

Original Research Article

Effect of Magnesium Sulphate on Hepatic Biomarkers in Pre-eclamptic Patients in Selected Tertiary Hospitals in Osun State South Western Nigeria

ABSTRACT

Aims: This study investigated the effect of Magnesium Sulphate ($MgSO_4$) on cardiac biomarkers in the management of pre-eclampsia in Osun State, Nigeria. This was with a view to providing scientific support for the use of $MgSO_4$ in the management of pre-eclampsia, and also to investigate likely adverse effects of $MgSO_4$ on the biological functions of the heart.

Study design: One-factor, two controls - six test groups quasi - experimental design.

Place and Duration of Study: Department of Biochemistry, Ekiti State University, Ado-Ekiti, Ekiti State, Nigeria, between November 2013 and July 2014.

Methodology: A total of two hundred and sixty (260) subjects were recruited for the study, and were grouped into normotensive pregnant women at 2nd and 3rd trimesters (n=20/trimester), pre-eclamptic women not on $MgSO_4$ at 2nd and 3rd trimesters (n=10/trimester) and pre-eclamptic women on $MgSO_4$ at 2nd and 3rd trimesters (n=60/trimester). Also normotensive pregnant women at post-partum (n=20) and pre-eclamptic women on $MgSO_4$ at post-partum (n=60). Blood samples (5 mL venous blood) were collected, centrifuged and stored as plasma before subjection to biochemical analysis. Blood plasma was analyzed for hepatic biomarker using standard Enzyme Linked Immunosorbent Assay (ELISA) and Spectrophotometric methods.

Results: The results also showed no significant difference in hepatic biomarkers of pre-eclamptic women on $MgSO_4$ compared with pre-eclamptic women not on $MgSO_4$ at both second and third trimesters. The results however showed that, comparing the hepatic biomarkers between normotensive pregnant women at post-partum and pre-eclamptic pregnant women on $MgSO_4$ also at post-partum, gave almost the same pattern with comparison between normotensive and pre-eclamptic women not on $MgSO_4$, but for albumin in which there was no significant difference in the mean plasma level

Conclusion: The results obtained from this work revealed that $MgSO_4$ exhibits safe and protective roles devoid of any adverse effects on the liver of pre-eclamptic women. This study further agrees with the existing usage of Magnesium Sulphate as an anti-hypertensive in the management of pre-eclampsia.

Keywords: Pre-eclampsia, Magnesium Sulphate, Hepatic Biomarkers.

1. INTRODUCTION

Pre-eclampsia is a pregnancy associated medical complication characterized by elevated blood pressure (hypertension), oedema, organ damage and significantly increased urine protein after 20th week of pregnancy, and various cardiovascular, hepatic, renal and haematological changes in an earlier normotensive pregnant woman. It is otherwise known as Pregnancy Induced Hypertension (PIH). It is a life threatening disorder that can occur during pregnancy and at post-partum, which if left untreated could progress and results into a very severe condition of 'eclampsia' known as occurrence of more severe seizures during pregnancy [1]. Meanwhile, eclampsia is a common cause of maternal mortality worldwide but particularly in the developing countries like Nigeria. It is estimated that every year eclampsia is associated with about 50,000 maternal deaths worldwide, most of which occur in developing countries [2,3].

Several management methods had been applied to prevent and treat the complications of hypertension in pregnancy. Therapeutic drug management has been a major prescription used by health providers in suppressing the effects of this pregnancy associated medical disorder at point of care in health institutions [4].

Magnesium sulphate (MgSO_4) is one of the possible therapeutic drugs used in the management and treatment of pregnancy induced hypertension. The dosage (intravascular injection) is 4g (20mL of 20% solution in saline) at a rate of 1g/5 min. over 5-20 min. Maintenance regime (intravascular injection) is 1 to 2g/ hour in 100 mL of maintenance solution following last convulsion [4]. It is an effective seizure prophylaxis that acts by causing fall in blood pressure and opposes calcium dependent arterial constriction to relieve vasoconstrictions. It increases cardiac output through its activity to antagonize increase in intracellular calcium concentration initiated by ischemia, and thus prevents cell damage and death. Magnesium sulphate is preferred because it possesses lower placental transfer thereby reversing the condition of placental abruption when taken by pre-eclamptic women [5]. The use of MgSO_4 in patients with severe pre-eclampsia reduced the risk of progression to eclampsia by more than half and reduced maternal mortality [6]. Also, the effect of MgSO_4 on perinatal outcomes has also been demonstrated to significantly improve outcomes for newborns compared to phenytoin [7].

