibited significant hepatoprotection, with substantial restoration of the hepatic architecture. The hepatocytes appeared polygonal with prominent nuclei, and the sinusoids were well-defined. Mild inflammatory infiltrates persisted, but there was minimal necrotic damage, suggesting efficient hepatocellular regeneration and membrane stabilization.

OIE (250 mg kg⁻¹) group (G-H)

Liver sections from the diclofenac + OIE (250 mg kg⁻¹) group showed moderate histological improvement, with reduced cellular degeneration and mild restoration of the hepatic architecture. However, some hepatocytes still exhibited cytoplasmic vacuolization and mild sinusoidal congestion was observed.

OIE (500 mg kg⁻¹) group (I-J)

A higher dose of the ethanolic extract (OIE, 500 mg kg⁻¹) exhibited notable hepatoprotective effects, comparable to silymarin. The liver sections displayed well-organized hepatic cords, reduced inflammatory infiltration, and minimal cytoplasmic vacuolization. Kupffer cell activation was evident, suggesting enhanced hepatic detoxification.

OIA (250 mg kg⁻¹) group (K-L)

The aqueous extract (OIA, 250 mg kg⁻¹)-co-treated group demonstrated partial hepatoprotection, with attenuated hepatocyte degeneration and mild sinusoidal dilation. However, moderate inflammatory infiltration and residual necrotic areas were also observed.

OIA (500 mg kg⁻¹) group (M-N)

In the OIA (500 mg kg⁻¹) group, histological recovery was more pronounced, with restored hepatocellular integrity, minimal inflammatory infiltration, and reduced necrotic lesions. However, the hepatic cords remained slightly disorganized compared to the OIE (500 mg kg⁻¹) and silymarin groups, indicating a slightly lower degree of hepatoprotection.

These results suggest that histopathological evaluation confirmed that diclofenac induced severe hepatic injury, characterized by necrosis, hepatocyte degeneration, and inflammatory infiltration. Co-treatment with *Oroxylum indicum* extracts demonstrated dose-dependent hepatoprotection, with OIE (500 mg kg⁻¹) providing the most substantial recovery, comparable to that of silymarin. The 500 mg kg⁻¹ OIA extract also showed significant hepatoprotective potential, although it was slightly less effective than its ethanolic counterpart. These findings support the cytoprotective role of *O. indicum* in mitigating drug-induced hepatotoxicity through membrane stabilization, anti-inflammatory action, and hepatic tissue regeneration.

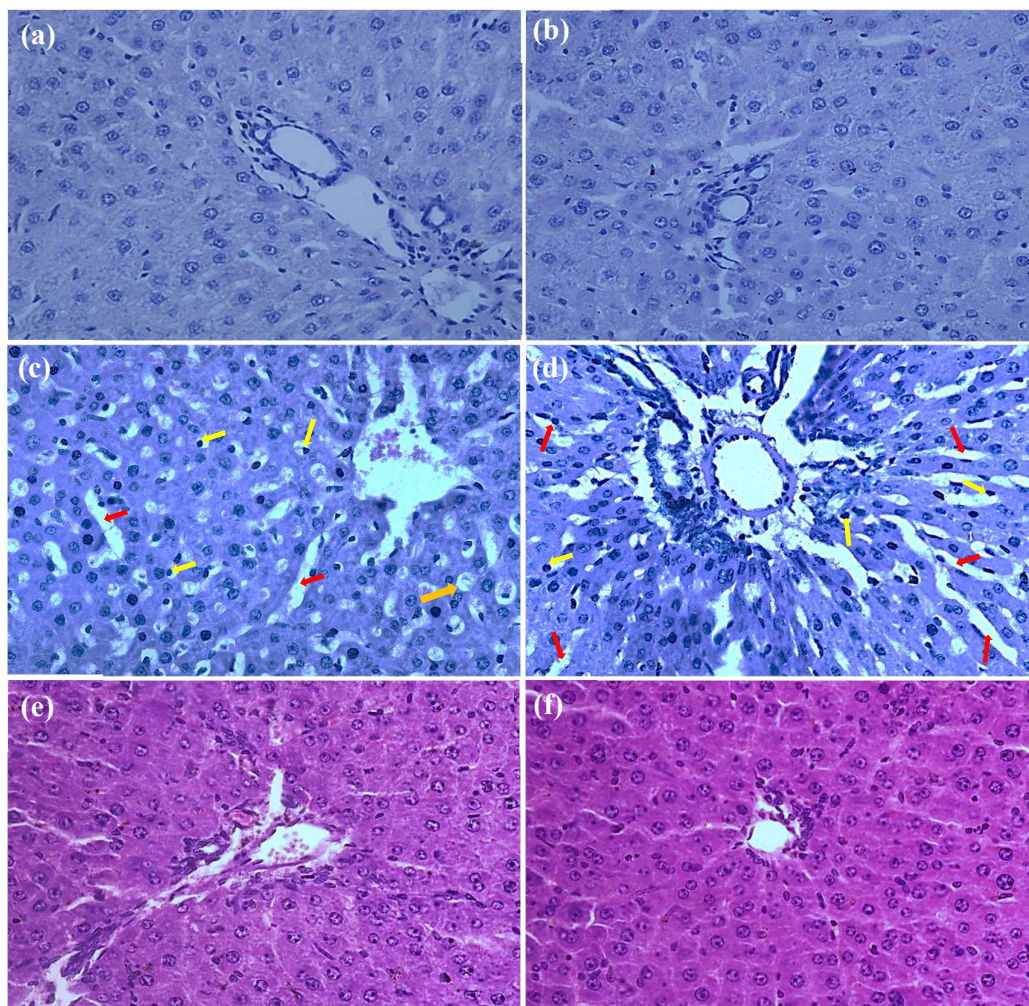


Figure 24a. The histopathology of hepatic tissue from Wistar albino rats was observed at 40x magnification. The results depict normal histoarchitecture of the liver in the control group (A-B). However, in comparison, the group treated with diclofenac at a dose of 100 mg kg^{-1} (C-D) displayed prominent histoarchitectural changes. In contrast, the groups treated with diclofenac combined with silymarin at 50 mg kg^{-1} (E-F).

