Biomedicine & Pharmacotherapy 127 (2020) 110227

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

Original article

Ketogenic diet improves and restores redox status and biochemical indices in monosodium glutamate-induced rat testicular toxicity

Omowumi T. Kayode[a], Damilare E. Rotimi[a], Tomilola D. Olaolu[a], Oluyomi Stephen Adeyemi[b,c,*]

[a] Department of Biochemistry, Medicinal Biochemistry, Nanomedicine & Toxicology Laboratory, Landmark University, PMB 1001, Omu-Aran, 251101, Nigeria
[b] Laboratory of Theoretical and Computational Biophysics, Ton Duc Thang University, Ho Chi Minh City, Viet Nam
[c] Faculty of Applied Sciences, Ton Duc Thang University, Ho Chi Minh City, Viet Nam

ARTICLE INFO

Keywords:
Flavor enhancer/food additive
Ketogenic diets
Low carb
Medicinal biochemistry
Testicular dysfunction

ABSTRACT

This study investigated the effect of ketogenic diet on monosodium glutamate (MSG)-induced testicular dysfunction. Forty-six male rats (180 ± 40 g) were grouped into two groups (23 rats each); control group and MSG-induced group (4 mg/kg bw) for 28 days. At the 29th day, 5 rats from both group were sacrificed to establish testicular dysfunction. The remaining animals from the control group was further divided into three sub-groups and treated for 42 days; untreated group, ketogenic diet only and curcumin only as the standard drug (150 mg/kg bw). In the pre-treatment, the administration of MSG resulted in a significant ($p < 0.05$) decrease in the testis-body weight ratio, alkaline phosphatase (ALP), acetylcholine esterase (AChE), cholesterol, triglycerides (TG), nitric oxide (NO), glycogen, protein and antioxidant enzymes in the testis. In the post treatment, the MSG only group significantly reduced testicular cholesterol, catalase (CAT) and NO. In contrast, MSG + ketogenic diet group showed a significant increase in levels of rat testicular acid phosphatase (ACP), ALP, cholesterol, HMG-CoA, TG, malondialdehyde (MDA), reduced glutathione (GSH) and NO. The ketogenic diet showed a significant increase ($p < 0.05$) in the levels of NO, ALP, cholesterol, HMG CoA reductase and (TG). In addition, significant increases in levels of rat testicular ACP, ALP, HMG-CoA, (CAT), SOD and GSH were recorded for MSG + Curcumin group. Taken together, the findings support the prospects of ketogenic diet to enhance the testicular function in rats.

1. Introduction

Testicular dysfunction is referred to as myriads of irregular occurrences in the testes, usually as a result of an extrinsic factor. Testicular dysfunction is a major cause of male sexual and reproductive dysfunctions. The testes are highly susceptible to damage from different exposures to chemicals which could lead to testicular dysfunction [1,2]. Examples of such toxic exposure include heavy metals like cadmium, lead, arsenite and also MSG. Fernandes et al. [3] reported that administration of MSG at 4.0 mg/g for 120 days exhibited testicular, prostatic and epididymis dysfunction. MSG triggers hemorrhage in the testis, deterioration of sperm production and cell structure which ultimately results in male infertility [4].

Monosodium glutamate (MSG) is a flavor enhancing food additive found in virtually every household in Western Africa and Asia. Sodium glutamate or monosodium glutamate contains glutamate as a major constituent. Manufactured MSG predominantly contain 99.6 % of L-glutamate enantiomer which gives its flavor enhancing property in foods worldwide. It is essential in metabolism and act as an excitatory neurotransmitter [5]. MSG is a major component of many proteins such as milk, meat, fish, and some vegetables. It has a daily consumption rate of 300–4000 mg/day in developed countries [4]. Repeated consumption of MSG has been linked with many deleterious effects in animals and human studies [6]. Some adverse effects of MSG include genotoxicity, hepatotoxicity, renal and reproductive toxicity. Neurotoxic effects induced by MSG caused pathological conditions such as Parkinson's diseases, depression, stroke, anxiety, addiction, Alzheimer's disease, brain trauma, and epilepsy [4,5].

Available treatment for testicular dysfunction includes the use of drugs, plant extracts, surgical operation, hormonal supplementation and the use of diet. Although different treatment procedures are currently available for the management of testicular dysfunction but problems of costs, efficacy, safety and ease of administration are often the challenges. Therefore, there is need to search for novel treatment particularly of natural origin that are cheap, locally available and with very little side effects. Ketogenic diet comprises of high fat, low carb and

* Corresponding author at: Ton Duc Thang University, Ho Chi Minh City, Viet Nam.
E-mail address: oluyomi.stephen.adeyemi@tdtu.edu.vn (O.S. Adeyemi).

https://doi.org/10.1016/j.biopha.2020.110227
Received 11 April 2020; Received in revised form 30 April 2020; Accepted 3 May 2020

moderate protein diet [7]. This results in high fat metabolism and lesser metabolism of carbohydrates and proteins. As a result, there are alteration in energy metabolism because of the high ketone bodies derived from fat and reduced blood glucose level [8]. Ketogenic diet however, has been used to treat disease conditions like cancer, inflammation, seizure, Parkinson's disease and Alzheimer [7]. In addition, ketogenic diet has been acclaimed to manage sexual activity [9], but there is a dearth of empirical evidence on its use as a pro-fertility to justify its acclaimed testicular enhancing property.

2. Material and methods

2.1. Assay kits and reagents

The assay kits for cholesterol, triglycerides and HMG-CoA reductase were obtained from Agappe Diagnostics Ltd, Switzerland. Other assays were carried out using analytical grade reagents prepared in glass wares and further stored at 37 °C in clean reagent bottles.

2.2. Low carbohydrate coconut-based diet formulation and preparation

A coconut-based ketogenic diet was formulated using the coconut fibre. Coconut was purchased from the general market in Omu-Aran (8.1402 °N, 5.0963 °E), Kwara State, Nigeria. The feed was formulated to mimic a ketogenic diet as follows; the coconut fibre from the coconut meal (50 %), coconut oil as fat source (20 %), crayfish as protein source (15 %), vitamix (10 %) and a binder in form of psyllum husk (5%).

The coconut fibre was obtained by first de-shelling the fruit. The fruit was then diced into small sizes and blended into a wet fiber using a mechanical blender. The coconut fiber was then dried overnight at room temperature and milled into a smooth fiber.

