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Protective Effects of Magnesium Chloride on Liver Enzymes and Biomarkers of Oxidative Stress in high fat diet fed Rats

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Summary: The excessive consumption of high cholesterol diet has been associated with an increased incidence of obesity. This is because obesity induced pathologies with high mortality, such as complications of dyslipidaemia, diabetes mellitus, arthritis, hypertension, myocardial infarction, and hepatocellular carcinoma. Although the associated, disease are enhanced by formation of oxidative stress, lipid peroxidation and hypercholesterolaemia**.** Magnesium chloride is found to be beneficial in a wide range of diseases. Magnesium is one of the most neglected mineral in human body. It is crucial for a healthy and lasting life. Magnesium is responsible for the activation of more than 300 enzymes in the body. The present study intends to determine the protective effect of magnesium chloride on liver enzyme and biomarker of oxidative stress in high fat diet fed rats. Twenty (20) adult Male Wistar rats weighing (100 – 150) grams randomly divided into three treatments and one control groups of five rats each (n = 5). Group I Normal control receive normal feed only for 6weeks, Group II received high fat diet only for 6weeks, Group III received high fat diet with 250 mg/kg for 6weeks of mgcl$_2$ and Group IV received 500 mg/kg for 6weeks of $MgCl_2$ respectively all treatments were administered via oral route, at the end of the sixth week rats were euthanized and blood samples were drawn from the heart by cardiac puncture and used to estimate oxidative stress biomarkers (Superoxide dismutase, Catalase and Glutathione peroxidase), lipid peroxidation biomarkers (Malondialdehyde) and liver enzymes. Analysis of variance and Turkey's post hoc test were used to analyze the data obtained. In relation to the liver enzyme, the showed that there was a significant ($p<0.05$) decrease in value of AST, ALT and ALP in the group co-administered with the doses of the Magnesium chloride to compared to the control. For the oxidative stress biomarkers assessed, the results showed that there was significant decrease ($P < 0.05$) in the SOD, CAT and GP_x level of the high fat diet fed groups, co-administered with 250 and 500 $MgCl_2$, when compared with the high fat diet fed group only. Also, the lipid peroxidation shows significant ($p<0.05$) decrease in the groups administered the two doses of Magnesium chloride (250 and 500 mg/kg) respectively as compared to control. In relation to the liver enzyme, the showed that there were significant ($p<0.05$) changes in value of AST, ALT and ALP in the group co-administered with the two doses of the Magnesium chloride compared to the control. The result showed that high-fat diet induces ROS, dyslipidaemia and release of biological metabolite, as evidenced by the rise in oxidative stress and activities of liver enzymes. $MgCl_2$ administration also protected the body against rise in the metabolites despite consumption of high-fat diet by the Wistar Rats.

Keywords*:* High fat diet, Liver enzymes, Oxidative stress, Magnesium chloride.

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INTRODUCTION

The first description of a 'high-fat diet' to induce obesity by a nutritional intervention was in 1959 (Masek and Fabry 1959). Subsequent studies have revealed that high-fat diets promote hyperglycemia and whole-body insulin resistance, and numerous researchers have examined their effects on muscle and liver physiology as well as insulin signal transduction. From this experience, it is generally accepted that high-fat diets can be used to generate a valid rodent model for the metabolic syndrome with insulin resistance and compromised β-cell function (Oakes *et al.,* 1997; Ahren *et al.,* 1999; Lingohr *et al.,* 2002). Most studies have employed only one high-fat formula in contrast with standard chow and did not analyze the influence of the specific fat component in the model. From the sparse data comparing different high-fat diets with respect to their metabolic effects, it is generally believed that diets based on saturated fatty acids induce the typical high-fat-diet phenotype, whereas diets containing polyunsaturated ω-3 fatty acids exert beneficial effects on body composition and insulin action (Storlien *et al.,* 1991).

