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

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Authors' contributions

This work was carried out in collaboration among all authors. The work emanated from the M.Sc. thesis of author SOA. Authors SOA and MFA conceived and designed the study. Author SOA carried out all laboratory works and wrote the first draft of the manuscript. Author JOI managed the literature searches, performed the statistical analysis and wrote the final draft of the manuscript. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Aims: This study investigated the effect of Magnesium Sulphate ($MgSO_4$) on cardiac biomarkers in the management of pre-eclampsia in selected tertiary hospitals in Osun State, Nigeria. This was with a view to provide scientific report 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.

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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 (10 mL venous blood) were collected, centrifuged and stored as plasma before subjection to biochemical analysis. Blood plasma was analyzed for cardiac biomarker using standard Enzyme Linked Immunosorbent Assay (ELISA) and Spectrophotometric methods.

Results: Results revealed that cardiac biomarkers (plasma troponin, c-reactive protein and creatine) were significantly decreased in pre-eclamptic women on $MgSO_4$ at both 2nd and 3rd trimesters compared to their counterparts not on $MgSO_4$, while creatine-kinase, lactate dehydrogenase aspartate aminotransferase, and myoglobin showed non-significant reduction in same comparison. Moreover, with exception of lactate dehydrogenase that showed non-significant reduction, all cardiac biomarkers at 3-6 days post-partum decreased significantly compared to Pre-eclamptic Women on $MgSO_4$ at 3rd trimester.

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

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 [1]. 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 'eclampsia' [1]. Eclampsia is a very severe condition during pregnancy characterized with more severe seizures (convulsion) in a woman suffering from high blood pressure, often followed by coma and posing a threat to the health of mother and baby [2]. Pre-eclampsia is a common cause of maternal mortality worldwide. It is estimated that per year, pre-eclampsia is associated with about 50,000 maternal deaths worldwide, most of which occur in developing countries like Nigeria [3].

Several management methods like habit, dietary control, immune factor administration, bed rest and delivery have been applied to prevent and treat complications of hypertension in pregnancy. Therapeutic drug management including anti-hypertensives, vasodilators and anticonvulsants administrations have 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 anticonvulsant therapeutic drugs used in the management and treatment of pregnancy induced hypertension. It is an effective seizure prophylaxis that resultantly causes fall in blood pressure and opposes calcium dependent arterial constriction to relieve vasoconstrictions. Its mode of action in heart is that of increasing cardiac output by being a unique calcium antagonist that acts on calcium channels in cardiac vascular smooth muscles, thereby inactivating the calmodulin dependent light chain kinase activity and decreasing contraction causing arterial relaxation that may subsequently lower intracellular calcium and peripheral resistance, relieve vasospasm and decrease arterial blood pressure, thus preventing cell damage, seizures and death [4].

The Magpie trial in 2001, tried to address the efficacy of $MgSO_4$ in preventing seizures. It was the largest study ever conducted for hypertensive disorder in pregnancy, recruiting 10,141 women from 175 centres in 33 countries between 1998 and 2001. Eligible pregnant women that had signs and symptoms of pre-eclampsia were

recruited and were randomised to either clinically receive magnesium sulphate or placebo. The dosage (intravascular injection) is 4 g (20 mL of 20% solution in saline) at a rate of 1 g/5 min. over 5-20 min. Maintenance regime (intravascular injection) is 1 to 2 g/ hour in 100 mL of maintenance solution following last convulsion [5]. The trial found out that magnesium sulphate halves the risk of eclampsia and probably reduced the risk of maternal death. Subsequent follow-up studies showed that there was no excessive death or disability attributable to the drug in women using $MgSO_4$ and it's preferred because it possesses lower placental transfer thereby reversing the condition of placental abruption when taken by pre-eclamptic women. Also, the effect of $MgSO_4$ on perinatal outcomes has also been demonstrated to significantly improve outcomes for newborns compared to phenytoin [6-8].

Cardiac biomarkers are substances (like chemicals, enzymes, molecules, indicators or signals) produced by the heart [9]. Cardiac biomarkers exhibit some characteristics such as specificity and sensitivity which make them significant in screening, diagnosis, treatment and monitoring the activity of the heart [10,11]. The heart releases the biomarkers into the blood stream when exposed to damage or foreign compounds and are measured to evaluate the state of the heart and its functionability [12].

