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The Impact of *Annona Muricata* on Semen Quality and Antioxidants Levels in Alcohol-Induced Testicular Dysfunction in Male Wistar Rats

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ABSTRACT

The study was aimed to determine the impact of *Annona muricata* on biochemical and histological profile on alcohol-induced testicular dysfunction in male Wistar Rat. Twenty-five male Wistar rats with a weight of 130-160 grams were employed. Group A received feed and distilled water only, group B received 40-ml of Alcohol, and group C received 200mg/kg of ethanolic leaf extract of *Annona muricata* (ELAM). Groups D and E received 40-ml of Alcohol and were treated with 200mg/kg and 400mg/kg of ELAM for three weeks. The administration was done through oral gavage daily for three weeks. Data obtained for testosterone, Malondialdehyde (MDA), Catalase (CAT), gonado-somatic index, and semen quality (total sperm count, motility, and morphology) were analyzed using ANOVA followed by post hoc LSD, and values were considered significant at $p<0.05$. The results showed a significant ($p<0.05$) decrease in testosterone level, total sperm count, activity motility, and normal sperm cells in-group B compared to A. However, in treatment groups, Annona muricata attenuated the effect on the parameters above, with a significant ($p<0.05$) increase compared to group B. Alcohol toxicity caused an increase in abnormal sperm cells and gonado-somatic index of the testes significantly ($p<0.05$), which was attenuated by the *Annona muricata*. Also, significant higher MDA activities were observed in group B; with no significant change in groups, C to E. CAT activities showed a significant decrease in group B and significantly attenuated CAT activities in-group E. Histoarchitectural results revealed spermatogenic arrest and necropsied spermatids in group B, while moderate healing and increased spermatogenic process by the extracts in the treated groups. The study revealed testicular dysfunction resulting in infertility by alcohol toxicity; however, Annona muricata showed improved fertility after reduced testicular function and improved CAT activities based on dose-dependent with no effect on MDA level.

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INTRODUCTION

The male reproductive system is a complex function of the continuity of life in a living organism, which involves the production of hormones and semen characteristics (1–3). Infertility in males is an issue of global concern, which results in a decline in semen quality with an upsurge among males (4). However,

the sperm acquire proactive mechanisms during spermatogenesis and their epididymal maturation and are still viable for toxic insult; though, decreased semen quality is a significant contributor to male infertility (4,5). Spermatogenesis is a complex process that involves numerous morphological, physiological, and biochemical changes resulting in the formation of male sexual function (1,6). Testosterone is responsible for developing primary sexual development, which includes testicular descent, spermatogenesis, enlargement of the penis and testes, and increasing libido (7).

A sedentary lifestyle is a critical factor in the aetiology of a more significant percentage of infertility in males, which is excessively affected by reactive oxygen species (ROS) production (5,8). ROS has a potentially toxic effect on semen quality and function, linked to alcohol consumption and environmental and sedentary lifestyle affecting spermatogenesis (8). Oxidative stress has been linked to sperm dysfunction, triggering ROS formation, leading to negative affluence on the spermatozoa and fertility capacity (5,9). ROS disrupts the Deoxyribonucleic acid (DNA) of testes, resulting in apoptosis, spermatic function alterations, and infertility as the end product (5,10,11). Malondialdehyde (MDA) is a stable lipid peroxidation product and, therefore, can be used as an indirect measure of cumulative lipid peroxidation (12). Catalase (CAT) is a crucial enzyme in the metabolism of H2O2 and reactive nitrogen species, and its expression and localization are markedly altered in tumours (13). CAT is known for maintaining an optimum molecule in the cell, which is essential for cellular signalling processes (14).

Alcohol is a crucial substance of abuse, a key target for testicular dysfunction capillaries, with Leydig cells in the interstitium are targets for cadmium and ethanol, respectively (4,15). Alcohol negatively affects semen quality, revealing a significant decline in sperm motility, count, and morphology (8). Reports has shown that alcohol abuse in acute and chronic form is associated with poor semen quality (16,17). Sequel to chronic consumption of alcohol, there was a significantly lowered levels of sperm count, motility, plasma testosterone, and atrophied seminiferous tubules (18).

