AMELIORATIVE ROLE OF CARNITINE ON MONOSODIUM GLUTAMATE INDUCED TESTICULAR TOXICITYIN WISTAR ALBINO RATS

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT

OF THE REQUIREMENTS FOR THE

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BY

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CERTIFICATE

This is to certify that **Ms. Lalrinzuali Sailo,** Department of Zoology, Mizoram University**,** has completed her dissertation work entitled "**Ameliorative Role of Carnitine on Monosodium Glutamate Induced Testicular Toxicity in Wistar Albino Rats"** in partial fulfilment of the requirement for the **Degree of Master of Philosophy in Zoology** from August 2015- December 2016 under my guidance and supervision. No part of this dissertation has been submitted for any other degree, diploma, associateship, fellowship or other similar titles in this University or any other University or institution of higher learning.

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DECLARATION

I, **Ms. Lalrinzuali Sailo**, an M.Phil scholar in Department of Zoology, Mizoram University, Aizawl, Mizoram, do hereby solemnly declare that the subject matter of thesis is the record of work done by me, that the contents of this dissertation did not form bias of the award of any previous degree to me or to do 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.

This is being submitted to the Mizoram University for the **Degree of Master of Philosophy in Zoology**.

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ABBREVIATIONS

%	percentage
µg	Microgram
µl	Microlitre
ALP	Alkaline Phosphatase
C	carnitine
CAT	catalase
DNB	1-chloro-2,4-dinitrobenzene
CHOD/ PAP-	cholesterol oxidase/ phenol + aminophenazone
CO	Carbon monoxide
$CuSO_4$	Copper sulphate
dl	decilitre
DNA	deoxyribonucleic acid
DNB	Dinitrobenzene.
Dpx	distyrene, plasticizer, xylene
DTNB	5,5 dithio 2-nitobenzoic acid (Dithionitrobenzene)
EDTA	Ethylenediaminetetraacetic acid
FAO	Food and Agriculture Organisations
FASEB	Federation of American Societies for Experimental Biology
FDA	Food and Drug Administration
FSANZ	Food Standards Australia New Zealand
G	gram
GSH	glutathione
GST	glutathione S–transferase
H_2O_2	Hydrogen peroxide

IFIC	International Food Information Council Foundation
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
K, fe (CN)	Potassium ferricynide
K_2HPO_4	Potassium Hydrogen Phosphate
Kg	kilogram
KH_2PO_4	Potassium dihydrogen phosphate
l	litre
MDA	malondialdehyde
mg	milligram
MSG	monosodium glutamate
MZU	Mizoram University
Na_2CO_3	Sodium carbonate
Na_2HPO_4	Sodium hydrogen phosphate
NaCl	Sodium chloride
NaoH	Sodium hydroxide
NBT	Nitrobluetetrzolium
^oC	Degree celsius
PBS	Phosphate buffer
PMS	Phenazenemethosulfate
SEM	Standard error of mean
SGOT (AST)	serum glutamic-oxaloacetic transaminase (Aspartate transaminase)
SGPT (ALT)	serum glutamate-pyruvate transaminase (Alanine transaminase)
SH	Sulphur, Hydrogen atom
SOD	Superoxide dismutase
TBA	Thiobarbituric acid

TBARS	Thiobarbituric acid
TCA	Trichloroacetic acid
U	Units
WHO	World Health Organisation

CHAPTER 1

INTRODUCTION

1.1. Monosodium glutamate

Monosodium glutamate (MSG) which is called as AJI – NOMOTO is the sodium salt of glutamic acid (Eweka, 2007). It is used worldwide as a flavour enhancing food additive especially in Chinese, Thainese and Japanese foods (Ikeda, 1917). As of 2009, the world production of MSG was estimated to be 2 million tonnes per year (Sahelian, 2014). The consumption of MSG had been reported to be associated with numerous adverse effects (Raiten, 1995; Food Standards Australia New Zealand, 2003). MSG is locally and globally used as favor enhancer; it may be present in packaged foods without appearing on the label. Glutamate is a naturally occurring amino acid that is one of the most abundant amino acid in the Central Nervous System (CNS). MSG contains 78% of glutamic acid and 22% of sodium water (Samuels, 1999).

MSG is abundant and excess in other food ingredients without otherwise appearing on the label. This shows that MSG can be taken unknowingly and without intention. It can remain subtle but the damage caused can never be neglected. Thus, it could be abused inadvertently .It is marketed under such trade names including A- One, Ajinomoto or Vedan and is a popular condiment in West African dishes (Obaseiki – Ebor *et al*., 2003).

1.2. MSG a flavour enhancer

MSG added to foods produces a flavouring function similar to the glutamate that occurs naturally in foods. It adds a fifth taste, called "umami" which is best described as a savoury, broth –like or meaty taste, although traditional East Asian cuisine had often used seaweed extract, which contains high concentrations of glutamic acid, it was not known until 1908 that MSG was isolated by Ikeda. It was subsequently patented by Ajinomoto Corporation of Japan in 1909. In its pure form, it appears as a white crystalline powder that, as a salt dissociates into sodium cations and glutamate anions while dissolving glutamate is the anionic form of glutamic acid (Sano,

2009). Modern commercial MSG is produced by fermentation of starch, sugar, beet sugarcane or molasses (Walker and Lupien, 2000).

Glutamate is one of the most common amino acids found in nature and is the main component of many proteins and peptides of most issues. Glutamate is also produced in the body and plays an essential role in human metabolism. It is a major component of many protein-rich food products either in free or bound state of animals such as meat, fish, milk and cheese or vegetable origins such as mushroom and tomato (IFIC, 1994) .

1.3. Daily intake of MSG

In the mid – 1950s The Joint FAO/WHO Expert Committee on Food Additives (JECFA) was established by the food and Agriculture Organisations of the United Nations (FAO) and the World Health Organisation (WHO) in order to assess the safety of chemical additives in food on an international basis. Its brief has been broadened subsequently to include contaminants and veterinary drug residues. Its primary aims were to protect the health of the consumers and facilitate international trade in food in matters relating to food additives. As MSG is a worldwide flavour enhancer food additive, it is also protected by JECFA. But MSG has a long history of use in foods as a flavour enhancer. The average daily intake of MSG is estimated to be 0.3 – 1.0 g in industrialized countries, but can be higher occasionally depending on the MSG content of individual food items and individual's taste preferences (Geha *et al*., 2000).

1.4. Effect of MSG

MSG is an exitotoxin, which may cause damage to the brain especially by oral intake ie, without food and other edibles. (Walker and Lupien, 2000) it is used mostly as a condiment in meats, soups and vegetables without food protection like carbohydrate food. MSG was toxic to humans and animals. It could produce symptoms such as numbness, weakness, flushing, sweating, dizziness and headache. Also ingestion of MSG has been alleged to cause condition

including asthma, urticarial, atopic dermatitis, abdominal discomfort etc. MSG has a toxic effect on the testis by causing a significant oligozoospermia and increases subnormal sperm morphology in a dose- dependent fashion in male Wistar rats. It has been implicated in male fertility by causing testicular hemorrage, degeneration and alteration of sperm cell of the hypothalamus is a pointer to the fact that it may alter the neutral control of reproductive; hormone secretion via the hypothalamic- pituitary- gonadal regulatory axis. Such alterations in reproductive hormone secretion may adversely affect the reproductive capacity of the effected animals.

In August 1995, the Federation of American Societies for Experimental Biology, or FASEB, published its findings regarding possible MSG-related reactions. Commissioned by the FDA, the report listed a range of possible symptoms associated with MSG consumption: a burning sensation in the back of the neck, forearms and chest; facial pressure or tightness; chest pain; heart palpitations; headache; nausea; numbness in the back of the neck radiating to the arms and back; tingling or warmth in the upper body; drowsiness; and weakness. FASEB referred to these symptoms collectively as MSG symptom complex. Monosodium glutamate is the sodium salt of glutamic acid. As University of Tokyo chemistry professor Ikeda discovered in 1908, MSG is the most stable salt formed from glutamic acid, and one that best delivers the sought-after 'umami' taste. 'Umami' – which translates as 'savoury' – is associated with a "meaty" flavour, and was also discovered by Ikeda, who pursued it believing that there was something more than the four basic tastes of sweet, salty, sour and bitter (Yamaguchi,1998).

1.5. L-carnitine

L-carnitine is a non-protein amino acid (β-hydroxy-γ-trimethyl-amino-butyric acid), that is synthesized from the essential amino acids lysine and methionine (Kendler , 1986). L- carnitine plays an important role in many biological processes and it can protect against a number of diseases. It reduced sperm abnormalities by chemicals (Fahmy *et al*., 2008). L-carnitine is an important antioxidant nutrient, is essential for normal testicular development, spermatogenesis and spermatozoa motility and functions (Messaoudi *et al*., 2010). Ursini *et al*. (1999) reported that L-carnitine supplementation in sub fertile men with low L-carnitine status could improve sperm motility and increase the chance of successful conception. L-carnitine has also been to have the protective effects against the toxicity of metals in the male reproductive system of experimental animals (Said *et al*., 2010). In spite of some studies on the effects of L-carnitine on spermatogenesis on rodent testis, little is known about the effects of L-carnitine on testicular testosterone. It has been suggested that L-carnitine have a protective role in peroxide damage to the sperm cell (Marin-Guzman *et al*., 2001).

1.6. L-carnitine and its derivative

L-Carnitine is quaternary ammonium compound biosynthesized from the amino acids lysine and methionine. It is a trimethylated amino acid roughly similar in structure to choline, facilitates the transfer of activated long-chain fatty acids from the cytoplasm to the mitochondria, where they are processed by oxidation to produce ATP (Steiber *et al*., 2004). The carnitine exerts a substantial antioxidant action, thereby providing a protective effect against lipid peroxidation of phospholipid membranes and against oxidative stress induced at the myocardial and endothelial cell level (Cavazza, 2002). L-carnitine also acts as an antioxidant to prevent the oxidative damage of sperms, improving sperm quality (Lenzi *et al*., 2003) and shown to have beneficial effects in the treatment of varicocele, a major cause of male infertility (Seo *et al*., 2003). Also, supplemental doses of L-carnitine effectively counteracts the toxic effects of chronic nicotine

administration on thyroid, liver, heart, bone, muscle, urinary bladder, and kidney functions and attenuates the oxidative damage possibly by its antioxidant action (Huang *et al*., 1999; Zadeh *et al*., 2008).

L-carnitine is an amino acid (a building block for proteins) that is naturally produced in the body. Carnitine is an amino acid derivative and nutrient involved in lipid (fat) metabolism in mammals and other eukaryotes. It is in the chemical compound classes of β-hydroxyacids and quaternary ammonium compounds, and because of the hydroxyl substituent, it exists in two stereoisomers, the biologically active enantiomer L-carnitine, and the essentially biologically inactive D- carnitine. Both are available through chemical synthesis, and the L-form is continuously biosynthesized in eukaryotic organisms from the proteinogenic aminoacids lysine and methionine. In such eukaryotic cells, it is specifically required for the transport of fatty acids from the intermembraneous space in the mitochondria into the mitochondrial matrix during the catabolism of lipids, in the generation of metabolic energy. Carnitine was originally found as a growth factor for mealworms and labelled vitamin B_T, although carnitine is not by biochemical definition a true vitamin. It is used efficaciously, clinically, in the treatment of some conditions, e.g. systemic primary carnitine deficiency and it is available over the counter as a nutritional supplement, though its efficacy for most conditions for which it is advertised is controversial or not yet established.

1.7. Importance of carnitine

L-carnitine helps the body produce energy. It is important for heart and brain function, muscle movement, and many other body processes**.** L-carnitine supplements are used to increase L-carnitine levels in people whose natural level of L-carnitine is too low because they have a genetic disorder, are taking certain drugs (valproic acid for seizures), or because they are undergoing a medical procedure (hemodialysis for kidney disease) that uses up the body's L-

carnitine. It is also used as are placement supplement in strict vegetarians, dieters, and low-weight or premature infants (Chan *et al*., 2007). L-carnitine is used for conditions of the heart and blood vessels including heart-related chest pain congestive heart failure (CHF), heart complications of a disease called diphtheria, heart attack, leg pain caused by circulation problems (intermittent claudication), and high cholesterol.

Carnitine is a substance found in almost every cell in the body, it is biosynthesized from the amino acids lysine and methionine. The compound plays a crucial role in energy production, as it is responsible for transporting fatty acids to the mitochondria. L-carnitine is often positioned as a weight loss aid. Although it definitely improves body composition in older people, many of the studies in younger people aren't so positive, probably because they already have optimal carnitine stores. It can, however, enhance exercise endurance and capacity in all age groups by increasing muscle carnitine levels and improving energy metabolism. L-carnitine is by no means a magic bullet, but if you're willing to exercise regularly—especially if you follow the mini-fast with exercise protocol—it may help. Carnitine, derived from an amino acid, is found in nearly all cells of the body. Its name is derived from the Latin *carnus* or flesh, as the compound was isolated from meat. Carnitine is the generic term for a number of compounds that include L-carnitine, acetyl-L-carnitine, and propionyl-L-carnitine (Rebouche *et al*., 1999).

Carnitine plays a critical role in energy production. It transports long-chain fatty acids into the mitochondria so they can be oxidized ("burned") to produce energy. It also transports the toxic compounds generated out of this cellular organelle to prevent their accumulation. Given these key functions, carnitine is concentrated in tissues like skeletal and cardiac muscle that utilize fatty acids as a dietary fuel (Rebouche *et al*., 1999).

The body makes sufficient carnitine to meet the needs of most people. For genetic or medical reasons, some individuals (such as preterm infants), cannot make enough, so for them carnitine is a conditionally essential nutrient (Rebouche *et al*., 1999).

1.8. Benefits of L-carnitine

Other health benefits of L-carnitine, especially acetyl-L-carnitine, include improved memory in older people and slowing of Alzheimer's progression. The acetyl group in this type of L-carnitine can be used to form acetylcholine, an important neurotransmitter that markedly declines as dementia advances. Both forms, however, increase mitochondrial energy production and power up energy-starved brain cells (Pettegrew *et al*., 1995).

More benefits of L-carnitine include relief from chronic fatigue syndrome, diabetic neuropathy, peripheral vascular disease and intermittent claudication, insulin resistance and type 2 diabetes, kidney disease and dialysis, and male infertility and erectile dysfunction. Patients with cancer will likely benefit from L-carnitine as well. Most people who undergo chemotherapy and/or radiation report significant fatigue. In addition, advanced disease is associated with cachexia, or muscle wasting and weight loss, and low carnitine levels may well contribute to this. L-carnitine is not a cancer therapy, but it has been reported to improve fatigue and quality of life—and that's reason enough to use it (Cruciani *et al*., 2004).

The body can convert L-carnitine to other amino acids namely acetyl-L-carnitine and propionyl-L-carnitine. But, no one knows whether the benefits of carnitine are interchangeable. Until more is known, one form of carnitine should not be substituted for another.

L-carnitine is an amino acid compound that performs three very important functions that enhance athletic performance:

1. L-carnitine is the nutrient, the "ferry" so to speak, that shuttles fatty acids from the blood into the mitochondria -- the energy producing "furnaces" in the cells -- so that they can be used as energy. If fat is used as a fuel efficiently and effectively as possible, L-carnitine is required.

2. L-carnitine also helps to improve endurance by inhibiting the build-up of lactic acid, which is one of the primary causes of fatigue.

3. L-carnitine has been shown to reduce the accumulation of metabolic wastes during exercise, helping increase workload output during exercise and enhancing recovery post-exercise. (Cha *et al*., 2001).

1.9. Sperm parameters

The sperm parameters include the sperm concentration in testis and epididymis. The percentage of motile sperms was counted in treatment groups with respect to control. Nayanatara *et al.* (2008) recorded MSG reduction in testicular weight and decrease in the sperm count in rats treated with MSG. Treating rats with MSG caused decrease in testicular weight, decrease in tubular diameter, reduction in germinal epithelium height, decrease in the spermatic count and abnormalities of sperms morphology (Nosseir *et al*., 2012).

1.10.1. Oxidative stress

Oxidative damage primarily occurs via production of reactive oxygen species such as superoxide anion, peroxides, and it can damage to lipids, proteins and DNA. Therefore, it may cause to loss of enzymatic activity and structural integrity of enzymes and activate inflammatory processes (Özyurt *et al*., 2004). Oxidative stress plays an important role in the

etiology of defective sperm formation, function, sperm count profile and male infertility (Acharya, 2008; Shen and Ong, 2000).

1.10.2. Antioxidant enzymes

The antioxidant enzymes include SOD, CAT, GSH and GST. These enzymes are also considered as an important indicator of the balance status between the first and second step of the enzymatic antioxidant pathway (Jihen *et al* ., 2009. The testis, epididymis, sperm and seminal plasma contain high activities of antioxidant enzymes (Aruldhas *et al*., 2005). Whereas SOD catalyzes the conversion of superoxide radicals to hydrogen peroxide, CAT converts hydrogen peroxide into water (Mansour and Mossa, 2009). Therefore, SOD–CAT system provides the first defense system against oxidative stress and these enzymes work together to eliminate active oxygen species (El-Demerdash, 2011 and Wafa *et al*., 2011). Glutathione peroxidases are antioxidant seleno enzymes that are present in the cytosol of cells. The major function of these enzymes, which use glutathione (GSH) as a substrate, is to reduce soluble hydrogen peroxide and alkyl peroxidases (Demir *et al*., 2011).

1.11. Testis histopathology

'Histology' term is derived from Greek: Histos means tissue while Logia means study of or knowledge. By refer to these terms, it actual refer to study of tissues for living organism. Histology is used loosely to include all subdivision of microscopic anatomy. The correlation between structure and function provide the evidence that show the histology is an intriguing and readily understandable subject. In the study of histology, preparation of tissue for microscopic viewing is an important consideration. This is because cell and tissue cannot be studied to advantage unless they are well prepared for microscopic examination. There are two subdivisions in whole method of tissue preparation: method involving direct viewing of living cell and method employed with dead cell (fixed and stained). However, different tissues that stacked together are

hard to be recognized and differentiated, as most of them appear as colourless compound. This study provided with a full processes which allow the observation of tissue become easier. (Boodnard *et al.,*2001) mentioned that administration of MSG to rats led to atrophic changes in enhancers widely used in many countries and is found in the testis and destruction of Sertolli cells and Leydig cell. (Das and Ghosh, 2010) observed loss of spermatogenic cells injected with MSG. Treating rats with MSG at short-term exhibited slight to moderate damaged seminiferous including cytoplasmic vacuolization of spermatogonia and loss of late spermatids. Long–term treatment of MSG caused severe damage of germ cells (Mohamed, 2012).

1.12. Biochemical parameters (ALT, AST, cholesterol, AP, Creatinine and Urea)

SGPT (ALT) is found in a variety of tissues but is mainly found in the liver. Increased levels are found in hepatitis, obstructive jaundice and other hepatic diseases. Slight elevation of the enzymes is also seen in myocardial infarction.

SGOT (AST) is an enzyme found mainly in heart muscle, liver cells, skeletal muscle and kidneys. Injury to these tissues results in the release of the enzyme in blood stream. Elevated levels are found in myocardial infarction, Cardiac operations, hepatitis, cirrhosis, acute pancreatitis, acute renal diseases, and primary muscle diseases. Decreased levels may be found in pregnancy, beri-beri and diabetic ketoacidosis.

Cholesterol is the main lipid found in blood, bile and brain tissues. It is the main lipid associated with arteriosclerotic vascular diseases. It is required for the formation of steroids and cellular membranes. The liver metabolizes the cholesterol and it is transported in the blood stream by lipoproteins. Increased levels are found in hypercholesterolemia, hyperlipidaemia,

hypothyroidism, uncontrolled diabetes, nephrotic syndrome and cirrhosis. Decreased levels are found in mal absorption, malnutrition, hyperthyroidism, anaemia and liver diseases.

Alkaline Phosphatase (ALP) is an enzyme of the hydrolase class of enzymes and acts in an alkaline medium. It is found in high concentrations in the liver, biliary tract epithelium and in the bones. Normal levels are age dependent and increase during bone development. Increased levels are associated mainly with liver and bone disease. Moderate increases are seen in Hodgkins disease and congestive heart failure.

Creatinine is the catabolic product of creatinine phosphate which is used by the skeletal muscle. The daily production depends on muscular mass and it is excreted out of the body entirely by the kidneys. Elevated levels are found in renal dysfunction, reduced renal blood flow (shock, dehydration, congestive heart failure) diabetes acromegaly. Decreased levels are found in muscular dystrophy.

Urea is the end production of protein metabolism. It is synthesized in the liver from the ammonia produced by the catabolism of amino acids. It is transported by the blood to the kidneys from when it is excreted. Increased levels are found in renal diseases, urinary obstructions, shock, congestive heart failure and burns. Decreased levels are found in liver failure and pregnancy.

1.13. PCNA

Proliferating cell nuclear antigen (PCNA) is a nuclear non histone protein, with a molecular weight of 36kD that plays a role in the initiation of cell prolifera tion by the augmenting of DNA polymerase; stains for proliferating cell nuclear antigen in tumors correlated with grade and mitotic activity. .PCNA was originally identified as an antigen that is expressed in the nuclei of cells during the DNA synthesis phase of the cell cycle. (Leonardi *et al.,* 1992).

CHAPTER 2

REVIEW

OF

LITERATURE

2.1. MSG

The intake of MSG under the name Ajinomoto is very common among people all over Mizoram. It is taken among both sexes. The excess risks confined to long term consumers and also to short term but adequate intake consumers. MSG is taken orally when mixed with food since it is a taste enhancer. Yamaguchi (1998) reported that when MSG is added to foods in small quantities, the palatability of those foods is increased by stimulating the sense of taste.

There are some reports which indicated that MSG was toxic to human and experimental animals. MSG could produce symptoms such as numbness, weakness, flushing, sweating, dizziness and headaches. In addition, ingestion of MSG has been alleged to cause or exacerbate numerous conditions, including asthma, urticaria, atopic dermatitis, ventricular arrhythmia, neuropathy and abdominal discomfort (Geha *et al*., 2001). MSG has a toxic effect on the testis by causing a significant oligozoospermia and increases abnormal sperm morphology in a dose-dependent fashion in male Wistar rats (Onakewhor *et al*., 1998). Machol *et al*. (1999) found that it changed several endocrine functions in neonatally treated rats.

It has been implicated in male infertility by causing testicular hemorrhage, degeneration and alteration of sperm cell population and morphology (Nayanatara *et al*., 2008; Das and Ghosh, 2010). MSG treatment induces neuronal damage by increasing lipid peroxidation (Babu *et al*., 1994) and degeneration in hippocampal CA1 pyramidal cells, which was associated with learning impairment (Ishikawa *et al*., 1997), hyper excitability and motor behavioural alterations (Kiss *et al*., 2007; López-Pérez *et al*., 2010).

MSG, a non-essential amino acid, widely used in food industry, has been the target of research because of its highly toxic potential. Kizer *et al*. (1977) demonstrated that the neurotoxic effect of MSG is more embracing to the neonatal period due to the immature blood–brain barrier;

besides this effect seems to be dose-dependent. Due to intake of MSG, the Chinese Restaurant Syndrome has been implicated by weakness, diarrhoea, vomiting, stomach ache and feeling of tightness of the chest. (Schaumberg *et al*., 1969).

2.2. MSG and dysfunction

MSG-exposed rats have a dysfunction in the serotonergic system which could be associated to the behavioral changes induced by MSG. Once MSG is ingested, skeletal muscles, including the masseter muscle, store most of the glutamate intramuscularly with a seven-to eightfold increase of concentration, although the elevation of the glutamate concentration is short lasting, high dietary MSG intake may elevate glutamate concentrations in skeletal muscles, which could be one lifestyle-related factor contributing to pain sensitivity in chronic musculoskeletal pain conditions including TMD and tension-type headache. There had been specific reports of adverse effects of MSG consumption oral administration on the male reproductive system and hormones, mostly when very high doses of MSG are acutely administered parenterally. (Ismail, 2012; Mohamed, 2012; Nosseir *et al*., 2012).

The consumption of MSG had been reported to be associated with numerous adverse effects (Raiten *et al.*, 1995; FSANZ, 2003). Apart from the well-known Chinese restaurant syndrome consisting of diarrhoea, vomiting, migraine, weakness, stomach ache and tightness of chest, MSG consumption and/or experimental administration had been associated with stunted skeletal development, obesity, hepatotoxicity, brain damage, deoxyribonucleic acid (DNA) damage, and neuroendocrine, reproductive, haematological and metabolic disorders among other things (JECFA – The Joint FAO/WHO Expert Committee on Food Additives, 1988; FASEB – Federation of American Societies for Experimental Biology, 1995; Gong *et al*., 1995; Bhattacharya *et al*., 2011; Igwebuike *et al*., 2011; Ismail, 2012; Meraiyebu *et al*., 2012). Farombi and Onyema found that MSG at a dose of 4 mg/g significantly induced the formation of

micronucleated polychromatic erythrocytes. This might also have been mediated through a deleterious effect on the hematopoietic stem cells in the bone marrow. MSG might cause increased oxidative stress in the tissues of animals. It was reported that MSG was associated with the production of oxygen free radicals and oxidative stress in different tissues of experimental animals (Oneyma *et al.,* 2012 ; Kumar and Bhandhari, 2013).

2.3. MSG and antioxidants

Antioxidants have been reported to play a significant role in the protection against lipid peroxidation. L-carnitine is antioxidants that are thought to have a protective effect by either reducing or preventing oxidative damage (Rinne *et al*., 2000). It is well known as non-enzymatic antioxidant (Al-Attar, 2011; Uzun *et al*., 2009). L-carnitine inhibits peroxidation of membrane lipids by scavenging lipid peroxylradicals (El-Demerdash *et al*., 2004; Uzunhisarcikli and Kalender, 2011) and it also inhibits oxidative damage in several tissues by heavy metals and pesticides in experimental animals (El-Demerdash *et al*., 2004; Acharya *et al*., 2008). Supplemental doses of L-carnitine effectively counteracts the toxic effects of MSG administration on thyroid, liver, heart, bone, muscle, urinary bladder, and kidney functions and attenuates the oxidative damage possibly by its antioxidant action (Zadeh *et al*., 2008). L-carnitine helps the body produce energy. It is important for heart and brain function, muscle movement, and many other body processes.

Patients with cancer are especially at risk for carnitine deficiency (Dodson *et al*., 1989; Visarius *et al*., 1999; Lancaster *et al*., 2010; Silverio *et al*., 2011). L-Carnitine supplementation has also been demonstrated to improve the patient's condition in several diseases, including senile dementia, metabolic neuropathies, HIV infection, myopathies, cardiomyopathies, and renal failure (Laviano *et al*., 2006).

2.4. L-carnitine supplementation

L-carnitine supplements are used to increase L-carnitine levels in people whose natural level of L-carnitine is too low because they have a genetic disorder, are taking certain drugs (valproic acid for seizures), or because they are undergoing a medical procedure (hemodialysis for kidney disease) that uses up the body's L-carnitine. It is also used as a replacement supplement in strict vegetarians, dieters, and low-weight or premature infants.

L-carnitine is used for conditions of the heart and blood vessels including heart-related chest pain, congestive heart failure (CHF), heart complications of a disease called diphtheria, heart attack, leg pain caused by circulation problems (intermittent claudication), and high cholesterol. Some people use L-carnitine for muscle disorders associated with certain AIDS medications, difficulty fathering a child (male infertility), a brain development disorder called Rett syndrome, anorexia, chronic fatigue syndrome, diabetes, overactive thyroid, attention deficit-hyperactivity disorder (ADHD), leg ulcers, Lyme disease, and to improve athletic performance and endurance. The carnitine content of seminal fluid is directly related to sperm count and motility, (Menchini-Fabris *et al* ., 1984; Matalliotakis *et al* ., 2000).

Several studies indicate that carnitine supplementation (2–3 grams/day for 3–4 months) may improve sperm quality (Costa *et al*., 1994; Vitali *et al*., 1995; Vicari *et al*., 2001) and one randomized, double-blind crossover trial found that 2 grams/day of carnitine taken for 2 months by 100 infertile men increased the concentration and both total and forward motility of their sperm (Lenzi *et al*., 2003). The reported benefits may relate to increased mitochondrial fatty-acid oxidation (providing more energy for sperm) and reduced cell death in the testes (Ng *et al*., 2004).

There has also been a report that L-carnitine is a small water-soluble molecule important for fat metabolism in mammalian (Bremer, 1983).

Boerrigter *et al*. (1993) reported that L-carnitine enhances the activity of DNA repairing enzyme poly (ADP-ribosyl) polymerase and also other related repair mechanisms.

CHAPTER 3

OBJECTIVES

3.0. Objectives

Based on the literature survey the following queries have been raised to validate the scientific finding:

Is MSG really a toxic substance or may be used as a flavour enhancer?

Is MSG causing organ toxicity?

If it causes organ toxicity what is the dose at which it impairs the organ function?

Is there any ameliorative measure for MSG toxicity?

Hence, the objectives of the present work is to study the effect of L-carnitine on reproductive parameters of MSG fed with Wistar albino rats and their role in amelioration of the testicular toxicity induced by MSG in relation to:

- Analysis of sperm parameters (sperm morphology, motility, daily sperm production, sperm concentration).

- Evaluation of the oxidative damage and antioxidant status in testes.

- Histopathological and immunohistochemical changes in testis.

CHAPTER 4

MATERIALS

AND

METHODS

4.1.1. Animals

This study was performed on mature male Wistar albino rats, weighing about 150–200g BW. They were bred in a well-ventilated room with the temperature ranging between 22 and 25°C and maintained under standardized conditions away from any stressful conditions with 12:12 hs light and dark cycle with free access to humidity and fed balanced meal for experimental animals provided. All experimental procedures and animal maintenance were conducted in accordance with the accepted standards of animal care per cage for the protection of vertebrate animals (Ethics Committee, Mizoram University, Permit No. MZUIAEC 16-17-06). One group served as control. Animals were weighed and randomly allocated into 6 groups (30 rats each) as following.

4.1.2. Experimental protocols

Rats were divided into six groups, each consisting of 30 rats. The experiment was performed during 30 days, 60 days and 90 days. The doses were fixed on the basis of LD_{50} of MSG - **15g/kg. (*Beyreuther,* 2006)**

30mg/kg	**1/500 times less than LD_{50}**
300 mg/kg	**1/50 times less than LD_{50}**
3000 mg/kg	**1/5 times less than LD_{50}**

The experimental design of this experiment was:

Group 1 – control

Group 2 – MSG treated rats (30 mg/kg BW per day in distilled water);

Group 3– MSG – medium dose treated rats (300 mg/kg BW per day in distilled water);

Group 4 – MSG – high dose treated rats (3000 mg/kg BW per day in distilled water);

Group 5-MSG-high dose treated rats (3000 mg/kg BW with 100 mg/kg L- carnitine);

Group 6-rats treated with 100 mg/kg L- carnitine.

Acute toxicity – 30 days trial

Dose	Chemicals	No of animals	No of days
control	0.9% saline	5	30
30 mg/kg	MSG	5	30
300 mg/kg	MSG	5	30
3000 mg/kg	MSG	5	30
3000+100 mg/kg	MSG + C	5	30
100 mg/kg	Carnitine	5	30

Chronic toxicity – 60 days trial

Dose	Chemicals	No of animals	No of days
control	0.9% saline	5	60
30 mg/kg	MSG	5	60
300 mg/kg	MSG	5	60
3000 mg/kg	MSG	5	60
3000+100mg/kg	MSG + C	5	60
100 mg/kg	Carnitine	5	60

Chronic toxicity – 90 days trial

Dose	Chemicals	No of animals	No of days
control	0.9% saline	5	90
30 mg/kg	MSG	5	90
300 mg/kg	MSG	5	90
3000 mg/kg	MSG	5	90
3000+100mg/kg	MSG + C	5	90
100 mg/kg	Carnitine	5	90

The doses were administered in the morning (between 9.30 and 10.30 h) to non-fasted rats. The first day, when the animals were treated was considered experimental day 0.

The body weight was recorded after every three days; from the date of experiment. Food and water consumption were also recorded till the rats were dissected. Rectal temperature had also been checked every week. Blood glucose level had also been recorded once a month during the experiment. At the end of the 30, 60 and 90 days of treatment, all animals were scarified and dissected. The testis tissues were quickly processed, dissected out, blotted free of blood, adhering tissues were removed and weighed and stored for light microscope investigations and biochemical examinations.

4.2. Sperm analysis

The technique of Wyrobek *et al*., (1984) was adopted for sperm abnormality. To evaluate the sperm abnormalities, the suspension was stained with eosin, dropped on slides to determine the motility, abnormality (using Olympus microscope by objective (40x). Abnormality was classified in head and tail. Abnormal sperm cells was counted and the percentage was calculated.

To assess motility, the sperms was classified as motile sperm (M) and non-motile (NM). To evaluate the daily sperm production in testes, spermatozoa was counted as per the standard protocol of WHO laboratory manual (1992). Motile sperm cells were counted and the percentage was calculated**.**

4.2.1. Sperm Motility

1. Sperm motility was evaluated immediately after sacrificing the animals.

2. Sperm suspension was dropped on a clean glass slide and covered with a coverslip (20mm ×20mm) and allowed to stand for about a minute.

3. The slides were examined under the microscope at 400x and score motility from different fields. Spermatozoa showing any degree of movement were considered motile.

Calculation:

Motility (%) = Number of motile spermatozoa/Total number of spermatozoa × 100

4.2.2. Sperm concentration analysis

A hemocytometer (Fein-optik, Jena, Germany) with improved Neubauer ruling was employed for counting the spermatozoa.

1. A 20- fold dilution was made by mixing the sperm suspension with the spermicidal solution ($NaHCO_3$:4g +phenol:1h in 100 ml distilled water).

2. The dilution was made using a white blood cell pipette, the sperm suspension was drawn to the 0.5 mark halfway up the stem and the spermicidal solution subsequently till the mark 11 at the top of the bubble chamber.

3. The preparation was then thoroughly mixed and one drop of it was added to both sides of the haemocytometer.

4. The spermatozoa were allowed to settle optimally by keeping the haemocytometer in a humid chamber for 30 minutes.

5. The humid chamber is constructed by placing a wet sponge inside a fairly tight ice box.

6. The number of spermatozoa was counted in the four corner squares of the haemocytometer under a microscope at 400 ×. When spermatozoa crossed the lines of the grid, only those at the top and right hand sides of the squares were counted.Spermatozoa on both sides of the haemocytometer were counted and the average number was recorded.

Concentration of spermatozoa= Average number of spermatozoa counted (N) ×Multiplication factor (11,000) × dilution factor (20) = N ×10,000 × 20 spermatozoa =N × 0.2 × 10^6 spermatozoa

4.2.3. <u>Daily sperm production</u>

1. Ten ml of sodium chloride (Na Cl) and 20 µl triton was mixed, of which 1ml was taken in test tube. One testis (without cover) was homogenised and mixed with it.

2. One ml of homogenate, 8.8 ml of distilled water and 200µl Trypan blue were mixed.

3. A drop was taken in hemocytometer for counting the daily sperm production in each square.

4.2.4. <u>Sperm morphology</u>

Sperm evaluation of sperm morphology, the same preparations as used for viability test is examined.

1. Spermatozoa are considered abnormal if they show any of the following types of abnormalities: amorphous head, banana-shaped head, detached head, coil tail and broken tail.

2. About one hundred spermatozoa both normal and abnormal are counted from different fields and the percent abnormality was calculated.

Calculation:

Abnormality (%) = Number of abnormal spermatozoa/Total number of spermatozoa × 100

4.3.1. Determination of oxidative stress (lipid peroxidation) and Antioxidant enzymes

4.3.1.1. Estimation of protein

Principle: The principle behind the Lowry method of determining protein concentrations lies in the reactivity of the peptide nitrogen[s] with the copper [II] ions under alkaline conditions and the subsequent reduction of the Folin Ciocalteau phosphomolybdic phosphotungstic acid to hetero polymolybdenum blue by the copper-catalyzed oxidation of aromatic acid.

The Lowry method is sensitive to pH changes and therefore the pH of assay solution should be maintained at 10 - 10.5. The Lowry method is sensitive to low concentrations of protein. Dunn (1992) suggests concentrations ranging from 0.10 - 2 mg of protein per ml while Price (1996) suggests concentrations of 0.005 - 0.10 mg of protein per ml. The major disadvantage of the Lowry method is the narrow pH range within which it is accurate. However, we have used very small volumes of sample, which have little or no effect on pH of the reaction mixture. A variety of compounds had interfered with the Lowry procedure. These include some amino acid derivatives, certain buffers, drugs, lipids, sugars, salts, nucleic acids and sulphydryl reagents (Dunn, 1992).

Price (1996) notes that ammonium ions, zwitter ionic buffers, non-ionic buffers and thiol compounds may also interfere with the Lowry reaction. These substances should be removed or diluted before running Lowry assays.

Reagents:

A) 4 g of Na_2CO_3 +4 g of NaoH dissolved in 100 ml.

B) i) 53 mg of sodium potassium tartarate dissolved in 500 µl of distilled water.

ii) 200 mg of $CuSO_4$ dissolved in 500 µl of distilled water.

50 ml of reagents A and 1 ml of reagent B were mixed.(analytical reagent)

C) Lowry's reagent---Folin (2 ml in each sample).

Procedure:

1. 990 µl of distilled water was added in each test tube.
2. 2 ml of analytical reagent was added in each test tube.
3. 10µl of sample was added to each test tube.
4. It was then incubated in dark for 40 min
5. 500 µl of Folin reagent was added to each test tube.
6. Then mixed and incubated for 20 min.
7. The O.D was taken at 750 nm.
8. Blank was set at 0.00
9. Protein concentration of the tissues was calculated based on the standard graph using bovine serum albumin.

4.3.1.2. Estimation of MDA level (Lipid Peroxidation) (Satoh *et al*., 1978)

Principle: This test is a calorimetric test which takes advantage of the ability of hydro peroxide to generate free radicals after reacting with transitional metals, when buffered chromogenic substance is added; a colored complex appeared. This colored complex was measured spectrophotometrically. Lipid peroxidation levels in the testis was measured using thio-barbituric acid reactive substances (TBARS).The testis was then homogenised in ice cold 1X PBS (10%) and the concentration of TBARS was expressed as nmol of MDA per mg protein. The absorbance was read at 540 nm.

