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Lemongrass diet improves petroleum fume-induced oxidative stress, spermatotoxicity and testicular histomorphological disorders in rats

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Abstract
Emerging evidence suggests that the consumption plant based foods and food products could be beneficial in terms of ameliorating adverse environmental exposures. The aim of this study was to evaluate the protective effect of Lemongrass leaf extracts (LGLEs) against petroleum fume (PF)-induced oxidative stress (OS) and reproductive dysfunction in male rats. Thirty-five mature male Wistar Albino rats were segregated into 5 groups, (n=7per group). Animals in group 1 served as unexposed control and were given standard feed and water for 6 weeks. Animals in group 2 received standard feed, water and were exposed to PF in an exposure chamber for 6 weeks. While animals in groups 3, 4 and 5 besides receiving standard feed, water and exposed to PF for 4 weeks were concomitantly fed 269.8mg/kg, 539.6mg/kg and 809.4mg/kg respectively of *LGLEs* for additional 2 weeks. After the exposure and treatment periods animals were sacrificed. Blood samples and testicular and epididymal tissues were obtained for analysis and histopathological examination respectively. There were significant (P<0.05) decreases in serum levels of CAT, testosterone, LH and FSH and significant (P<0.05) increase in MDA in animals exposed to PF alone compared to levels in the unexposed control. Histomorphological changes observed in the testicular and epididymal structures of animals exposed to PF alone included degenerative changes in seminiferous tubules, depleted germ cells, congested blood vessels and low luminal sperm volume. Supplementation with different concentrations of LGLEs caused dose dependent changes in the biochemical and histomorphological indices of male reproductive dysfunction. *LGLE* has a dose-dependent protective effect against PF-induced OS and male reproductive disorders due to its varied bio-constituents and their activities.

Keywords: Hydrocarbon, male reproductive disorders, lemongrass, protection, antioxidant

1. Introduction
The incidence of male reproductive system dysfunction leading to infertility is common and evidences abound in the literature [1]. Among etiologic factors, exposure to workplace chemicals/solvents have been shown to impair both male [2, 3] and female [4] fertility. The extent of reproductive dysfunction could range from histo-morphological distortion to bio-chemical alterations and clinical manifestations.

Cases of solvent-induced low sex hormone levels, low sperm count and quality, low sex drive, erectile dysfunction and low sperm quality have been reported in male workers exposed to workplace chemicals. Likewise, solvent-induced menstrual disorders, altered sex hormone profile, decreased fertility, reduced birth weight, intrauterine growth retardation, embryonic malformation have been reported in female exposed workers [5].

Worst still, information on the potential for negative reproductive health outcomes from many workplace exposures is scanty. Petroleum retailing/dispensing is one such occupation that has recently experienced influx of young people of both genders, and in particular those of reproductive age. Petroleum constituents especially benzene, toluene, ethylbenzene and xylene (BTEX compounds) are known to cause injury to reproductive organs including the ovaries and testis, hence are referred to as reproductive toxicants/endocrine disrupters [6].

Most human studies are limited to changes in biochemical abnormalities because it is difficult to perform histomorphological evaluation in human. Also, a recent study found that plant molecules can regulate ovarian cell function and attenuate the toxic effect of petroleum constituents on these cells [7]. Given these, this study was aimed to assess the effect of Lemongrass leaf extracts (LGLEs) on PF-induced biochemical and testicular histomorphological distortions in male rats.

2. Materials and Methods

Lemongrass leaves (LGLs) were obtained from the medicinal farm of the Department of Pharmacognosy and Natural Medicine Faculty of Pharmacy University of Uyo in the month of March, 2016 a day prior to utilization. The leaves were identified and authenticated by a taxonomist and sample was preserved in the herbarium unit of the Department of Botany, University of Uyo. The specimen was assigned a voucher number UUPH 334. Thereafter, the matured fresh *LGL*s were sliced into smaller sizes and pulverized into powdery form with a manual blender. Extraction using ethanol as the solvent was performed by macerating 2kg of the powdered extract into 95% ethanol for 72hrs. The solution was then filtered through a Whatman No 2 filter paper and the filtrate was concentrated to dryness at 45 ^{o}C in water bath. The final solid extract was weighed with an electronic weighing scale (Mettler Toledo) model AG204. The total yield of 28% was obtained. The extract was stored in glass bottles in a refrigerator at temperature of 4 ^{o}C and was dissolved in physiological saline at concentration of 100mg/ml for use.