2.3. Animal treatment

The rats were procured from the animal holding unit, Department of Biochemistry, University of Ilorin, Nigeria. They were housed in clean, well-ventilated cages and received *ad libitum* access to water and food for two weeks until experimentation. The animals were maintained at a laboratory temperature of 22 ± 3 °C, optical cycle 12 h of darkness and 12 h of brightness and relative humidity of 50 ± 5%. The procedure for the handling and treatment of animals were approved by the Landmark University Ethical Committee. Healthy forty-six (46) male rats (180 ± 40 g) were grouped into two groups (23 rats each). The Control group were fed with standard chow and MSG-induced group were administered 4 mg/kg bw MSG (orally, once daily at 24 h interval) for twenty-eight days to induce testicular dysfunction [6]. At the 29th day, five rats were randomly selected from both groups and sacrificed to establish induction of testicular dysfunction. The animals remaining in the control group were further sub-divided into three group; untreated group (standard rat chow), ketogenic diet only and curcumin only as standard drug (150 mg/kg bw). The MSG-induced group were also subdivided into three groups; MSG recovery (untreated), ketogenic diet and curcumin (150 mg/kg bw). The rats were daily treated for 42 days. The rats were sacrificed 24 h after last treatment.

2.4. Preparation of testes homogenate

Twenty-four hours after the cessation of treatment (29th day and 71st day), the animals were sacrificed following mild diethyl ether anesthesia. The rats were quickly dissected and testes carefully excised using the method described by Yakubu et al., [10] The right testes were later homogenized using homogenizer in ice-cold 0.25 M sucrose solution (1:5 w/v). The homogenates were centrifuged at 5000 rpm for 10 min to obtain supernatants. It was stored frozen until required for biochemical assays

2.5. Biochemical estimation of antioxidant status, lipid profile and testicular indices

The superoxide dismutase (SOD) assay was as described by Misra and Fridovich [11]. Catalase (CAT) was assayed at 240 nm using method described by Kayode et al. [12] at 30 s intervals for about 3 min. Reduced glutathione (GSH) was determined at 412 nm by the procedure described by Jollow et al. [13]. The Malondialdehyde (MDA) assay was based on the reaction of MDA with thiobarbituric acid (TBA) under acidic condition and assessed at 531 nm as described by Satoh [14]. The DNA fragmentation was determined using the diphenylamine (DPA) activities as described by Perandones et al. [15].

Activity of HMG-CoA reductase was measured as described by Kayode et al. [12]. The level of total cholesterol was determined at 546 nm using the method described by Fredrickson et al. [16]. Triglyceride (TG) concentration was determined at 630 nm as described by Jacobs and Vandenmark [17]. The activity of acetylcholine esterase (AChE) was determined using method described by Ellman et al. [18]. Nitric oxide (NO) level was determined at 546 nm using the method described by Havarasan et al. [19].

The testicular glycogen level was measured as described by Kemp et al. [20]. The protein level in the testis was measured at 540 nm by the procedure of Gornall et al. [21]. Determination of alkaline phosphatase (ALP) activity was as described by Wright et al. [22]. The procedure established by Wright et al. [23] was used to determine the activity of acid phosphatase (ACP).

2.6. Statistical analysis

Results were analysed by using a one-way analysis of variance (ANOVA), complemented with an unpaired Student's ***t*-test** on a GraphPad Prism 6 (GraphPad Software Inc., San Diego, California, USA) and were expressed as mean ± standard error of mean (SEM). Comparisons among group mean values were performed by Tukey's post-hoc test and p value < 0.05 was considered to indicate a significant difference.

3. Results

3.1. MSG decreased testis-body weight ratio

In the pre-treatment with MSG, there was a significant ($p < 0.05$) decrease in testis-body weight ratio when compared to the control. In the post treatment, there is no significant difference ($p > 0.05$) across all the group compared with the control (Fig. 1).

This result correlates with the decreased in the semen qualities observed in the pre-treatment with MSG (not reported).

3.2. MSG caused a reduction in rat testicular glycogen levels

In the pre-treatment with MSG, there was a significant decrease ($p < 0.05$) in the testicular glycogen. In the post treatment, there was a significant ($p < 0.05$) increase in the group given curcumin only and significant ($p < 0.05$) decrease in the group fed with ketogenic diet only in testicular glycogen when compared to the control group (Fig. 2).

3.3. MSG administration led to decreased levels of rat testicular protein

In the pre-treatment, MSG administration resulted in a significant ($p < 0.05$) decrease of the testicular protein. There was a significant decrease ($p < 0.05$) observed in the group fed with ketogenic diet only and curcumin only when compared to the control group (Fig. 3).

3.4. MSG altered the activities of rat testicular ACP and ALP

In the pre-treatment, the administration of MSG resulted in a

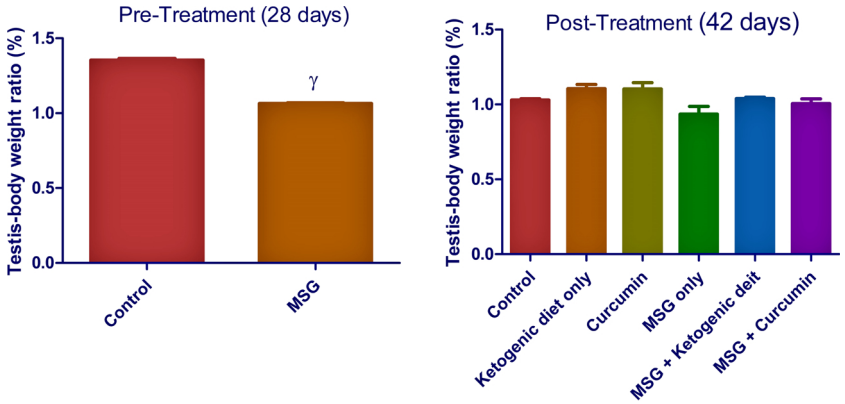

Fig. 1. Effect of pre-treatment with MSG and post-treatment with ketogenic diet and curcumin on rat testis-body weight ratio. Values are presented as mean ± SEM, n = 6. γ is significant at $p < 0.001$ versus control.

significant decrease ($p < 0.05$) of the testicular ALP when compared to the control (Fig. 4), while there was no significant increase ($p > 0.05$) in the administration of MSG in testicular ACP (Fig. 5). In the post-treatment, activities of ACP and ALP significantly increased ($p < 0.05$) in the ketogenic diet only group, curcumin only, MSG + ketogenic diet group and MSG + Curcumin group when compared to control (Figs. 4 and 5).

3.5. MSG administration caused a reduction in rat testicular acetylcholine esterase (AChE)

In the pre-treatment, there was a significant decrease ($p < 0.05$) in testicular AChE activity when compared to the control after the administration of MSG. In the post treatment, there is a significant decrease ($p < 0.05$) across the entire group compared with the control (Fig. 6).

3.6. Rat testicular lipid profile was altered following oral exposure to MSG

In the pre-treatment, administration of MSG to the rats significantly decreased ($p < 0.05$) the level of cholesterol and TG in the testis, while testicular HMG-CoA reductase activity showed no significant ($p > 0.05$) decrease when compared to the control (Figs. 7–9). In the post–treatment, groups fed with ketogenic diet alone and the MSG + ketogenic diet showed significantly increased ($p < 0.05$) levels of cholesterol, HMG CoA and TG. There was also a significant increase ($p < 0.05$) in the activity of HMG-CoA reductase for the MSG + curcumin group. Meanwhile, there was a significant decrease ($p < 0.05$) in the level of HMG-CoA reductase in groups fed with curcumin, MSG + curcumin and MSG only (Figs. 7–9).