The Magpie trial tried to address the main fear of toxicity in the use of MgSO_4 . Toxicity of MgSO_4 was monitored using clinical parameters. The parameters that were monitored are the knee jerk, respiratory rates (which should normally be more than 16/minute), and urine output (which should normally be more than 25 ml/l). The first warning of toxicity is loss of the knee jerk which occurs at serum magnesium level of 3.5 to 5 mmol/l. Respiratory paralysis occurs at 5 to 6.5 mmol/l, cardiac conduction is altered at more than 7.5 mmol/l while cardiac arrest occurs when serum magnesium exceeds 12.5 mmol/l [8].

Biomarkers are substances (like chemicals, enzymes, molecules, indicators or signals) produced by a cell, tissue and organ normally, or produced for the purpose of monitoring response to foreign compounds (like drugs, chemicals or substances), and treatment progress [9]. Furthermore, biomarkers exhibit some characteristics that make them significant such as specificity and sensitivity. Specificity in the sense that their presence or changes is an indicative of a particular effect, and sensitivity to show that something must have happen to the cell, tissue and organ before the biomarkers' appearance [10]. These characteristics have made biomarkers significant in screening, diagnosis, treatment and monitoring the activity of tissue and organ [11]. The liver is an organ in the body that performs many functions essential for life. It is a major and body's largest organ that receives processes, and stores metabolites like amino acids, carbohydrates, lipids, vitamins and minerals. In addition, many of the proteins, coagulation factors and transport proteins are synthesized and processed by the liver. Liver is the primary site of detoxification of exogenous compounds such as drugs, toxins and endogenously the site for conjugation and metabolism [12 Dereck et al., 1986].

Liver biomarkers are used as an important medical aid to detect any injury or damage the liver is exposed to, and they are of significant importance in the development of new therapeutics for prevention, treatment and management of a broad range of diseases the liver is exposed to as hepatotoxicity or liver injury and dysfunctions [13 Obembe et al., 2000]. Some of the biomarkers of importance include total protein, albumin, prothrombin, alanine aminotransferase, alkaline phosphatase, alphafetoprotein, bilirubin, gamma-glutamyl transferase, fibrinogen, platelets count, and amylase [14 Macfarlene, 1961].

This study investigated the effects of MgSO_4 on hepatic biomarkers in the management of pre-eclampsia in Osun State, Nigeria. This was with a view to further provide, or otherwise, scientific support for the use of MgSO_4 in the management of pre-eclampsia, and also to investigate likely adverse effects of MgSO_4 on the biological functions of the liver.

2. MATERIAL AND METHODS / EXPERIMENTAL DETAILS / METHODOLOGY

2.1 Materials

2.1.1 Reagents and chemicals

The reagents and chemicals used for this work were of analytical grade from sigma, and they include: total protein biuret reagent [NaOH (8g), alkaline tartrate (9g), $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (3g), potassium iodide (5g)], albumin bromocresol green reagent [sodium citrate (10.16g), citric acid (13.7g), sodium azide (0.2g), bromocresol green dye (0.698g), solid brij 35 (25g)], bilirubin reagent [sodium benzoate (75g), caffeine (50g), hydrated sodium acetate (125g) ethylene di-thymic acid (1g), sulphanilic acid (5g), H_2SO_4 (15ml), sodium nitrite (5g), NaOH (50g), sodium potassium tartrate (175g), ascorbic acid (200mg)], and platelet reagent [ammonium oxalate (1mL), NaCl (8.5g)] to mention a few.

2.2 Methods

2.2.1 Experimental design and grouping of subjects

Quasi-experimental design method was utilized in this study and subjects were divided into eight (8) groups:

- i. Group 1 was 2nd Trimester Normotensive Pregnant Women;
- ii. Group 2 was 3rd Trimester Normotensive Pregnant Women;
- iii. Group 3 was 2nd Trimester Pre-eclamptic Women not on MgSO_4 ;
- iv. Group 4 was 3rd Trimester Pre-eclamptic Women not on MgSO_4 ;
- v. Group 5 was 2nd Trimester Pre-eclamptic Women on MgSO_4 ;
- vi. Group 6 was 3rd Trimester Pre-eclamptic Women on MgSO_4 ;
- vii. Group 7 was Post-partum Normotensive Pregnant Women;
- viii. Group 8 was Post-partum Pre-eclamptic Women on MgSO_4 .