Procedure:

1. Homogenised tissue in 1X PBS (10%) if weight of tissue is 50 mg, 500µl of PBS was added. (wt of tissue × 10).

2. 500µl of homogenate tissue and PBS amount taken µl of 10% TCA in one eppendorf tube and centrifuged.

3. The supernatant was taken out carefully.

4. The amount of supernatant taken should be known.

5. 0.8%TBA was added in 1(protein):2 (TBA) ratio.

If more pink is observed, it contains more malondialdehyde

6. 1 ml of supernatant and 2 ml of TBA (1:2) was taken.

7. Boiled for 45 min and were observed for the formation of colored complex.

8. Slight pink color is expected to come.

9. O.D. at 540 nm was taken.

4.3.1.3. Estimation of Superoxide Dismutase (SOD) enzyme activity (Asada *et al*., 1974)

Principle: SOD is an enzyme that catalyses dismutation of two superoxide anion into hydrogen peroxide and molecular oxygen.

SOD is one of the most important enzyme in the front line of defence against oxidative stress.

Reagents:

1. Phenazenemethosulfate (PMS): 0.06 mg dissolved in 1 ml of distilled water.

2. Nitrobluetetrzolium (NBT): 2.5 mg dissolved in 1 ml of distilled water.

3. NADH: 0.6 mg dissolved in 1 ml of distilled water.

4. Acetic acid

5. n-Butanol

Procedure:

Reagents	Volume
PMS (Phenazinemethosulfate)	**50µl**
NBT(Nitrobluetetrzolium)	**15µl**
NADH	**100µl**
Sample	**50µl**
Incubated for 90 sec at 30 ^{0}C, stopped the reaction by adding Acetic acid	**500µl**
n-Butanol	**2ml**
The absorbance was taken at 560nm	

Calculation:

% inhibition = {Blank-Test/Blank} x 100

50% inhibition = 1 unit

1% inhibition = 1/50

SOD unit = 1/50 × % inhibition

4.3.1.4. Estimation of Catalase activity (Aebi, 1984)

Principle: The assay is performed as described by Aebi *et al*, (1974). Catalase catalyses the dismutation of H_2O_2 and thus competes with GSHPs for the common substrate. It is considered to be the primary scavenger of intracellular H_2O_2. In the UV range H_2O_2 absorbs maximally at 240nm.Catalase rapidly breaks down H_2O_2 leading to a decrease in absorbance. A difference in the absorbance at 240 nm per unit time is measured of Catalase activity.

Reagents:

1. Fifty nM phosphate buffer, pH 7 was made by mixing A and B in a 1:1.5 v/v ratio.

A) 6.8 g----KH_2PO_4

B) 8.9 g----Na_2HPO_4

2. 30 mM H_2O_2 made by diluting 340µl 30% H_20_2 to 100 ml with Phosphate buffer pH-7.

3. Sample used: 10% (w/v) homogenates of tissue, cell line.

Procedure:

Pipette successively into the cuvettes	**Blank**	**Sample**	**Concentration in assay mixture 50 m mol/l**
Phosphate buffer	**1 ml**		
Sample	**2 ml**	**2 ml**	
H_2O_2	**-**	**1ml**	**10 m mol/l**
The reaction was started by adding H_2O_2.The O.D was taken at 10 sec intervals upto 30 sec.			

Calculation:

$0.23 \times \log A_1/A_2$

A_1 is A_2 at 0 sec

And A_2 is A_2 at15 sec

4.3.1.5. Estimation of Glutathione (Rahman *et al*., 2007).

Principle: Glutathione is measured by its reaction with DTNB 5, 5 dithio 2-nitobenzoic acid (Ellmans reaction) to give a compound that absorbs light at 412 nm.

Reagents:

1.0.2 M Na2HPO4:28.392 g was weighed and dissolved in 1l distilled water.

-----0.425 mg in 15 ml.

2.10 mM DTNB:39.63 mg DTNB was dissolved in 10 ml of 0.2M Na_2HPO_4.

-----Dithionitrobenzene 4 mg in 1 ml.

Procedure:

Reagents	**Blank**	**Test**
Na_2HPO_4	**900µl**	**900 µl**
DTNB	**20 µl**	**20 µl**
Distilled water	**80 µl**	**-**
Sample	**-**	**80 µl**
Absorbance taken at 412 nm within 5 min		

4.3.1.6. Estimation of Glutathione-S-Transferase (GST) (Habig *et al*., 1974):

Principle: Glutathione-S-Transferase catalyses the reaction of CDNB with the SH group of glutathione.

GSH (reduce)+CDNB----------DNB-S-Glutathione

Reagents:

1. Phosphate Buffer: 0.1M, pH 6.5.

a) K_2HPO4---0.1M. (Solution A)

8.709g of $K_2HPO_4$was dissolved in 500 ml of distilled water.

b) KH_2PO_4---0.1 M.(solution B)

6.084g of $KH_2PO_4$was dissolved in 500 ml of distilled water.

164 ml of Solution A was mixed with 336 ml of Solution B and adjust the pH to 6.5.

2. CDNB (F.W=202.6)-20mM.

20.26 mg of CDNB was made upto 5 ml in 95% ethanol. Stored in brown bottle in refrigerator.

3. GSH (F.W=307.3)-20mM.

3.073 mg of reduced GSH was dissolved in 0.5 ml of distilled water. It was prepared fresh.

4. Sample was then added.

Procedure:

Reagents	**Volume**
Distilled water	**4.4 ml**
Phosphate buffer	**250µl**
CDNB	**50µl**
Incubate at 37^0C for 10 mins	
GSH	**250µl**
Extract	**50µl**
Read absorbance at 340 nm at 1 min interval	

For blank distilled water was taken.

Calculation:

GST activity=Absorbance of test-absorbance of blank ×1000IU/L

9.6 × vol of test (µl)

(where 9.6 is the molar extinction coefficient of GST)

4.3.1.7. Estimation of SGPT (ALT) activity (Reitman &Frankel's method)

For the determination of SGPT (ALT) activity in serum.

Principle: SGPT converts L-Alanine and α-Ketoglutarate to Pyruvate and Glutamate. The Pyruvate formed reacts with 2,4Dinitrophenyl hydrazine to produce a hydrazone derivative, which in an alkaline medium produces a brown coloured complex whose intensity is measured. The reaction does not obey Beer's law and hence a calibration curve is plotted using a Pyruvate standard. The activity of SGPT (ALT) is read off this calibration curve.

Contents	40 assays	80 assays
L1:Substrate Reagent	**25 ml**	**50 ml**
L2:DNPH Reagent	**25 ml**	**50 ml**
L3:NaOH Reagent (4N)	**25 ml**	**50 ml**
S:PyruvateStandard(2mM)	**5 ml**	**5 ml**

Storage /stability

Contents are stable at 2-8 ^{0}C at R.T.

Reagent Preparation:

All reagents are ready to use except NaOH Reagent (4N) which has to be diluted 1:10 with distilled/deionised water.

Working NaOH reagent: Sodium Hydroxide was diluted to 250ml or for every 1.0 ml of NaOH. Reagent (4N) 9.0 ml of water was added. The Working Sodium Hydroxide reagent is stable at R.T.

Sample material: Serum

Procedure:

Wavelength/filter : 505 nm (Hg 546 nm)/Green

Temparature ` : 37 ^{0}C & R.T.

Light path : 1 cm

Plotting of the Calibration curve:

Pipette into five clean dry test tubes labeled as 1,2,3,4, & 5:

Addition sequence	**1**	**2**	**3**	**4**	**5**
Enzyme Activity (U/ml)	**0**	**28**	**57**	**97**	**150**
	(ml)	**(ml)**	**(ml)**	**(ml)**	**(ml)**
Substrate Reagent (L1)	**0.50**	**0.45**	**0.40**	**0.35**	**0.30**
Pyruvate Standard (S)	**-**	**0.05**	**0.10**	**0.15**	**0.20**
Distilled Water	**0.10**	**0.10**	**0.10**	**0.10**	**0.10**
DNPH Reagent (L2)	**0.50**	**0.50**	**0.50**	**0.50**	**0.50**
Mixed well and allowed to stand at R.T for 20 minutes.					
Working NaOH Reagent (L 3)	**5.00**	**5.00**	**5.00**	**5.00**	**5.00**

Mixed well and allowed to stand at R.T for 10 min. The absorbances of the tubes 2 – 5 against tube 1 (Blank) was measured. Plot a graph of the absorbances of tubes 2 – 5 on the 'Y' axis versus the corresponding Enzyme activity on the 'X' axis.

Assay :

Pipette into clean dry test tubes labeled as Blank (B) & (T)

Addition Sequence	**B (ml)**	**(T) (ml)**
Substrate Reagent (L 1)	**250µl**	**250 µl**
Incubate at 37 C for 3 minutes		
Sample	-	**50 µl**
Mix well and incubate 37 C for 30 minutes	-	-
DNPH Reagent (L2)	**250µl**	**250 µl**
Mixed well and allowed to stand at R.T for 20 minutes		
Distilled Water	**50µl**	**-**
Working NaOH Reagent (L3)	**250µl**	

Mixed well and allowed to stand at R.T for 10 min. The absorbances of the Test (T) was measured against Blank,(Blank) and the activity of the test was read from the calibration curve plotted earlier.

4.3.1.8. Estimation of SGOT (AST) activity (Reitman & Frankel's method)

For the determination of SGOT (AST) activity in serum.

Principle: SGOT converts L-Aspartate and α Ketoglutarate to Oxaloacetate and Glutamate. The Oxaloacetate formed reacts with 2, 4, Dinitrophenyl hydrazine to produce a hydrazone derivative, which in an alkaline medium produces a brown coloured complex whose intensity is measured. The reaction does not obey Beer's law and hence a calibration curve is plotted using a Pyruvate standard. The activity of SGOT (ASAT) is read off this calibration curve.

Contents	**40 assays**	**80 assays**
L-1 : Substrate Reagent	**25 ml**	**50 ml**
L-2 : DNPH Reagent	**25 ml**	**50 ml**
L-3 : NaOH Reagent (4N)	**25 ml**	**50 ml**
S:PyruvateStandard (2mM)	**5 ml**	**5 ml**

Reagent Preparation:

All reagents are ready to use except NaOH Reagent (4N) which has to be diluted 1:10 with distilled/deionised water.

Working NaOH reagent: Sodium Hydroxide was diluted to 250ml or for every 1.0 ml of NaOH Reagent (4N), 9.0 ml of distilled water was added. The Working Sodium Hydroxide reagent was stable at R.T.

Sample material: Serum.

Procedure:

Wadvelength : 505 nm (Hg 546 nm)/Green

Temparature : 37^{0} C & R.T.

Light path : 1 cm

Plotting of the Calibration curve :

Pipette into five clean dry test tubes labeled as 1,2,3,4, & 5:

Addition sequence	**1**	**2**	**3**	**4**	**5**
Enzyme Activity (U/ml)	**0**	**24**	**61**	**114**	**190**
	(ml)	**(ml)**	**(ml)**	**(ml)**	**(ml)**
Substrate Reagent (L1)	**0.50**	**0.45**	**0.40**	**0.35**	**0.30**
Pyruvate Standard (S)	**-**	**0.05**	**0.10**	**0.15**	**0.20**
Distilled Water	**0.10**	**0.10**	**0.10**	**0.10**	**0.10**
DNPH Reagent (L 2)	**0.50**	**0.50**	**0.50**	**0.50**	**0.50**
Mixed well and allowed to stand at R.T. for 20 minutes.					
WorkingNaOH Reagent (L3)	**5.00**	**5.00**	**5.00**	**5.00**	**5.00**

Mixed well and allowed to stand at R.T for 10 min. The absorbances of the tubes 2 – 5 against tube 1 (Blank) was measured. A graph of the absorbances of tubes 2-5 on the 'Y'axis versus the corresponding Enzyme activity on the 'X' axis was plotted.

Assay:

Pipette into clean dry test tubes labeled as Blank (B) & Test (T) :

Addition Sequence	**(B) (ml)**	**(T) (ml)**
Substrate Reagent (L1)	**250 µl**	**250 µl**
Incubated at 37 C for 3 minutes		
Sample	**-**	**50 µl**
Mixed well and incubated at 37^0C for 60 minutes		
DNPH Reagent (L2)	**250 µl**	**250 µl**
Mix well and allow to stand at R.T for 20 minutes		
Distilled Water	**50µl**	**-**
Working NaOH Reagent (L3)	**250 µl**	**250 µl**

Mixed well and allowed to stand at R.T. for 10 min. The absorbances of the Test (T) against Blank (Blank) was measured and the activity of the test from the calibration curve plotted earlier was read.

4.3.1.9. Estimation of Cholesterol (CHOD/ PAP method) (Allain *et al*., 1974)

For the determination of Cholesterol in serum

Principle: Cholesterol esterase hydrolyses esterified cholesterols to free cholesterol. The free cholesterol is oxidised to form hydrogen peroxide which further reacts with phenol and 4-animoantipyrine by the catalytic action of peroxidase to form a red coloured quinoneimine dye complex. In tensity of the colour formed is directly proportional to the amount of cholesterol present in the sample.

$$\text{Cholesterol esters} + H_20 \xrightarrow{\text{Cholesterol Esterase}} \text{cholesterol} + \text{Fatty acids}$$

$$\text{Cholesterol} + 0_2 \xrightarrow{\text{Cholesterol Oxidase}} \text{cholesterol} + H_20_2$$

$$H_20_2 + 4\ \text{Aminoantipyrine} + \text{Phenol} \xrightarrow{\text{Peroxidase}} \text{Red Quinoneimine dye} + H_20$$

Contents	75 ml	2 X 75ml	2 X 150ml	2 X 250ml
L1:Enzyme reagent1	60 ml	2 X 60ml	2 X 120ml	2 X 200ml
L2:Enzyme reagent 2	15 ml	2 X 15ml	2 X 30ml	2 X 50ml
S:Cholesterol Standard (200 mg/ dl)	5 ml	5ml	5ml	5ml

Storage/ stability

Contents are stable at 2 -8^0 C.

Reagent Preparation:

Working Reagent : The contents of 1 bottle of L2 (Enzyme Reagent 2) was poured into 1 bottle of L1 (Enzyme reagent 1). This working reagent is stable for at least 8 weeks when stored at 2-8^0 C. Upon storage the working reagent may develop a slight pink colour however this does not affect the performance of the reagent.

Alternatively for flexibility as much of working reagent may be made as and when desired by mixing together 4 parts of L1 (Enzyme Reagent 1) and part 1 of L2 (Enzyme Reagent 2).Alternatively 0.8 ml of L1 and 0.2ml of L2 may also be used instead of 1 ml of the working reagent directly during the assay.

Sample material: Serum

Procedure:

Wavelength/ filter : 505 nm (Hg 546 nm) / Green

Temperature : 37^{0}C / R.T.

Light path : 1 cm

Then pipetted into clean dry test tubes labelled as Blank (B), Standard (S), and Test (T) :

Additon Sequence	**B (ml)**	**S (ml)**	**T (ml)**
Working reagent	**1.0**	**1.0**	**1.0**
Distilled water	**0.01**	**-**	**-**
Cholesterol Standard(S)		**0.01**	**-**
Sample			**0.01**

Mixed well and incubated at 37^0C for 5 min. or at R.T. (25^0C) for 15 min. The absorbance of the Standard (Abs.S), and Test Sample (Abs.T) was measured against the Blank, within 60 Min.

Calculations

Cholesterol in mg/dl = Abs.T/ Abs.S× 200 mg/dl

4.3.1.10. ALKALINE PHOSPHATASE (Mod. Kind & King'2 method)

For the determination of Alkaline Phosphatase activity in serum.

Principle: ALP at an alkaline pH hydrolyses di Sidium Phenylphosphate to form phenol. The phenol formed reacts with 4 – Aminoantipyrine in the presence of Potassium Ferricyanide, as an oxidising agent to form a red coloured complex. The intensity of the colour formed is directly proportional to the activity of ALP present in the sample.

Contents	15 Tests	30 Tests
L1 : Buffer Reagent	**60 ml**	**120ml**
L2 : Substrate reagent	**6ml**	**12 ml**
L3 : Colour Reagent	**60ml**	**120ml**
S : Phenol Standard (10 mg/ dl)	**5ml**	**5ml**

Sample material : Serum.

Procedure:

Wavelength/ filter : 510 nm (Hg 546 nm) Green

Temperature : 370 C

Light path : 1 cm

Assay :

Pipette into four clean dry test tubes labelled as Blank (B), Standard (S), Control (C), Test (T)

Addition Sequence	B (ml)	S (ml)	C (ml)	T (ml)
Distilled water	**525µl**	**500 µl**	**500 µl**	**500 µl**
Buffer Reagent (L1)	**500µl**	**500µl**	**500 µl**	**500 µl**
Substrate Reagent (L2)	**50µl**	**50 µl**	**50 µl**	**50 µl**
Mixed well and allowed to stand at 37^0C for 3 minutes				
Sample	-	-	**25 µl**	**25 µl**
Phenol Standard (S)	-	**25 µl**	-	
Mixed well and allowed to stand at 37^0C for 15 minutes and add.				
Colour Reagent (L3)	**500µl**	**500µl**	**500µl**	500µl
Sample	-	-	**0.025**	-

It was mixed well after each addition. The absorbances of the Blank (Abs.S), Control (Abs.C) and Test (Abs.T) was measured against distilled water.

Calculations:

Total ALP activity in K.A. Units = Abs. T- Abs.C / Abs.s – Abs.B×10 K.A. Units

4.3.1.11. Estimation of Creatinine (Alkaline Picrate method)

For the determination of Creatinine in serum and urine

Principle: Picric acid in an alkaline medium reacts with creatinine to form an orange coloured complex with the alkaline picrate. Intensity of the colour formed is directly proportional to the amount of creatinine present in the sample.

Creatinine + Alkaline Picrate →Orange Coloured Complex

Contents	15 Tests	35 Tests	70 Tests
L1:Picric Acid Reagent	**60 ml**	**140 ml**	**2x140 ml**

L2:Buffer Reagent	**5 ml**	**12 ml**	**25 ml**
S:Creatinine Standard (2 mg/dl)	**5 ml**	**5 ml**	**10 ml**

Storage/stability

All reagents are stable at R.T.

Reagent Preparation

Sample material: Serum

.

Procedure

Wavelength/filter : 520 nm (Hg 546 nm)/Green
Temperature : R.T
Light path : 1 cm

Deproteinization of specimen

Pipetted into a clean dry test tube

Picric acid reagent (L1)	**2.0 ml**
Sample	**0.2 ml**

Mixed well and centrifuged at 2500 – n3000 rpm for 10 min and a clear supernatant was observed.

Pipette into clean dry test tubes labelled as Blank (B), Standard (S), and Test (T)

Addition Sequence	**B (ml)**	**S (ml)**	**T (ml)**
Supernatant	**-**	**-**	**550µl**
Picric Acid Reagent (L1)	**500 µl**	**500 µl**	**-**
Distilled water	**50 µl**	**-**	**-**
Creatinine Standard(S)	**50 µl**	**50 µl**	**50 µl**
Buffer Reagent (L2)			

Mixed well and kept the test tubes at R.T. for exactly 20 minutes. The absorbance of the Standard (Abs.S), and Test Sample (Abs.T) against the Blank was measured.

Calculations:

Creatinine in mg% = mg/dl

4.3.1.12. Estmation of Urea (Mod.Berdthelodt method, 1859)

For the determination of Urea in serum, plasma and urine.

Principle: Urease hydrolyses urea to ammonia and CO. The ammonia formed further reacts with a phenolic chromogen and hypochlorite to form a green coloured complex. Intensity of the colour formed is directly proportional to the amount of urea present in the sample.

$$\text{Urea} + H_2O \xrightarrow{\textit{Urease}} \text{Ammonia} + CO_2$$

Ammonia + Phenolic chromogen → Green coloured complex + Hypochlorite

Contents	75 Assays	3x75 Assays	2x150Assays
L1: Buffer Reagent	75 ml	3x 75 ml	2x150 ml
L2: Enzyme Reagent	7.5 ml	3 x 7.5 ml	30ml
L3:Chromogen Reagent	15 ml	45 ml	60 ml
S : Urea Standard (40 mg/dl)	5 ml	5 ml	5 ml

Working Enzyme Reagent : For the flexibility and convenience in performing large assay series, a working enzyme reagent was made by pouring 1 bottle of L2 (Enzyme Reagent) into 1 bottle of L1 (Buffer Reagent). For smaller series combine 10 parts of L1 (Buffer Reagent) and 1 part of L2 (Enzyme Reagent). 1ml of the working reagent per assay was used instead of 1 ml of L1 and 0.1 ml of L2 as given in the procedure. The working enzyme reagent is stable for at least 4 weeks when stored at 2-8 C.

Cuveties, 1 part of L3 (Chromogen Reagent) was diluted with 4 parts of fresh ammonia free distilled/deionised water. 1ml of working chromogen was used instead of 0.2 ml in the assay. The working chromogen reagent is stable for atleast 8 weeks when stored at 2-8 ^{0}C in a tightly stopper plastic bottle.

Sample material: Serum

Procedure

Wavelength/filter : 570 nm (Hg 578 nm)/Yellow

Temparature : 37 ^{0}C /R.T

Light path : 1 cm

Pipetted into clean dry test tubes labelled as Blank (B), Standard (S), and Test (T):

Addition sequence	**B (ml)**	**S (ml)**	**T (ml)**
Buffer reagent (L1)	**1ml**	**1ml**	**1ml**
Enzyme reagent (L2)	**100 ml**	**100 ml**	**100 ml**
D.H_2O	**10 ml**	**-**	**-**
Urea std	**-**	**10µl**	**-**
Sample	**-**	**-**	**10µl**
Mixed well and was incubated for 5 min at 37^{0}C.			
Chromogen Reagent (L3)	**200µl**	**200µl**	**200µl**

Calculations:

Urea in mg/dl = AbsT/Abs S × 40 mg/dl

Urea nitrogen in mg/dl = Urea in mg/dl × 0.467 mg/dl

4.4.1. Histopathological study of the testes

One testis of each rat was removed and quickly fixed in Bouins fluid for at least 24 hs. The fixed specimens was processed through the conventional paraffin embedding technique, sectioned at 5µm and stained with hematoxylin and eosin (Bancroft and Gamble, 2002) for histopathological studies. Histological specimens was used for immunohistochemical studies by using respective antibodies as per the manufacturer`s instructions.

4.4.1.1. Histology

After necropsy, the testis samples from all the animals of each group were preserved in Bouins fluid for at least 24 h for histopathological examination. Bouins fixed samples were processed by the standard paraffin wax technique, and sections of 5µm thickness were cut and stained with hematoxylene and eosin (H and E) and then examined microscopically for histological analysis.

Block preparation

1. One of the testis which had been preserved in 70% alcohol was used for histology.
2. From 70% alcohol it was then changed to 90% alcohol for 1 hour.
3. From 90% alcohol it was changed again to 90% alcohol for 1 hour.
4. From 90% alcohol it was changed to 100% alcohol for 1 hour.

5. From 100% alcohol it was changed again to 100% alcohol for 1 hour.

6. The testis was then changed to xylene for 5-10 min.

7. Followed by xylene + wax for 40 min at 60^0C.

8. It was changed to wax-1 for 40 min at 60^0C.

9. It was again changed to wax-2 for 40 min at 60^0C.

10. Then finally it was changed to wax-3 for 40 min at 60^0C.

11. Melted wax was poured into paper boat and the tissue was fixed and dried.

12. After dried, the tissues were cut and fixed in the woods, then cut using microtome and attached to the gelatin coated slides for further staining.

Hematoxylene staining

1. Xylene one change for 10 min each.

2. 100% alcohol one change for 10 min each.

3. 90% alcohol one change for 10 min each.

4. 70% alcohol for 10-15 min.

5. Then dipped in distilled water for 10 min.

6. Stained in hematoxylene for 5 min.

7. It is then kept under running water for 5 min.

8. Dipped in 50% alcohol for 10 min.

9.70 % alcohol for 10 min.

10. Kept in eosin for 1-2 min.

11. Dehydrated in 90% alcohol for 10mins each with one change.

12. One change of 100% alcohol for 10 min each.

13. Then changed to xylene for one change, 10 min each.

14. Mounted in Dpx and observed under the microscope. (Leica, Germany)

4.4.2. Immunohistochemical study of the testes

1. The processed testis slides were dipped in xylene for 10 min each with one change.

2. Changed in 100% alcohol two times for 10 min.

3. Changed in 90% alcohol two times for 10 min.

4. Changed in 70% alcohol for 10 min.

5. Changed in methanol + H_20_2 for 10 min.

6. Changed to distilled water for 5 min

7. Changed to 1X PBS for 10 min.

8. Changed to blocking serum + 1X PBS (1:100) for 30 min.

9. Primary antibody + 1 X PBS 100 µl (1:75).

10. Overnight incubation (4^0C).

11. 1 X PBS-Primary washed two times for 10 min.

12. Block serum +secondary antibody +1 X PBS 100 µl one time for 40 min.

13.1 X PBS-Secondary washed two times for 10 min.

14. A+B+PBS 100µl for 40 mins (1:40).DAB incubation for color development.

15. Diaminobenzolene +H2O2+tris-pinch +5 ml of Tris +50 ml of PBS.

16. Washed with distilled water for 5 min.

17. Changed to hematoxylene for 1min.

18. Washed with distilled water.

19. Kept in 50% alcohol for 5 min.

20. Changed in 70% alcohol for 5 min.

21. Changed to 90% alcohol two times for 5 min.

22. Changed to 100% alcohol two times for 5 min.

23. Changed to xylene two times for 5 min.

24. The slide is finally mounted with Dpx, covered with coverslip and observed under microscope.

4.5. Statistical analysis

Data was collected, arranged and reported as mean ± standard error of mean (SEM) of six groups, and was analyzed using the computer program (SPSS/version15.0). The statistical method was one way analyzes of variance ANOVA test, and if significant differences between means will be found, Duncan's multiple range test (Whose significant level was defined as $P < 0.05$) according to (Snedecor and Cochran, 1982) to estimate the effect of different treated groups.

CHAPTER 5

RESULTS

5.1. Evaluation of acute toxicity of MSG against Wistar albino rats for 30 days exposure and the ameliorative role of L-carnitine

5.1.1. Food and water consumption

Food and water consumption analysis of 30 days exposure of MSG to Wistar albino rats showed no significant differences ($p > 0.05$) in terms of their food and water consumption in comparison with control. The average daily food intake of control rats was 92.67 g while 93.29 g was noted in the highest dose of 3000mg/kg MSG (Table 1). These results corroborate with recent findings by (Maluly *et al*., 2013).

Table 1: Effect acute treatment for 30 days at different doses (30 mg/kg, 300 mg/kg, 3000 mg/kg MSG, 3000 mg/kg MSG+100 mg/kg carnitine and 100 mg/kg carnitine) on food and water consumption of rats.

Parameters	Experimental groups					
Food and water consumption	control	30mg/kg MSG	300mg/kg MSG	3000mg/kg MSG	3000+100 mg/kg MSG + carnitine	100mg/kg carnitine
Initial (g)	96.87 ± 2.63	96.87 ± 2.63	96.87 ± 2.63	96.87 ± 2.63	96.87 ± 2.63	96.87 ± 2.63
Final (g)	5.14 ± 0.61a	3.72 ± 0.25b	6.21 ± 1.58c	3.26 ± 2.64b	3.43 ± 0.32b	3.29 ± 0.26b
Food consumed (g)	92.67 ± 2.22	92.83 ± 2.39	92.50 ± 2.35	93.29 ± 2.45	93.46 ± 2.74	93.58 ± 2.51
Initial (ml)	350 ± 0.00	350 ± 0.00	350 ± 0.00	350 ± 0.00	350 ± 0.00	350 ± 0.00
Final (ml)	258.12 ± 2.89a	277.96 ± 3.00b	220 ± 3.62c	219.06 ± 5.12c	198.43 ± 5.53d	220.31 ± 2.59c
Water consumption (ml)	91.87 ± 2.95a	70.46 ± 2.76b	130 ± 3.62c	131.25 ± 5.11c	151.56 ± 5.56d	129.68 ± 2.59c

Data are expressed in Mean ± SEM, N=5 for control; N = 5 for treated group; P > 0.05 indicates a non-significant difference between the compared means. No symbol = non-significant, a, b, c, d, indicated significant values.

5.1.2. Rectal temperature and blood glucose level

Rectal temperature and blood glucose level did not show significant changes in acute (30 days) treatment. No stress behaviour was observed in treatment groups compared to control which may be the cause of non - significant (P>0.05) changes in body temperature. Since there is no significant changes in food consumption, blood glucose levels are in normal range when treatment groups were compared to control (Table 2 and 3).

Table 2: Effect of acute treatment for 30 days at different doses (30 mg/kg, 300 mg/kg, 3000 mg/kg MSG, 3000 mg/kg MSG+100 mg/kg carnitine and 100 mg/kg carnitine) on rectal temperature of rats.

Parameters	Experimental groups					
Rectal temperature	control	30 mg/kg MSG	300 mg/kg MSG	3000 mg/kg MSG	3000+10 0 mg/kg MSG+ Carnitine	100 mg/kg carnitine
Initial ^{0}F	97.72 ± 0.43	98.4 ± 0.00	97.02 ± 0.65	97.62 ± 0.35	93.6 ± 0.72	98.26 ± 0.22
Final ^{0}F	98.24 ± 0.44	98.14 ± 0.18	98.1 ± 0.30	97.78 ± 0.42	95.98 ± 1.27	98.56 ± 0.27

Data are expressed in Mean ± SEM, N=5 for control; N = 5 for treated group; P > 0.05 indicates a non-significant difference between the compared means. No symbol = non-significant.

Table 3: Effect of acute treatment for 30 days at different doses (30 mg/kg, 300 mg/kg, 3000 mg/kg MSG, 3000 mg/kg MSG+100mg/kg carnitine and 100 mg/kg carnitine) on blood glucose levels of rats.

Parameters	Experimental groups					
Blood glucose	control	30 mg/kg MSG	300mg /kg MSG	3000 mg/kg MSG	3000+100 mg /kg MSG+ carnitine	100 mg/kg carnitine

Initial (mg/dl)	118.6 ± 0.24	118.4 ± 0.24	118.6 ± 0.67	117.8 ± 1.77	118.4 ± 0.24	118.6 ± 0.24
Final (mg/dl)	128.2 ± 3.92	121.8 ± 2.59	119.8± 1.24	119.8 ± 1.06	116.4 ± 1.72	117.4 ± 1.20

Data are expressed in Mean ± SEM, N=5 for control; N = 5 for treated group; P > 0.05 indicates a non-significant difference between the compared means. No symbol = non-significant.

5.1.3. Body weight and organ weight

In the first phase of the study, evaluation of body weight and organ weight was done in both control and treated groups after 30 days of treatment**.** The steady weight gained by rats were the effect of MSG but no significant changes were observed in the organ weight of treatment groups with respect to control may possibly be due to shrinkage in subsequent toxicity by compounds which was investigated by (Hamaoka and Kusunok.,1986). In table 4, significant increase in body weight was observed in treatment groups in comparison to control ($P<0.05$) whereas non-significant differences ($P>0.05$) in the organ weight and their respective relative weight were observed in control and treatment groups.

Table 4: Effect of acute treatment for 30 days at different doses (30mg/kg, 300 mg/kg, 3000 mg/kg MSG, 3000 mg/kg MSG+100mg/kg carnitine and 100 mg/kg carnitine) on body weight, organ weight and the relative weight of rats.

Parameters	Experimental groups					
	Control	30 mg/kg MSG	300 mg/kg MSG	3000 mg/kg MSG	3000+100 mg/kg MSG+ carnitine	100 mg/kg carnitine
Initial body weight (g)	110.8 ± 2.99 a	111.8 ± 3.23 a	108.8 ± 1.96 a	166.8 ± 249 b	132.8 ± 3.83 c	120 ± 7.74 d
Final body weight (g)	117 ± 3.08 a	129.6 ± 3.42 b	123.8 ± 4.74 c	209.2 ± 5.21 d	154 ± 10.96 e	130.6 ± 6.03 b

Testis (g)	1.5 ± 0.10	0.46 ± 0.16	0.88 ± 0.13	0.4 ± 0.10	0.98 ± 0.07	1.34 ± 0.17
Testis (g/100g)	1.41 ± 0.12	0.51 ± 0.3	0.75 ± 0.12	0.31 ± 0.07	0.48 ± 0.10	0.86 ± 0.15
Liver(g)	6.82 ± 0.54	5.12 ± 1.21	4.18 ± 0.57	4.38 ± 0.93	4.4 ± 0.66	6.64 ± 0.89
Liver (g/100g)	6.28 ± 0.56	3.95 ± 1.05	3.62 ± 0.61	3.5 ± 0.70	3.38 ± 0.52	5.4 ± 0.32
Kidney (g)	0.88 ± 0.09	0.67 ± 0.34	0.46 ± 0.10	0.38 ± 0.05	0.46 ± 0.08	0.60 ± 0.19
Kidney (g/100g)	0.80 ± 0.08	0.45 ± 0.21	1.07 ± 0.67	0.30 ± 0.04	0.35 ± 0.06	0.46 ± 0.14
Seminal vesicles (g)	0.48 ± 0.11	0.46 ± 0.23	0.32 ± 0.12	0.22 ± 0.03	0.26 ± 0.09	0.46 ± 0.09
Seminal vesicles (g/100g)	0.43 ± 0.11	0.38 ± 0.16	0.27 ± 0.10	0.17 ± 0.03	0.2 ± 0.07	0.35 ± 0.07
Vas deferens (mg)	`0.22 ± 0.07	0.5 ± 0.24	0.24 ± 0.05	0.14 ± 0.02	0.16 ± 0.04	0.26 ± 0.09
Vas deferens (g/100g	0.17 ± 0.04	0.24 ± 0.15	0.20 ± 0.05	0.11 ± 0.02	0.12 ± 0.02	0.20 ± 0.07
Cauda (g)	0.68 ± 0.14	0.50 ± 0.11	0.46 ± 0.10	0.46 ± 0.10	0.56 ± 0.12	0.76 ± 0.12
Cauda (g/100g)	0.59 ± 0.09	0.37 ± 0.08	0.39 ± 0.10	0.35 ± 0.07	0.35 ± 0.07	0.57 ± 0.07
Caput (g)	0.62 ± 0.18	0.24 ± 0.05	0.50 ± 0.23	0.40 ± 0.27	0.52 ± 0.13	0.84 ± 0.10
Caput (g/100g)	0.56 ± 0.15	0.17 ± 0.03	0.41 ± 0.19	0.32 ± 0.05	0.41 ± 0.12	0.65 ± 0.09

Data are expressed in Mean ± SEM, N=5 for control; N = 5 for treated group; P > 0.05 indicates a non-significant difference between the compared means. No symbol = non-significant, a, b, c, d, indicated significant values.

5.1.4. Sperm parameters

The sperm concentration in treated groups (MSG) decreased compared to control and increased in carnitine treated groups. Table 5 and Fig 1 shows the % of motile sperms in treatment groups in comparison to control. Fig 2 shows sperm concentration and daily sperm production in different groups compared to control. MSG treatment resulted in a significant decrease in sperm count in testis (daily sperm production) and epididymis (caput and cauda). Furthermore, there was a reduction in daily sperm production per testis in MSG treated rats. Decreased spermatozoa production, cauda concentration count and caput concentration count caused after MSG treatment were improved by carnitine (100 mg/kg). Furthermore, there was a reduction in daily sperm production per testis, but slightly improved in carnitine treated groups.

Table 5: : Effect of acute treatment for 30 days at different doses (30mg/kg, 300 mg/kg, 3000 mg/kg MSG, 3000 mg/kg MSG+100mg/kg carnitine and 100 mg/kg carnitine) on sperm parameters.

Parameters	Experimental groups					
Sperm count	control	30 mg/kg MSG	300 mg/kg MSG	3000 mg/kg MSG	3000+100 mg/kg MSG+ carnitine	100 mg/kg carnitine
Testis						
Sperm number ($\times 10^6$)	287.64 ± 2.90a	240.24 ± 2.89b	160.46 ± 3.63c	140.35 ± 2.06d	282.6 ± 2.65a	293.45 ± 3.67e
Sperm number ($\times 10^6$/g)	462.71 ± 17.49a	323.96 ± 73.40b	263.85 ± 62.70c	192.74 ± 15.04d	360.34 ± 45.32e	484.14 ± 67.43f
DSP ($\times 10^6$/testis/day)	44.69 ± 0.66a	39.34 ± 0.47b	26.69 ± 0.67c	22.84 ± 0.39d	41.94 ± 2.91e	46.32 ± 0.43a
DSPr	75.28	52.13 ±	43.24	31.71	57.46	179.58

($\times 10^6$/testis/day/g)	± 18.18a	12.16b	± 10.27c	± 2.42d	± 21.85e	± 72.46f
Epididymis						
Caput						
Sperm number ($\times 10^6$)	62.96 ± 1.72a	60.59 ± 0.90a	49.34 ± 2.99b	33.12 ± 1.44c	41.72 ± 2.76d	63.97 ± 1.40a
Sperm number ($\times 10^6$/g)	254.80± 88.04a	139.06 ± 73.89 b	119.36 ± 22.92c	80.93 ± 9.91d	155.91 ± 51.65e	315.44 ± 82.11f
Sperm transit time (days)	1.35 ±0.04	1.35 ± 0.03	1.26 ± 0.07	1.44 ± 0.07	1.38 ± 0.10	1.54 ± 0.11
Cauda						
Sperm number ($\times 10^6$)	67.10 ± 0.51a	62.95 ± 0.50b	59.61 ± 0.89c	38.01 ± 0.93d	48.13 ± 1.00e	76.95 ± 1.39
Sperm number ($\times 10^6$/g)	151.2 ± 28.92a	115.36 ± 36.14 b	103.84 ± 25.33c	99.68 ± 18.70d	119.67 ± 22.41e	164.08 ± 40.61f
Sperm transit time (days)	1.65 ± 0.02a	1.40 ± 0.02b	1.51 ± 0.02c	1.47 ± 0.02d	1.62 ± 0.10e	1.83 ± 0.06f

Data are expressed in Mean ± SEM, N=5 for control; N = 5 for treated group; P > 0.05 indicates a non-significant difference between the compared means. No symbol = non-significant. a, b, c, d, e, f, indicated significant values

Regarding the sperm motility, we observed decreased type M sperm (motile with progressive movement) accompanied by increased in type IM (immotile) in 30 days MSG treated groups. The percentage of motile sperms in treated groups (MSG) decreased compared to control and increased in carnitine treated groups. Fig 1 shows the % of motile sperms.