2.1 Phytochemical screening of *LGL* ethanolic extracts

Phytochemical (qualitative and quantitative) screening of the leaf extracts was carried out using standard procedures to test for the following phyto-constituents; alkaloids, flavonoids, phenols, tannins, terpenes, saponins, anthraquinones, reducing sugars, cardiac glycosides and others as described by Trease and Evans [8].

2.2 Dose determination

The experimental doses of the extracts were calculated from the median Lethal dose (LD_{50}). LD_{50} was estimated with 40 Albino rats using Lorke's methods [9]. LD_{50} was calculated as the square root of the maximum dose producing 0% death and the minimum dose producing 100% death.

$$LD50\sqrt{AB}$$

Where A = maximum dose producing 0% death.
B = minimum dose producing 100% death.

$$LD_{50} = \sqrt{AB} = \sqrt{2600 \text{ X } 2800} = 2698.10 mg/kg$$

From the median lethal dose, minimum, medium and maximum doses of 1/10, 2/10 and 3/10 respectively were calculated.

2.3 Experimental Animals

Thirty five mature Wistar Albino rats weighing between 200 and 250g were obtained from the animal house of the Department of Pharmacology, Faculty of Pharmacy, University of Uyo, Nigeria. They were kept in well ventilated cages for 7 days to acclimatize. They were allowed access to food and water *ad libitum*. All animals were fed rat chow (Vital Feeds, Grand Cereal Ltd, Jos).

2.4 Segregation of Animals

The animals were randomly divided into 5 groups (n=7 per group).
Group 1 served as unexposed control and was orally gavaged 2ml of normal saline for 6wks.
Group 2 was exposed to PFs alone for 6wks.
Group 3 was exposed to PFs for 4 weeks and orally gavaged 269.8mg/kg of *LGLEs* for the next 2 wks.
Group 4 was exposed to PFs for 4 wks and orally gavaged 539.6mg/kg of *LGLEs* for the next 2wks.
Group 5 was exposed to PFs and orally gavaged 809.4mg/kg of *LGLEs*.
All animals in groups 3 to 5 were exposed to PFs in the exposure chambers (60 x 80 x 100cm^3) for 4 weeks and orally gavaged *LGLEs* in their respective doses for the next 2 weeks after exposure, whereas, animals in group 2 were exposed to PF alone for 6wks.

2.5 Collection of experimental samples for analysis

After 2 weeks of *LGLEs* administration, the animals were weighed and anaesthesized with chloroform soaked in a swab of cotton wool in a desiccator. Blood sample was collected by cardiac puncture and emptied into labeled specimen bottles, for biochemical evaluation including determination of follicle stimulating hormone (FSH), Luteinizing hormone (LH) activities, testosterone level, catalase (CAT) and malondialdehyde (MDA) levels.
Animals were sacrificed by cervical dislocation and reproductive organ (testis) was carefully removed and fixed in a suitably treated formalin reagent and thereafter, subjected to normal routine histological procedures/examination.

3. Biochemical Analysis

3.1 Estimation of CAT and MDA activities

CAT activity was determined by Titrimetric method. Tissue lipid peroxidation was quantified by estimating the plasma concentration of MDA using thiobarbituric acid reactive substance (TBARS) method and measured spectrophotometrically at 532nm. Serum testosterone, LH and FSH levels were determined by Enzyme-Linked Immuno-sorbent assay (ELISA) as described by Tietz [10].