2.2.2 Sampling areas

Six major hospitals located in various parts of Osun State, South Western Nigeria were used for the purpose of this research. Majority of pregnant women in Osun State attend these hospitals because of their proximity and qualitative health care delivery. The hospitals included:

- i. Obafemi Awolowo University Teaching Hospital Complex, Ile-Ife.
- ii. Wesley Guild Hospital, Ilesa.
- iii. Ladoke Akintola University Teaching Hospital, Osogbo.
- iv. Osun State General Hospital, Asubiaro, Osogbo.
- v. The General (Osun State) Hospital, Iwo.
- vi. Seventh- Day Hospital, Ile-Ife.

2.2.3 Recruitment of subjects

A total of two hundred and sixty (260) subjects comprising of normotensive pregnant women at 2nd and 3rd trimesters (n=20/trimester), pre-eclamptic women not on MgSO_4 at 2nd and 3rd trimesters (n=10/trimester),

pre-eclamptic women on MgSO_4 at 2nd and 3rd trimesters (n=60/trimester), normotensive pregnant women at post-partum (n=20) and pre-eclamptic women on MgSO_4 at post-partum (n=60) were recruited for this research and their samples were processed for analysis within 72 hours of collection employing standard diagnostic techniques.

2.2.4 Criteria for selection of subjects

The subjects used for this project were selected according to the following criteria:

- i. Visiting antenatal clinic to seek consent of the subject groups 1-6 (Pregnant and Pre-eclamptic women), and post-natal ward for subject groups 7-8 (Post-partum subjects);
- ii. Obtaining brief clinical history from all subjects using questionnaire;
- iii. Anthropometric measurements of the subjects blood pressure was done using sphygmomanometer, and ensuring subject groups 3-6 and 8 have readings greater or around 140 mmHg systolic and 90 mmHg diastolic measurement, while subject groups 1-2 and 7 have normal blood pressure readings;
- iv. Ensuring subject groups 5-6 and 8 were on magnesium sulphate drug while subject groups 3-4 were not on magnesium sulphate drug, and subject groups 1-2 and 7 were not on any anti-hypertensives therapy;
- v. Noticing the presence of swelling for oedema and proteinuria through rapid urine check for subject groups 3-6 and 8 which is absent in other subject groups.

2.2.5 Collection of blood samples

2.2.5.1 Blood sample

About 10mL of venous blood was collected from each subject and distributed into lithium heparin bottle (6mL), sodium citrate bottle (2mL) and Ethylene Diamine Tetra-acetic Acid bottle (2mL) respectively.

2.2.5.2 Preparation of blood plasma

The blood samples (in lithium heparin bottle) were separated as plasma into plain labeled bottles after spinning in a centrifuge at 4,000 rpm for 20 min. The haematological samples were stored at 4^{oC} for analysis of platelets counts, prothrombin, and fibrinogen.

2.2.6 Determination of concentration of Plasma Total Protein

Plasma total protein concentration was determined spectrophotometrically using biuret method [15].

139 **2.2.6.1 Assay procedure**

140 Plasma and total protein standard (60g/L) was taken with pipette in equal volume of 0.05mL into
 141 appropriately labelled test tubes. Biuret reagent (4mL) was added to each tube. The reaction mixture in
 142 each tube was thoroughly mixed and incubated for 20 min. at room temperature. The absorbance of the
 143 test and standard was read against reagent blank at 540 nm. The coloured complex absorbance was
 144 directly proportional to the amount of total protein in the plasma.

145 **Calculation:**

146 Concentration of Plasma = $\frac{\text{Absorbance of test} \times \text{Concentration of standard}}{\text{Absorbance of standard}}$

147 Total protein (g/L)

148 **2.2.7 Determination of concentration of Plasma Albumin**

149 Plasma albumin concentration was determined spectrophotometrically using dye binding method [15].

150 **2.2.7.1 Assay procedure**

151 Plasma and albumin standard (30g/L) was taken with pipette in equal volume of 0.02mL into appropriately
 152 labelled test tubes. Bromocresol green reagent (0.15g/L) of 4mL was added to each tube. The reaction
 153 mixture in each tube was thoroughly mixed. The absorbance of the test and standard was read against
 154 reagent blank at 630 nm. The coloured complex absorbance was directly proportional to the amount of
 155 albumin in the plasma.

156 **Calculation:**

157 Concentration of Plasma = $\frac{\text{Absorbance of test} \times \text{Concentration of standard}}{\text{Absorbance of standard}}$

158 Albumin (g/L)

159

160 **2.2.8 Determination of Prothrombin Time**

161 Prothrombin time was determined according to the procedure reported by Quick [16].

162 **2.2.8.1 Assay procedure**

163 Plasma (0.1mL) was taken with pipette into test tubes, and incubated at 37°C for 5 min. in a water bath.
 164 Stop watch (timer) was started on addition of 0.2mL of liquid calcium thromboplastin (25mM) to record the
 165 time required for clot formation as value of prothrombin.