3.7. MSG exposure altered rat testicular redox status

After 28-day pre-treatment of rat with MSG, there were significant increases ($p < 0.05$) in the level of rat testis MDA. In the post treatment, the MDA level decreased ($p < 0.05$) in the curcumin only group, while the MSG + ketogenic diet group showed a significant increase ($p < 0.05$) when compared to the control. However, compared to the MSG only group, the MSG + ketogenic diet and MSG + curcumin groups showed a significant decrease ($p < 0.05$) in MDA level (Fig. 10).

In the pre-treatment, MSG administration resulted in a significant decrease ($p < 0.05$) in the levels of rat testis SOD, GSH and CAT. In the post treatment, SOD level was significantly increased ($p < 0.05$) in the group fed with ketogenic diet only as well as the curcumin only and MSG + curcumin when compared to the MSG only group. Also in the post treatment, the GSH level was significantly ($p < 0.05$) increased in groups given curcumin, MSG only, MSG + ketogenic diet, MSG + curcumin. CAT level was decreased significantly ($p < 0.05$) compared to the control in MSG only group and MSG + ketogenic diet group (Figs. 11–13).

In the pre-treatment with MSG, there was a significant increase ($p < 0.05$) in the level of rat testicular DNA fragmentation when compared to the control. However, in the post treatment, there is a significant decrease ($p < 0.05$) in the groups given ketogenic diet, MSG + ketogenic diet as well as MSG + curcumin (Fig. 14).

3.8. MSG exposure decreased rat testicular nitric oxide level

In the pre-treatment with MSG, there was a significant decrease ($p < 0.05$) in the level of rat testicular NO when compared to the control. In the post treatment, there is a significant increase ($p < 0.05$)

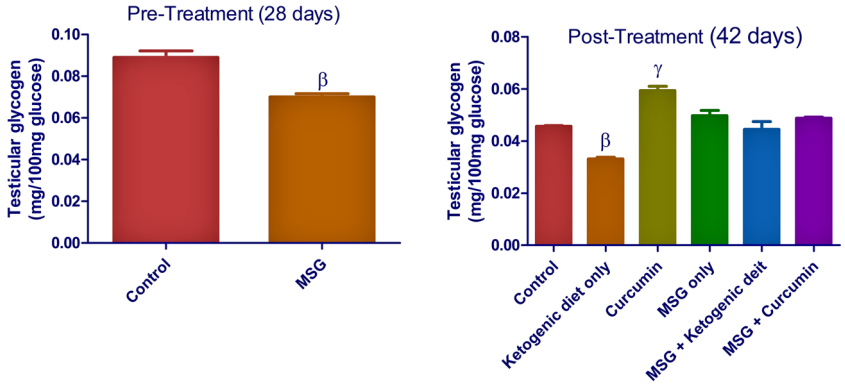

Fig. 2. Effect of pre-treatment with MSG and post-treatment with ketogenic diet and curcumin on rat testicular glycogen. Values are presented as mean ± SEM, n = 6. For pre-treatment, β is significant at $p < 0.01$ versus control. For post-treatment, β is significant at $p < 0.01$ and γ at $p < 0.001$ versus control.

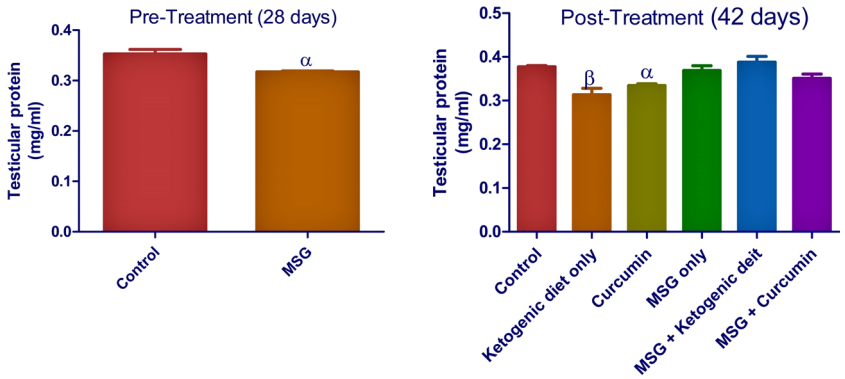

Fig. 3. Effect of pre-treatment with MSG and post-treatment with ketogenic diet and curcumin on rat testicular protein. Values are presented as mean ± SEM, n = 6. For pre-treatment, α is significant at $p < 0.05$ versus control. For post-treatment, α is significant at $p < 0.05$and β at $p < 0.01$ versus control.

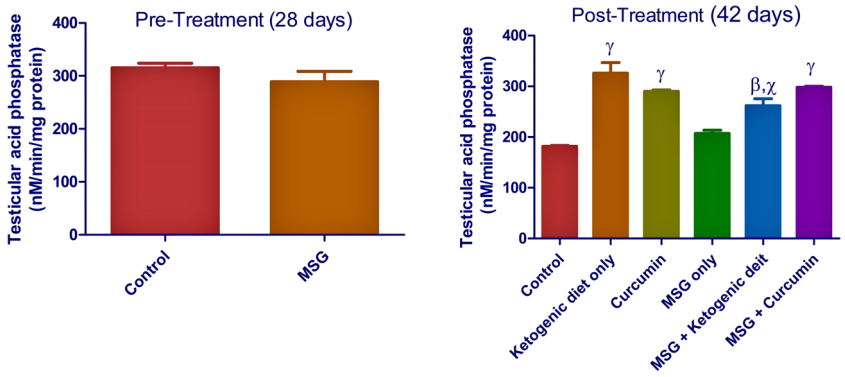

Fig. 4. Effect of pre-treatment with MSG and post-treatment with ketogenic diet and curcumin on rat testicular ACP level. Values are presented as mean ± SEM, n = 6. For post-treatment, β is significant at $p < 0.01$ versus control, γ is significant at $p < 0.001$ versus control. χ is significant at $p < 0.05$ versus MSG only.

