*Niger. J. Physiol. Sci. 33(December 2018) 195-200*
www.njps.com.ng

Niger. J. Physiol. Sci.

# Sub-Acute Toxicity Study of Ethylene Glycol Monomethyl Ether on the Antioxidant Defense System of the Testes and Epididymes of Wistar Rats

**Adeyemo-Salami, O. A. and Farombi, E. O.***
*Department of Biochemistry, University of Ibadan, Ibadan, Oyo State, Nigeria.*

Summary: Ethylene glycol monomethyl ether is a toxicant with wide industrial applications. This study is aimed at investigating its effect on the antioxidant system of the reproductive organs of male rats. Fifty male Wistar rats were distributed into five groups. Group I received distilled water, Groups II-V received EGME at 100, 200, 300 and 400 mg/kg body weight respectively. All administrations were done orally for fourteen days and the weight was monitored weekly. On day fifteen, the animals were sacrificed and reproductive organs were collected and weighed. The testes and epididymes were processed for the biochemical estimations, histopathology and spermatozoa analysis. The percentage body weight gained weekly and the relative weight of the testes reduced significantly *($p < 0.05$)* in the treatment groups. The spermatozoa analysis showed decreases in the treatment groups. In the testis and epididymis, various antioxidant parameters such as superoxide dismutase and glutathione-S-transferase were affected. The histopathology results confirmed the biochemical findings. The study suggests that EGME exerts deleterious effects on the testes and epididymes by increasing the oxidative load in rats.

**Keywords***:* Ethylene glycol monomethyl ether, Antioxidant defense, Spermatozoa, epididymes, testes.



**Address for correspondence*: olatunde_farombi@yahoo.com

*Manuscript Accepted: December, 2018*

## INTRODUCTION

Ethylene glycol monomethyl ether (EGME) is also known as methyl cellosolve (commercially), 2-methoxy ethanol, monomethyl ether, methyl glycol, monomethyl glycol, monomethyl ethylene glycol ether or methyl oxitol. Due to its hydrophilic and lipophilic properties, it has wide consumer and industrial applications. EGME is used as an anti-freeze additive in hydraulic fluids and jet fuel. It is also used in paints, stains, inks and surface coating, lacquers, photographic and photo lithographic processes, production of food-contact plastics, textile and leather finishing, and silk-screen printing as well as in the semi-conductor industry (Johanson, 2000, Takei *et al.*, 2010).

In humans and several other species, exposure to EGME either by inhalation, ingestion and/or dermal absorption has been reported to cause reproductive, hematopoietic and developmental toxicities with emphasis on testicular damage (Bagchi and Waxman, 2008). The evidences of reproductive toxicities as a result of exposure to EGME or the active oxidation product include gene expression changes in germ cells and mouse Leydig cells i*n-vitro* (Bagchi *et al.*, 2010); activation of caspases leading to apoptosis triggered by oxidative stress in spermatocytes (Bagchi and Waxman, 2008); prolonged estrus cycle, hypertrophy of corpora lutea evidenced by the presence of round to polygonal luteal cells with abundant vacuolated cytoplasm and ovulatory inhibition in rats (Dodo *et al.*, 2009, Taketa *et al.*, 2011); altered androgen-dependent processes in mouse Leydig cells *in-vitro* (Bagchi *et al.*, 2011); affects microRNAs expression in the testes of rats (Fukushima *et al.*, 2011); caused spermatocyte toxicity correlated with decreased expression of spermatocyte-specific genes (Matsuyama *et al.*, 2018); affects the antioxidant system and increase lipid peroxidation in the rat testes when treated dermally (Malik and Gupta, 2013). However, there is dearth of knowledge on the effect of EGME toxicity on the antioxidant defence system of the epididymes and testes, and report of the effect on the morphology of the spermatozoa is scarce.

According to IIRT (2017), the essence of a sub-acute toxicity study is to unravel the toxic effect of a drug/substance as a result of constant exposure and taking into consideration doses of EGME that have been worked with in previous studies, the duration of administration, mode of administration and the doses used for the study were determined.

This study was therefore designed to investigate the toxic effects of EGME on antioxidant components of the male reproductive organ of Wistar rats.

## MATERIALS AND METHODS

### *Reagents*

EGME and trichloro acetic acid were products of LobaChemie (Mumbai, India). All other reagents were either SureChem, U.K. or Sigma Aldrich. St. Louis, MO, U.S.A. products.