166 **2.2.9 Assay of plasma Alkaline Phosphatase (ALP) activity**

167 Alkaline phosphatase activity was assayed spectrophotometrically according to enzymatic method of
 168 Henry [17].

169 **2.2.9.1 Assay procedure**

170 Plasma (0.02mL) was pipetted into appropriately labelled cuvette and 1mL of alkaline phosphatase
 171 reagent [containing magnesium chloride (0.5mmol/L), diethanolamine buffer (1 mol/L at pH 9.8) and para-
 172 nitrophenyl phosphate (Na salt) (10mmol/L)] was added respectively to each cuvette. The content of each

173 cuvettes was thoroughly mixed. Timer was started and absorbance of plasma recorded kinetically after
174 1,2 and 3 min. at 405 nm.

175 **Calculation:**

176 Plasma Alkaline phosphatase = Mean ALP absorbance change per min. X 2760 U/L Activity (U/L)

177 **2.2.10 Estimation of Plasma Alpha-fetoprotein (AFP) Concentration**

178 Concentration of alpha-fetoprotein was estimated using enzyme linked immunosorbent assay (ELISA)
179 method [18].

180

181 **2.2.10.1 Assay procedure**

182 The microplate wells for each calibrator, tetramethylbenzidine blank, standard and plasma to be assayed
183 in duplicate, were firstly formatted. 0.1mL of conjugate [containing horseradish peroxidase with preserving
184 agent (0.05%), proclin 300 (0.05%), gentamycin sulphate (0.004%) and phenol (0.1%)] was pipetted into
185 each well, except that of blank. Thereafter, 0.025mL of each calibrator standard (10 IU/L) and plasma
186 were put into appropriate wells with new disposable tips. The microplates well were covered and swirled
187 gently for 40 sec. to thoroughly mix. The reaction mixtures were then incubated for 120 min. at room
188 temperature, and the microplate supernatant discarded by decantation. The residue was later washed
189 five times with 0.3mL of washing solution (automatic/manual plate washer can be used). 0.1mL of
190 tetramethylbenzidine-substrate [containing H₂SO₄ (1%) in TMB-citric acid buffer] was dispensed into each
191 well of washed residue at 1 min. interval, and was incubated for 25 - 30 min. at room temperature in a
192 dark place. After incubation, 0.15mL of stopping reagent [containing 0.2M sulphuric acid solution] was
193 appropriately taken into each well at same time interval used for tetramethylbenzidine-substrate, and
194 gently mixed for 5 sec. The microplate reader was engaged to read the microplates of
195 tetramethylbenzidine standard and plasma against reagent blank at 450 nm within 20 min. The mean
196 absorbance of the duplicates for each set of standards and plasma from the graph-curve and charts
197 generated by the reader gave the AFP concentration.

198 **2.2.11 Assay of plasma Alanine Aminotransferase (ALT) activity**

199 This was assayed spectrophotometrically using enzymatic method [19].

200 **2.2.11.1 Assay procedure**

201 Phosphate buffer (100mmol/L at pH 7.4) of 0.5mL was taken with pipette into test- tubes meant for tests
202 and blank. To the sample tubes, plasma (0.1mL) was added and mixed. The reaction mixture was then
203 incubated for 30 min. at 37°C in a water bath. On removal, 2,4 Dinitrophenyl hydrazine reagent (2mmol/L)
204 of 0.5mL was pipetted into all the tubes. Afterward, plasma (0.1mL) was taken to the tubes of blank (as
205 sample blank), with the content mixed. All the tubes were then allowed to stand for exactly 20 min. at
206 room temperature. Sodium hydroxide (0.4N) of 5ml was added to each tube of test and blank with the
207 content mixed carefully. The absorbance of test at 546 nm were read and recorded against the sample
208 blank after 5 min. incubation at room temperature. The absorbances were compared with the calibrated
209 chart of the reagent as the activity of alanine aminotransferase in the plasma.

210 **2.2.12 Estimation of plasma levels of Bilirubin [total and conjugated]**

211 Plasma bilirubin concentration was estimated spectrophotometrically according to Jendrassik's method of
212 Cheesbrough [15].