3.2 Histopathological Tissue Processing

The fixed tissues were dehydrated in different grades of alcohol as follows; two changes of 70% and 95% alcohol for a period of 2hrs each, two changes of 100% also known as absolute alcohol for a period of 2hrs. Dehydrated tissues were cleared using xylene. Tissues were impregnated with two changes of paraffin wax in the oven at the temperature of 60 ^{o}C for 1hr 30mins) each to ensure they were fully embedded. Tissues were transferred from the final wax bath to moulds filled with molten wax, allowed to solidify and thereafter, properly oriented for sectioning. The paraffin block was sectioned at 5µm after cooling the surface of the tissues with ice bar. Ribbons were gently picked with Carmel brush and dropped in a water bath containing water at 60 ^{o}C to enable ribbons float, expand and flatten out. Slides were rubbed with thymol containing egg albumen, and gently dipped into the bath to pick up the flattened out tissue ribbons [11].
Haematoxylin and Eosin (H&E) staining tecniques [11] were applied in staining the tissue sections.

3.3 Haematoxylin and Eosin Staining procedures

Tissue sections were deparaffinized in two changes of xylene and hydrated through graded series of alcohols in descending order and were rinsed in water and stained with Haematoxylin for 10mins. Tissue sections were rinsed and differentiated in one percent (1%) acidic alcohol and blued in running water using saturated lithium carbonate solution until sections appear sky blue. The blued section was counterstained in Eosin solution for 3mins. Tissues were washed in water and dehydrated in ascending grades of alcohol, cleared in xylene

and mounted in DPX covered with cover slips and observed under microscope.

3.4 Microscopy
Processed slides were viewed under light microscope at magnification (X400), and photomicrographs obtained were linked to the computer using the microscope's camera.

4. Statistical Analysis
Statistical analysis was carried out using Statistical Package for Social Sciences (SPSS), version 20.0 and M. S. Excel. The one-way analysis of variance (ANOVA) and post-hoc Tukey least significant difference (LSD) test were used to analyze the data and to determine the significance respectively. Data are expressed as Mean ± Standard Error of Mean (S.E.M) and tables were used to illustrate the variations in the numerical values across the experimental groups. The P. values <0.05 were considered statistically significant.

5. Results
Phytochemical analysis of *LGLEs* revealed high concentrations of saponins (45%), alkaloids (22.5%), phlobatannins (19%), cardiac glycoside (8.7%), flavonoids (0.19%), moderate concentration of phenols (0.11%).

5.1 Antioxidant activity
Assessment of serum levels of antioxidant enzyme (CAT) and a marker of lipid peroxidation (MDA revealed that serum CAT and MDA levels significantly decreased (P<0.05) and increased respectively in animals exposed to PF alone compared to levels in the unexposed control. However, administration of different concentrations of LGLEs reversed these changes (Figures1 A & B).
Serum testosterone level decreased significantly in animals exposed to PFs alone (2.17±0.84ng/mc), compared to level (3.37 ± 0.75ng/mc) in the unexposed group.
Furthermore, dose dependent significant decreases were observed in animals treated with low (G3) and medium doses of *LGLEs* (1.75±0.56ng/mc) and 0.94±0.55ng/mc respectively) compared to levels in G1 and G2. However, treatment with high dose regimen caused a significant (*P*<0.05) increase in serum testosterone level (3.87±1.55ng/mc) compared to levels in G1 and G2 (Figure 2).

5.2 Luteinizing Hormone (LH)
Mean serum levels of LH decreased significantly (*P*<0.05) in animals exposed to PFs alone (0.13±0.17mμ/ml) compared to levels in the unexposed group (G1) (0.37±0.15mμ/ml). Significant (*P*<0.05) decreases were also observed in animals treated with low and high doses of *LGLEs* (0.06±0.046mμ/ml or 0.05+0.04) compared to levels in G1 and G2. However, animals in G4 (treated with medium dose of the *LGLEs* had significant (*p*<0.05) increase in serum level of LH (0.39±0.18mμ/ml) compared to levels in G1 and G2 (0.37±0.15 and 0.13±0.17 respectively) (Figure 3A).