Plants have gained strong evidence in the management of male fertility globally because of their rich phytochemicals (19,20). However, medicinal plants are known for their better therapeutic approach in improving fertility issues arising from environmental and sedentary lifestyles (19,21) through numerous phytochemicals, they possess. *Annona muricata* L. is an evergreen tropical tree of the Annonaceae family with phytotherapeutic bioactive compounds known for their medicinal value (22,23). The Anxiolytic, anti-stress, anti-inflammatory, contraceptive, anti-tumoral, antiulcer, wound healing, hepato-protective, anti-icteric, and hypoglycemic activities In-vivo study of the crude extracts and isolated compounds of *A. muricata* (23,24). Despite its therapeutic effects on the different testicular models of toxicity following treatments with *Annona muricata*, there is a dearth of literature on the anti-infertility effect of *Annona muricata* on the alcohol testicular dysfunction in males Wistar rats.

Materials and Methodology

Area of Study: The experiment was carried out in the Department of Physiology, College of Health Sciences, Nnamdi Azikiwe University, and Nnewi Campus.

Ethical Approval: Ethical approval was obtained from the Faculty of Basic Medical Science, College of Health Science, Nnamdi Azikiwe University, Nnewi campus. Rats handling and treatments conform to the National Institute of Health guidelines for laboratory animal care and use (25).

Materials: Twenty-five male Wistar rats, Alcohol, *Anonna muricata* leaf, Absolute Ethanol (JHD Chemicals, Guangdong China), distilled water, Whatman qualitative filter paper no. 1, oral cannula, Automatic Water distiller (SZ-1 Search Tech Instrument), (Olympus XSZ-107BN), heparinized capillary tube, and Pelletized laboratory chow (Jos, Nigeria). Neubauer Counting Chamber (England), Centrifuge 90(1) (Alpin Medical, England), and Plain Blood tube (Fantastik, China). Standard cages with water can, Slide, Rotary evaporator (Digital) TT-52 (Techmel & Techmel, USA), Thermostat Oven (DHG-9023A, PEC MEDICAL USA), chloroform, heparinized capillary tube, Electronic weighing balance (M-Metallar M311), 2ml hypodermic sterile syringe, and animal weighing balance (Camry LB11).

Reagents and Chemicals: Hydrogen Peroxide (Sigma Aldrich, USA), sodium-potassium phosphate buffer (Analytic graded in the laboratory), Thiobarbituric acid (TBA) 99% pure will be purchased from BDH (BDH, England); malondialdehyde tetrabutylammonium salt (MDA salt) 96% pure and methanol 99.8% pure were from Sigma-Aldrich (Steinheim, Germany). Glacial acetic acid (99–101% pure) was purchased from Sigma-Aldrich (USA).

Methodology

Identification and preparation of *Anonna muricata* leaf: The leaves of *Anonna muricata* were obtained from the college premises, identified by the

Department of Botany, Nnamdi Azikiwe University, Awka Campus, and the herbarium was deposited in the catalogue. The leaves of *Anonna muricata* were air-dried under room temperature, after rinsed under tap water. The dried formed of *Anonna muricata* was milled using a local grinder and 500g of the coarse form were macerated in 1000mls of 95% absolute ethanol for 48hours. Thereafter, the solute was filtered using a clean handkerchief and further filtration using Whatman No 1 filter paper. The filtrate was concentrated using a rotatory evaporator and further dried using a Thermostat oven at 45°c into a gel-like form.

Experimental Animals and Design: In-breed twenty-five male Wistar rats with weight of 130-160 gram were employed in the study, and housed in the animal house, Department of Physiology, College of Health Sciences, Nnewi campus. The animals were sustained with standard pellet laboratory chow and distilled water in a standard cage at ambient temperature *ad libitum*. Acclimatization of the animals was done two weeks prior to the start of experiment following alcohol-inducement and administration of the *Annona muricata*.

