RESEARCH ARTICLE

ISSN: 0331 - 670X

https://doi.org/10.51412/psnnjp.2023.25

The Nigerian Journal of PHARMACY
AS MEN OF HONOUR WE JOIN HANDS
THE OFFICIAL ORGAN OF THE PHARMACEUTICAL SOCIETY OF NIGERIA FOUNDED 1927

Alcohol-Induced Reproductive Toxicity in Male Rats: Protective Effects of Psidium guajava (Myrtacaea) Ethanol Leaf Extract

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ARTICLE INFO

Article history:

Received 28 April 2023
Revised 18 June 2023
Accepted 30 June 2023
Online Published 30 September 2023

Keywords:

Psidium guajava,
chronic alcohol consumption,
male factor infertility,
male reproductive parameters.

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ABSTRACT

Background: Alcohol is a known male reproductive toxicant, while *Psidum quajava* is a potent male fertility enhancer with antioxidant properties. This study aimed at determining the possible ameliorative effect of the ethanolic leaf extract of *P. guajava* against alcohol-induced repro-toxicity in male rats.

Methods: The study was an *in-vivo* designed investigation wherein group 1 animals served as the negative control group and received 2 mL of distilled water following ethanol administration. Animals in groups 2, 3, and 4 received 50, 100, and 200 mg/kg of ethanol leaf extract (ELE) of *P. guajava* respectively. Group 5 animals served as the positive control and were administered 50 mg/kg Vitamin C. All administrations were par oral and lasted for 21 days. Animals were sacrificed after an overnight fast and blood collected from animals was analyzed for serum concentration of testosterone, follicle stimulating hormone (FSH) and luteinizing hormone (LH). Equally, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) antioxidant enzyme as well as malondialdehyde (MDA) serum levels were determined. Sperm analysis and histological evaluation were performed on the semen and testes respectively. GC-MS analysis of the extract was carried out to identify compounds present in it.

Results There was a dose dependent and significant ($p<0.001$) increase in serum levels of testosterone and FSH in 100 mg/kg treated rats. Serum levels of SOD and GPx were significantly ($p<0.0001$) increased while a decrease in levels of MDA was observed in the 200 mg/kg extract treated group. A dose independent increase in normal sperm forms and progressive motility respectively were recorded in all extract treated and positive control groups. Animals in treatment groups had leydig cell regeneration and normal sequential maturation. Sixty-seven compounds were identified in the extract with β - caryophyllene having the highest relative abundance (18.67%).

Conclusion: Ethanolic leaf extract of *P. guajava* has potential ameliorative effects against ethanol-induced repro-toxicity in male rats.

1. Introduction

Infertility, defined as the inability to achieve conception after one year of unprotected sexual intercourse is the cause of substantial psychological and social distress in concerned patients[1,2]. It imposes considerable economic burden on patients and health care systems[3] and affects 8 – 12% of couples worldwide with male factor infertility (MFI) responsible for 40 -50% of all reported cases[4]. Majorly, (MFI) can be due to low sperm production, abnormal sperm function and/or blockages that prevent the delivery of sperm. Numerous stressors, including medical ignorance, improper and uncontrolled medication usage, metabolic problems, vitamin and mineral deficiencies are identified risk factors for MFI. Also, the avoidable effects of industrial pollution, addiction diseases, injuries, illnesses, chronic health problems and unhealthy lifestyle choices are contributors to MFI[5]. Of particular importance is the fact that substance use can predispose to MFI; alcohol being one

of the most recognized substances of abuse that can cause infertility in males[6]. Alcohol causes the increased production of reactive oxygen species, while also inducing a reduction in the levels of antioxidants present in sperm cells. This leads to oxidative stress induced sperm cell damage and invariably infertility[7]. In the pituitary gland, alcohol decreases the production, release, and/or activity of FSH and LH; both of which are needed for proper male reproductive function[8]. Sadly, chronic alcohol consumption by men is a common social vice that cannot be completely eradicated, though it has grave negative consequence on reproductive parameters and fertility. According to the World Health Organization[9], alcohol consumption represents the third largest risk factor for disease burden in high income countries with smoking and hypertension standing in first and second place.

Over the years, medicinal plants and their constituents have been used in the management of sub fertility in males; especially that linked to oxidative stress[10]. *Psidium guajava* commonly known as "guava" is a known tropical plant abundantly grown for its fruits. Local names of the plant include; Yoruba – 'guaba', 'gilofa', Hausa – 'goba', and Igbo – 'ugwoba' [11]. It is an aromatic tree which is native to tropical areas of America, Mexico to Brazil and cultivated widely in hot climates zones of Africa and Asia[12]. It is reported to have antimicrobial, anti-diarrheal, anti-inflammatory, wound healing, anti-hyperglycemic, hypotensive and anti-cancer activities[12-18]. Of note is its reported male fertility enhancing and beneficial antioxidant properties[19]. Hence this study aimed at assessing the possible ameliorative effects of the leaf extract of the plant on alcohol-induced oxidative stress on reproductive parameters and in-vivo antioxidant enzyme levels in male rats. The study also sought to identify compounds present in the extract using Gas Chromatography- Mass -Spectroscopy (GC-MS). The study will add to the body of knowledge that already exist about the activity of *P. guajava* in the management of MFI.