213 **2.2.12.1 Assay procedure**

To test tubes of total bilirubin, distilled water (1mL), plasma (0.1mL), 0.5mL of diazo reagent (containing 5g/L of both sulphanilic acid and Na nitrite) and 1mL of caffeine (0.28mol/L) were pipetted. The reaction mixtures were incubated at room temperature for 10 min. This was followed by adding 0.1ml of ascorbic acid (4g%) and 1.5mL of alkaline tartrate reagent (0.99mol/L). Conjugated bilirubin and sample blank differs in procedure to that of total bilirubin. Addition of caffeine was after incubation in conjugated bilirubin tubes while in sample blank tubes, diazo reagent and caffeine were added after incubation. The absorbances of total and conjugated bilirubin were read against the sample blank at 600 nm.

221 **Calculation:**

222 Concentration of Plasma Bilirubin = $\frac{\text{Absorbance of test} \times \text{Concentration of standard}}{\text{Absorbance of standard}}$

223 (total or conjugated in umol/L)

224 **2.2.13 Assay of Plasma Gamma-Glutamyl Transferase (GGT) activity**

225 This was assayed using enzymatic method [18].

226 ***2.2.13.1 Assay procedure***

227 Plasma and standard (2mM) of 0.1mL each were pipetted into their test tube. 1mL of gamma-glutamyl transferase reagent [containing tris buffer (180mM), glycylglycine (100mM) and gamma-glutamyl-p-nitroanilide (4.79mM)] was added to all tubes and the reaction mixture was incubated in a waterbath for 1 min. at 37°C. After incubation, absorbance of plasma and standard at 405 nm were recorded kinetically against reagent blank after 1, 2 and 3 min.

232 **Calculation:**

233 Gamma-Glutamyl Transferase = Mean GGT absorbance change per min. X 1640 U/L Activity (U/L).

234 **2.2.14 Determination of plasma Fibrinogen concentration**

235 Plasma levels of fibrinogen in all the samples collected were carried out according to clot-weight method [20].

237 ***2.2.14.1 Assay procedure***

238 Plasma (1mL) was taken with pipette into the test tube and 1mL of prewarmed calcium chloride (0.28%) was added. The mixture was thoroughly mixed, and an applicator stick was inserted, and this was incubated for 30 min. at 37°C in a water bath. When all the fibrin had wound round the applicator stick, the adhered fibrin was removed from the applicator stick and washed for 4 times, each was carefully removed into a clean petridish and left for 4 days at room temperature to dry. The dried fibrin was then weighed, and the plasma fibrinogen concentration calculated.

244 **Calculation:**

245 Concentration of Plasma Fibrinogen (g/L) = $\frac{\text{Dry weight of fibrin clot} \times 10 \text{ g/L}}{\text{Volume of plasma}}$

246

247 **2.2.15 Estimation of Platelets**

248 ***2.2.15.1 Assay procedure***

Whole blood (0.02mL) was taken with pipette into test tubes, and diluted with 1% ammonium oxalate fluid (0.38mL). The dilution was mixed well and incubated at room temperature for 7 min to permit other cell lysis except platelets. Then, the dilution was mounted on a haemocytometer (with cover glass). The cells were allowed to settle for 5 min., and the platelets were counted in a specific area of the haemocytometer chamber under the microscope with inclusion of hand counter. The number of platelet was calculated per mm³.

2.2.16 Assay of Plasma Amylase activity

This was assayed using enzymatic method [21].

2.2.16.1 Assay procedure

Sodium phosphate buffer (0.02M) with sodium chloride (6.7mM) and soluble starch solution (1%) of 0.5mL equal volume were pipetted into tubes of test and standard, and was placed in water bath at 37°C for 5 min. On removal from the water bath, plasma and standard (0.2%) of 0.02mL each was added to their tubes. The reaction mixture was placed again in water bath at 37°C for 7.5 min. Then, distilled water (4mL) and iodine colour solution (1mL) were added to all tubes. The tubes were removed from the water bath and incubated at room temperature for 15 min., and the absorbance was read at 590 nm against reagent blank.

Calculation:

Plasma Amylase Activity = $\frac{\text{Absorbance of test} \times \text{Concentration of standard}}{\text{Absorbance of standard}}$

(I.U/L)

2.3 Statistical analysis

Results are expressed as mean $\pm$ SEM. Statistical difference was determined by one-way analysis of variance (ANOVA) followed by a post hoc test (Student Newman-Keuls Test (SNK)). Difference was considered statistically significant with $p < 0.05$. Computer software Graph pad PRISM[®] version 3.00 was used for the analysis.

3. RESULTS

This study showed that pre-eclamptic women at 2nd and 3rd trimesters had significant increase in plasma prothrombin, alkaline phosphatase, alphafetoprotein and gamma-glutamyl transferase, and significant decrease in albumin and platelet when compared with normotensive pregnant women. There was no significant difference in same comparison in other investigated hepatic biomarkers (see tables 1 and 2 below).