Group A received feed and distilled water only, group B received 40-mls of alcohol only, and group C received 200mg/kg of ethanolic leaf extract of *Anonna muricata* (ELAM). Groups D and E received 40-mls of Alcohol and were treated with 200mg/kg and 400mg/kg of ELAM for three-weeks. The administration of the extract lasted for three-weeks and was done for through oral gavage between the hours of 7:00 to 8:00 AM daily.

Sample Collection: Animals were anaesthetized with chloroform in an enclosed container 24 hours after the last administered dose of the ELAM and Alcohol, and blood were collected through heparinized capillary tube and put into a plain container as described by Parasuraman et al., (26), which was allowed to cool for 5-minutes. The blood was centrifuge for 15-minutes at 3000 RMP, and the serum were retrieve using micropipette and placed in a plain bottle, which was used for estimating serum testosterone, MDA, and CAT. Semen was obtained from the epididymal gland immediately and fixed in a cleaned, labeled slide. The testes were weighed and documented and stored in bouin's fluid for proper fixation for histopathological studies.

Hormonal Assay for testosterone: The serum retrieved was used to assay testosterone levels in the different groups using the ELISA Method described by the Manufacturer's manual.

Principle and Method: The normal range was standardized and then 25 µl serum samples were placed in the well plates. Hundred-(100) µl of enzyme conjugate was added in each well. After that, it was left for incubation at 370C in an incubator for 1 hour. Then, the wells were washed with 300 µl-distilled water for at least 3-times and blotted. Then, 100 µl TMB solution was added as a substrate in each well plate and will again be left for the incubation for 15 minutes for the colour. Finally, a 100 µl stop solution was added in each well to stop the reaction. Reading was taken at 630nm through Merck ELISA reader in ng/ml value.

Oxidative Marker: MDA was assayed using the method described by Aguilar Diaz De Leon and Borges, (27).

Principle: The standard MDA solution (1 mL) was taken in a 10 mL test tube and mixed with TBA (1 mL). The mixture was heated in a boiling water bath at 95°C for 60 minutes. The test tubes was cooled at room temperature and absorbance and was measured at 532 nm using UV-visible spectrophotometer. A repeat of the procedure occurred 5-times. The TBARS was calculated using the formula as M/g of the sample: TBARS (µM/g) = (Ac x V) / W. Where 'Ac' is the amount determined from the calibration curve, and 'W' is the weight of the sample taken while 'V' is the volume in mL or dilution factor of the total extract prepared.

Antioxidant Marker (Catalase Activity): Catalase was determined according to the standard method described by Hadwan (2018) by using a UV-VIS spectrophotometer.

Principle: Catalase activity was assessed by incubating the enzyme sample in substrate hydrogen peroxide in the sodium-potassium phosphate buffer, (pH 7.4) at 37°C for three minutes. The reaction was stopped with ammonium molybdate. The absorbance of the yellow complex of molybdate and hydrogen peroxide were measured at 374 nm against the blank. This is equivalent to the catalase activities in the serum.

Semen Quality: The semen quality includes sperm motility, sperm count, and sperm morphology.

Sperm motility was determined according to the method Badkoobeh et al. (29). Sperm cells were obtained from the epididymis' caudal end. It was placed in a clean glass slide, and mixed with a physiological solution of 990 µL (paraformaldehyde and sodium citrate) in the ration of 1-20. About 5.0 µL of supernatant containing the sperm was placed between the slide and coverslip and observed at 100x in a negative phase contrast microscopy (XSZ-107BN). The motile sperm was counted based on the movement in the different fields.

Total Sperm Count: Approximately 10 µL of the diluted contents was transferred hemocytometer (Neubauer chamber) and taken in light microscopy at 400 x. The pelleted cells were counted on the surface of the chamber. The sperm concentration calculation was performed according to the number of counted cells and hemocytometer dimensions. The concentration were expressed in millions of sperm per mL (29).