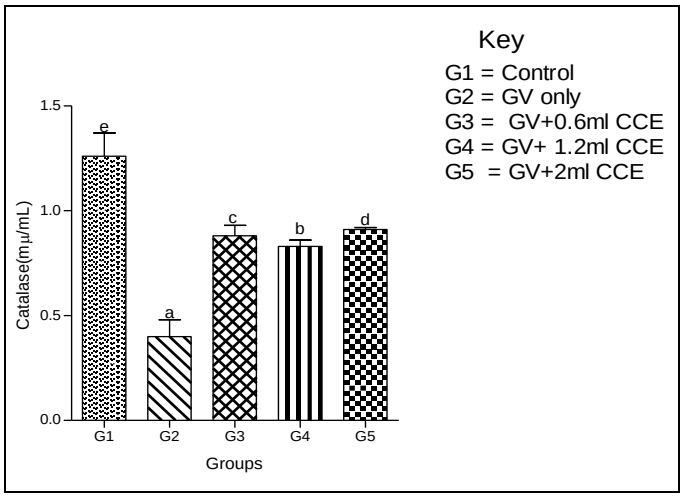
5.3 Follicle stimulating hormone (FSH) concentrations.
Exposure to PFs alone caused a significant (*P*<0.05) decrease in serum FSH level (0.12±0.17iμ/L) compared to level in the unexposed control (0.16±0.03 iμ/L). However, administration of the low or high dose of the LGLEs caused further decrease in serum levels of FSH except the level in the group treated with medium dose of LGLEs which showed a non-significant decrease compared to the level in PF alone group (Figure 3B).

5.4 Histological analysis.
Histological analysis showed that animals in the unexposed group had normal cytoarchitectural array in the testis (normal seminiferous tubules, germinal layer made up of spermatogonia and different stages of spermatogenesis progressing from the germinal layer to mature spermatozoa) and the epididymis (presence of matured sperm cells and normal epididymal epithelium) Figures 4A &4B.
Several stages of degenerative changes in seminiferous tubules and leading to depleted germ cells and congested blood vessels between the tubules were observed in PF alone group. Likewise decrease in sperm cell number and volume were found in PF alone group compared to the unexposed group (Figures 5A & B).

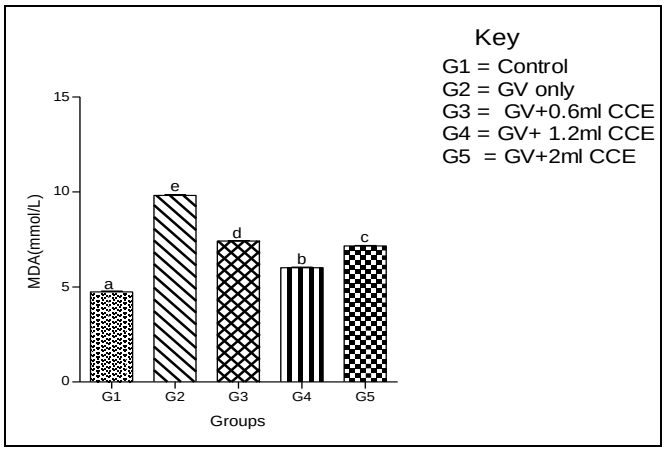
5.5 Low dose CCLEs (269.8mg/kg)
Administration of different concentrations of LGLEs caused a progressive but dose dependent improvements in the damaged testicular and epididymal structures. For instance, administration of low dose of LGLEs (269.8mg/kg) stimulated the germinal epithelium and triggered on spermatogenesis that caused the germinal epithelium to appear at different stages of spermatogenesis. The seminiferous tubules were found to contain spermatogonia and sertoli cells.
The epididymal tissues had normal cytoarchitectural and epithelial cells arrangements. However, spermatozoa decreased in number and volume, and were more compacted with little or no space between the inner walls of the epididymal ducts (Figures 6A & B).

5.6 Medium dose of LGLEs (539.6mg/kg)
Treatment with medium dose (539.6mg/kg) of LGLEs caused no further improvement in the cyto-architecture of the testis and epididymis, rather there was a complete loss of connective tissues in ductus epididymis. Depletion of germ cells was also observed in the seminiferous tubules.