### *Experimental Animals and Care*

The protocol for the animal study was approved by the Animal Care and Use Research Committee of the University of Ibadan, Nigeria and the number UI-ACUREC/ APP/ 10/2016 /003 was assigned. Fifty (50) nine weeks old male Wistar rats weighing 140-190 g were obtained from the Primate Colony of the Department of Biochemistry, University of Ibadan and randomly distributed into five groups of ten animals each. They were kept in appropriate laboratory cages and given feed (Ladokun Feed, Nigeria) and laboratory water *ad libitum* in the Animal house of the same Department where they were acclimatized for a week. The 12 hour light/dark cycle was maintained.

### *Experimental Design*

All mandatory laboratory health and safety procedures where adhered to while conducting the experiment. Administrations were carried out orally daily at the same time for fourteen consecutive days and the weight was monitored weekly. Stock solution of EGME was prepared using distilled water as the vehicle. The protocol for administration is as follows:

Group I received distilled water only (Control)
Group II received EGME at 100 mg/kg body weight dosage
Group III received EGME at 200 mg/kg body weight dosage
Group IV received EGME at 300 mg/kg body weight dosage
Group V received EGME at 400 mg/kg body weight dosage

On day 15, the animals were euthanized by cervical dislocation after an overnight fast. The animals were then dissected and the testes, epididymes, prostate gland and seminal vesicles were removed into ice- cold 1.15 % potassium chloride, blotted with Whatman no.2 filter paper and weighed using a Mettler balance. The right testes and epididymes samples were used for spermatozoa analysis and the ones for histopathology were fixed in Bouin's solution, processed, sectioned, mounted on slides and stained for histological examination. The left testis and epididymis were used for the biochemical analyses of antioxidant markers.

### *Spermatozoa analysis*

**(a)** *Assessment of the characteristics of epididymal sperm*

The method of Zemjanis (1970) was employed to evaluate the motility of the sperm. The improved Neubauer chamber haemocytometer (LABART, Munich, Germany) was used to count the sperm according to Pant and Srivastava (2003). Employing the method of Wells and Awa (1970), a total of 400 sperm/ rat were used for examination of the morphology and application of nigrosin-eosin-sodium citrate dehydrate solution was used for assessing viability.

*(b)Testicular sperm number (TSN) and Daily sperm production (DSP) determination*

The method of Blazak *et al.*(1993) was employed. To calculate the DSP, the number of spermatids at stage 19 was divided by 6.1 (6.1 is the period of seminiferous cycle in which the spermatids are present in the seminiferous epithelium).

### *Biochemical analysis*

The left testis and epididymis of each animal were homogenized in 4 and 20 volumes respectively of tris-HCl/KCl buffer (pH 7.4) and the homogenates were centrifuged at 4 $^{o}$C for 10 mins at 10,000 g. The supernatant was then decanted and used for the antioxidant assays as follows: Protein content was determined using the method of Lowry *et al.*(1951) and was used to normalize the antioxidant indices; malondialdehyde formed was estimated using the method of Varshney and Kale (1990) for the determination of lipid peroxidation (LPO); the method of Claiborne (1985) was used to determine catalase (CAT) activity; superoxide dismutase (SOD) activity was evaluated using the Misra and Fridovich (1972) method; glutathione-S-transferase (GST) activity was determined using the method of Habig *et al.*(1974); vitamin C concentration was assessed using the Jakota and Dani (1982) method; the method of Beutler *et al.* (1963) was employed in determining the concentration of reduced glutathione (GSH); glutathione peroxidase (GPx) was assessed using the method of Rotruck *et al.* (1973) and lactate dehydrogenase (LDH) activity was determined using the method of Vassault (1983). It is important to note that in the assays for GPx, LPO and vitamin C, the trichloroacetic acid was weighed into glass beakers because it is very corrosive.

### *Statistical analysis*

All data, as appropriate, were expressed as mean ± standard error of mean (S.E.M). Statistical analyses were carried out using one- way analysis of variance (ANOVA). Values of $p < 0.05$ were considered to be significant and post- hoc tests were carried out using the least significant difference.

## RESULTS

Figure 1 shows the percentage weight gained weekly over the fourteen day treatment period. The groups of the control, 100, 200 and 300 mg/kg doses showed an increase in weight weekly over the period of study while the 400 mg/kg dose group decreased from 12% to 10% between day 7 and day 14.

Table 1 reveals that the weight of the testis decreased significantly ($p < 0.05$) in the 200, 300 and 400 mg/kg

dosage groups compared to the control. This is also presented in the relative weight.