The excessive consumption of high cholesterol diet has been associated with an increased incidence of obesity. This is because obesity induced pathologies with high mortality, such as complications of dyslipidaemia and diabetes mellitus (Kohli *et al.,*

2010; Buettner *et al.*, 2006; Kim et al, 2011). Although the associated, disease are enhanced by formation of oxidative stress, lipid peroxidation and hypercholesterolaemia **(**Misra *et al.*, 2010) Magnesium chloride is found to be beneficial in a wide range of diseases but one of the most neglected mineral in human body. It is crucial for a healthy and lasting life and is responsible for the activation of more than 300 enzymes in the body. Excessive accumulation of body fat is one of the leading causes of death worldwide. Paststudieshave shown that dietary modifications such as low fat diets, high-fiber diets, diets rich in flavonoids and phenolic acids can reduce metabolic syndrome risk factors (Minich and Bland, 2008; Lyer *et al.*, 2009).

Cholesterol is a soft waxy substance found in animal cell membranes used for the synthesis of digestive bile acids, vitamin D and certain steroid hormones and in plant it is known as phytosterols which are believed to compete with cholesterol for absorption in the intestines (Ostlund et al.,2003; Weingartner et al.,2008). High density lipoprotein cholesterol (HDL) also known as good cholesterol which picks up excess cholesterol dropped off by low density lipoproteins, and transports it to the liver for excretion. High amount of HDL is usually of more health significance than the LDL cholesterol (Lewis and Rader2005). Since HDL helps remove cholesterol from the blood, it thus, keeps cholesterol from building up in the arterial walls, Low density lipoprotein cholesterol (LDL) also known as bad cholesterol is the major blood cholesterol carrier from the liver to the tissues. It transports lipids such as phospholipid and triglyceride within the extracellular fluids. Too much of LDL cholesterol in the body can lead to the build-up of plaque on the arterial walls (Tymoczko et al., 2002).

Magnesium is a cofactor in more than 300 enzyme systems that regulate diverse biochemical reactions in the body, including protein synthesis, muscle and nerve function, blood glucose control, and blood pressure regulation (Rude 2012). It was reported that Mg supplementation has beneficial effects on blood levels of HDL-C (Djurhuus et al.,1999), cholesterol and/ or triglycerides (Guerrero-Romero et al., 2000). Oral intake of magnesium also has beneficial effects on lipid metabolism and efficiency of insulin in maintaining glucose homeostasis in human subjects (Saris et al., 2000; Barbagallo 2003). Mg deficiency is known to decrease the level of GSH in erythrocytes (Hsu et al., 1982; Weglicki et al., 1996) and even inhibit its biosynthesis (Mills et al., 1984), and in agreement with these findings, magnesium supplementation was shown to induce a significant increase in GSH in kidney of mice treated with cadmium (Djukić-Ćosić et al., 2007). Magnesium intake is capable of decreasing the blood concentration of vanadate in rats (Ścibior et al., 2012) and the cadmium level in blood, kidney, spleen, and bone marrow in rabbits (Bulat et al., 2008). In addition, both oral and intraperitoneal supplementation of magnesium acetate were effective against cadmium toxicity (Matović et al., 2012). Other findings suggest that magnesium chenodeoxycholic acid (Mg-CUD) may prevent liver fibrosis induced by CCl_4 (Kang et al., 2012). Moreover, magnesium has been shown to have protective effects against oxidative stress observed in experimental animals with different pathologies (Hans et al., 200; Zhang et al., 2003). Magnesium supplementation appears to attenuate the hepatotoxicity of CCl_4 as reported by Eidi et al. (2014) and prevent liver fibrosis ((Kang et al., 2012). The nephroprotective effect of Magnesium has also been well documented (Bulat et al., 2008) and seems to be related to its property of scavenging free radicals before the occurrence of damage to cellular macromolecules.

The aim of this research is to investigate the protective effects of Magnesium chloride on liver enzymes and biomarkers of oxidative stress in rats fed on high fat diet.

MATERIALS AND METHODS

Chemical used

All chemicals were obtained commercially and were of analytical grade: (Cholesterol: Sigma chemical Company St. Louis USA) and Magnesium Chloride (Sigma Aldrich).