5.7 High dose of LGLEs (809.4mg/kg)
Treatment with high dose of LGLEs (809.4mg/kg) progressively restored the damaged testicular and epididymal cytoarchitecture observed in PF alone group. These included the restoration of normal cytoarchitecture of the ducts epididymis lumen with partially filled matured sperm cells and normal epididymal epithelium.
There were also improvements in some degenerative changes observed in other groups (Figure 7A & B).



A)



B)

Fig 1A&B: Changes in serum levels of (A) CAT and (B) MDA, following exposure to PFs, and treatment with different concentrations of LGLEs. Values are expressed as Mean ± SEM at P < 0.05 relative to control. Different letters indicate significance while similar letters represent non-significance.

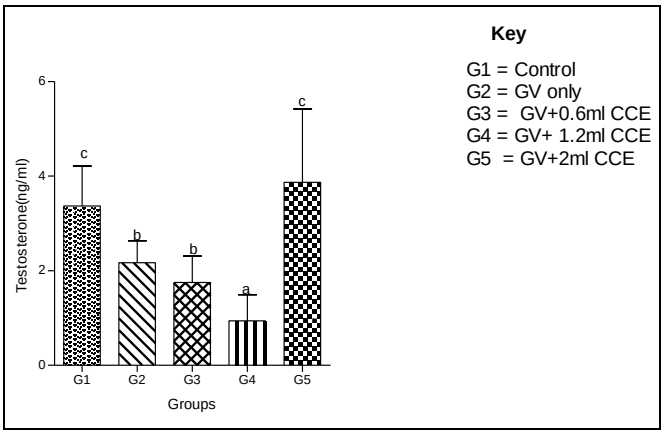
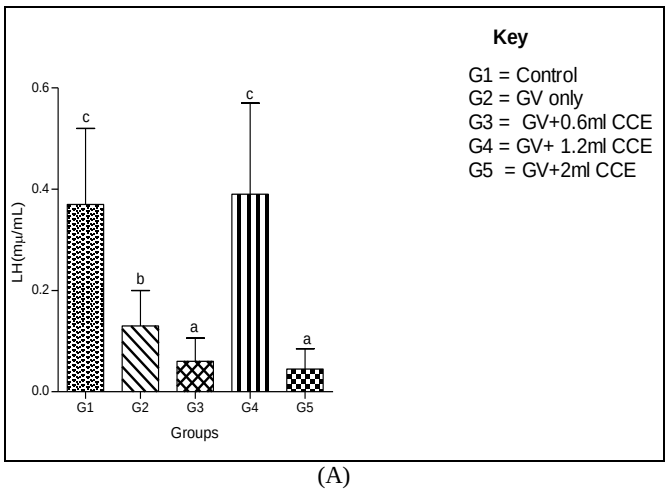
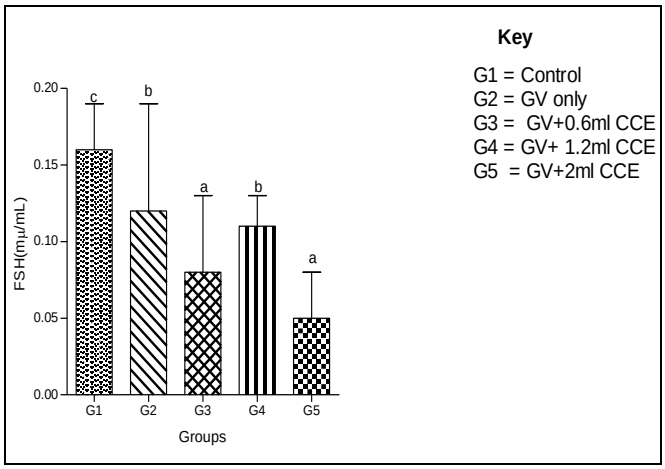


Fig 2: Changes in serum testosterone levels following exposure to PFs, and treatment with different concentrations of LGLEs. Values are expressed as Mean ± SEM at P < 0.05 relative to control. Different letters indicate significance while similar letters represent non-significance



