

Modulatory role of Magnesium and Copper Sulphates on Serum Lipid profile and Serum Liver Enzymes in Fructose-induced Diabetic Wistar Rats

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ARTICLE INFO

Article history:

Received on: 08/06/2013

Revised on: 02/07/2013

Accepted on: 09/09/2013

Available online: 18/09/2013

Key words:

Diabetes, magnesium sulphate, copper sulphate, lipid profiles, liver enzymes, fructose.

ABSTRACT

The aim of this study is to determine the effect of magnesium and copper sulphates on serum lipid profile and serum liver enzymes in fructose-induced diabetic Wistar rats. Diabetes was induced by administration of 20% (20g/100ml) of fructose dissolved in distilled water and administered to the animals for a period of six (6) weeks. After which the animals were randomly assigned into 4 groups of 6 rats each. Group I served as diabetic negative control were administered 1ml distilled water. Group II were administered Magnesium sulphate (250 mg/kg b w). Group III were administered Copper sulphate (250 mg/kg b w) and Group IV administered Metformin (250 mg/kg b w) served as positive control. All treatments were given orally for a period of seven days. The results obtained showed a statistically significant decrease ($p < 0.05$) in the serum total cholesterol and triglyceride levels in groups administered with 250mg/kg b w of magnesium and copper sulphate when compared to diabetic control group. However, high density lipoprotein serum level was significantly increased ($p < 0.05$) in groups administered with 250mg/kg b w of magnesium and copper sulphate when compared to diabetic control group. The results also showed that magnesium and copper sulphates at dose of 250mg/kg b w produced a significantly decreased ($p < 0.05$) serum levels of liver enzymes (AST, ALT and ALP) in the treated groups when compared to diabetic untreated control group.

INTRODUCTION

Diabetes is a condition primarily defined by the level of hyperglycaemia giving rise to risk of microvascular damage (retinopathy, nephropathy and neuropathy). It is associated with reduced life expectancy, significant morbidity due to specific diabetes related microvascular complications, increased risk of macrovascular complications (ischaemic heart disease, stroke and peripheral vascular disease), and diminished quality of life. Recent estimates indicate there were 171 million people in the world with diabetes in the year 2000 and this is projected to increase to 366 million by 2030 (WHO, 2006).

Type 2 diabetes mellitus is a metabolic disorder, with hyperglycemia as the dominant feature, as a result of reduced secretion of insulin and increased insulin resistance (Salgueiro *et al.*, 2001). Trace element status may be altered in diabetic patients (Kamata and Kobayashi, 1996).

Locally produced reactive oxygen intermediates (ROI's) are involved in the effector mechanism of pancreatic β cell destruction (Lenzen *et al.*, 1996) and they are known to disrupt the antioxidant enzymes by releasing trace elements (Bond *et al.*, 1983; Seelig and Heggtveit, 1974). Some trace elements like copper and zinc may act as antioxidants and prevent membrane peroxidation, others like magnesium act directly on glucose metabolism through its role as a cofactor in the phosphorylation of glucose (McNair *et al.*, 1978). Copper acts as a catalytic and structural cofactor for various enzymes involved in energy generation, iron procurement, oxygen transport, cellular metabolism, hormone (peptide) maturation, blood clotting, signal transduction and in many other processes (Kim *et al.*, 2008). Previous studies reported derangements of these metals in diabetic patients with cardiovascular complications (Ceriello *et al.*, 1980; Walter *et al.*, 1991; Blissé and Abraham, 1985; Mooradian and Morley, 1987). Rats fed a high-fructose diet (>60% of total calories) provide a useful animal model of insulin resistance (Dai *et al.*, 1994; Thornburn *et al.*, 1989; Bezerra *et al.*, 2000).

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The sites of fructose-induced insulin resistance are documented to be the liver (Thornburn *et al.*, 1989), skeletal muscle (Zavaroni *et al.*, 1980) and adipose tissue (Vrana *et al.*, 1974). The rats also develop a cluster of abnormalities, which include hypertension, hypertriglyceridaemia and glucose intolerance in addition to hyperinsulinaemia (Reaven, 1988). Therefore, this research study was aimed at evaluating the effects of magnesium and copper sulphates on serum lipid profiles and serum liver enzymes in fructose-induced diabetic wistar rats.

MATERIALS AND METHODS

Drugs and Chemicals

All drugs and chemicals used were of analytical grades.