2. Materials and Methods

2.1 Materials

Ethanol (Pharmatrend, Benin city), vitamin C (Emzor pharmaceuticals, Lagos), enzyme linked immunosorbent assay (Elisa) kits for testosterone, FSH and LH (Elab Science, China), Ransod® assay kit (Randox Laboratory, UK), GPx and MDA assay kits (Abcam Laboratory, UK).

2.2 Methods

2.2.1 Plant Collection and Identification

The leaves of *Psidium guajava* (guava) were obtained from cultivated species around Ugbowo area of Benin City, Edo State Nigeria, geolocated at latitude 6.339°N and longitude 5.617°E. Collected plant was identified and authenticated by Dr Henry Akinnibosun of the herbarium unit, Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria. The herbarium number (UBH-P378) was assigned to the plant specimen and the prepared voucher sample was deposited at the herbarium unit for future reference.

2.2.2 Plant Preparation and Extraction

The fresh leaves were dried under shade for four days pulverized using an attrition mill. A weighed amount (1.80 kg) of the powdered plant material was extracted in 2.5 L of ethanol with the aid of the Soxhlet apparatus at 60^{o}C for 12 hours. The extract obtained was concentrated *in vacuo* with the rotary evaporator and the dried extract (220.30 g) was preserved in glass jars at 4^{o}C until needed.

2.2.3 Experimental animals

Thirty 12 weeks old male albino rats (210±6.5 g) were used for this study. They were obtained from and housed in the Animal house of the Department of Pharmacology, Faculty of Pharmacy, University of Benin throughout the study period in clean plastic cages with wood shavings as bedding. They were allowed to acclimatize for one week prior to the study, allowed access to clean drinking water *ad libitum* and fed pelletized animal feed (Premier Feeds, Ibadan). Ethical care and handling of experimental animals was always observed according to guidelines of the National Institute of Health Public Health Policy on the Human use and care of laboratory animals[20,] and the study was approved by the University of Benin, Faculty of Pharmacy Ethical Committee vide reference number EC/FP/022/18.

2.2.4 Experimental Procedure

The protocol according to Ofeimun et al[21] with a slight modification was adopted for the study. The animals were randomly divided into 5 groups of 6 animals each. Animals in all the groups received an oral dose of 2 mL ethanol (20% v/v). Animals in group 1 which served as the negative control group further received 2 mL of distilled water following administration of ethanol. Animals in groups 2, 3, and 4 received 50 mg/kg, 100 mg/kg, and 200 mg/kg received ELE of *P. guajava* leaf respectively. Animals in group 5 served as the positive control and were

administered 50 mg/kg of ascorbic acid. All administration was done by means of oral gavage daily for 21 days and the body weight of animals in each group was determined every 7 days.

2.2.5 Specimen collection

At the end of the treatment period, the rats were fasted overnight, anesthetized in a chloroform saturated chamber, sacrificed, and dissected. Blood was collected through the inferior vena cava into plain bottles, centrifuged at 3000 rpm for 10 minutes to obtain the serum as the supernatant which was transferred into plain bottles. The testes were surgically removed and placed in sterile tissue bottles containing Bouin's solution for histopathological evaluation. The caudal epididymis was also surgically extracted for the sperm analysis.

2.2.6 Hormonal assay

The collected serum samples were used to analyze for levels of testosterone, Follicle Stimulating hormone (FSH) and Luteinizing hormone (LH) using commercial Enzyme Linked Immuno-Sorbent Assay (ELISA®) kits following manufacturer's instructions.

2.2.7 Antioxidant Enzyme assay

The *in-vivo* antioxidant enzyme activity as measured by serum level of SOD, GPx and MDA were determined using appropriate commercial assay kits and following manufacturer's instructions (Randox Laboratory, UK), while the activity of CAT was determined by spectrophotometric method as described by Hedwan[22].

2.2.8 Semen analysis

2.2.8.1 Sperm morphology evaluation

The procedure of Seed et al[23] was adopted for this evaluation. A suspension of sperm was obtained from the epididymis following incision. This was placed on a slide and stained with eosin from which smears were made and air dried to make it permanent. The slides were examined under the light microscope at a magnification of x 400 and the percentage of normal and abnormal sperm was calculated.

2.2.8.2 Sperm count evaluation

The sperm count was determined using the Neubauer improved haemocytometer. Epidermal fluid ratio of 1:20 was prepared by adding 0.1 mL of fluid to 0.9 ml of normal saline. After mixing the dilution thoroughly and scoring both sides of the counting chamber, spermatozoa within five of the red blood cells squares including those lying across the outermost lines at the top and right sides was counted, while those at the bottom and left-hand sides were left out[24].