Sperm Morphology: About 20 µL of the sperm suspension was placed on the slides and swiped with 95% v/v ethanol for proper fixation for 5-10 minutes, and allowed to air-dry. The smear was washed with sodium bicarbonate solution (reagent no. 72) to remove any mucus, which may be present. Rinse smear with several distilled water changes, after allowing to air-dry and cover smear with carbon fuchsin (1 in 20) and allow staining for 3 minutes and wash off with distilled water. After that, the counterstain was done by covering the smear with dilute Loeffler's methylene blue (1 in 20) for 2 minutes. Allow to dry and wash off with distilled water (29,30).

Histopathological Procedure: Tissues (testes) was fixed in bouin's fluid and was dehydrated in four (4) concentrations of Isopropyl alcohol, i.e. 70%, 80%, 90%, 100% for 1hr each and then cleared in xylene before embedded in molten paraffin wax to remove the isopropyl alcohol. Micro sections of 5micrometer using Leica RM 212 Rt. Rotary Microtome, tissues were stained using Haematoxylin and Eosin (H&E) to demonstrate general tissue structure. Tissues sectioned was be examined and interpreted using Leica DM 750 binocular microscope with photomicrographic facilities and then photomicrographed by a histopathologist (31).

Statistical Analysis: Data obtained from this study was subjected to Statistical Package for Social Sciences (SPSS) version 25. Data obtained for antioxidant activity (CAT), oxidative stress markers (MDA), hormonal profile (testosterone), gonado-somatic index, and semen quality (total sperm count, motility, and morphology) were analyzed using ANOVA followed by post hoc LSD. Data was considered significant at $p<0.05$.

RESULTS

Table 1: effect of *Annona muricata* on testosterone and total sperm count following alcohol-induced testicular dysfunction

Groups	Testosterone (ng/ml)	Total sperm count (x10^6/mls)
	MEAN±SEM	MEAN±SEM
Group A (Positive control)	4.06±0.29[a]	566.67±8.82 [a]
Group B (Alcohol only)	1.57±0.18	259.67±25.49
Group C (200mg/kg of ELAM)	2.80±0.35 [b]	600.00±5.77 [a]
Group D (Alcohol + 200mg/kg of ELAM)	3.40±0.30 [a]	488.33±20.48[a]
Group E (Alcohol + 400mg/kg of ELAM)	4.23±0.17 [a]	520.67±15.71 [a]

Data was analyzed using ANOVA followed by Post-Hoc LSD and values were considered significant at $p<0.05$. $p<0.001$[a], $p<0.01$[b], and $p<0.05$[c]; ELAM: ethanolic leaf extract of *Annona muricata*; SEM: standard error of mean

Table 1 result revealed a significant ($p<0.05$) decline in testosterone level in-group B compared to A; groups C, D, and E had a significant ($p<0.05$) higher testosterone level compared to group B. Also, the total sperm count result showed a significant ($p<0.05$) decrease in-group B compared to A; groups C, D, and E had a significant ($p<0.05$) increase compared to group B.

Table 2: effect of *Annona muricata* on active and sluggish motility following alcohol-induced testicular dysfunction

Groups	Active motility (%)	Sluggish motility (%)
	MEAN±SEM	MEAN±SEM
Group A (Positive control)	83.33±3.33[a]	16.67±3.33 [a]
Group B (Alcohol only)	60.00±5.77	40.00±5.77
Group C (200mg/kg of ELAM)	81.67±1.66 [a]	18.33±1.67 [a]
Group D (Alcohol + 200mg/kg of ELAM)	80.00±2.88 [b]	20.00±2.88[a]
Group E (Alcohol + 400mg/kg of ELAM)	81.67±1.66 [a]	18.33±1.67 [a]

Data was analyzed using ANOVA followed by Post-Hoc LSD and values were considered significant at $p<0.05$. $p<0.001$[a], $p<0.01$[b], and $p<0.05$[c]; ELAM: ethanolic leaf extract of *Annona muricata*; SEM: standard error of mean.

Table 2 result revealed a significant ($p<0.05$) decrease in active motility in-group B compared to A; groups C, D, and E had a significant ($p<0.05$) increase compared to group B. Further, the sluggish motility result showed a significant ($p<0.05$) increase in-group B compared to A; groups C, D, and E had a significant ($p<0.05$) decrease compared to group B.