Serial tests of more than one cardiac biomarker are necessary to ensure the correct state of the heart. Only a few cardiac biomarker tests are routinely recommended by the health providers, the current and specific tests include troponin, creatine kinase, myoglobin, aspartate aminotransferase, C - reactive protein, creatine and lactate dehydrogenase [13].

This study investigated the effects of $MgSO_4$ on cardiac biomarkers in the management of pre-eclampsia in selected tertiary hospitals in Osun State, Nigeria. This was with a view to provide scientific report 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.

2. MATERIALS AND METHODS

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: creatine reagents [picric acid (9.3 g), NaOH (30 g), H_2SO_4 (18.8 ml), sodium tungstate (50 g)], Phosphate buffer (100 mmol/L at pH 7.4), 2,4 Dinitrophenyl hydrazine reagent (2 mmol/L), Sodium hydroxide (0.4N), lactate dehydrogenase reagents [containing tris buffer (50 mmol/L), pyruvate (0.6 mmol/L) and NADH (0.18 mmol/L)], latex reagent coated with anti-CRP antibodies (0.20%) and 0.08 mL of diluent [containing tris buffer (20 mmol/L at pH 7.3), merthiolate (0.05 g/L) and sodium azide (0.90 g/L)], enzyme conjugate reagent [containing horseradish peroxidase with preserving agent (0.05%)], tetramethylbenzidine reagent [containing H_2SO_4 (1%) in TMB-tris buffer (20 mmol/L at pH 7.3)], HCl, Picric acid, reflotron test strip, diluent [containing bovine serum (1%) and proclin (1%)], enzyme conjugate reagent [containing horseradish peroxidase with preserving agent (0.05%)], tetramethylbenzidine reagent solution [containing H_2SO_4 (1%) in TMB-tris buffer (20 mmol/L at pH 7.3)].

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 Women;
viii. Group 8 was Post-partum Pre-eclamptic Women on $MgSO_4$.

2.2.2 Sampling areas

Six major tertiary hospitals located in various parts of Osun State, South Western Nigeria were selected for the purpose of this research. These hospitals are recognised referral centres for pregnancy complications such as pre-eclampsia. The hospitals include:

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 Selection of subjects

The subjects for this project were selected as follow:

i. Visiting antenatal clinic to seek consent of the subjects in groups 1-6 (Pregnant and Pre-eclamptic women), and post-natal ward for subjects in groups 7-8 (Post-partum subjects);
ii. Obtaining brief clinical history from all subjects using questionnaire to take care of age, body mass index, gestational age (20 weeks and above) and other personal information;
iii. Measurement of the subjects' blood pressure was done using sphygmomanometer, and ensuring subjects in groups 3-6 and 8 have readings greater or around 140 mmHg systolic and 90 mmHg diastolic measurement, while subjects in groups 1,2 and 7 have normal blood pressure readings;
iv. Ensuring subjects in groups 5,6 and 8 were on magnesium sulphate drug while subjects in groups 3 and 4 were not on magnesium sulphate drug, and subject in groups 1,2 and 7 were not on any anti-hypertensives therapy;
v. Noticing the presence of oedema and proteinuria through rapid urine check for

subjects in groups 3,6 and 8 which are absent in subjects in other groups.

2.2.5 Collection of blood samples

2.2.5.1 Blood sample

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

2.2.5.2 Preparation of blood plasma

The blood samples 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°C for analysis of platelets counts, prothrombin, and fibrinogen.

2.2.6 Estimation of plasma troponin concentration

Concentration of troponin was estimated using enzyme linked Immunosorbent assay (ELISA) method [14].