Table 1: Hepatic Biomarkers in Normotensive Pregnant Women at Second Trimester [A] and Pre-eclamptic Women not on MgSO₄ at Second Trimester [B]

GROUP	TP (g/L)	ALB (g/L)	PROTH (sec)	ALP (IU/L)	AFP (IU/mL)	ALT (IU/L)	TB (umol/L)	CB (umol/L)	GGT (U/L)	FIB (g/L)	PLT (/mm ³)	AMY (IU/L)
A (n=20)	76.20 ±2.69	35.30 ±1.04	22.50 ±0.47	171.10 ±6.20	41.60 ±2.06	18.60 ±1.80	36.20 ±4.21	7.90 ±0.98	34.50 ±2.53	4.90 ±0.16	194,200.00 ±7,948.16	195.40 ±18.72
B (n=10)	73.40 ±2.40	30.60 ±0.40	24.60 ±0.48	193.00 ±4.03	124.60 ±5.80	21.20 ±1.65	41.40 ±1.25	8.40 ±0.51	51.00 ±1.61	5.50 ±0.22	104,600.00 ±1,802.37	250.60 ±19.36
Differences between means	A>B	A>B	A<B	A<B	A<B	A<B	A<B	A<B	A<B	A<B	A>B	A<B
Levels of significance	P> 0.05	P< 0.001	P< 0.05	P< 0.01	P< 0.001	P> 0.05	P> 0.05	P> 0.05	P< 0.05	P> 0.05	P< 0.001	P> 0.05

GROUP	TP (g/L)	ALB (g/L)	PROTH (sec)	ALP (IU/L)	AFP (IU/mL)	ALT (IU/L)	TB (umol/L)	CB (umol/L)	GGT (U/L)	FIB (g/L)	PLT (mm ³)	AMY (IU/L)
C (n=20)	79.70 ±1.26	36.70 ±0.92	22.50 ±0.47	150.30 ±8.76	48.00 ±3.42	17.10 ±2.15	34.40 ±3.67	8.50 ±0.85	42.20 ±3.42	4.90 ±0.16	208,800.00 ±8,567.12	217.40 ±24.79
D (n=10)	81.40 ±1.50	31.00 ±0.71	25.70 ±0.44	224.20 ±12.73	90.00 ±2.80	33.80 ±1.96	42.20 ±2.67	9.60 ±0.51	68.80 ±3.42	5.60 ±0.23	108,400.00 ±2,416.52	271.20 ±11.38
Differences between means	C<D	C>D	C<D	C<D	C<D	C<D	C<D	C<D	C<D	C<D	C>D	C<D
Levels of significance	P>0.05	P<0.001	P<0.001	P<0.05	P<0.001	P>0.05	P>0.05	P>0.05	P<0.001	P>0.05	P<0.001	P>0.05

The results also showed no significant difference in hepatic biomarkers of pre-eclamptic women on MgSO₄ compared with pre-eclamptic women not on MgSO₄ at both second and third trimesters (see tables 3 and 4 below).

[illegible]

Table 4: Hepatic Biomarkers in Pre-eclamptic Women not on MgSO_4 at Third Trimester [D] and Pre-eclamptic Women on MgSO_4 at Third Trimester [F]

[illegible]

significance	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
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Table showed Means $\pm$ Standard error of mean (SEM), Differences between means and the Level of significance ($P < 0.05$). TP = Total protein, ALB = Albumin, PROTH = Prothrombin, ALP = Alkaline phosphatase, AFP = Alpha-fetoprotein, ALT = Alanine aminotransferase, TB = Total bilirubin, CB = Conjugated bilirubin, GGT = Gamma-glutamyl transferase, FIB = Fibrinogen, PLT = Platelet, AMY = Amylase.

The results however showed that, comparing the hepatic biomarkers between normotensive pregnant women at post-partum and pre-eclamptic pregnant women on $MgSO_4$ also at post-partum, gave almost the same pattern with comparison between normotensive and pre-eclamptic women not on $MgSO_4$, but for albumin in which there was no significant difference in the mean plasma level (see table 5 below).