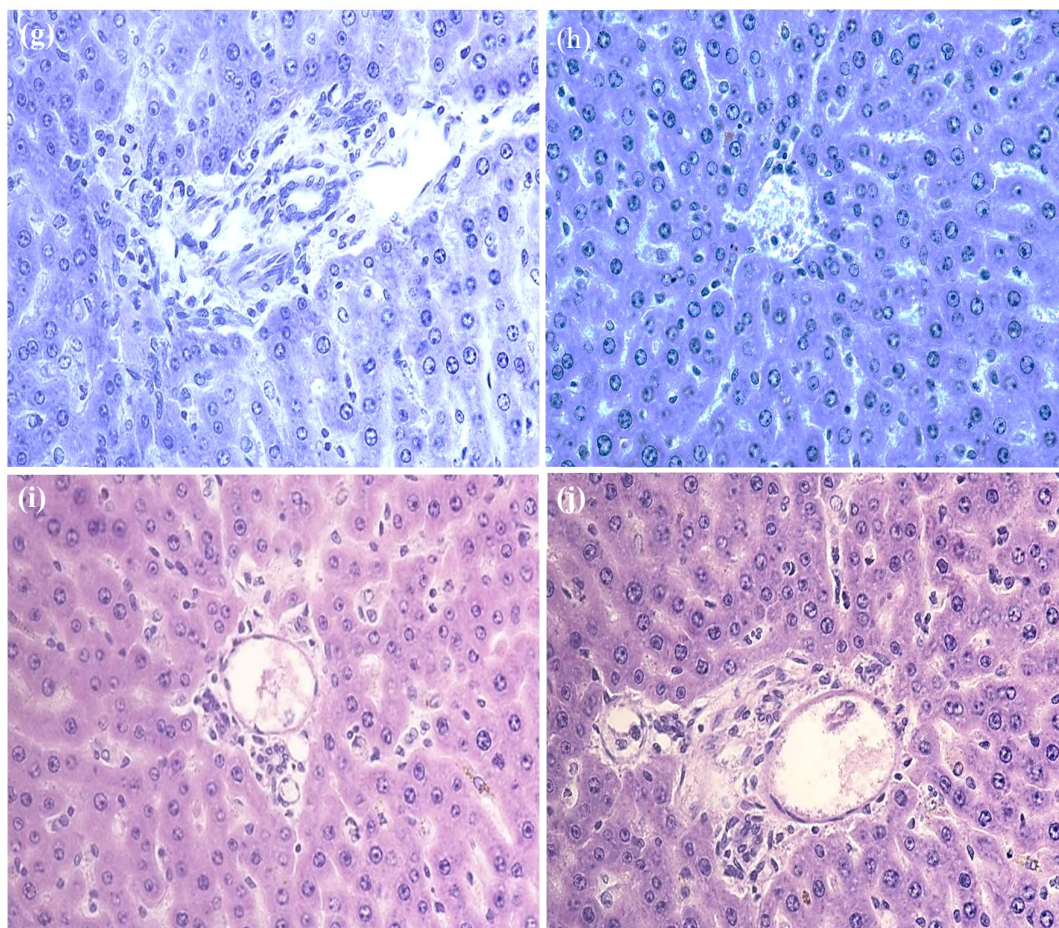


Figure 24b. Diclofenac combined with OIE at 250 mg kg⁻¹ (G-H) and diclofenac combined with OIE at 500 mg kg⁻¹ (I-J).

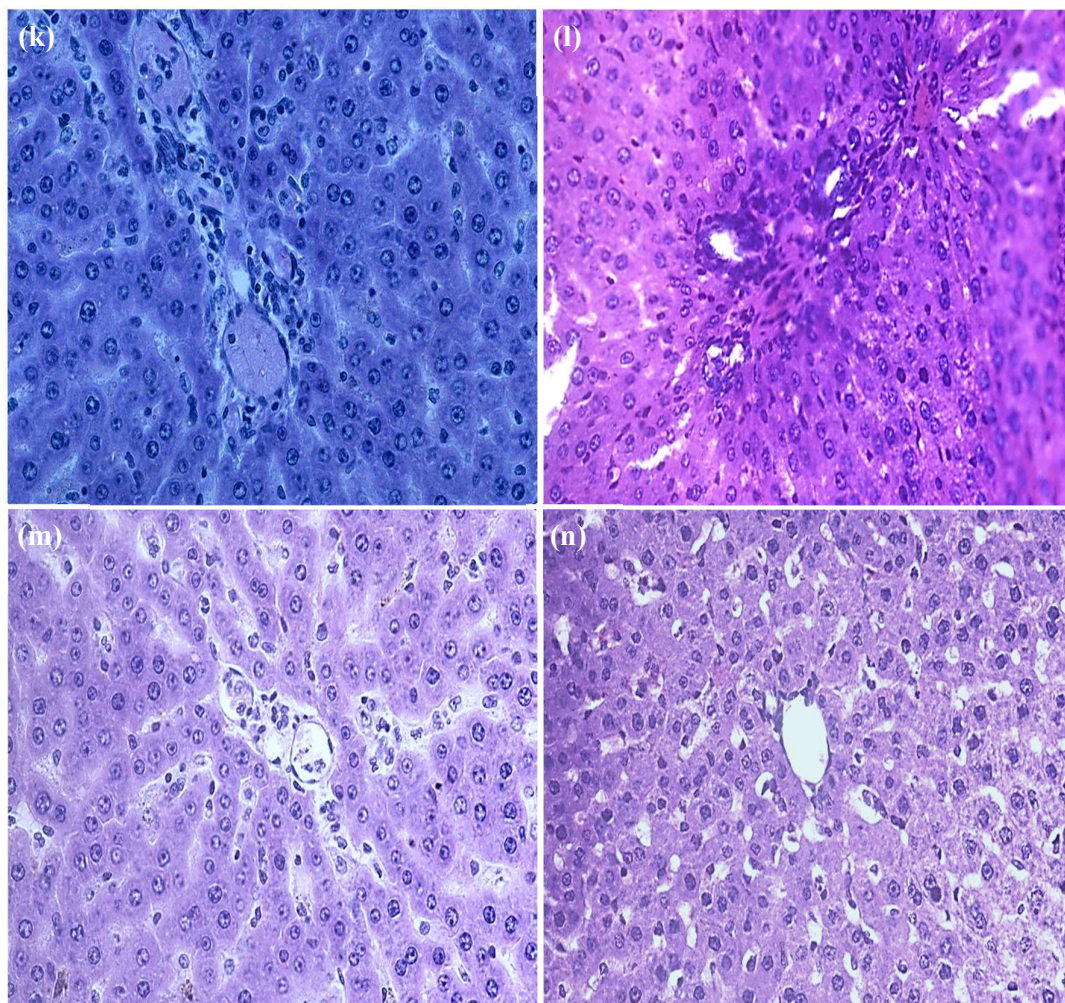


Figure 24c. Diclofenac combined with OIA at 250 mg kg⁻¹ (K-L), and diclofenac combined with OIA at 500 mg kg⁻¹ (M-N).

DISCUSSION

Previous studies have shown that diclofenac causes liver toxicity. In some cases, it causes severe liver damage. The formation of protein adducts from reactive metabolites (Hargus et al., 1994) is believed to be the primary mechanism underlying toxicity. These metabolites of diclofenac irreversibly bind to select hepatic proteins such as dipeptidyl peptidase IV, which may lead to harmful consequences in the liver (Hargus et al., 1995). Importantly, the mechanisms underlying diclofenac hepatotoxicity are multifactorial. Recent studies have shown that diclofenac induces lysosomal dysfunction dependent on reactive oxygen species (ROS) leading to impaired autophagy (HCC: Hepatitis C Virus Hepatocellular Carcinoma). This disrupts mitochondrial function, contributing to the induction of ROS in cells (Jung et al., 2020). Moreover, immunological factors may also contribute to diclofenac-induced toxicity. For example, Aithal et al. (2004) detected antibodies against diclofenac metabolite-modified liver protein adducts in patients with hepatic injuries. It has been suggested that diclofenac have a greater tendency to cause liver toxicity than other NSAIDs (Schmeltzer et al., 2016; Sriuttha et al., 2018). Typical signs of diclofenac-induced hepatotoxicity include nausea, jaundice, and dark urine (Schmeltzer et al., 2016). Although the exact frequency of diclofenac-induced hepatotoxicity is unknown, it occurs in approximately 3–23 cases per 100,000 patients per year (Aithal and Day, 2007). Based on these studies, this drug should be prescribed with the most care (O’connor et al., 2003; Schmeltzer et al., 2016).