Animals

Twenty four (24) healthy albino Wistar rats of both sexes weighing between 150g-200g were purchased from the Department of Pharmacology and Therapeutics Animal House Ahmadu Bello University Zaria. The animals were housed in stainless steel cages under standard laboratory condition and fed on commercial feed (grower mash) and drinking water *ad libitum*. They were acclimatized to laboratory environment for a period of two weeks before the commencement of the experiment.

Experimental design

Group I: Served as diabetic control and received 1ml distilled water.

Group II: Received Magnesium sulphate 250 mg/kg b w.

Group III: Received Copper sulphate 250 mg/kg b w.

Group IV: Received Metformin 250 mg/kg b w.

All regimens were given orally for a period of seven days.

Serum sample preparation

After the last day of administration the animals were euthanized and blood samples were drawn from the heart of each by cardiac puncture into plain tubes and were allowed to clot and the serum separated by centrifugation using Denley BS400 centrifuge (England) at 3000 r p m for 15minutes and the serum collected and then subjected to biochemical assays.

Determination of serum total cholesterol

The serum level of total cholesterol was quantified after enzymatic hydrolysis and oxidation of the sample as described by method of Stein (1987). 1000µl of the reagent was added to each of the sample and standard. This was incubated for 10 minutes at 20-25 °C after mixing and the absorbance of the sample (A_{sample}) and standard (A_{standard}) was measured against the reagent blank within 30 minutes at 546nm. The value of TC present in serum was expressed in the unit of mmol/L.

Determination of serum triglyceride

The serum triglyceride level was determined after enzymatic hydrolysis of the sample with lipases as described by method of Tietz (1990). 1000µl of the reagent was added to each of the sample and standard. This was incubated for 10 minutes at 20-25 °C after mixing and the absorbance of the sample (A_{sample}) and standard (A_{standard}) was measured against the reagent blank within 30 minutes at 546nm. The value of triglyceride present in the serum was expressed in the unit of mmol/L.

Determination of serum high-density lipoprotein cholesterol

The serum level of HDL-C was measured by the method of Wacnic and Albers (1978). Low-density lipoproteins (LDL and VLDL) and chylomicron fractions in the sample were precipitated quantitatively by addition of phosphotungstic acid in the presence of magnesium ions. The mixture was allowed to stand for 10 minutes at room temperature and centrifuged for 10 minutes at 4000 rpm. The supernatant represented the HDL-C fraction. The cholesterol concentration in the HDL fraction, which remained in the supernatant, was determined. The value of HDL-C was expressed in the unit of mmol/L.

Evaluation of serum liver enzymes

The serum enzymes Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP) were determined spectrophotometrically, using enzymatic colometric assay kits according to the laboratory procedures of Randox Laboratories Limited kits, United kingdom.

Statistical analysis

Data obtained were expressed as mean \pm SEM. The data were analysis using one-way analysis of variance (ANOVA) and Tukey's post hoc test was used to determine the level of significance between control and the experimental groups. The value of $P < 0.05$ were considered significant.

RESULTS

Effects of magnesium and copper sulphates on serum lipid profiles in fructose-induced diabetic wistar rats

Table 1 shows the mean serum lipid profile values of control and experimental groups of magnesium and copper sulphate treated animals of fructose-induced diabetic rats. Data generated revealed that the serum total cholesterol and serum triglyceride levels were significantly decreased ($p > 0.05$) in groups that received magnesium and copper sulphate at a dose of 250mg/kg b w when compared to diabetic control group. However, the serum high density lipoprotein significantly increased ($p < 0.05$) in groups padministered with 250mg/kg b w of magnesium and copper sulphate when compared to diabetic control group.

Table 1: Effects of Magnesium and Copper sulphates mean \pm SEM on serum lipid profiles in fructose-induced Diabetic in rats.

Treatment Given	Serum total cholesterol (mmol/L)	Serum triglyceride (mmol/L)	Serum high density lipoprotein (mmol/L)
Diabetic control + distilled water	2.90 \pm 0.13	1.14 \pm 0.08	0.52 \pm 0.09
Magnesium sulphate (250 mg/kg b w)	2.20 \pm 0.11 ^a	0.40 \pm 0.43 ^a	1.42 \pm 0.04 ^a
Copper sulphate (250mg/kg)	2.06 \pm 0.10 ^a	0.36 \pm 0.08 ^a	1.20 \pm 0.07 ^a
Metformin (250 mg/kg b w)	1.94 \pm 0.07 ^a	0.42 \pm 0.04 ^a	1.00 \pm 0.03 ^a

$P < 0.05$ is statically significant when compared to the control group while ns= significant and ns = non significant.