2.2.8.3 Sperm motility evaluation

The right caudal epididymis of the rats was incised and a drop of the epidermal fluid was delivered onto a glass slide which was covered quickly to prevent drying up. Slide was examined under a light microscope at the highest magnification using different fields of view. Sperm cells were classified as progressively motile, non-progressively motile or immotile. The relative percentage of motile sperm was estimated and recorded to the nearest 5% using subjective determination of motility[23].

2.2.9 Histological Evaluation

The effect of the extract on the histology of the testes was determined according to the method of Drury and Wellington[25]. Briefly, harvested testes from animals were preserved in Buoin's fluid and thereafter fixed in 4% paraformaldehyde in 0.1 M phosphate buffer solution. Tissue was dehydrated with upgraded ethanol, cleared with xylene and finally embedded in paraffin. Sectioning was done by use of a microtone (5 mm thickness) and counter-stained with hematoxylin and eosin (H and E). Sections obtained were examined under a microscope at X100 and photographed.

2.2.10 GC-MS Analysis of Extract

Gas -Chromatography/Mass- Spectroscopy analysis ELE of *P. guajava* was carried out using Shimadzu QP2010 with specification listed as follows: injection temperature: 250.00°C, Column oven temperature: 60.0°C, Column flow: 1.03ml/min, Pressure: 59.7kPa, Linear velocity: 37.0 cm/sec, sampling time: 1.0 min, injection mode: split less, purge flow: 3.0ml/min, injection volume: 8.00 and total flow rate of 24.6ml/min. The mass peak was subsequently generated at a scan speed of 2500 and the mass to charge ratio of the peaks were obtained within range 50.00-700.00amu. Compounds showing the best percentage similarity index were tentatively denoted as the queried compound. Furthermore, the peak obtained were analyzed by direct comparison of the m/z and retention time to the compounds present on the National Institute of Standards and Technology (NIST) library. The relative abundance of identified compounds was obtained as shown in Equation 1 below.

Relative abundance = $\frac{a}{b} \times 100$

Equation 1:

Where:

a = height of identified compound in sample

b = Total Height of identified compounds

2.2.12 Statistical analysis

The results obtained are expressed as Mean ±Standard Error of Mean (SEM) and analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's comparison test. Analysis was done using GraphPad Prism version 8.0.2. Data were considered statistically significant at $P<0.05$

1. Result

3.1 Effect of extract on hormonal profile

Compared to the negative control group, testosterone levels in serum of animals in the different treatment groups were considerably enhanced by the extract. Increases seen in animals administered 100 mg/kg and 200 mg/kg doses respectively were statistically significant. Although there was increase in testosterone level seen in the standard (vitamin C) and 50 mg/kg extract groups, these were not statistically significant compared to the negative control group. Equally, ELE of *P. guajava* dose dependently, significantly increased the serum level of FSH, compared to the negative control group. The standard (vitamin C) also effected an increase in serum level of FSH, which was non-significant. The extract at studied doses significantly increased the serum level of luteinizing hormone in a dose dependent manner when compared to the negative control group. The standard (vitamin C) also demonstrated a significant increase in luteinizing hormone levels, although this increase was not as significant as those of the extract groups (Figure 1a – 1c).

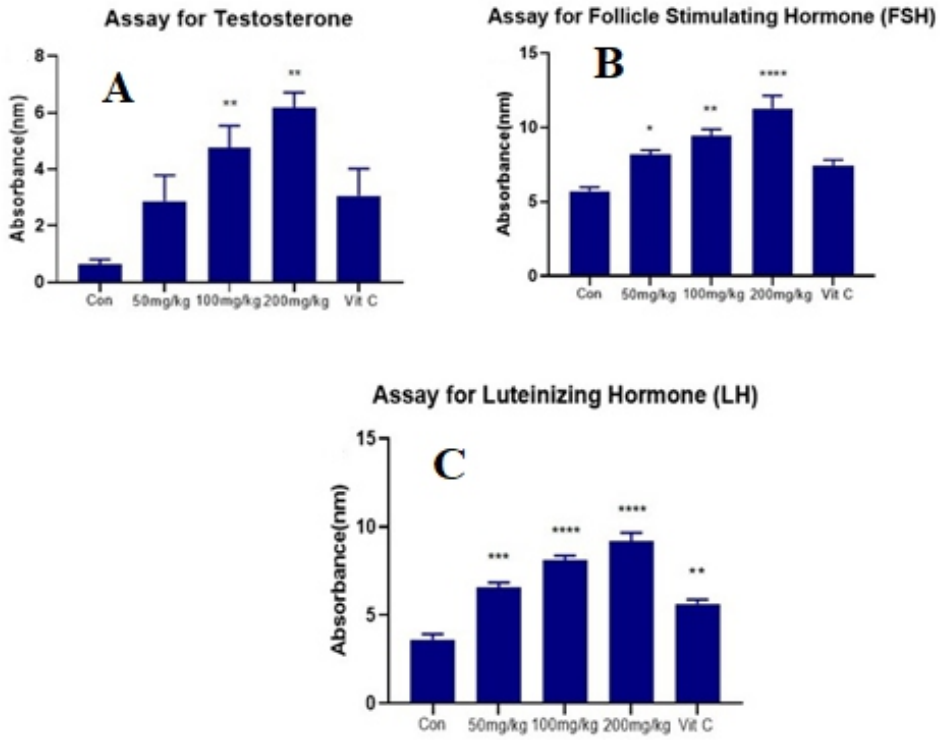

Fig 1A-C: Effect of ELE of *P. guajava* on serum levels of (A)Testosterone (B) FSH (C) LH (*p<0.05, **p<0.01, ***p<0.001 ****p<0.0001, Con = Negative control)