The spermatozoa analysis (Table 2) shows that the daily spermatozoa production, testicular spermatozoa number, epididymal spermatozoa number, sperm motility and sperm viability decreased significantly ($p < 0.05$), especially at the 200, 300 and 400 mg/kg doses compared to the control. The percentage sperm abnormalities increased significantly ($p < 0.05$) in the 200, 300 and 400 mg/kg doses (Table 2).

The details of the abnormalities observed in the spermatozoa are displayed in Table 3. There was significantly ($p<0.05$) increased prevalence of curved- and bent- mid-pieces, curved- and bent- tails in the spermatozoa at the 200 and 400 mg/kg dose of EGME.

Table 4 reveals the effect of EGME on testicular antioxidant parameters. The activities of CAT, GST and GPx increased significantly ($p<0.05$) at the 200, 300 and 400 mg/kg doses compared to the control. The

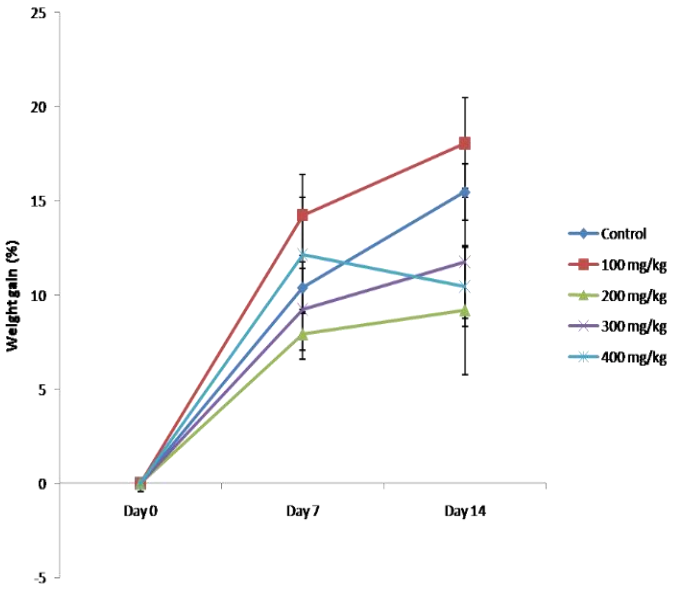


**Figure 1:** Percentage weight gain of male Wistar rats over fourteen day treatment of ethylene glycol monomethyl ether

**Table 1:** Effect of EGME on weight and relative weight of organs in male Wistar rats after a fourteen day treatment

| | Weight of organs (g) | | | | Relative weight of organs (%) | | | |
|---|---|---|---|---|---|---|---|---|
| **Group** | **Testes** | **Epididymes** | **Seminal Vesicles** | **Prostate Gland** | **Testes** | **Epididymes** | **Seminal Vesicles** | **Prostate Gland** |
| **I** | 2.10± 0.06 | 0.16± 0.01 | 0.75± 0.10 | 0.28± 0.03 | 1.07± 0.02 | 0.08± 0.01 | 0.38± 0.05 | 0.14± 0.01 |
| **II** | 1.94± 0.11 | 0.16± 0.01 | 0.66± 0.05 | 0.26± 0.02 | 1.02± 0.05* | 0.09± 0.00 | 0.35± 0.02 | 0.14± 0.01 |
| **III** | 1.28± 0.06* | 0.16± 0.00 | 0.66± 0.13 | 0.23± 0.03 | 0.69± 0.03* | 0.08± 0.01 | 0.35± 0.07 | 0.12± 0.02 |
| **IV** | 1.13± 0.04* | 0.14± 0.01 | 0.49± 0.12 | 0.21± 0.04 | 0.63± 0.02* | 0.08± 0.00 | 0.27± 0.05 | 0.10± 0.02 |
| **V** | 1.12± 0.05* | 0.12± 0.01 | 0.35± 0.03 | 0.22± 0.04 | 0.65± 0.07* | 0.07± 0.01 | 0.19± 0.02 | 0.12± 0.02 |

Note: n = 10; *- significant at $p < 0.05$; values are mean±standard error of mean