Currently, the available therapeutic strategies for diclofenac-induced hepatic toxicity mainly concentrate on supportive care and drug withdrawal. No specific treatment is available for drug-induced liver injury. Routine practice is the immediate cessation of diclofenac and supportive treatment to reduce symptoms and avoid further hepatic injury. These interventions include IV fluid therapy to provide hydration, vitamin K therapy to normalize coagulopathy, and N-acetylcysteine therapy to restore glutathione levels, which may help overcome oxidative stress. Liver transplantation may be necessary in severe cases. Efforts have been made to study possible hepatoprotective agents, including silymarin and N-acetylcysteine. However, their

efficacy remains essentially incompletely studied. However, there is an increasing demand for specific therapeutic interventions that target the fundamental pathways of diclofenac-induced hepatocellular injury. Antioxidants and ROS scavengers have been found to attenuate diclofenac-mediated cellular toxicity (Pourahmad et al., 2011). Additionally, reducing mitochondrial dysfunction and lysosomal membrane damage may be protective (Chao et al., 2018). The synthesis of inhibitors such as CYP2E1 and CYP2C9 can dramatically reduce the formation of toxic metabolites (Chao et al., 2018). Moreover, the importance of autophagy may open a new avenue for therapeutic intervention in diclofenac-induced hepatotoxicity (Chao et al., 2018). In addition, new drug delivery systems and hepatoprotective preparations may help improve the effectiveness of treatment (and potentially prevent liver injury) in chronic diclofenac therapy in long-term patients.

While synthetic drugs are commonly linked to several detrimental effects, plant-derived compounds show significant activities against diclofenac-induced liver injuries (Rani et al., 2024). Herbal drugs act on a variety of biochemical and molecular pathways, including antioxidant, anti-inflammatory, and immunomodulatory mechanisms (Maran et al., 2022; Tasneem et al., 2019). The liver-shielding properties of plants are fundamentally ascribed to a variety of phytochemicals such as flavonoids, polyphenols, and alkaloids (Shawon et al., 2024; Ugwu and Suru, 2021). Various plant species, including *Silybum marianum*, *Taraxacum officinale*, and *Andrographis paniculata*, display strong hepatoprotective activity (Karakuş et al., 2017; Mahboubi and Mahboubi, 2020; Maran et al., 2022). A study conducted in Sudan documented 21 plant species with liver-shielding activities (Ali et al., 2019). This study showed that 90.5 % of plant species have considerable activity in animals, highlighting the importance of overlooked medicinal plants in protecting liver health (Ali et al., 2019). A study conducted in Malaysia further indicated the global diversity of hepatoprotective plants (Maran et al., 2022). For instance, *Sanguisorba officinalis* L. has strong hepatoprotective properties (Meng et al., 2021).

A range of bioactivities of *Oroxylum indicum* extracts has been documented (Abdulhafiz et al., 2022). *O. indicum* extracts have been found to be effective in promoting wound healing, curing diabetes (Singh and Kakkar, 2013), reducing

allergies (Lee et al., 2016), and cancer treatment (Costa-Lotufo et al., 2005). However, reports on the hepatoprotective activity of this plant are limited. In their review, Chaudhary et al. (2011) mentioned the hepatoprotective properties of *O. indicum* along with other beneficial effects. Dinda et al. (2015) corroborated the hepatoprotective activity of *O. indicum* extracts and suggested that *O. indicum* extracts are mostly non-toxic. However, investigations with different extract types are required to establish the non-toxic nature of *O. indicum* extract. The same study also suggested that flavonoids are primarily responsible for the bioactivity of *O. indicum* (Dinda et al., 2015).

This study aimed to examine the liver-protective properties of *O. indicum* (L.) Kurz in diclofenac-treated rat model. The results showed that *O. indicum* had a rich antioxidant profile. Our results showed that the ethanolic extract (OIE) contains higher levels of bioactive compounds than the aqueous extract (OIA). Similar to the present study, other studies have advocated the suitability of ethanol for the extraction of phytochemicals (Sun et al., 2015; Teixeira et al., 2022; Wu et al., 2019). The effectiveness of ethanol is mainly attributed to its high-to medium-range polarity (Guenau et al., 2021; Kaewseejan and Siriamornpun, 2015). This property makes ethanol very effective for the extraction of both polar and nonpolar phytochemicals from plant materials. Ethanol provides a more comprehensive extraction of phenolic compounds and flavonoids. Additionally, ethanol offers advantages in terms of safety and environmental impacts. Compared with other solvents, ethanol is less toxic and more environmentally benign. However, the effectiveness of ethanol may vary depending on the target compound and plant material. In some cases, solvent combinations result in better extraction of phytochemicals. For example, in the extraction of caffeine and catechins from *Camellia sinensis*, supercritical CO₂ with ethanol as a modifier and water as an additive has shown promising results (Atwi-Ghaddar et al., 2023).

In the present study, we found a sufficient amount of total phenolic compounds. Phenolics are known for their high free radical scavenging capacity (Maisuthisakul et al., 2007; Villano et al., 2007). Similarly, Kumar and Goel, (2019) also reported the strong antioxidant property of phenolics and suggested that phenolics can neutralize various types of free radicals. Another study showed that the ethyl acetate extract of

leaves of *Jerusalem artichokes* exhibits high concentrations of total phenolics and, subsequently, strong free radical scavenging abilities (Yuan et al., 2012). Similarly, barley grains contain abundant phenolic compounds with considerable antioxidant activities (Ge et al., 2021). Notably, the OH groups of phenols play a critical role in determining their ability to scavenge radicals. Ortho-structured phenols show the highest rate coefficients for free radical scavenging (Thavasi et al., 2009).

The current study further validated the free radical scavenging capacity of aqueous and ethanolic extracts of *O. indicum* using DPPH and ABTS radical scavenging assays. OIE was lower IC₅₀ value than that of OIA, suggesting that the ethanolic extracts possess a higher free radical scavenging potential. Furthermore, in the present study, we found that the antioxidant potential of the extracts was comparable to that of BHT. This further confirmed the strong antioxidant potential of *O. indicum*. Tannins were detected in both the extracts. The tannins present in both extracts may contribute to the antioxidant potential of *O. indicum*; however, they could also negatively impact the bioavailability of other compounds (Molino et al., 2023; Zhang et al., 2023). Further investigations are required to evaluate the specific functions of tannins.

In the present study, the hydroxyl radical-scavenging activity of *O. indicum* extracts was determined. Hydroxyl radicals are highly reactive, and multiple studies have documented their role of hydroxyl radicals in alcoholic liver disease (ALD) and non-alcoholic fatty liver disease (NAFLD) (Albano, 2002; Sumida et al., 2013). In the present study, both OIE and OIA effectively inhibited hydroxyl radical levels in vitro. These results were in good agreement with those reported by (Chaudhary et al., 2011). This study shows the hydroxyl radical-scavenging potential of *O. indicum*. Rai et al. (2022) isolated endophytic *Colletotrichum gloeosporioides* from *O. indicum* leaves. These studies further showed that the ethyl acetate extract of the endophytic fungus possesses high hydroxyl radical-scavenging properties. The above study reported an EC₅₀ value of 61.55 µg mL⁻¹ for the hydroxyl scavenging property of the endophytic extract. Therefore, in the present study, hydroxyl radical scavenging may be attributed to the endophytes associated with *O. indicum*. As it was out of the scope of the present study, further attempts were not made in the present study to isolate and characterize

endophytes associated with this plant. Earlier studies have concluded that phytochemicals effectively scavenge hydroxyl radicals at lower concentrations. Phytochemicals may act as pro-oxidants at relatively high concentrations (Giacomo et al., 2023; Supruniuk et al., 2023). Further studies are recommended to identify the active compounds of *O. indicum* responsible for its hydroxyl radical-scavenging activities.

In the present study, experiments were conducted to investigate the reducing power of both *O. indicum* extracts. The results showed that the extracts of *O. indicum* possessed considerable reducing power. The ethanol extract exhibited reducing power comparable to that of ascorbic acid. This suggests that metabolites present in the extracts may function as electron donors (Andrés et al., 2023; Gulcin, 2020). This observation aligns with the results of previous studies (Saad et al., 2021; Yusoff et al., 2022).