2.2.6.1 Assay procedure

Plasma and standard (75 ng/mL) of 0.1mL each were pipetted appropriately into secured coated wells in a holder. 0.1mL of enzyme conjugate reagent [containing horseradish peroxidase with preserving agent (0.05%)] was also added into each well and thoroughly mixed for 30 sec. The reaction mixture was incubated at room temperature for 90 min. and the supernatant of the content discarded. The microtiter wells residues were washed 5 times with deionized water and the residual water droplets was removed by absorbent paper towels. 0.1 mL of tetramethylbenzidine reagent [containing H_2SO_4 (1%) in TMB-tris buffer (20 mmol/L at pH 7.3)] was added to each well, and the reaction mixture were gently mixed for 5 sec. The mixture was incubated at room temperature for 20 min., and the reaction was stopped by adding 0.1 mL of HCl (1N). The new content was thoroughly mixed for 30 sec. and the absorbance was read against reagent blank at 450 nm with a microtiter well reader within 15 min. The mean absorbance of the duplicates for each set of standard and plasma from the graph-curve and charts generated by the reader gave the troponin concentration.

2.2.7 Assay of plasma Creatine-kinase (CK) activity

Creatine-kinase activity was assayed according to reflotron analytical method [14].

2.2.7.1 Assay procedure

Plasma (0.03 mL) was applied using reflotron pipette to the centre of red application zone on the reflotron test strip. The reflotron sliding cover was opened, and the test strip was placed on to the guide. The strip was ensured to be locked within its guide position, thereafter the reflotron sliding cover was closed for 15 sec. Within this time interval, the instrument displayed creatine-kinase code on its visual centre, which gave allowance for enzymatic reactions to commence and stop at the peak of dye formation, after which the absorbance was read against reagent blank at 642 nm in the reflotron instrument and result displayed. The plasma creatine-kinase activity was calculated with a conversion factor of 0.63 U/L.

2.2.8 Determination of plasma C- reactive protein (CRP) concentration

Plasma C - reactive protein concentration was determined spectrophotometrically using turbidimetric method [14].

2.2.8.1 Procedure

Plasma and standard (0.5 mg/L) of 5 μL each were pipetted into their cuvettes; 0.02 mL of latex reagent coated with anti-CRP antibodies (0.20%) and 0.08 mL of diluent [containing tris buffer (20 mmol/L at pH 7.3), merthiolate (0.05 g/L) and sodium azide (0.90 g/L)] were added to them respectively. The reaction mixtures were properly mixed and initial absorbance (Abs.1) of plasma and standard read against reagent blank at 540 nm. This was then followed by the second absorbance (Abs.2) exactly after 2 min. at same 540 nm.

Calculation:

Concentration of Plasma C - reactive protein (mg/L) = [(Absorbance 2 - Absorbance 1) of test X Concentration of standard / (Absorbance 2 - Absorbance 1) of standard]

2.2.9 Assay of plasma lactate dehydrogenase (LDH) activity

Lactate dehydrogenase (LDH) activity was assayed using enzymatic method [14].

2.2.9.1 Assay procedure

Plasma and standard (2 mM) of 0.02 mL each were pipetted into their cuvettes. 1 mL of lactate dehydrogenase reagent [containing tris buffer (50 mmol/L), pyruvate (0.6 mmol/L) and NADH (0.18 mmol/L)] was added to all cuvette, and the reaction mixture was incubated in a water bath for 1 min. at 37°C. After incubation, absorbance of plasma and standard at 340 nm were recorded kinetically against reagent blank after 1, 2 and 3 min.

Calculation:

Plasma Lactate dehydrogenase = Mean LDH absorbance change per min. X 8095 U/L Activity (U/L)

2.2.10 Estimation of creatine concentration

Creatine concentration was estimated spectrophotometrically according to Modified Jaffe - Sloth's heat reaction method [14].

2.2.10.1 Procedure

The preformed creatinine was determined according to Jaffe's reaction procedures for plasma creatinine estimation. To determine the total creatinine, 3.5 mL, and 2.0 mL of water was added to tubes of standard and test. Thereafter, 2.0 mL of protein-free filtrate plasma and standard (1 mg/mL) from preformed creatinine were taken to tubes of test and standard. Picric acid (0.04 mol/L) of 1.0 mL was in like manner added to all tubes. The reaction mixtures were thoroughly mixed and heated for 1 hour at 100°C in water bath, after this, they were cooled and the volume made-up to 5.0 mL (with distilled water if any evaporation is noticed). To all tubes, 0.75M NaOH (1 mL) was added, mixed again and allowed to stand for 15 min. The absorbance was taken at 500 nm against reagent blank and correlated with values from the standard curves to obtain the total creatinine. The preformed creatinine was subtracted from the total creatinine and the difference multiplied by 1.16 to obtain the concentration of creatine in mg/dL of plasma.

