Duration effects of alcohol graded concentrations on the extent of lipid peroxidation, testis morphology and sperm quality assessment in Wistar rats

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ABSTRACT

Alcohol consumption is known to cause an array of alcohol-induced biochemical changes in a biological system. This study investigated the durations effects of different alcohol concentrations (30%, 40%, and 50%) on malondialdehyde levels, testes histology, and sperm characteristics in matured male Wistar rats. The rats were divided into four groups namely thus; control, 30%, 40% and 50%. Control group was orally administered 0% alcohol while, group 30%, 40% and 50% received orally 30%, 40% and 50% of alcohol concentrations (3.20 g/Kg body weight) respectively for maximum durations of 28 days. On the day 1, 7, 14, 21, and 28, five rats from each group (control, 30%, 40% and 50% alcohol) were sacrificed, and malondialdehyde levels, testes histology, and sperm characteristics were examined.

Graded alcohol concentrations caused different detrimental effects on sperm characteristics and induced pathological lesions in the testes. Significant increases in serum, liver and testes malondialdehyde levels were durations independent but almost entirely concentrations dependent.

Ultimately, administration of alcohol graded concentration led to loss of sperm motility and testicular degeneration in concentration and durations dependent manner without a concomitant increase in the malondialdehyde levels.

1. Introduction

Excessive alcohol intake affects virtually every organ and tissue in the body, with multi-factorial actions on cellular and molecular functions by altering biological function either via direct interaction with cellular components or direct biochemical effect of alcohol metabolism [1–5]. The effects of excessive alcohol consumption on the male reproductive system are of rising concern [2,3,6,7]. Researchers have reported the detrimental effects of excessive consumption of alcohol on male fertility [1–3,7]. Some also suggested that alterations of sperm cells morphology by excessive alcohol intake suppresses spermatogenesis, which directly leads to male infertility [2,3,7,8].

It is well known that part of alcohol metabolism in the liver generates acetaldehyde which directly affects the complete metabolism of ethanol, causes hepatocyte injury and indirectly promotes lipid peroxidation [3,4,7,8]. High concentrations of lipid peroxidation byproducts such as malondialdehyde (MDA) can further contribute to cellular and organ damage [1,3,7,9,10]. It has been suggested that lipid peroxidation may play an essential role in the mechanism that stimulates testicular pathogenesis and the toxic effects caused by excessive alcohol intake [1,3,7,9]. Siervo et al. [7] showed that administration of ethanol (25%, 2 g/Kg body weight) caused an increase in lipid peroxidation in the testes, as

Abbreviations: MDA, malondialdehyde; FUNAAB, Federal University of Agriculture, Abeokuta; %, percentage; g/Kg, gram per kilogram; TBARS, thiobarbituric acid reactive substance; nmole/L, nanomole per litre; PUFAS, polyunsaturated fatty acids; TD, Tissue Damage; N, Normal tissue; C, control administered distilled water; T, administered 30% ethanol; F, administered 40% ethanol; V, administered 50% ethanol.

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evidenced by the increased levels of testes MDA, which may be related to the damages observed in sperm morphology and motility.

Schlöffl et al. [11] reported that acute ethanol ingestion perturbs plasma antioxidant status and induces lipid peroxidation in a dose (2, 4, and 6 g/Kg body weight) and time (0, 1, 1.5, 2, 4, and 6 h) -dependent manners. Furthermore, Akinloye et al. [5] investigated and reported lipid homoeostasis disruptions, renal and hepatic dysfunctions induction in response to different concentrations of alcohol administration in male rats. Although, several experimental studies have addressed different biochemical effects of alcohol at different specific concentrations and times, it is still unclear if graded concentrations of alcohol over a certain period of time will influence or affect the relationship among tissues morphologies, MDA levels, and sperm characteristics. Thus, this research was conducted to examine the duration effects of alcohol graded concentrations on the extent of tissue (liver and testis) lipid peroxidation, tissues (brain, stomach, liver, kidney and testis) morphologies and sperm quality relationship.

2. Materials and methods

2.1. Chemicals

All chemicals used were of analytical grade and were obtained from Sigma- Aldrich Chemical (Missouri, USA).