Animals and induction of Diabetes

Wistar rats, weighing between 100 – 150g, were used for the study. They were bred and purchased in the animal house of the Department of Human Physiology, Ahmadu Bello University (ABU), Zaria and according to the Principle of Laboratory Animal Care, ABU, Zaria, Nigeria. The animals were kept in well-aerated laboratory cages at room temperature (25-26°C) in the animal house. They were fed with growers' and starters' mash (Vital Feeds Company, Kaduna, Nigeria), and given access to drinking water during the stabilizing period. High fat diet diet was induced by feeding the rats with standard animal feed + high- fat diet (10% groundnut oil, 20% groundnut meal and 2% cholesterol) according to Kolawole et al, (2012) with slight modification. The animals were fed with the high fat diet for a period of eight (8) weeks.

Experimental design

Twenty (20) Wistar rats weighing between 100g-150g were used for the study. The rats were randomly divided into 4 groups of five (n = 5) animals in each

Group 1: Normal control received normal feed only

Group 2: High fat diet control untreated received high fat diet only, for a period of six weeks

Group 3: High fat diet + 250mg/kg $MgCl_2$ orally for a period of six weeks (Ige *et al.*, 2016)

Group 4: High fat diet + 500mg/kg $MgCl_2$ orally for a period of six weeks

Blood Sample Collection and Serum Preparation
At the end of the six weeks of administration period, the rats were euthanized by cervical dislocation and blood samples were collected from the animals through cardiac puncture. About 5 mL of blood were collected into specimen bottles and allowed to clot and separated by centrifugation at 3,000g for 10 minutes using Centrifuge Hitachi (Universal 32). The supernatant obtained were used for the determinations of oxidative stress biomarkers and liver enzyme.

Determination of Liver Enzyme Activity
Activities of serum alanine amino transaminase (ALT) was estimated by method adopted by Tietz (I995), aspartate amino transaminase (AST) was determined by method of Henderson and Moss (2001), while alkaline phosphatase (ALP) was determined according to the method of Scherwin (2003). All tests were carried out using ELITECH clinical system kits.

Determination of Biomarkers of Oxidative Stress assay:

Superoxide Dismutase Activity
Activity of SOD in the rat serum was determined using NWLSS SOD assay kit (Product NWK-SOD02, Specificity: Cu/Zn, Mn and Fe Superoxide Dismutase, Sensitivity: 5 U/mL). The assay kit is based on the principle of superoxide inhibition of auto oxidant of hematoxylin as described by Martin et al. (1987).

Catalase Activity
Catalase (CAT) activities were assayed by the method of Sinha (1972). 0.1 mL of Plasma and 1.5 mL of phosphate buffer were added. To this, 0.4 mL of hydrogen peroxide was added and the reactions were arrested after 30 and 60 second by the addition of 2.0 mL dichromate acetic acid reagent. A control was also carried out simultaneously. All the tubes were heated in a boiling water bath for exactly 10 min, cooled and absorbance read at 620 nm. Standards in the range of 2-10 mmoles were taken and processed as the test. The activities of catalase were expressed as μmoles of hydrogen peroxide consumed/min/mg of protein (unit per milligram of protein).

Glutathione Peroxidase
The NWLSSTM Glutathione Peroxidase Assay kit was used which is an adaptation of the method of Paglia and Valentine (1967). Glutathione peroxidase catalyzes the reduction of hydrogen peroxide (H_2O_2), oxidizing reduced glutathione (GSH) to form oxidized glutathione (GSSG). GSSG was then reduced by glutathione reductase (GR) and β-nicotinamide adenine dinucleotide phosphate (NADPH) forming $NADP^+$ (resulting in decrease absorbance at 340nm) and recycling the GSH. Since GPx is limiting, the decrease in absorbance at 340nm is directly proportional to the GPx concentration. The absorbance was read at 1,2 and 3 minutes against reagent blank. The absorbance for blank was subtracted from the sample reading to give the corrected value. Thus, GPx activity was calculated using 8.412 as the extinction coefficient:

$GPx(U/L) = 8.412 \times \Delta A\ 340/min$

U/L = unit activity per liter

Δ A 340/min = change in absorbance at 340 per minute.

