EFFECT OF AQUEOUS EXTRACT OF *TETRAPLEURA TETRAPTERA* ON ALCOHOL INDUCED TESTICULAR INJURY IN MALE SPRAGUE-DAWLEY RAT

Introduction

Infertility affects around 17.5% of the global population, it has been generally reported that 8%–12% of couples are infertile, with approximately one in six people experiencing infertility in their lifetimes (WHO, 2023). Male infertility can have various causes, including genetic factors, physical abnormalities, injuries, the use of certain drugs or alcohol, infections of the genital tract, exposure to radiation, toxins, or reasons that are currently unexplained. (Uadia *et al.,* 2015).

Tetrapleura tetraptera is a flowering medicinal plant classified under the Leguminosae family. This deciduous tree is predominantly found in the western regions of Africa and is commonly known as the Aidan fruit in Yoruba Uhio in Igbo and Dawo in Hausa languages in Nigeria. It is readily available in countries such as Ghana and Nigeria. In Ghana, it is referred to as "Prekese" in the Twi language, (Uyoh *et al.,* 2013). "It is a popular seasoning spice in Southern and Eastern Nigeria (Odesanmi *et al.,* 2011). The plant is a robust, single-stemmed perennial tree characterized by dark green leaves, a thick woody base, and spreading branches. It is widely distributed across tropical Africa, particularly within the rainforests of West, Central, and East Africa. The fruit consists of a fleshy pulp containing small, brownish-black seeds, and is known for its fragrance, which is believed to have insect repellent properties (Ojewole *et al*., 2004). When unripe, the fruits are green, but they turn dark red-brown upon ripening. The fruits are approximately 22–27 cm long, with a pod 4–5 cm wide, and feature four longitudinalcv, fleshy ridges about 2 cm broad, two of which are hard and woody. *Tetrapleura tetraptera* is a tree species with green fruits that turn dark red-brown upon ripening. The fruits measure 22–27 cm long with pods about 4–5 cm wide, featuring four fleshy ridges. The tree can grow up to 30m tall, with a grey/brown bark that may be smooth or rough, and young branchlets that are hairless. Its bipinnate leaves have 5–7 pinnae with 6–11 pairs of small leaflets, which are either opposite or alternate. The tree's flowers are yellow or pink, forming racemes. Its reddish wood is hard and dense, used for firewood, building poles, and carvings. The tree is commonly found in lowland forests. (Adesina *et al*., 2016). Studies have reported the effects of *Tetrapleura tetraptera* pods on reproductive hormones. *Tetrapleura tetraptera* has been shown to decrease estrogen levels, exhibit aphrodisiac potential, and impact spermatogenesis (Agbai *et al.,* 2019; Adelakun *et al.,* 2021). The *Tetrapleura tetraptera* pods is rich in proteins, lipids, and minerals compared to popular spices such as onion and ginger (Adesina *et al.,* 2016). It also contains essential oils, fatty acids, carbohydrates, minerals, phytochemicals, and crude fiber (Tsala *et al.,* 2014; Adesina *et al.,* 2016). This fruit has various pharmacological benefits, including antimalarial, anticonvulsant, antibacterial, molluscicidal, antidepressant, analgesic, and anti-inflammatory properties (Tsala *et al.,* 2014; Adesina *et al.,* 2016). Research has shown that *Tetrapleura tetraptera* extracts have antioxidant and protective effects on enzymes involved in oxidative stress. They also contain a higher concentration of polyphenols such as eugenol, quercetin, tyrosol, and catechin (Irondi *et al.,* 2013; Atawodi *et al.,* 2014 and Moukette *et al*., 2015).

Alcohol consumption entails the ingestion of beverages containing ethanol, a psychoactive compound that profoundly impacts the central nervous system (CNS). Ethanol is derived from the fermentation of sugars by yeast and is commonly present in various alcoholic beverages, including beer, wine, and spirits. Upon consumption, alcohol is swiftly absorbed from the gastrointestinal tract, with peak blood-alcohol concentration (BAC) typically attained between 10 and 60 minutes after ingestion (Jones, 2023). The effects of alcohol consumption are influenced by several factors, including the quantity consumed, individual metabolic variations, genetic predispositions, and the presence of food in the stomach (Jones & Jonsson, 1994). For instance, consuming alcohol on an empty stomach can lead to a higher and quicker BAC compared to consuming alcohol with food, as food can decelerate gastric emptying and consequently delay absorption (Jones, 2020). Alcohol consumption significantly impacts reproductive hormonal regulation by disrupting the hypothalamic-pituitary-gonadal (HPG) axis, which is essential for the secretion of reproductive hormones such as testosterone. The HPG axis functions through a feedback loop where the hypothalamus releases gonadotropin-releasing hormone (GnRH), stimulating the adenohypophysis to produce luteinizing hormone (LH) and follicle-stimulating hormone (FSH**)** (Stamatiades *et al.,* 2018). Research shows that drinking alcohol can hinder the release of GnRH from the hypothalamus, leading to lower levels of LH and testosterone (McCann *et al.*, 1999). Long-term alcohol f has been linked to changes in Leydig cell structure and a decrease in their quantity, which affects testosterone production. Furthermore, alcohol boosts the activity of aromatase, an enzyme that turns testosterone into estradiol, causing higher estrogen levels and possible hormonal imbalances (Muthusami & Chinnaswamy 2005).