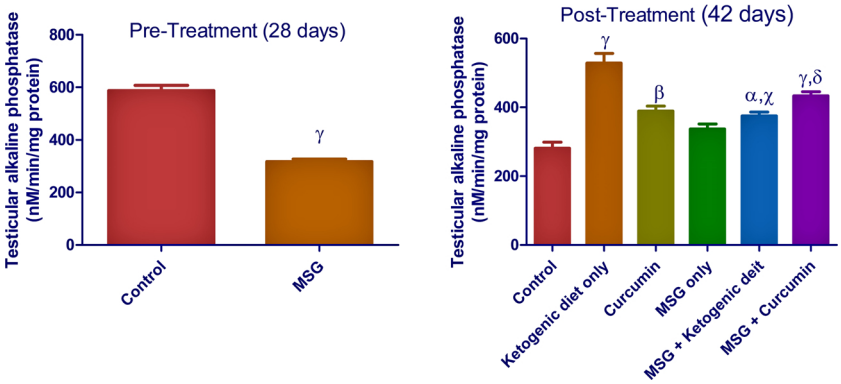

Fig. 5. Effect of pre-treatment with MSG and post-treatment with ketogenic diet and curcumin on rat testicular ALP level. Values are presented as mean ± SEM, n = 6. For pre-treatment, γ is significant at $p < 0.001$ versus control. For post-treatment, α is significant at $p < 0.05$ versus control β at $p < 0.01$ versus control and γ at $p < 0.001$ versus control. χ is significant at $p < 0.01$ and δ at $p < 0.01$ versus MSG only.

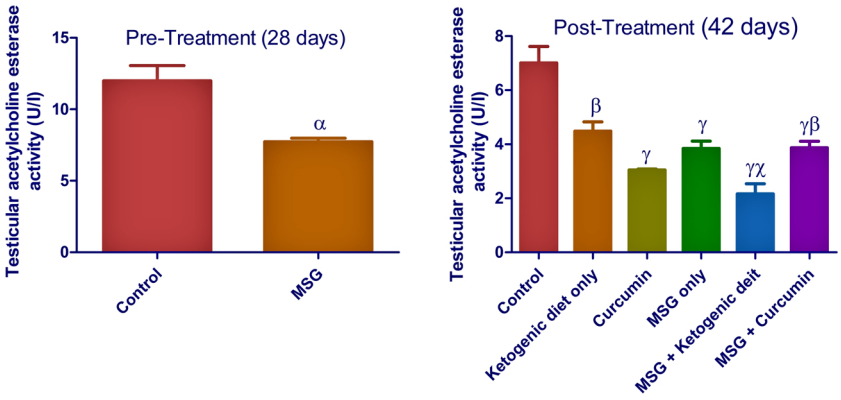

Fig. 6. Effect of pre-treatment with MSG and post-treatment with ketogenic diet and curcumin on rat testicular AChE. Values are presented as mean ± SEM, n = 6. For pre-treatment, α is significant at $p < 0.05$ versus control. For post-treatment, β is significant at $p < 0.01$ and γ is significant at $p < 0.001$ versus control, χ is significant at $p < 0.05$ versus MSG only.

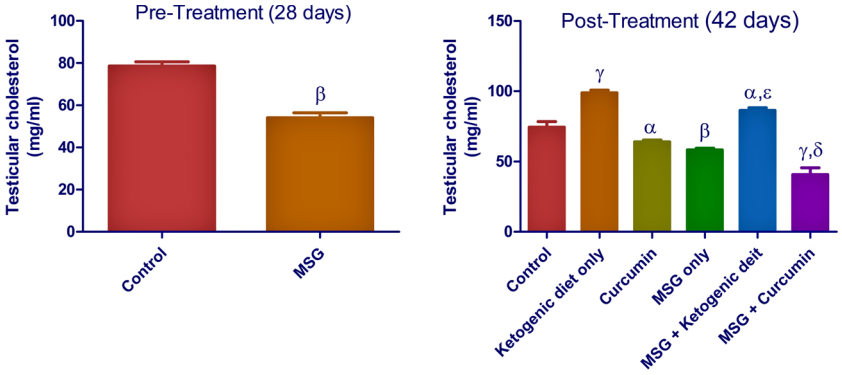

Fig. 7. Effect of pre-treatment with MSG and post-treatment with ketogenic diet and curcumin on rat testicular cholesterol level. Values are presented as mean ± SEM, n = 6. For post-treatment, α is significant at $p < 0.05$, β at $p < 0.01$ and γ at $p < 0.001$ versus control. δ is significant at $p < 0.01$and ε at $p < 0.001$ versus MSG only.

in the group fed with ketogenic diet and MSG + ketogenic diet. There was a significant decrease ($p < 0.05$) in NO level for groups fed with MSG only and MSG + curcumin (Fig. 15).

4. Discussion

Testicular function indices are used to evaluate the testicular functional capability [1]. The testicular functional indices investigated in this study included ALP, ACP, cholesterol, protein, glycogen and testicular lipid profile.

The testis-body weight ratio may suggest the toxic or otherwise effects on the reproductive system. The significant reduction in the testis-body weight ratio in MSG treated group after 28 days of administration may indicate testicular toxicity, and this fact agrees with a previous report [3]. The post treatment revealed improvement in testis-body weight ratio; there was no significant change compared with control.

Testicular glycogen provides energy source for effective functioning of the testicular tissue and spermatogenetic processes in the seminiferous tubular cells [24,25]. In this study, MSG administration after 28 days caused decline in rat testicular glycogen content. The decrease in glycogen which is the glucose source for the testes could be as a result of interruption in glucose metabolism and/or glucose transport. Additionally, testicular glycogen serves as the main source of energy for the synthesis of protein in the germinal cells, therefore, the decrease in glycogen content of testis after 28 days of MSG administration might impose testicular dysfunction. In post-treatment with ketogenic diet and the reference treatment curcumin, increased testicular glycogen content may indicate restoration of glucose transport and/or metabolism as well as improved the synthesis of enzymes involved in hormonal production. Such increased testicular glycogen content may promote steroidogenesis and spermatogenesis by enhancing the availability of substrates required for the normal functioning of germ cells [25].

Testicular proteins are required for sperm production and maturation [1,12]. In the present study, the treatment with MSG after 28 days reduced the testicular protein which might subsequently affect the production of spermatozoa. However, the reduced testicular protein concentration in MSG only group was restored suggesting reversal of effect after MSG treatment cessation. In the ketogenic diet only group, the protein content increased significantly which may indicate enhanced sperm maturation as required for the fertilization process in animals.

ALP is involved in androgen transfer between seminiferous tubules and peritubular interstitial. ALP also facilitates active transport of substances between the sperm membrane and luminal fluid [26]. Meanwhile, ACP enhances sperm development by facilitating the material exchange between sertoli cells and germinal cells [27]. In the present study, the decreased phosphatase activities caused by MSG may indicate a reduction in rat testicular steroidogenesis as a result of decreased androgen production and bioavailability. The post treatment resulted in increased activities of rat testicular phosphatases in groups given ketogenic diet, curcumin only, MSG + curcumin and MSG + Ketogenic diet. Together, these may indicate an androgen dependent elevation in metabolic and functional activities of the testis. The resultant increase could also enhance the transportation of vital substances across the testes.

AChE hydrolyzes acetylcholine and thereby terminates the action of this neurotransmitter at the cholinergic neuroeffector junctions. AChE catalyses the breakdown of acetycholine in the cholinergic nerves of the corpus cavernosum smooth muscle cells and penile vasculature [28]. The observable decreases across all groups contradict earlier reports [29] which showed that increased testicular AChE activity could be linked with increased testosterone synthesis.