Table 5: Hepatic Biomarkers in Normotensive Pregnant Women at Post-partum [G] and Pre-eclamptic Women on $MgSO_4$ at Post-partum [H]

GROUP	TP (g/L)	ALB (g/L)	PROTH (sec)	ALP (IU/L)	AFP (IU/mL)	ALT (IU/L)	TB (umol/L)	CB (umol/L)	GGT (U/L)	FIB (g/L)	PLT (/mm ³)	AMY (IU/L)
G (n=20)	66.50 ± 3.00	34.00 ± 1.36	20.60 ± 0.60	105.20 ± 8.22	32.50 ± 2.66	9.30 ± 2.40	27.20 ± 1.93	5.80 ± 0.60	32.50 ± 2.47	2.80 ± 0.28	191,700.00 $\pm 7,161.71$	139.00 ± 16.48
H (n=60)	71.40 ± 1.25	34.50 ± 0.49	23.10 ± 0.18	192.30 ± 10.70	69.50 ± 2.26	17.90 ± 0.66	31.50 ± 1.17	7.40 ± 0.70	34.60 ± 1.42	5.40 ± 0.06	166,850.00 $\pm 4,242.63$	159.30 ± 9.24
Differences between means	G<H	G<H	G<H	G<H	G<H	G<H	G<H	G<H	G<H	G<H	G>H	G<H
Levels of significance	P> 0.05	P> 0.05	P< 0.001	P< 0.001	P< 0.001	P> 0.05	P> 0.05	P> 0.05	P> 0.05	P< 0.001	P< 0.01	P> 0.05

Table showed Means $\pm$ Standard error of mean (SEM), Differences between means and the Level of significance ($P < 0.001$, $P < 0.01$, $P < 0.05$). TP = Total protein, ALB = Albumin, PROTH = Prothrombin, ALP = Alkaline phosphatase, AFP = Alpha-fetoprotein, ALT = Alanine aminotransferase, TB = Total bilirubin, CB = Conjugated bilirubin, GGT = Gamma-glutamyl transferase, FIB = Fibrinogen, PLT = Platelet, AMY = Amylase.

4. DISCUSSION

Results obtained from this study revealed a non-significant decrease in plasma concentrations of total protein and albumin in pre-eclamptic pregnant women at 2nd trimester, while at 3rd trimester, total protein was non-significantly raised when compared with the normotensive groups. Similar observations have been reported by Gray and Baron [22] and Edward et. al., [23]. This result could be due to coupling effects of increased synthesis of proteins (endogenous, plasma and glycoproteins) by hepatocyte and proteinuria in pre-eclamptic individuals. However, plasma concentration of total protein was non-significantly decreased while albumin was non-significantly raised in pre-eclamptic pregnant women treated with $MgSO_4$ at both 2nd and 3rd trimesters, as well as 3-6 days post-partum when compared with same groups that were not on $MgSO_4$ treatment. This could be due to effect of $MgSO_4$ as a seizure prophylaxis that helps in prevention of endothelial damages to the hepatocytes that normally occur in pre-eclampsia to oppose transcellular transport of proteins through the tight junctions leading to reduced total protein and increased synthesis of albumin [24].

The formation time of prothrombin was observed to be significantly prolonged in pre-eclamptic women when compared with the normotensive pregnant women. The prolonged prothrombin formation could be linked to possible hepatocyte damage and necrosis from endothelial dysfunctions in pre-eclampsia. This is as reported by Edward, et al., [23]. However, the prothrombin time was non-significantly reduced in pre-eclamptic pregnant women treated with $MgSO_4$ at both 2nd and 3rd trimesters, and at post-partum when compared with similar groups not on $MgSO_4$ treatment. This reduction in prothrombin formation could be due to effects of $MgSO_4$ in the prevention of endothelial damages to the hepatocytes that normally occur as a result of pre-eclampsia, by opposing transcellular transport of proteins (glycoproteins) through the tight junctions leading to quick prothrombin formation. This gives similar observation with the report of Munjuluri et al., [24].

The plasma activities of alkaline phosphatase and gamma-glutamyl transferase were significantly increased while that of alanine aminotransferase was non-significantly raised when compared with the normotensive pregnant groups. This result could be linked to possible hepatic necrosis in pre-eclampsia [25]. In other way round, non-significant decreased activities of plasma alkaline phosphatase, gamma-glutamyl transferase and alanine aminotransferase were seen in pre-eclamptic pregnant women treated with $MgSO_4$ at both 2nd and 3rd trimesters, as well as 3-6 days post-partum when compared with similar group not on $MgSO_4$ treatment. The reduced enzyme activities again confirm $MgSO_4$ as angiotensin converting enzyme inhibitor that opposes the action of angiotensin converting enzyme by preventing hepatic necrosis to decrease plasma alkaline phosphatase, gamma-glutamyl transferase and alanine aminotransferase activities [26].