Lipid peroxidation biomarker (MDA)
Lipid peroxidation can be evaluated by the thiobarbituric acid reactive substances method (Gallou *et al*., 1993). Plasma malondialdehyde (MDA) levels were measured by the double heating method of Draper and Hadley (1990) using Malondialdehyde Assay kits from Northwest Life Sciences Specialities (NWLSSTM, product NWK-MDA01). Butylated hydroxytoluene (BHT) in methanol reagent was used as the control. The method is based on the spectrophotometric measurement of the purple color generated by the reaction of thiobarbituric acid (TBA) with MDA at 532nm. The MDA formed will therefore be quantified using an extinction coefficient of 1.56 x 10_5/mole/cm (Yagi, 1987). The amount of MDA formed in the control samples is subtracted from the amount in the experimental samples to obtain the amount of MDA in each sample. Since absorbance is directly proportional to the concentration, thus; concentration of MDA in each sample = Absorbance in sample – Absorbance in control x 10_5 nmol/ml ÷ 1.56 x 10_5 $M^{-1}CM_1$

Statistical Analysis
The data obtained were expressed as mean ± standard error of mean (SEM) and data were statistically analyzed using analysis of variance (ANOVA) followed by Tukey's *post hoc* test. The values of $p \leq 0.05$ were considered as significant.

RESULTS

Liver enzyme assay

Aspartate aminotransferase (AST): Figure 1 shows a significant($p<0.05$) increase in the group that were fed on high fat diet alone, as compared with the group that was fed on normal feed. However, administration of 250 mg/kg mGcL2 significantly decrease ($p<0.05$) the AST level as compared to the high fat diet control group. Consequently, 500 mg/kg $MgCl_2$ significantly increase the activity of the enzymes as compared to the high fat diet control group.

Alanine Amino Transferase (ALT) and Alkaline phosphatase: Figure 2 shows a significant decrease in the group that were fed on high fat diet as compared with the control with the normal control group. With regards to the 250 mg/kg $MgCl_2$ significantly increase($p<0.05$) the levels of the ALT. In relation to the 500 mg/kg $MgCl_2$ there was a significant increase as compared to the high fat diet control group.

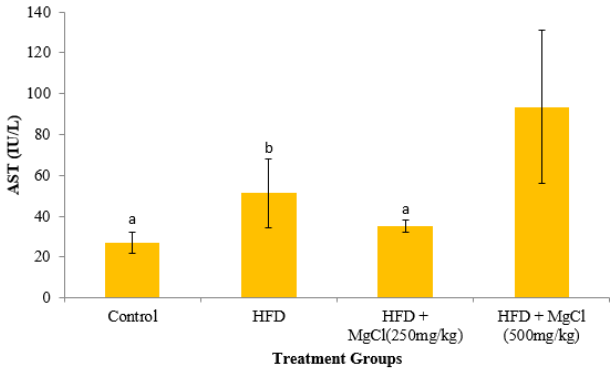

Figure 1:
Effect of co-administration of magnesium chloride and high-fat diet on serum AST level in rats as compared with normal and high fat diet fed control group. Bars with different superscript letter (a, b) differ significantly (P < 0.05).

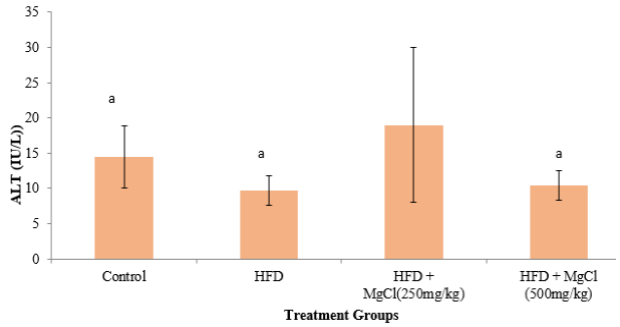

Figure 2:
Effect of co-administration of magnesium chloride and high-fat diet on serum ALT level in rats as compared with normal and high fat diet fed control group. Bars with different superscript letter (a, b) differ significantly (P < 0.05).