**Table 2:** Effect of fourteen day treatment of EGME on the spermiogram in Wistar rats

| | Group | | | | |
|---|---|---|---|---|---|
| **Parameter** | **I** | **II** | **III** | **IV** | **V** |
| **Daily Spermatozoa Production** (x $10^6$ / gm testis) | 19.31± 0.61 | 15.06± 1.68 | 15.05± 0.96 | 16.54± 2.16 | 14.80± 1.14 |
| **Testicular Spermatozoa Number** (x $10^6$/ gm testis) | 46.00± 3.61 | 33.33± 3.53 | 26.67± 3.28* | 24.67±2.40* | 21.00± 1.73* |
| **Epididymal Spermatozoa Number** (x $10^6$/ml) | 132.33± 3.62 | 123.00±3.14 | 107.57± 6.93* | 102.57±5.98* | 107.14±5.75* |
| **Motility (%)** | 92.22± 0.88 | 80.00± 2.11* | 71.25± 2.95* | 70.00± 3.78* | 65.00± 3.42* |
| **Viability (%)** | 97.33± 0.44 | 96.50± 0.50 | 93.78± 1.72 | 95.25± 1.56 | 86.57± 7.94* |
| **Abnormalities (%)** | 10.60± 0.46 | 11.53± 0.27 | 12.28± 0.60* | 12.05± 0.59* | 12.52± 0.40* |

Note: n = 10; *- significant at $p < 0.05$; values are mean±standard error of mean

**Table 3:** Constituents of spermatozoa abnormalities

| **Group** | **Head (%)** | **Mid piece (%)** | | **Tail (%)** | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Tailless** | **Curved** | **Bent** | **Headless** | **Rudimentary** | **Bent** | **Curved** | **Looped** |
| **I** | 1.14± 0.11 | 2.03± 0.13 | 2.10± 0.13 | 1.02± 0.07 | 0.47± 0.08 | 1.98± 0.08 | 1.98± 0.08 | 0.47± 0.07 |
| **II** | 1.23± 0.09 | 2.21± 0.11 | 2.26± 0.08 | 1.14± 0.08 | 0.42± 0.07 | 2.24± 0.09 | 2.19± 0.09 | 0.42± 0.07 |
| **III** | 1.32± 0.06 | 2.28± 0.12 | 2.37± 0.12 | 1.07± 0.10 | 0.47± 0.09 | 2.34± 0.14* | 2.29± 0.11* | 0.41± 0.07 |
| **IV** | 1.24± 0.09 | 2.29± 0.15 | 2.16± 0.13 | 1.24± 0.07* | 0.43± 0.08 | 2.29± 0.15 | 2.19± 0.14 | 0.37± 0.06 |
| **V** | 1.28± 0.10 | 2.62± 0.11* | 2.41± 0.11* | 1.10± 0.11 | 0.50± 0.09 | 2.45± 0.08* | 2.55± 0.11* | 0.39± 0.07 |

Note: n = 10; *- significant at $p < 0.05$; values are mean±standard error of mean

**Table 4:** Effect of EGME on testicular antioxidant parameters in Wistar rats

| **Group** | **LPO** | **CAT** | **SOD** | **GST** | **VIT.C** | **GSH** | **GPx** | **LDH** |
|---|---|---|---|---|---|---|---|---|
| **I** | 4.22± 0.13 | 49.33± 1.02 | 8.83± 0.61 | 6.39± 0.25 | 34.50± 2.01 | 4.84± 0.12 | 238.01± 7.51 | 23.05± 1.00 |
| **II** | 4.50± 0.12 | 49.32± 1.82 | 3.44± 0.57* | 7.13± 0.51 | 41.83± 3.17 | 4.50± 0.08 | 260.32± 14.25 | 16.27± 1.43* |
| **III** | 6.86± 0.30* | 79.96± 3.23* | 3.74± 0.75* | 9.97± 0.60* | 41.00± 3.71 | 4.49± 0.25 | 395.00± 18.69* | 14.06± 1.54* |
| **IV** | 6.18± 0.39* | 105.79±6.57* | 4.69± 0.70* | 11.83± 0.28* | 40.25± 3.12 | 4.85± 0.16 | 439.77± 15.21* | 8.24± 0.17* |
| **V** | 7.14± 0.60* | 101.75± 14.43* | 2.85± 0.16* | 12.38± 0.92* | 35.67± 1.20 | 4.57± 0.10 | 464.22± 22.65* | 6.24± 0.45* |

Note: n = 10; *- significant at $p < 0.05$; values are mean±standard error of mean; KEY: Parameter (unit):- LPO- Lipid peroxidation (µmol malondialdehyde formed/ mg protein); CAT- Catalase (mmoles $H_2O_2$ consumed/min/mg protein); SOD- Superoxide dismutase (unit/mg protein); GST- Glutathione-S-Transferase (µmol/min/mg protein); VIT. C- Ascorbic acid (µg ml-1); GSH- reduced glutathione (µmol/ g tissue); GPx- Glutathione peroxidase (µg/ml/mg protein); LDH- Lactate dehydrogenase (unit/ mg protein)