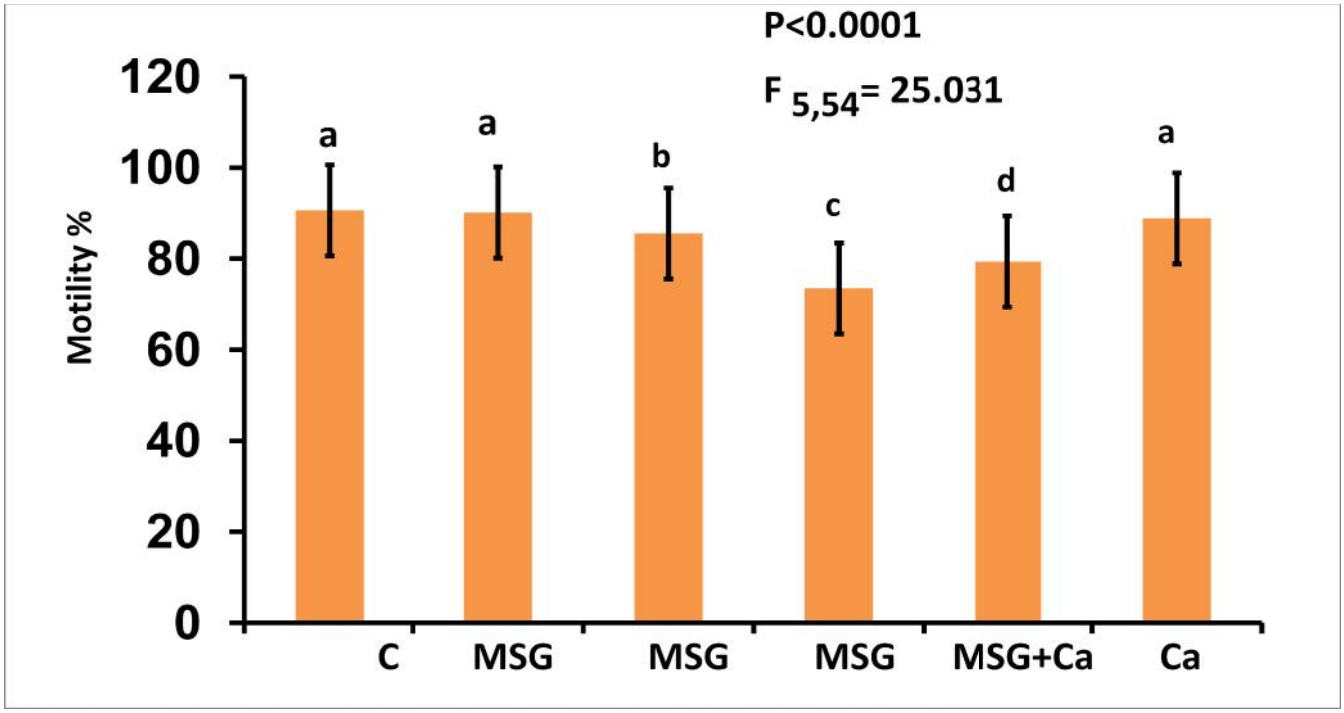


Fig 1: Effect of 30 days treatment of MSG and carnitine on % of motile sperms (Control, MSG 30 mg/kg, MSG 300 mg/kg, MSG 3000 mg/kg, MSG 3000 + C 100 mg/kg and C100 mg/kg). Data represents the means ± SEM of 5 samples in each group

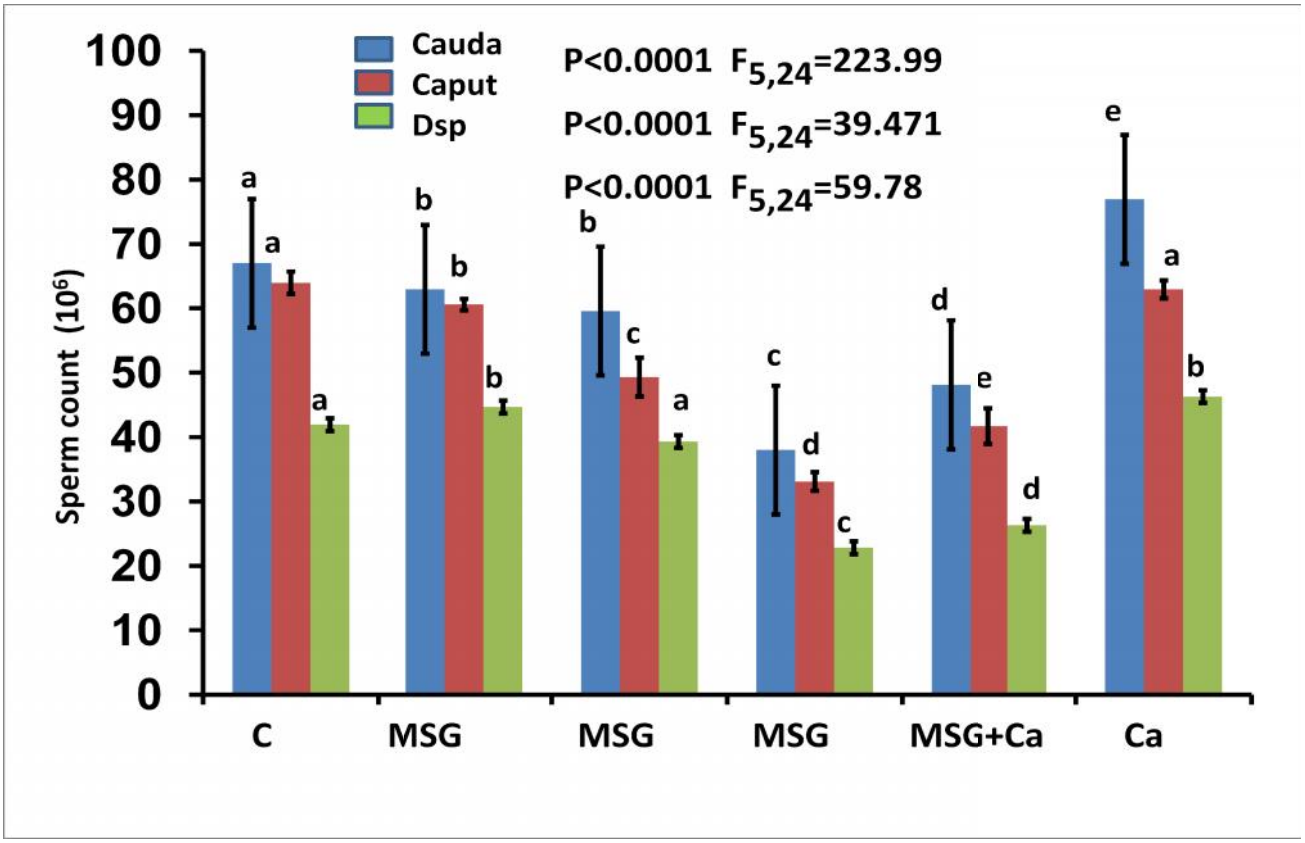


Fig 2: Effect of 30 days treatment of MSG and carnitine on sperm parameters and daily sperm production of rats, (Control, MSG 30 mg/kg, MSG 300 mg/kg MSG 3000 mg/kg, MSG 3000 + C 100 mg/kg and C 100mg/kg). Data represents the means ± SEM of 5 samples in each group. a, b, c, d indicates the significant differences between the groups

5.1.5 Sperm Morphology: Sperm morphology analysis revealed a significant increase in sperm abnormalities in rats treated for 30 days**.** However, the acute treatment of MSG did not have significant effect on the sperm morphology of treated rats compared to control. Sperm were classified as normal and abnormal sperms i.e., tail (amorphous, banana, detached) abnormality and head (coil or broken) abnormality. As shown in table 6, the normal sperms were found maximum in control groups and abnormality increased with MSG treated groups. Carnitine improved the sperm morphology even if treated with MSG.

Table 6: Effect of 30 days treatment at different doses (30mg/kg, 300 mg/kg, 3000 mg/kg MSG, 3000 mg/kg MSG+100mg/kg carnitine and 100 mg/kg carnitine) on sperm morphology of rats.

Table 6: Effect of 30 days treatment at different doses (30mg/kg, 300 mg/kg, 3000 mg/kg MSG, 3000 mg/kg MSG+100mg/kg carnitine and 100 mg/kg carnitine) on sperm morphology of rats.

5.2. Biochemical Parameters

Under normal conditions, the acute toxicity of glutamate is very low. No significant changes were observed in the biochemical parameters studied. These results are in agreement with the work of Maluly *et al* .,2013.

Table 7: Effect of a chronic treatment for 90 days at different doses (30mg/kg, 300 mg/kg, 3000 mg/kg MSG, 3000 mg/kg MSG+100mg/kg carnitine and 100 mg/kg carnitine) on biochemical parameters of rats.

Parameters	Experimental groups					
	Control	30 mg/kg MSG	300 mg/kg MSG	3000 mg/kg MSG	3000+100 mg/kg MSG+ carnitine	100 mg/kg carnitine
ALT (IU/L)	94.4 ± 0.87	101.66 ±1.33	98.76± 0.39	112.5± 0.76	106.33 ± 0.88	97.24 ± 0.53
AST (IU/L)	101.54 ± 0.99	104.43 ± 1.10	103.46 ± 1.00	110.3 ± 0.88	113.73 ± 0.93	99.25 ± 1.84
Urea (mg/dl)	30.28 ± 0.46	23.43 ± 0.97	26.5±1. 04	31.66 ± 0.66	28.5 ± 1.60	29.4 ± 0.30
Creatinine (mg/dl)	33.4 ± 0.87	36.33 ± 0.88	29.8 ± 1.33	38.41 ± 1.52	29.83 ± 1.36	15.33 ± 2.02
AP(IU/L)	111.16 ± 0.44	107 ± 1.52	124.2 ± 2.44	106.8 ± 3.68	106.33 ± 1.45	121.8 ± 0.41
Cholesterol (mg/dl)	60.06 ± 0.58	85.66 ± 1.85	55.08 ± 0.64	53.13 ± 0.63	78.83 ± 0.44	80.06 ± 0.63

Data are expressed in Mean ± SEM, N=5 for control; N = 5 for treated group; P > 0.05 indicates a non-significant difference between the compared means. No symbol = non-significant.

5.3.1 Effect on lipid peroxidation and antioxidant status

The MDA level which is a bio-indicator of oxidative stress had increased with MSG treated groups resulting in higher lipid peroxidation and decreased with carnitine treated groups. The antioxidants ie.SOD, GSH, GST levels decreased with MSG treated groups and increased with carnitine treated groups (Fig3-7) after 30 days of treatment.

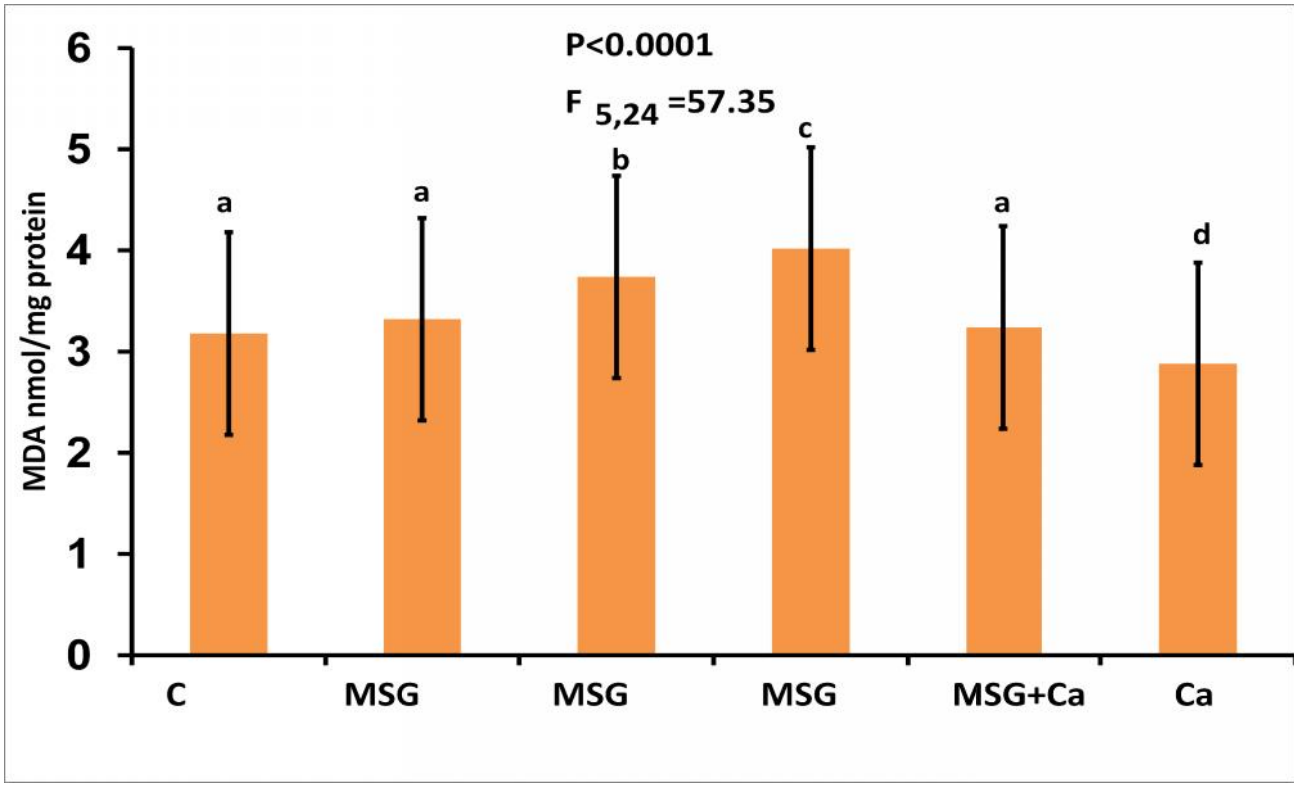


Fig 3: Estimation of 30 days treatment of MDA level on Control, MSG 30 mg/kg, MSG 300 mg/kg, MSG 3000 mg/kg, MSG 3000 + C 100 mg/kg and C 100 mg/kg. Data represents the means ± SEM of 5 samples in each group

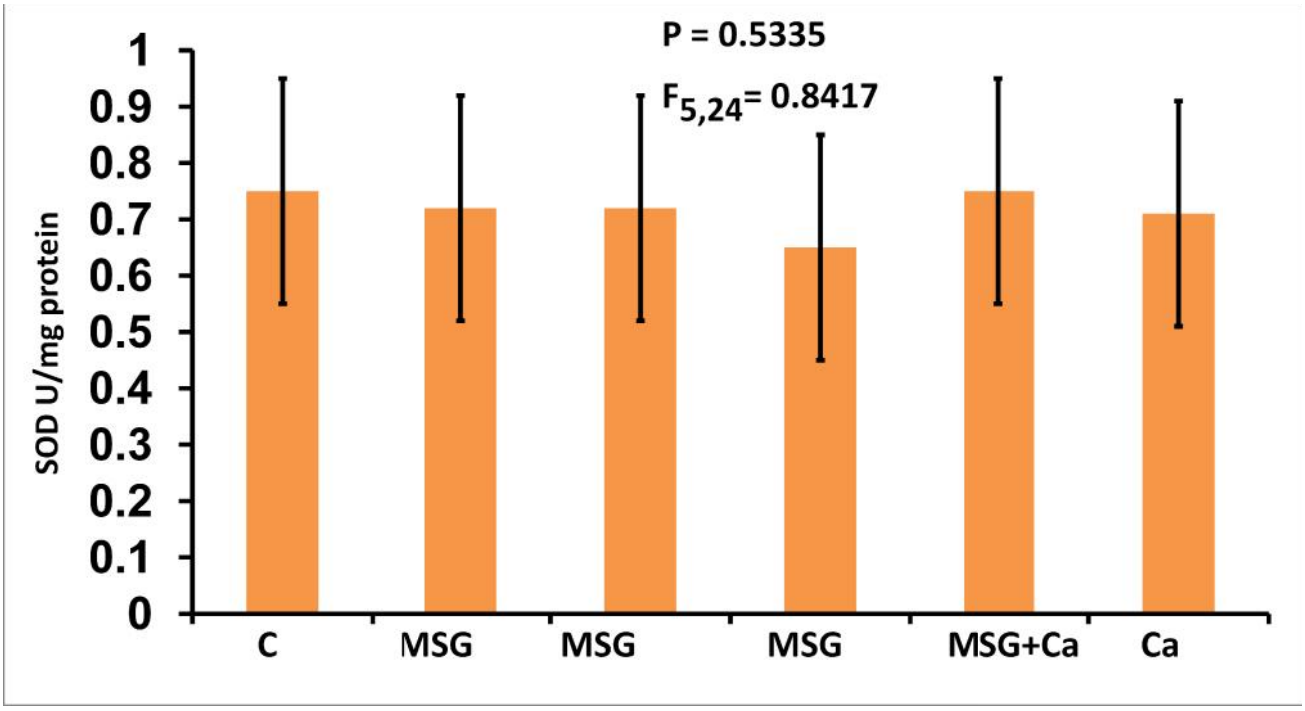


Fig 4: Estimation of 30 days treatment of SOD activity on Control, MSG 30 mg/kg, MSG 300 mg/kg, MSG 3000 mg/kg, MSG 3000 + C 100 mg/kg and C 100 mg/kg. Data represents the means ± SEM of 5 samples in each group

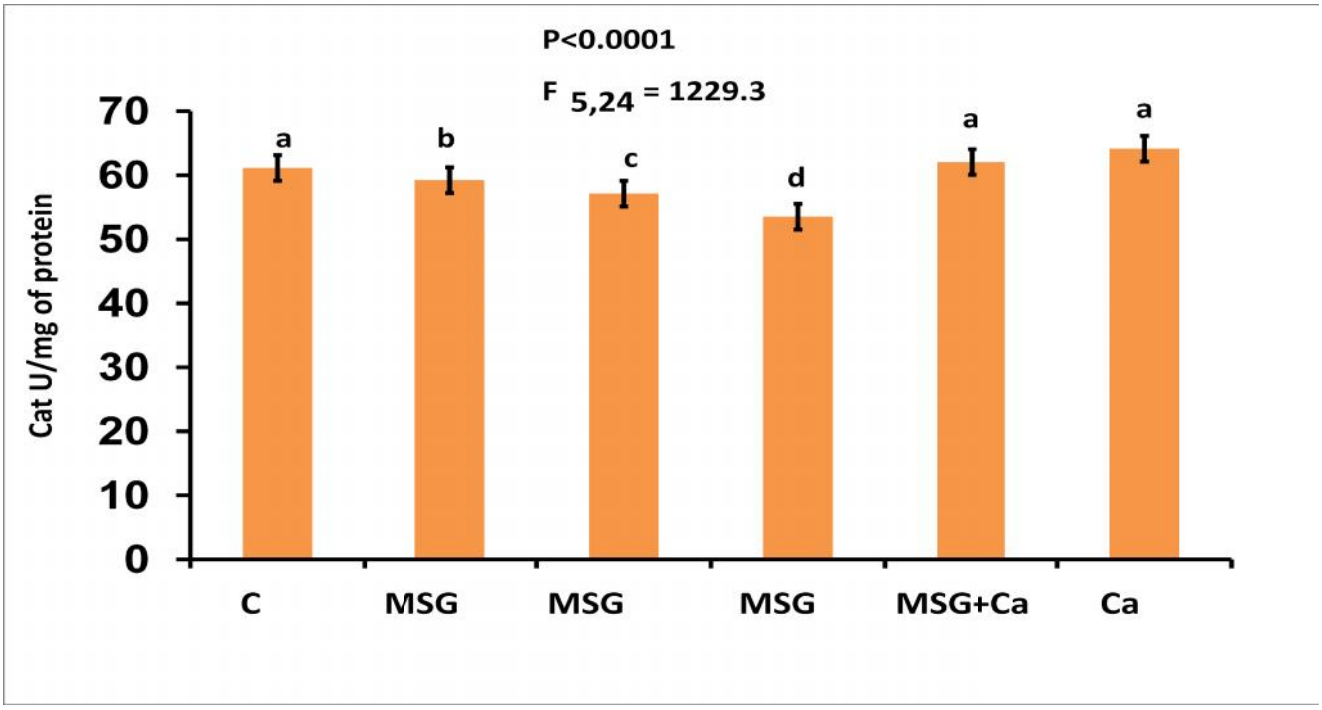


Fig 5: Estimation of Catalase activity on Control, MSG 30mg/kg, MSG 300 mg/kg, MSG 3000 mg/kg, MSG 3000 + C 100 mg/kg and C 100mg/kg. Data represents the means ± SEM of 5 samples in each group

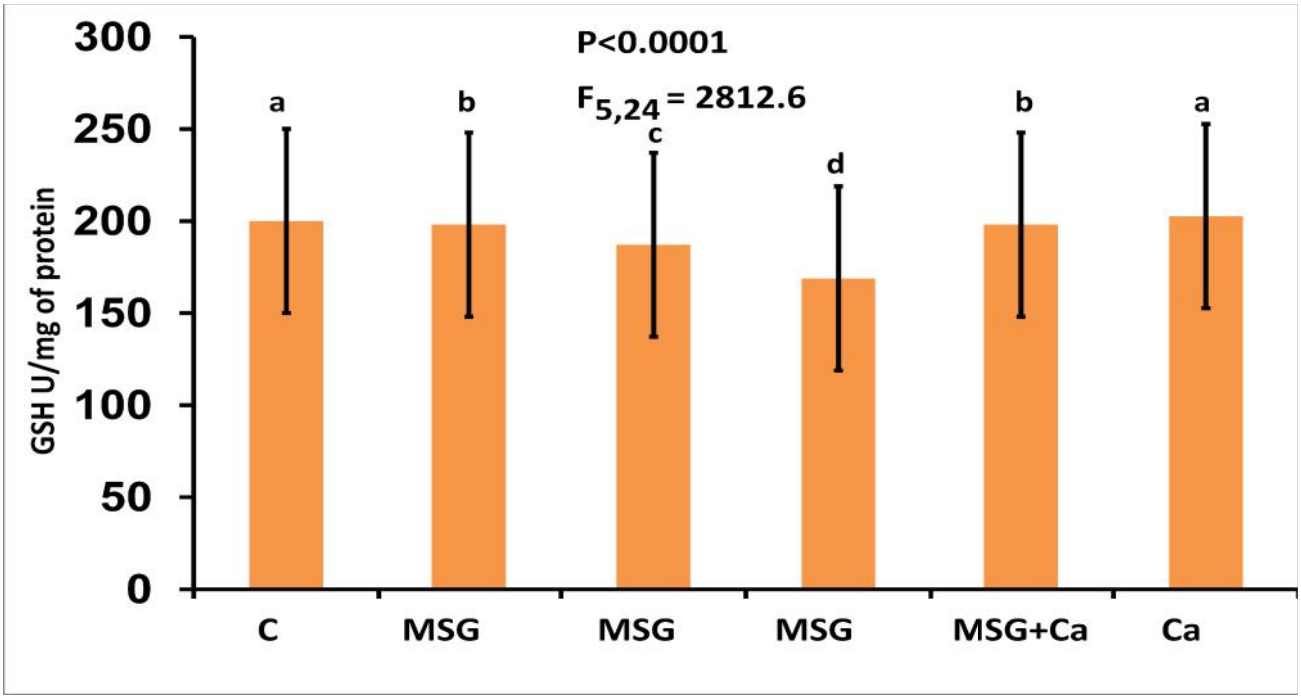


Fig 6: Estimation of GSH activity on Control, MSG 30mg/kg, MSG 300mg/kg, MSG 3000mg/kg, MSG 3000 + C 100 mg/kg and C 100mg/kg. Data represents the means ± SEM of 5 samples in each group

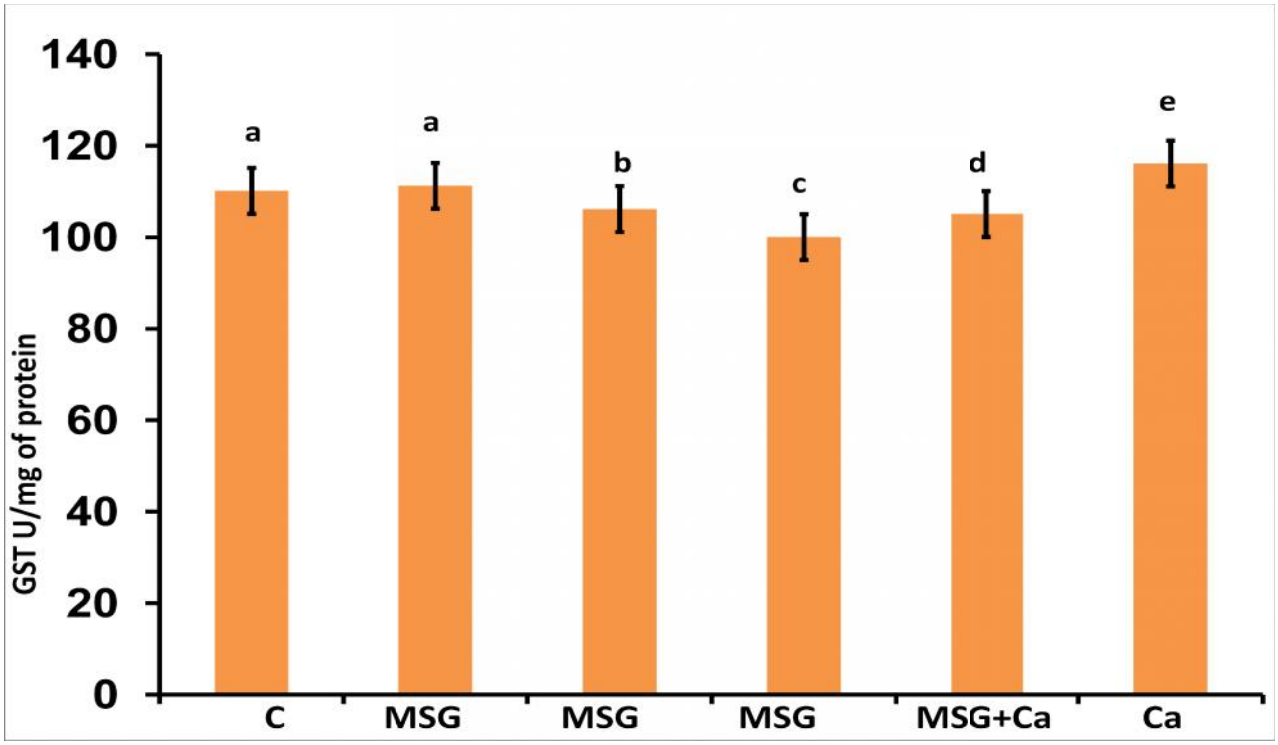


Fig 7: Estimation of 30 days treatment of GST activity on Control, MSG 30 mg/kg MSG 300 mg/kg, MSG 3000 mg/kg, MSG 3000 + C 100 mg/kg and C 100 mg/kg. Data represents the means ± SEM of 5 samples in each group. P<0.0001, $F_{5,12}$ = 416.14

Histological analysis of testis in 30 days treatment of MSG and the ameliorative role of carnitine.

5.4 Histopathology

At the end of 30 days of treatment, the spermatogenic cells in the seminiferous tubules appeared to have normal histological structures in control groups. On histopathological examination, control rat testes showed normal sperm morphology and spermatogenesis, containing abundant amounts of spermatids and sperm in the lumen. In contrast to control, the arrangement of the cells was distributed in the seminiferous tubules of MSG treated rats. Normal spermatogenesis occurs in the control and treated groups. Slight decrease in the number of spermatids was observed in the higher doses of MSG (3000mg/kg). Seminiferous tubules filled with spermatogenic cells with normal sperm formation. Fig8-11 shows the observations in the histological changes.

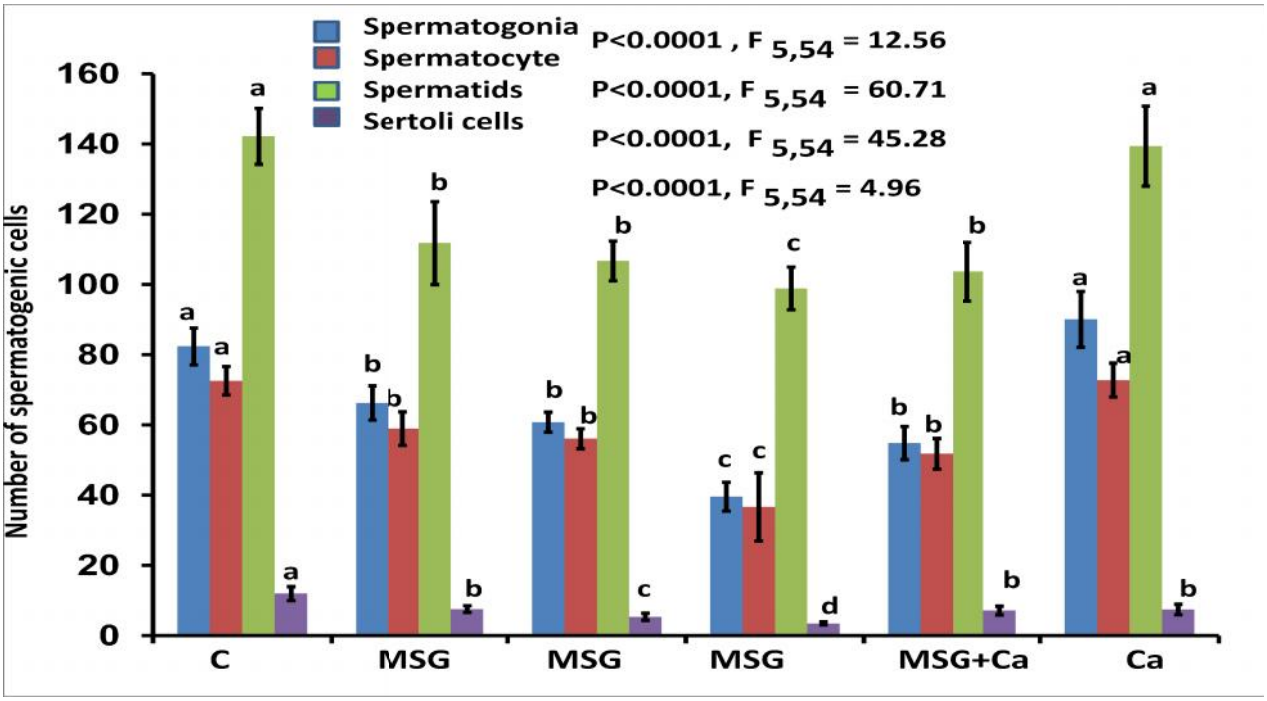


Fig 8: Effect of 30 days treatment of MSG and carnitine on spermatogenic cells of Control, MSG 30 mg/kg, MSG 300 mg/kg, MSG 3000 mg/kg, MSG 3000 + C 100 mg/kg and C 100 mg/kg .Data represents the means ± SEM of 5 samples in each group

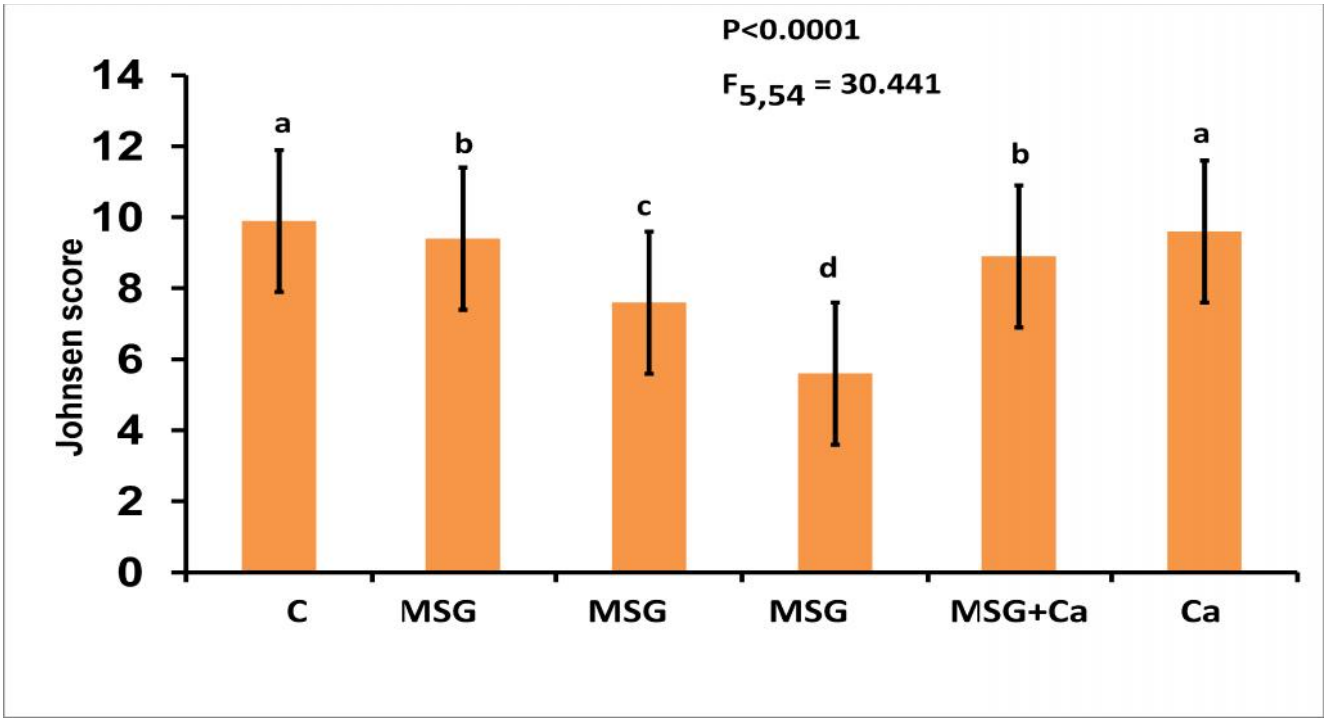


Fig 9: Effect of 30 days treatment of MSG and carnitine on Johnsen score of Control, MSG 30mg/kg, MSG 300mg/kg, MSG 3000mg/kg, MSG 3000 + C 100 mg/kg and C 100mg/kg. Data represents the means ± SEM of 5 samples in each group

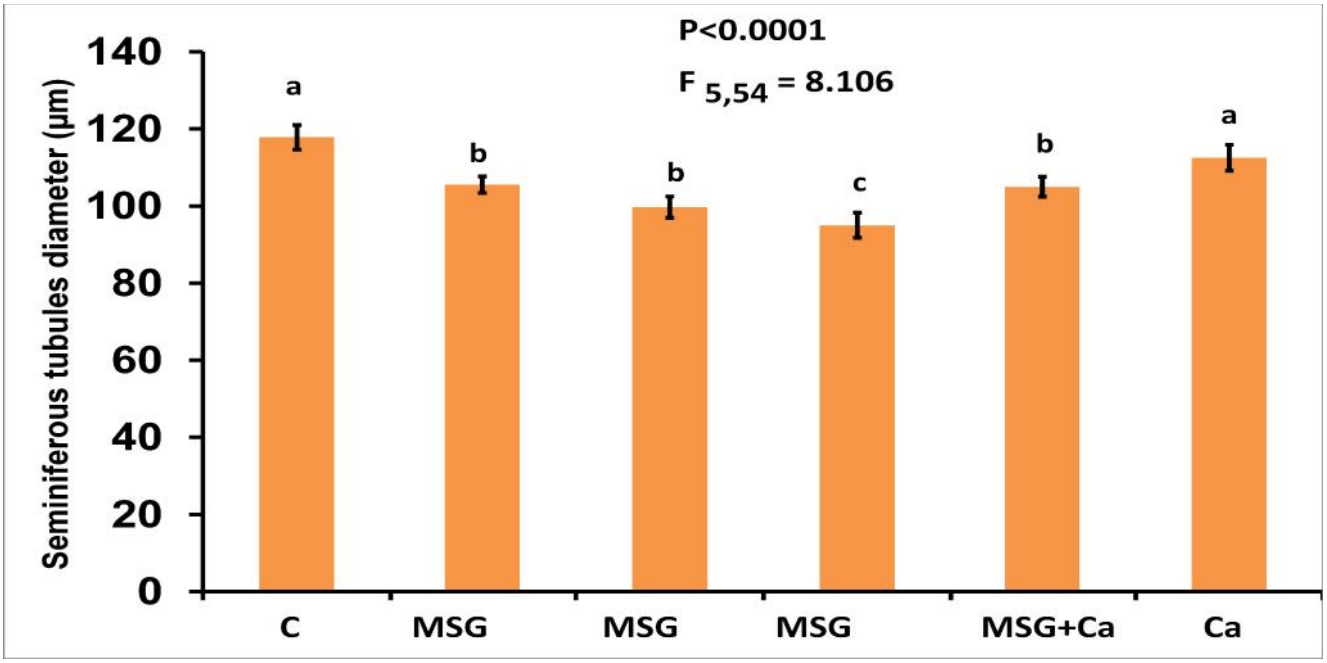


Fig 10: Effect of 30 days treatment of MSG and carnitine on seminiferous tubules diameter of Control, MSG 30mg/kg, MSG 300mg/kg, MSG 3000mg/kg, MSG 3000 + C 100 mg/kg and C 100mg/kg. Data represents the means ± SEM of 5 samples in each group

The figures below are the testicular sections of control and different groups showing histological changes within 30 days treatment.