2.2. Animals and treatment

Male Wistar rats (adults) weighing 200–250 g were used for the experiment. The experiment was conducted under the approved (FUNAAB- BCH- D1 016c) laboratory animal care and ethical guidelines of the Department of Biochemistry, College of Biosciences, Federal University of Agriculture, Abeokuta (FUNAAB) Nigeria. All animals were maintained in polypropylene cages with ad libitum access to rat chow and drinking water. After two weeks of acclimatization, five rats were sacrificed prior to the commencement of the experiment to get the baseline values for the analyses and the experimental design was carried out as described by Akinloye et al. [5]. The animals were divided into four groups (n = 5) which are:

- Control (0% Alcohol) = C
- 30% Alcohol (3.20 g/Kg body weight) = T
- 40% Alcohol (3.20 g/Kg body weight) = F and
- 50% Alcohol (3.20 g/Kg body weight) = V

In order to know the actual concentration of alcohol consumed, administrations were done once daily (every morning) using oral gavage [6] throughout the experiment. At the end of the day(s) 1, 7, 14, 21 and 28, twenty rats (five from each group) were anaesthetised and disected. Sperm and blood were collected and organs excised for sperm characterisation, tissue morphology and biochemical analysis.

2.3. Analysis and tissue morphology

Lipid peroxidation was evaluated using thiobarbituric acid reactive substance (TBARS) as malondialdehyde (MDA) levels according to the method of Fernandez et al. [12] as described by Siervo et al. [7]. The concentration of MDA was calculated using the extinction coefficient of 1.56 × 10^5 M^-1 cm^-1.

Portions of brain, stomach, liver, kidney and testis were fixed separately in 10% neutral buffered formalin for histological studies as described by Rehan et al. [13].

Sperm motility was evaluated by counting the motile and the non-motile sperms and assessed up to 200 sperms under a 10x magnification of a light microscope [7]. Data are shown as the percentage of sperm motility. Percentage of sperm motility and morphological analysis were carried out as described by Siervo et al. [7].

2.4. Statistical analysis

Data generated were analysed with one-way analysis of variance (ANOVA) and presented as mean ± standard error mean. Duncan multiple range tests (DMRT) of Statistical Package for Social Sciences version 16.0. was used to determine the significance level at p < 0.05.

3. Results

Fig. 1 represents serum (A), testes (B) and liver (C) malondialdehyde concentrations. An increased serum MDA (nmole/L) concentration of 53%, 54%, and 50% was observed after day 7 of administration for 30%, 40%, and 50% alcohol dose, respectively, compared with the control. Serum MDA concentration increased after 14 days of administration by 63%, 54%, and 57% at 30%, 40%, and 50% alcohol dose, respectively, compared with the control. After day 21 of administration, serum MDA concentration increased by 55% at 30% and 40% alcohol dose and 41% at 50% alcohol dose compared with the control. Serum MDA concentration increased after 28 days of administration at 30%, 40% and 50% alcohol dose by 10%, 29% and 23% respectively, compared with the control.

The MDA (nmole/g) concentration in the testes increased at 30%, 40% and 50% alcohol dose (32%, 24% and 46% respectively) when compared with the control. The concentration of testes MDA increased at 30%, 40% and 50% alcohol dose by 45%, 14% and 47%, respectively, when compared with the control after day 14 of administration. A dose-dependent increase of 50%, 56% and 67% (at 30%, 40% and 50% alcohol dose, respectively) was observed in testes MDA compared with the control after day 28 of administration.

After seven days of the administration, there was an increase in liver MDA (nmole/g) concentration by 12%, 17% and 14% at 30%, 40% and 50% alcohol doses compared with the control, respectively. Similarly, after 21 days of the administration, liver MDA levels increased by 11%, 23% and 7.5% at 30%, 40% and 50% doses compared with the control, respectively. A dose-dependent increase (45%, 49%, 62%) of liver MDA levels was observed after day 28 of administration of 30%, 40% and 50% alcohol concentration, respectively, compared with the control.