**Table 5:** Effect of EGME on epididymal antioxidant parameters in Wistar rats

| Group | LPO | CAT | SOD | GST | VIT.C | GSH | GPx | LDH |
|---|---|---|---|---|---|---|---|---|
| I | 17.62± 0.17 | 127.48± 12.14 | 16.67± 2.38 | 9.18±0.75 | 3.50± 0.50 | 12.64± 1.26 | 3.05± 0.20 | 30.92± 3.75 |
| II | 17.79±1.68 | 120.32± 13.20 | 19.05± 2.38 | 14.24±1.99* | 4.67± 0.67 | 12.84± 0.77 | 3.15± 0.09 | 2.66± 0.12* |
| III | 17.40±1.28 | 135.97± 15.08 | 21.43± 5.05 | 13.09±1.05 | 3.50± 0.22 | 10.80± 1.17 | 3.16± 0.10 | 3.37± 0.56* |
| IV | 24.60± 3.21* | 140.27±18.89 | 28.57± 7.14 | 20.36± 2.84* | 7.20± 0.58* | 15.89± 1.24 | 3.68± 0.26* | 2.99± 0.92* |
| V | 24.42± 2.18* | 135.06± 7.14 | 30.95± 2.38* | 21.27± 3.44* | 6.00± 0.41* | 16.25±1.37* | 4.02± 0.28* | 0.97± 0.10* |

Note: n = 10; *- significant at $p < 0.05$; values are mean±standard error of mean; KEY: Parameter (unit):- LPO- Lipid peroxidation (µmol malondialdehyde formed/ mg protein); CAT- Catalase (mmoles $H_2O_2$ consumed/min/mg protein); SOD- Superoxide dismutase (unit/mg protein); GST- Glutathione-S-Transferase (µmol/min/ mg protein), VIT. C- Ascorbic acid (µg mol-1); GSH- reduced glutathione (µmol/ g tissue); GPx- Glutathione peroxidase (µg/ml/mg protein); LDH- Lactate dehydrogenase (unit/ mg protein)

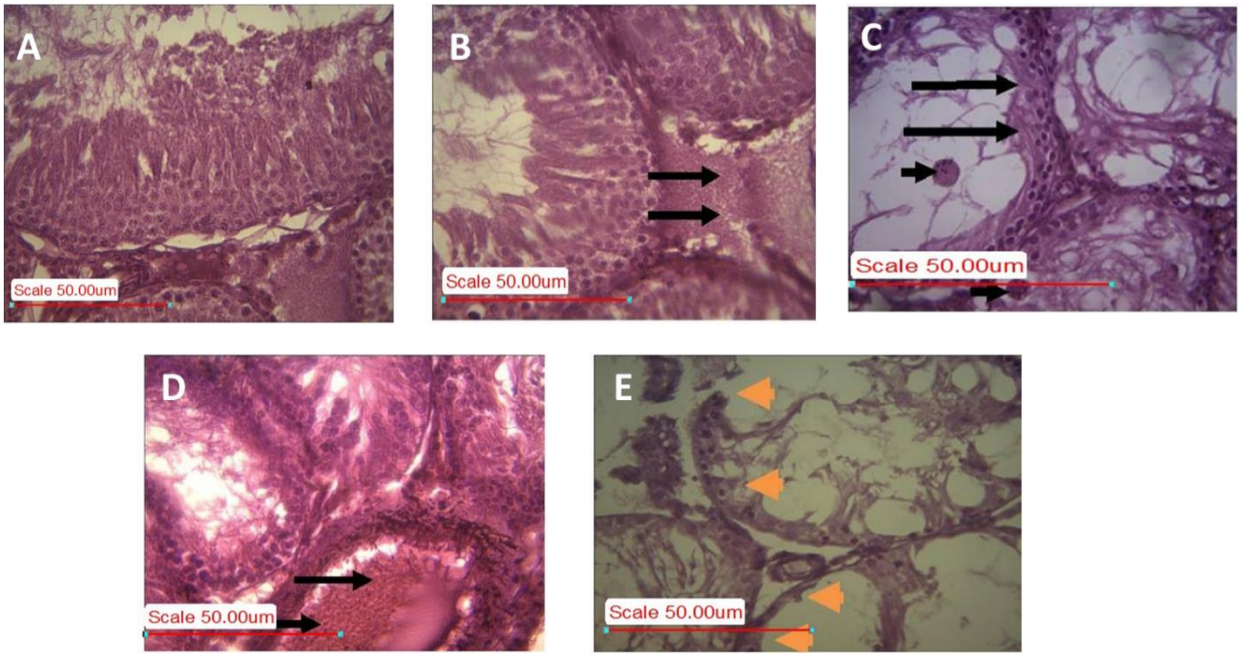


**Figure 2**: Photomicrographs of Testis in the control and treatment groups after 14 day treatment of EGME. H&E, Mag. x400. A: Control- No lesions; B: 100mg/kg- Mild quantity of pink staining (oedema) fluid in the interstitium; C: 200 mg/kg- Some sections of the seminiferous tubules have a greatly reduced germinal epithelial height (*long arrows*). Late maturing stages appear absent and there are few cellular clumps in the lumina (*short arrows*); D: 300 mg/kg- Mild congestion of the interstitial vessels; E: 400 mg/kg- Severe epithelial erosion.