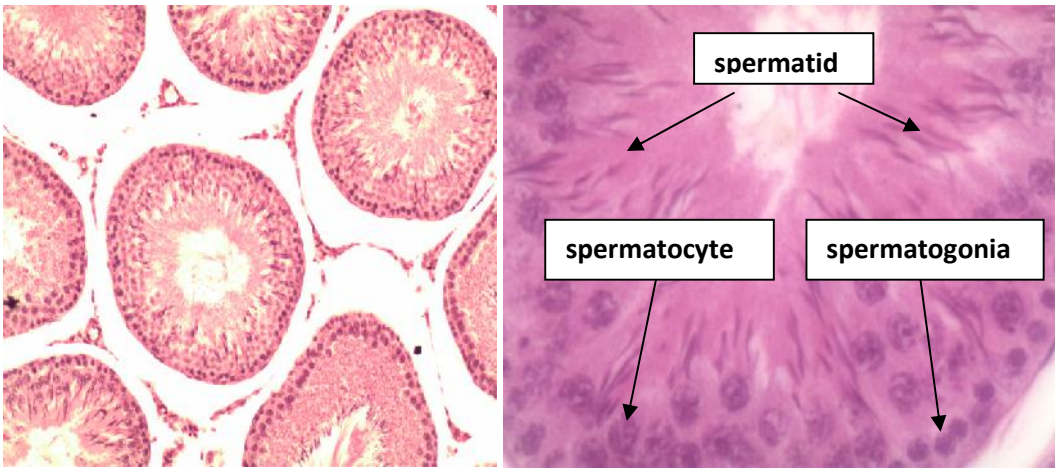


Fig 11 (A): Section of the testis of rat control group of 30 days (10X and 40X) showing seminiferous tubules filled with spermatogenic cells, development of spermatocyte with normal sperm formation.

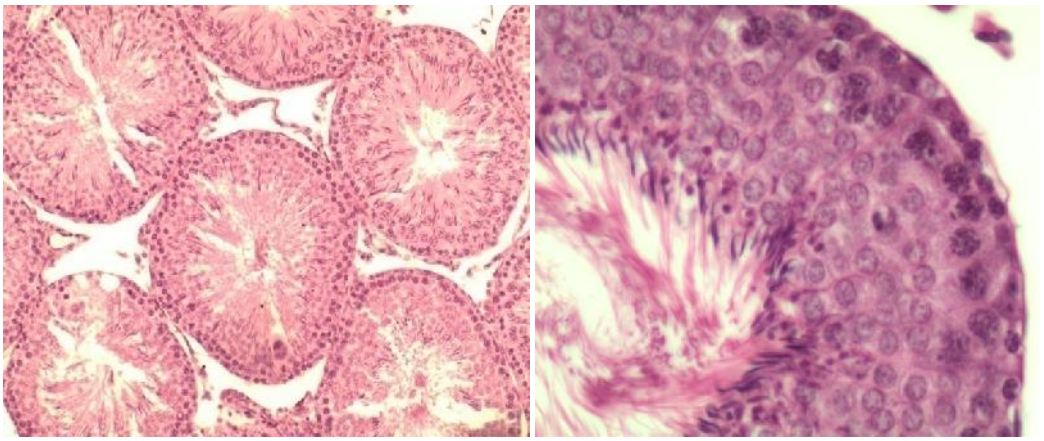


Fig 11 (B): Section of the testis of rat administered for 30 days (10 X and 40 X) at 30mg/kg with MSG, seminiferous tubules filled with spermatogenic cells.

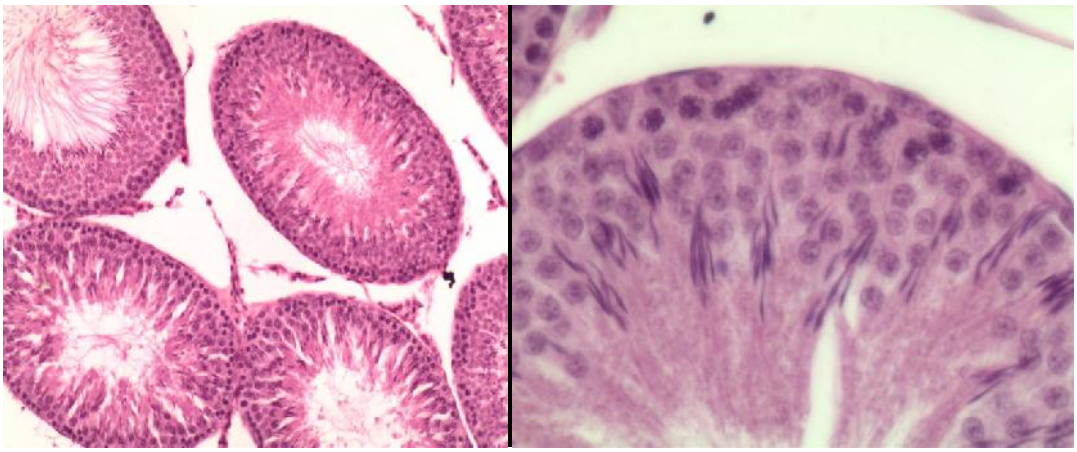


Fig 11 (C): Section of the testis of rat administered for 30 days at a dose of 300mg/kg MSG. Seminiferous tubules lined by spermatogenic cells, slight degeneration of Leydig cells.

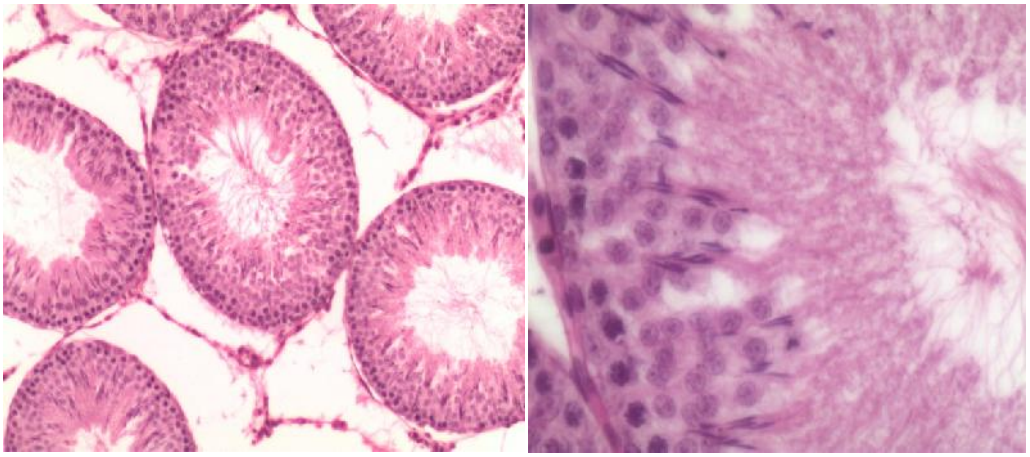


Fig 11 (D): Section of the testis of rat administered for 30 days (10X and 40 X) at a high dose of 3000 mg/kg MSG. Seminiferous tubules lined by spermatogenic cells, small vacoules and fewer sperm formation.

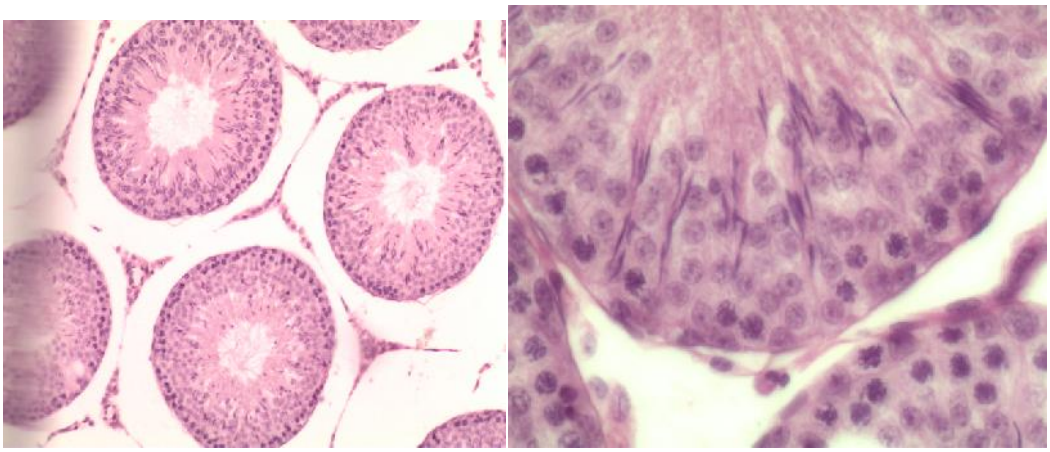


Fig 11 (E): Section of the testis of rat administered for 30 days (10 X and 40 X) at 3000 mg/kg with MSG+100mg/kg of carnitine. Seminiferous tubules filled with spermatogenic cells and development of spermatocyte with normal sperm formation, degeneration of vacoules and moderate sperm formation.

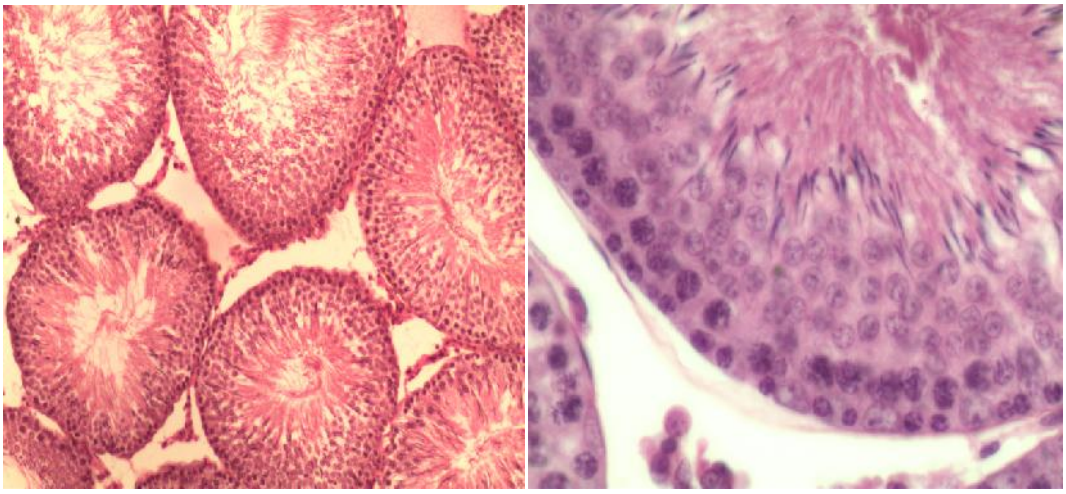


Fig 11 (F): Section of the testis of rat administered for 30 days (10 X and 40 X) at a dose of 100 mg/kg carnitine. Seminiferous tubules filled with spermatogenic cells and development of spermatocyte with normal sperm formation and degeneration of vacuoles.

60 DAYS

6.1. Evaluation of acute toxicity of MSG against Wistar albino rats for 60 days exposure and the ameliorative role of L-carnitine

6.1.1. Food and water consumption: Food and water consumption analysis Table 8 shows the results referring to food and water consumption. Significant changes were not observed in food consumption when the treatment groups were compared with control during 60 days of treatment. However, water consumption was found to increase in MSG and carnitine treated groups.

Table 8: Effect of treatment for 60 days at different doses (30 mg/kg, 300 mg/kg, 3000 mg/kg MSG, 3000 mg/kg MSG+100 mg/kg carnitine and 100 mg/kg carnitine) on food and water consumption of rats.

Parameters	Experimental groups					
Food and water consumption	control	30 mg/kg MSG	300 mg/kg MSG	3000 mg/kg MSG	3000+100 mg/kg MSG + carnitine	100 mg/kg carnitine
Initial (g)	142.09 ± 4.01	142.09 ± 4.01	142.09 ± 4.01	142.09 ± 4.01	142.09 ± 4.01	142.09 ± 4.01
Final (g)	6.86 ± 0.57	6.45 ± 0.51	6.45 ± 0.50	5.64 ± 0.46	5.54 ± 0.44	4.79 ± 0.48
Food consumed (g)	135.18 ± 3.89	135.63 ± 3.91	135.6 ± 3.94	136.41 ± 4.03	136.47 ± 4.01	136.34 ± 4.60
Initial (ml)	350 ± 0.00	350 ± 0.00	350 ± 0.00	350 ± 0.00	350 ± 0.00	350 ± 0.00
Final (ml)	226.20 ± 3.96	221.20 ±4.03	215 ± 4.24	212.25 ± 3.36	209.51 ± 4.18	206.14 ± 4.27
Water consumption (ml)	123.62 ± 3.98 a	129.43 ± 4.01 b	134.35 ± 4.18 c	137.74 ± 3.36 d	141.29 ± 4.16 e	143.85 ± 4.27 f

Data are expressed in Mean ± SEM, N=5 for control; N = 5 for treated group; P > 0.05 indicates a non-significant difference between the compared means. No symbol = non-significant, a, b, c, d, e, f, indicated significant values.

6.1.2. Rectal temperature and blood glucose level

Rectal temperature increased with treatment groups due to stress and blood glucose level was also higher in treatment groups. Due to behavioural stress observed the rectal temperature may have increased with treatment groups. After the glucose load, plasma insulin concentration reached the highest values in MSG-obese rats. The higher glucose levels may be due to increased in body weight. Our result is similar to the findings of (Marmo *et al.,* 1994). The long term treatment may also have increased the blood glucose levels in the MSG treated rats.

Table 9: Effect of 60 days treatment at different doses (30mg/kg, 300 mg/kg, 3000 mg/kg MSG, 3000 mg/kg MSG+100mg/kg carnitine and 100 mg/kg carnitine) on rectal temperature of rats.

Parameters	Experimental groups					
Rectal temperature	control	30 mg/kg MSG	300 mg/kg MSG	3000 mg/kg MSG	3000+100 mg/kg MSG+ carnitine	100 mg/kg carnitine
Initial (˚F)	98.56 ± 0.29	96.72 ± 0.76	96.68 ± 1.07	97.12 ± 0.94	96.82 ± 0.64	97.36 ± 0.34
Final (˚F)	97.62 ± 0.44	96.96 ± 0.72	97.24 ± 0.84	97.9 ± 0.58	98.34 ± 0.24	98.72 ± 0.20

Data are expressed in Mean ± SEM, N=5 for control; N = 5 for treated group; P > 0.05 indicates a non-significant difference between the compared means. No symbol = non-significant.

Table 10 : Effect of a chronic treatment for 60 days at different doses (30mg/kg, 300 mg/kg, 3000 mg/kg MSG, 3000 mg/kg MSG+100mg/kg carnitine and 100 mg/kg carnitine) on blood glucose levels of rats.

Parameters	Experimental groups					
Blood glucose	control	30 mg/kg MSG	300 mg/kg MSG	3000 mg/kg MSG	3000+100 mg/kg MSG+ carnitine	100 mg/kg carnitine

Initial (mg/dl)	118.6 ± 2.54	118.4 ± 0.24	118.2 ± 0.20	118.6 ± 0.24	119.4 ± 1.40	119.2 ± 0.48
Final (mg/dl)	121.6 ± 1.60	120.2 ± 0.96	121.2 ± 1.14	121.8 ± 1.59	120.2 ± 0.80	120.6 ± 1.43

Data are expressed in Mean ± SEM, N=5 for control; N = 5 for treated group; P > 0.05 indicates a non-significant difference between the compared means. No symbol = non-significant.

6.1.3. Body weight and organ weight: In the first phase of the study, evaluation of body weight and organ weight was done in both control and treated groups after 60 days of treatment**.** MSG increased the body weight and organ weight with respect to control. The increased in body weight may be due to long term treatment of MSG in rats. The steady weight gained by treated groups were the effect of MSG, but no significant changes were observed in the organ weight of treatment groups with respect to control Significant increase in body weight, organ weight and their respective relative weight were observed in treatment groups as compared to control which is shown in table 11.

Table 11: Effect of a chronic treatment for 60 days at different doses (30mg/kg,300 mg/kg, 3000 mg/kg MSG, 3000 mg/kg MSG+100mg/kg carnitine and 100 mg/kg carnitine) on body weight, organ weight and the relative weight of rats.

Parameters	Experimental groups					
	Control	30 mg/kg MSG	300 mg/kg MSG	3000 mg/kg MSG	3000+100 mg/kg MSG+ carnitine	100 mg/kg carnitine
Initial body weight (g)	120.8 ± 1.85a	141.4 ± 2.24b	140.4 ± 15.90b	128.2 ± 12.8a	95 ± 4.69f	143.6 ± 8.43b
Final body weight (g)	155 ± 2.88 a	192.6 ± 5.49b	172.2 ± 15.70c	149.8 ± 12.81d	131.6 ± 4.57e	181.8 ± 5.35f
Testis(g)	0.82 ± 0.03a	0.68 ± 0.09b	0.52 ± 0.08c	0.4 ± 0.13d	0.43 ± 0.15d	1.02 ± 0.14e

Testis (g/100g)	0.56 ± 0.06a	0.35 ± 0.05b	0.31 ± 0.05b	0.24 ± 0.06c	0.44 ± 0.01d	0.70 ± 0.12e
Liver(g)	6.88 ± 0.79	6.32 ± 0.23	6.38 ± 0.29	5.28 ± 0.52	5.6 ± 0.58	7.34 ± 0.29
Liver (g/100g)	4.28 ± 0.44	3.93 ± 0.64	3.53 ± 0.26	3.29 ± 0.20	4.04 ± 0.49	4.33 ± 0.55
Kidney(g)	0.68 ± 0.23a	0.64 ± 0.20a	0.52 ± 0.11b	0.36 ± 0.06c	0.56 ± 0.10b	0.98 ± 0.30d
Kidney (g/100g)	0.42 ± 0.08a	0.29 ± 0.05b	0.23 ± 0.04b	0.21 ± 0.06b	0.37 ± 0.12c	0.61 ± 0.19d
Seminal vesicles(g)	0.36 ± 0.06a	0.32 ± 0.03a	0.32 ± 0.08a	0.24 ± 0.06b	0.24 ± 0.07b	0.58 ± 0.18c
Seminal vesicles (g/100g)	0.27 ± 0.05a	0.19 ± 0.02b	0.16 ± 0.01b	0.13 ± 0.03c	0.20 ± 0.05d	0.31 ± 0.09e
Vas deferens(mg)	0.28 ± 0.09a	0.26 ± 0.04a	0.22 ± 0.05b	0.20 ± 0.04b	0.24 ± 0.05b	1.05 ± 0.98c
Vas deferens (mg/100g)	0.15 ± 0.05a	0.14 ± 0.05a	0.08 ± 0.02b	0.03 ± 0.00c	0.13 ± 0.03a	0.17 ± 0.04d
Cauda(mg)	0.58 ± 0.11a	0.46 ± 0.04b	0.4 ± 0.10c	0.36 ± 0.08d	0.66 ± 0.12e	0.66 ± 0.08e
Cauda (g/100g)	0.35 ± 0.04a	0.30 ± 0.06b	0.27 ± 0.03c	0.25 ± 0.05c	0.21 ± 0.02d	0.51 ± 0.11e
Caput(mg)	0.54 ± 0.10a	0.48 ± 0.12b	0.42 ± 0.05c	0.38 ± 0.13d	0.40 ± 0.11c	0.56 ± 0.09a
Caput (g/100g)	0.29 ± 0.06a	0.25 ± 0.03b	0.25 ± 0.03b	0.17 ± 0.05c	0.28 ± 0.04a	0.36 ± 0.09d

Data are expressed in Mean ± SEM, N=5 for control; N = 5 for treated group; $P < 0.05$ indicates significant difference between the compared means. No symbol = non-significant, a, b, c, d, e, f, indicated significant values.

6.1.4. Sperm Parameters: The sperm concentration decreased with MSG treated groups and increased with carnitine treated groups. Moreover, the daily sperm production decreased with treatment groups and increased with carnitine treated groups.The sperm concentration in treated groups (MSG) decreased compared to control and increased in carnitine treated groups. Table 12 and fig 13 shows sperm concentration and daily sperm production in different groups compared to control. MSG treatment resulted in a significant decrease in sperm count in testis and epididymis (caput and cauda). Furthermore, there was a reduction in daily sperm production per testis in MSG treated rats. Decreased spermatozoa production, cauda concentration count and caput concentration count caused after MSG treatment were improved by carnitine (100 mg/kg). Furthermore, there was a reduction in daily sperm production per testis, but slightly improved in carnitine treated rats

Table 12: Effect of a chronic treatment for 60 days at different doses (30mg/kg, 300 mg/kg, 3000 mg/kg MSG, 3000 mg/kg MSG+100mg/kg carnitine and 100 mg/kg carnitine) on sperm counts and epididymis of rats.

Parameters	Experimental groups					
Sperm count	control	30 mg/kg MSG	300 mg/kg MSG	3000 mg/kg MSG	3000+100 mg/kg MSG+ Carnitine	100 Mg/kg carnitine
Testis						
Sperm number ($\times 10^6$)	175.6 ± 1.21a	165.49 ± 2.17b	162.49 ± 2.81b	157.57 ± 2.31c	168.21 ± 1.17b	183.90 ± 3.78d
Sperm number ($\times 10^6$/g)	352.06 ± 2.9a	268.55 ± 3.71b	239.66 ± 2.18c	100.98 ± 4.1d	170.99 ± 3.9e	430.43 ± 3.05f
DSP ($\times 10^6$/testis/ day)	32.74 ± 0.78a	27.12 ± 0.35b	26.63 ± 0.46b	25.82 ± 0.37b	27.57 ± 0.19b	32.91 ± 0.62a

DSPr ($\times 10^6$/testis/ day/g)	44.01 ± 7.48a	39.7 ± 1.84b	39.27 ± 4.65b	15.02 ± 1.9c7	32.84 ± 4.7d	57.71 ± 0.49e
Epididymus						
Caput						
Sperm number ($\times 10^6$)	55.71 ± 4.44a	47.46 ± 4.39b	40.29 ± 2.75c	37.54 ± 1.84d	44.17 ± 2.59e	56.64 ± 1.92f
Sperm number ($\times 10^6$/g)	172.30 ± 81.85a	121.45 ± 30.19b	108.79 ± 18.18c	73.08 ± 13.22d	144.81 ± 24.33e	180.13 ± 66.22c
Sperm transit time(days)	1.66 ± 0.13	1.74 ± 0.17	1.43 ± 0.15	1.44 ± 0.06	1.59 ± 0.09	3.78 ± 2.53
Cauda						
Sperm number ($\times 10^6$)	58.73 ± 2.68a	51.20 ± 3.99b	41.78 ± 3.27c	40.81 ± 2.37c	45.53 ± 3.01d	61.59 ± 5.01a
Sperm number ($\times 10^6$/g)	146.15 ± 37.22a	122.96 ± 20.08b	93.62 ± 10.20c	81.72 ± 18.08d	108.83 ± 28.27e	150.85 ± 27.24f
Sperm transit time(days)	1.97 ± 0.06	1.64 ± 0.10	1.57 ± 0.07	1.56 ± 0.01	1.84 ± 0.15	5.37 ± 3.94

Data are expressed in Mean ± SEM, N=5 for control; N = 5 for treated group; $P < 0.05$ indicates significant difference between the compared means. No symbol = non-significant, a, b, c, e, f, indicated significant values.

Regarding the sperm motility, we observed decreased type M sperm (motile with progressive movement) accompanied by increased in type IM (immotile) in 60 days MSG treated rats. The percentage of motile sperms in treated groups (MSG) decreased compared to control and increased in carnitine treated groups. Fig 12 shows the % of motile sperms.

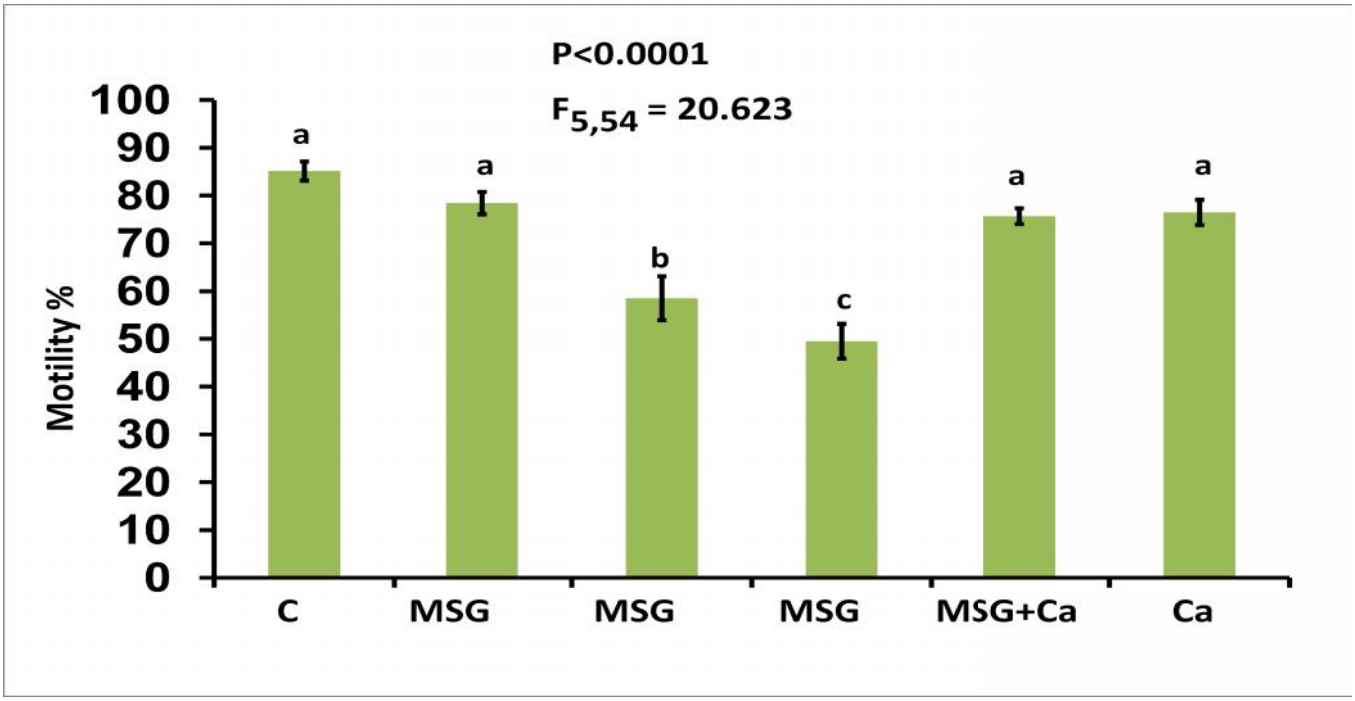


Fig 12: Effect of 60 days treatment of MSG and carnitine on % of motile sperms (Control, MSG 30 mg/kg, MSG 300 mg/kg, MSG 3000 mg/kg, MSG 3000 + C 100 mg/kg and C 100 mg/kg). Data represents the means ± SEM of 5 samples in each group

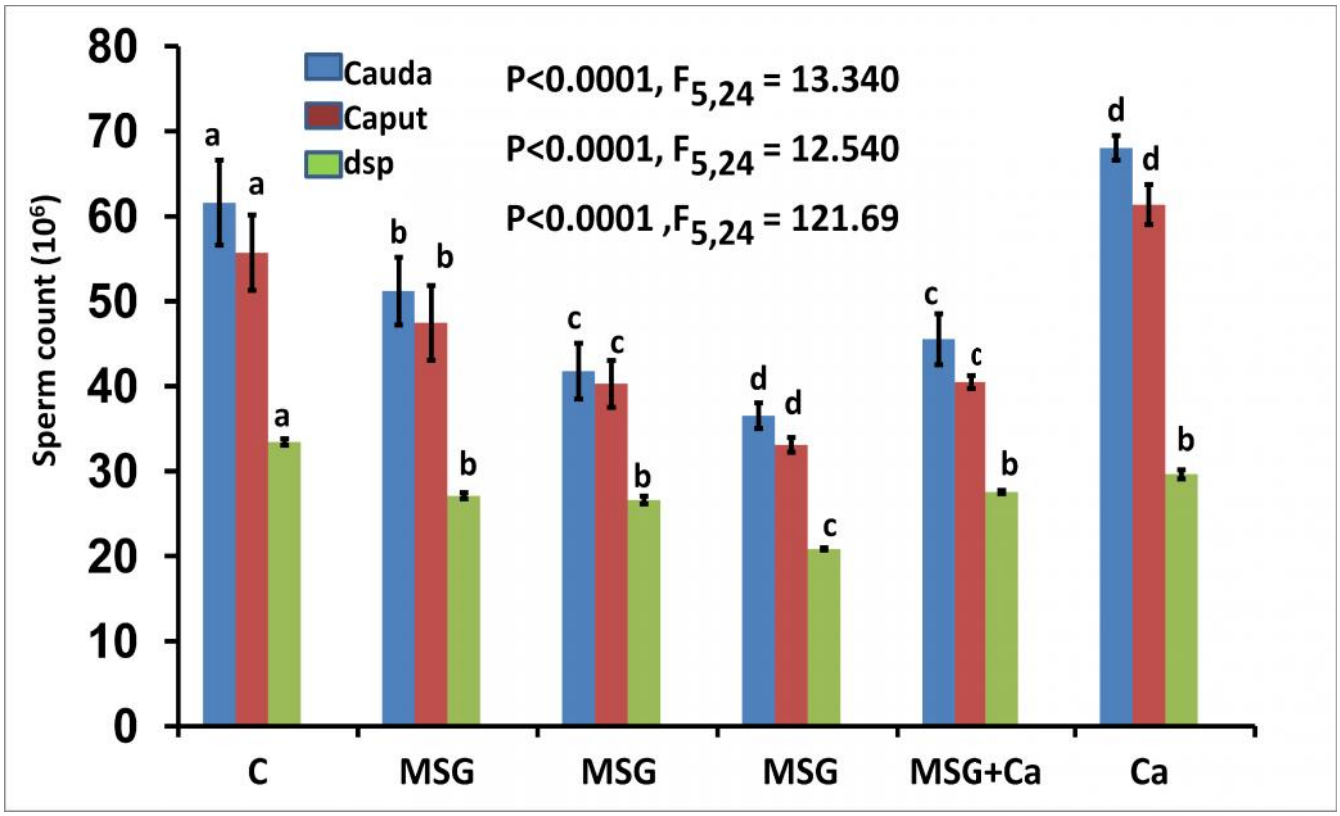


Fig 13: Effect of 60 days treatment of MSG and carnitine on sperm parameters and daily sperm production of rats, (Control, MSG 30mg/kg, MSG 300 mg/kg, MSG 3000 mg/kg, MSG 3000 + C 100 mg/kg and C 100mg/kg).Data represents the means ± SEM of 5 samples in each group

6.1.5. Sperm Morphology: Sperms were classified as normal and abnormal sperms., tail (amorphous, banana, detached) abnormality and head (coil or broken) abnormality. The normal sperms were found maximum in control groups and abnormality increased with MSG treated groups and decreased with carnitine treated groups. Table 13 shows the abnormalities observed in chronic treatment of MSG.

Table 13: Effect of 60 days treatment at different doses (30mg/kg, 300 mg/kg, 3000 mg/kg MSG, 3000 mg/kg MSG+100mg/kg carnitine and 100 mg/kg carnitine) on sperm morphology of rats.

Table 13: Effect of 60 days treatment at different doses (30mg/kg, 300 mg/kg, 3000 mg/kg MSG, 3000 mg/kg MSG+100mg/kg carnitine and 100 mg/kg carnitine) on sperm morphology of rats.

6.2.1. Biochemical Parameters

Under normal conditions, the toxicity of glutamate is very low in 60 days treatment of MSG. The oral median lethal doses for rats are 1.5 g/kg (Walker and Lupien, 2000). So, no significant changes were observed in ALT which signifies that there is no observable damage in the liver and cholesterol levels in treated groups. The increased levels in AST may be caused by brain, heart and lung damage as previously reported by (Bain, 2003). AP, Creatinine and Urea also show significant changes in the treatment groups and decreased in carnitine treated groups. These results are in agreement with the work of (Egbuonu 2009).

Table 14 : Effect of a chronic treatment for 60 days at different doses (30mg/kg, 300 mg/kg, 3000 mg/kg MSG, 3000 mg/kg MSG+100mg/kg carnitine and 100 mg/kg carnitine) on biochemical parameters of rats.

Parameters	Experimental groups					
	Control	30 mg/kg MSG	300 mg/kg MSG	3000 mg/kg MSG	3000+100 mg/kg MSG+ carnitine	100 mg/kg carnitine
ALT (IU/L)	95.96 ± 0.31a	104.36 ± 1.72b	97.95 ± 0.96a	113.63 ± 2.69c	106.33 ± 0.88b	96.23 ± 0.72a
AST (IU/L)	112.53 ± 1.86a	119.83 ± 1.01b	125.66 ± 1.66c	135.4 ± 1.40d	110.6 ± 1.45a	114.46 ± 0.77a
Cholesterol (mg/dl)	65.16 ± 0.52a	70.33 ± 0.44b	64.67 ± 0.88a	52.33 ± 1.45c	68.96 ± 0.57a	72.91 ± 0.63b
AP (IU/L)	127.86 ± 0.13a	130.73 ± 0.89a	137.5 ± 1.04b	130.06 ± 0.63a	134.73 ± 1.50a	132.66 ± 1.20a

Creatinine (mg/dl)	5.99 ± 0.98a	6.33 ± 0.35b	6.74 ± 1.13b	13.42 ± 0.62c	5.36 ± 0.70a	6.19 ± 0.37b
Urea(mg/dl)	93.8 ± 0.75a	97.56 ± 0.74b	102.73 ± 0.37c	123.66 ± 0.33d	107.83 ± 0.92c	93.43 ± 0.33a

Data are expressed in Mean ± SEM, N=5 for control; N = 5 for treated group; $P < 0.05$ indicates a non-significant difference between the compared means. No symbol = non-significant, a, b, c, d, indicated significant values.

6.3.1. Effect on lipid peroxidation and antioxidant status

The MDA level which is a bio-indicator of oxidative stress was significantly elevated with MSG treated groups resulting in higher lipid peroxidation respect to control groups, and decreased with carnitine treated groups. The antioxidants ie., SOD, CAT, GSH, GST are considered to be an important indicator of the balance status between the first and second step of the antioxidant pathways. The testis and epididymis contains high activities of enzymes whereas SOD catalyses the conversion of superoxide radicals to hydrogen peroxide, CAT converts hydrogen peroxide to water. GSH reduce soluble hydrogen peroxide and alkyl peroxidises (Demir,2011).Therefore, their levels decreased with MSG treated groups as compared to control groups and increased with carnitine treated groups. Fig (14-18) shows the effect of MSG on oxidative stress and antioxidant status.