Effects of alcohol graded doses on rats’ percentage (%) sperm motility are shown in Fig. 2. An irregular trend of percentage sperm motility with no progressive percentage sperm motility decrease was observed in the control group only. However, groups administered graded doses of alcohol (30%, 40% and 50%) showed a significant decrease in the percentage of sperm motility as the durations increased compared with the control groups. Significant decrease (P < 0.05) in progressive percentage sperm motility were observed at the end of day 28 alcohol graded doses administrations with 50% alcohol producing the highest level of decrease.

Fig. 3 shows the effects of alcohol graded doses on rats’ percentage (%) of life (A) and dead (B) sperm cells. There was no progressive sperm loss observed in the control group regardless of the durations. On day one, groups administered graded doses of alcohol (30%, 40% and 50%) showed no significant difference in the levels of sperm loss but were significantly higher than the control group. The levels of sperm loss were time and dose-dependent from day seven to twenty-one, while there was no significant difference in the levels of sperm loss on day twenty-eight between groups of rats administered 40% and 50% alcohol.

Administrations of 30% and 40% alcohol showed no adverse effects on sperm cells from day one to seven. However, as the number of days increased from day fourteen to twenty-eight, the alcohol (30% and 40%) effects were in concentrations and time dependents. There were no significant effects shown by 50% alcohol administration from day one to seven. Nevertheless, as the time of administration increases, the effects of 50% alcohol administration increase compared with the control percentage of abnormal sperm cells (Fig. 4).

The histological studies of brain sections of different groups of rats administered graded doses of ethanol (30%, 40%, and 50%) are
presented in Fig. 5. The rat brain of control groups (NI-1 C, 7 C, 14 C, 21 C and 28 C, respectively) revealed normal hippocampus (white arrow) and normal cortex; the cortex showed normal neuronal cells (blue arrow) and normal stroma (slender arrow). Also, the cerebellum showed a standard granular layer and a normal Purkinje cell layer with normal Purkinje cells. The brain section of the rat representing groups given a graded dose of ethanol for one day (N2-1 T, 1 F and 1 V, respectively) revealed no pathological lesion. The cerebellar cortex showed normal folia, the molecular cell layer (white arrow), the Purkinje cells layer (black arrow), the granular layer appeared normal (red arrow), and the white matter also appeared normal. Group administered with 30% ethanol for seven days (TD1-7 T) showed a hippocampus with degenerated and depleted cells at the cornu ammonis-CA3 (white arrow) with normal cortex showing normal neuronal cells and stroma. The brain of the groups administered 40% ethanol for seven days and 30% ethanol for fourteen days (TD2-7 F and 14 T, respectively) showed a hippocampus with degenerated and depleted cells at the CA3, the cortex showed normal neuronal cells and normal stroma, while the cerebellum showed standard Purkinje cells layer with normal Purkinje cells (black arrow). The groups administered 50% ethanol for seven days and 30% ethanol for twenty-one days (TD3-7 V and 21 T, respectively) showed normal hippocampus and normal cortex with normal neuronal cells and stroma, while the cerebellum showed standard Purkinje cells layer with normal Purkinje cells (black arrow) while the molecular cell layer showed vaculations on the stroma. Group administered 50% ethanol for fourteen days (TD4-14 F) revealed a normal cerebrum, the cortex showed normal neuronal cells and normal stroma, while the cerebellum showed a Purkinje cells layer with several Purkinje cells that revealed chromatolysis and hyalinisation (black arrow). The groups administered 50% ethanol for fourteen days, 40% ethanol for twenty-one days, and 30% ethanol for twenty-eight days (TD5-14 V, 21 F and 28 T, respectively) showed the hippocampus degeneration of neuronal cells; the cells are depleted at the cornu ammonis-CA2 and appeared non-compact. The cerebellum also showed some Purkinje cells layer with chromatolysis (black arrow). The groups administered 50% ethanol for twenty-one days and 40% ethanol for twenty-eight days (TD6-21 V and 28 F, respectively) revealed cerebellum Purkinje cells layer with several chromatolysis and hyalinisation (black arrow). Histology of the brain of a group of rats administered 50% ethanol for twenty-eight days (TD7-28 V) showed focal neuronal necrosis and perivascular lymphocytic cuffing (arrowed).
The results of the histological studies of liver sections of different groups are presented in Fig. 7. The rat liver of control groups (NI-1 C, 7 C, 14 C, 21 C and 28 C, respectively) revealed standard gastric mucosa architecture. The stomach section of the group administered graded doses of ethanol for one day (N2-1 T, 1 F and 1 V, respectively) revealed standard gastric mucosa architecture with mild gastric congestion. The stomach section of the group administered 30% ethanol for seven days (TD1-7 T) showed moderate congestion of superficial mucosa. Groups administered 40% ethanol for seven days and 30% ethanol for fourteen days (TD2-7 F and 14 T, respectively) showed mild erosive gastritis. The groups administered 50% ethanol for seven days and 30% ethanol for twenty-one days (TD3-7 V and 21 T, respectively) showed gastric erosion. Group administered 40% ethanol for fourteen days (TD4-14 F) showed moderate focal erosive gastritis. While the groups administered 50% ethanol for fourteen days, 40% ethanol for twenty-one days and 30% ethanol for twenty-eight days (TD5-14 V, 21 F and 28 T, respectively) revealed focal erosive gastritis. The groups administered 50% ethanol for twenty-one days and 40% ethanol for twenty-eight days (TD6-21 V and 28 F, respectively) showed acute fibrous-haemorrhagic gastritis. The group-administered 50% ethanol histological section for twenty-eight days (TD7-28 V) showed focal necrotic gastritis.