Table 3: effect of *Annona muricata* on sperm morphology following alcohol-induced testicular dysfunction

Groups	Normal sperm cells (%)	Abnormal sperm cells (%)
	MEAN±SEM	MEAN±SEM
Group A (Positive control)	83.33±3.33^{b}	16.67±3.33^{b}
Group B (Alcohol only)	63.33±4.40	36.67±4.40
Group C (200mg/kg of ELAM)	81.66±1.67^{b}	18.33±1.67^{b}
Group D (Alcohol + 200mg/kg of ELAM)	79.33±0.67^{b}	20.67±0.67^{b}
Group E (Alcohol + 400mg/kg of ELAM)	83.33±4.41^{a}	16.67±4.41^{a}

Data was analyzed using ANOVA followed by Post-Hoc LSD and values were considered significant at $p<0.05$. $p<0.001^{a}$, $p<0.01^{b}$, and $p<0.05^{c}$; ELAM: ethanolic leaf extract of *Annona muricata*; SEM: standard error of mean

Table 3 result revealed a significant ($p<0.05$) decrease in normal sperm cells in-group B compared to A; groups C, D, and E had a significant ($p<0.05$) increase compared to group B. Further, the abnormal sperm cells result showed a significant ($p<0.05$) increase in-group B compared to A; groups C, D, and E had a significant ($p<0.05$) decrease compared to group B.

Table 4: effect of *Annona muricata* on MDA, Catalase, and gonado-testicular index following alcohol-induced testicular dysfunction

Groups	Catalase (umol/L)	MDA (umol/L)	Gonado-testicular index (IU/L)
	MEAN±SEM	MEAN±SEM	MEAN±SEM
Group A (Positive control)	336.17±4.18^{a}	2.63±0.60^{c}	0.65±0.02^{b}
Group B (Alcohol only)	237.33±2.18	5.01±1.32	1.33±0.03
Group C (200mg/kg of ELAM)	232.50±0.28^{d}	4.50±0.31^{d}	0.43±0.04^{a}
Group D (Alcohol + 200mg/kg of ELAM)	250.00±5.77^{d}	4.12±0.30^{d}	0.60±0.06^{b}
Group E (Alcohol + 400mg/kg of ELAM)	258.67±7.31^{b}	3.73±0.34^{d}	0.53±0.03^{a}

Data was analyzed using ANOVA followed by Post-Hoc LSD and values were considered significant at $p<0.05$. $p<0.001^{a}$, $p<0.01^{b}$, and $p<0.05^{c}$ ELAM: ethanolic leaf extract of *Annona muricata*; SEM: standard error of mean.

Table 4 result demonstrated a significant ($p<0.05$) decrease in the CAT level in-group B compared to A; groups C had an insignificant decrease; group D showed an insignificant ($p>0.05$) increase and group E significant ($p<0.05$) increase compared to group B. The MDA result indicated a significant ($p<0.05$) increase in-group B compared to A, groups C, D, and E had an insignificant ($p>0.05$) decline compared to group B. The gonado-somatic index (testicular weight) result showed a significant ($p<0.05$) increase in-group B compared to A; groups C, D, and E had a significant ($p<0.05$) decrease compared to group B.