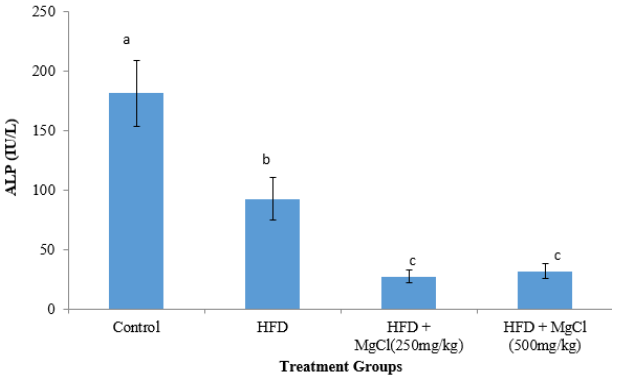

Figure 3:
Effect of co-administration of magnesium chloride and high-fat diet on serum ALP level in rats as compared with normal and high fat diet fed control group. Bars with different superscript letter (a,b) differ significantly (P < 0.05).

Alkaline Phosphatase (ALP)
In figure 3, a significant ($p \leq 0.05$) increase in values of alkaline phosphatase (ALP) obtained in the group that was fed on normal feed when compared to high fat diet control. Administration of 250 and 500 mg/kg $MgCl_2$ significantly ($p<0.05$) decreases the ALP level as compared to the high fat diet control group.

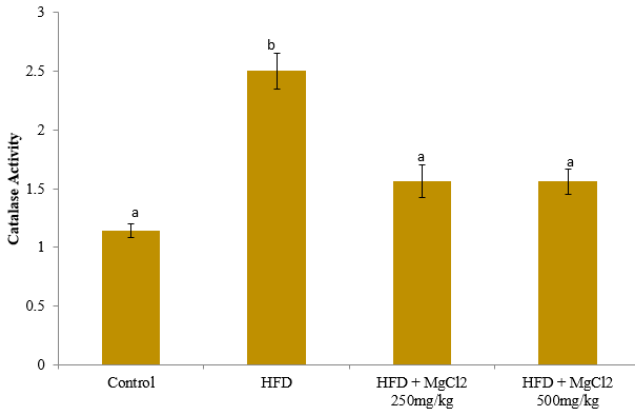

Figure 4:
Effect of co-administration of Magnesium chloride and high-fat diet on serum Catalase level in rats as compared with normal and high fat diet fed control group. Bars with different superscript letter (a,b) differ significantly (P < 0.05).

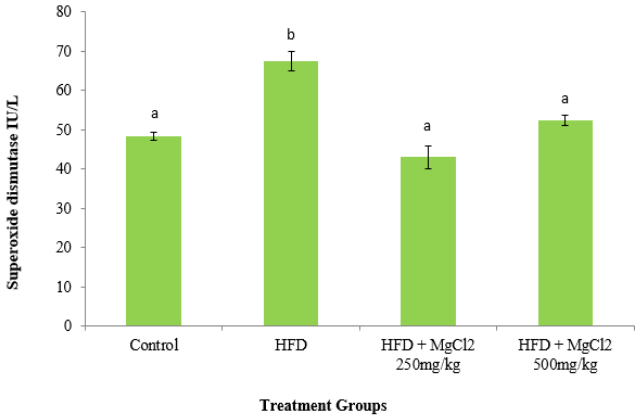

Figure 5:
Effect of co-administration of magnesium chloride and high-fat diet on serum Superoxide dismutase level in rats as compared with normal and high fat diet fed control group. Bars with different superscript letter (a,b) differ significantly (P < 0.05).