Cholesterol is a required precursor for the production of steroid hormone and a requirement for normal testicular physiology. Cholesterol serves as a protective barrier against environmental shock

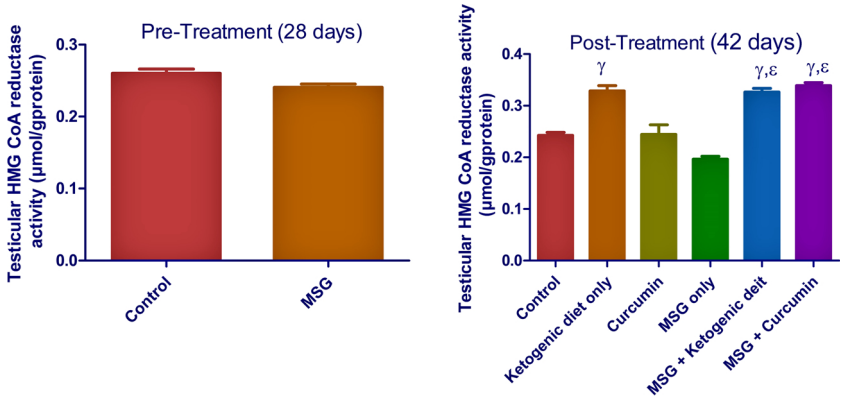

Fig. 8. Effect of pre-treatment with MSG and post-treatment with ketogenic diet and curcumin on rat testicular HMG CoA reductase activity. Values are presented as mean ± SEM, n = 6. For post-treatment, γ is significant at $p < 0.001$ versus control, while, ε is significant at $p < 0.001$ versus MSG only.

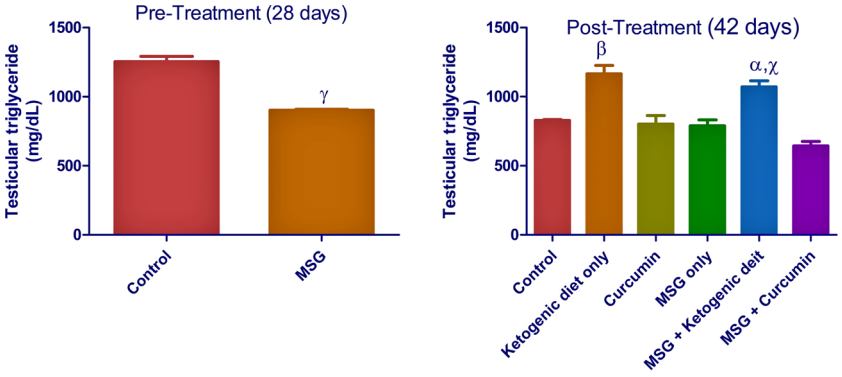

Fig. 9. Effect of pre-treatment with MSG and post-treatment with ketogenic diet and curcumin on rat testicular TG level. Values are presented as mean ± SEM, n = 6. For pre-treatment, γ is significant at $p < 0.001$ versus control. For post-treatment, α is significant at $p < 0.05$ and β is significant at $p < 0.01$ versus control, χ is significant at $p < 0.05$ versus MSG only.

for the sperm cells [1]. HMG-CoA reductase is an enzyme intermediate in cholesterol synthesis and its enhanced activity also increase testicular cholesterol production [26]. The significant decrease in testicular cholesterol, TG and HMG-CoA by MSG in this study could be as a result of the decreased cholesterol secretion in the prostate or reduced testicular membrane mobilization or alteration in gonadal lipid distribution [25]. These alterations by MSG were reversed by the administration of the ketogenic diet, especially in the MSG + ketogenic diet group. The improvement in rat testicular cholesterol, TG and HMG CoA in groups given ketogenic diets highlights the restoration potential of the diet. The group fed with ketogenic diet alone also improved the rat lipid profile. Therefore, the increased testicular cholesterol in the present study may be one of the mechanisms by which the diet acts as an enhancer of the testicular function. The improvement in rat testicular lipid profile given the ketogenic diet might be linked to increased rat testosterone content which may indicate that the availability of synthesized cholesterol for androgens biosynthesis. Furthermore, the observed improvement in rat testicular cholesterol, HMG CoA reductase and TG levels in the ketogenic diet group may point to the likely action mechanism by the ketogenic diet in improving and/or restoring rat testosterone synthesis.

Lipid peroxidation measured as the levels of MDA is an assessment factor for oxidative stress level. The high level of lipid peroxidation suggests alterations of the lipid structure of sperm membranes, and may hinder sperm motility [30]. In this study, increased levels of rat testicular MDA caused by MSG indicates that this flavor enhancer might not only predispose to oxidative stress, but facilitate production of free radicals in rat testes. The observed increase in MDA concentration could result from promotion of peroxidation of membrane lipids in the testis by MSG. Induction of oxidative stress by MSG through production of free radicals have been shown to cause oxidative DNA damage, peroxidation of membrane biomolecules and cell death [5].

Antioxidants facilitate defense mechanism against oxidation of biomolecules such as lipid, nucleic acids and protein. Redox status of the cells can be measured using antioxidant enzymes (CAT, SOD and GSH) and the lipid peroxidation end product, MDA [4]. SOD mops up cellular superoxide anion and hinders the peroxidation of membrane lipid. SOD must conjugate with catalase or glutathione peroxidase to fight against H_2O_2 [31,32]. SOD acts against the action of superoxide radicals that induces premature hyperactivation and sperm capacitation before ejaculation [3,32]. CAT detoxifies cellular H_2O_2 to oxygen and water. Also, CAT activates nitric oxide-induced sperm capacitation mechanism involving H_2O_2 [33]. GSH is a tripeptide of glutamic acid, glycine and cysteine and it is maintained in a reduced state by an efficient glutathione peroxidase/glutathione reductase system. Glutathione is a scavenging antioxidant for a number of free radicals. Earlier reports on the administration of MSG at 4 mg/g bw indicated the correlation between the increase in lipid peroxidation and GSH reduction [34]. This support the observed decrease in GSH noted in the MSG pre-treatment group. Meanwhile, increased GSH level recorded in groups given curcumin only as well as the MSG + curcumin may be due to the radical scavenging properties of curcumin.

The increase in the SOD and CAT activity in the testes by ketogenic diet only, Curcumin only and MSG + curcumin in this study could represent a first line of adaptive mechanism to combat the sudden rise in free radicals. Hence increased activities of antioxidant enzymes may represent a strategy to offset oxidative stress due to the action of MSG in rat tissues. The effect of the reference treatment, curcumin, is similar and comparable to the action of the diet, as it also led to increases in the activities of the antioxidant enzymes. Alterations in rat testicular redox status could be a likely mechanism underlying the induction of reproductive toxicity by MSG exposure. It is also probable that the increases in the activities of antioxidant enzymes recorded in this study might be associated with the upregulation and/or induction of antioxidant enzyme synthesis by the ketogenic diet, so as to effectively neutralize the circulating free radicals and hence reduce oxidative stress [35].