In the present study, both the OIE and OIA extracts significantly reduced the oxidative stress caused by diclofenac in rats. The results of the present study show that diclofenac administration considerably increased lipid peroxidation (TBARS) in hepatic cells. These findings are consistent with those reported by Cantoni et al. (2003) and Jung et al. (2020). These studies have shown that diclofenac exposure increases the production of reactive oxygen species (ROS) and lipid peroxidation in liver cells.

Interestingly, this study demonstrated the downregulation of antioxidant enzymes (SOD, GPx, CAT, and GSH) in rats pre-administered *O. indicum* extract. The lowered activity of antioxidant enzymes in the extract-treated group of animals can be attributed to multiple reasons. First, prolonged exposure to oxidative stress can downregulate enzyme activity through a feedback mechanism (Lopez et al., 2007; W. Sun et al., 2013). Second, direct inhibition of enzymes cannot be ruled out. The flavonoids and tannins present in *O. indicum* can form complexes with metal cofactors (e.g., Mn, Fe, Cu, and Zn) of SOD and CAT proteins, leading to decreased enzyme activity (Slabbert, 1992). Zhang et al. (2023) also reported that tannins can form complexes with transition metals through ortho-dihydroxyphenolic groups, which, in turn, can affect various biological processes. The ortho-dihydroxyl group on the flavonoid B-ring of tannins mainly forms complexes with metal ions (Slabbert, 1992).

Furthermore, phenolics, flavonoids, and other metabolites scavenge free radicals more effectively than enzymes. This could result in a decreased demand for antioxidant enzymes. In contrast, baicalein present in *O. indicum* may upregulate antioxidant enzymes, such as NQO-1 and HO-1, via the Nrf2 signaling pathway in HaCaT keratinocytes (Kim et al., 2023). Lee et al. (2011) reported the recovery of MnSOD protein expression by the baicalein via activating Nrf2-mediated MnSOD expression. Menon et al. (2018) reported increased activity of SOD, GSH, and GPx in extract-treated groups of rats.

The present study examined the acute oral toxicity of *O. indicum* extract. The results showed negligible toxicity of the extract at elevated doses (up to 5000 mg kg⁻¹). Rats treated with concentrations above the extract did not show any mortality or atypical behavioral alterations. In agreement with the present results, *Psidium guajava* bark extract showed no mortality or signs of toxicity at 5000 mg kg⁻¹ in an acute toxicity study (Manekeng et al., 2019). Similarly, the ethanol extract of *Oncoba spinosa* at 5000 mg kg⁻¹ did not cause mortality in an acute toxicity experiment (Prasanth et al., 2015). Similarly, S'hih et al. (2023) reported that the extract of *Coriandrum sativum* seeds did not cause death in rats at 5000 mg kg⁻¹. In contrast to the observed lack of mortality and behavioral changes in rats exposed to higher extract concentrations, some studies have reported adverse effects in similar experimental settings. For instance, Kale et al. (2019) showed that *Acridocarpus smeathmannii* root extract exhibited considerable toxicity at doses as low as 250 mg kg⁻¹ in subacute and subchronic studies. The above studies suggest that toxicity profiles vary significantly between different plant extracts and study designs. Further research is needed to fully characterize the toxicity of *O. indicum* extracts at high doses across different species and exposure durations.

FTIR and LC-MS analyses provided comprehensive insights into the bioactive constituents of these plants. FTIR studies carried out in the present study identified several functional group characteristics of phenolic compounds, flavonoids, and other metabolites.

LC-MS analysis revealed the presence of multiple bioactive compounds that have been proven to exert hepatoprotective effects in *O. indicum* extracts. Extracts

prepared using both ethanolic and aqueous solvents demonstrate potent antioxidant, anti-inflammatory, and antifibrotic properties, which contribute to liver protection and recovery from hepatotoxic conditions.

In the ethanolic extract, two known compounds were also found: Scutellarein and Hispidulin (Table 6). The anti-inflammatory and antifibrotic properties of scutellarein have been suggested to counteract liver damage caused by oxidative stress (Lin et al., 2019; Miao et al., 2020). The antioxidant and anti-inflammatory properties of hispidulin have also been reported. Inhibition of the PI3K/Akt signaling pathway and mitochondrial dysfunction in HepG2 cells has been demonstrated by hispidulin to induce pro-apoptotic effects (Gao et al., 2014). According to the above study, the ethanolic extract of *O. indicum* has the potential to reduce both liver damage caused by oxidative stress and hepatoblastoma, as well as other types of cancer.

Quercetin, luteolin, chlorogenic acid, and sinapic acid were also identified in *O. indicum* extracts (Table 6). These metabolites have been identified as hepatoprotective agents. Quercetin protects the liver from NAFLD by reducing oxidative stress and inflammation (Chen et al., 2021). Luteolin demonstrates hepatoprotective action against liver injury caused after exposure to chemicals. Domitrović et al. (2009) reported that luteolin pre-treatment reduced CCl₄-induced hepatotoxicity in mice in a dose- and time-dependent manner. This study showed that luteolin pretreatment decreased serum aminotransferase levels and improved liver histopathology.

Chlorogenic acid was identified in the *O. indicum* extract. Several studies have demonstrated the hepatoprotective effects of this compound. It has been shown to be hepatoprotective against liver injury from various sources. Chlorogenic acid exhibits dramatic protective effects against liver damage induced by carbon tetrachloride (CCl₄), α -naphthyl isothiocyanate (ANIT), aflatoxin B1 (AFB1), and lipopolysaccharide (LPS) (Cheng et al., 2023; Kapil et al., 1995; Tan et al., 2016; Zhou et al., 2016). Kapil et al. (1995) stated that CGA provided better protection than silymarin in murine models administered CCl₄. CGA regulates important signaling pathways, including Nrf2, TLR4/NF- κ B, and AMPK (Xue et al., 2023).

As shown in Table 6, *O. indicum* was rich in sinapic acid. Studies have reported that this compound is effective against dimethylnitrosamine-induced liver injury and thioacetamide-induced liver fibrosis (Jabbar et al., 2023; Pandi and Kalappan, 2021; Shin et al., 2013). Pre-exposure to sinapic acid significantly reduced the serum levels of liver enzymes. Sinapic acid decreases hepatic hydroxyproline levels and the expression of fibrosis-related factors (Shin et al., 2013). Sinapic acid exhibits free radical scavenging and increases antioxidant enzymes (SOD, CAT) (Jabbar et al., 2023). Additionally, sinapic acid had anti-inflammatory effects and reduced pro-inflammatory cytokines like TNF- α and IL-6. It had increased anti-inflammatory IL-10 levels (Jabbar et al., 2023). It also modulates the NF- κ B p65 and proinflammatory cytokine expression in carbon tetrachloride (CCl₄)-induced severe hepatic damage (Shin et al., 2013).

Biochanin A, an isoflavone, possesses potent hepatoprotective, antioxidant, and immunomodulatory activities. Biochanin A protects against liver injury by decreasing oxidative stress and inflammation, and it can also modulate immune responses (Breikaa et al., 2013; Ibrahim et al., 2023). its possible application as a therapeutic agent in liver diseases. It further supports that biochanin modulates critical signaling pathways like MAPK, PI3K, NRF2, and NF- κ B (Yan et al., 2021). Silymarin, a widely recognized hepatoprotective drug, has been tested previously in various types of extracts, demonstrating comparable benefits from both ethanolic and aqueous extracts against diclofenac-induced alterations in body weight, biochemical indices, and liver weight. This similarity implies that *O. indicum* extracts would possess therapeutic effects in the liver, especially against DIC-induced hepatotoxicity. The results suggest that the extracts of *O. indicum* may be used as an effective natural hepatoprotective agent in place of the traditional drug silymarin. The active principles for the protective effects of *O. indicum* extracts in hepatic damage are yet to be confirmed. These extracts would need to undergo clinical trials to test for their safety and efficacy in humans before potential application as a treatment for liver disease.