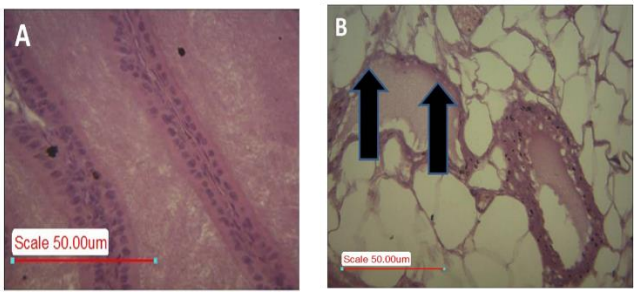


**Figure 3:** Photomicrographs of Epididymis in the control and 400 mg/kg dose group after 14 day treatment of EGME. H&E, Mag. x400. A: Control- No lesion; B: 400 mg/kg- Severe diffuse germinal cell erosion

activities of SOD and LDH decreased significantly ($p < 0.05$) and LPO increased significantly ($p > 0.05$) in the 200, 300 and 400 mg/kg dosage groups compared to the control. The levels of vitamin C and GSH were not affected (Table 4).

The antioxidant analysis in the epididymis (Table 5) showed that there were increases in LPO; in the activities of CAT, SOD, GST and GPx; and in the levels of vitamin C and GSH compared to the control. These increases were significant ($p < 0.05$) at the 300 and 400 mg/kg doses. There was significant ($p < 0.05$) decrease in the activity of LDH in all doses compared to the control. Figure 2 shows the histopathology of the testis in the control and treatment groups. The control group (A) had no lesions and the 100 mg/kg group (B) showed oedema in the interstitium. The 200 mg/kg dose group (C) showed that some of the sections of the seminiferous tubules have a greatly reduced germinal epithelial height, late maturing stages were absent and there were few cellular clumps in the lumina. The 300 mg/kg dose group (D) showed mild congestion of the interstitial vessels while the 400 mg/kg dose group (E) showed severe epithelial erosion. Figure 3 shows the histopathology of the epididymis in the control and treatment groups. No lesions were observed in the control group (A) and the treatment groups, but the 400 mg/kg dose group (B) showed severe diffuse germinal cell erosion.

## DISCUSSION

EGME elicits gonadotoxicity by inducing oxidative stress in the testes and epididymes, as shown by the effect on the enzymatic and non-enzymatic antioxidants, and alterations in the morphology of spermatozoa.

The decrease in body weight in the 400 mg/ kg dose of EGME is a sign of toxicity. The decrease in weight and relative weight of testis in the treatment groups especially 200, 300 and 400 mg/kg doses of EGME is similar to the observations of Welsch (2005) and Malik and Gupta (2013) in rats and humans.

Daily spermatozoa production (DSP) decreased insignificantly in all the treatment groups compared to the control. The testes are the sites for spermatogenesis and androgen production. The gradual significant decrease in testicular spermatozoa number shows a disturbance in spermatogenesis. The epididymis is the site for sperm maturation as they traverse the different regions of the epididymis from the testis. Significant decrease in epididymal spermatozoa number is therefore expected since the testicular spermatozoa number is decreased. The epididymis is also the site where sperm acquires significant motility. Significant decrease in sperm motility in the treatment groups suggests that the integrity of internal milieu of the epididymis is compromised.

The spermatozoa viability decreased gradually in a dose-dependent manner in the treatment groups and was significant at the 400 mg/kg dose of EGME. This implies that there is a larger number of dead spermatozoa compared to live spermatozoa with increased dosage of EGME. Thus, showing that EGME had adverse effect on the spermatozoa. Moreover, the percentage abnormalities which increased significantly in the treatment groups showed that there was increased presence of deformed sperms in the treatment groups. These deformities included tailess- head, curved- and bent- mid-pieces, and headless-, bent- and curved- tails which were significantly present at the 400 mg/kg dose.