Antioxidant enzyme assay:

Catalase activity (CAT): CAT activities in the magnesium chloride co-administered with high fat diet, control group alone and HFD group only are shown in figure 4. 250 mg/kg and 500 mg/kg magnesium chloride co-administered with high fat diet showed significant ($p < 0.05$) decrease in CAT activity with values of 1.56 ± 0.14 IU/L and 1.56 ± 0.11 IU/L when compared to the high fat diet group only with a value of 2.50 ± 0.15 respectively.

Superoxide dismutase (SOD): Figure 5 shows the activity of SOD in the magnesium chloride administered with HFD, control group alone and high fat diet group only. Magnesium chloride co-administered with cholesterol diet showed significant

($p < 0.05$) decrease in SOD activity of 250 mg/kg and 500 mg/kg magnesium chloride with values of 43.00 ± 2.81 IU/L and 52.40 ± 1.36 IU/L when compared to the high fat diet group only with a value of 67.40 ± 2.48 IU/L respectively. The result shows that the activity of SOD was decreased despite consumption of high fat diet in groups treated with magnesium chloride.

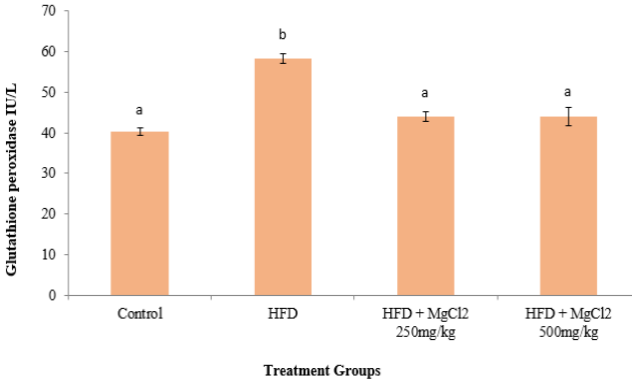

Figure 6:
Effect of co-administration of Magnesium chloride and high-fat diet (HFD) on serum Glutathione peroxidase level in rats as compared with normal and high fat diet fed control group. Bars with different superscript letter (a,b) differs significantly ($P < 0.05$).

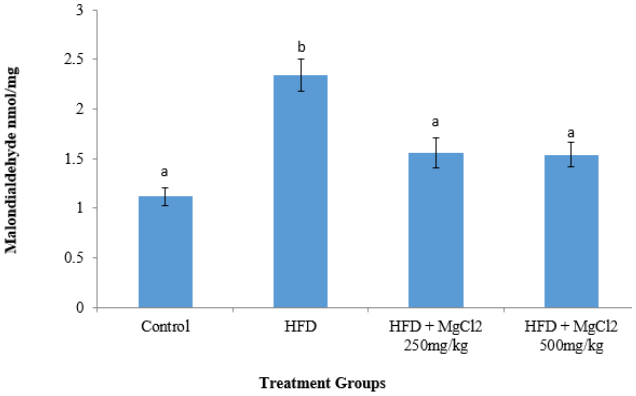

Figure 7:
Effect of co-administration of Magnesium chloride and high-fat diet on serum Malondialdehyde concentration level in rats as compared with normal and high fat diet fed control group. Bars with different superscript letter (a,b) differ significantly ($P < 0.05$).

Glutathione peroxidase activity (GPx): Figure 6 shows the activity of GPx in the magnesium chloride administered with high fat diet and high fat diet group only. Magnesium chloride co-administered with high fat diet revealed a significant ($p < 0.05$) decrease in GPx activity with a value of 44.00 ± 1.30 IU/L for 250 mg/kg magnesium chloride and 44.00 ± 2.17IU/L for 500 mg/kg magnesium chloride when compared to the high fat diet group only with a value of 58.40 ± 1.21 IU/L respectively. Magnesium chloride administration ameliorates GPx activity despite consumption of high fat diet.