The results of the histological studies of liver sections of different groups are presented in Fig. 6. The rat stomach of control groups (NI-1 C, 7 C, 14 C, 21 C and 28 C, respectively) revealed standard gastric mucosa architecture. The stomach section of the group-administered graded dose of ethanol for one day (N2-1 T, 1 F and 1 V, respectively) revealed normal liver tissue with mild hepatic degeneration, a slight distortion of the hepatic cord and slight swelling of hepatocytes. Group administered 30% ethanol for seven days (TD1-7 T) showed moderate hepatic degeneration and pericholangitis, distorted hepatic cords, hepatocyte swelling, and mononuclear infiltrates around the bile ducts (arrowed). Groups administered 40% ethanol for seven days and 30% ethanol for fourteen days (TD2-7 F and 14 T, respectively) showed pericholangitis and numerous mononuclear cellular infiltrates (arrowed) around the bile duct. The groups were given 50% ethanol for seven days and 30% ethanol for twenty-one days (TD3-7 V and 21 T, respectively) revealed pericholangitis, moderate bile duct hyperplasia and numerous bile ducts (arrowhead) surrounded by mononuclear cellular infiltrates. The group was given 40% ethanol for fourteen days (TD4-14 F) and showed bile duct hyperplasia and numerous bile duct epitheliums (arrowed) in the portal area. The groups administered 50% ethanol for fourteen days, 40% ethanol for twenty-one days and 30% ethanol for twenty-eight days (TD5-14 V, 21 F and 28 T, respectively) revealed hepatic degeneration and bile duct hyperplasia and pericholangitis. The groups administered 50% ethanol for twenty-one days and 40% ethanol for twenty-eight days (TD6-21 V and 28 F, respectively) showed focal acute hepatitis and focus of inflammatory cells (neutrophils) surrounding necrotic hepatocytes. A histological section of the liver of group-administered 50% ethanol for twenty-eight days (TD7-28 V) revealed bile duct hyperplasia and pericholangitis. Numerous mononuclear cellular infiltrates (arrowed) around bile ducts in the portal areas and focus on inflammatory cells (neutrophils) surrounding necrotic hepatocytes (arrowhead).