2.2.11 Assay of plasma Aspartate Aminotransferase (AST) activity

This was assayed spectrophotometrically using enzymatic method [15].

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2.2.11.1 Assay procedure

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

2.2.12 Estimation of plasma myoglobin concentration

Concentration of myoglobin was estimated using enzyme linked Immunosorbent assay (ELISA) method [14].

2.2.12.1 Assay procedure

Plasma and standard (500 ng/mL) was diluted 10 fold with diluent [containing bovine serum (1%) and proclin (1%)] and the desired number of coated wells was secured in their holder. 0.02 mL of myoglobin standard and plasma with 0.2 mL of enzyme conjugate reagent [containing horseradish peroxidase with preserving agent (0.05%)] was appropriately dispensed into each well. The content was completely mixed for 30 sec., and incubated at room temperature for 45 min. Incubated mixture supernatant was removed by flicking plate contents into a waste container, thereafter the residue was washed 5 times with de-ionized water. All residual water droplets were removed with absorbent paper, and 0.1 mL of tetramethylbenzidine reagent solution [containing H_2SO_4 (1%) in TMB-tris buffer (20mmol/L at pH 7.3)] was added into each well. The reaction mixture was gently mixed for 5 sec. and incubated at room temperature for 20 min. The reaction was stopped after incubation, by adding 0.1mL of HCl (1N) to each well, and the new content was thoroughly mixed for 30 min. to make sure that all the blue colour changes to yellow colour completely. The absorbance was read at 450 nm with a microtiter well reader within 15 min., as the concentration of myoglobin.

2.3 Statistical Analysis

Results are expressed as mean ± 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

The results of this study showed that pre-eclamptic women that were not on $MgSO_4$, irrespective of the trimesters, showed significant increase ($P<0.001$, $P<0.01$ or $P<0.05$) in the investigated cardiac biomarkers (except in LDH) when compared with normotensive pregnant women (see Tables 1 and 2).

Pre-eclamptic women at both 2nd and 3rd trimesters that were on $MgSO_4$ however, showed significantly reduced level of plasma TRP, CRP and CRE ($P<0.05$) compared to those that were not on $MgSO_4$ (see Tables 3 and 4).

Furthermore, While Table 5 showed a significant reduction ($P<0.001$, except in LDH) in plasma level of investigated cardiac biomarkers in post-partum pre-eclamptic subjects that were on $MgSO_4$ compared to pre-eclamptic subjects that were on $MgSO_4$ at 3rd trimester, Table 6 however, showed that there was significant reduction ($P<0.01$ or $P<0.05$) in cardiac biomarkers (except in LDH) in normotensive women at post-partum compared to pre-eclamptic women on $MgSO_4$ at post-partum.

4. DISCUSSION

The results obtained from this study showed a significant increase in plasma troponin concentration of pre-eclamptic women compared to the normotensive group. This increase in troponin could be due to vasoconstriction in pre-eclamptic women. This is in line with findings of Jullian and Mauro [16]. However, there was a significant decrease in concentration of troponin in pre-eclamptic pregnant women treated with $MgSO_4$ at both 2nd and 3rd trimesters when compared with their counterparts that were not on $MgSO_4$. Moreover, troponin concentration at

Table 1. Cardiac biomarkers in normotensive pregnant women at second trimester [A] and pre-eclamptic women not on MgSO₄ at second trimester [B]

Group/Biomarkers	TRP (ng/mL)	CK (U/L)	CRP (mg/L)	LDH (U/L)	CRE (mg/dL)	AST (IU/L)	MYO (ng/mL)
A (n=20)	0.60±0.03	127.50±10.63	7.70±0.51	213.60±7.97	0.67±0.05	13.70±0.79	101.30±3.98
B (n=10)	1.20±0.15	192.00±10.63	11.20±0.86	232.80±17.60	1.10±0.13	23.60±1.80	157.80±5.26
Differences between means	B>A	B>A	B>A	B>A	B>A	B>A	B>A
Levels of significance	P<0.001	P<0.01	P<0.05	P>0.05	P<0.001	P<0.05	P<0.001