The DNA's structural integrity serve as a reproductive function marker that evaluate sperm function and morphological changes [36]. The present study reveals that MSG administration caused testicular

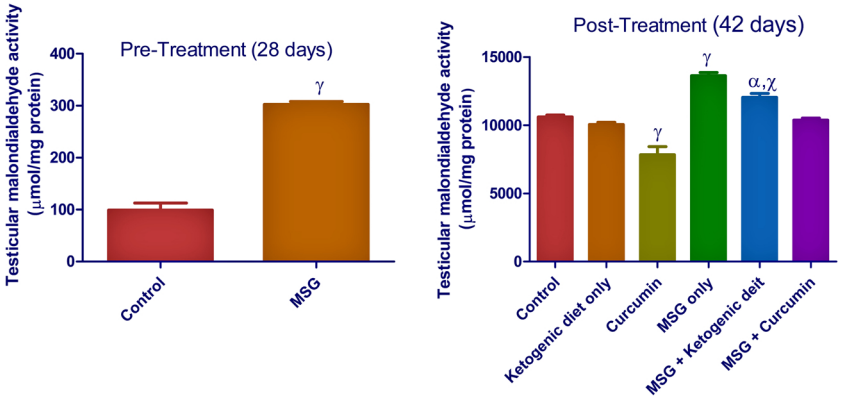

Fig. 10. Effect of pre-treatment with MSG and post-treatment with ketogenic diet and curcumin on rat testicular MDA level. Values are presented as mean ± SEM, n = 6. For pre-treatment, γ is significant at $p < 0.001$ versus control. For post-treatment, α is significant at $p < 0.05$ and γ at $p < 0.001$ versus control, χ is significant at $p < 0.05$ and δ at $p < 0.01$ versus MSG only.

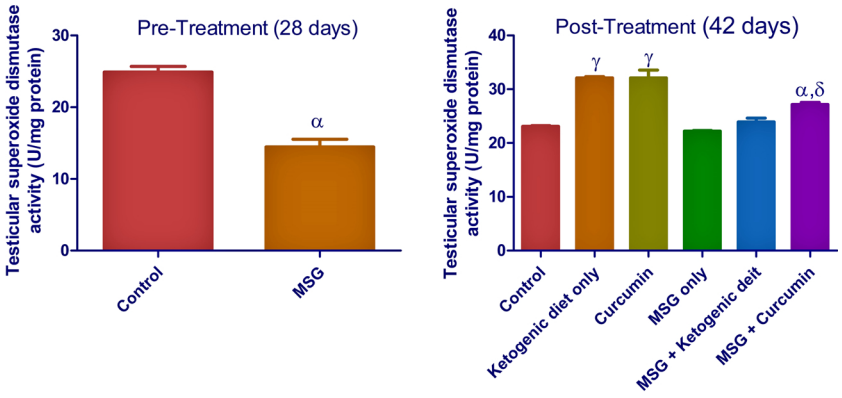

Fig. 11. Effect of pre-treatment with MSG and post-treatment with ketogenic diet and curcumin rat testicular SOD level. Values are presented as mean ± SEM, n = 6. For pre-treatment, α is significant at $p < 0.05$ versus control. For post-treatment, α is significant at $p < 0.05$ and γ at $p < 0.001$ versus control. δ is significant at $p < 0.01$ versus MSG only.

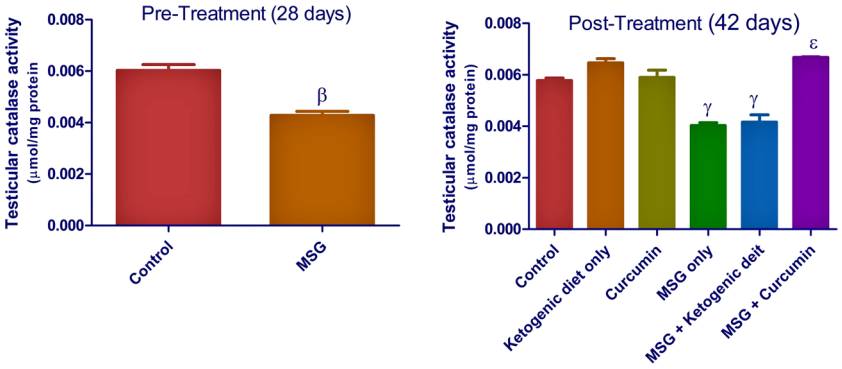

Fig. 12. Effect of pre-treatment with MSG and post-treatment with ketogenic diet and curcumin on rat testicular CAT activity. Values are presented as mean ± SEM, n = 6. For pre-treatment, β is significant at $p < 0.01$ versus control. For post-treatment, γ is significant at $p < 0.001$ versus control and ε at $p < 0.001$ versus MSG only.

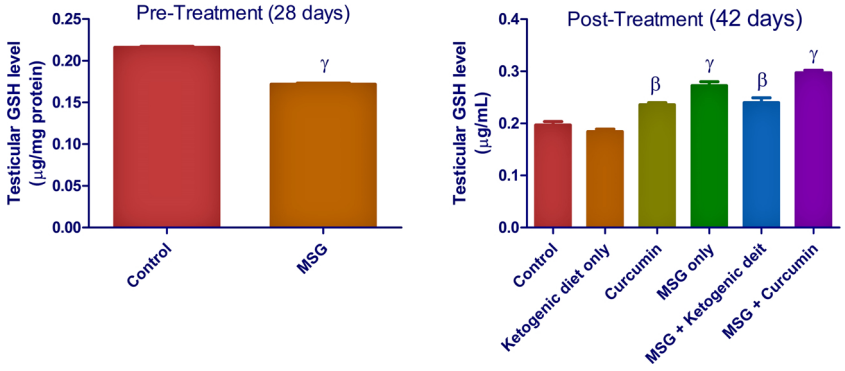

Fig. 13. Effect of pre-treatment with MSG and post-treatment with ketogenic diet and curcumin on rat testicular GSH level. Values are presented as mean ± SEM, n = 6. For pre-treatment, γ is significant at $p < 0.001$ versus control. For post-treatment, β is significant at $p < 0.01$ and γ at $p < 0.001$ versus control.

DNA damage after 28 days. Generally, the testicular DNA is prone to injury during spermatogenesis due to overproduction of reactive oxygen species, limited DNA repair mechanisms, and condensation of chromatin [37]. But, the antioxidant defense mechanisms help protect against oxidative damage. The post treatment with ketogenic diet only, MSG + ketogenic diet and MSG + curcumin reduced the DNA damage and this may not be unconnected with the enhanced antioxidant status in these groups.