Results obtained from this study indicated that alpha-fetoprotein concentration was significantly raised in pre-eclamptic women than the normotensive groups. Phyllis et al., [1] has given similar reports. The increase in alpha-fetoprotein might be due to the production of alpha-fetoprotein as a response to foetal and neonatal development by liver in pre-eclampsia. However, the plasma concentration of alpha-fetoprotein was non-significantly reduced in pre-eclamptic pregnant women treated with $MgSO_4$ at both 2nd and 3rd trimesters, and at post-partum when compared with pre-eclamptic pregnant women that were not on $MgSO_4$ treatment. The decrease is in agreement with report of Munjuluri et al., [24]. This reduction in alpha-fetoprotein could be due to effect of $MgSO_4$ as a seizure prophylaxis in the prevention of alpha-fetoprotein production by liver that normally occur as a result of pre-eclampsia, thereby opposing transcellular transport of proteins (glycoproteins) through the tight junctions leading to decreased synthesis of alpha-fetoprotein.

Plasma bilirubin (total and conjugated) concentration was non-significantly elevated in pre-eclamptic women when compared with the normotensive groups. This hyperbilirubinaemia may be due to hepatic necrosis in pre-eclampsia causing continuous red cell haemolysis for bilirubin synthesis and decreased conjugated bilirubin excretion [27]. However, a non-significant decreased bilirubin concentration (total and conjugated) was observed in pre-eclamptic pregnant women treated with MgSO_4 at both 2nd and 3rd trimesters, as well as 3-6 days post-partum when compared with pre-eclamptic pregnant women that were not on MgSO_4 treatment. The hypobilirubinaemia may be due to the effect of MgSO_4 as a good cell membrane protector (neuroprotective) agent that decrease cellular disruption and oedema from pinocytosis associated with pre-eclampsia whose actions also causes reduced erythrocyte destruction for bilirubin synthesis and conjugation [5].

Plasma concentration of fibrinogen was significantly raised in pre-eclamptic women during pregnancy and at post-partum when compared with the normotensive groups. The elevated fibrinogen concentration might be due to acute inflammation or cell injury, immunological and biological injury (which include infection) to hepatocytes in pre-eclampsia. Similar observation has been reported by Bolarin and Bolarin, [28]. However, plasma concentration of fibrinogen was decreased non-significantly in pre-eclamptic pregnant women treated with MgSO_4 at both 2nd and 3rd trimesters, and after delivery when compared with their counterparts that were not on MgSO_4 treatment. The reduction in fibrinogen concentration confirm that MgSO_4 treatment may help in the prevention of endothelial damages to the hepatocytes that normally occur as a result of pre-eclampsia by opposing transcellular transport of proteins (glycoproteins) through the tight junctions leading to reduced fibrinogen concentration [24].

Platelet count was significantly reduced in pre-eclamptic women when compared with normotensive pregnant women. Similar observation has been reported by Furuhielm, [29]. This reduced platelet count could be attributed to hepatocyte damage from endothelial dysfunction in pre-eclamptic women that facilitates reduced fibrinolytic activity by the liver cells to initiates thrombocytopenia. On the other hand, platelet count was non-significantly raised in pre-eclamptic pregnant women treated with MgSO_4 at both 2nd and 3rd trimesters, and after delivery when compared with their counterparts. From this result, it can be deduced that MgSO_4 may help in preventing endothelial damages to the hepatocytes that normally occur as a result of pre-eclampsia leading to increased fibrinolytic activity by the liver cells for increase platelets synthesis [30].

Amylase activity was non-significantly raised in pre-eclamptic women when compared with the normotensive groups. Similar report has been made by Bolarin and Bolarin, [28]. This could be attributed to hepatic vascular obstruction in pre-eclampsia for increase plasma amylase activity. Findings afterward revealed a non-significant decreased plasma activity of amylase in pre-eclamptic pregnant women treated with MgSO_4 at both 2nd and 3rd trimesters, and at post-partum when compared with pre-eclamptic pregnant women that were not on MgSO_4 treatment. This is in agreement with the report of Witlin et al., [26]. This finding shows that magnesium sulphate may prevent vascular blockages and damages by stimulating production of prostacyclin (a vasodilator) by endothelial cells causing vasodilation for reduced plasma activity of amylase.

5. CONCLUSION

The results obtained from this work revealed that MgSO_4 exhibited safe and protective roles devoid of any adverse effects on the liver, and also further agrees with the existing usage of magnesium sulphate drugs as an anti-hypertensive in the management and treatment of pre-eclampsia.

CONSENT

All authors declare that written informed consent was obtained from all the subjects studied in this work. A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal.

ETHICAL APPROVAL

The Postgraduate Ethical Committee of the Faculty of Science, Ekiti State University, Ado-Ekiti, Ekiti State, Nigeria, approved the research work.

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