3.2 Effect of extract on serum antioxidant enzymes profile

At studied doses, the extract significantly ($p<0.05$; $p<0.001$; $p<0.0001$) increased the serum SOD levels dose dependently compared to the negative control group. The standard (vitamin C) equally evoked an increase in serum level of SOD, although this was not statistically significant ($p<0.05$). Compared to the negative control group, the ELE of *P. guajava* at doses of 100 and 200 mg/kg significantly ($p< 0.05$; 0.005) increased the serum level of catalase. The standard drug and the 50 mg/kg dose equally caused similar increases which were not significant. The GPx serum level was significantly increased in treated animals with the highest increase observed in animals treated with 200 mg/kg. The standard drug equally caused an increase in the serum levels of GPx in animals in the positive control group which was not superior to that observed in the extract treated groups. The extract at various doses caused a significant ($p<0.05$; 0.01; 0.000) dose-dependent decrease in the malondialdehyde serum levels when compared to the negative control group. The standard was also observed to cause a significant ($p<0.05$) decrease in malondialdehyde levels (Figure 2a -2d).

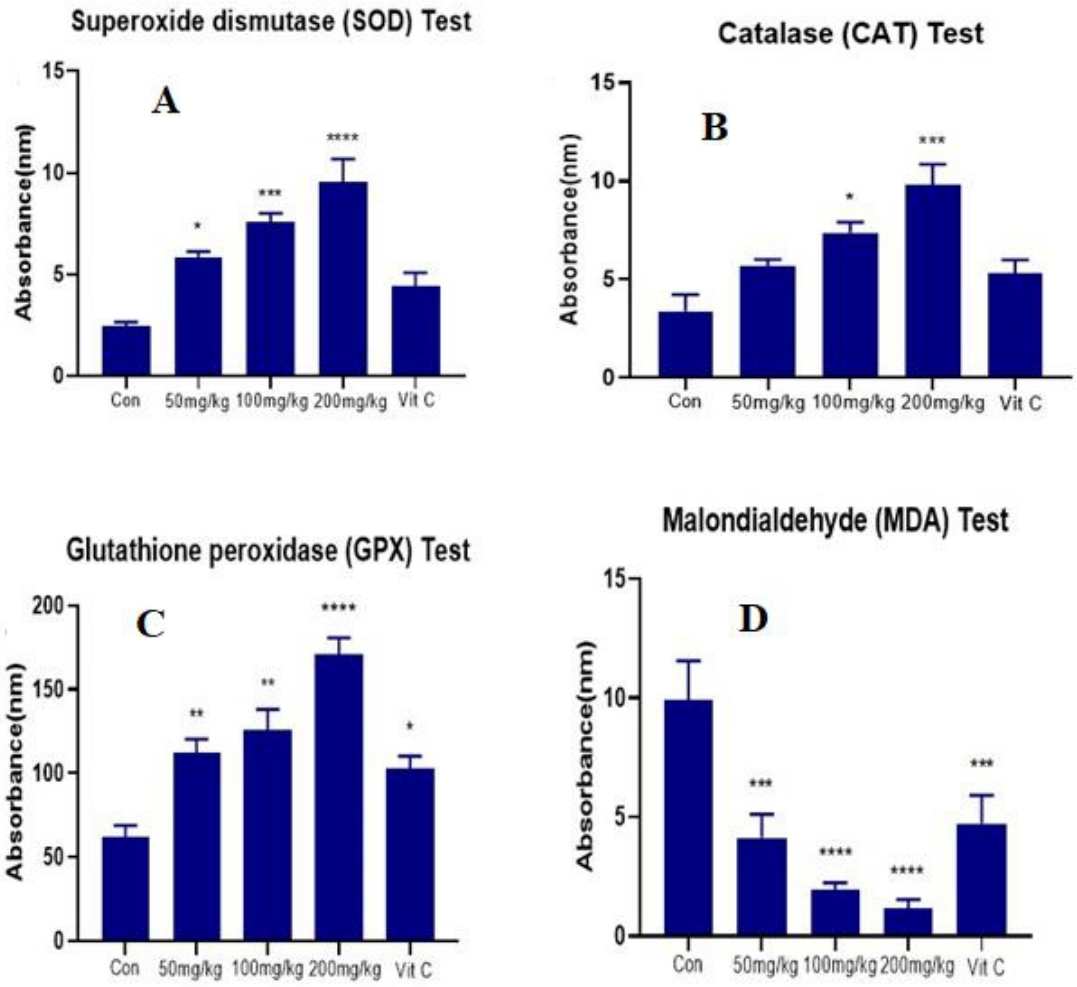

Fig 2A-D: Effect of ELE of *P. guajava* on serum levels of (A) sodium dismutase (B)catalase (C) glutathione peroxidase (D) Malondialdehyde *($p<0.05$, **$p< 0.01$, ***$p<0.001$, ****$p<0.0001$, Con = negative control)