There is conflicting evidence regarding the effects of alcohol on FSH and LH levels. Some studies report increased levels of these hormones in heavy drinkers, while others indicate lower levels in alcoholic men This inconsistency may stem from variations in study designs, populations, and methods of classifying drinking habits (Maneesh *et al.,* 2006; Jensen *et al.,* 2014). *Tetrapleura tetraptera* pod has already been demonstrated by several researches to possess medicinal as well as pro-fertility properties. However, there are limited studies on its effects on alcohol induced testicular damage. Thus, this study was designed to investigate the ameliorative potential of aqueous extract of *tetrapleura tetraptera* on alcohol-induced testicular damage in male Sprague Dawley rat.

Material and Methods

Plants Materials

Dried *Tetrapleura Tetraptera* pods were purchased from the local market in Shomolu, Lagos, Nigeria. *Tetrapleura Tetraptera* pods were identified and authenticated at the University of Lagos herbarium (Voucher no-LUH100183).

Preparation of Plant material

Tetrapleura Tetraptera pods were washed well to remove foreign particles, air dried and pulverized into powdery form. Four hundred grams (400g), of the milled plant sample was later soaked in 2.8L of phosphate-buffered saline (PBS) for 72 hour at room temperature and filtered

with a cheesecloth and through a Whatman #1 filter paper. The filtrate was concentrated using a rotary evaporator at 42-47ºC to form a suspension and was administered orally using oral cannula (Adelakun *et al.,* 2021).

Alcohol

The alcohol used was 99.9% alcohol (woldoclean) obtained from a Boluke pharmacy in Mushin Lagos. To create a 30% v/v alcohol solution, 30 ml of alcohol was diluted in 70 ml of distilled water and it was given orally at the dose of 2 ml/kg thrice a week for 8 weeks (Oremosu & Akang, 2015).

Animals

Thirty (30) adult male Sprague-Dawley rats with average weight of 160g-200 were procured from the Animal house, College of Medicine of the University of Lagos, Nigeria. They were housed in the Anatomy Department animal room in well-ventilated plastic cages. They were acclimatized for two weeks to get them accustomed to the new environment. The rats were fed with standard chow and water was provided *ad libitum*. All procedures were carried out following National Academy of Science's Guide for care and Use of laboratory Animals (1996).

Acute toxicity studies

The extract's Oral acute toxicity (LD_{50}) was assessed following the up and down method of acute. Twelve adult Sprague Dawley rats weighing an average of 160g were used and placed in three (3) groups, each group having four (4) rats, each group received an oral dose of 250mg, 500mg, and 1000mg per kg (body weight) of extract respectively. Then monitored for 48 hours for signs of toxicity and mortality. Another dose of 1000, 2500 and 5000mg/kg of the extract was administered and the animals were monitored for toxicity and mortality then the LD_{50} was determined.

Experimental Design and sample collection

Thirty adult male Sprague Dawley rats weighing an average of 160g-200g, the extract was orally administered daily for fifty-six days. Dosage of administration was adapted with modification from Adelakun *et al.,* (2021) and Oremosu & Akang, (2015). The assigned experimental groups are:

Group A: received 1ml/kg b. wt of *Tetrapleura Tetraptera.*

Group B: received 2 ml/kg b. wt Alcohol+1ml/kg/b.wt of *Tetrapleura Tetraptera*

Group C received 2 ml/kg b. wt Alcohol + 0.5ml/kg/b.wt of *Tetrapleura Tetraptera.*

Group D: received 2 ml/kg b. wt Alcohol+ 0.25ml/kg/b.wt of *Tetrapleura Tetraptera.*

Group E: received 2 ml/kg b. wt Alcohol only thrice in a week

Group F: served as a control group and received distilled water

All investigation were conducted 24 hours after the administration of the final dose of treatment. Sperm parameter investigation was conducted first. Blood was obtained through ocular puncture and collected in EDTA bottles then centrifuged at 3500 rpm for 10 minutes and the serum was obtained for testosterone, Follicle stimulating hormone (FSH) and Luteinizing hormone (LH) estimation using Elabscience Rat enzyme-linked immunosorbent assay (ELISA) kit Microwells and the procedure were as presented by the Kit manufacturer.