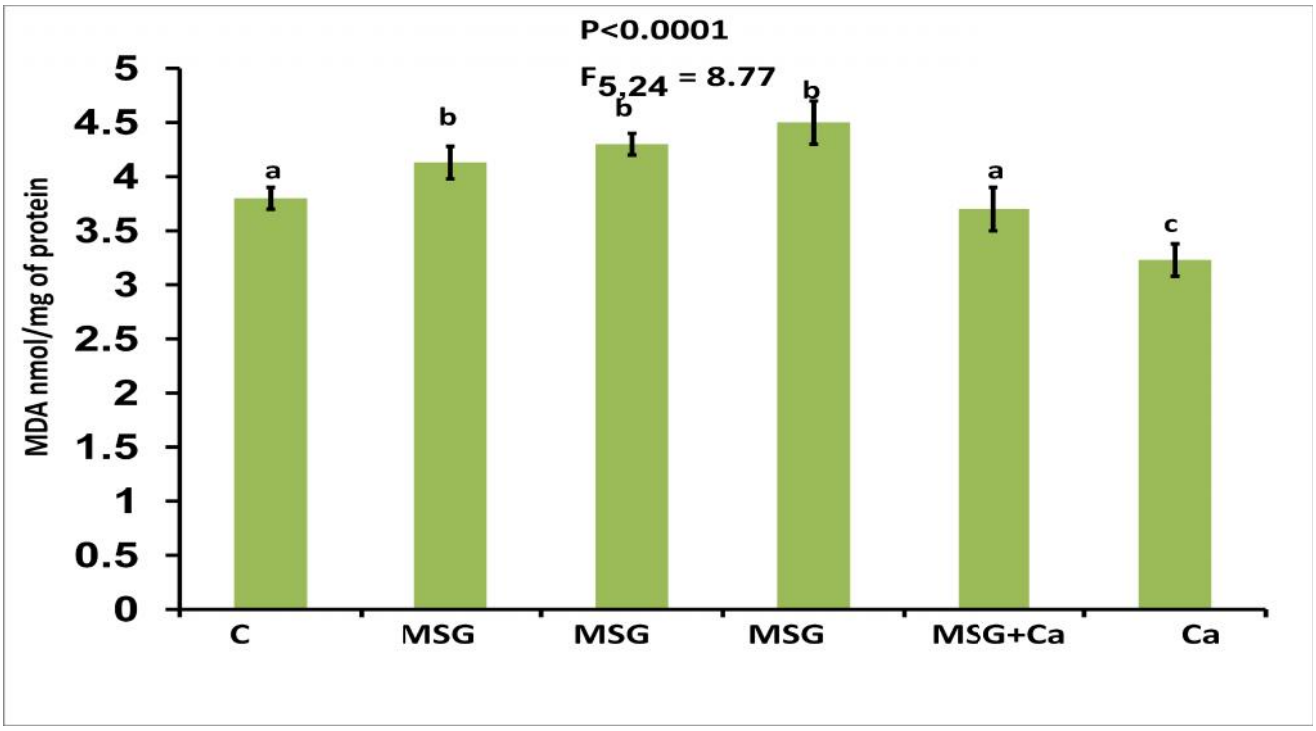


Fig 14: Estimation of 60 days treatment of MDA level on Control, MSG 30mg/kg MSG 300mg/kg MSG 3000mg/kg MSG 3000 + C 100 mg/kg and C 100mg/kg. Data represents the means ± SEM of 5 samples in each group

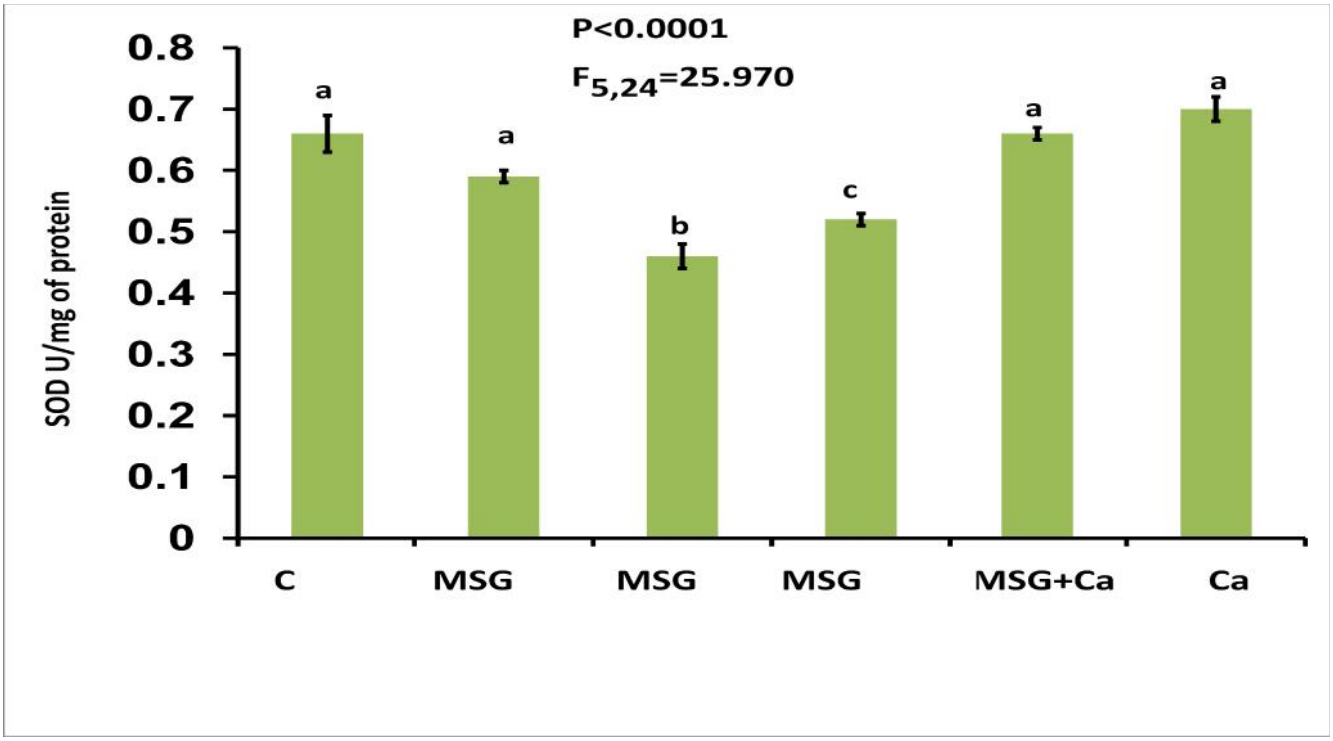


Fig 15: Estimation of 60 days treatment of SOD level on Control, MSG 30 mg/kg, MSG 300 mg/kg, MSG 3000 mg/kg, MSG 3000 + C 100 mg/kg and C 100 mg/kg. Data represents the means ± SEM of 5 samples in each group

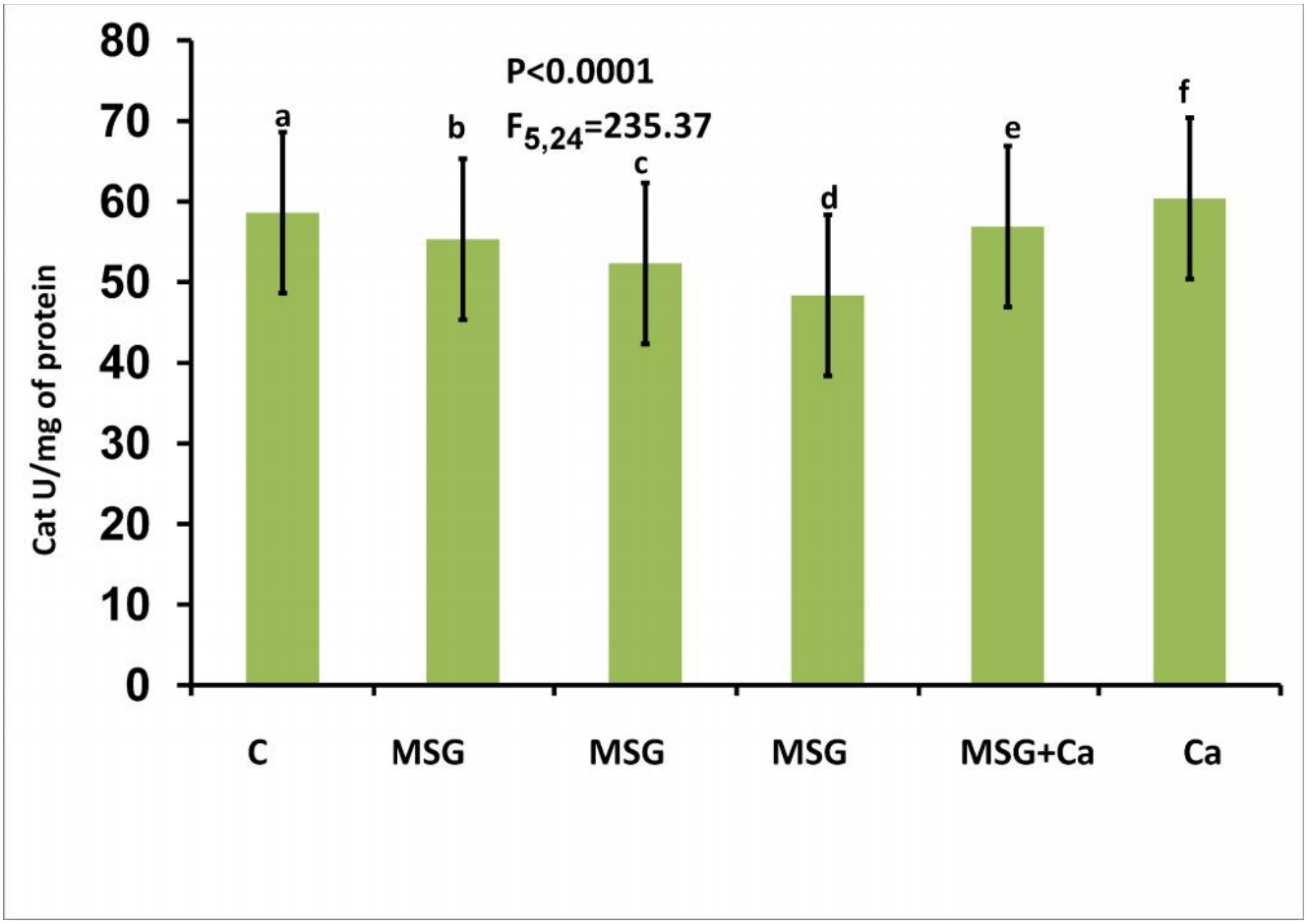


Fig 16: Estimation of 60 days treatment of Catalase activity on Control, MSG 30 mg/kg, MSG 300 mg/kg, MSG 3000 mg/kg, MSG 3000 + C 100 mg/kg and C 100mg/kg. Data represents the means ± SEM of 5 samples in each group

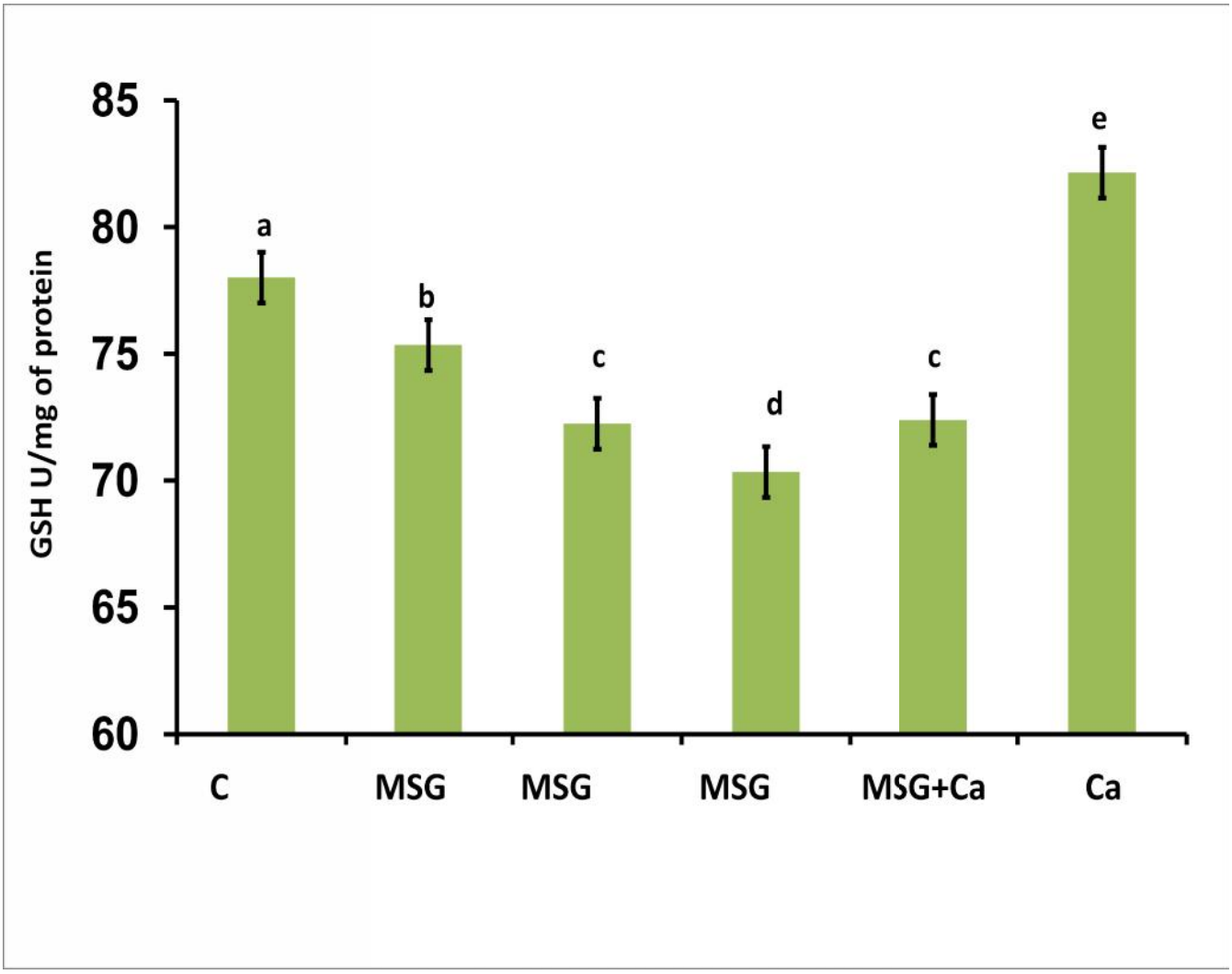


Fig 17: Estimation of 60 days treatment of GSH activity on Control, MSG 30 mg/kg, MSG 300mg/kg, MSG 3000mg/kg, MSG 3000 + C 100 mg/kg and C 100 mg/kg. Data represents the means ± SEM of 5 samples in each group

$P<0.0001$, $F_{5,24} = 417.68$

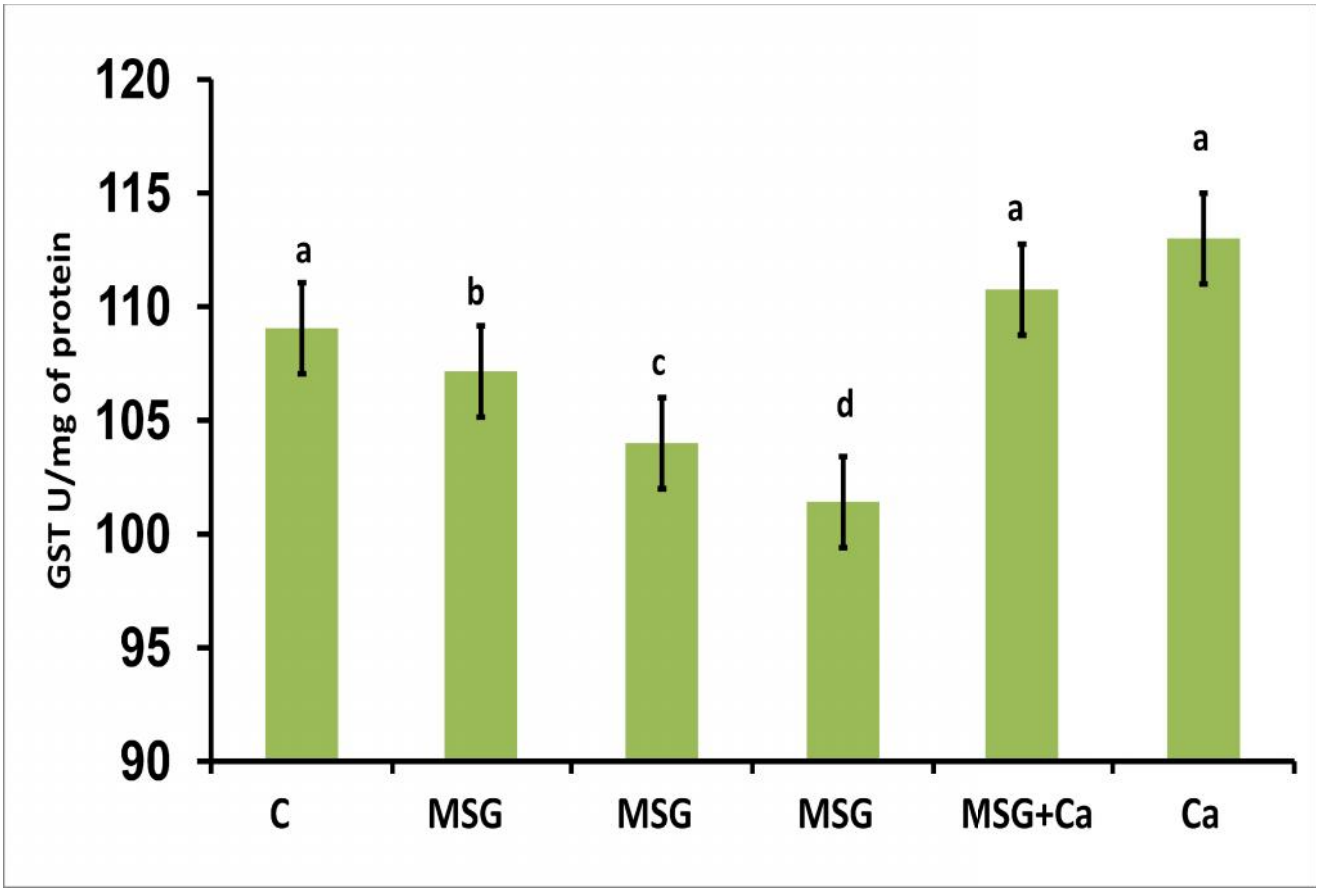


Fig 18: Estimation of 60 days treatment of GST activity on Control, MSG 30 mg/kg, MSG 300 mg/kg, MSG 3000 mg/kg, MSG 3000 + C 100 mg/kg and C100 mg/kg. Data represents the means ± SEM of 5 samples in each group

$P<0.0001$, $F_{5, 24} = 51.372$

6.4. Histological analysis of testis in 60 days treatment of MSG and attenuation of carnitine

On histopathological examination, control rat testes showed normal morphology and spermatogenis, containing abundant amounts of spermatids and sperm in the lumen. In contrast to control, the arrangement of the cells was distributed in the seminiferous tubules of MSG treated rats. Vacoulization was observed in the higher doses of MSG treated rats. Disruptions of the Leydig cells were observed in the treated groups. No significant changes were observed in Johnsen score and seminiferous tubules.

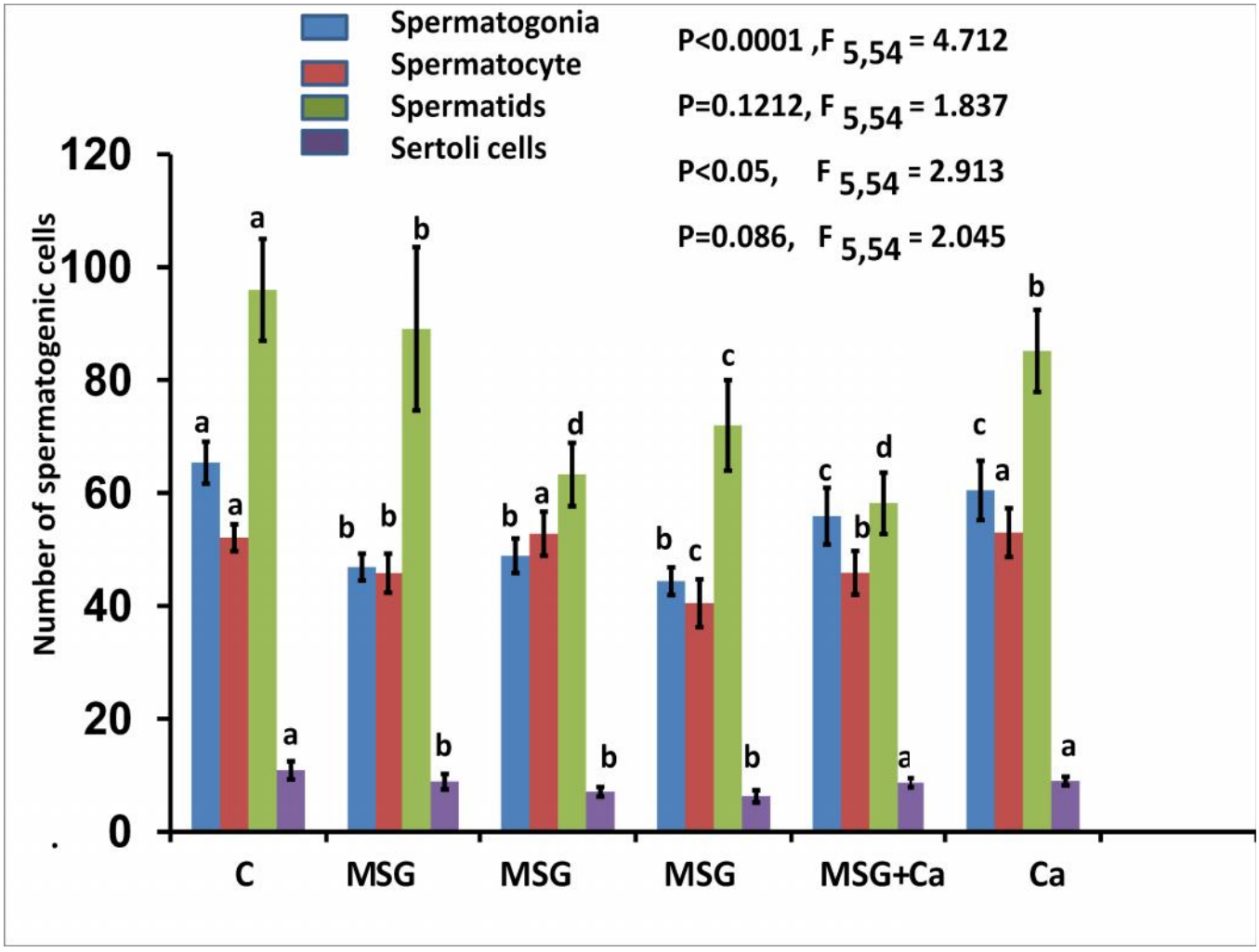


Fig 19: Effect of 60 days treatment of MSG and carnitine on spermatogenic cells of Control, MSG 30mg/kg, MSG 300mg/kg, MSG 3000mg/kg, MSG 3000 + C 100 mg/kg and C 100mg/kg. Data represents the means ± SEM of 5 samples in each group.

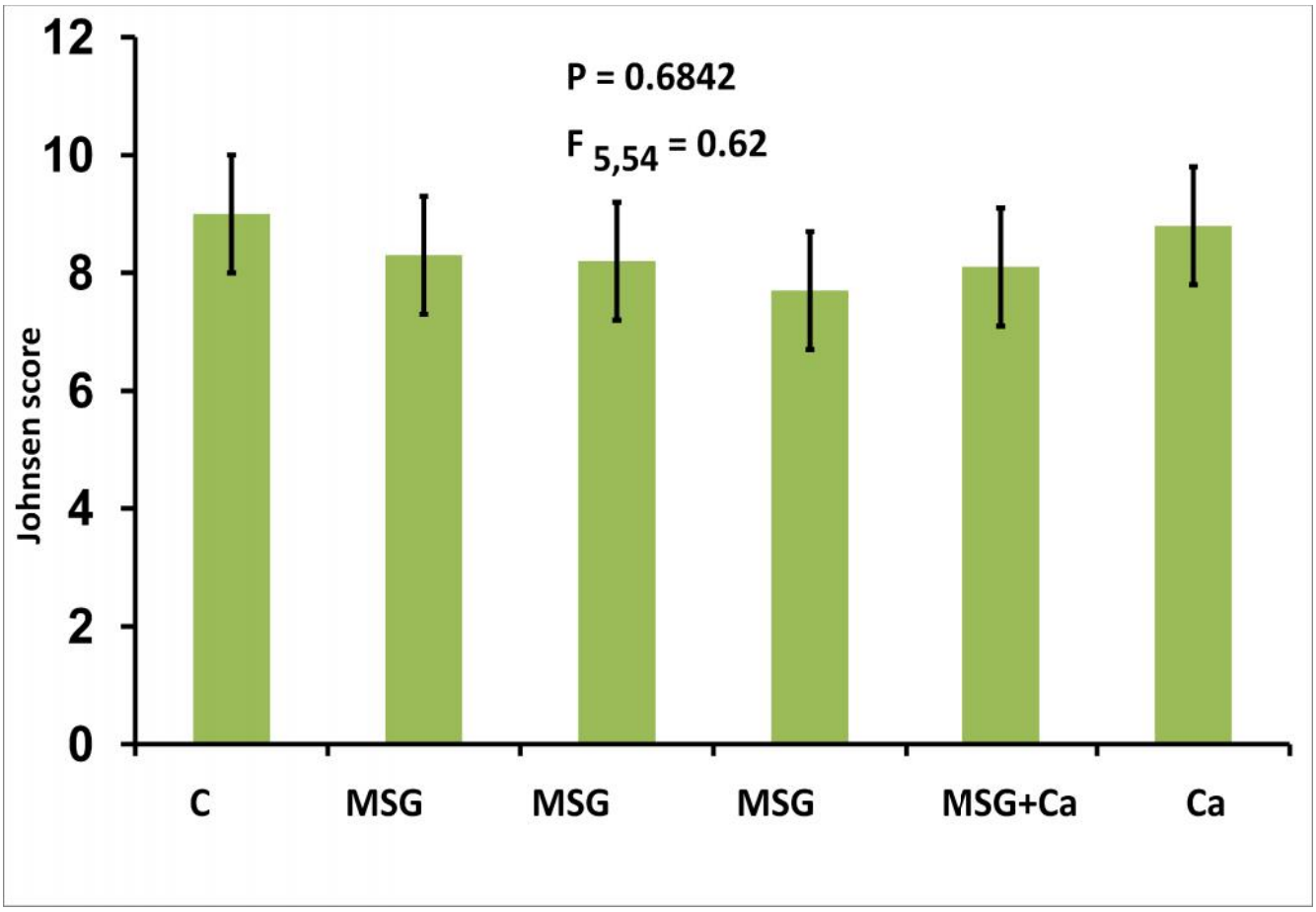


Fig 20: Effect of MSG and carnitine on Johnsen score on Control, MSG 30mg/kg, MSG 300mg/kg, MSG 3000mg/kg, MSG 3000 + C 100 mg/kg and C100 mg/kg. Data represents the means ± SEM of 5 samples in each group.

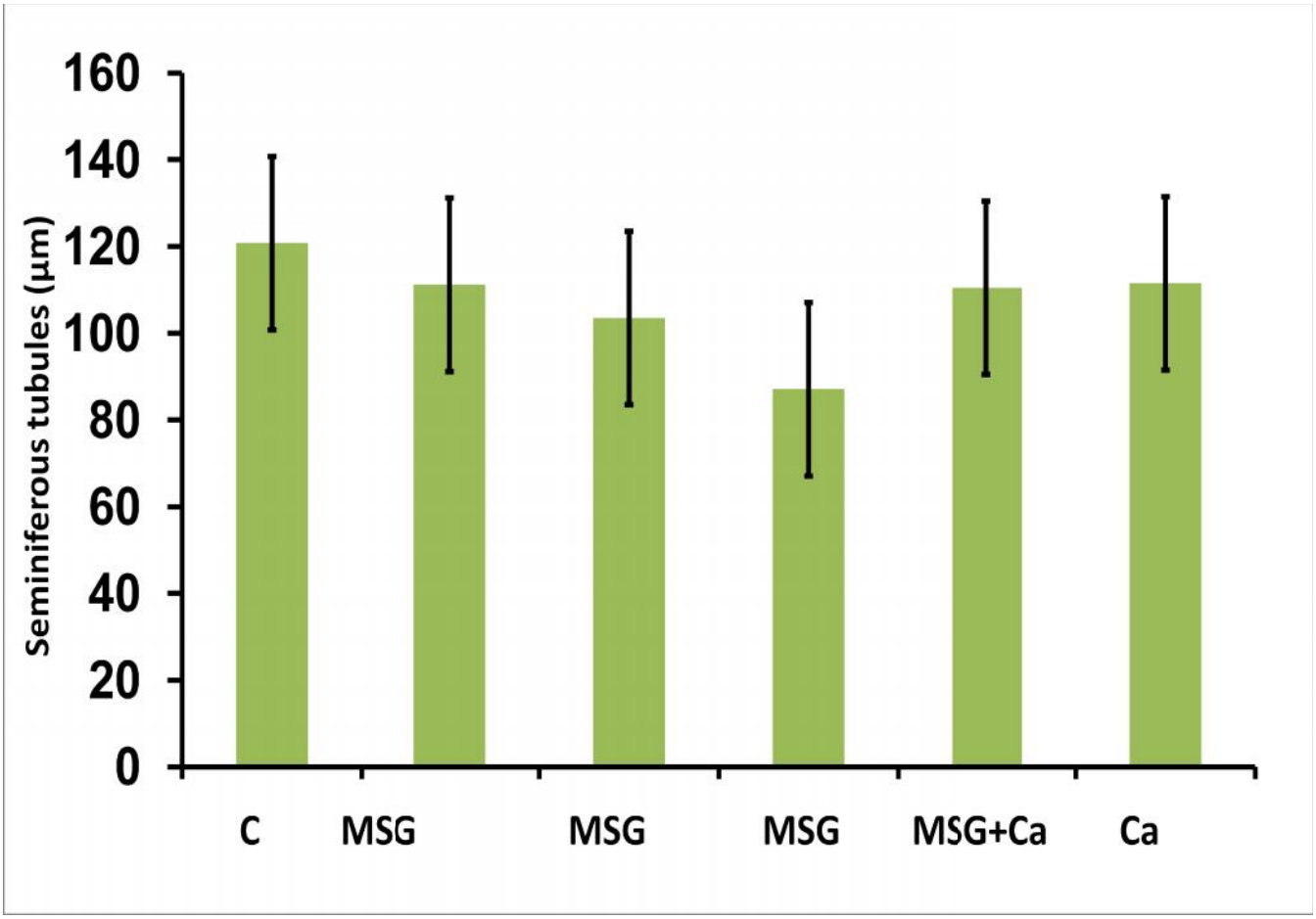


Fig 21: Effect of MSG and carnitine on seminiferous tubules diameter (STD) of Control, MSG 30mg/kg, MSG 300mg/kg, MSG 3000mg/kg, MSG 3000 + C 100 mg/kg and C 100mg/kg. Data represents the means ± SEM of 5 samples in each group. P = 0.0155, $F_{5,54}$ = 3.106

The figures below are the testicular sections of control and different groups showing histological changes within 60 days treatment.

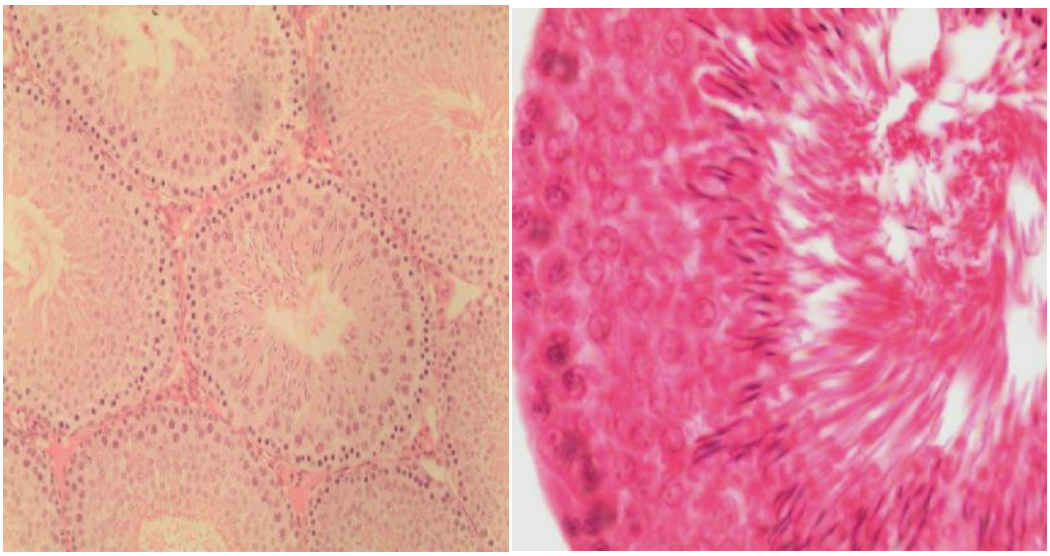


Fig 22 (A): Section of the testis of rat control group in 60 days (10 X and 40 X) showing seminiferous tubules filled with spermatogenic cells, development of spermatocyte with normal sperm formation.

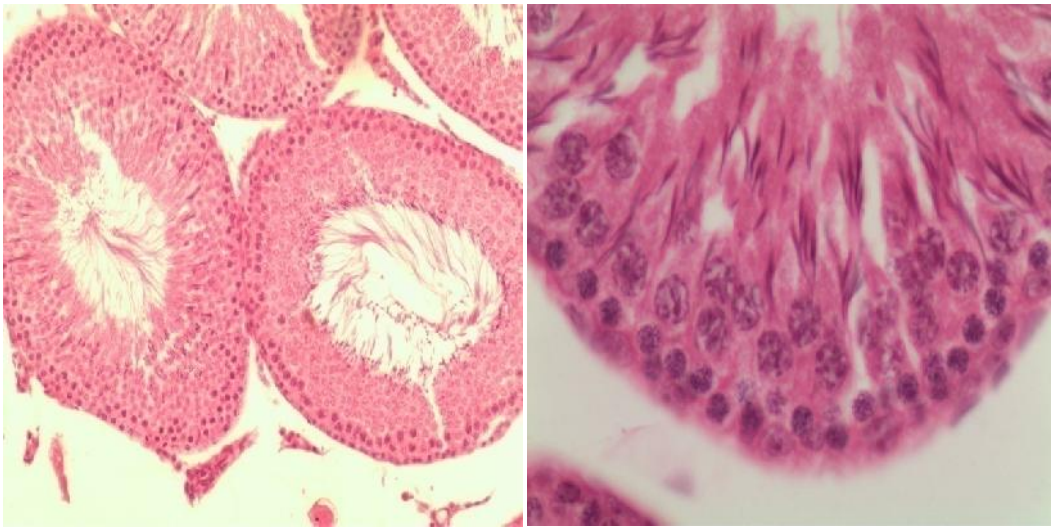


Fig 22 (B): Section of the testis of rat administered for 60 days (10 X and 40 X) at 30mg/kg with MSG, seminiferous tubules filled with spermatogenic cells, alteration of few sperms, formation of vacuoles.

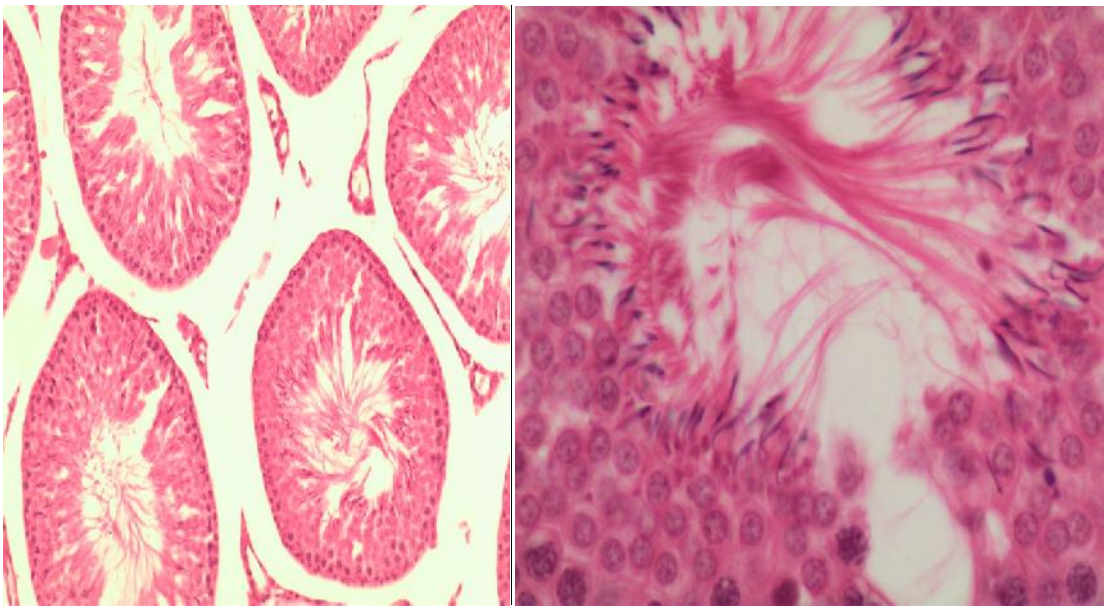


Fig 22 (C): Section of the testis of rat administered for 60 days (10 X and 40 X) at a dose of 300mg/kg MSG. Seminiferous tubules lined by spermatogenic cells, degeneration of Leydig cells with formation of small vacoules.

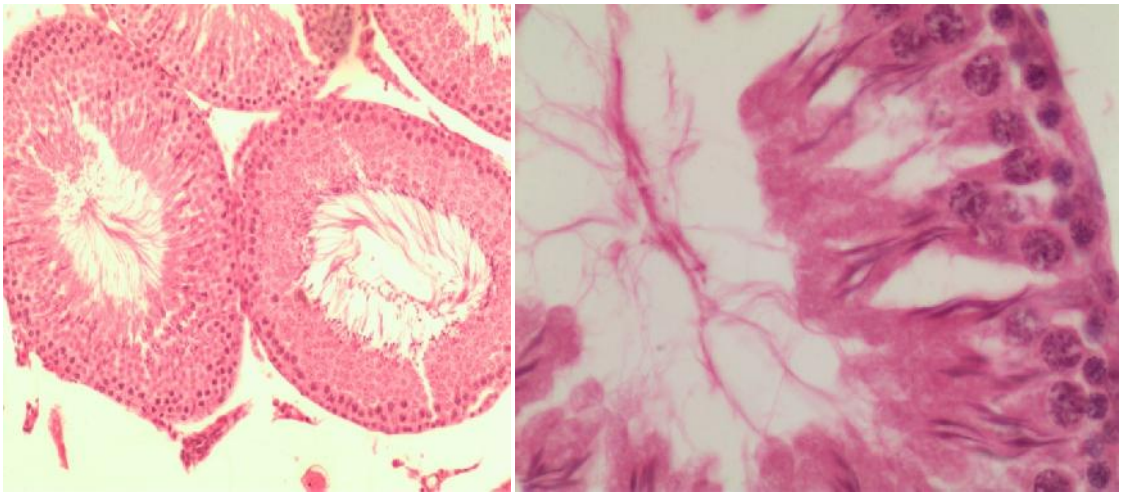


Fig 22 (D) : Section of the testis of rat administered for 60 days (10 X and 40 X) at a high dose of 3000mg/kg MSG. Seminiferous tubules lined by spermatogenic cells, larger vacoules and lesser sperm formation.

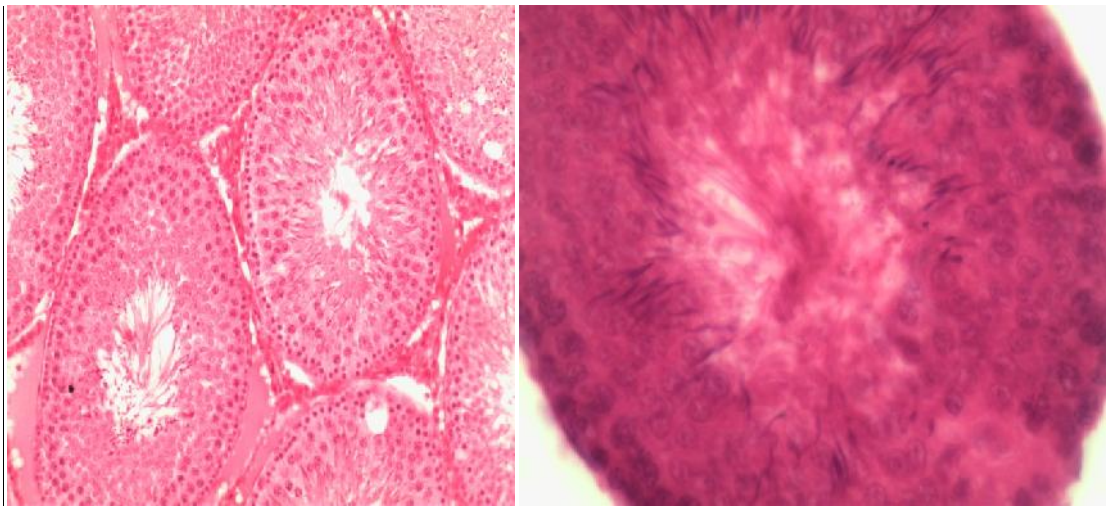


Fig 22 (E): Section of the testis of rat administered for 60 days (10 X and 40 X) at 3000 mg/kg with MSG+100mg/kg of carnitine. Seminiferous tubules filled with spermatogenic cells and development of spermatocyte with normal sperm formation, degeneration of vacoules and moderate sperm formation.