Histological studies of kidney sections of different groups are presented in Fig. 8. The rat kidney of control groups (NI-1 C, 7 C, 14 C, 21 C and 28 C, respectively) revealed standard renal tubular and glomerular architecture. The rat-representing groups administered graded dose of ethanol for one day (N2-1 T, 1 F and 1 V, respectively) revealed normal renal tissue and cellular architecture. Group administered 30% ethanol for seven days (TD1-7 T) showed renal congestion. Also, the kidney showed prominent and engorged capillaries. Groups were given 40% ethanol for seven days, and 30% ethanol for fourteen days (TD2-7 F and 14 T, respectively) and showed mild renal tubular degeneration and numerous intra-tubular eosinophilic hyaline cast (arrowed). The groups were given 50% ethanol for seven days, and 30% ethanol for fourteen days (TD3-7 V and 21 T, respectively) showed focal renal hyperplasia.
ethanol for twenty-one days (TD3-7 V and 21 T, respectively) and showed moderate renal degeneration and intra-tubular eosinophilic hyaline cast (arrowed) within renal tubules. Group administered 40% ethanol for fourteen days (TD4-14 F) showed renal tubular degeneration characterised by the intra-tubular eosinophilic hyaline cast (arrowed). The groups administered 50% ethanol for fourteen days, 40% ethanol for twenty-one days and 30% ethanol for twenty-eight days (TD5-14 V, 21 F and 28 T, respectively) showed focal interstitial nephritis with a focal aggregate of mononuclear inflammatory cells (arrowed) surrounding an atrophied tubule. The groups were given 50% ethanol for twenty-one days and 40% ethanol for twenty-eight days (TD6-21 V and 28 F, respectively) showed chronic interstitial nephritis and mononuclear inflammatory cell infiltration of the inter-tubular connective tissue. The histological section of the kidney group given 50% ethanol for twenty-eight days (TD7-28 V) revealed chronic interstitial nephritis: mononuclear cellular infiltrates and tubular necrosis.

Histological studies of testes sections of different groups are presented in Fig. 5. The rat testes of control groups (NI-1 C, 7 C, 14 C, 21 C and 28 C, respectively) revealed normal testes with standard architecture and cellularity of seminiferous tubules. The testis section of the rat representing groups was given a graded dose of ethanol for one day (N2-1 T, 1 F and 1 V, respectively) revealed normal testis with mild testicular degeneration, mild variation in sizes, and cellularity seminiferous tubules. Group administered 30% ethanol for seven days (TD1-7 T) showed mild testicular degeneration characterised by a few seminiferous tubules (arrowed) lined by a single layer of germinal epithelium. Groups administered 40% ethanol for seven days and 30% ethanol for fourteen days (TD2-7 F and 14 T) showed mild testicular degeneration with several degenerated tubules (arrowed) and a single layer of germinal epithelium with a wide lumen. The groups were given 50% ethanol for seven days and 30% ethanol for twenty-one days (TD3-7 V and 21 T, respectively) showed moderate testicular degeneration and hypoplasia. Degenerate tubules (arrowed) are distorted and contain an intraluminal mass of exfoliated germ cells. Group administered 40% ethanol for fourteen days (TD4-14 F) revealed testicular hypoplasia and seminiferous tubules containing a single (arrowed) or fewer layer of germinal epithelium. The groups administered 50% ethanol for fourteen days, 40% ethanol for twenty-one days, and 30% ethanol for twenty-

Fig. 5. Photomicrograph of different group sections of the brain tissue stained by Haematoxylin and Eosin (x 400). KEY NOTE: TD-Tissue Damage (different forms), N- Normal tissue.

NI-1 C, 7 C, 14 C, 21 C and 28 C; N2-1 T, 1 F and 1 V; TD1-7 T; TD2-7 F and 14 T; TD3-7 V and 21 T; TD4-14 F; TD5-14 V, 21 F and 28 T; TD6-21 V and 28 F; TD7-28 V White arrow – hippocampus, blue arrow- neuronal cell, slenser arrow – stroma, black arrow- purkinje cells, red arrow – granular layer, and slenser arrow – normal stroma Where C- control administered distilled water, T- administered 30% alcohol, F- administered 40% alcohol and V- administered 50% alcohol. Numeric number 1, 7, 14, 21, and 28 stands for the day(s) rate were sacrificed.(For interpretation of the references to colour in this figure, the reader is referred to the web version of this article.)
eight days (TD5-14 V, 21 F and 28 T, respectively) showed testicular degeneration and variation in shape low cellularity of seminiferous tubules. The groups administered 50% ethanol for twenty-one days and 40% ethanol for twenty-eight days (TD6-21 V and 28 F, respectively) showed testicular degeneration, distended interstitial space, and the distorted architecture of seminiferous tubules (arrowed) and lining of seminiferous tubules by a single layer of germinal epithelium. The histological section of the testis of group-administered 50% ethanol for twenty-eight days (TD7-28 V) revealed severe testicular degeneration, distorted architecture, shrunken appearance and hypocellularity of seminiferous tubules.