3.3 Effect of extract on sperm parameters.

The extract at the studied doses of 50 and 200 mg/kg respectively, significantly increased normal forms (NF) sperm when compared to the negative control group. The standard (vitamin C) also demonstrated a significant increase in sperm normal forms (NF). The extract at the dose of 100mg/kg did not show any significant change in sperm morphology when compared to the negative control group. Also, no significant difference in sperm count was observed between the extract treated groups and the negative control group Table 1. The standard, and the extract at 50 and 200 mg/kg respectively, significantly increased the percentage of progressive motile sperm compared to the negative control group as shown in Figure 3. The standard (vitamin C) and 50 mg/kg extract equally caused a significant decrease in the percentage of non-progressive motile sperm. Overall, there was no significant change in the percentage of immotile sperm across the different treatment groups compared to the negative control.

Table 1: Effect of ELE of *P. guajava* on sperm morphology and count

Group	Abnormal forms	Normal forms	Sperm count
Negative control	28.33 ± 1.67	71.67 ± 1.67	$21.10\pm6.48x10^6$
50 mg/kg extract	6.67 ± 1.67***	93.33 ± 1.67***	$23.50\pm1.29x10^6$
100 mg/kg extract	26.67 ± 1.67	73.33 ± 1.67	$24.40\pm7.37x10^6$
200 mg/kg extract	16.67 ± 3.33**	83.33 ± 1.33**	$27.10\pm6.11x10^6$
Vitamin C	11.67 ± 1.67***	88.33 ± 1.67***	$25.80\pm5.30x10^6$

Results are expressed as Mean ± S.E.M (**p<0.01, ***p<0.001) where abnormal form represent percentage of abnormal sperm cells and normal form signify percentage of normal sperm cells.

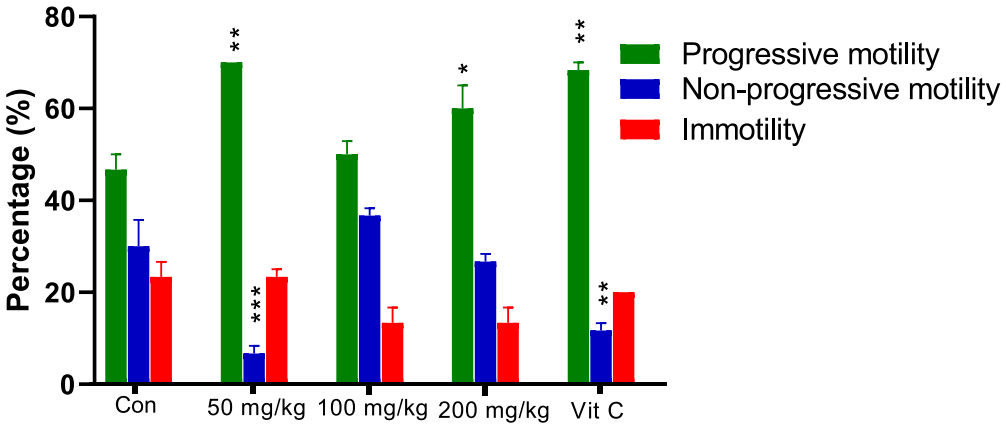

Fig 3: Effect of ELE of *P. guajava* on sperm motility (*p<0.05, **p<0.01, ***p<0.001, Con = negative control).

3.4 Effect of extract on histopathology of testes

Figure 4a-4e reveals the effect of the extract and the standard drug on the histoarchitecture of the testes in animals compared to the negative control. Testes of animals in the negative control group revealed leydig cell degeneration, spermatogenic maturation arrest, and sertoli cell degeneration. Animals in treatment group of dose 50 mg/kg and 100 mg/kg showed leydig cell regeneration, normal sequential maturation and active interstitial congestion. While animals in treatment group of 200 mg/kg and standard drug group although showed normal sequential maturation also showed maturation arrest.