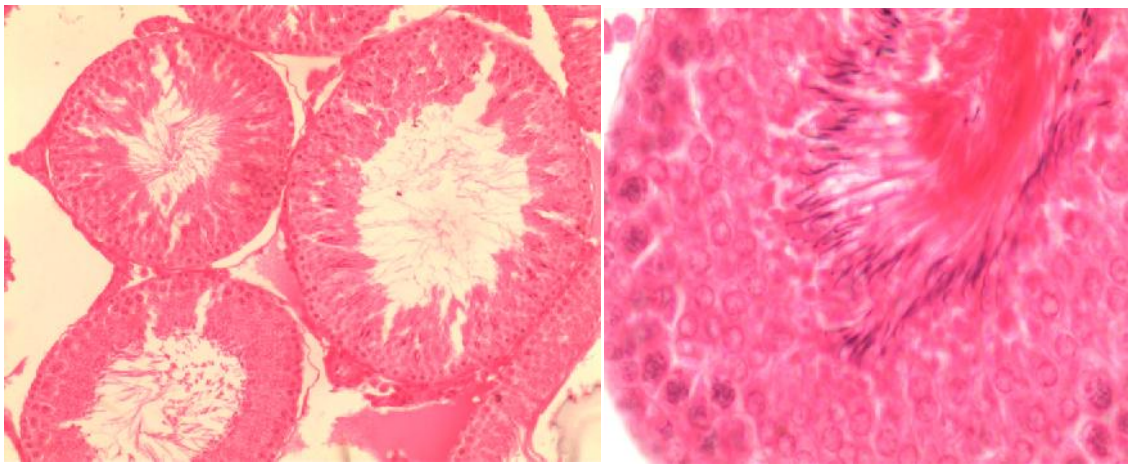


Fig 22 (F): Section of the testis of rat administered for 60 days (10 X and 40 X) at a dose of 100mg/kg carnitine. Seminiferous tubules filled with spermatogenic cells and development of spermatocyte with normal sperm formation and degeneration of vacuoles. Sertolli cells were also found enormously.

90 DAYS

7.1. Evaluation of acute toxicity of MSG against Wistar albino rats for 90 days exposure and the ameliorative role of L-carnitine

7.1.1. Food and water consumption

Table 15 shows the results referring to food and water consumption. Significant differences were observed in food consumption when the treatment groups were compared with control. Since MSG is a taste enhancer and a food additive, higher doses were observed to gain more weight than the control groups. However, there were no significant changes with water consumption.

Table 15: Effect of a chronic treatment for 90 days at different doses (30mg/kg, 300 mg/kg, 3000 mg/kg MSG, 3000 mg/kg MSG+100mg/kg carnitine and 100 mg/kg carnitine) on food and water consumption of rats.

Parameters	Experimental groups					
	control	30 mg/kg msg	300 mg/kg msg	3000 mg/kg msg	3000+100 mg/kg msg+ carnitine	100 mg/kg carnitine
Initial (g)	260.86 ± 2.99	259.89 ± 2.93	259.89 ± 2.93	259.89 ± 2.93	259.89 ± 2.93	259.89 ± 2.93
Final(g)	11.81 ± 1.18a	7.65 ± 0.64b	9.33 ± 0.81c	6.91 ± 0.55d	7.23 ± 0.62b	7.33 ± 0.69b
Food consumed (g)	245.92 ± 4.15a	249.63 ± 3.89b	250.55 ± 2.87b	260.73 ± 3.89c	252.64 ± 2.85b	252.54 ± 2.92b
Initial (ml)	350.00 ± 0.00	350.00 ± 0.00	350.00 ± 0.00	350.00 ± 0.00	350.00 ± 0.00	350.00 ± 0.00
Final (ml)	181.30 ± 5.35a	177.88 ± 4.87b	183.54 ± 13.49b	160.27 ± 5.10c	164.59 ± 6.05c	160.83 ± 6.83c
Water consumed (ml)	167.6 ± 5.35a	172.98 ± 4.90b	179.60 ± 5.12c	189.40 ± 5.16d	185.94 ± 6.05d	186.98 ± 6.87d

Data are expressed in Mean ± SEM, N=5 for control; N = 5 for treated group; P > 0.05 indicates a non-significant difference between the compared means. No symbol = non-significant, a, b, c, d, indicated significant values.

7.1.2. Rectal temperature and blood glucose level

Rectal temperature increased with treatment groups due to stress and blood glucose level was also higher in treatment groups. Long term exposure to MSG resulted in higher stress which shows higher rectal temperature. The increase in blood glucose levels may be due to the increased body weight in MSG treated groups.

Table 16: Effect of a chronic treatment for 90 days at different doses (30mg/kg, 300 mg/kg, 3000 mg/kg MSG, 3000 mg/kg MSG+100mg/kg carnitine and 100 mg/kg carnitine) on rectal temperature of rats.

Parameters	Experimental groups					
Rectal temperature	control	30 mg/kg MSG	300 mg/kg MSG	3000 mg/kg MSG	3000+100 mg/kg MSG + carnitine	100 mg/kg carnitine
Initial ^{0}F	96.54 ± 1.34	96.54 ± 1.34	96.54 ± 1.34	96.54 ± 1.34	96.54 ±1 .34	96.54 ± 1.34
Final ^{0}F	97.78 ± 0.42	96.8 ± 1.02	97.6 ± 0.97	98 ± 0.89	96.6 ± 1.16	96.54 ± 1.34

Data are expressed in Mean ± SEM, N=5 for control; N = 5 for treated group; P = 0.912 indicates a non-significant difference between the compared means. No symbol = non-significant.

Table 17 : Effect of a chronic treatment for 90 days at different doses (30mg/kg, 300 mg/kg, 3000 mg/kg MSG, 3000 mg/kg MSG+100mg/kg carnitine and 100 mg/kg carnitine) on blood glucose levels of rats.

Parameters	Experimental groups					
Blood glucose level	control	30 mg/kg MSG	300 mg/kg MSG	3000 mg/kg MSG	3000+100 mg/kg MSG+carnitine	100 mg/kg carnitine
Initial (mg/dl)	118.2 ± 0.58	117.6 ± 0.97	118.6 ± 0.50	120 ± 0.89	119 ± 0.00	127 ± 5.14
Final (mg/dl)	161.6 ± 1.16a	162.8 ± 1.02a	167.8 ± 0.48b	169.4 ± 0.67b	156.6 ± 4.77c	157.6 ± 6.78c

Data are expressed in Mean ± SEM, N=5 for control; N = 5 for treated group; $P < 0.05$ indicates significant difference between the compared means. No symbol = non-significant, a, b, c, indicated significant values.

7.1.3. Body weight and organ weight

In the first phase of the study, evaluation of body weight and organ weight was done in both control and treated groups after 60 days of treatment**.** MSG increased the body weight and organ weight with respect to control. The increased in body weight may be due to long term treatment of MSG in rats. The steady weight gained by rats were the effect of MSG but no significant changes were observed in the organ weight of treatment groups with respect to control may possibly be due to shrinkage in subsequent toxicity by compounds which was investigated by (Hamaoka and Kusunok.,1986). Significant increase in body weight, organ weight and their respective relative weight were observed in treatment groups as compared to control. Table 18 shows that MSG increased the body weight and organ weight with respect to control. This is due to the increased food consumption of MSG treated rats. Ekaluo *et al.* ,(2013) MSG-treatment caused reduction in testes and epididymis weight.

Table 18: Effect of a chronic treatment for 90 days at different doses (30mg/kg, 300 mg/kg, 3000 mg/kg MSG, 3000 mg/kg MSG+100mg/kg carnitine and 100 mg/kg carnitine) on body weight, organ weight and the relative weight of rats.

Parameters	Experimental groups					
	Control	30 mg/kg MSG	300 mg/kg MSG	3000 mg/kg MSG	3000+100 mg/kg MSG + carnitine	100 mg/kg carnitine
Initial body weight (g)	180.4 ± 4.41a	119.2 ± 3.15b	120.2 ± 3.59c	99.6 ± 6.98d	125.8 ± 11.25e	127.6 ± 9.49e
Final body weight (g)	215 ± 8.07a	209 ± 4.72b	218.8 ± 1.53c	201.6 ± 6.49d	197.8 ± 3.69e	218.6 ± 1.83c
Testis(g)	0.66 ± 0.10a	0.63 ± 0.13b	0.46 ± 0.10c	0.36 ± 0.06d	0.50 ± 0.10e	0.66 ± 0.16a
Testis (g/100g)	0.29 ± 0.07a	0.26 ± 0.07b	0.20 ± 0.06c	0.17 ± 0.03d	0.20 ± 0.06c	0.30 ± 0.04a
Liver(g)	7.26 ± 0.45a	6.04 ± 0.94 b	5.5 ± 0.59c	4.48 ± 0.68c	6.66 ± 0.57b	7.5 ± 0.64a
Liver (g/100g)	3.31 ± 0.20	2.91 ± 0.49	2.52 ± 0.28	2.25 ± 0.39	3.3 5± 0.26	3.49 ± 0.29
Kidney(g)	0.56 ± 0.0a	0.5 ± 0.07a	0.46 ± 0.10a	0.36 ± 0.06b	0.56 ± 0.09a	0.76 ± 0.15c
Kidney (g/100g)	0.26 ± 0.03a	0.23 ± 0.03b	0.20 ± 0.04c	0.17 ± 0.0d	0.27 ± 0.04a	0.34 ± 0.06e
Seminal vesicles(g)	0.84 ± 0.18a	0.66 ± 0.16b	0.5 ± 0.21c	0.32 ± 0.08d	0.76 ± 0.17e	1.00 ± 0.15f
Seminal vesicles (g/100g)	0.37 ± 0.08a	0.30 ± 0.07b	0.22 ± 0.09c	0.15 ± 0.03d	0.37 ± 0.08a	0.46 ± 0.07e

Vas deferens(mg)	0.22 ± 0.05a	0.18 ± 0.05b	0.16 ± 0.04c	0.16 ± 0.04c	0.18 ± 0.05b	0.26 ± 0.09d
Vas deferens (g/100g	0.11 ± 0.04a	0.07 ± 0.02b	0.07 ± 0.01b	0.06 ± 0.01b	0.10 ± 0.02a	0.31 ± 0.10c
Cauda(g)	0.32 ± 0.08a	0.30 ± 0.08b	0.28 ± 0.06c	0.22 ± 0.04d	0.32 ± 0.08a	0.56 ± 0.13e
Cauda (g/100g)	0.19 ± 0.04a	0.15 ± 0.04b	0.16 ± 0.04b	0.13 ± 0.03c	0.25 ± 0.06d	0.25 ± 0.06d
Caput(g)	0.40 ± 0.09a	0.22 ± 0.05b	0.22 ± 0.05b	0.22 ± 0.03b	0.30 ± 0.07c	0.48 ± 0.05d
Caput (g/100g)	0.16 ± 0.03a	0.10 ± 0.02b	0.12 ± 0.02c	0.10 ± 0.01b	0.13 ± 0.03c	0.18 ± 0.04a

Data are expressed in Mean ± SEM, N=5 for control; N = 5 for treated group; $P < 0.05$ indicates significant difference between the compared means. No symbol = non-significant, a, b, c, d, e, indicated significant values.

7.1.4. Sperm Parameters

The sperm concentration in chronic (90 days) treatment decreased with MSG treated groups and increased with carnitine treated groups. Moreover, the daily sperm production decreased with treatment groups and increased with carnitine treated groups .Ekaluo *et al,* (2013) MSG-treatment caused reduction in sperm count. The sperm concentration decreased with MSG treated groups and increased with carnitine treated groups. Moreover, the daily sperm production decreased with treatment groups and increased with carnitine treated groups. The sperm concentration in treated groups (MSG) decreased compared to control and increased in carnitine treated groups. Table 19 and fig 24 shows sperm concentration and daily sperm production in different groups compared to control. MSG treatment resulted in a significant decrease in sperm count in testis and epididymis (caput and cauda). Furthermore, there was a reduction in daily sperm production per testis in MSG treated rats. Decreased spermatozoa production, cauda concentration count and

caput concentration count caused after MSG treatment were improved by carnitine (100 mg/kg). Furthermore, there was a reduction in daily sperm production per testis, but slightly improved in carnitine treated rats.

Table 19: Effect of a chronic treatment for 90 days at different doses (30mg/kg, 300 mg/kg, 3000 mg/kg MSG, 3000 mg/kg MSG+100mg/kg carnitine and 100 mg/kg carnitine) on sperm counts and epididymis of rats.

Parameters	Experimental groups					
Sperm count	control	30 mg/kg MSG	300 mg/kg MSG	3000 mg/kg MSG	3000+100 mg/kg MSG+ Carnitine	100 mg/kg Carnitine
Testis						
Sperm number (×10)	168.6 ± 2.58	118.4 ± 3.31 b	98.82 ± 0.89 c	56.66 ± 1.86 d	126.6 ± 3.07 e	176.6 ± 1.74 a
Sperm number (×10/g)	324.33 ± 86.83a	285.41 ± 100.80 b	274.01 ± 68.97 c	179.87 ± 33.26 d	311.88 ± 74.43e	329.25 ± 76.51 f
DSP (×10/testis/day)	27.63 ± 0.42a	19.40 ± 0.54 b	16.19 ± 0.14 b	9.61 ± 0.52 c	20.74 ± 0.50 b	28.94 ± 0.28 a
DSPr (×10/testis/day/g)	51.12 ± 12.20 a	45.87 ± 8.71 b	44.90 ± 11.30b	31.10 ± 6.93 c	52.53 ± 8.17 a	54.56 ± 12.77 a
Epididymis						
Caput						
Sperm number ($\times 10^6$)	42.44 ± 2.92 a	22.7 ± 10.71 b	21.02 ± 0.13 b	18.53 ± 0.08 b	23.46 ± 1.04 b	44.93 ± 0.71 a
Sperm number ($\times 10^6$/g)	172.48 ± 71.91	142.01 ± 39.01 b	129.57 ± 33.81 c	98.82 ± 22.66 d	113.45 ± 33.65 c	180.56 ± 43.59 a

Sperm transit time(days)	1.62 ± 0.03	1.17 ± 0.05	1.29 ± 0.00	1.94 ± 6.10	1.12 ± 0.06	1.45 ± 0.09
Cauda						
Sperm number ($\times 10^6$)	49.57 ± 0.18	25.83 ± 0.87b	23.28 ± 0.38b	20.03 ± 0.11c	26.44 ± 0.63b	75.73 ± 1.97 a
Sperm number ($\times 10^6$/g)	133 ± 0.05a	122.05 ± 36.39b	109.98 ± 35.29c	89.99 ± 29.51d	119.33 ± 38.66e	358.42 ± 102.38f
Sperm transit time(days)	2.62 ± 0.06	1.38 ± 25.33	1.43 ± 0.02	2.10 ± 0.10	1.27 ± 0.05	1.79 ± 0.03

Data are expressed in Mean ± SEM, N=5 for control; N = 5 for treated group; $P < 0.05$ indicates significant difference between the compared means. No symbol = non-significant, a, b, c, d, e, f, indicated significant values.

Regarding the sperm motility, we observed decreased type M sperm (motile with progressive movement) accompanied by increased in type IM (immotile) in 30 days MSG treated rats. The percentage of motile sperms in treated groups (MSG) decreased compared to control and increased in carnitine treated groups. Fig 23 shows the % of motile sperms.

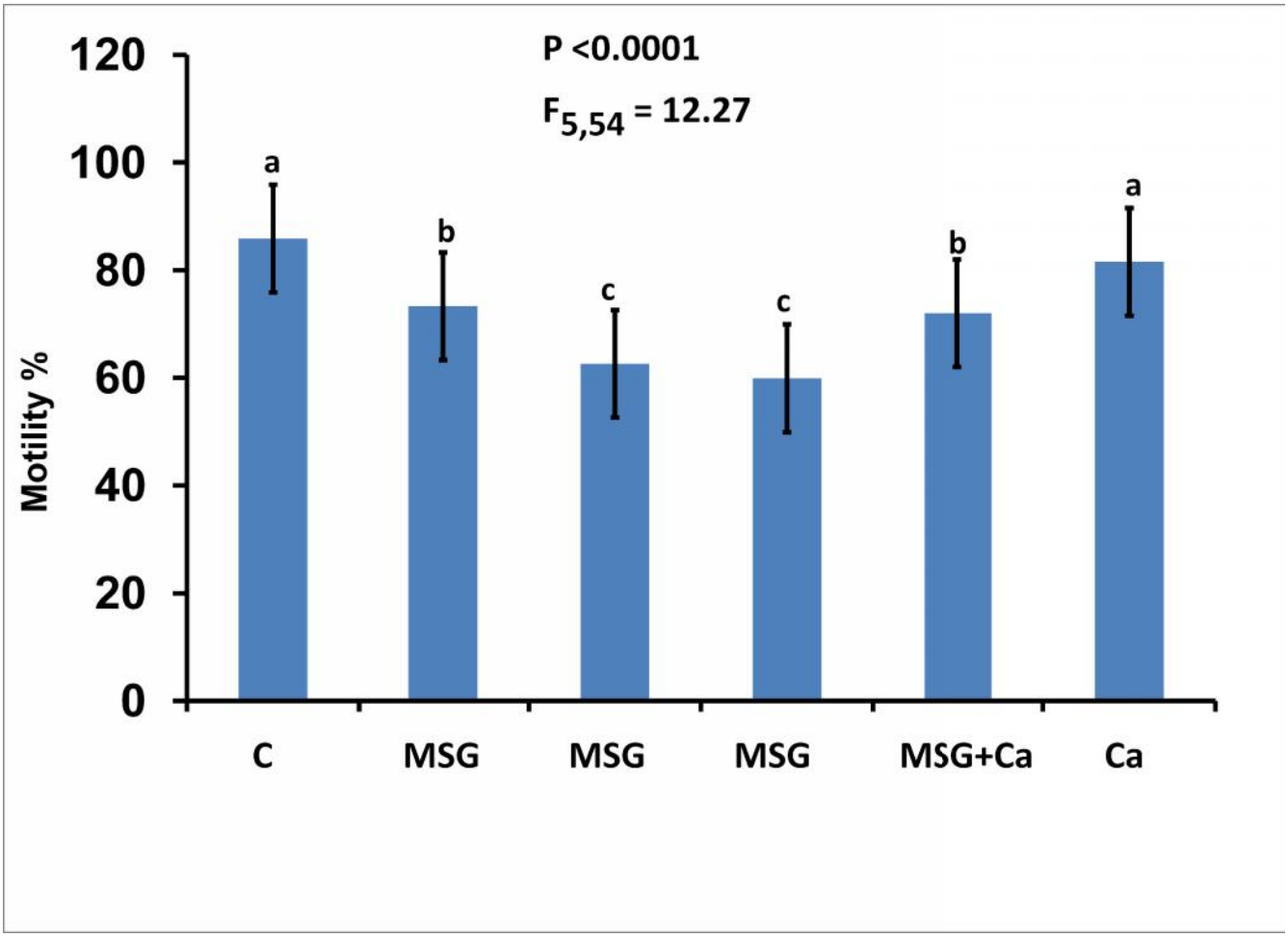


Fig 23: Effect of 90 days treatment of MSG and carnitine on % of motile sperms (Control, MSG 30 mg/kg, MSG 300 mg/kg, MSG 3000 mg/kg , MSG 3000 + C 100 mg/kg and C100mg/kg). Data represents the means ± SEM of 5 samples in each group.

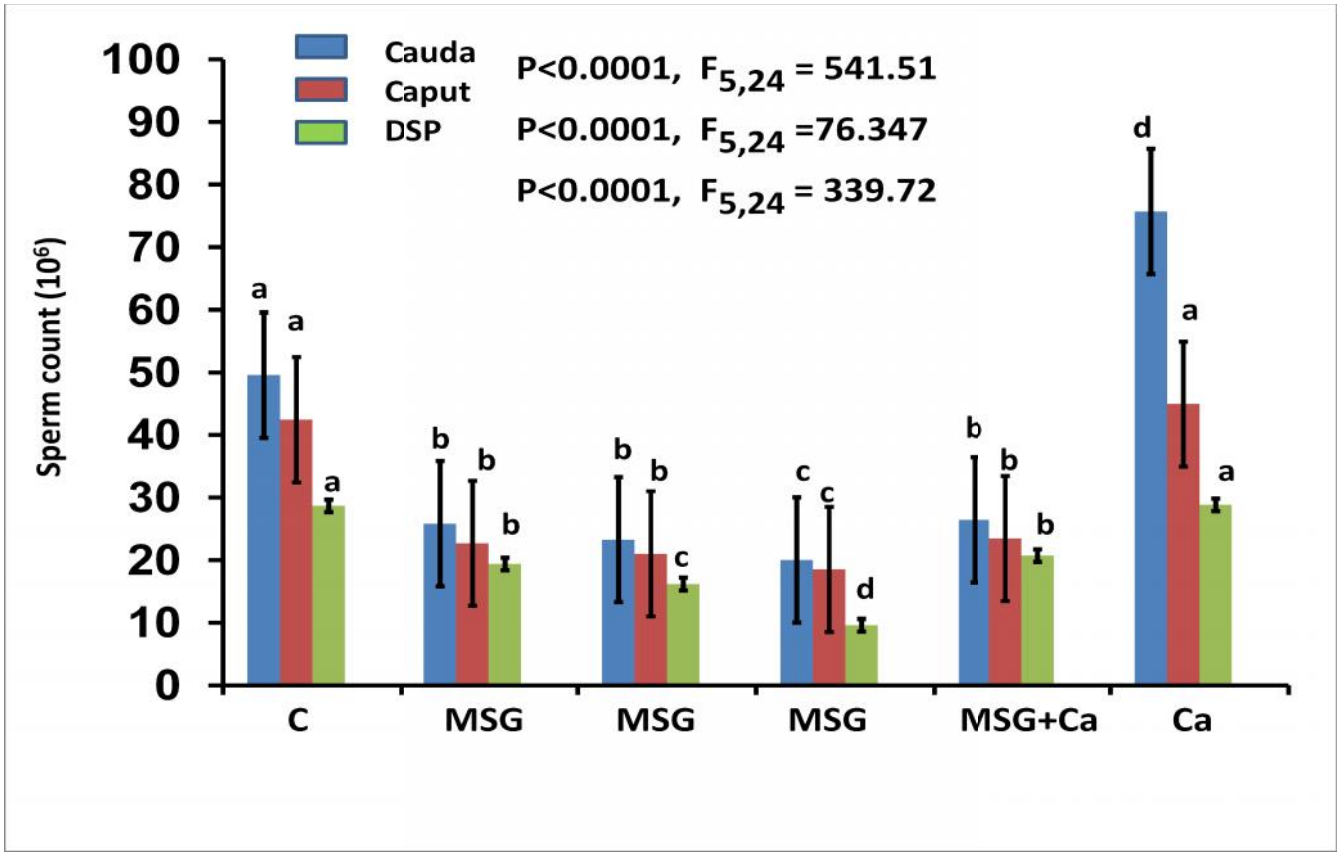


Fig 24: Effect of 90 days treatment of MSG and carnitine on sperm parameters and daily sperm production of rats, (Control, MSG 30 mg/kg, MSG 300 mg/kg ,MSG 3000 mg/kg, MSG 3000 + C 100 mg/kg and C 100 mg/kg). Data represents the means ± SEM of 5 samples in each group

7.1.5. Sperm Morphology

Sperms were classified as normal and abnormal sperms., tail (amorphous ,banana, detached) abnormality and head (coil or broken) abnormality. The normal sperms were found maximum in carnitine groups and abnormality increased with MSG treated groups and decreased with carnitine treated groups as shown in table 20. Ekaluo *et al* (2013) MSG-treatment caused reduction testes and epididymis weight, sperm count and increase in sperm abnormalities.

Table 20: Effect of a chronic treatment for 90 days at different doses (30mg/kg, 300 mg/kg, 3000 mg/kg MSG, 3000 mg/kg MSG+100mg/kg carnitine and 100 mg/kg carnitine) on sperm morphology of rats.

Table 20: Effect of a chronic treatment for 90 days at different doses (30mg/kg, 300 mg/kg, 3000 mg/kg MSG, 3000 mg/kg MSG+100mg/kg carnitine and 100 mg/kg carnitine) on sperm morphology of rats.

7.2.1. Biochemical Parameters

ALT, creatinine, AP, urea and cholesterol and AST increased with MSG treated groups and decreased with control and carnitine treated groups. Serum hepatic marker enzymes (ALT, AST and ALP) were evaluated for hepatotoxicity. The testis is one of the most sensitive organ to pre-oxidative damage because it is rich in oxidizable substances. The more severe the testis damages the higher the release of the testis enzymes (El-Khayat et al., 2009). Increase in serum level of ALT, AST and ALP as observed in groups induced with these additives may reflect damage of testis cells and cellular degeneration or destruction in this organ and the increase in the activities of ALP in plasma might be due to the increased permeability of plasma membrane or cellular necrosis. When the testis cell membrane is damaged, varieties of enzymes normally located in the cytosol are released into the blood stream. Elevation of AST and ALT indicates the utilization of amino acids for the oxidation or for gluconeogenesis and is used to determine testis damage (Etim et al., 2006). Also, the elevation in ALP level in case of MSG suggests an increase in lysosomal mobilization and cell necrosis due to toxicity (Kalender et al., 2005). The increased levels of serum enzyme such as AST and ALT indicate the increased permeability and necrosis of hepatocytes (Pari and Arumugam, 2008).

Table 21: Effect of a chronic treatment for 90 days at different doses (30mg/kg, 300 mg/kg, 3000 mg/kg MSG, 3000 mg/kg MSG+100mg/kg carnitine and 100 mg/kg carnitine) on biochemical parameters of rats.

Parameters	Experimental groups					
	Control	30 mg/kg MSG	300 mg/kg MSG	3000 mg/kg MSG	3000+100 mg/kg MSG+ carnitine	100 mg/kg carnitine
ALT (IU/L)	124.33 ± 2.72a	148.66 ± 2.84b	181 ± 1.15c	183.33 ± 3.52c	163 ± 4.61d	145.66 ± 5.60e
AST (IU/L)	118.66 ± 2.60a	217.33 ± 7.44b	307.33 ± 9.70c	313.33 ± 21.32b	268 ± 17.15d	199.66 ± 17.05b
Cholesterol (mg/dl)	45 ± 2.08a	33 ± 1.73b	12.66 ± 1.76c	10.33 ± 1.20d	29.66 ± 2.84b	33.66 ± 1.45b
AP(IU/L)	126.33 ± 17.34a	210.66 ± 21.78b	224.33 ± 24.91b	594.66 ± 26.82c	203.33 ± 19.09b	217.66 ± 54.30b
Creatinine (mg/dl)	5.866 ± 1.19 a	6.623 ± 2.18a	11.51 ± 2.89 b	13.39 ± 10.11c	6.11 ± 4.65 a	4.776 ± 2.74 a
Urea (mg/dl)	88.33 ± 5.56a	84.52 ± 4.63a	40.58 ± 2.23b	34.73 ± 2.23b	48.89 ± 9.00b	56.17 ± 6.60b

Data are expressed in Mean ± SEM, N=5 for control; N = 5 for treated group; $P < 0.05$ indicates significant difference between the compared means. No symbol = non-significant, a, b, c, d, e, indicated significant values.

7.3.1. Lipid Peroxidation and Antioxidant status

In this study, the cleared decrease of SOD, CAT, GSH and GST enzymes in MSG treated group may be due to the consumption during the breakdown of free radicals and high level of H_2O_2 or the inhibition of these enzymes by these radicals. Thus, the changes in oxidative defense systems and increase the level of oxidants in the testis tissues associated with MSG exposure leading to increased lipid peroxidation.(Kanburet al., 2009).The MDA level which is a bio-indicator of oxidative stress increased with MSG treated groups resulting in higher lipid peroxidation and decreased with carnitine treated groups. The activity of antioxidants ie., SOD, Cat, GSH, GSTdecreased with MSG treated groups and increased with carnitine treated groups MSG caused significant decrease in SOD, CAT, GSH and GST activities and these findings are greatly in accordance with (Fábio et al., 2012) and significant amelioration in these parameters after combination with carnitine.

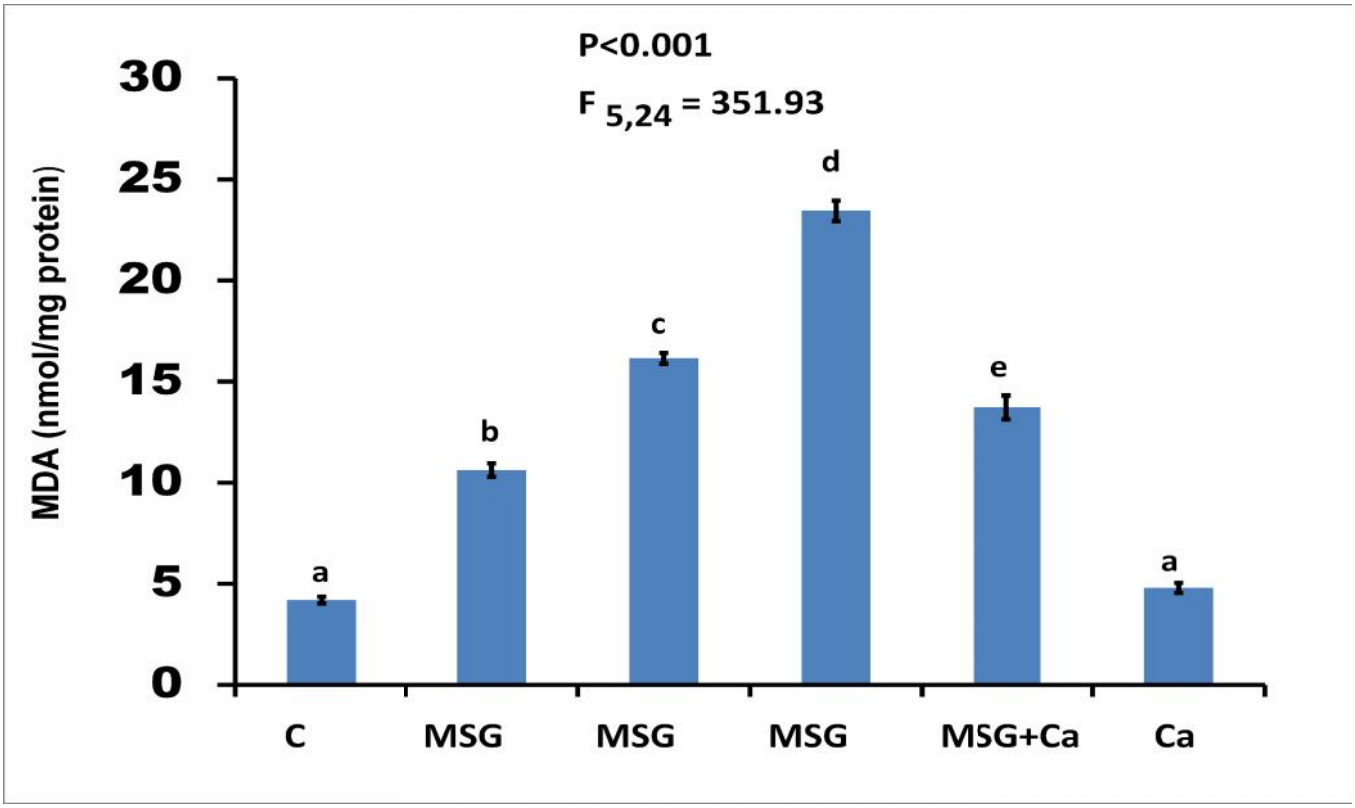


Fig 25: Estimation of 90 days treatment of MDA level on Control, MSG 30 mg/kg, MSG 300 mg/kg, MSG 3000 mg/kg, MSG 3000 + C 100 mg/kg and C 100mg/kg. Data represents the means ± SEM of 5 samples in each group

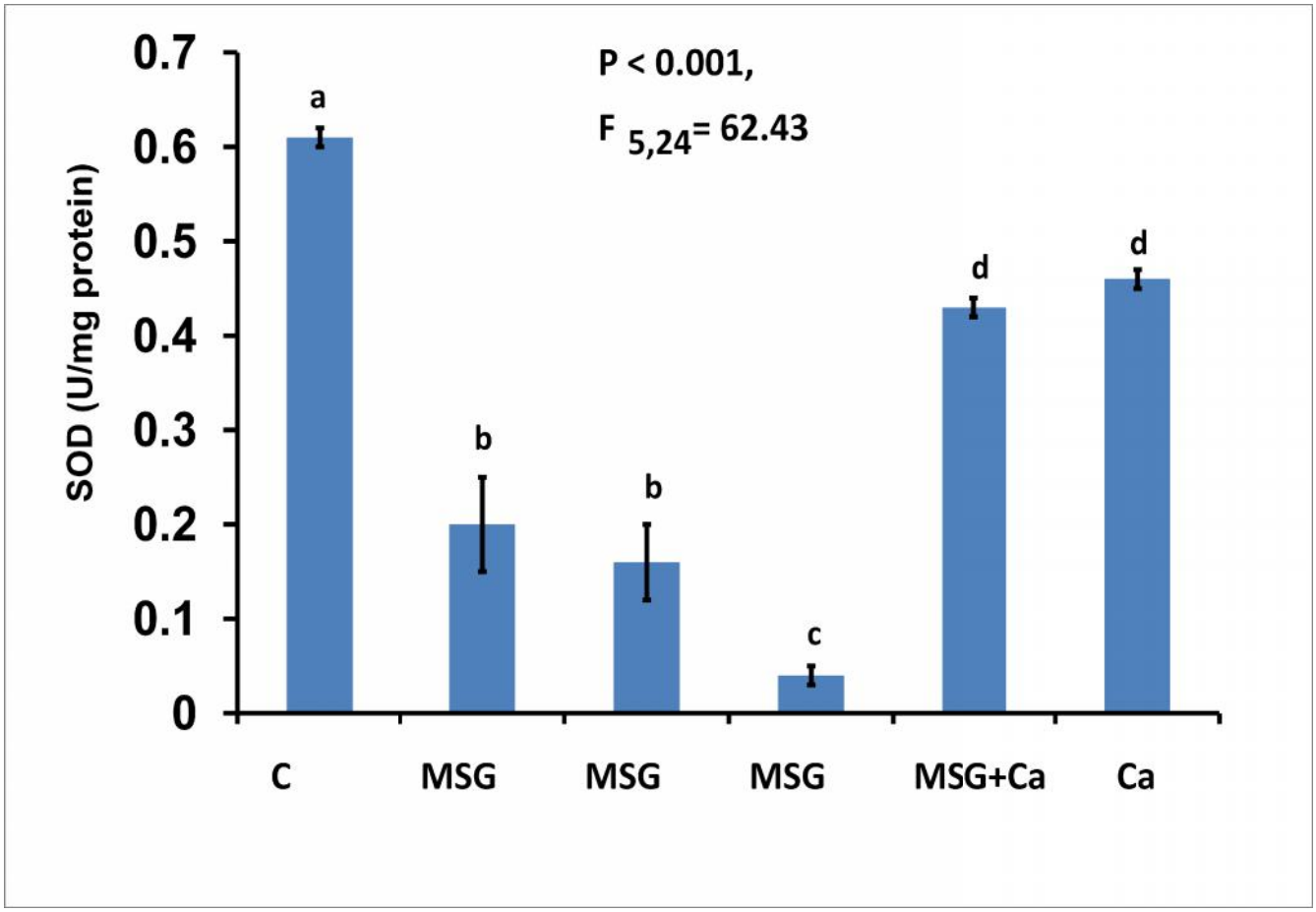


Fig 26: Estimation of 90 days treatment of SOD activity on Control, MSG 30 mg/kg, MSG 300 mg/kg, MSG 3000 mg/kg, MSG 3000 + C 100 mg/kg and C 100mg/kg. Data represents the means ± SEM of 5 samples in each group