Table showed Means ± Standard error of mean (SEM), Differences between means and the Levels of significance (P<0.001, P<0.01, P<0.05 and P>0.05). TRP = Troponin, CK = Creatine-kinase, CRP = C-reactive protein, LDH = Lactate dehydrogenase, CRE = Creatine, AST = Aspartate aminotransferase, MYO = Myoglobin

Table 2. Cardiac biomarkers in normotensive pregnant women at third trimester [C] and pre-eclamptic women not on MgSO₄ at third trimester [D]

Group	TRP (ng/mL)	CK (U/L)	CRP (mg/L)	LDH (U/L)	CRE (mg/dL)	AST (IU/L)	MYO (ng/mL)
C (n=20)	0.70±0.03	154.40±7.94	9.10±0.70	235.90±5.47	0.85±0.08	13.60±1.36	118.40±4.40
D (n=10)	1.40±0.11	271.20±9.79	15.40±1.08	255.00±7.17	1.56±0.08	49.20±5.30	193.40±6.28
Differences between means	D>C	D>C	D>C	D>C	D>C	D>C	D>C
Levels of significance	P<0.001	P<0.001	P<0.001	P>0.05	P<0.001	P<0.001	P<0.001

Table showed Means ± Standard error of mean (SEM), Differences between means and the Levels of significance (P<0.001, P<0.01, P<0.05 and P>0.05). TRP = Troponin, CK = Creatine-kinase, CRP = C-reactive protein, LDH = Lactate dehydrogenase, CRE = Creatine, AST = Aspartate aminotransferase, MYO = Myoglobin

Table 3. Cardiac biomarkers in pre-eclamptic women not on MgSO₄ at second trimester [B] and pre-eclamptic women on MgSO₄ at second trimester [E]

Group/Biomarkers	TRP (ng/mL)	CK (U/L)	CRP (mg/L)	LDH (U/L)	CRE (mg/dL)	AST (IU/L)	MYO (ng/mL)
B (n=10)	1.20±0.15	192.00±10.63	11.20±0.86	232.80±17.60	1.10±0.13	23.60±1.80	157.80±5.26
E (n=60)	1.00±0.03	186.40±5.69	10.30±0.46	229.80±5.19	0.92±0.03	21.80±1.96	154.60±2.35
Differences between means	E<B	E<B	E<B	E<B	E<B	E<B	E<B
Levels of significance	P<0.05	P>0.05	P<0.05	P>0.05	P<0.05	P>0.05	P>0.05

Table showed Means ± Standard error of mean (SEM), Differences between means and the Level of significance (P>0.05). TRP = Troponin, CK = Creatine-kinase, CRP = C-reactive protein, LDH = Lactate dehydrogenase, CRE = Creatine, AST = Aspartate aminotransferase, MYO = Myoglobin

Table 4. Cardiac biomarkers in pre-eclamptic women not on MgSO₄ at third trimester [D] and pre-eclamptic women on MgSO₄ at third trimester [F]

Group/Biomarkers	TRP (ng/mL)	CK (U/L)	CRP (mg/L)	LDH (U/L)	CRE (mg/dL)	AST (IU/L)	MYO (ng/mL)
D (n=10)	1.40±0.11	271.20±9.79	15.40±1.08	255.00±7.17	1.56±0.08	49.20±5.30	193.40±6.28
F (n=60)	1.30±0.04	258.20±7.69	13.20±0.48	249.80±5.19	1.07±0.04	48.00±4.91	188.70±4.85
Differences between means	F<D	F<D	F<D	F<D	F<D	F<D	F<D
Levels of significance	P<0.05	P>0.05	P<0.05	P>0.05	P<0.001	P>0.05	P>0.05

Table showed Means ± Standard error of mean (SEM), Differences between means and the Level of significance (P<0.001, P>0.05). TRP = Troponin, CK = Creatine-kinase, CRP = C-reactive protein, LDH = Lactate dehydrogenase, CRE = Creatine, AST = Aspartate aminotransferase, MYO = Myoglobin

Table 5. Cardiac biomarkers in pre-eclamptic women on MgSO₄ at third trimester [F] and post-partum [H]