(A)



(B)

Fig 3A&B: Changes in serum LH (A), FSH (B) levels following exposure to PFs, and treatment with different concentrations of LGLEs. Values are expressed as Mean ± SEM at P < 0.05 relative to control. Different letters indicate significance while similar letters represent non-significance

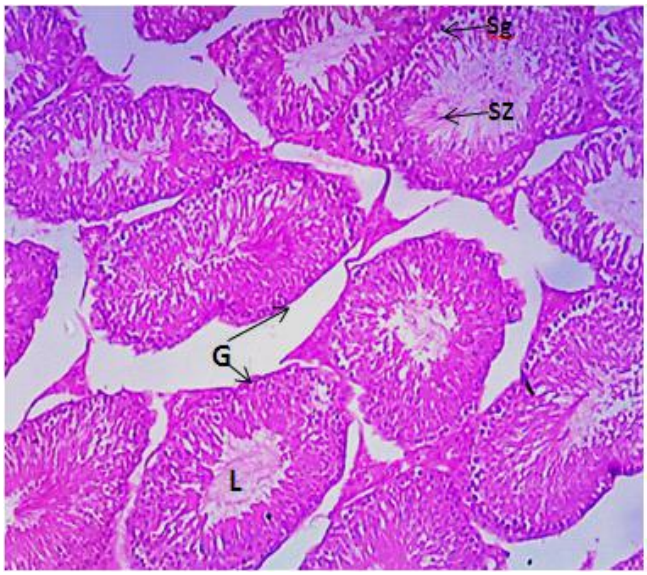


Fig 4A: Section of Testis (Control Group)

Histological section of the testis (H & E Stain) of animals in G1 (unexposed control) at magnification (X400) showing normal testicular architecture and normal size seminiferous tubules with germinal cell layers composed of spermatogonia (Sg) and spermatogenesis progressing from the basal germinal layer (G) to mature spermatozoa (Sz) with their tail extending into the lumen (L).

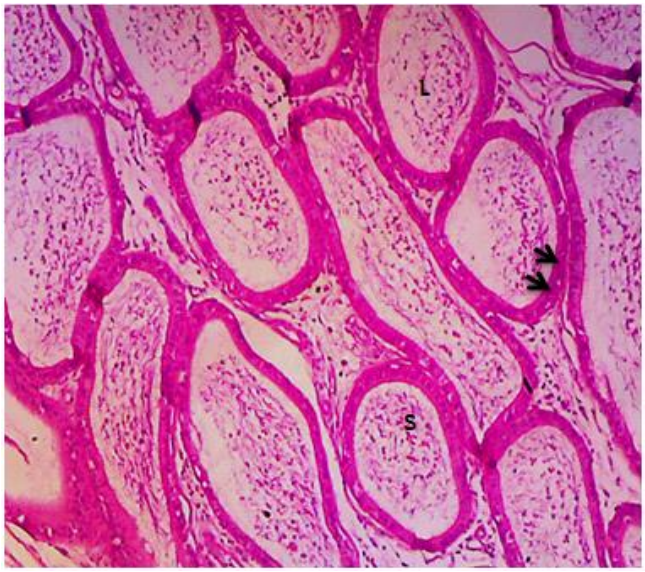


Fig 4B: Section of Epididymis (Unexposed Control)

Histological section of epididymis (H & E Stain) of animals in G1(unexposed control) at magnification (X400) showing normal architecture of the ductus epididymis lumen (L) filled with mature sperm cell (S).