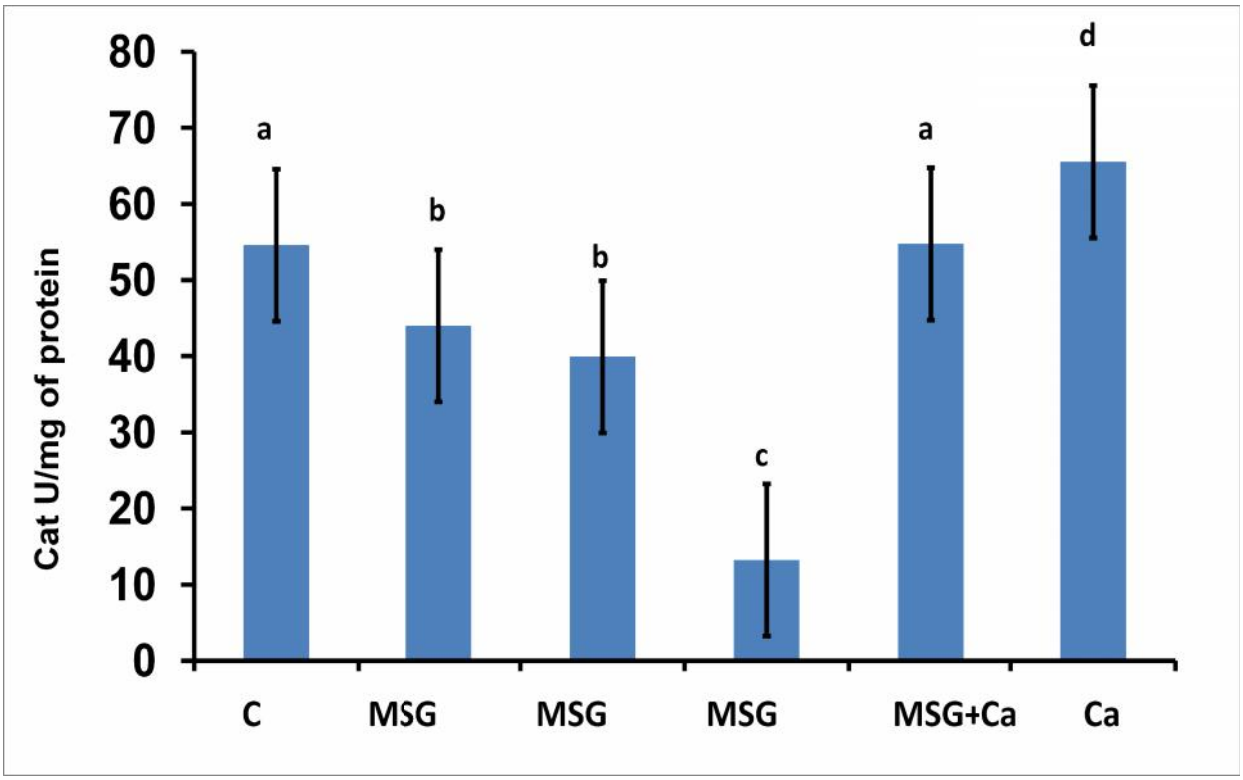


Fig 27: Estimation of 90 days treatment of Catalase activity on Control, MSG 30 mg/kg, MSG 300 mg/kg, MSG 3000 mg/kg, MSG 3000 + C 100 mg/kg and C 100mg/kg. Data represents the means ± SEM of 5 samples in each group.P<0.0001, $F_{5,12}$ = 3010.0

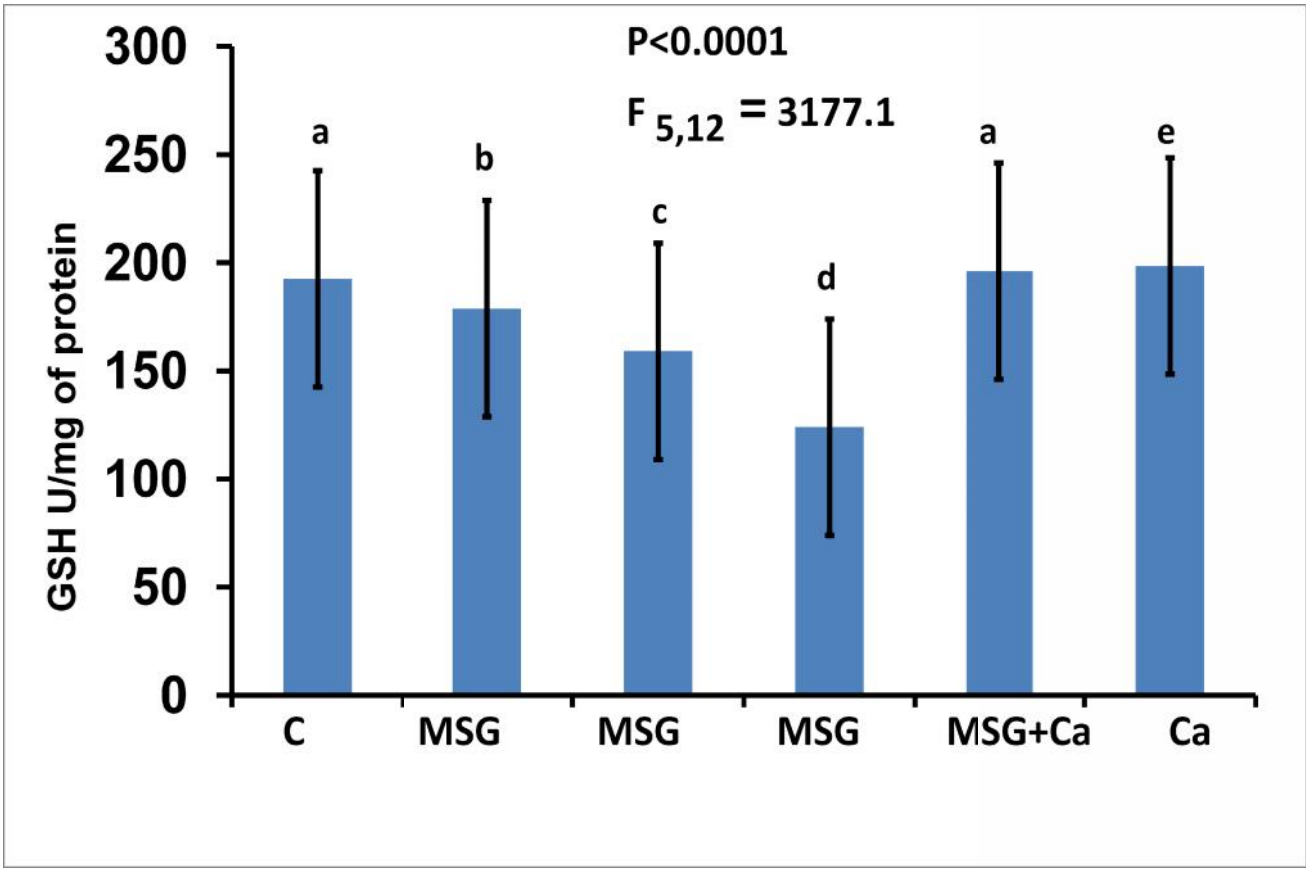


Fig 28: Estimation of 90 days treatment of GSH activity on Control, MSG 30 mg/kg, MSG 300 mg/kg, MSG 3000 mg/kg, MSG 3000 + C 100 mg/kg and C 100 mg/kg. Data represents the means ± SEM of 5 samples in each group

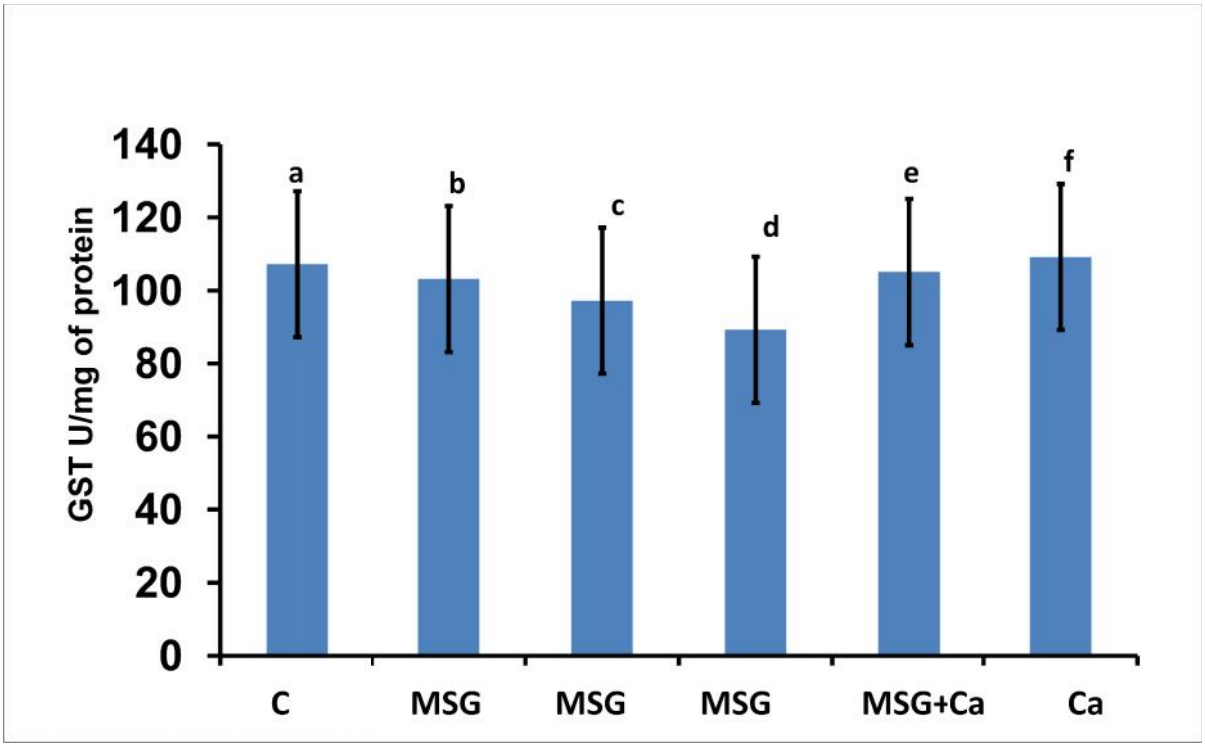


Fig 29: Estimation of 90 days treatment of GST activity on Control, MSG 30 mg/kg, MSG 300 mg/kg, MSG 3000 mg/kg, MSG 3000 + C 100 mg/kg and C 100 mg/kg. Data represents the means ± SEM of 5 samples in each group $P<0.0001$, $F_{5,12} = 371.87$

7.4. Histopathology

On histopathological examination, control rat testes showed normal morphology and spermatogenis, containing abundant amounts of spermatids and sperm in the lumen. In contrast to control, the arrangement of the cells was distributed in the seminiferous tubules of MSG treated rats . Vacoulization of spermatogonia was increased. Germinal epithelial cells were separated from each other and the tubular basement membrane. There was desquamation of

germinal cells and consequent appearance of irregular spaces in the epithelium and spermatogenic cells were decreased. The numbers of spermatozoa in the lumen were significantly low. However, seminiferous tubule basement membranes in this group of animals were observed to be normal.MSG may also affect male reproductive function (Alalwani 2013) . In this study MSG caused several histopathological changes like spermatogenic arrest, edema, and hypospermia. It may be related to oxidative effects of MSG on testis cell membrane and also testis tissues.

Carnitine treatment improved MSG induced histological changes in rat testis. The number of spermatogenic cells in the carnitine-treated rats was higher compared to the MSG group and the disturbance in the arrangement of the cells was slight in this group. In the 90 day MSG treated animals, a large amount of sloughed cells and cellular debris was noticed in the tubular lumen of many seminiferous tubule sections; some of these sections contained cells with degenerative characteristics. Accentuated germ cell depletion was frequently noticed in the seminiferous epithelium of rats from this group, sometimes characterizing the 'Sertoli cell-only' phenomenon, which were considered severe damage. On the other hand, some sections showed only spermatogonia and Sertoli cells. Thus, the Carnitine treated showed lower quantity of sections with severe damage and with moderate germ cell depletion. We considered as moderate the tubular sections displaying reduced numbers of germs cell layers, but containing at least two layers. Thus, a lower frequently of tubular sections with germ cell depletion (severe and moderate) was noticed in Carnitine group in comparison to the MSG. The hypotrophic tubular sections in the subgroup were usually surrounded by morphologically normal tubules. Germ cell depletion from seminiferous epithelium was also observed in the MSG.

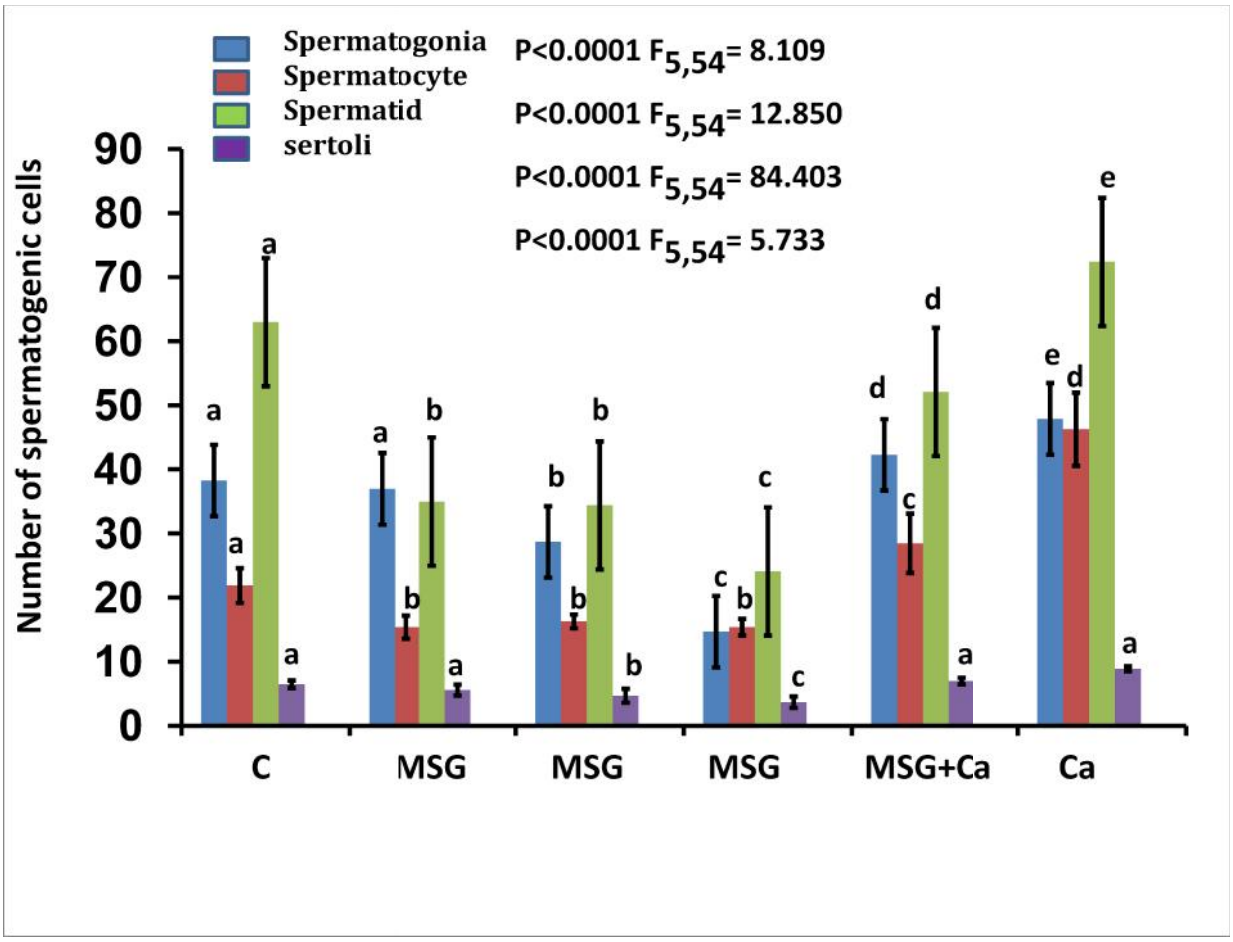


Fig 30: Effect of 90 days treatment of MSG and carnitine on spermatogenic cells of rats, (Control, MSG 30 mg/kg, MSG 300 mg/kg MSG 3000 mg/kg, MSG 3000 + C 100 mg/kg and C 100 mg/kg). Data represents the means ± SEM of 5 samples in each group

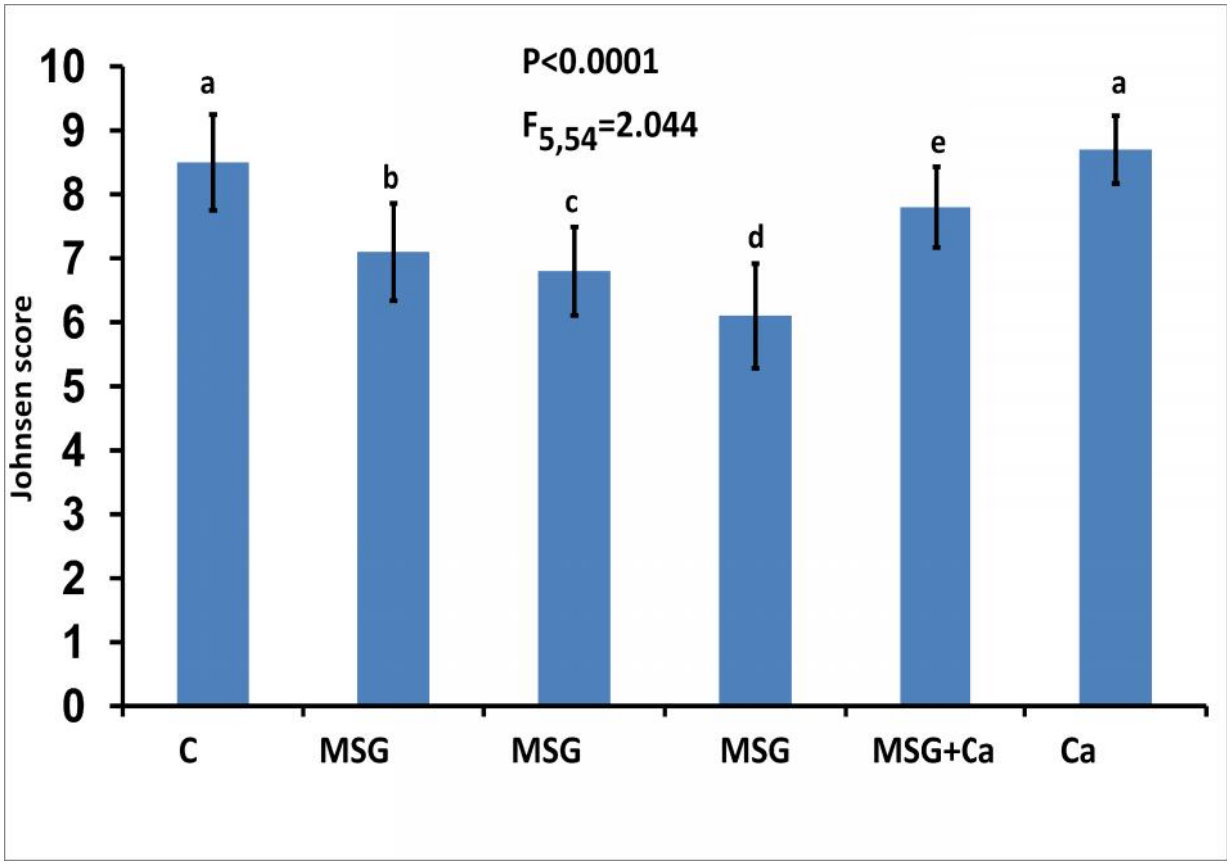


Fig 31: Effect of 90 days treatment of MSG and carnitine on Johnsen score of Control, MSG 30 mg/kg, MSG 300 mg/kg, MSG 3000 mg/kg, MSG 3000 + C 100 mg/kg and C 100 mg/kg. Data represents the means ± SEM of 5 samples in each group

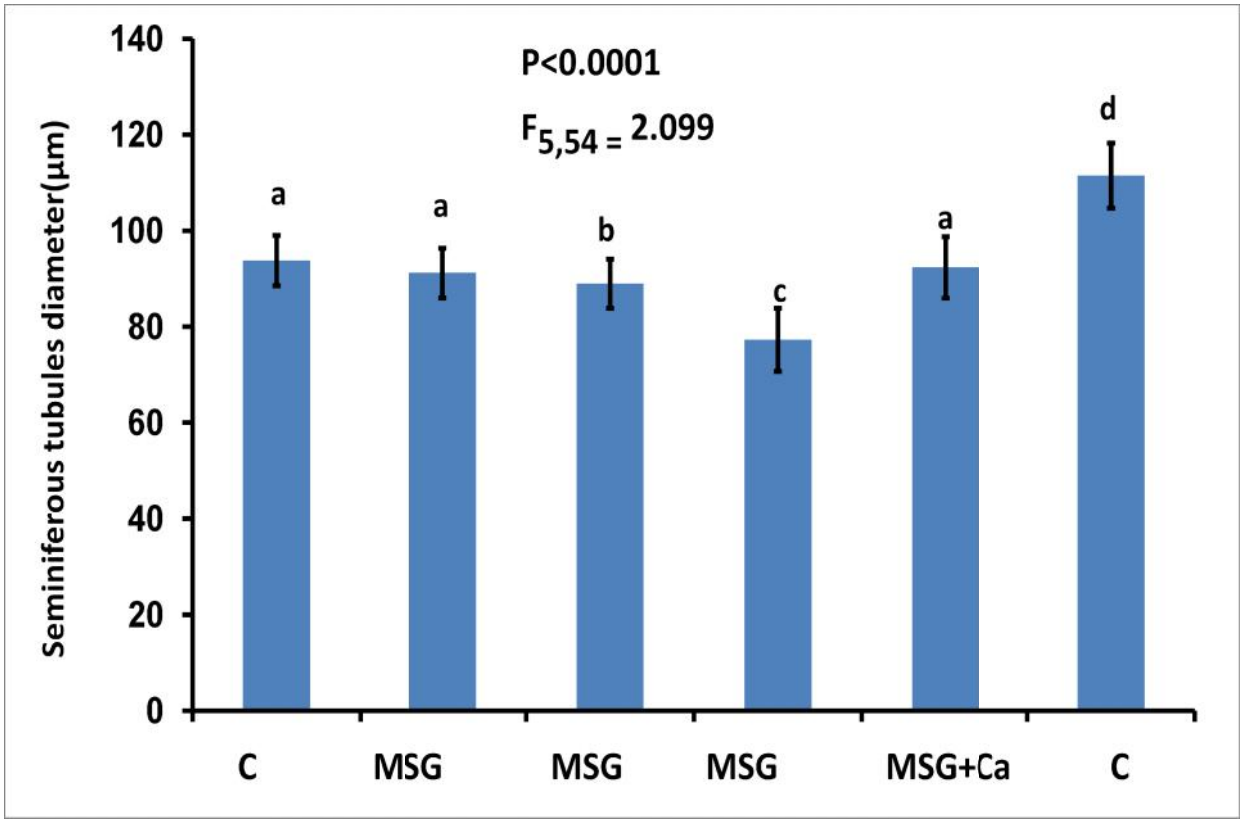


Fig 32: Effect of 90 days treatment of MSG and carnitine on spermatogenic cells of rats, (Control, MSG 30 mg/kg, MSG 300 mg/kg MSG 3000 mg/kg, MSG 3000 + C 100 mg/kg and C100 mg/kg). Data represents the means ± SEM of 5 samples in each group

The figures below are the testicular sections of control and different groups showing histological changes within 90 days treatment.

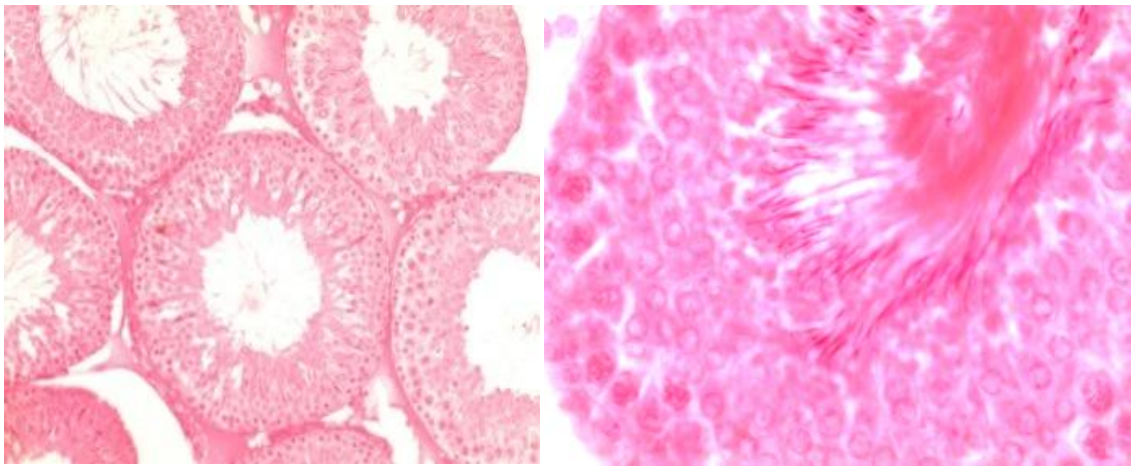


Fig 33 (A): Section of the testis of rat control group (10 X and 40 X) showing seminiferous tubules filled with spermatogenic cells, development of spermatocyte with normal sperm formation.

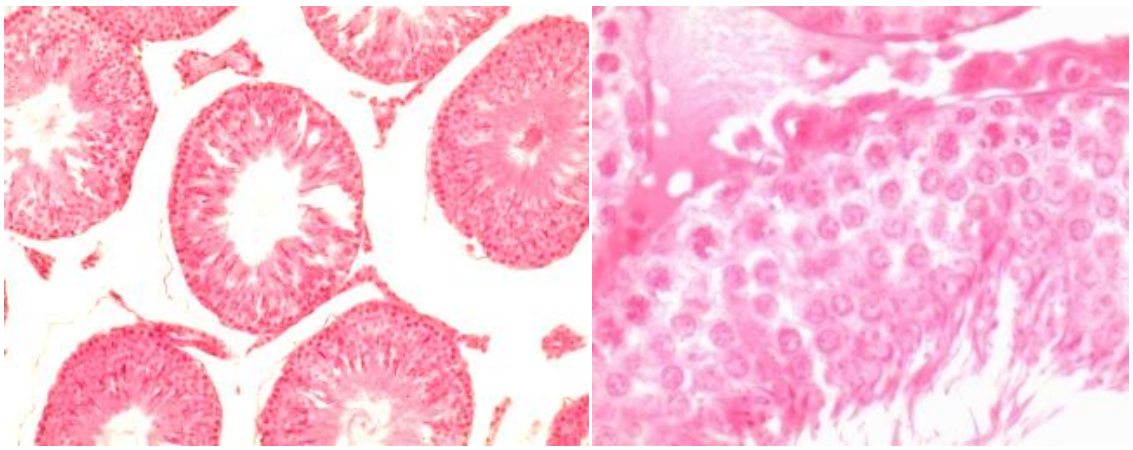


Fig 33 (B): Section of the testis of rat administered for 90 days (10 X and 40 X) at 30mg/kgwith MSG, seminiferous tubules filled with spermatogenic cells, alteration of few sperms, formation of vacuoles.

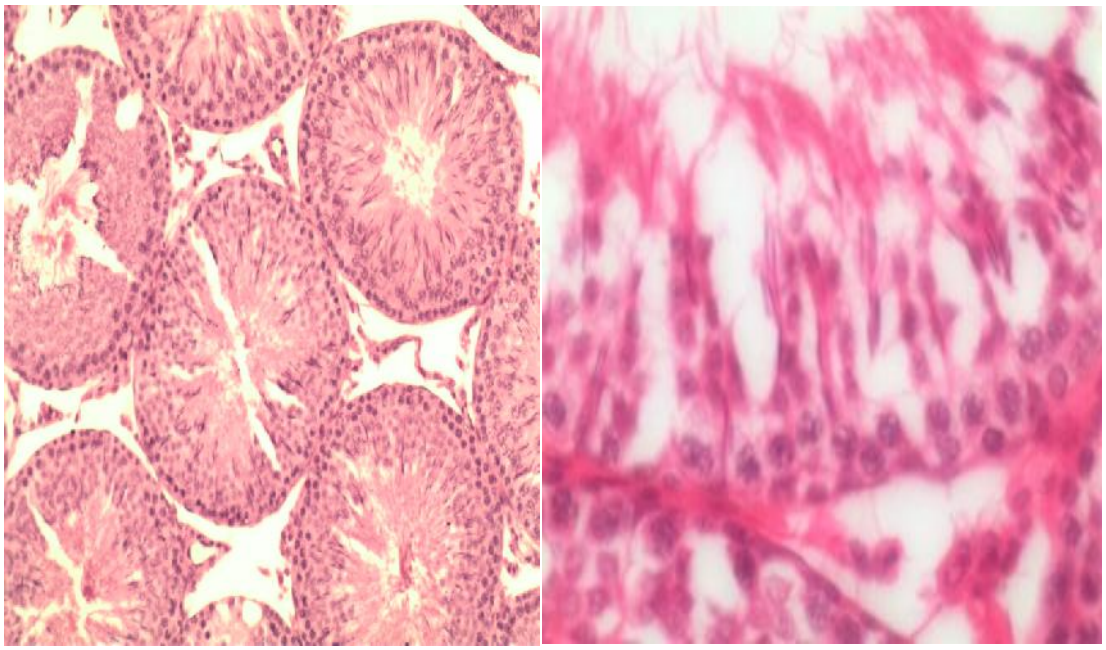


Fig 33 (C): Section of the testis of rat administered for 90 days (10 X and 40 X) at a dose of 300mg/kg MSG. Seminiferous tubules lined by spermatogenic cells, degeneration of Leydig cells with formation of numerous vacoules.

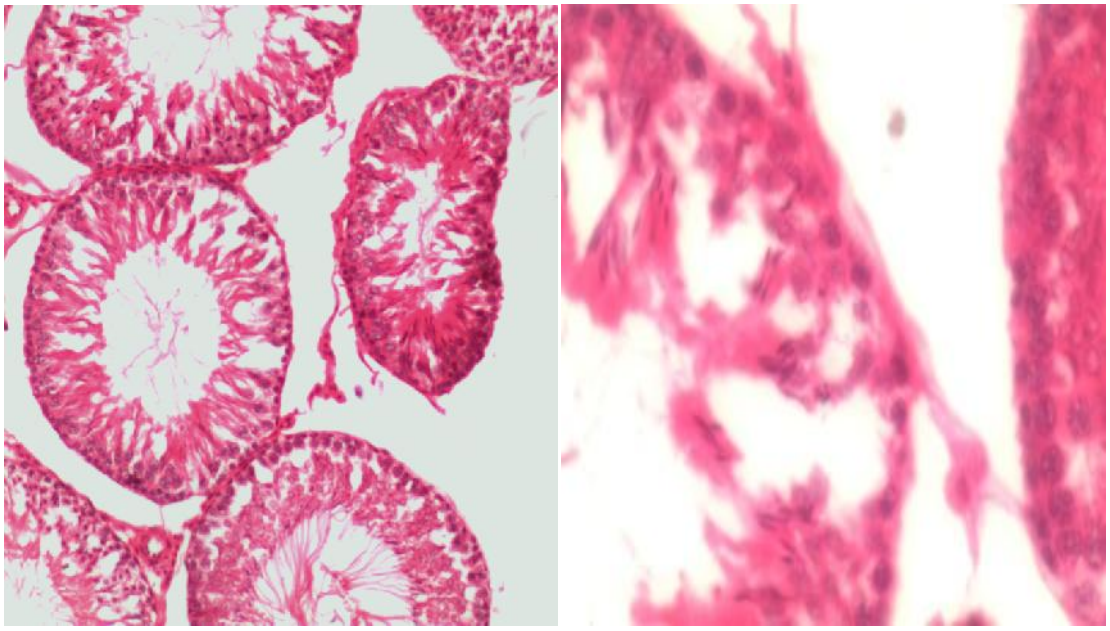


Fig 33 (D): Section of the testis of rat administered for 90 days (10 X and 40 X) at a high dose of 3000mg/kg MSG. Seminiferous tubules lined by spermatogenic cells, numerous vacoules and few sperm formation.

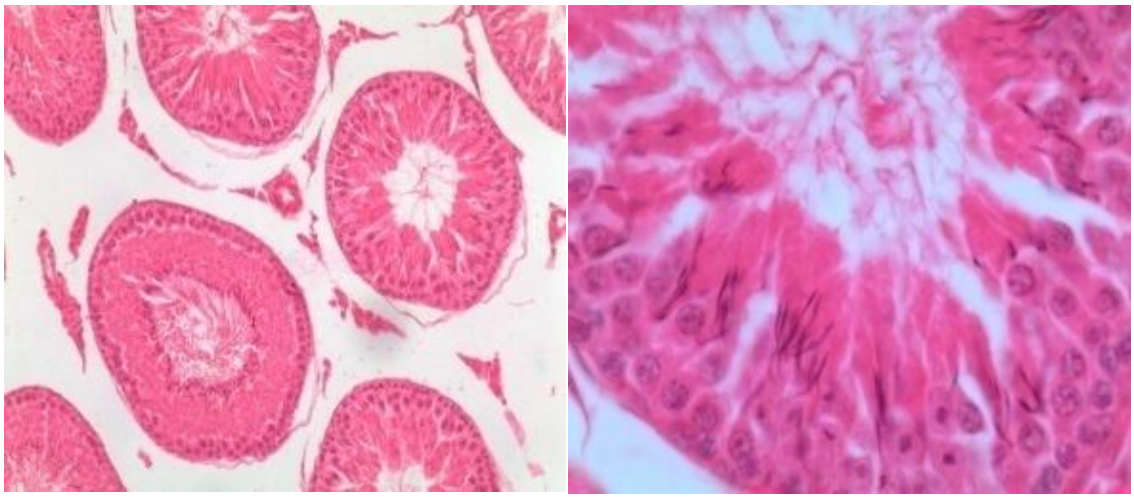


Fig 33 (E): Section of the testis of rat administered for 90 days (10 X and 40 X) at 3000mg/kg with MSG+100mg/kg of carnitine. Seminiferous tubules filled with spermatogenic cells and development of spermatocyte with normal sperm formation, degeneration of vacoules and moderate sperm formation.

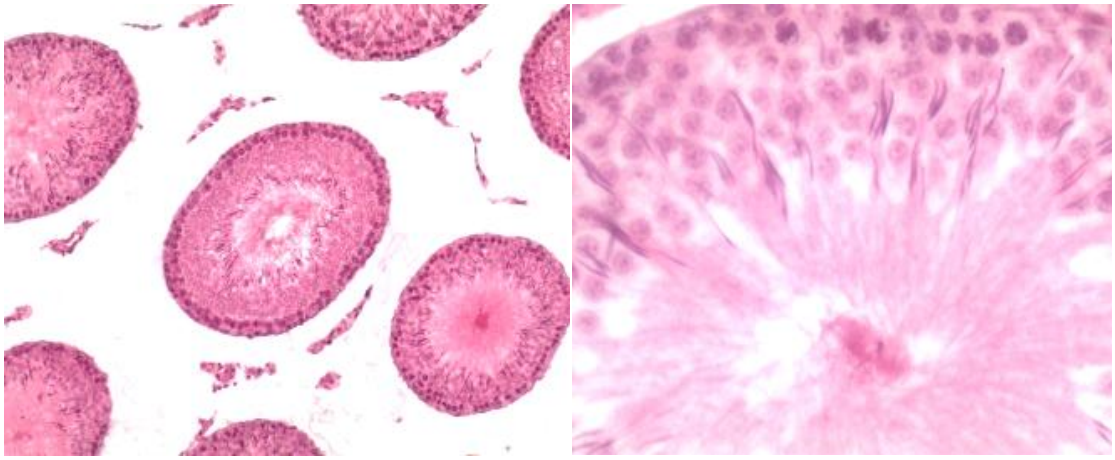


Fig 33 (F): Section of the testis of rat administered for 90 days (10 X and 40 X) at a dose of 100mg/kg carnitine. Seminiferous tubules filled with spermatogenic cells and development of spermatocyte with normal sperm formation and degeneration of vacuoles.

7.5. Immunohistochemical observations

The immunuhistochemical evaluation showed great differences in the positivity of staining among the experimental groups. PCNA was used as the marker where the testis sections of the MSG treated groups showed slightly faint staining nuclei indicating mild cell division of the spermatogonia. However, there was a strong immunoreactivity in the testis section of control and carnitine treated groups. Fig below shows the immunohistochemical changes of treatment groups with respect to control.