Group/Biomarkers	TRP (ng/mL)	CK (U/L)	CRP (mg/L)	LDH (U/L)	CRE (mg/dL)	AST (IU/L)	MYO (ng/mL)
F (n=60)	1.30±0.04	258.20±7.69	13.20±0.48	249.80±5.19	1.07±0.04	48.00±4.91	188.70±4.85
H (n=60)	0.80±0.03	148.30±4.44	8.20±0.35	237.00±5.01	0.72±0.02	24.10±1.45	128.40±1.45
Differences between means	H<F	H<F	H<F	H<F	H<F	H<F	H<F
Levels of significance	P<0.001	P<0.001	P<0.001	P>0.05	P<0.001	P<0.001	P<0.001

Table showed Means ± Standard error of mean (SEM), Differences between means and the Level of significance (P<0.001, P>0.05). TRP = Troponin, CK = Creatine-kinase, CRP = C-reactive protein, LDH = Lactate dehydrogenase, CRE = Creatine, AST = Aspartate aminotransferase, MYO = Myoglobin

Table 6. Cardiac biomarkers in normotensive women at post-partum [G] and pre-eclamptic women on MgSO₄ at post-partum [H]

Group/Biomarkers	TRP (ng/mL)	CK (U/L)	CRP (mg/L)	LDH (U/L)	CRE (mg/dL)	AST (IU/L)	MYO (ng/mL)
G (n=20)	0.60±0.03	121.00±9.71	7.10±0.44	228.20±5.88	0.64±0.04	11.90±2.25	107.10±2.72
H (n=60)	0.80±0.03	148.30±4.44	8.20±0.35	237.00±5.01	0.72±0.02	24.10±1.45	128.40±1.45
Differences between means	H>G	H>G	H>G	H>G	H>G	H>G	H>G
Levels of significance	P<0.05	P<0.05	P<0.05	P>0.05	P<0.05	P<0.05	P<0.01

Table showed Means ± Standard error of mean (SEM), Differences between means and the Level of significance (P<0.01, P>0.05). TRP = Troponin, CK = Creatine-kinase, CRP = C-reactive protein, LDH = Lactate dehydrogenase, CRE = Creatine, AST = Aspartate aminotransferase, MYO = Myoglobin

3-6 days post-partum decreases significantly compared to Pre-eclamptic Women on $MgSO_4$ at 3rd trimester.The reduction in troponin concentration might be due to the effect of $MgSO_4$ as a calcium antagonist that acted on calcium channels in vascular smooth muscle to decrease intracellular calcium, thereby causing inactivation of calmodulin-dependent myosin light chain kinase activity and decreased contraction which may subsequently lower troponin synthesis [5].

The plasma activity of creatine-kinase was raised significantly in pre-eclamptic women than the normotensive pregnant women. This result is in agreement with the findings of Burns [17], and Daniel [18]. The increase in creatine-kinase activity may be due to cell membrane disruptions from hypoxia in pre-eclampsia. However, a non-significant decrease was observed in activity of creatine-kinase in pre-eclamptic pregnant women treated with $MgSO_4$ at both 2nd and 3rd trimester when compared with their counterparts that were not on $MgSO_4$ treatment. Moreover, activity of creatine-kinase at 3-6 days post-partum decreased significantly compared to Pre-eclamptic Women on $MgSO_4$ at 3rd trimester. The reduction in creatine-kinase activity may be due to the effect of $MgSO_4$ as a good cell membrane protector that decrease cellular disruption and oedema formation from pinocytosis associated with pre-eclampsia whose actions also causes reduced blood-brain barrier permeability for creatine-kinase enzyme reduction. Similar observations have also been reported by Sibai [5].

C-reactive protein concentration in plasma of pre-eclamptic women was elevated significantly compared to the normotensive groups. The increase in C-reactive protein might be due to inflammations in pre-eclamptic women [19]. However, observation revealed significant reduction in plasma concentration of C-reactive protein in pre-eclamptic pregnant women treated with $MgSO_4$ at both 2nd and 3rd trimester compared to the groups that were not on $MgSO_4$. Moreover, C-reactive protein concentration at 3-6 days post-partum decreased significantly when compared with Pre-eclamptic Women on $MgSO_4$ at 3rd trimester. The reduction in C-reactive protein could be due to the effect of $MgSO_4$ as a good anti-inflammatory drug that initiates reduction in inflammation in pre-eclampsia by increasing production of prostaglandin which will inhibits platelets aggregation and macrophages factors release for inflammation to enhance

decreased C-reactive protein. This agrees well with the report of Sibai [5].