Histopathological Findings

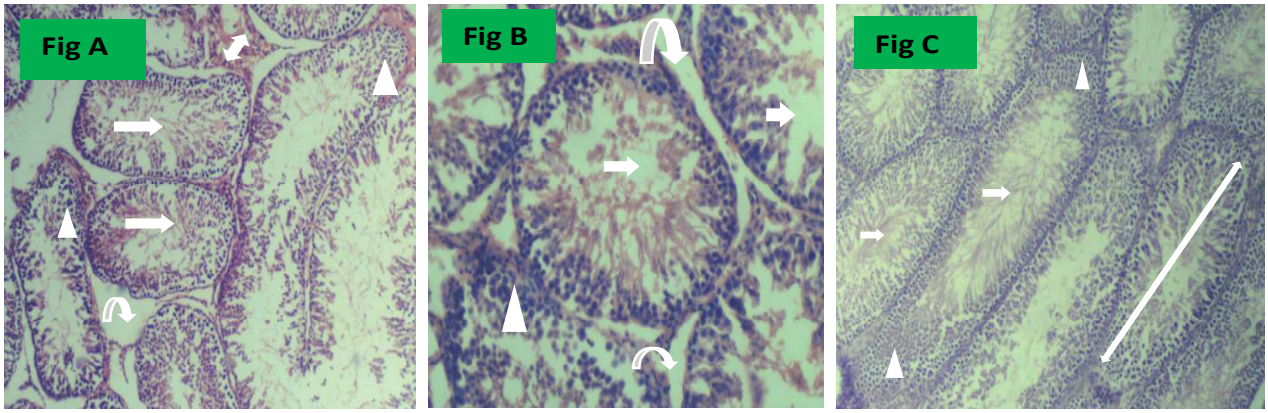

Fig A (positive control): Photomicrograph section of the testes shows active spermatogonia and spermatids (arrow head), ductus epididymis (curved arrow), spermatozoa (arrow), and connective tissue (both arrow). Testicular morphology was consistent and stained using H & E (x400).

Fig B (received 40mls of Alcohol only): Photomicrograph section of the testes shows moderate spermatogenic arrest (arrow), with necrosis of spermatid cells and atrophy (arrow head), and increase diameter of seminiferous tubules (curved arrow). Stained by H & E (x400).

Fig C (received 200mg/kg of ELAM only): Photomicrograph section of the testes shows increased active spermatogenesis (arrow), active spermatid and spermatogonia (arrow head), and intact seminiferous tubules and ductus epididymis (both side arrow). Testicular morphology was consistent. Stained by H & E (x400).

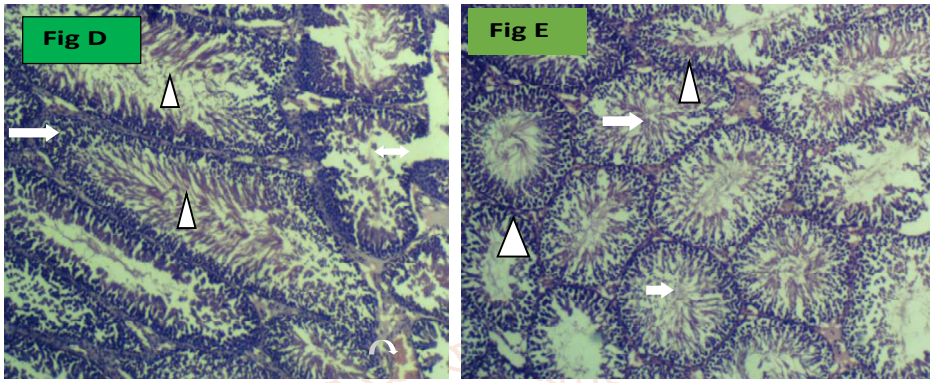

Fig D (received 40mls of Alcohol and treated with 200mg/kg of ELAM): Photomicrograph section of the testes show moderate healing with active spermatogonia (arrow), increased spermatogenesis (arrow head), mild fatty change (arrow both side) and mild hemorrhage (curved arrow). Stained by H & E (x400).

Fig E (received 40mls of Alcohol and treated with 400mg/kg of ELAM). Photomicrograph section of the testes shows healing with increased spermatogenesis process (arrow), active spermatogonia and spermatid (arrowhead), and intact seminiferous tubules. Stained by H & E (x400).

Discussion

Infertility is considered a threat to global fertility in males, which results from environmental and sedentary lifestyles affecting about 30-50% of the men populace globally (32,33). However, its aetiology is idiopathic in some cases (30-50%) and is heavily linked to chemicals and environmental toxins leading to oxidative stress (34,35). However, male infertility is a multifaceted pathological condition with 7% of the population being affected (35,36), genetic factors affecting 25% of the male population. Alcohol intake has increased globally, resulting from emotional distress, which is significantly used as a medium to ease stress (37).