Effect of magnesium and copper sulphates on serum liver enzymes in fructose-induced diabetic wistar rats

Table 2 shows the mean serum liver enzyme values of control and experimental groups of magnesium and copper sulphate treated animals of fructose-induced diabetic rats. Data generated revealed that the liver enzymes significantly decreased ($p > 0.05$) in groups that received magnesium and copper sulphates (250mg/kg b w) when compared to diabetic control group.

Table 2: Effects of Magnesium and Copper sulphates mean \pm SEM on serum liver enzymes in fructose-induced Diabetic in Wistar rats.

Treatment Given	Serum (iu/L)	AST Serum (iu/L)	ALT Serum (iu/L)	ALP (iu/L)
Diabetic control + distilled water	25.60 \pm 0.51	74.06 \pm 2.18	34.60 \pm 1.40	
Magnesium sulphate (250 mg/kg b w)	16.60 \pm 0.21 ^a	41.20 \pm 2.0 ^a	20.20 \pm 0.32 ^a	
Copper sulphate (250mg/kg b w)	18.20 \pm 0.73 ^a	38.0 \pm 1.02 ^a	17.10 \pm 0.26 ^a	
Metformin (250 mg/kg b w)	20.60 \pm 0.51 ^a	42.60 \pm 1.02 ^a	20.20 \pm 0.37 ^a	

P < 0.05 is statically significant when compared to the control group while ns= significant and ns = non significant.

DISCUSSION

Minerals have been shown to influence hormones at several levels, including hormone secretion and activity, and binding to the target tissue. Moreover, hormones have been shown to influence trace metal metabolism at several levels, including excretion and transport of trace metals (Henkin 1976). Magnesium is a macro mineral which is known to play an important role in carbohydrate metabolism, and its imbalance has been implicated in diabetes mellitus both as a cause and a consequence (American Diabetic Association 1992; Mooradian and Morley 1987). Magnesium deficiency has been associated with type II diabetes and may reduce insulin sensitivity and impair glucose tolerance (Wälti *et al.* 2003).

Chronic uncontrolled hyperglycemia can cause significant alterations in the status of various minerals, and conversely some of these substances can directly modulate glucose homeostasis (Sjogren *et al.*, 1988). Serum magnesium, zinc and copper were found to be deficient in diabetic children (Maher and Shaaban, 2002).

The result of this study showed a significant decrease in serum total cholesterol and serum triglycerides and a significant increase in serum high density lipoprotein in the magnesium and copper sulphate treated groups when compared with the control group. Copper has been associated with lipid metabolism since 1973 (Klevay, 1973). Work on rats, and on monkeys, has shown that copper deficiency can markedly increase (sometimes double) the plasma cholesterol concentration (Klevay, 1973). Furthermore, the deficiency can decrease the percentage of the total plasma cholesterol that was bound to high density lipoprotein and increase the percentage bound to low density lipoprotein (Klevay, 1980).

This work agrees with the work of Aly *et al.*, 2011, which indicated that there are good results with lipid profiles among rat groups supplemented with minerals (Zn, Mg and Cr) at individual and combined high levels and also Altura *et al.*, 1990 that showed magnesium modulates blood lipid levels.

High serum levels of AST and ALT are usually indicative of liver damage in animals (Gil *et al.*, 1988) and humans (El Demerdash *et al.*, 2005). This study showed a significant decrease in serum liver enzymes in the magnesium and copper sulphates treated groups when compared with the control group, which is in consistence with the work of Aly *et al.*, 2011, that indicated a significant reduction in liver enzymes in rat groups supplemented with individual minerals (Zn, Mg and Cr) at high levels. Copper is an essential element, and adverse effects can arise from copper deficiency as well as excess copper intake (WHO, 1998). Liver damage is the critical endpoint for intake of high levels of copper in animal and human studies (WHO, 1998). The decrease in liver enzymes seen with the dose of copper sulphate used in this study may suggest an improved liver enzyme activity, indicating a beneficial and not deleterious effect on the liver.

CONCLUSION

In conclusion, the results of this study demonstrated that magnesium and copper sulphates at a dose of 250mg/ kg bw possessed a beneficial effect on improving liver enzyme activity and anti - hyperlipidemic effect in fructose induced diabetic wistar rats.

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How to cite this article:

Tanko Y, Alhassan M, Mohammed K. A, Eze E.D, Jimoh A, Sada N. M, Muhammad A and Mohammed A. Modulatory role of Magnesium and Copper Sulphates on Serum Lipid profile and Serum Liver Enzymes in Fructose-induced Diabetic Wistar Rats. *J App Pharm Sci.* 2013; 3 (8 Suppl 1): S64-S67.