Malondialdehyde (MDA) Concentration: Figure 7 shows the activity of MDA in the magnesium chloride administered with high fat diet, control group alone and high fat diet group only. high fat diet significantly ($P< 0.05$) elevated the serum MDA level when compared with the normal control animals. Magnesium chloride at doses of 250 mg/kg and 500 mg/kg significantly ($P < 0.05$) reduced the serum MDA levels with values of 1.56 ± 0.15IU/L and 1.54 ± 0.12 when compared with the high fat diet group only with a value of 2.34 ± 0.16IU/L respectively, despite the consumption of high fat diet.

DISCUSSION

Metabolic disturbances both in obese and experimental animals, obesity has been shown to be accompanied by an increase in oxidative stress markers (Diniz *et al.*, 2006). An augmented oxidative stress, if coupled with an attenuated antioxidant capacity tends to disrupt the normal redox homeostasis leading to irreversible damage to membranes and other macromolecules (Kamata and Hirata, 1999; Levine and Stadtman, 2001). High fat diet administration is routinely employed in experimental models of obesity and metabolic syndrome. It has been argued that excessive accumulation of fat leads to enhanced production of Reactive oxygen species in adipocytes and systemic tissues (Furukawa *et al.*, 2004). Obesity, insulin resistance and hyperglycemia, develop over a period of several weeks of Hhigh fat diet administration and it has been demonstrated that increased Oxidative stress precedes these changes (Matsuzawa *et al.*, 2008). Furthermore, an increase in liver biomarkers such as AST, ALT, and ALP in the plasma of rats fed with high cholesterol dietcould be an indication of liver damage resulting in the injury of hepatocytes which may have caused a leakage of cytosolic enzymes (AST, ALT, and ALP) from the cell into circulation, thus, leading to an increase in the levels of these enzymes in the plasma (Pratt and Kaplan, 2000). The result shows a reduction in the function of liver biomarker enzymes due to the increase in AST, ALT, and ALP levels as compared to the HFD control. Supplementing with magnesium chloride caused a significant decrease in plasma AST, ALT and ALP levels when compared with the control ($P<0.05$). Generally, hypercholesterolemia is considered to be an increase in both the abnormal hepatic and serum cholesterol and triglyceride levels (Wang et al., 2010). The administration of dietary cholesterol has been shown to influence hepatic lipid metabolism in rats (Wang et al., 2010). Also, an increase in serum total cholesterol may result in impairment of triglyceride metabolism which causes deposition or accumulation of free fatty acids in the liver, thereby leading to a condition otherwise known as fatty liver (Wang et al., 2010). This expanded liver

fatty acid pool results in an increase in peroxisomal and mitochondrial β-oxidation which leads to the formation of reactive oxygen species. This may, in turn, result in the progression of liver injury via the process of a local proinflammatory state (Schwimmer et al., 2008). Hence, our result showed that magnesium chloride is able to protect the liver from oxidative damage due to its phenolic contents. This work agrees with the findings of Adekiya *et al.* (2018).