The left testis were homogenized and the homogenate was centrifuged to obtain the supranatant to assay for tissue malondialdehyde (MDA), Superoxide dimutases (SOD), glutathione (GSH) and catalase (CAT). MDA was determined by measuring the thiobituric acid reactive substance produced as described by Varshney and Kale (1990), The level of SOD was determined as previously described by Sun *et al*., (1998), CAT level was determined as previously described by Sinha *et al.,* (1971) and GSH was determined previously described by Rukkumani *et al.,* (2004). The right testis were standard histological procedure and were processed into the slide and viewed under the microscope for histological observation and interpretation.

Statistical analysis

The results were analysed using GraphPad Prism 9 software. Data was reported as mean ± standard deviation. Differences between means and the main effects of treatment groups were determined by one-way analysis of variance (ANOVA), and multiple comparisons were conducted using LSD post-hoc tests. The mean difference is significant at the 0.05 level ($p < 0.05$).

RESULTS

General Observation

Three animals were reported dead in the study during the administration of the *Tetrapleura tetraptera* and alcohol in the 7th week of administration in Group A, Group C and Group D.

Body weight

There was a significant increase ($P<0.05$) in the body weight of all groups when comparing between pre-administration and post-administration (Table 1).

Table 1: Effect of Tetrapleura tetraptera pods co-administered with alcohol on body weight in male Sprague Dawley rats

GROUPS	PRE-ADMINISTRATION BODY WEIGHT(g)	POST-ADMINISTRATION BODY WEIGHT(g)	% Differences in body weight
GROUP A	**150.4±10.46**	**179.5±8.89** $^{\alpha}$	**16.2**
GROUP B	**160.2±12.36**	**192.8±11.47** $^{\alpha}$	**16.9**
GROUP C	**153.0±16.05**	**186.0±17.99** $^{\alpha}$	**18.9**
GROUP D	**143.0±15.25**	**178.2±11.73** $^{\alpha}$	**18.0**
GROUP E	**131.2±12.13**	**161.4±4.39** $^{\alpha}$	**18.7**

GROUP F	**132.4±9.71**	**160.6±5.51** $^{\alpha}$	**16.5**

TESTICULAR WEIGHT ANALYSIS

There were no significant changes between groups (table 2)

Table 2: Effect of Tetrapleura tetraptera pod co-administered with alcohol on testicular weight in male Sprague Dawley rats

GROUPS	**TESTICULAR WEIGHT(g)**
GROUP A	1.16±0.17
GROUP B	1.21±0.32
GROUP C	1.33±0.13
GROUP D	1.25±0.13
GROUP E	1.14±0.12
GROUP F	1.25±0.05

Table 3: Effects of *Tetrapleura tetrapleura* administration with Alcohol on the sperm indices in adult male Sprague-Dawley rats

GROUPS	**MOTILITY(%)**	**COUNTS($\times10^6$/ml)**	**MORPHOLOGY(%)**
GROUP A	94.25±1.71	110.6±3.89	0.50±0.58
GROUP B	85.25±1.71$^{¥\beta}$	85.96±5.44$^{\beta\alpha}$	3.00±0.82 $^{\beta}$
GROUP C	75.75±3.09$^{¥\beta}$	73.75±7.78$^{¥\beta}$	4.50±1.29 $^{\beta}$
GROUP D	57.40±2.88$^{¥\beta\alpha}$	57.00±5.05$^{¥\beta\alpha}$	9.00±1.58 $^{\alpha}$
GROUP E	31.20±3.63$^{¥\alpha}$	32.35±3.59$^{¥\alpha}$	13.40±1.82 $^{¥\alpha}$
GROUP F	83.00±5.19	94.00±6.46	1.00±1.00

Table 4: The effects of *Tetrapleura tetraptera* and alcohol on the reproductive hormones of adult male Sprague Dawley rats.