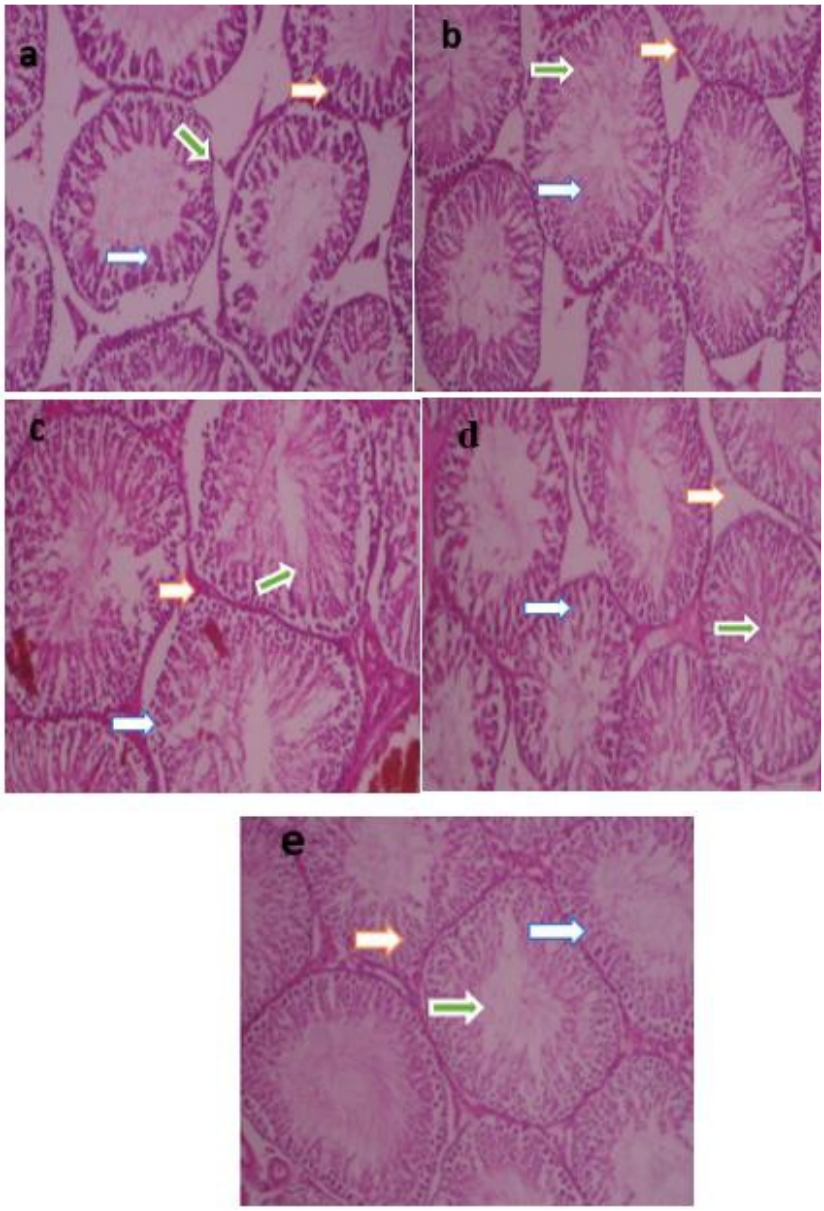

Figure 4a – e**:** Photomicrograph of histology of testes of rats treated with alcohol only (a); 50 mg/kg(b) 100 mg/kg (c); 200 mg/kg (d) and Vitamin C (e). Arrow pointing to interstitial spaces (cells) ⟹ ; Arrow pointing to leydig cells ⇨ ; Arrow pointing to Sertoli cells ➡

3.4 GC-MS analysis result of ELE of *P. guajava*

Several secondary metabolites belonging to different class of compounds were identified in the ELE of *P. guajava*, chief of which was β – caryophyllene with the highest relative abundance. Others were Bicyclo [5.3.0]decane, 2-methylene-5-(1-methyl-vinyl)-8-methyl and Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-

dimethyl-2-(1-methylethenyl)-, [2R-(2.alpha.,4a). In all, sixty-seven compounds were identified in the extract.

Table 2: GC-MS analysis result of ELE of *P. quajava*

S/No	Name of compound	RT (Mins)	Peak Height	Relative Abundance (%)
1	o-Cymene	5.37	131266	0.30
2	Cyclobutane, 1,2-bis(1-methylethenyl)-trans-	5.42	103828	0.24
3	Eucalyptol	5.46	355560	0.82
4	α.-Terpineol	7.72	140048	0.32
5	Phenol, 2-methoxy-3-(2-propenyl	9.97	292824	0.68
6	Cedrene	10.09	195399	0.45
7	α.- Copaene	10.15	780671	4.13
8	9-Eicosene (E)-	10.22	127535	0.30
9	1-Octadecanesulphonyl chloride	10.32	112952	0.26
10	α-Caryophyllene	10.55	252081	0.58
11	Cyclohexene, 4-ethenyl-4-methyl-3-(1-methylethenyl)-1-(1-methylethyl)-, (3R-trans)	10.65	141558	0.33
12	β - Caryophyllene	10.76	8045233	18.67
13	Bicyclo[7.2.0]undec-4-ene,4,11,11-trimethy l-8-methylene	10.86	275129	0.64
14	β - curcumene	11.03	344397	0.80
15	1R,3Z,9s-4,11,11-Trimethyl-8-methylenebicyclo[7,2,0] undec-3-ene	11.13	198201	0.46
16	1,5,9,9-Tetramethyl-1,4,7-cycloundecatriene	11.23	1126576	2.61
17	1H-Benzocycloheptene, 2,4a,5,6,7,8-hexahydro-3,5,5,9-tetramethyl-(R)	11.32	476184	1.10
18	Iso - Caryophyllene	11.41	809632	1.88
19	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	11.46	1361256	3.16
20	Andrographolide	11.61	925173	2.15
21	Bicyclo [5.3.0]decane, 2-methylene-5-(1-methyl-vinyl)-8-methyl	11.66	3460334	8.03
22	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2.alpha.,4a.alpha	11.74	3056963	7.09
23	β -Bisabolene	11.79	1929729	4.48
24	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl	11.95	1206914	2.80
25	Trans-calamenene	12.03	1407903	3.27
26	Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-	12.17	460760	1.07
27	Cadala-1(10),3,8-triene	12.30	236926	0.55
28	Cyclohexane, 1,5-dimethyl-2,3-divinyl-	12.51	697795	1.62
29	Naphthalene, 1,4,6-trimethyl-	12.67	198696	0.46
30	Propanoic acid, 3-(3-hydroxybicyclo [2.2.1] hept	12.73	174272	0.40
31	Caryophyllenyl alcohol	12.78	230579	0.53
32	Caryophyllene oxide	12.85	707880	1.64
33	9- β-Acetoxy-3,5,8-trimethyltricyclo [6.3.1.0	13.04	172239	0.40
34	Viridiflorol	13.13	225191	0.52
35	Epiglobulol	13.21	230203	0.53
36	(7S)-trans-syn-cis-Tricyclo [7.3.0.0(2,6)]dodecan	13.28	165611	0.38
37	Isoaromadendrene epoxide	13.34	452303	1.05
38	Isocaryophyllene	13.53	1037609	2.41
39	Selina-6-en-4-ol	13.78	868153	2.01
40	β - bisabolol	13.81	859375	1.99