Nitric oxide is synthesized from L-arginine by nitric oxide synthase. It is a post-ganglionic neurotransmitter released from autonomic nerve terminals that diffuses into the vascular and cavernosal smooth muscle [38]. In the smooth muscle, NO activates guanyl cyclase and this increases cyclic guanosine monophosphate (*c*GMP) concentration. *c*GMP activates certain intracellular protein kinases that phosphorylate receptor proteins. Activated protein kinases open the potassium channels, increase the influx of potassium and block the influx of calcium by inhibiting calcium channels [39]. This leads to hyperpolarization and relaxation of smooth muscle. Reduced arteriolar resistance leads to sinusoidal spaces filled with blood. These enlarged sinusoids further increase the intracavernosal pressure by blocking the venous return and producing a rigid erection [38]. Moreover, the increase in the levels of rat NO in the ketogenic diet group only and MSG + ketogenic diet group makes it plausible that the diet might have enhanced the activity of NO synthase leading to increased cyclic GMP and ultimately improving rat testicular functions. Taken together, MSG exposure in rats might altered testicular redox status. However, sustenance of rats on ketogenic diet improved rat testicular redox status and by extension testicular function.

5. Conclusion

This study revealed that MSG administration after 28 days caused

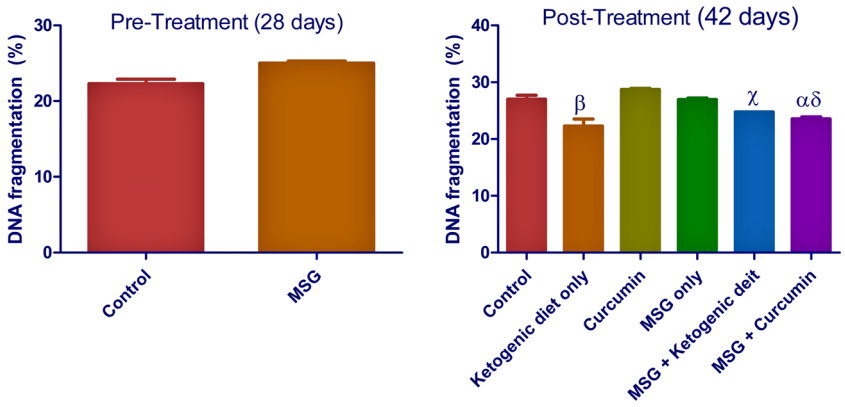

Fig. 14. Effect of pre-treatment with MSG and post-treatment with ketogenic diet and curcumin on rat testicular DNA fragmentation. Values are presented as mean ± SEM, n = 6. For post-treatment, α is significant at $p < 0.05$ and β at $p < 0.01$ versus control. χ is significant at $p < 0.05$ and δ at $p < 0.01$ versus MSG only.

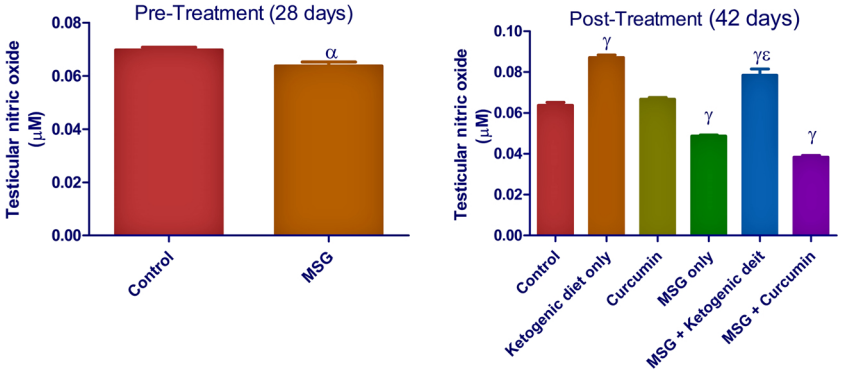

Fig. 15. Effect of pre-treatment with MSG and post-treatment with ketogenic diet and curcumin on rat testicular NO level. Values are presented as mean ± SEM, n = 6. For pre-treatment, α is significant at $p < 0.05$ versus control. For post-treatment, γ is significant at $p < 0.001$ versus control and ε at $p < 0.001$ versus MSG only.

testicular dysfunction *viz-a-viz* alteration of redox status, reduced testicular glycogen, decreased NO level and altered lipid profiling. In contrast, post treatment with ketogenic diet improved the rat biochemical parameters as well as the testicular functional indices. Together, findings support the prospect of ketogenic diet to restore and improve testicular functions in rats.

Funding source

None.

Declaration of Competing Interest

The authors have no competing interests.

Acknowledgements

The authors wish to thank the laboratory staff in the Department of Biochemistry, Landmark University, Omu-Aran, Nigeria. Also, the Ton Duc Thang University, Ho Chi Minh City is appreciated.

References

[1] T.D. Olaolu, D.E. Rotimi, A.P. Olaolu, Effect of alcohol infusion of *Cissus populnea* root on testicular function and serum hormone of male Wistar rats, Asian Pac. J. Reprod 7 (3) (2018) 117–122.
[2] S.A. Prihatno, I. Padeta, A.D. Larasati, B. Sundari, A. Hidayati, Y.H. Fibrianto, T. Budipitojo, Effects of secretome on cisplatin-induced testicular dysfunction in rats, Vet. World 11 (9) (2018) 1349–1356.
[3] G.S. Fernandes, A.C. Arena, K.E. Campos, G.T. Volpato, J.A. Anselmo-Franci, D.C. Damasceno, W.G. Kempinos, Glutamate induced obesity leads to decreased sperm reserves and acceleration of transit time in the epididymis of adult male rats, Reprod. Biol. Endocrinol. 10 (2012) 105–111.
[4] L. Sailo, M.K. Murthy, K. Pratima, V.K. Roy, G. Gurusubramanian, Monosodium glutamate toxicity and the possible protective role of l–carnitine, Sci. Technol. J. 6 (2018) 2321–3388.
[5] O.T. Kayode, D.E. Rotimi, A.A.A. Kayode, T.D. Olaolu, O.S. Adeyemi, Monosodium glutamate (MSG)-induced male reproductive dysfunction: a mini review, Toxics 8 (2020) 7, https://doi.org/10.3390/toxics8010007.
[6] S.A. Sakr, G.M. Bada, Protective effect of curcumin on monosodium glutamate-induced reproductive toxicity in male albino rats, Glob. J. Pharmacol. 7 (4) (2013) 416–422.
[7] S. Vidalia, S. Aminzadeha, B. Lambert, T. Rutherford, W. Sperl, B. Kofler, R. Feichtingera, Mitochondria: the ketogenic diet-a metabolism-based therapy, Int. J. Biochem. Cell Biol. 63 (2015) 55–59.
[8] D. Kulak, A. Polotsky, Should the ketogenic diet be considered for enhancing fertility? Maturitas 74 (2013) 10–13.
[9] A.I. Castro, D. Gomez-Arbelaez, A.B. Crujeiras, R. Granero, Z. Aguera, S. Jimenez-Murcia, I. Sajoux, P. Lopez-Jaramillo, F. Fernandez-Aranda, F.F. Casanueva, Effect of a very low-calorie ketogenic diet on food and alcohol cravings, physical and sexual activity, sleep disturbances, and quality of life in obese patients, Nutrients 10 (10) (2018) E1348.
[10] M.T. Yakubu, M.A. Akanji, T.A. Oladiji, Effect of oral administration of aqueous extract of *Fadogia agrestis* stem on some testicular function indices of male rats, J. Ethnopharmacol. 111 (2008) 288–292.
[11] H.P. Misra, I. Fredovich, The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase, J. Biol. Chem. 247 (1972) 3170–3175.
[12] O.T. Kayode, A.A.A. Kayode, C.O. Nwonuma, Alcoholic bitters modulates sex hormones and some biochemical parameters of testicular function in male Wistar rats, F1000Research 7 (2018) 1838.
[13] D.J. Jollow, J.R. Mitchell, N. Zampaglione, J.R. Gillette, Bromobenzene-induced liver necrosis. Protective role of glutathione and evidence for 3,4-bromobenzene oxide as the hepatotoxic metabolite, Pharmacology 11 (1974) 151–169.
[14] K. Satoh, Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method, Clin. Chim. Acta 90 (1978) 37–43.
[15] C.E. Perandones, V.A. Illera, D. Peckham, L.L. Stunzl, R.F. Ashman, Regulation of apoptosis in vitro in mature murine spleen T cells, J. Immunol. 151 (1993)