GROUPS	**FSH (ng/ml)**	**LH (ng/ml)**	**TST (ng/ml)**
GROUP A	1.69±0.01	0.63±0.01	4.93±0.16
GROUP B	1.21±0.05$^{¥\beta}$	0.57±0.03$^{\beta}$	3.02±0.03$^{¥\beta\alpha}$
GROUP C	1.11±0.09$^{¥\beta\alpha}$	0.57±0.01$^{¥\beta\alpha}$	2.91±0.96$^{¥\beta\alpha}$
GROUP D	0.74±0.36$^{\alpha}$	0.53±0.02$^{¥\alpha}$	2.75±0.05$^{¥\beta\alpha}$
GROUP E	0.43±0.02$^{¥\alpha}$	0.47±0.02$^{¥\alpha}$	2.51±0.04$^{¥\alpha}$
GROUP F	1.61±0.03	0.61±0.00	4.74±0.17

Table 5: The effects of *Tetrapleura tetraptera* and alcohol on the oxidative stress markers of adult male Sprague Dawley rats

GROUPS	GSH(µ/mg)	MDA(µ/mg)	CAT(µ/mg)	SOD(µ/mg)
GROUP A	1.23±0.04	1.02±0.02 $^{\alpha\beta}$	5.51±0.24	0.97±0.12 $^{\beta}$
GROUP B	1.19±0.08 $^{\alpha}$	0.94±0.04 $^{\alpha\beta}$	5.56±0.17	1.04±0.17
GROUP C	1.17±0.08	0.86±0.15 $^{\beta}$	5.78±0.54	0.97±0.22 $^{\beta}$
GROUP D	1.15±0.64 $^{\alpha}$	0.96±0.07 $^{\alpha\beta}$	5.43±0.41 $^{\alpha}$	0.89±0.18 $^{\beta}$
GROUP E	0.96±0.20	2.04±0.23 $^{\alpha}$	4.92±0.75	1.48±0.15
GROUP F	1.39±0.12	1.39±0.05	6.12±0.51	0.95±0.07 $^{\beta}$

Values are expressed as mean ± Standard Deviation (SD). $^{\alpha}$ P<0.05 Significant when compared to control (Group F); β P<0.05 Significant when compared to Alcohol only (Group E); $^{¥}$P<0.05 Significant when compared to *Tetrapleura tetraptera* only (Group A). Group A=*Tetrapleura tetraptera* only; Group B= *Tetrapleura tetraptera* High dose + Alcohol; Group C= *Tetrapleura tetraptera* Medium dose + Alcohol; Group D= *Tetrapleura tetraptera* Low dose + Alcohol; Group E= Alcohol only; Group F= Control

Histological observations

Plate 1: Photomicrograph of the testes control rat showing the normal testes with typical seminiferous tubule (ST), lumen (L) with basement membrane (black arrow) and interstitial space (blue arrow). It also shows tubules lined by spermatogenic series cells (spermatogonia (SP) spermatocyte (ST) and containing numerous luminal spermatozoa. No abnormalities are seen. (H&E stain) x100