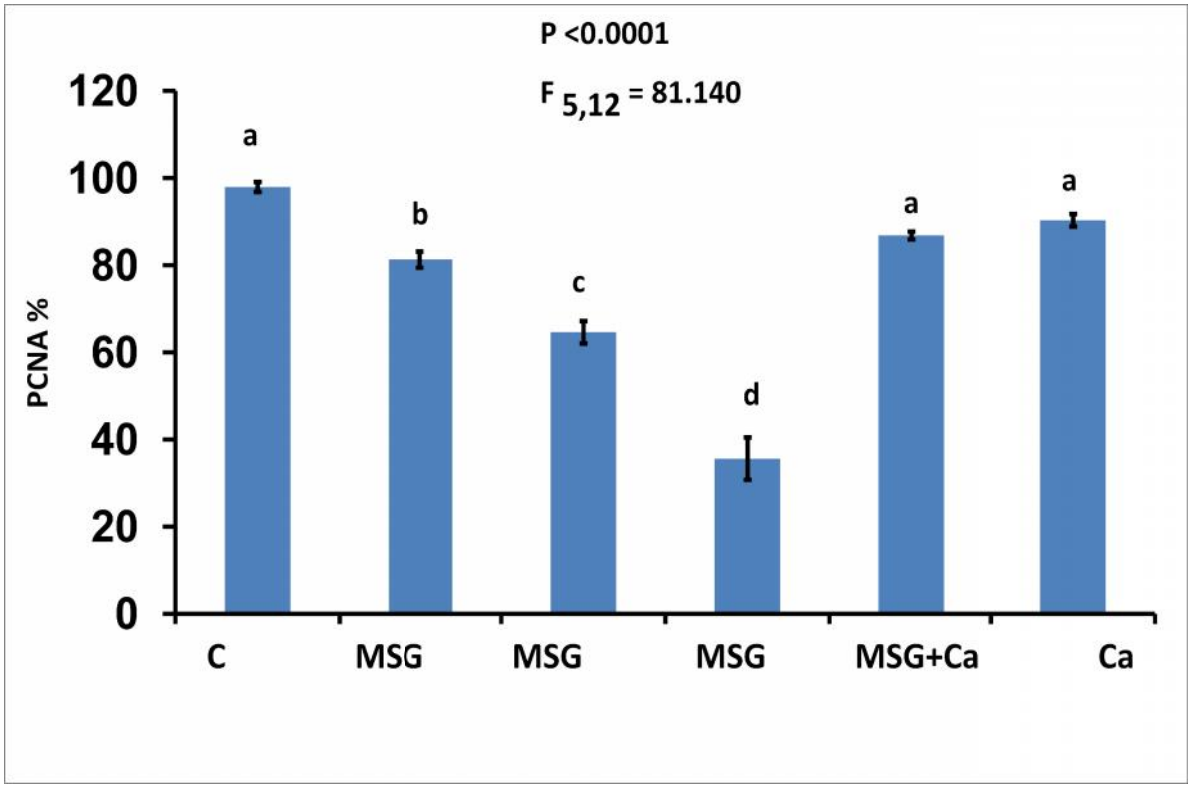
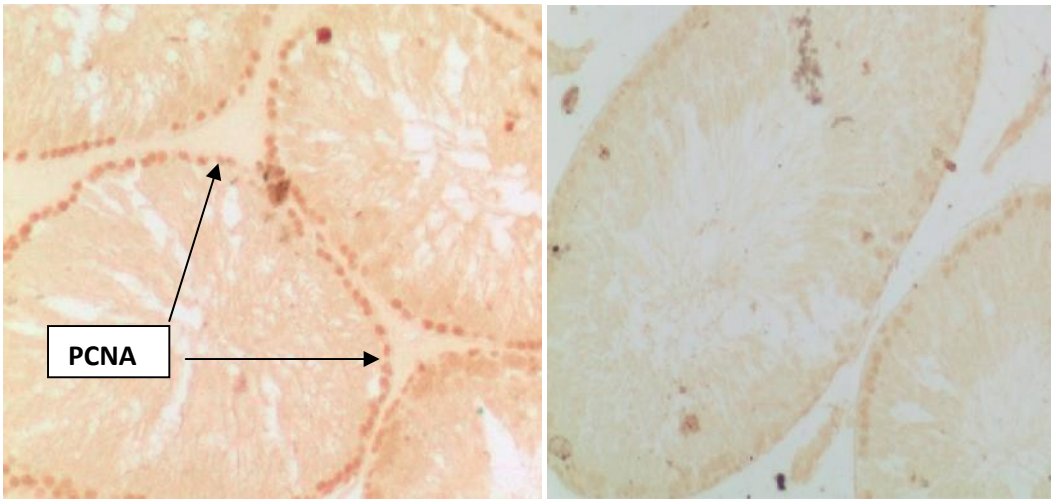
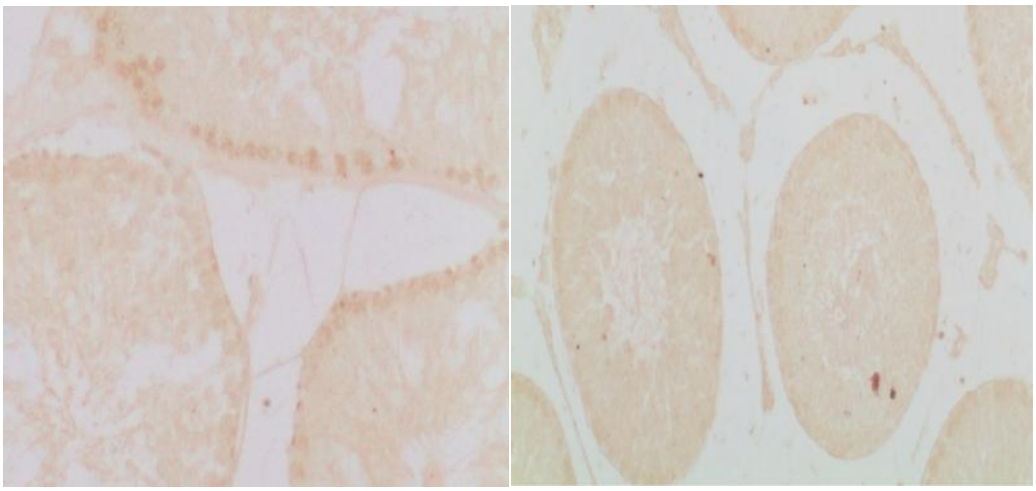


Fig 34 (A): Effect of 90 days treatment of MSG and carnitine showing staining of PCNA (%) with decreased staining of positive cells. Control, MSG 30 mg/kg, MSG 300 mg/kg, MSG 3000 mg/kg, MSG 3000 + C 100 mg/kg and C 100 mg/kg. Data represents the means ± SEM of 5 samples in each group.



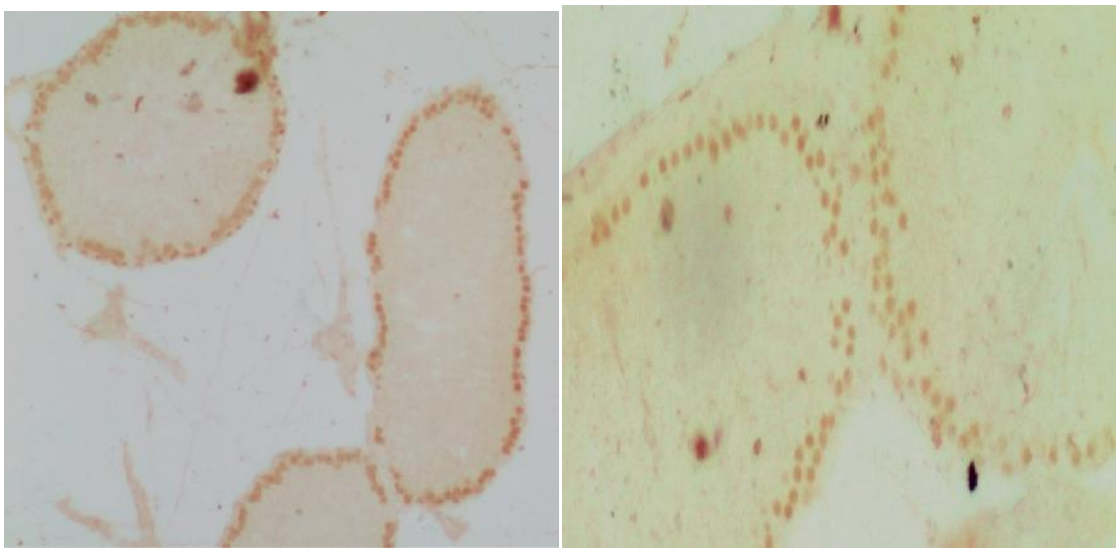
Control 30 mg/kg MSG

Fig 34 (B): Testicular section of control rats (10x) showing positive staining of PCNA and 30 mg/kg MSG with decreased positive cells.



300 mg/kg MSG 3000 mg/kg MSG

Fig 34 (C): Testicular section of MSG rats 300 mg/kg (10x) and 3000 mg/kg MSG with decreasing of positive cells and in 3000 mg/kg there is no mark of proliferating cells.



3000 mg/kg MSG+ 100 mg/kg carnitine **100 mg/kg carnitne**

Fig 34 (D): Testicular section of MSG rats 3000 mg/kg MSG +100 mg/kg carnitine (10x) with regeneration of positive cells and 100 mg/kg carnitine seminiferous tubules lined with proliferating cells.

CHAPTER 6

DISCUSSION

8.1. MSG food additive

To enjoy good quality of life, consumption of healthy and nutritious food is important. Eating food should also be pleasurable. It is therefore a common practice all over the world to amplify the taste and flavour of foods by means of food additives, especially monosodium glutamate(MSG).Potential consumers are usually attracted to industrially prepared foods because of characteristic umami taste and typical flavour imparted by MSG (Schiffman,2000). However when enhancing palatability, it is also of paramount importance to ensure that food additives will not cause harm to the consumers in acute exposure.

8.2. Effect of MSG on food and water consumption

Under normal circumstances, the acute toxicity of glutamate is very low. The oral median lethal dose (LD50) for mice and rats are 1.5 and 1.8g/kg respectively (Beyreuther,2006).The literature is full of contradictory reports on the level of toxicity of MSG in animal and human experiments. (Kondoh and Torii, 2008) reported that MSG- treated rats had a significantly higher weight gain, increased stress and high consumption of food. Hermanussen *et al*., (2006) reported that the ingestion of MSG increased body weight gain food consumption in rats, implying that MSG could cause obesity and metabolic disorders. The sperm count had decreased with increased treatment of MSG groups. The sperm abnormality had increased with increased MSG doses and decreased with the carnitine treated groups. No significant changes were observed in acute treatment.

8.3. Lipid peroxidation and antioxidant status

Lipid peroxidation is one of the main process of oxidative damage, which plays a critical role in the toxicity of many xenobiotic (Ongjanovic *et al*., 2010).It was evaluated by assessment of TBARS (Qiao*et al*., 2005). In the present study, TBARS levels also increased in the MSG

produces reactive oxygen species. Therefore, antioxidant enzymes could play a crucial role on MSG toxicity (Fabio *et al*., 2012) Our results was in harmony with (Tezcan *et al*., 2003) who declared that MDA is one of the final decomposition of the cyclooxygenase reaction in prostagland in metabolism and this assure our finding who conclude the presence of oxidative stress in rats treated with MSG in which there was a high level of MDA.

We demonstrated that the major reason for damage of testicular tissues is the increasing level of lipid peroxidation and these findings was in parallel with (Aitken *et al*., 1989) who reported that the increased lipid peroxidation led to oxidative damage to sperms DNA, alter membrane functions, impair motility and possibly have a significant effect on the development of spermatozoa. Possibly, the toxic effects of MSG on the spermatozoa physiology and biochemical parameters might be related to the increased production of free radicals in the rat reproductive organs.

There is a defense system which consists of antioxidant enzymes such as SOD and CAT (Celik *et al*., 2009; Uzun *et al*., 2010; Demir *et al*., 2011). The present investigation revealed that MSG caused significant decrease in SOD, CAT activities and these findings are greatly in accordance with (Fabio *et al*.,2012) who reported reduction in SOD after administration in these parameters after combination with carnitine. These enzymes are also considered as an important indicator of the balance status between the first and second step of the enzymatic antioxidants pathway (Jihen *et al*., 2009). The testis, epididymis, sperm and seminal plasma contain hydrogen peroxide; CAT converts hydrogen peroxide into water (Mansour and Mossa. 2009). Therefore, SOD-CAT system provides the first defense system against oxidative stress and these enzymes work together to eliminate active oxygen species (El-Demerdash., 2011; Wafa *et al*., 2011).

In this study, the cleared decrease of SOD, CAT, GSH and GST enzymes in MSG treated group may be due to the consumption during the breakdown of free radicals and high level of H_2O_2 or the inhibition of these enzymes by these radicals. Thus, the changesin oxidative defense systems and increase the level of oxidants in the testis tissues associated with MSG exposure leading to increase lipid peroxidation.

MSG may also affect male reproductive function (Alalwani *et al*., 2013). In this study MSG caused several histopathological changes like spermatogenic arrest, edema, and hypospermia. It may be related to oxidative effects of MSG on testis cell membrane and also testis tissues.

Oxidative damage primarily occurs via production of reactive oxygen species such as superoxide anion, peroxides, and it can damage o lipids, proteins and DNA. Therefore, it may cause to loss of enzymatic activity and structural integrity of enzymes and activate inflammatory processes (Ozyurt *et al*., 2004). It is suggested that toxic effects of MSG lead to alterations in the structural integrity of mitochondrial inner membrane, resulting in the depletion of mitochondrial GSH levels and increased formation of hydrogen peroxide by the mitochondrial electron transport chain (Sener *et al*., 2003). Oxidative stress plays formation, function, sperm count profile and male infertility (Acharya *et al*., 2008; Shen and Ong., 2007). Decreased reduced glutathione (GSH) level and decreased the activities of glutathione-s-transferase (GST), catalase and superoxide dismutase (SOD) in the liver of the animals; these were observed in MSG treated groups. The activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were also significantly increased in the serum on MSG administration. sCarnitine co-administered with MSG, significantly increased the GSH level, catalase and SOD. Farombi and Oneyma, 2006). Simultaneous administration of carnitine to MSG-treated rats significantly reduced this increase in MDA induced by MSG. Carnitine reduced lipid peroxidation most in the testis. (Oneyma *et al*., 2006).

8.4. Effects on biochemical parameters

Administration of 100 mg/kg L-carnitine can significantly increase carnitine levels in young rat serum, liver, kidney, heart, and skeletal muscle. A recent study showed that a daily dose of 100 mg/kg body weight ALC in drinking water elevated total carnitine levels in plasma, heart, skeletal muscle, and cerebral cortex in rats.

As cholesterol is known to be a precursor of steroid hormones (Stocco, 1998; Hu el al ., 2010), the lowered serum cholesterol levels maybe due to lowered testosterone levels in the MSG treated rats.

The significantly lower serum cholesterol recorded for the rats treated with MSG in this study is thought to be related to the reported damage to the arcuate nucleus of the hypothalamus in animals given MSG as the arcuate nucleus of the hypothalamus had been reported to partly function in the regulation of fat metabolism- 136 (Dieguez *et al*., 2011). This lowered serum cholesterol is believed to be partly responsible for the significantly lower serum testosterone recorded for the MSG-treated rats, as testosterone is one of the steroid hormones synthesised from cholesterol (Stocco, 1998; Hu *et al*., 2010). The significantly lower serum cholesterol recorded for rats given MSG in this study is in agreement with the report of (Bazzano *et al*., 1970) on rats and gerbils.

The serum urea nitrogen is measure of renal function. Normally, the serum urea nitrogen level rises in the heart failure, dehydration, or a high protein diet and urea nitrogen level can be seen in liver and renal damage or in liver diseases (Johnson *et al*., 1972).A change in serum creatinine level is also an indicator of kidney function. The significant increase in the creatinine content of the serum following the administration of MSG may be attributed to compromise of renal function capacity. MSG might have either interfered with creatinine metabolism leading to increased synthesis or the tissue might have compromised all or part of its function which might be due to oxidative stress induced by MSG on the renal tissue. AST increased with MSG treated

groups and decreased with control and carnitine treated groups.ALT increased with increased treatment with MSG groups and decreased with control and carnitine treated groups. AP increased with MSG treated groups and decreased with control and carnitine treated groups. (Maluly *et al*., 2013). No significant changes were observed in acute treatment.

8.5. Histological and Immunohistochemical analysis

H&E stain contain haemotoxylin and eosin. Haemotoxylin is basic stain. It will stain basophilic component, such as nuclei as dark blue (Kiernan, 2008). Meanwhile, eosin, which is acidic stain, will stain acidophilic substrate as pinky red in different degree (Kiernan, 2008). For instance, cytoplasm which fulfilled with granular mass is stained pink (Kiernan, 2008); collagen and muscle is stained pink (Kiernan, 2008) and erythrocyte (red blood cell) is stained intensely red (Kiernan, 2008).The fixation, processing, embedding and staining process make the tissue more suitable to be observed microscopically. Fixation keeps the cell in their original form while halted the effect of autolysis and putrefaction. Processing and embedding process alter the hardness of tissue, give sufficient support on tissue and allow the smooth cutting on tissue substrate. Staining process attach coloured compound on specific tissue substrates, rise different colours on the different parts of tissue based on their tissue affinity, so that viewing and distinguish of tissue substrates become clearer. Suitable fixative agent and staining solution was chosen so it caused irreversible effect on tissue substrate. The immunuhistochemical evaluation showed great differences in the positivity of staining among the experimental groups. PCNA was used as the marker where the testis sections of the MSG treated groups showed slightly faint staining nuclei indicating mild cell division of the spermatogonia. No significant changes were observed in acute treatment.

CHAPTER7

Summary

- The present study was designed to examine the sperm parameters, oxidative stress and antioxidant effects, biochemical parameters, histological and immunohistochemical analysis of carnitine on MSG induced testicular toxicity in acute (30 days) and chronic (60 and 90 days) treatment.

- The higher the dose of monosodium glutamate intake by the rats, the higher is the food and water consumption, increased in body weight and organ weight, decreased in motility % of sperms. In 30 days treatment no significant changes were observed in food and water consumption.

- The rectal temperature was effected with either of the treatment groups, also the blood glucose levels was significantly higher in the MSG treated rats. In 30 days treatment no significant changes were observed in rectal temperature and blood glucose levels.

- Elevated results of lipid peroxidation was observed in MSG treated groups and decreased in carnitine treated groups which results in the decrease of the activity of the antioxidant enzymes CAT, SOD, GSH and GST in the acute and chronic treatment.

- AST increased with MSG treated groups and decreased with control and carnitine treated groups. ALT increased with increased treatment with MSG groups and decreased with control and carnitine treated groups. AP increased with MSG treated groups and decreased with control and carnitine treated groups. Their increased may be mainly due to

liver damage in 90 days treatment. No significant changes were observed in chronic treatment (30 days).

- The significant increase in the creatinine and urea content of the serum following the administration of MSG may be attributed to compromise of renal function capacity. Cholesterol levels decreased in MSG treated groups which may be due to decrease in the level of testosterone. No significant changes were observed in acute exposure.

- Carnitine is an essential nutrient which plays a major role in cellular energy production and has been considered a promising cytoprotective agent.

- Carnitine was orally induced to rats at 100 mg/kg dose for 30 days, 60 days and 90 days which greatly affected the sperm motility, sperm count and daily sperm production (DSP).

- The sperm count and daily sperm production decreased with increase in the MSG treated groups since it has toxic effect in the testis, which may ultimately lead to reduced fertilitybut increased with the carnitine treated groups.

- Carnitine is a very important antioxidant which helps in DNA repair, germ cell recovery and sertoli cell metabolism.

- The sperm abnormalities in MSG group was significantly higher as compared with MSG (3000 mg/kg) + Carnitine (100 mg/kg) and Carnitine (100 mg/kg).

- Oxidative stress was reduced by carnitine and antioxidant enzymes were elevated in acute and chronic treatment groups.

- The changes caused by MSG with respect to biochemical parameters was attenuated by carnitine exposure.

- Carnitine treatment greatly improved MSG induced histopathological changes in rat testis in the acute (30 days treatment) and chronic (60 and 90 days treatment).

- Immunohistochemical changes were observed in 90 days treatment resulting in the degeneration of proliferating cells in MSG treated groups and regeneration of positive cells in carnitine treated groups

CHAPTER 8

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Workshop attended

- **SEMINAR ON SCIENCE FOR NATION BUILDING** catalysed and supported by the National Council for Science & Technology Communication, Department of Science and Technology, New Delhi on 1 October 2015 at Pi Zaii Hall, Synod Conference Centre, Aizawl, Mizoram. Mizo Academy of Sciences in collaboration with the Directorate of Science and Technology, Government of Mizoram.

- International Workshop on Snakebite Management held during 27th June – 3rd July organized by Department of Zoology and Biotechnology, Mizoram University sponsored by State Biotech-Hub facility, Department of Biotechnology (DBT), New Delhi.

- One Week Course on Research Methodology for Research Scholars held from 20th – 26th June, 2016, University Grants Commission Human Resource Development Centre, Mizoram University UGC – sponsored Short Term Course.

Table 6: Effect of 30 days treatment at different doses (30mg/kg, 300 mg/kg, 3000 mg/kg MSG, 3000 mg/kg MSG+100mg/kg carnitine and 100 mg/kg carnitine) on sperm morphology of rats.

Parameters	Experimental groups					
	control	30 mg/kg MSG	300 mg/kg MSG	3000 mg/kg MSG	3000+100 mg/kg MSG + carnitine	100mg/kg carnitine
Normal	90.22 (3.49-19.43) a	78.11 (4.54-11.98)b	61.89 (2.26-9.07)c	61.37 (0.72-7.97)c	75.23 (0.05-11.22)d	81.88 (5.47-10.04)e
Head abnormalities						
Amorphous	0.99 (0.00-0.33)a	2.88 (0.00-0.73)b	3.27 (0.10-0.90)c	6.17 (0.16-1.23)d	2.61 (0.00-0.63)b	1.88 (0.08-0.35)e
Banana	0.77 (0.00-0.22)a	1.90 (0.00-0.73)b	3.78 (0.00-0.90)c	4.37 (0.00-0.89)d	2.96 (0.00-0.75)e	2.06 (0.00-0.62)f
Detached	0.88 (0.00-0.33)	1.78 (0.00-0.73)	4.58 (0.00-0.95)	2.41 (0.00-0.67)	2.09 (0.00-0.52)	0.80 (0.00-0.26)
Total	2.66 (0.11-0.44)	6.57 (0.06-1.04)	11.64 (0.40-1.91)	12.97 (0.72-1.74)	7.67 (0.11-1.74)	4.75 (0.26-0.98)

Tail abnormalities						
Coil	1.44 (0.00-0.44)	1.96 (0.00-0.55)	5.69 (0.15-0.90)	2.75 (0.00-0.67)	2.67 (0.00-0.69)	2.60 (0.00-0.71)
Broken	0.77 (0.00-0.16)	2.39 (0.00-0.49)	2.21 (0.00-0.55)	3.59 (0.11-0.72)	2.03 (0.00-0.46)	1.70 (0.00-0.35)
Total	2.22 (0.05-0.44)	4.36 (0.18-0.73)	7.40 (0.35-1.10)	6.34 (0.11-1.06)	4.70 (0.11-1.41)	4.30 (0.00-0.07)

Data are expressed as Mean ± SEM. N = 5 for the control; N = 5 for treated group; Mean of treated group is compared with the corresponding mean of the control. P > 0.05 indicates indicate a non-significant difference between the compared means. No symbol = non-significant. No symbol = non-significant, a, b, c, d, e, f indicate significant values.

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Table 13: Effect of 60 days treatment at different doses (30mg/kg, 300 mg/kg, 3000 mg/kg MSG, 3000 mg/kg MSG+100mg/kg carnitine and 100 mg/kg carnitine) on sperm morphology of rats.

Parameters	Experimental groups					
	control	30 mg/kg MSG	300 mg/kg MSG	3000 mg/kg MSG	3000+100 mg/kg MSG+ carnitine	100 mg/kg carnitine
Normal	79.60 (4.09-12.91)a	69.13 (3.62-10.25)b	62.69 (4.20-7.05)c	52.17 (0.74-13.90)d	71.72 (4.87-9.41)e	72.73 (2.16-11.73)e
Head abnormalities						
Amorphous	1.89 (0.00-0.43)a	1.86 (0.00-0.46)a	3.36 (0-0.77)b	4.49 (0.23-0.74)c	3.26 (0.00-0.58)b	3.71 (0.00-0.97)b
Banana	2.37 (0.00-0.60)a	1.96 (0.00-0.56)b	3.95 (0-1.03)c	5.00 (0.00-1.01)d	3.90 (0.14-0.78)c	4.07 (0.00-1.28)e
Detached	1.55 (0.00-0.52)a	3.78 (0.00-0.67)b	4.08 (0.12-1.10)c	5.23 (0.13-0.83)d	3.07 (0.00-0.78)b	1.50 (0.00-0.35)a

Total	5.85 (0.13-1.08)a	7.61 (0.15-1.24)b	11.39 (0.32-2.07)c	14.73 (0.50-2.03)d	10.23 (0.34-1.90)e	9.29 (0.2-2.43)f
Tail abnormalities						
Coil	1.42 (0.00-0.52)a	4.24 (0.10-1.13)b	4.08 (0-0.77)b	4.12 (0.00-0.83b	2.53 (0.00-0.78)c	2.21 (0.00-0.39)c
Broken	2.93 (0.00-0.82)a	3.57 (0.15-0.62)b	3.17 (0.12-0.71)b	5.05 (0.13-0.83)c	1.36 (0.00-0.29)d	2.12 (0.00-0.53)a
Total	4.35 (0.01-1.03)a	7.81 (0.36-1.76)b	7.25 (0.19-1.23)b	9.17 (0.23-1.52)c	3.90 (0.09-1.07)d	4.33 (0.04-0.92)a

Data are expressed as Mean ± SEM. N = 5 for the control; N = 5 for treated group; Mean of treated group is compared with the corresponding mean of the control. $P < 0.05$ indicates a significant difference between the compared means.

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Table 20: Effect of a chronic treatment for 90 days at different doses (30mg/kg, 300 mg/kg, 3000 mg/kg MSG, 3000 mg/kg MSG+100mg/kg carnitine and 100 mg/kg carnitine) on sperm morphology of rats.

Parameters	Experimental groups					
	control	30 mg/kg MSG	300 mg/kg MSG	3000 mg/kg MSG	3000+100 mg/kg MSG+ carnitine	100 mg/kg carnitine
Normal	68.90 (2.30-10.05)a	64.83 (4.59-9.87)b	55.85 (2.89-14.59)c	48.64 (0.66-8.52)d	71.63 (2.57-8.87)e	74.13 (4.01-10.56)f
Head abnormalities						
Amorphous	4.46 (0.15-0.87)a	3..25 (0.00-0.59)b	6.86 (0.28-1.22)c	4.48 (0.05-1.16)a	3.01 (0.04-0.61)b	4.18 (0.00-1.22)a
Banana	3.33 (0.00-0.66)a	4.09 (0.14-0.79)b	5.85 (0.21-0.86)c	4.64 (0.00-0.66)b	1.66 (0.00-0.34)d	3.33 (0.00-0.67)a
Detached	2.61 (0.15-0.41)a	3.80 (0.14-0.69)b	4.47 (0.00-0.86)c	5.81 (0.27-0.94)d	3.10 (0.00-0.78)b	1.35 (0.00-0.38)e
Total	10.41 (0.41-1.69)a	11.16 (0.59-1.62)a	17.19 (1.22-2.23)b	15.48 (1.05-2.60)c	7.91 (0.34-1.26)d	8.87 (0.59-1.47)d

Tail abnormalities						
Coil	2.10 (0.00-0.46)a	3.40 (0.14-0.93)b	6.43 (0.00-1.15)c	4.03 (0.00-0.83)d	3.62 (0.08-0.69)b	2.83 (0.04-0.50)a
Broken	3.02 (0.00-0.82)a	3.01 (0.00-0.59)a	4.69 (0.21-1.15)b	6.41 (0.38-0.88)c	2.53 (0.00-0.52)d	1.22 (0.00-0.38)e
Total	5.13 (0.05-1.07)a	6.41 (0.29-1.33)b	11.12 (0.72-1.73)c	10.04 (0.38-1.66)d	6.51 (0.43-1.22)b	4.05 (0.08-0.76)a

Data are expressed as Mean ± SD. N = 5 for the control; N = 5 for treated group; Mean of treated group is compared with the corresponding mean of the control. $P < 0.05$ indicates a significant difference between the compared means. No symbol = non-significant, a, b, c, d, e, f indicate significant values.

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INTRODUCTION:

Monosodium glutamate

Monosodium glutamate (MSG) which is called as AJI – NOMOTO is the sodium salt of glutamic acid (Eweka, 2007). It is used worldwide as a flavour enhancing food additive especially in Chinese, Thainese and Japanese foods (Ikeda, 1917). As of 2009, the world production of MSG was estimated to be 2 million tonnes per year (Sahelian, 2014). The consumption of MSG had been reported to be associated with numerous adverse effects (Raiten, 1995; Food Standards Australia New Zealand,2003). MSG is locally and globally used as favor enhancer; it may be present in packaged foods without appearing on the label. Glutamate is a naturally occurring amino acid that is one of the most abundant amino acid in the Central Nervous System (CNS). MSG contains 78% of glutamic acid and 22% of sodium water (Samuels, 1999).

MSG is abundant and excess in other food ingredients without otherwise appearing on the label. This shows that MSG can be taken unknowingly and without intention. It can remain subtle but the damage caused can never be neglected. Thus, it could be abused inadvertently. It is marketed under such trade names including A- One, Ajinomoto or Vedan and is a popular condiment in West African dishes (Obaseiki – Ebor *et al*., 2003).

MSG added to foods produces a flavouring function similar to the glutamate that occurs naturally in foods. It adds a fifth taste, called "umami" which is best described as a savoury, broth – like or meaty taste, although traditional East Asian cuisine had often used seaweed extract, which contains high concentrations of glutamic acid, it was not until 1907 that MSG was isolated by Ikeda. It was subsequently patented by Ajinomoto Corporation of Japan in 1909. In its pure form, it appears as a white crystalline powder that, as a salt dissociates into sodium cations and glutamate anions

while dissolving glutamate is the anionic form of glutamic acid.(Sano, 2009). Modern commercial MSG is produced by fermentation of starch, sugar, beet sugarcane or molasses (Walker and Lupien, 2000).

L-Carnitine

L-Carnitine is quaternary ammonium compound biosynthesized from the amino acids lysine and methionine. It is a trimethylated amino acid roughly similar in structure to choline, facilitates the transfer of activated long-chain fatty acids from the cytoplasm to the mitochondria, where they are processed by oxidation to produce ATP (Steiber *et al*., 2004). The carnitines exert a substantial antioxidant action, thereby providing a protective effect against lipid peroxidation of phospholipid membranes and against oxidative stress induced at the myocardial and endothelial cell level (Cavazza, 2002). L-carnitine also acts as an antioxidant to prevent the oxidative damage of sperms, improving sperm quality (Lenzi *et al*., 2003) and shown to have beneficial effects in the treatment of varicocele, a major cause of male infertility (Seo *et al*., 2003). Also, supplemental doses of L-carnitine effectively counteracts the toxic effects of chronic nicotine administration on thyroid, liver, heart, bone, muscle, urinary bladder, and kidney functions and attenuates the oxidative damage possibly by its antioxidant action (Huang *et al*., 1999; Zadeh *et al*., 2008).

The sperm parameters include the sperm concentration in testis and epididymus. The percentage of motile sperms were counted in treatment groups with respect to control. Nayatara et al., (2008) recorded MSG reduction in testicular weight and decrease in the sperm count in rats treated with MSG.

Oxidative damage primarily occurs via production of reactive oxygen species such as superoxide anion, peroxides, and it can damage to lipids, proteins and DNA. Therefore, it may cause

to loss of enzymatic activity and structural integrity of enzymes and activate inflammatory processes (Özyurt et al 2004).

The antioxidant enzymes include SOD, CAT, GSH and GST. These enzymes are also considered as an important indicator of the balance status between the first and second step of the enzymatic antioxidant pathway (Jihen *et al* .,2009.

'Histology' term is derived from Greek: Histos means tissue while Logia means study of or knowledge. By refer to these terms, it actual refer to study of tissues for living organism.

Biochemical parameters include (ALT, AST, cholesterol, AP, creatinine and Urea,) in which blood sample have been used in the experiment.

Proliferating cell nuclear antigen (PCNA) is a nuclear non histone protein, with a molecular weight of 36kD that plays a role in the initiation of cell prolifera tion by the augmenting of DNA polymerase; stains for proliferating cell nuclear antigen in tumors correlated with grade and mitoticactivity. .PCNA was originally identified as an antigen that is expressed in the nuclei of cells during the DNA synthesis phase of the cell cycle. (Leonardi *et al.,*1992).

Review of Literature

There are some reports which indicated that MSG was toxic to human and experimental animals. MSG could produce symptoms such as numbness, weakness, flushing, sweating, dizziness and headaches. In addition, ingestion of MSG has been alleged to cause or exacerbate numerous conditions, including asthma, urticaria, atopic dermatitis, ventricular arrhythmia, neuropathy and abdominal discomfort (Geha *et al*., 2001). MSG has a toxic effect on the testis by causing a significant oligozoospermia and increases abnormal sperm morphology in a dose-dependent fashion in male Wistar rats (Onakewhor *et al*., 1998).

Antioxidants have been reported to play a significant role in the protection against lipid peroxidation. L-carnitine is antioxidant that are thought to have a protective effect by either reducing or preventing oxidative damage (Rinne *et al*., 2000). It is well known as non-enzymatic antioxidant (Al-Attar, 2011; Uzun *et al*., 2009). L-carnitine inhibits peroxidation of membrane lipids by scavenging lipid peroxylradicals (El-Demerdash *et al*., 2004; Uzunhisarcikli and Kalender, 2011) and it also inhibits oxidative damage inseveral tissues by heavy metals and pesticides in experimental animals (El-Demerdash *et al*., 2004; Acharya *et al*., 2008).

Objectives

The main objectives of the present work is to study the effect of L-carnitine on reproductive parameters of MSG fed with Wistar albino rats and their role in amelioration of the testicular toxicity induced by MSG in relation to:

- Analysis of sperm parameters (sperm morphology, motility, daily sperm production, sperm concentration).
- Evaluation of the oxidative damage and antioxidant status in testes .
- Histopathological and immunohistochemical changes in testis.

MATERIALS AND METHODS

Animals

This study was performed on mature male Wistar rats, weighing about 150–200g BW. They was breeding in a well-ventilated room with the temperature ranging between 22 and 25°C and maintained under standardized conditions away from any stressful conditions with 12:12 light and dark cycle with free access to humidity and was fed balanced meal for experimental animals provided. All experimental procedures and animal maintenance was conducted in accordance with the accepted standards of animal care per cage for the protection of vertebrate animals (Ethics Committee, Mizoram University, Permit No. MZUIAEC 16-17-06). One group served as control. Animals were weighed and randomly allocated into 6 groups (20 rats each) as following.

Experimental protocols

Rats were divided into six groups consisting of 5 animals each. The experiment was performed during 30, 60 and 90 days.

Group 1-control rats;

Group 2-MSG treated rats (30mg/kg BW per day in distilled water);

Group 3-MSG-medium dose treated rats (300 mg/kg BW per day in distilled water);

Group 4-MSG-high dose treated rats (3000 mg/kg BW per day in distilled water);

Group 5-MSG-high dose treated rats (3000 mg/kg BW with 100 mg L- carnitine);

Group 6-rats were treated with 100 mg L- carnitine.

The doses were administered in the morning (between 9.30 and 10.30 h) to non-fasted rats. The first day, when the animals was treated was considered experimental day 0. At the end of the 90 days of treatment, all animals was scarified and dissected. The testis tissues was quickly processed for light microscope investigations and biochemical examinations.

Sperm analysis

The technique of Wyrobek et al., (1984) was adopted for sperm abnormality. To evaluate the sperm abnormalities, the suspension was stained with eosin, dropped on slides to determine the motility, abnormality (using Olympus microscope by objective (40x). Abnormality was classified in head and tail. Abnormal sperm cells was counted and the percentage calculated. To assess motility, the sperms was classified as motile sperm (M) and non-motile (NM). To evaluate the daily sperm production in testes, spermatozoa was counted as per the standard protocol of WHO laboratory manual (1992). Motile sperm cells was counted and the percentage was calculated**.**

Determination of oxidative stress (lipid peroxidation) and Antioxidant enzymes

Lipid peroxidation (MDA) (Satoh et al., 1978), superoxide dismutase (SOD) (Asada et al., 1974), catalase (CAT) (Aebi, 1984), reduced glutathione (GSH) (Rahman et al., 2007), and glutathione S–transferase (GST) (Habig et al., 1974) was estimated by following standard protocols.

Analysis of biochemical parameters

Estimation of SGPT (ALAT) activity: (Reitman &Frankel‘s method)

Estimation of SGOT (ASAT) activity: (Reitman & Frankel’s method)

Estimation of Cholesterol: (CHOD/ PAP method)

Alkaline Phosphatase: (Mod. Kind & King’2 method)

Estimation of Creatinine: (Alkaline Picrate method)

Estmation of Urea: (Mod.Berdthelodt method)

Histopathological and immunohistochemical study of the testes

One testis of each rats was removed and quickly fixed in Bouins fluid for at least 24 hs. The fixed specimens was processed through the conventional paraffin embedding technique, sectioned at 5µm and stained with hematoxylin and eosin (Bancroft and Gamble, 2002) for histopathological studies. Histological specimens was used for immunohistochemical studies by using respective antibodies as per the manufacturer`s instructions.

Statistical analysis

Data was collected, arranged and reported as mean ± standard error of mean (SEM) of six groups, and will be analyzed using the computer program (SPSS/version15.0). The statistical method was one way analyzes of variance ANOVA test, and if significant differences between means will be found, Duncan's multiple range test (Whose significant level was defined as $P < 0.05$) according to (Snedecor and Cochran, 1982) to estimate the effect of different treated groups.

Summary of results

- The present study was designed to examine the sperm parameters, oxidative stress and antioxidant effects, biochemical parameters, histological and immunohistochemical analysis of carnitine on MSG induced testicular toxicity in acute (30 days) and chronic (60 and 90 days) treatment.

- The higher the dose of monosodium glutamate intake by the rats, the higher is the food and water consumption, increased in body weight and organ weight, decreased in motility % of sperms. In 30 days treatment no significant changes were observed in food and water consumption.

- The rectal temperature was effected with either of the treatment groups, also the blood glucose levels was significantly higher in the MSG treated rats. In 30 days treatment no significant changes were observed in rectal temperature and blood glucose levels.

- Elevated results of lipid peroxidation was observed in MSG treated groups and decreased in carnitine treated groups which results in the decrease of the activity of the antioxidant enzymes CAT, SOD, GSH and GST in the acute and chronic treatment.

- AST increased with MSG treated groups and decreased with control and carnitine treated groups. ALT increased with increased treatment with MSG groups and decreased with control and carnitine treated groups. AP increased with MSG treated groups and decreased with control and carnitine treated groups. Their increased maybe mainly due to liver damage in 90 days treatment. No significant changes were observed in chronic treatment (30 days).

- The significant increase in the creatinine and urea content of the serum following the administration of MSG may be attributed to compromise of renal function capacity. Cholesterol levels decreased in MSG treated groups which may be due to decrease in the level of testosterone. No significant changes were observed in acute exposure.

- Carnitine is an essential nutrient which plays a major role in cellular energy production and has been considered a promising cytoprotective agent.

- Carnitine was orally induced to rats at 100 mg/kg dose for 30 days, 60 days and 90 days which greatly affected the sperm motility, sperm count and daily sperm production (DSP).

- The sperm count and daily sperm production decreased with increase in the MSG treated groups since it has toxic effect in the testis, which may ultimately lead to reduced fertility but increased with the carnitine treated groups.

- Carnitine is a very important antioxidant which helps in DNA repair, germ cell recovery and sertoli cell metabolism.

- The sperm abnormalities in MSG group was sifnificantly higher as compared with MSG (3000 mg/kg) + Carnitine (100 mg/kg) and Carnitine (100 mg/kg).

- Oxidative stress was reduced by carnitine and antioxidant enzymes were elevated in acute and chronic treatment groups.

- The changes caused by MSG with respect to biochemical parameters was attenuated by carnitine exposure. No observable changes were observed in 30 days treatment.

- Carnitine treatment greatly improved MSG induced histopathological changes in rat testis in the acute (30 days treatment) and chronic (60 and 90 days treatment).

- Immunohistochemical changes were observed in 90 days treatment resulting in the degeneration of proliferating cells in MSG treated groups and regeneration of positive cells in carnitine treated groups.