4. Discussion

The lipid peroxidation cascade forms different intermediates and byproducts, one of which is malondialdehyde (MDA), which is usually relatively abundant during lipid peroxidation [3,9,10,14]. Thus, MDA levels are used mainly in pre-clinical and human studies to monitor the degree of peroxidative damage caused by reactive species to cells and tissues [1-4,10,14]. In the present study, different alcohol concentrations administration significantly increased the testes’ MDA levels, and this result indicates that the lipid peroxidation processes in the testicular tissues were due to the generation of free radicals. These observations agreed with several researchers who reported an increase in testicular lipid peroxidation (MDA levels) in rats exposed to a single specific concentration of ethanol ranging from 25% to 50% [1-6,7].

Low to moderate reactive species are required in the physiological fertilisation process, but an excessive amount is detrimental [15,16]. Good sperm motility is a prerequisite for sperm function in fertilisation [3,8,15]. The administration of graded alcohol concentrations to rats in this study caused adverse effects on the sperm characteristics by significantly decreasing sperm motility percentage and increasing the percentage of sperm dead cells with abnormal morphology. These observations could be due to the increased generation of reactive species induced by excessive alcohol consumption. This observation agrees quite very well with previous documentation by some researchers that alcohol intoxication affected the spermatozoa of the rats adversely, thereby lowering sperm motility and decreasing live sperm and sperm
with normal morphology [2,3,8].

Spermatozoa are susceptible to oxidative stress-induced damage because of large content of polyunsaturated fatty acids (PUFAS) in their membrane, especially phospholipids esterified with two PUFAS commonly found in sperm [3,9,15,16]. The abnormal morphology of the sperm structure can be attributed to the lipid peroxidation damage to the PUFAS component caused by excessive reactive species production [3,9,17]. Therefore, abnormal sperm quality observed in this study might be due to the disruption of spermatogenesis by excessive alcohol consumption. This study observed that concentrations and durations dependent increase in testes MDA levels, compared with control after day 14–28 of alcohol administration, corresponded only with the rate of sperm death and loss of sperm motility. These findings demonstrated an alcohol-induced reduction in sperm quality through free radical-mediated lipid peroxidation and pathological lesions in the testes. Also, high levels of MDA, as observed especially in the testes, might be due to the presence of easily peroxidisable fatty acids in their membranes. Our findings are consistent with the conclusion of the previous researchers that lipid peroxidation could be considered, at least in part, a mechanism of action for ethanol on testicular and sperm parameter alterations [2,3,7].

It has long been known that the chronic consumption of ethanol may cause alterations in the male reproductive tract of mammals [2,8]. Experimental studies have found evidence of testicular lesions as the main alterations resulting from abusive and prolonged ethanol intake [2,3,7,8]. In this study, the histopathological findings of the testes ranged from mild testicular degeneration, degenerated tubules with distortion, the intraluminal mass of exfoliated germ cells, testicular hypoplasia and seminiferous tubules containing a fewer layer of germinal epithelium. In addition, cellularity of seminiferous tubules, distended interstitial space, severe testicular degeneration, distorted architecture, shrunken appearance and hypocellularity of seminiferous tubules were observed in a duration and concentration-dependent manner. These testicular damages could be due to increased generation of acetaldehyde produce, which attacked the polyunsaturated fatty acids (PUFA) in the testes’ membrane, generating more reactive species like MDA and thus leading to the loss of testicular structure and functions. Therefore, the testicular structural disruption and damage of the testis’s function might have ensued from excessive alcohol-induced oxidative damage. These histopathological findings on the testes agree with previous report that oral gavage administration of alcohol intoxication induced different forms of testicular damage [2,3,7,8].