Oxidative stress is a condition where the enzymatic and/or non-enzymatic scavengers of reactive oxygen species (ROS) are overwhelmed by the level of ROS in the system (Willcox *et al.*, 2004). Catalase (CAT), glutathione-S-transferase (GST), superoxide dismutase (SOD) and glutathione peroxidase (GPx) are enzymatic ROS scavengers while reduced glutathione and vitamin C are non-enzymatic ROS scavengers. In the testes, the activities of CAT, GST and GPx were increased significantly in the treatment groups especially at 200, 300 and 400 mg/kg doses of EGME. This is a sign of adaptive response. However, the activity of SOD was significantly reduced in the treatment groups thus confirming oxidative stress. Lactate dehydrogenase is an enzyme of the glycolytic pathway and is used to identify the location, and severity of tissue damage. The significant dose-dependent decreased activity of this enzyme in the treatment groups may be as a result of its inhibition by the metabolite of EGME. These observations are at variance with the findings of Malik and Gupta (2013) who administered EGME dermally to rats for 28 days at a dose of 2g/ kg body weight. This may be because the route of administration was different as well as the duration was longer and the dose higher.

Lipid peroxidation, which is an indication of oxidative degradation of lipids by free radicals, was significantly elevated in a dose-dependent manner in the testes of the treatment groups. This shows damage to the membrane of the cells of the testis. The histopathology confirmed the results showing varying degrees of lesions in the treatment groups with the 400 mg/kg dose of EGME group reflecting severe epithelial erosion.

In the epididymis, the activities of GPx, GSH, CAT, SOD, GST and vitamin C level were elevated and were significant at the 400 mg/kg dose of EGME. Again, this shows adaptive response. LPO was also increased significantly at the 300 and 400 mg/kg dose while the activity of LDH was decreased significantly in a dose-dependent manner in the treatment groups. This again suggests that LDH may be inhibited by the metabolite of EGME. The histopathology showed that there was severe diffuse germinal cell erosion only at the 400 mg/kg dose of EGME thus buttressing the elevated lipid peroxidation.

All these findings support the results of the spermatozoa analysis. The toxicity of EGME was further made obvious by the survival rate which was observed to be 90% at 200 mg/kg, 80% at 300 mg/kg and 70 % at 400 mg/kg.

The data show that Ethylene glycol monomethyl ether effects its gonadotoxic potential in the epididymes and testes by inducing oxidative stress, as shown by the effect on the antioxidant defence system. It also shows the effects on the morphology of spermatozoa.

**Acknowledgement:** The authors appreciate the Federal Government of the Republic of Nigeria for the National Earned Allowance postgraduate study grant awarded to Oluwatoyin A. Adeyemo- Salami, which was utilized in conducting this study.

## REFERENCES

Bagchi, G. and Waxman, D.J. (2008). Toxicity of ethylene glycol monomethyl ether: impact on testicular gene expression. Int. J. Androl. (Oxford). 31: 269 – 274.

Bagchi, G., Zhang Y. and Waxman D.J. (2010). Impact of methoxy acetic acid on mouse Leydig cell gene expression. Reprod. Biol. Endocrin. 8: 65.

Bagchi G., Zhang Y., Stanley K.A. and Waxman D.J. (2011). Complex modulation of androgen responsive gene expression by methoxy acetic acid. Reprod. Biol. Endocrin. 9: 42.

Beutler, E., Duron, D.J and Kelly, M.B. (1963). Improved method for the determination of blood glutathione. J. Lab. Clin. Med. 61: 882 – 888.

Blazak, W.F., Trienen, K.A. and Janiewicz, P.E. (1993). Application of testicular sperm head counts in the assessment of male reproductive toxicity. In: Chapin, R.E. and Hendel, J. (eds.). Methods in Toxicology. Male Reproductive Toxicology, Vol. 3A. Academic Press, San Diego, pp. 86-94.

Claiborne, A. (1985). Catalase activity. In: Greenwald, R.A. (ed.). Handbook of Methods for Oxygen Radical Research. CRC Press, Boca Raton, FL, pp. 243 – 247.

Dodo, T., Taketa, Y., Sugiyama, M., Inomata, A., Sonoda, J., Okuda, Y., Mineshima, H., Hosokawa, S. and Aoki, T. (2009). Collaborative work on evaluation of ovarian toxicity. II) two – or 4 – week repeated dose studies and fertility study of ethylene glycol monomethyl ether in female rats. J. Toxicol. Sci. 34: SP 121 – 128.