The oxidative stress biomarkers, superoxide dismutase, glutathione peroxidase, and catalase activity groups fed with high fat diet expressed the highest level of activity as compared with the control groups. There was a statistical significant ($p<0.05$) decrease in superoxide dismutase activity as compared to control. Similar observations with a corresponding decrease in enzymatic antioxidants (SOD and GPx) have been reported in number of studies on an HFD (Noeman et al., 2011; Rahman et al., 2017). The groups administered with 250mg/kg and 500mg/magnesium chloride. Had an equal increase in activity, this shows that the activity of oxidative biomarkers is not dose dependent. This result agrees with the finding of Halliwell, (2007). Who demonstrated an increase in biomarkers of oxidative stress with administration of substance that cause release of free radicals in the body.It has been reported that hypercholesterolemia enhanced the production of oxidative stress and increased Lipid peroxidation (LPO) (Cox and Cohen, 1996). Studies have shown that a diet rich in high cholesterol concentration results in an increase in the levels of LPO by free radicals and aggravates hypercholesterolemia (Lee et al., 2006). The increase in cholesterol diet also caused a marked elevation in the levels of plasma MDA; an initial outcome of LPO. However, an observable decrease in the levels of plasma MDA of hypercholesterolemic rats treated with magnesium chloride clearly indicates a great significant regulation of cholesterol metabolism by lowering the MDA level. Therefore, magnesium chloride supplementation can be considered as important antioxidant therapeutic diet in hypercholesterolemic state; due to their great significant regulatory effect in the plasma cholesterol concentration by lowering the plasma MDA which in turn results in the inhibition of oxidative stress. Superoxide (SOD) is an antioxidant enzyme that catalyzes the conversion of two superoxides into H_2O_2 and oxygen. It acts as a major defense system against the cytotoxic effects of superoxide radicals (Caldwell *et al.,* 2008). SOD is metal-containing enzyme that depends on bound trace metals for antioxidant activity. They are of two types: copper/zinc (Cu/Zn) SOD and manganese (Mn) SOD and each type of SOD plays a different role in keeping cells healthy. Different isoforms of SOD are located at different sites within the cells (Caldwell *et al.,* 2008). Bohr *et al.* (2004) showed a significant increase in small intestine SOD activity of diabetic rats. Increased activity of SOD in the brain of diabetic rats has also been reported (Genet *et al.,* 2002). Decrease in the activity of SOD in diabetes could possibly be a response to increased generation of H_2O_2 and O_2 by the autoxidation of glucose and non-enzymatic glycation (Pari and Latha, 2004). Kumawat *et al.* (2005) has also reported that the reduced activity of SOD in the erythrocytes of diabetic rats could be due to ageing or an increase in the glycation of SOD.

With regards to the catalase there was a significant increase in the group that was fed on high fat diet. However, administration of Magnesium chloride at the doses tested (250 and 500 mg/kg significantly decrease the levels. Catalase (CAT) is an antioxidant enzyme that is produced naturally in the body and found in peroxisomes in eukaryotic cells. It is particularly important in conditions where glutathione (GSH) is limited or the activity of GPx is diminished (Caldwell *et al.,* 2008). CAT allows for important cellular processes which produce H_2O_2 as a by-product to occur by preventing excessive buildup of hydrogen peroxide and also protect against hydrogen peroxide mediated oxidative damage. In the small intestine, CAT activity was significantly increased in the diabetic rats (Bohr *et al.,* 2004). CAT activity has been shown to be significantly high in diabetic patients (Kumawat *et al.,* 2005) The uncontrolled generation of H_2O_2 as a result of the auto-oxidation of glucose, protein glycation and lipid oxidation in diabetes is markedly responsible for the decline in catalase activity (Saravanan and Ponmurugan, 2012).

In relation to the glutathione peroxidase, administration of 250 and 500mg/kg magnesium chloride significantly decrease in the level as compared with the control. Glutathione peroxidase (GPx) is a group of enzymes of which most contain selenium. It helps to protect the cell from damage due to free radicals like hydrogen and lipid peroxides and its actions take place in the presence of glutathione, the master antioxidant. They act like catalase by degrading hydrogen peroxide. GPx metabolizes hydrogen peroxide to water with the usage of reduced glutathione as a hydrogen donor (Caldwell *et al.,* 2008). They also reduce organic peroxides to alcohols, providing another way for the removal of toxic oxidants. A decrease in the activity of GPx in the pancreas of diabetic rats has also been reported (Babujanarthanam *et al.,* 2011). Reduced activity of GPx could be due to low content of glutathione in diabetic state, since glutathione serves as a substrate and cofactor of GPx (Saravanan and Ponmurugan, 2012). Decrease in GPx activity could be a result of a number of deleterious effects due to the accumulation of toxic products (Saravanan and Ponmurugan 2012).

The outcome of this present study suggests that magnesium chloride is able to protect the liver from oxidative damage. It also revealed that the treatment of

hypercholesterolemic rats with magnesium chloride inhibited the generation of MDA in the plasma, which in turn resulted in the formation of lipid peroxidation. Additionally, the scavenging activities and the hypocholesterolemic effects of magnesium chloride after the administration of high cholesterol diet were also established by the study.

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