Analysis of the extracts of *Oroxylum indicum* by LC-MS (Table 7) also showed the presence of many other metabolites. Based on the dataset, flavonoids with antioxidant, anti-inflammatory and hepatoprotective properties, such as oroxylin A,

baicalein, quercetin, and acacetin were identified. All these metabolites have been reported from *O. indicum* in several studies (Chaudhary et al., 2011; Chen et al., 2003; Muniyasamy and Manjubala, 2024; Peng et al., 2019; Rai et al., 2022). The sulfur-containing amino acid is also detected in the present study and demonstrates anti-inflammatory and wound-healing properties (Chang et al., 2010; Weimann et al., 2018) in addition to essential fatty acids such as gamma-linolenic acid and palmitoleic acid found from extracts. In point of interest, the presence of 2'-deoxyguanosine monohydrate and D-glucosamine-6-phosphate sodium salt suggest roles in nucleic acid metabolism (Matsuda et al., 1999) and cartilage protection (Fenton et al., 2000), respectively. The ATRA (17.25 min) detection suggests possible applications in the dermatological and anticancer fields. The findings overall justify the pharmacological importance of *O. indicum* which could be a potential tool in therapeutics.

Hematological and antioxidant parameters and liver function markers also confirmed these protective effects. There was significant preservation of serum enzyme (SGPT, SGOT, and ALP) and total bilirubin levels with *O. indicum* extracts as compared to the pamidronate-induced DIC group, indicating the hepatoprotective action of *O. indicum* extracts. These results corroborate those of earlier studies that showed ameliorative effects of antioxidant and anti-inflammatory plant extracts on liver function and oxidative damage (Gupta et al., 2021; Zhang et al., 2023).

Histopathological examination of liver samples also confirmed the protective effects of *O. indicum* extracts, indicating a marked reversal of diclofenac-induced liver damage, represented by necrosis, steatosis, and congested blood vessels observed in the liver sections of the treated groups. These findings are consistent with those of previous studies showing hepatoprotective effects of *O. indicum* extracts in animal models (Gera et al., 2024; Tenpe et al., 2009). Similar to the present study, the study reported in Baravalia et al. (2011) showed that a metabolite occurring in plants reverses diclofenac-induced hepatic damage in rats. This study found that the methanol extract of *Woodfordia fruticosa* Kurz flowers (WFM) protected against diclofenac sodium-induced hepatotoxicity in rats (Baravalia et al., 2011).

This study demonstrated the antioxidant and hepatoprotective effects of *O. indicum* extracts, demonstrating protection against DIC-induced liver injury (Figure

23b, c). The extracts had effects similar to that of the well-characterized hepatoprotective agent silymarin, suggesting therapeutic application. Clinical trials and further studies are necessary to understand the mechanism of action and the safety and efficacy of *O. indicum* extracts in liver disease.

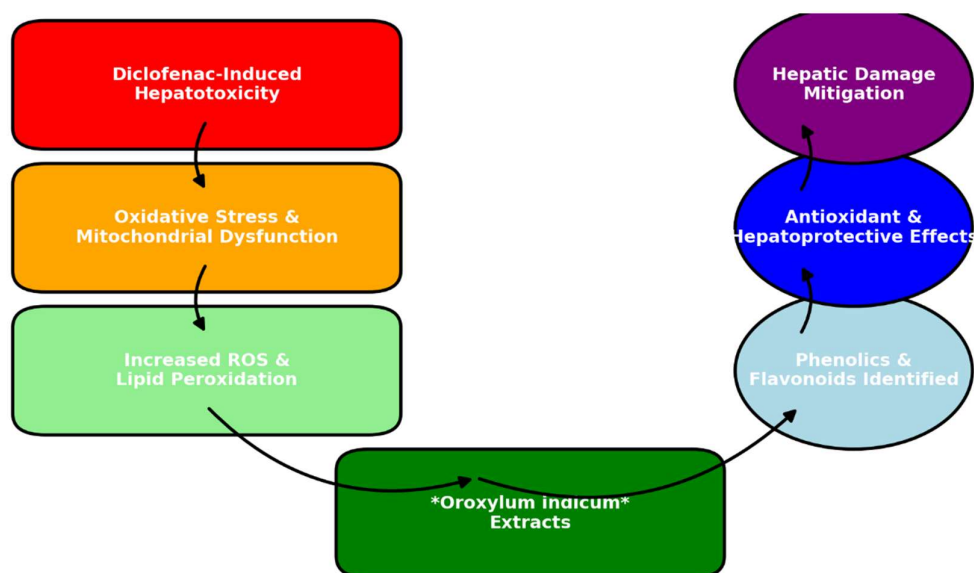


Figure 24. Hepatoprotective effects of *Oroxylum indicum* against diclofenac-induced toxicity. The image illustrates the sequence of events in diclofenac-induced hepatotoxicity, commencing with oxidative stress, mitochondrial dysfunction, and elevated reactive oxygen species (ROS), which culminate in lipid peroxidation and hepatic injury. Administration of *O. indicum* extracts, which are rich in phenolic compounds and flavonoids, counteracts these deleterious effects through its antioxidant and hepatoprotective properties. These plant extracts mitigated oxidative stress, restored mitochondrial function, and enhanced antioxidant enzyme activity, ultimately attenuating hepatic damage, and improving liver function. The pathological processes leading to hepatotoxicity are represented by rectangular shapes, whereas the protective effects of *O. indicum* are depicted in circular forms. Arrows indicate sequential progression of events.

SUMMARY

Oroxylum indicum (L.) Kurz is a widely recognized medicinal plant known for its extensive use in traditional medicine. It has been the subject of various phytochemical and pharmacological studies to validate its therapeutic applications, particularly in liver disease management. The present study focuses on the phytochemical composition, antioxidant properties, and hepatoprotective effects of *O. indicum* stem bark extracts, with a specific emphasis on their ability to counteract diclofenac-induced hepatotoxicity in Wistar albino rats. By employing both ethanolic (OIE) and aqueous (OIA) extracts, the study evaluates their effectiveness in mitigating oxidative stress and liver damage while elucidating their safety and bioactive compound profiles.

Qualitative and quantitative phytochemical screening of *O. indicum* extracts confirmed the presence of vital bioactive compounds such as flavonoids, alkaloids, saponins, phenols, and tannins. The extraction yield was 8 % for OIE and 9.7 % for OIA, indicating that water was a more effective solvent for polar compounds. However, the ethanolic extract exhibited higher concentrations of phenolic (557.21 ± 6.8 GAE mg g⁻¹) and flavonoid (355.1 ± 1.6 QE mg g⁻¹) content compared to the aqueous extract. Tannin levels remained consistent across both extracts, suggesting similar secondary metabolite profiles.

To further characterize the bioactive compounds responsible for the plant's pharmacological effects, FTIR and LC-MS analyses were conducted. These revealed the presence of key flavonoids, including scutellarein, hispidulin, and quercetin, which contribute significantly to the plant's antioxidative and hepatoprotective properties. Additional phytochemicals such as chlorogenic acid, luteolin, and sinapic acid were also identified, further supporting the therapeutic potential of *O. indicum*.