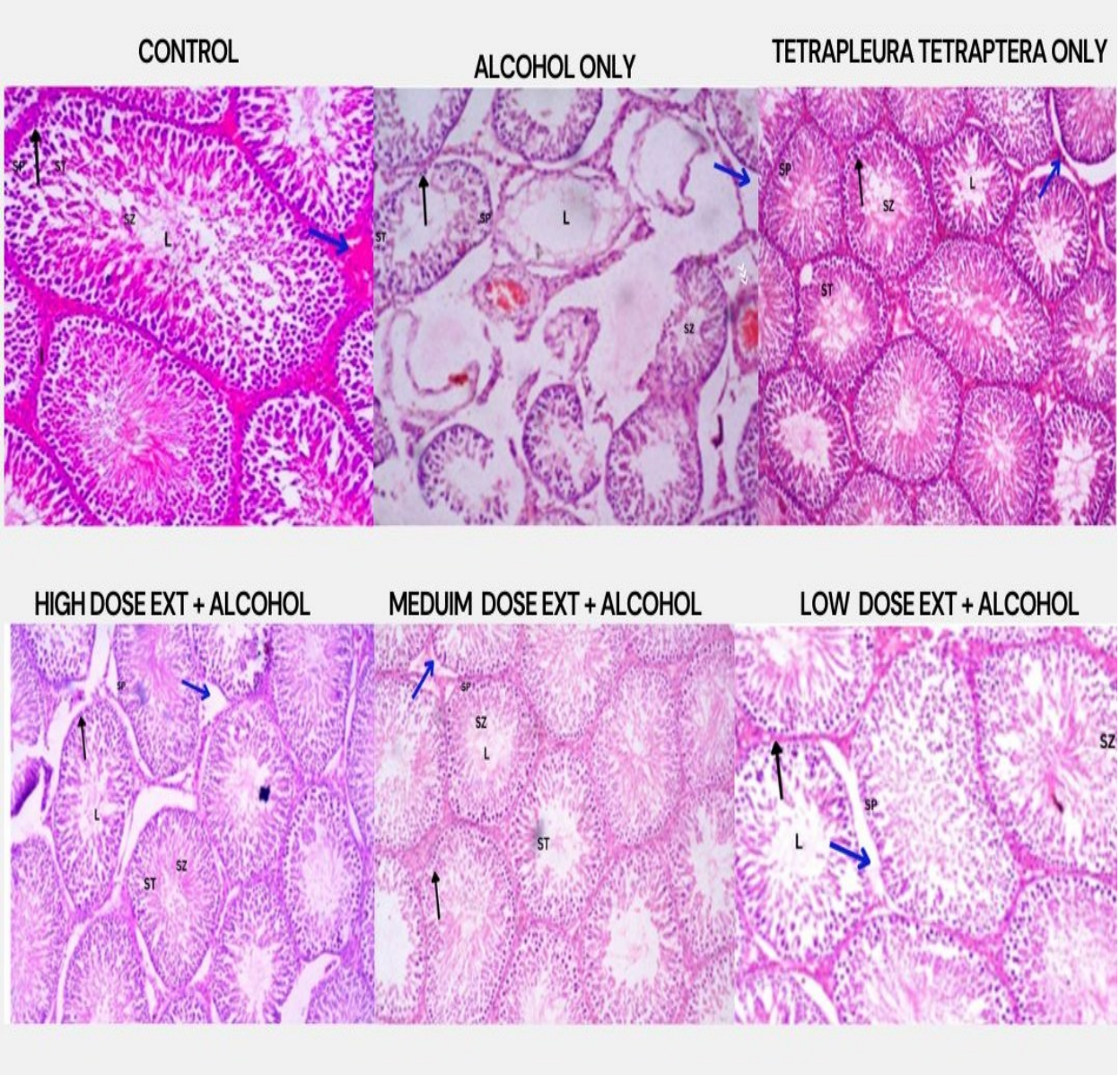
Plate 2: Photomicrograph of the testes of ethanol-only rat showing ghost outlines of some tubules with necrotic cells, degenerated interstitial space and also containing numerous distorted luminal spermatozoa and dilated blood vessels, and aggregates of red blood cells congestion of the intertubular blood vessel (repeated arrow head) are also seen. lumen (L) with basement membrane (black arrow) and interstitial space (blue arrow). (H&E stain) x100

Plate 3: Photomicrograph of the testes for *Tetrapleura Tetraptera* only rat showing the normal testes with typical seminiferous tubule (ST), lumen (L) with basement membrane (black arrow) and interstitial space (blue arrow). It also shows tubules lined by spermatogenic series cells (spermatogonia (SP) spermatocyte (ST) and containing numerous luminal spermatozoa (SZ). No abnormalities are seen. (H&E stain) x100

Plate 4: Photomicrograph of the testes for *Tetrapleura Tetraptera* high dose co-administered with alcohols rat showing the normal testes with typical seminiferous tubule (ST), lumen (L) with basement membrane (black arrow) and interstitial space (blue arrow). It also shows tubules lined by spermatogenic series cells (spermatogonia (SP) spermatocyte (ST) and containing numerous luminal spermatozoa (SZ). No abnormalities are seen. (H&E stain) x100

Plate 5: Photomicrograph of the testes for *Tetrapleura Tetraptera* medium dose co-administered with alcohol rat showing the normal testes with typical seminiferous tubule (S), lumen (L) with basement membrane (black arrow) and interstitial space (blue arrow). It also shows tubules lined by spermatogenic series cells (spermatogonia (SP) spermatocyte (ST) and containing numerous luminal spermatozoa (SZ). No abnormalities are seen. (H&E stain) x100

Plate 6: Photomicrograph of the testes for *Tetrapleura Tetraptera* low dose co-administered with alcohols rat showing the normal testes with typical seminiferous tubule (S), lumen (L) with basement membrane (black arrow) and interstitial space (blue arrow). It also shows tubules lined by spermatogenic series cells (spermatogonia (SP) spermatocyte (ST) and containing numerous luminal spermatozoa (SZ). No abnormalities are seen. (H&E stain) x100



Discussion

For thousands of years, people have used aromatic and medicinal plants, particularly those having ethnopharmacological applications, as a natural source of cures and medical treatment (Ansari *et al.,* 2023). It is important to understand the effects of those plants on the anatomical and physiological status of the reproductive system. Therefore, this study was conducted to investigate the enhancing fertility effect of *Tetrapleura tetraptera* in alcohol-treated male Sprague-Dawley rats. *Tetrapleura tetraptera* has been investigated and found to be effective in the treatment of several form of diseases (Adeshina *et al.,* 2016) and even as an aphrodisiac (Adienbo & Krukru, 2021).