3521–3529.
[16] D.S. Fredrickson, R.I. Levy, R.S. Lees, Fat transport in lipoproteins- an integrated approach to mechanisms and disorders, N. Engl. J. Med. 276 (1967) 148–156.
[17] N.J. Jacobs, P.J. Vandenmark, Colorimetric method for determination of triglycsrides, Arch. Biochem. Biophys. 88 (1960) 250–255.
[18] G.L. Ellman, K.D. Courtney, V. Andres Jr, R.M. Featherstone, A new and rapid colorimetric determination of acetylcholinesterase activity, Biochem. Pharmacol. 7 (1961) 88–90.
[19] R. Havarasan, M. Mallika, S. Venkataramanan, Anti-inflammatory and free radical scavenging activity of *Ricinus communis* root extract, J. Ethnopharmacol. 103 (3) (2006) 478–480.
[20] A. Kemp, J. Adrienne, K. Heijningen, A colorimetric micro-method for the determination of glycogen in tissues, Biochem. J. 56 (1954) 646–648.
[21] A.C. Gornall, C.J. Bardawill, M.M. David, Determination of serum protein by means of biuret reaction, J. Biol. Chem. 177 (1949) 751–756.
[22] P.J. Wright, P.D. Leathwood, D.T. Plummer, Enzymes in rat urine. Alkaline phosphatase, Enzymologia 42 (1972) 317–327.
[23] P.J. Wright, P.D. Leathwood, D.T. Plummer, Enzymes in rat urine. Acid phosphatase, Enzymologia 42 (1972) 459–462.
[24] N. Choudhary, R. Goyal, S.C. Joshi, Effect of malathion on reproductive system male rats, J. Environ. Biol. 29 (2) (2008) 259–262.
[25] M.T. Yakubu, R.O. Jimoh, *Carpolobia lutea* roots restore sexual a performance in paroxetine-induced sexually impaired male rats, J. Androl. 12 (3) (2014) 89.
[26] Q.O. Nurudeen, T.O. Ajiboye, Aqueous root extract of *Lecaniodiscus cupanioides* restores the alterations in testicular parameters of sexually impaired male rats, Asian Pac. J. Reprod. 1 (2) (2012) 120–124.
[27] R.L. Peruquetti, S.R. Tobaqa, M.T.V. Aziredu-Olivera, Expression of acid phosphatase in the seminiferous epithelium of vertebrates, Genetic J. Mol. Responses 9 (2) (2010) 620–628.
[28] I.A. Adedara, A.O. Abolaji, J.B.T. Rocha, E.O. Farombi, Diphenyl diselenide protects against mortality, locomotor deficits and oxidative stress in drosophila melanogaster model of manganese-induced neurotoxicity, Neurochem. Res. (2016) 9, https://doi.org/10.1007/s11064-016-1852-x.
[29] P. Hedlund, L. Ny, P. Alm, K.E. Andersson, Cholinergic nerves in human corpus cavernosum and spongiosum contain nitric oxide synthase and heme oxy-genase, J. Urol. 164 (2000) 868–875.
[30] A. Bustos-Obregón, E. González, Melatonin as protective agent for the cytotoxic effects of diazinon in the spermatogenesis in the earthworm *Eisenia foetida*, Ital. J. Anal. Embryol. (2005) 159–165.
[31] H.B.I. El-Sawy, M.M. Soliman, S.A. El-Shazly, H.A. Ali, Protective effects of camel milk and vitamin E against monosodium glutamate induced biochemical and testicular dysfunctions, Prog. Nutr. 20 (2018) 76–85.
[32] E.N.A. Hanipah, N.J. Yahya, E.M. Ajik, N.A. Yusoff, I.S. Taib, Monosodium glutamate induced oxidative stress in accessory reproductive organs of male sprague-dawley rats, J. Sains Kesihat. Malays. (2018) 67–73.
[33] S.P. Dandekar, G.D. Nadcarni, V. Kulkarni, S. Punekar, Lipid peroxidation and antioxidant enzymes in male infertility, J. Postgrad. Med. 48 (2002) 186–189.
[34] B.J. Kim, B.L. Hood, R.A. Aragon, J.P. Hardwick, T.P. Conrads, T.D. Veenstra, et al., Increased oxidation and degradation of cytosolic proteins in alcohol-exposed mouse liver and hepatoma cells, Proteomics 6 (2006) 1250–1260.
[35] V. Ragini, K. Prasad, K. Bharathi, Antidiabetic and antioxidant activity of *Shorea tumbuggaia* Rox, Int. J. Innov. Pharm. Res. 2 (2) (2011) 113–121.
[36] P. Talwar, S. Hayatnagarkar, Sperm function test, 2015, J. Hum. Reprod. Sci. 8 (2) (2015) 61–69.
[37] P. Sabeti, S. Pourmasumi, T. Rahiminia, et al., Etiologies of sperm oxidative stress, Int. J. Reprod. Biomed. 14 (4) (2016) 231–240.
[38] S. Moncada, R.M.J. Palmer, E.A. Higgs, Nitric oxide: physiology, pathophysiology and pharmacology, Pharmacol. Rev. 43 (1991) 109–142.
[39] A.D. Seftel, K.A. Viola, S.E. Kasner, M.B. Ganz, Nitric oxide relaxes rabbit corpus cavernosum smooth muscle via a potassium-conductive pathway, Biochem. Biophys. Res. Commun. 219 (1996) 382–338.