41	Tricyclo [7.2.0.0(2,6)]undecan-5-ol, 2,6,10,10-tetramethyl	13.90	467454	1.08
42	2,2,6,7-Tetramethyl-10-oxatricyclo [4.3.1.0(1,6)	13.91	501081	1.16
43	α -Bisabolol	14.03	260349	0.60
44	1-Tetradecene	14.29	141742	0.33
45	6-epi-shyobunol	15.28	104492	0.24
46	Phytol – acetate	15.49	991846	2.30
47	Ergostane-3,5,6,12,25-pentol- 25-acetate (3. Beta	15.61	233576	0.54
48	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	15.75	200254	0.46
49	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	15.95	358404	0.83
50	4,4,8-Trimethyltricyclo [6.3.1.0(1,5)] dodecane-2	16.39	157381	0.60
51	Pentadecanoic acid	16.90	504800	1.17
52	Hexadecanoic acid, ethyl ester	17.11	956273	2.22
53	Phytol	18.28	436381	1.01
54	Fumaric acid - tetradec-3-enyl tridecyl ester	18.61	137547	0.32
55	2,7-Dioxatricyclo [4.4.0.0(3,8)] decan-4-one-oxime	18.65	120958	0.28
56	n-Propyl 9,12-octadecadienoate	18.72	262969	0.61
57	Dichloroacetic acid, tridec-2-ynyl ester	18.78	438266	1.02
58	Nonadecanoic acid, ethyl ester	19.01	329297	0.76
59	Pyridine, 1,2,3,6-tetrahydro-1-benzyl-4-phenyl-	19.68	126074	0.29
60	Silane, diphenyldecyloxy (1,1,1-trifluoroprop-2-yloxy)-	21.85	157011	0.36
61	1H-Benzoimidazole, 1-(2-phenoxyethyl)-2-phenyl-	21.91	157235	0.36
62	2-(Ethylenedioxy) ethylamine,N-methyl-N-[4- (1-pyrrolidinyl)-2-butynyl]-	21.96	102550	0.24
63	Diisooctyl phthalate	22.13	162182	0.38
64	2-(4-Fluorophenyl)-5-methoxy-2H-benzo [g]indazole	22.25	145639	0.34
65	Phenyl-(5-phenyl-cyclopent-1-enyl)-methanone	22.42	151050	0.35
66	(R-(R*,R*))-4-(1,5-Dimethylhexyl)-1-cyclohexenecarboxylic acid	22.48	118234	0.27
67	2-Propen-1-one, 1-(2,6-dihydroxy-4-Methoxyphenyl-3-phenyl-(E)-	22.76	301503	0.70
			43099486	100.00

RT= Retention time

4. Discussion

Alcohol is a recognized male reproductive toxin; acute and chronic use of which can impair how the hypothalamus, the anterior pituitary, and the testes' function, leading to impotence, infertility, and a decline in the secondary male sexual traits[7]. The effect of alcohol induced male toxicity follows a pattern of increased intracellular oxidative stress hence the use of vitamin C – a known antioxidant molecule in this study as a standard.

Male puberty, fertility, and sexual function depend on optimal production and secretion of androgens. The leydig cells release high quantities of intratesticular testosterone, which is required for spermatogenesis[25]. Chronic alcohol consumption results in production of ROS that damage the leydig cells hence interfering with the production of intratesticular testosterone. Result from this study showed the ability of the extract to reverse/reduce the effect of alcohol consumption on serum testosterone level. This finding agrees with report from a similar study with virgin coconut oil [26].