Histological examination was done to evaluate the histopathological changes in the treatment groups. The histological section of the normal control group showed normal testicular histo-architecture, revealing the basement membrane, lumen of the seminiferous tubules, interstitial space and the germinal epithelium of the Leydig cells. Alcohol treated group showed ghost outlines of some tubules with necrotic cells and also containing numerous distorted luminal spermatozoa, dilated blood vessels, and aggregates of red blood cells congestion of the intertubular blood vessel which correlates with the reduction in testicular weight, testosterone level and increased MDA level. In addition, the enlarged and degenerated interstitial space would lead to loss of spermatogenic cells due to the low testosterone levels which was observed in table 4. It was observed that *Tetrapleura tetraptera* ameliorated the deleterious effect of alcohol in the testis because the group that received *Tetrapleura tetraptera* showed no histopathological alteration. This also agree with previous studies Akang *et al.,* (2011); Oremosu *et al.,* (2013)

This study shows a significant increase in post-administration body weight compared to each group's pre-administration body weight. The group treated with *tetrapleura tetraptera* increased in weight, contrary to the findings of Adelakun *et al*., (2021). The group treated with alcohol only increased significantly which is in agreement with the finding of Maclnnis *et al*., (2014), who reported that heavy intake of alcohol has been more consistently associated with weight gain.

The organ weight depends on the mass of differentiated spermatogenic cells, and its structural and functional integrity requires the adequate biosynthesis of male sex hormones. Therefore, increase in testicular weight in *Tetrapleura tetraptera* treated rats reveals increased spermatogenesis and steroidogenesis function. Also, increase in testicular weight can be linked to higher sperm production (Ekaluo, *et al.,* 2015; Adelakun *et al*., 2021). This study establishes that the effect of *tetrapleura tetraptera* on the testicular weight is dose-dependent and there is no significant difference between rat treated with high dose and extract only (Table 3). However, there was a reduction in the testicular weight of the rat treated with alcohol only, This weight reduction can be correlated to the histological changes in plate 2 where there is degeneration of interstitial space, distorted seminiferous tubules and necrotic cells which was also in agreement with the findings of Dosunmu *et al*., (2014) which clarified the possibility of using *Tetrapleura tetraptera* aqueous extract to mitigate the effects of alcoholic testicular injury in males Sprague-Dawley rat.

According to this study, rats that were given alcohol alone had significantly lower sperm motility than rats that were given *Tetrapleura tetraptera* alone (Table 3). This indicates that the spermatozoa's structural integrity was depleted, which was avoided in all groups by treating them

with *Tetrapleura tetraptera.* Comparing the rats treated with *Tetrapleura tetraptera* at high, medium, and low doses to the alcohol-only group revealed a significant increase ($P<0.05$), indicating that *Tetrapleura tetraptera* was able to reverse sperm alcohol deletion. This was likely due to the fact that *Tetrapleura tetraptera* contains antioxidant vitamins A, C, and E (Adelakun *et al.,* 2021), which shield the sperm from lipid peroxidation caused by alcohol. The rats treated with alcohol only showed a significant reduction in sperm count when compared to the control group and the *Tetrapleura tetraptera* only groups. *Tetrapleura tetraptera* increased the number of sperm, and its high dosage levels demonstrated its impressive testosterone levels, which drive spermatogenic cells to mature and the accessory gland to provide its best secretory activity.

As with oligospermia, the group that received alcohol treatment only displayed a modest increase in aberrant sperm morphology, which was indicative of the structural breakdown of the sperm head brought on by lipid peroxidation. However, the harmful effects of alcohol could be reversed by *Tetrapleura tetraptera*. The study's findings agree with those of Dosunmu *et al.,* (2014), Ekaluo *et al.,* (2015), and Akang *et al.,* (2011).

According to the study's hormonal measurement, the alcohol group's testosterone level was considerably lower than that of the *Tetrapleura tetraptera* alone group. *Tetrapleura tetraptera,* however, was able to counteract the effects of alcohol damage on the hormonal level, especially in the group that received a high dose of the treatment. FSH and LH levels decreased in the alcohol only group. There were significant differences between treatment groups and control and alcohol-only groups. This also suggests that low level of testosterone does not lead to an increase which agrees with the work of Akang *et al*., (2011); Dosunmu *et al*., (2014) and Oremosu, & Akang, (2014)