To evaluate the antioxidative potential of *O. indicum* extracts, various *in vitro* free radical scavenging assays, including DPPH, ABTS, hydroxyl, superoxide, and nitric oxide radical scavenging tests, were conducted. The results indicated that OIE exhibited superior antioxidant activity, with IC₅₀ values of 23.7 ± 1.8 µg mL⁻¹ (DPPH) and 42.5 ± 1.9 µg mL⁻¹ (ABTS), comparable to standard antioxidants. These findings suggest that the ethanolic extract has stronger free radical neutralizing capabilities,

likely due to its higher concentration of bioactive compounds. While OIA also demonstrated antioxidant activity, its efficacy was slightly lower than that of OIE.

The hepatoprotective potential of OIE and OIA was assessed using a Wistar albino rat model of diclofenac-induced hepatotoxicity. Diclofenac, a commonly used nonsteroidal anti-inflammatory drug (NSAID), is known to cause liver damage through oxidative stress, mitochondrial dysfunction, and immune-mediated responses. Rats treated with diclofenac exhibited significant mortality, weight loss, and reductions in liver weight, confirming the hepatotoxic effects. Histological examination further revealed hepatocellular degeneration, necrosis, and inflammation.

Co-administration of OIE and OIA resulted in a dose-dependent protective effect, with OIE at 500 mg kg⁻¹ demonstrating the most substantial hepatoprotective activity. Rats treated with OIE exhibited restored body weight, relative organ weight, liver weight, and organ integrity. Additionally, hematological parameters, including WBC counts, RBC counts, hemoglobin levels, and hematocrit values, were significantly improved following treatment with OIE, closely resembling the effects of silymarin, a well-known hepatoprotective agent.

Biochemical assays provided further validation of the hepatoprotective properties of *O. indicum*. Diclofenac-induced hepatotoxicity was associated with elevated serum glutamate-pyruvate transaminase (SGPT/ALT) and total bilirubin (TB) levels, along with reduced total protein (TP) concentrations. By Day 16, treatment with OIE (500 mg kg⁻¹) significantly normalized TP levels and reduced TB concentrations, indicating improved hepatic function and metabolic stability.

Oxidative stress was a key contributing factor to diclofenac-induced liver damage, as evidenced by increased lipid peroxidation (TBARS) and suppressed antioxidant enzyme activities, including superoxide dismutase (SOD), glutathione (GSH), glutathione peroxidase (GPx), and catalase (CAT). OIE (500 mg kg⁻¹) demonstrated the most potent antioxidant activity, effectively restoring oxidative markers to near-normal levels. While OIA (500 mg kg⁻¹) also displayed antioxidative properties, its efficacy was slightly lower than that of OIE.

Histopathological assessments further supported the hepatoprotective potential of *O. indicum* extracts. Liver sections of diclofenac-treated rats displayed severe hepatocellular damage, including extensive necrosis and inflammatory infiltration. However, treatment with OIE led to marked improvements in hepatic architecture, with reduced cellular degeneration, vacuolation and inflammation. OIA also demonstrated protective effects, albeit to a lesser extent.

Acute toxicity studies were conducted to assess the safety of *O. indicum* extracts. No adverse effects were observed at doses up to 5000 mg kg⁻¹, confirming the extracts' safety profile. The absence of significant hepatic deviations in treated groups further validates the plant's therapeutic potential. Given the limitations of conventional hepatoprotective strategies, the study suggests that natural alternatives such as *O. indicum* could provide safer and more effective solutions for liver disease management.

This study highlights the potent hepatoprotective and antioxidant properties of *O. indicum*, particularly its ethanolic extract, in mitigating diclofenac-induced hepatic toxicity. The higher concentration of bioactive compounds in OIE contributes to its superior radical scavenging activity and hepatoprotective effects. These findings underscore the significant therapeutic promise of *O. indicum* as a natural alternative to conventional hepatoprotective agents.

Future studies should focus on isolating specific bioactive compounds and elucidating their mechanistic pathways to optimize their therapeutic applicability. Clinical trials and formulation development will be crucial in translating these findings into practical applications for liver disease treatment. Additionally, exploring synergistic interactions between *O. indicum* extracts and other hepatoprotective compounds may further enhance its efficacy. By advancing research in this area, *O. indicum* could emerge as a valuable natural hepatoprotective agent for managing liver diseases.

Overall, this study establishes a strong foundation for the therapeutic use of *O. indicum* and encourages further investigation into its bioactive compounds to unlock its full medicinal potential.

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Bio-Data

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EDUCATION

Examination	Board/University	Year of passing	Division	(%/Grade)
M.Sc (Botany)	Mizoram University	2018	Distinction	80.23 %
B.Sc (Botany)	Govt. Kolasib College	2017	First	77.25 %
HSSLC (Science)	BHSEC, Daga Higer Secondary School	2014	Second	53.25 %
SSLC (Science)	BCSEA, Daga Higer Secondary School	2012	First	71.2 %
Diploma in Software Development	Mizoram University	2019	First	74.3 %

LIST OF PUBLICATIONS

1. Mehta, S. K., & **Pradhan, R. B.** (2024). Phytochemicals in antiviral drug development against human respiratory viruses. *Drug Discovery Today*, 29(9), 104107. <https://doi.org/10.1016/j.drudis.2024.104107> (IF: 6.5)
2. Momin, S. C., **Pradhan, R. B.**, Nath, J., Lalmuanzeli, R., Kar, A., & Mehta, S. K. (2024). Metal sequestration by *Microcystis* extracellular polymers: A promising path to greener water treatment. *Environmental Science and Pollution Research*, 31(7), 11192–11213. <https://doi.org/10.1007/s11356-023-31755-3> (IF: 5.8)
3. Ngamlai, E. V., **Pradhan, R. B.**, Lalbiaknii, P. C., Ralte, V., Lalnunmawia, F., Vanlalhluna, P. C., & Mehta, S. K. (2024). Diuretic activity evaluation and chemical composition analysis of *Hedyotis scandens* extract from Mizoram, India, in rat models. *Journal of Ethnopharmacology*, 319, 117079. <https://doi.org/10.1016/j.jep.2023.117079> (IF: 5.4)
4. Momin, S. C., Nath, J., Mahana, A., **Pradhan, R. B.**, & Mehta, S. K. (2024). Chemical activation of *Microcystis aeruginosa* biomass: A promising approach for enhanced diclofenac sorption and water treatment. *Journal of Chemical Technology & Biotechnology*, 99(1), 149–163. <https://doi.org/10.1002/jctb.7517> (IF: 3.4)
5. R. Lalremdika, **Pradhan, R. B.**, Ch Momin, S., Nath, J., Lalmuanzeli, R., & Mehta, S. K. (2024). Carbon dioxide sequestration and sustainable domestic sewage bioremediation by *Microcystis aeruginosa* and *Chlorella vulgaris*. *Ecology, Environment and Conservation*, 30(10), S108–S119. <https://doi.org/10.53550/EEC.2024.v30i06s.017>

Patents

Mehta S. K, Momin S Ch, **Pradhan R. B** (2024). Methods for removing heavy metals from aqueous solutions using extracellular polymeric substance from *Microcystis aeruginosa*. Application No. 202431032536 A.