Furthermore, findings from this study are indicative of the positive effects of *P. guajava* on the pituitary-testicular axis, as there was an increase in serum levels FSH and LH. Both hormones are the prime regulators of germ cell development[25]. Studies consistent with the present research show that rats given *P. guajava* leaf extracts had higher

levels of the reproductive hormones; testosterone, LH, and FSH, as well as testicular and epididymal weight, motility, and epididymal sperm concentration[19].

Generally, infertile men have higher levels of serum reactive oxygen species (ROS) than do fertile men[27]. An imbalance in antioxidant to ROS with elevated levels of serum ROS overwhelming the antioxidants are cytotoxic leading to oxidative stress that can cause sperm dysfunction, sperm DNA damage and reduced male reproductive potential. Enzymes known to mop up these free radicals to retain cell integrity include GPx, SOD, and CAT. The significant increase in the serum levels of these enzymes in the extract treated and positive control groups is suggestive of the potential of the extract to counter alcohol induced ROS damage in male rats. *Ocimum canum* have been shown to have similar protective effect by increasing SOD, CAT and GPx levels in alcohol-induced oxidative stress in experimental male rats[28]. One of the final products of polyunsaturated fatty acid peroxidation in the cells is MDA. Overproduction of MDA due to chronic alcohol consumption results in an increase in reactive oxygen species[29]. The significant reduction in serum MDA levels observed in the extract and vitamin C treated groups suggest a potential in reducing lipid peroxidation and cell damage as measured by MDA.

Oxidative damage to sperm cells can alter sperm morphology, motility and count [30]. The significant increase in the percentage of sperms cells with normal form and decrease in abnormal form reveals further reveals the protective and positive effects of the extract and vitamin C on sperm damage due alcohol consumption. This summation is corroborated by the positive effect of the extract and Vitamin C on sperm progressive motility in the extract and vitamin C treated groups compared to the negative control group.

The bulk of each testis histologically consists of seminiferous tubules embedded in relatively sparse interstitial tissue. The tubules are lined by a complex epithelium which is most easily understood as consisting of two very different cell populations, Sertoli cells and germ cells[33,34]. Results from the histopathological evaluation shows that animals in the standard and extract treated groups showed preservation of the integrity of the testis by regeneration of Leydig cell, and normal sequential maturation compared to the negative control group that revealed Leydig and Sertoli cell degeneration leading to spermatogenic maturation arrest. This could be associated with the ability of the extract to reduce the ROS levels in the testes resulting in better histological manifestations. The studied doses showed ameliorative effects similar to the standard group with the optimum dose and best effect of integrity offered at 100 mg/kg.

Gas Chromatography-Mass Spectroscopy (GCMS) is a powerful, quick and convenient analytical tool for the quantitative and qualitative analysis of organic samples such as the extract used in this study[35]. The system outputs a quantitative representation of the chemicals presents in a sample. In the current study, sixty-seven constituents were identified in the ELE of *P. guajava*, with β-caryophyllene ($C_{15}H_{24}$) being the most abundant. β-caryophyllene is natural bicyclic sesquiterpene found in essential oils of various plants, spices and food, including *Syzigium aromaticum.* It often occurs in a mixture with iso-caryophyllene and α-caryophyllene as seen in this study [36]. It is a potent antioxidant compound with confirmed immune-modulatory and anti-inflammatory activities[37]. Andrographolide, another compound identified in the ELE of *P. quajava* is a diterpene lactone linked with antioxidant, anti-inflammatory, immune-modulatory and anti-tumor activities. It is the major compound found in *Andrographis paniculata* - a plant with a long history of use and diverse activities[38]. Phytol is an acyclic diterpene alcohol with potent antioxidant, antimicrobial, anxiolytic, cytotoxic, metabolism-modulating, autophagy and immune modulating, antinociceptive and anti-inflammatory activities[39]. It is a component of chlorophyll present in vitamin E, K and other tocopherols[40]. It was identified as a component of ELE of *P. quajava* in combination with its derivative; phytol acetate in this study. Medicinal plants in general owe their activities to the compounds they contain. It can thus be assumed that the ameliorative effects of ELE *P. quajava* in alcohol induced repro-toxicity male rats is due to the presence of some or all of the compounds identified in the extract, in isolation or in combination, as some of the identified compounds have been shown to possess the observed effects. Finding from this suggest a need for further research to isolate and characterize compound/s that may be responsible for observed activity.

5. Conclusion

The ethanolic leaf extract of *P. guajava* showed a significant ameliorative effect against alcohol-induced reproductive toxicity in male rats. The ethanol extract of the leaf of *P. guajava* showed a positive impact on the in vivo antioxidant effect as shown with improved anti-oxidative enzyme levels. Hormonal parameters and sperm motility were also improved in the alcohol-induced reproductive toxicity following the administration of the ethanol extract

of *P. guajava*. Histopathological evaluation showed normal sequential maturation and Leydig cell regeneration. Sixty-seven constituents were identified from the GC-MS analysis of the ethanol extract of *P. guajava*, with the most abundant of the constituents being caryophyllene.

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