Increased plasma MDA levels could directly reflect a rat’s vital organs or tissues injuries following excessive ethanol ingestion [4,11]. Based on the observations in this study, one could attribute tissues damages, morphological alterations of the brain, stomach, kidney, liver, and testes as well as the reduction in sperm quality to lipid peroxidation effects of excessive alcohol consumption. This study validated that graded alcohol concentrations caused different detrimental effects on sperm characteristics and induced pathological lesions in the testes, brain, liver, kidney and stomach. However, as observed in this study, the increase in serum, liver and testes MDA levels were durations independent but almost entirely concentrations dependent. These observations corroborate the duration effects of 30% alcohol intoxication on
sperm motility but not with MDA levels. Oremosu and Akang [6] reported an increase in the duration of 30% alcohol intoxication in rats induced a decrease in percentage sperm motility with a concomitant increase in MDA levels. The inconsistency observed in the results of MDA levels might be due to difference in the methods used to estimate the extent of lipid peroxidation in tissues. This observation agreed with the conclusion of Tsikas [14] and Altomare et al. [10] that MDA levels measurement using thiobarbituric acid-reactive substances (TBARS), though famous but lacks selectivity, which dramatically limits its usefulness for detecting actual differences in the extent of lipid peroxidation in clinical studies.

5. Conclusions

Detrimental effects of graded concentration of alcohol over a certain period on testes morphologies were validated. Furthermore, the alcohol effects led to loss of sperm motility and testicular degeneration in concentration and duration dependent manner without a concomitant increase in MDA levels. This study further added to existing knowledge that excessive alcohol consumption causes interrelationship between tissues (brain, stomach, kidney, liver, and testis) pathological lesions, and sperm motility loss, apart from increased testicular malondialdehyde levels correlation. However, further studies are necessary to investigate other underlying mechanisms associated with toxic alcohol effects with increased concentration and duration consumption.

Ethics approval and consent to participate

The manuscript does not contain clinical studies or patient data. Ethics approval and consent to participate in this study were approved and obtained after the presentation of the proposal to the Departmental Animal Use and Care Ethical Committee of the Department of Biochemistry College of Biosciences, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria with ethical reference number FUNAAB-BCH – DI 016c. All authors approved the project proposal presented to the Ethical Committee, and the experiment was performed under the ethical standards laid down by the Committee.

Consent for publication

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AKINLOYE Dorcas Ibukun: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization, Project administration, Funding acquisition; UGBAJA Regina Ngozi: Investigation, Resources, Writing – review & editing, Supervision; ADEBIBI Adekunle Adeniyi: Methodology, Investigation, Resources, Writing – review & editing; IDOWU Olusegun Mark Obafemi: Investigation, Resources, Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Not applicable.

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References

[1] O.O. Dosumu, O.B. Akinola, E.N. Akang, Alcohol-induced testicular oxidative stress and cholesterol homeostasis in rats—the therapeutic potential of virgin coconut oil, Middle East Fertil. Soc. J. 17 (2) (2012) 122–128, https://doi.org/10.1016/j.mefs.2011.12.005.
[2] O.A. Adaramoye, M. Arisekola, Kolaviron, a biflavonoid complex from Garcinia kola seeds, ameliorates ethanol-induced reproductive toxicity in male Wistar rats, Nigerian J. Physiol. Sci. 28 (1) (2013) 9–15. (https://www.ajol.info/index.php/njps/article/view/95095).
[3] R. Uygur, M. Yagmurca, O.A. Alkoc, A. Genc, A. Songur, K. Ucok, O.A. Ozen, Effects of quercetin and fish n-3 fatty acids on testicular injury induced by ethanol in rats, Andrologia 46 (4) (2013) 356–369, https://doi.org/10.1111/andr.12085.
[4] D.I. Akinloye, R.N. Ugba, O.A. Dosumu, Appraisal of the antioxidative potential of Aloe Barbadensis M. on alcohol-induced oxidative stress, Folia Veterinaria 63 (3) (2019) 34–46, https://doi.org/10.2478/fv-2019-0025.