Testes are more susceptible to oxidative stress due to their increased membrane lipid content (Al-Shaikh *et al*., 2013). Decreasing testicular MDA and increasing testicular SOD, GSH, and CAT in a dose-dependent way, the *Tetrapleura tetraptera* bioactive component in this study lowers the membrane lipid content of the testes. lipid buildup in non-adipose tissues may cause cell death and malfunction (Schaffer, 2003). Based on this investigation, we can thus hypothesize that *Tetrapleura teraptera* pods can efficiently scavenge reactive oxygen species ROS and prevent the development of oxidative damage in the testis, which can be caused by any toxin. Lipid peroxidation can be reduced by *tetrapleura tetraptera* that can scavenge free radicals (Erukainure *et al.,* 2017) MDA levels were significantly reduced in all *Tetrapleura tetraptera* treatment groups when compared to alcohol only group (Table 4). According to studies, ethanol reduces GSH levels by blocking the mitochondrial glutathione transporter and producing oxidants. Following ethanol consumption, the mitochondrial pool of GSH is reduced when the transport of GSH from the cytosol into the mitochondria is inhibited (Colell *et al.,* 1998 and Yurkiv *et al.,* 2018) An increase of GSH was observed between the groups and this increase were dose-dependent. There was also increase in testicular SOD and CAT levels between the groups.

There was an observed increase in seminiferous diameter and gonado-somatic index between the *Tetrapleura tetraptera*-treated groups when compared to alcohol alcohol-only group but not significant.

Conclusion

This study demonstrates the protective benefit aqueous benefits of *Tetrapleura tetraptera* had on the testicular and spermatic parameters in enhancing sex performance. This study revealed further that higher doses of *Tetrapleura tetraptera* (1ml/kg/b.w.) is more effective in ameliorating testicular and sperm parameters which can be attributed to the presence of more antioxidants such as vitamin C and E which helps in protecting testicular tissues.

REFERENCES

Adebayo A. S., Gbadamosi I. A., Adewunmi C. O. (2000). Formulation of antimicrobial dried powdered herbs in soap bases. In Phytomedicine in Malaria and Sexually Transmitted Diseases: Challenges for the New Millennium (Eds. Adewunmi C. O., Adesina S. K.), Obafemi Awolowo University, Ile – Ife; 97.

Adeshina G. O., Ojo O. A., & Oduola T. (2020). Effects of *Tetrapleura tetraptera* on reproductive parameters in male rats. African Journal of Biomedical Research , 23(3), 245–252.

Adienbo, O. M., & Krukru, E. E. (2021). Hydromethanol Extract Of *Tetrapleura Tetraptera* Fruit Enhances Sexual Behaviour But Impairs Fertility In Male Wistar Rats. *European Journal of Advanced Research in Biological and Life Sciences*, *9*(1).

Akang, E., Oremosu, A., Dosumu, O., & Ejiwunmi, A. (2011). *Telfairia Occidentalis*, a Prophylactic Medicine for Alcohol's Damaging Effect on the Testis. *Macedonian Journal of Medical Sciences*, *4*(4).

Atawodi, S. E. O., Yakubu, O. E., Liman, M. L., & Iliemene, D. U. (2014). Effect of methanolic extract of *Tetrapleura tetraptera* (Schum and Thonn) Taub leaves on hyperglycemia and indices of diabetic complications in alloxan–induced diabetic rats. Asian Pacific Journal of Tropical Biomedicine, 4(4), 272-278.

Ayala A., Muñoz M. F., & Argüelles S. (2014). Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxidative Medicine and Cellular Longevity, 2014(1), 360438.

Colell, A., García-Ruiz, C., Miranda, M., Ardite, E., Marí, M., Morales, A., ... & Fernández-Checa, J. C. (1998). Selective glutathione depletion of mitochondria by ethanol sensitizes hepatocytes to tumor necrosis factor. *Gastroenterology*, *115*(6), 1541-1551.

Dosumu, O. O., Osinubi, A. A. A., & Duru, F. I. O. (2014). Alcohol induced testicular damage: Can abstinence equal recovery?. Middle East Fertility Society Journal, 19(3), 221-228.

Ekaluo, U. B., Ikpeme, E. V., Etta, S. E., & Ekpo, P. B. (2015). Effect of aqueous extract of tigernut (Cyperus esculentus L.) on sperm parameters and testosterone level of male albino rats. *Asian Journal of Biotechnology*, *7*(1), 39-45.

Erukainure, O. L., Onifade, O. F., Odjobo, B. O., Olasehinde, T. A., Adesioye, T. A., Tugbobo-Amisu, A. O., & Okonrokwo, G. I. (2017). Ethanol extract of *Tetrapleura tetraptera* fruit peels: Chemical characterization, and antioxidant potentials against free radicals and lipid peroxidation in hepatic tissues. *Journal of Taibah University for Science*, *11*(6), 861-867.

Irondi, A. E., Anokam, K. K., Chukwuma, P. C., Akintunde, J. K., & Nurain, I. O. (2013). Variation in nutrients composition of *Tetrapleura tetraptera* fruit at two maturity stages.