PRESENTATION, WORKSHOP AND CONFERENCE

1. Presented a paper (oral) entitled “Bioactivity and antioxidant activities of *Oroxylum indicum* bark extracts” and phytochemical profile of aqueous and ethanolic bark extracts of *Oroxylum indicum*” in international conference on the theme biodiversity, biogeochemistry and ecosystem, sustainability in changing environment jointly organized by Indian Ecological Society and Department of Forestry at Mizoram University, 14-16 June, 2023
2. Presented a paper (oral) on Green filamentous alga *Cladophora sp.* as a source of cellulose nanofiber (CNF) in two-day national seminar on “Environment and climate change in North East India; challenges & sustainability” organised by Sibsgar college, Joysagar (Autonomous) Sivasagar, Assam from 22 - 23 August, 2022.
3. Presented a paper (oral) entitled “Biodegradation of petroleum hydrocarbon by some strains of cyanobacteria isolated from Mizoram” in national conference on recent advances in plant biology with special reference to North-East India organized by Department of Botany, School of life Sciences, Mizoram University, India from 20-21 August, 2023.
4. Attended webinar on “Biomedical sciences: a touch of medicinal plants” organised by Department of HAMP, Mizoram University, Aizawl.
5. Attended the webinar on “career in banking sector: opportunities and challenges” held on 16th June, 2020 organised by Department of Management, Mizoram University.
6. Attended webinar on impact of COVID 19 lock down on trade and commerce 19 June 2020, jointly organised by Mizoram University and Sikkim University.
7. Attended webinar on “Managing stress and relationships during covid-19 pandemic” organised by Department of Psychology, Mizoram University Aizawl, Mizoram.
8. Completed the “NRDMS-DST” winter school on geospatial technologies (Level-1) conducted from 29th November, 2019 to 19th December, 2019 in the Department of

Civil Engineering, National Institute of Technology Agartala and supported by natural resource data management system - Department of Science and Technology, Government of India, New Delhi.

9. Completed one week online short-term course on LaTeX, from 29th June, 2020 to 3rd July, 2020, conducted by the Department of Information Technology, Mizoram University in association with spoken tutorial, IIT Bombay, MHRD. Govt. of India
10. Attended webinar on “Mending the broken heart: evolution of novel therapeutic approach” organised by the school of life science, Mizoram University, Aizawl India.
11. Attended webinar on “Microwave antenna measurement yesterday, today and tomorrow” and design of antennas through simulation using tarang: 19.1” organised by Department of Electronic and Communication Engineering, Mizoram University.
12. Participated in the online webinar on research publication and citations: perspective of highly cited researcher conducted by Mizoram university on 16th June 2020.
13. Attended webinar on “Tree improvement at a glance and wildlife conservation- why and how? On 30th June 2020.
14. Attended webinar on “Tropical food for metabolic syndrome” organised by Department of Food Technology, Mizoram University, Aizawl on 3rd July, 2020.
15. Attended the webinar on “The role of science and technology in the times of war, pandemic and natural disasters and systematic and scientific exploration of medicinal plants of NE region: a translation for the ethano to modern medicine.

ANNEXURE-I

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Ph.D. registration Number	MZU /Ph.D./1211 of 31.08.2018	
Title of Ph.D. Thesis	Hepatoprotective Potential of <i>Oroxylum indicum</i> (L.) Benth. Ex. Kurz Against Non-Steroidal Anti-Inflammatory Drug (Diclofenac) - Induced Hepatotoxicity in Rat	
Name & Institutional Address of the Supervisor	Prof. Surya Kant Mehta, Department of Botany, Mizoram University, Aizawl, Tanhril-796004	
Name of the Department and school	Department of Botany, School of Life Sciences	
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Ran Bahadur Pradhan

**Hepatoprotective Potential of Oroxylum indicum (L.) Benth. Ex.
Kurz Against Nonsteroidal Anti-Inflammatory Drug (Diclofena...**

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NAME : Ran Bahadur Pradhan

DEGREE : Ph.D.

DEPARTMENT : Botany

TITLE OF THESIS : Hepatoprotective Potential of *Oroxylum indicum* (L.) Benth. Ex. Kurz Against Non-Steroidal Anti-Inflammatory Drug (Diclofenac)-Induced Hepatotoxicity in Rat

DATE OF ADMISSION : 31st August, 2018

DATE OF APPROVAL OF RESEARCH PROPOSAL

1. DRC : 02.05.2019

2. BOS : 03.05.2019

3. SCHOOL BOARD : 17.05.2019

MZU REGISTRATION NO. : 3012 of 2013

Ph.D. REGISTRATION NO. and DATE : MZU/Ph.D./1211 of 31.08.2018

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Head

Department of Botany

ABSTRACT

HEPATOPROTECTIVE POTENTIAL OF *OROXYLUM INDICUM* (L.) BENTH. EX. KURZ AGAINST NON-STEROIDAL ANTI- INFLAMMATORY DRUG (DICLOFENAC) - INDUCED HEPATOTOXICITY IN RAT

**AN ABSTRACT SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF
PHILOSOPHY**

RAN BAHADUR PRADHAN

MZU REGISTRATION NO.: 3012 OF 2013

Ph.D. REGISTRATION NO.: MZU/Ph.D./1211 OF 31.08.2018



**DEPARTMENT OF BOTANY
SCHOOL OF LIFE SCIENCES**

MARCH, 2025

**HEPATOPROTECTIVE POTENTIAL OF *OROXYLUM INDICUM* (L.)
BENTH. EX. KURZ AGAINST NONSTEROIDAL ANTI - INFLAMMATORY
DRUG (DICLOFENAC) - INDUCED HEPATOTOXICITY IN RAT**

BY

**RAN BAHADUR PRADHAN
DEPARTMENT OF BOTANY**

SUPERVISOR

PROF. SURYA KANT MEHTA

SUBMITTED

**IN PARTIAL FULFILLMENT OF THE REQUIREMENT OF THE DEGREE
OF DOCTOR OF PHILOSOPHY IN BOTANY OF MIZORAM UNIVERSITY,
AIZAWL**

ABSTRACT

The liver, a vital organ responsible for metabolic regulation and detoxification, is particularly susceptible to drug-induced toxicity. Among nonsteroidal anti-inflammatory drugs (NSAIDs), diclofenac is widely used but poses a significant risk of hepatotoxicity. Diclofenac-induced liver injury is primarily mediated through oxidative stress, mitochondrial dysfunction, and inflammatory pathways. Despite its clinical efficacy, the hepatotoxic potential of diclofenac necessitates the search for safer alternatives.

Oroxylum indicum (L.) Kurz, a member of the Bignoniaceae family, is traditionally used in various indigenous medicinal systems for its hepatoprotective properties. The present study aimed to evaluate the hepatoprotective efficacy of *O. indicum* stem bark extracts against diclofenac-induced hepatotoxicity in a rat model, focusing on its biochemical, histopathological, and molecular protective mechanisms. The stem bark of *O. indicum* was collected, authenticated (ACS No. MZUBOT0088), and subjected to ethanolic and aqueous extractions, yielding 8 % and 9.7 % (w/w), respectively.

Phytochemical screening confirmed the presence of flavonoids, phenols, tannins, alkaloids, glycosides, and saponins, with variations in abundance between the ethanolic and aqueous extracts. Quantitative assessments revealed higher phenolic (557.21 ± 6.8 GAE mg g⁻¹) and flavonoid (355.1 ± 1.6 QE mg g⁻¹) content in the ethanolic extract compared to the aqueous extract (80.61 ± 0.64 GAE mg g⁻¹ and 241 ± 1.3 QE mg g⁻¹, respectively). Tannin levels remained consistent across both extracts.

In vitro antioxidant activity assays demonstrated that the ethanolic extract exhibited stronger radical scavenging activity across DPPH (IC₅₀: 23.7 ± 1.8 µg mL⁻¹), ABTS (42.5 ± 1.9 µg mL⁻¹), hydroxyl radical (27.8 ± 0.8 µg mL⁻¹), superoxide radical (36.2 ± 1.5 µg mL⁻¹), and nitric oxide inhibition (43.0 ± 5.4 µg mL⁻¹) assays compared to the aqueous extract. The reducing power assay further confirmed the superior electron-donating ability of the ethanolic extract, exhibiting a dose-dependent increase in absorbance at 700 nm. In addition, the total antioxidant activity of the ethanolic extract was recorded at 100.9 ± 1.6 AAE mg g⁻¹, significantly higher than

the aqueous extract (77.24 ± 1.98 AAE mg g⁻¹). These results align with the higher phenolic and flavonoid content in the ethanolic extract, reinforcing its potent antioxidant properties. The observed antioxidant effects suggest that the extract can effectively counteract oxidative damage by scavenging free radicals and enhancing cellular redox balance.