Alcohol toxicity has been linked with oxidative stress, a critical potential for testicular dysfunction affecting the continuity of species among living organisms (38). The current study investigates the impact of *Annona muricata* on biochemical and histological profiles following alcohol-induced testicular dysfunction in male Wistar rats. The study results showed a significant ($p<0.05$) decline in testosterone level in-group B compared to A; groups C, D, and E had a significant ($p<0.05$) higher testosterone level compared to group B. Also, the total sperm count result showed a significant ($p<0.05$) decrease in-group B compared to A; groups C, D, and E had a significant ($p<0.05$) increase compared to group B. Testosterone is an influential hormone in the continuity of species along with the semen quality (sperm motility, count, and morphology). However, an alteration in testosterone production could cause infertility in the male populace, acting through a distinct mechanism by generating ROS. Alcohol is known to affect testosterone formation through the inhibition of gonadotropin-releasing hormone (GnRH) suppression through oxidative stress (39). However, the study corroborated the findings of Oremosu and Akang (40), Dosumu et al. (16,18), Talebi et al. (17), revealing a significant decline following alcohol ingestion in Wistar rats. Also, Muthusami and Chinnaswamy (41) reported a significant decline in testosterone levels following alcohol consumption, similar to the study report. Sermondade et al. (42) contradicted this study revealing a non-significant change in testosterone levels following alcohol consumption. However, treatment with *Annona muricata* leaf extract revealed attenuated changes in the testosterone levels compared to group B. This is attributed to the presence of flavonoids, which has the potency of combating ROS activities caused by Alcohol. Also, Ekaluo et al. (43) showed a significant attenuated effect on testosterone following a significant increase

in sequel *Annona muricata* ingestion, which corresponds to the study findings. Reports from Alsenosy et al. (44), Anacletus et al. (45), Adeleye et al. (46) had similarities to the study results, revealing the attenuative effect of *Annona muricata* on testosterone following testicular dysfunction. The total sperm count result revealed a significant decline in the alcohol group only, which is attributed to ROS disruption of DNA sperm cells in the seminiferous and epididymal gland (11,47). Reports by Ricci et al. (47), Ekaluo et al. (43), Bai et al. (48), Boeri et al. (49), Jensen et al. (50), Sansone et al. (51) has accordance with the study findings, which revealed a significant decline in sperm count following alcohol ingestion. Further, the study revealed significantly higher levels of sperm count with respect to *Annona muricata* administration in the treated groups; which has correspondence to Eyeghre et al. (52), Alsenosy et al. (44), Anacletus et al. (45), Adeleye et al. (46), and Ekaluo et al. (43), which revealed significant higher sperm count following *Annona muricata* administration in other testicular model toxicity.

Further, the result obtained in the study showed a significant ($p<0.05$) decrease in active motility in-group B compared to A; groups C, D, and E had a significant ($p<0.05$) increase compared to group B. Further, the sluggish motility result showed a significant ($p<0.05$) increase in-group B compared to A; groups C, D, and E had a significant ($p<0.05$) decrease compared to group B. Alcohol interferes with sperm motility acting through ROS production leading to impairment of testicular function, causing apoptosis and DNA integrity dysfunction negatively through the interference of the testicular antioxidants (53,54). However, reports by Ogedengbe et al. (55), Enebeli et al. (56), Talabi et al. (57), Adaramoye and Arisekola (58), Sansone et al. (51), Bai et al. (48), Boeri et al. (49) corroborate the study findings, which showed decreased sperm motility. However, treatment with *Annona muricata* showed a significant increase in sperm motility, attributed to the flavonoids present. The effects of flavonoids help alter the deteriorated antioxidant system in the testicular tissue by reducing ROS production. The study findings have similarities to Alsenosy et al. (44), Anacletus et al. (45), Adeleye et al. (46), revealing an attenuated effect on sperm motility. The study results showed that the sperm morphology had a significant decline in alcohol-treated groups compared to the control; however, treatment sequel to *Annona muricata* had significantly higher levels of normal sperms and a significant decline in abnormal sperm cells shown. The significant decline in the morphology of the sperm is associated with disruption of the DNA integrity through excessive ROS production. The study has accordance with the report of Oremosu and Akang (40), Dosumu et al. (16,18), Talebi et al. (17), Anacletus et al. (45), Adeleye et al. (46), Donnelly et al. (59), and contradict the findings of Sermondade et al. (2010). Also, the study results corroborated the reports of Alsenosy et al. (44), Anacletus et al. (45), Adeleye et al. (46), following treatments with *Annona muricata* on testicular insults.