Jones, A. W. (2020). Pharmacokinetics of Ethanol: A Primer for Forensic Practitioners. *Alcohol, Drugs, and Impaired Driving*, 275-346.

Jones, A. W., & Jönsson, K. Å. (1994). Food-induced lowering of blood-ethanol profiles and increased rate of elimination immediately after a meal. *Journal of forensic sciences*, *39*(4), 1084-1093.

Jensen T. K., Swan S., Jørgensen N., Toppari J., Redmon B., Punab M., Drobnis E. Z., Haugen T. B., Zilaitiene B., Sparks A. E. 2014). Alcohol and male reproductive health: A cross-sectional study of 8344 healthy men from Europe and the USA. Human Reproduction, 29(8), 1801–1810. https://doi.org/10.1093/humrep/deu118

Maneesh, M., Dutta, S., Chakrabarti, A., & Vasudevan, D. M. (2006). Alcohol abuse-duration dependent decrease in plasma testosterone and antioxidants in males. Indian journal of physiology and pharmacology, 50(3), 291.

McCann S. M., Mastronardi C., Walczewska A., Karanth S., Rettori V., & Yu W. H. (1999). The role of nitric oxide in reproduction. Brazilian Journal of Medical and Biological Research , 32(11), 1367–1379.

McGovern P. E., Zhang J., Tang J., Zhang Z., Hall G. R., Moreau R. A., ... & Wang C. (2004). Fermented beverages of pre-and proto-historic China. Proceedings of the National Academy of Sciences , 101(51), 17593–17598.

Moukette, B. M., Pieme, A. C., Biapa, P. C. N., Njimou, J. R., Stoller, M., Bravi, M., & Yonkeu Ngogang, J. (2015). In vitro ion chelating, antioxidative mechanism of extracts from fruits and barks of *Tetrapleura tetraptera* and their protective effects against fenton mediated toxicity of metal ions on liver homogenates. Evidence-Based Complementary and Alternative Medicine, 2015(1), 423689.

Muthusami K. R., & Chinnaswamy P. (2005). Effect of chronic alcoholism on male fertility hormones and semen quality. Fertility and Sterility , 84, 919–924.

Oremosu A. A., & Akang E. N. (2015). Impact of alcohol on male reproductive hormones, oxidative stress, and semen parameters in Sprague–Dawley rats. Middle East Fertility Society Journal , 20(2), 114–118.

Oyeyemi, M. O., & Fayomi, A. P. (2011). Gonadosomatic index and spermatozoa morphological characteristics of male wistar rats treated with graded concentration of Aloe vera gel. *Int J Anim Vet Adv*, *3*(2), 47-53.

Rukkumani, R., Aruna, K., Varma, P. S., Rajasekaran, K. N., & Menon, V. P. (2004). Comparative effects of curcumin and an analog of curcumin on alcohol and PUFA induced oxidative stress. *J Pharm Pharm Sci*, *7*(2), 274-283.

Sinha, A. K. (1972). Colorimetric assay of catalase. *Analytical biochemistry*, *47*(2), 389-394.

Sun, Y. I., Oberley, L. W., & Li, Y. (1988). A simple method for clinical assay of superoxide dismutase. *Clinical chemistry*, *34*(3), 497-500.

Tsala, D. E., Habtemariam, S., Simplice, F. H., Thierry, B. N. M., Abraham, J. A., & Theophile, D. (2014). Topically applied *Tetrapleura tetraptera* stem-bark extract promotes healing of excision and incision wounds in rats. Journal of intercultural ethnopharmacology, 3(2), 63.

Uadia, P. O., & Emokpae, A. M. (2015). Male infertility in Nigeria: A neglected reproductive health issue requiring attention. Journal of Basic and Clinical Reproductive Sciences, 4(2), 45-53.

Ujihara I., Hitomi S., One K., Kakinoki Y., Hashimoto H., Ueta Y., & Inegana K. (2015). The ethanol metabolite acetaldehyde induces water and salt intake via two distinct pathways in the central nervous system of rats. Neuropharmacology, 99, 589–599. https://doi.org/10.1016/j.neuropharm.2015.08.001

World Health Organization. (2023). Infertility prevalence estimates, 1990–2021. World Health Organization.

Yurkiv, M., Kurylenko, O., Vasylyshyn, R., Dmytruk, K., Fickers, P., & Sibirny, A. (2018). Gene of the transcriptional activator MET4 is involved in regulation of glutathione biosynthesis in the methylotrophic yeast Ogataea (Hansenula) polymorpha. *FEMS yeast research*, *18*(2), foy004.