Fourier-transform infrared spectroscopy (FTIR) and liquid chromatography-mass spectrometry (LC-MS) profiling identified major bioactive compounds, including baicalein, quercetin, chrysin, scutellarein, and biochanin A, known for their hepatoprotective properties. FTIR analysis revealed the presence of diverse functional groups, including alcohols, phenols, alkanes, alkenes, amines, and halogenated compounds, in both extracts. The ethanolic extract exhibited distinct peaks for carbonyl groups and conjugated alkenes, while the aqueous extract showed evidence of hydroxylated compounds, alkynes, and conjugated dienes. Halogenated species, such as iodinated and chlorinated compounds, were identified in both extracts, suggesting the presence of terpenoids or alkaloids. The presence of chlorogenic acid and sinapic acid in the aqueous extract suggests potential anti-inflammatory and liver-protective mechanisms. Additionally, LC-MS detected flavonoid glycosides such as baicalin and oroxylin A, known for their antioxidative and anti-inflammatory activities. The ethanolic extract contained a higher concentration of baicalein and chrysin, contributing to its enhanced radical scavenging ability. The identification of phenolic acids, including caffeic acid and ferulic acid, further reinforces the extract's role in modulating oxidative stress pathways and improving hepatic function. These findings highlight the potential of *O. indicum* bioactive compounds in mitigating diclofenac-induced hepatotoxicity through multiple biochemical and molecular mechanisms.

Acute oral toxicity studies confirmed the safety of both extracts across doses of 1000, 3000, and 5000 mg kg⁻¹ body weight of rats. Clinical observations showed no mortality or severe adverse effects in female albino Wistar rats, with only transient hypoactivity and mild gastrointestinal irritation noted at higher doses. The absence of systemic toxicity markers or neurological impairments suggests a high acute safety threshold for *O. indicum* extracts. In contrast, diclofenac administration demonstrated

dose-dependent toxicity, with higher doses (500 mg kg⁻¹, 250 mg kg⁻¹, and 100 mg kg⁻¹) leading to earlier mortality, while all subjects survived at 50 mg kg⁻¹.

Histological examination further supported the safety profile, revealing intact hepatic architecture with no indications of necrosis, inflammation, or fibrosis. These findings establish the high tolerability and non-toxic nature of *O. indicum* extracts at the tested doses, reinforcing their potential for safe therapeutic application.

The protective effect of *O. indicum* extracts against diclofenac-induced hepatotoxicity was evaluated by administering the extracts alongside diclofenac (50 mg kg⁻¹) for four days. Hematological analysis revealed that diclofenac significantly altered WBC counts, RBC counts, hemoglobin levels, and hematocrit values. On days 5 and 16 revealed significant alterations in the DIC-treated group, including elevated white blood cell (WBC) counts, reduced red blood cell (RBC) counts, decreased hemoglobin (Hb) and hematocrit (HCT) levels, and increased mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH), indicative of macrocytosis. Pretreatment with OIE (500 mg kg⁻¹) and silymarin demonstrated significant recovery by day 16, normalizing WBC counts, RBC counts, Hb, and HCT levels, and reversing macrocytosis. While all treatment groups showed partial improvements by day 5, OIE (500 mg kg⁻¹) exhibited superior efficacy, comparable to silymarin, in mitigating diclofenac-induced hematotoxicity.

Biochemical assays revealed a significant reduction in serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) levels in extract-treated groups compared to the diclofenac-only group ($p < 0.05$). The ethanolic extract at 500 mg kg⁻¹ exhibited superior hepatoprotective efficacy, closely resembling the effect of the standard hepatoprotective agent, silymarin (50 mg kg⁻¹). Additionally, bilirubin levels, both total and direct, were significantly reduced in the extract-treated groups, suggesting improved liver function. The levels of total protein and albumin, which were decreased in the diclofenac-only group, showed significant restoration in the extract-treated groups, further supporting hepatoprotective potential. Furthermore, oxidative stress markers such as lipid peroxidation (LPO) were significantly decreased, while endogenous antioxidant levels such as glutathione (GSH) and enzymatic antioxidants were enhanced, demonstrating

a robust defense against oxidative liver damage. The ability of the ethanolic extract to mitigate diclofenac-induced alterations in biochemical markers highlights its therapeutic potential in preserving liver integrity and function.

Histopathological analysis of liver tissues revealed that diclofenac administration caused severe hepatocellular injury, characterized by hepatocyte degeneration, cytoplasmic vacuolization, sinusoidal congestion, and inflammatory infiltration. In contrast, the control group exhibited normal hepatic histoarchitecture, while the silymarin-treated group demonstrated significant restoration of liver structure, confirming its hepatoprotective efficacy. Treatment with OIE at 250 mg kg⁻¹ showed moderate improvement, with reduced cellular degeneration but persistent vacuolization and congestion, whereas the higher dose of OIE (500 mg kg⁻¹) provided substantial hepatoprotection, comparable to silymarin, with well-organized hepatic cords, minimal inflammation, and reduced vacuolization. Similarly, OIA at 250 mg kg⁻¹ exhibited partial protection, while the 500 mg kg⁻¹ dose showed significant recovery, though it was slightly less effective than the ethanolic extract. *Oroxylum indicum* extracts, particularly OIE at 500 mg kg⁻¹, offer dose-dependent hepatoprotection against diclofenac-induced hepatotoxicity, likely through mechanisms such as membrane stabilization, anti-inflammatory action, and tissue regeneration.

The molecular mechanism of hepatoprotection was explored through antioxidant enzyme activity analysis, showing increased superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activity in extract-treated groups. The ethanolic extract exhibited a 35 % increase in SOD and a 29 % enhancement in GPx levels compared to the diclofenac-only group ($p < 0.05$), indicating a strong antioxidant defense response. The extracts also exhibited downregulation of oxidative stress markers, including malondialdehyde (MDA), with a reduction of 42 % in the ethanolic extract group, thereby confirming their antioxidative role in hepatic protection. These findings suggest an immunomodulatory role in liver protection, potentially contributing to reduced hepatic inflammation and improved liver function. Additionally, the restoration of glutathione (GSH) levels by 28 % further supports the antioxidative and cytoprotective role of the extracts against diclofenac-induced oxidative damage.

The findings of this study provide substantial evidence supporting the hepatoprotective potential of *O. indicum* stem bark extracts against diclofenac-induced liver injury. The study highlights the ethanolic extract as a more potent antioxidant and hepatoprotective agent, likely due to its higher concentration of bioactive flavonoids and polyphenols. The research underscores the potential of *O. indicum* as a natural alternative to conventional hepatoprotective agents, offering a promising therapeutic strategy for mitigating drug-induced liver toxicity. Critical discussion on the differential bioavailability of the bioactive compounds in aqueous and ethanolic extracts highlights the need for further pharmacokinetic studies. Future studies focusing on detailed mechanistic pathways, dose optimization, and clinical validation could further establish *O. indicum* as a viable hepatoprotective supplement in pharmaceutical applications. Additionally, long-term studies exploring potential side effects and synergistic interactions with existing hepatoprotective drugs will enhance its translational potential in clinical settings.