The study findings showed the gonado-somatic index of the testes result, which revealed a significant ($p<0.05$) increase in-group B compared to A; groups C, D, and E had a significant ($p<0.05$) decrease compared to group B. The relative testicular weight or gonado-somatic index weight is used as a diagnostic tool for the extent of the injury on testes structurally (60,61). The study showed that alcohol intoxication had a significantly higher relative testicular weight in the alcohol group compared to the control. The precise mechanism of action is not well understood but suggests ROS production, which triggers hypertrophy and necropsied seminiferous tubules. The study findings have a contradiction with Dosumu et al. (16), Osabuohien and Emokpae (62), Akbari et al. (63), who revealed a significant lowered testicular weight following alcohol consumption. Treatment with *Annona muricata* revealed a significant decline in the gonado-testicular index, attributed to the presence of flavonoids. Uno et al. (64) reported a non-significant change in testicular weight sequel to *Annona muricata* administration following caffeine toxicity, which disagrees with the study findings.

Oxidative stress is an imbalance between the oxidant and antioxidant system, which results in depletion of the antioxidant system resulting in ROS production, causing damage to the various organs and tissues (65). ROS production plays a vital role in the onset of male infertility because of the very high rate of mitosis in cells and mitochondrial oxygen consumption in testicular tissue that is accompanied by higher levels of unsaturated fatty acids than in other tissues (38). The study findings showed a significant increase in MDA levels in Alcohol treated group compared to the control. However, the significance shown in the MDA levels following alcohol ingestion is attributed to high ROS production, which affects DNA integrity. The study result aligned with Dosumu et al. (66) and Akbari et al. (63), revealing higher MDA activities following Alcohol ingestion. The report of Shukry et al. (67) had dissimilar to the study's finding, revealing that *Annona muricata* (Graviola) had a significant decline in MDA level. Also reported in the current

study is the antioxidant effect of CAT; It was shown that alcohol ingestion caused a significant decline in CAT levels compared to positive control. The mechanism attributed to CAT decline is ROS formation through oxidative stress pathways. The study has corroboration with Dosumu et al. (66), revealing increased CAT levels significantly following alcohol intoxication. Olakunle et al. (68), Shukry et al. (67) had similar findings to the study's report revealing a significant decline in CAT levels following *Annona muricata*.

The histoarchitectural report shows moderate spermatogenic arrest, with necrosis of spermatid cells and atrophy and increased diameter of seminiferous tubules in the alcohol-treated group compared to positive control. Alcohol, a well-known testicular toxicant, has a deleterious effect on the testes' spermatogenic cells and micromorphological features (16,69). Its' lethal effect is ROS production, which increases the activation of caspase-3 through the activation of survival kinases essential for cell growth (70). The study has similarities to Dosumu et al. (16) and Albadri et al. (54), revealing testicular lesions following alcohol ingestion. Although, treatment with *Annona muricata* extract showed an ameliorative effect on the testicular architecture showing moderate healing with active spermatid and spermatogonia and increased spermatogenesis in the treated groups. The physiology behind these activities of the extract is flavonoids, which has an antioxidant effect. Uno et al. (64) had similar findings to this study report, revealing *that Annona muricata* syrup had an ameliorative effect following caffeine intoxication. Also, findings from Oladipo et al. (71) had contradictory reports revealing testicular lesions following *Annona muricata* bark extract.

Conclusion

The study revealed testicular dysfunction resulting in infertility by alcohol toxicity; however, *Annona muricata* leave extract showed improved fertility parameters and improved CAT activities based on dose-dependent with no effect on MDA level.

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Conflict of Interest

There was no conflict among the authors.

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