There was a non-significant increase in plasma activity of lactate dehydrogenase in pre-eclamptic women compared to normotensive pregnant women. This increase in lactate dehydrogenase might be due to release from red blood cells, enhanced by myocardial cell membrane disruption from hypoxia in pre-eclampsia [17]. Observations however showed that plasma activity of lactate dehydrogenase reduced non-significantly in pre-eclamptic pregnant women treated with $MgSO_4$ at both 2nd and 3rd trimester than the same groups that were not on $MgSO_4$ treatment. Moreover, activity of lactate dehydrogenase at 3-6 days post-partum decreases non-significantly when compared with Pre-eclamptic Women on $MgSO_4$ at 3rd trimester. The decrease in lactate dehydrogenase deduced that $MgSO_4$ helps in the prevention of endothelial damages to the myocardial cells that normally occur as a result of pre-eclampsia leading to decreased release of lactate dehydrogenase enzyme [20].

Result revealed significant elevation of plasma creatine concentration in pre-eclamptic women when compared with normotensive groups. The elevated plasma creatine could be due to vasoconstriction triggering vessel degradation for raised creatine in pre-eclampsia [21]. However, significant decrease of plasma creatine concentration was observed in pre-eclamptic pregnant women treated with $MgSO_4$ at both 2nd and 3rd trimester when compared with pre-eclamptic pregnant women that were not on $MgSO_4$. Moreover, creatine concentration at 3-6 days post-partum decreases significantly than the Pre-eclamptic Women on $MgSO_4$ at 3rd trimester. The reduction in creatine concentration is in agreement with the report of Livingston et al. [20]. This finding could be due to effect of magnesium sulphate to inhibit muscular and vascular damages by stimulating production of prostacyclin (a vasodilator) by endothelial cells causing vasodilation for reduction in plasma creatine.

Plasma aspartate aminotransferase activity was significantly raised in pre-eclamptic women compared to normotensive groups. This observation could be attributed to myocardial cell damage and injury in pre-eclampsia which increase plasma aspartate aminotransferase activity. This agrees well with the findings of Burns [17] and Luanne [22]. However, the activity

of plasma aspartate aminotransferase was non-significantly reduced in pre-eclamptic pregnant women treated with $MgSO_4$ at both 2nd and 3rd trimester when compared with those that were not on $MgSO_4$. Moreover, aspartate aminotransferase activity at 3-6 days post-partum decreased significantly when compared with Pre-eclamptic Women on $MgSO_4$ at 3rd trimester. The reduction in aspartate aminotransferase may be due to effect of $MgSO_4$ as angiotensin converting enzyme inhibitor that opposes the action of angiotensin converting enzyme by preventing myocardial cell damage and injury to significantly decrease aspartate aminotransferase. Similar observation has been reported by Witlin et al. [23].

Myoglobin concentration was elevated significantly in pre-eclamptic women when compared with normotensive pregnant women. The increase in plasma concentration of myoglobin could be due to cardiac hypoxia in pre-eclampsia demanding for more myoglobin to supply oxygen for recuperation [24]. On the other hand, the concentration of myoglobin was non-significantly decreased in pre-eclamptic pregnant women treated with $MgSO_4$ at both 2nd and 3rd trimester compared to those that were not on $MgSO_4$. Moreover, Myoglobin concentration at 3-6 days post-partum decreased significantly when compared with Pre-eclamptic Women on $MgSO_4$ at 3rd trimester. This decrease in myoglobin concentration suggest that $MgSO_4$ treatment may prevent hypoxia by increasing production of nitric oxide (a vasodilator), thus reducing myoglobin [20].

5. CONCLUSION

The results obtained from this work suggest that $MgSO_4$ may be exhibiting safe and protective roles devoid of any adverse effects on the heart, and also further agrees with the existing usage of magnesium sulphate drugs as an anti-convulsant 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.

ETHICAL APPROVAL

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

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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    PMID 9323061.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/14126