Fukushima, T., Taki, K., Ise, R., Horii, I. and Yoshida, T. (2011). Micro RNAs expression in the Ethylene Glycol Monomethyl Ether-induced testicular lesion. J. Toxicol. Sci. 36: 601- 611.

Habig, W.H., Pabst, M.J. and Jakoby, W.B. (1974). Glutathione – S – transferase: The first enzymatic step in mercapturic acid formation. J. Biol. Chem. 249: 7130 – 7139.

Institute for Industrial Research and Toxicology (IIRT) (2017). Toxicological studies. www.toxicityindia.org/category/toxicology/sub-acute-toxicity-studies.html.

Jakota, S.K. and Dani, H.M. (1982). A colorimetric technique for the estimation of vitamin C using folin phenol reagent. Anal. Biochem. 127: 178 – 182.

Johanson, G. (2000). Toxicity review of ethylene glycol monomethyl ether and its acetate. Crit. Rev. Toxicol. 30: 307 – 345.

Lowry, O.H., Rosenbrough, N.J., Farr, A.I. and Randall, R.J. (1951). Protein measurement with Folin phenol reagent. J. Biol. Chem. 193: 265.

Malik, T. and Gupta, G.D. (2013). Effects of ethylene glycol monomethyl ether on testicular antioxidant system in adult rats. Research and Reviews: A Journal of Toxicology. 3: 1 – 6.

Matsuyama, T., Yabe, K., Kuwata, C., Ito, K., Ando, Y., Iida, H. and Mori, K. (2018). Transcriptional profile of ethylene glycol monomethyl ether-induced testicular toxicity in rats. Drug Chem Toxicol. 41:105-112. doi: 10.1080/01480545.2017.1320406.

Misra, H.P. and Fridovich, I. (1972). The role of superoxide anion in the autooxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem. 247: 3170 – 3175.

Pant, N. and Svivastava, S.P. (2003). Testicular and spermatotoxic effects of quinophosin rats. J. Appl. Toxicol. 23: 271 – 274.

Rotruck, J.T., Pope, A.L., Ganther, H.E., Swanson, A.B., Hafeman, D.G. and Hoekstra, W.G. (1973). Selenium: biochemical role as a component of glutathione peroxidase. Science. 179: 588 – 590.

Takei, M., Ando, Y., Saitoh, W., Tanimoto, T., Kiyosawa, N., Manabe, S., Sanbuissho, A., Okazaki, O., Iwabuchi, H., Yamoto, T., Adam, K-P., Weiel, J.E., Ryals, J.A., Milburn, M.V. and Gno, L. (2010). Ethylene glycol monomethyl ether – induced toxicity is mediated through the inhibition of flavoprotein dehydrogenase enzyme family. Toxicol. Sci. 118: 643 – 652.

Taketa, Y., Inomata, A., Hosokawa, S., Sonoda, J., Hayakawa, K., Nakano, K., Momozawa, Y., Yamate, J., Yoshida, M., Aoki, T. and Tsukidate, K. (2011). Histo-pathological characteristics of luteal hypertrophy induced by ethylene glycol monomethyl ether with a comparison to normal luteal morphology in rats. Toxicol. Pathol. 39: 372 – 380.

Varshney, R. and Kale, R.K. (1990). Effect of calmodulin antagonists on radiation – induced lipid peroxidation in microsomes. Int. J. Radiat. Biol. 58: 733 – 743.

Vassault, A. (1983). Lactate dehydrogenase. UV – method with pyruvate and NADH. In: H.U. Bergmeyer, ed. Methods of Enzymatic Analysis, 3rd edition. Plenum, New York, pp. 118 – 125.

Wells, M.E. and Awa, O.A. (1970). New technique for assessing acrosomal characteristics of spermatozoa. J. Dairy Sci. 53: 227.

Welsch, F. (2005). The mechanism of ethylene glycol ether reproductive and developmental toxicity and evidence of advance effects in humans. Toxicol. Lett. 156: 13 – 28.

Willcox, J.K., Ash, S.L. and Catignani, G.L. (2004). Antioxidants and prevention of chronic disease. Crit. Rev. Food Sci. Nutr. 44: 275 – 295.

Zemjanis, R., 1970. Collection and evaluation of semen. In Zemjanis, R. (ed.) Diagnostic and Therapeutic Technique in Animal reproduction. 2nd edition. Waverly Press, Baltimore, pp. 139-153.

Fernandez-Lopez, D., Serena, J., Castillo-Melendez, M., Moro, M.A. and Lizasoain, I. (2011). Neurological tests for functional outcome assessment in rodent models of ischaemic stroke. *Rev Neurol*, 53(10): 607-618.