[5] D.I. Akinloye, R.N. Ugba, O.A. Dosumu, S.A. Rahman, E.I. Ugwor, A.S. James, O. O. Oyesile, M.B. Bada, A time-course study on the dose-response relationship between alcohol exposure and its effects on lipid profile and biomarkers of tissue damage, Biochem. Biophys. Rep. 26 (2021) 1–8, https://doi.org/10.1016/j.bbrep.2021.100927.

[6] A.A. Oremosu, E.N. Akang, Impact of alcohol on male reproductive hormones, oxidative stress and semen parameters in Sprague–Dawley rats, Middle East Fertl. Soc. J. 20 (2) (2015) 114–118, https://doi.org/10.1016/j.mefs.2014.07.001.

[7] G.E. Siervo, H.R. Vieira, F.M. Ogo, C.D. Fernandez, G.D. Gonçalves, S.F. Mesquita, J.A. Anselmo-Franci, R. Cecchini, F.A. Guarnier, G.S. Fernandes, Spermatic and testicular damages in rats exposed to ethanol: influence of lipid peroxidation but not testosterone, Toxicology 330 (2015) 1–8, https://doi.org/10.1016/j.tox.2015.01.016.

[8] I.M. El-Ashmawy, A. Saleh, O.M. Salama, Effects of marjoram volatile oil and grape seed extract on ethanol toxicity in male rats, Basic Clin. Pharmacol Toxicol 101 (5) (2007) 320–327, https://doi.org/10.1111/j.1742-7835.2007.00125.x.

[9] S. Dutta, A. Majzoub, A. Agarwal, Oxidative stress and sperm function: a systematic review on evaluation and management, Arab J. Urol. 17 (2) (2019) 87–97, https://doi.org/10.1080/2096598X.2019.1599624.

[10] A. Altomare, G. Baron, E. Gianazza, C. Banfi, M. Carini, G. Aldini, Lipid peroxidation derived reactive carbonyl species in free and conjugated forms as an index of lipid peroxidation: limits and perspectives, Redox Biol. 42 (2021) 1–14, https://doi.org/10.1016/j.redox.2021.101899.

[11] E.C. Schlöff, K. Husain, S.M. Somani, Dose- and time-dependent effects of ethanol on plasma antioxidant system in rat, Alcohol 17 (2) (1999) 97–105, https://doi.org/10.1016/S0741-8329(98)00039-1.

[12] J. Fernandez, J.A. Pérez-Álvarez, J.A. Fernández-López, Thiobarbituric acid test for monitoring lipid oxidation in meat, Food Chem. 59 (3) (1997) 345–353, https://doi.org/10.1016/S0308-8146(96)00114-8.

[13] M.R. Rehan, A.M. Zedan, S.A. El-Hashash, M.A. Farid, G.A. El-Shafie, Protective effects of some fruit juices with low-fat diet on rat testis damaged by carbon tetrachloride: A genetic and histological study, African J. Biotechnol. 15 (36) (2016) 1977–1985, https://doi.org/10.5897/AJB2016.15456.

[14] D. Tsikas, Assessment of lipid peroxidation by measuring malondialdehyde (MDA) and relatives in biological samples: analytical and biological challenges, Analit. Biochem. 524 (2017) 13–30, https://doi.org/10.1016/j.ab.2016.10.021.

[15] T. Takeshima, K. Usui, K. Mori, T. Asai, K. Yasuda, S. Kuroda, Y. Yamura, Oxidative stress and male infertility, Reprod. Med. Biol. 20 (1) (2021) 41–52, https://doi.org/10.1002/rmb2.12353.

[16] R.K. Sharma, A. Agarwal, Role of reactive oxygen species in male infertility, Urology 48 (6) (1996) 835–850, https://doi.org/10.1016/S0090-4295(96)00313-5.

[17] A. Agarwal, T.M. Said, Role of sperm chromatin abnormalities and DNA damage in male infertility, Hum. Reprod. Update 9 (4) (2003) 331–345, https://doi.org/10.1093/humupd/dmg027.