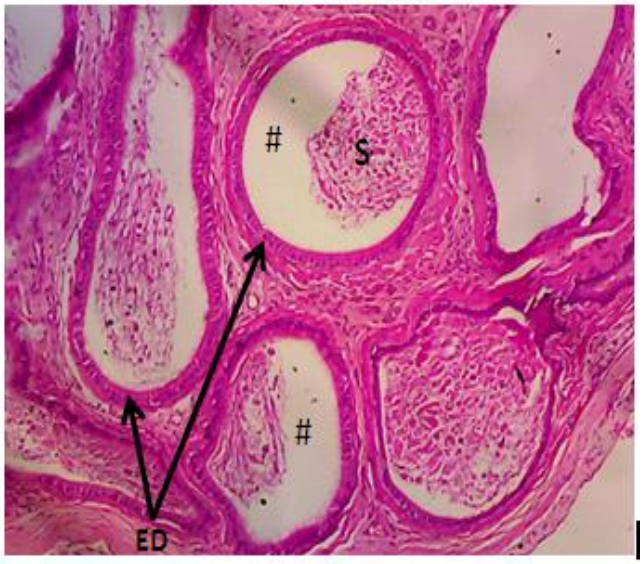


Fig 5B

Histological section of ductus epididymis (ED) (H & E Stain) of animals in G2 (PFs alone group) at magnification (X400) showing a reduction in matured sperm cells (S) volume (L) and decreased spermatozoa number (#) within the lumen

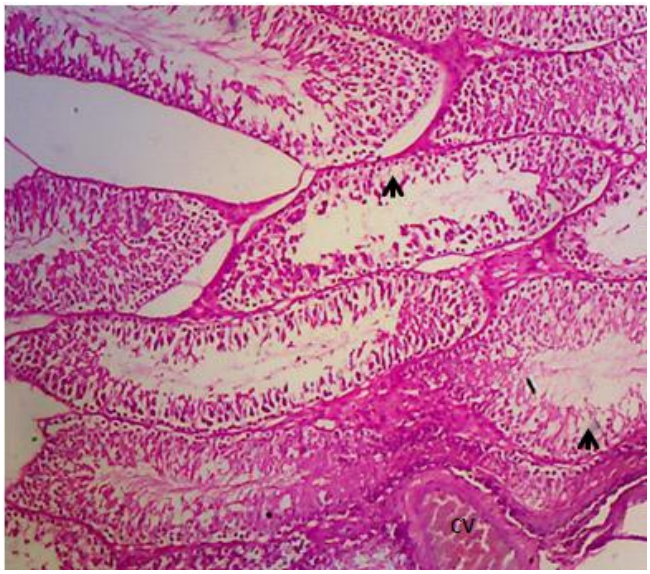


Fig 5A: Histological section of Testis of animals in G1 at magnification (X400)

(H-E Stain) showing testicular cytoarchitecture with multiple foci areas of degenerated seminiferous tubules, depleted germ cells (arrowheads), and congested blood vessels (CV) between the tubules.

The epididymal tissue shows decrease volume within the lumen (L). The clear area (H) indicates a reduction in spermatozoa within the lumen.

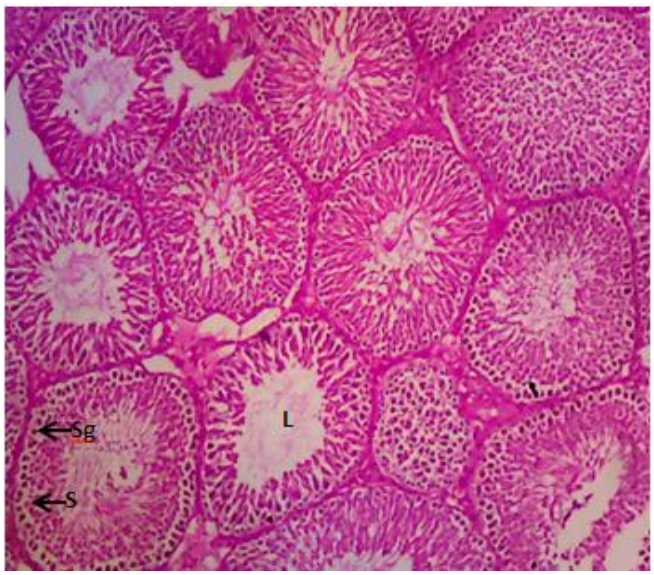


Fig 6A: Histological Section of Testis (Seminiferous tubules) of animals (H & E Stain) in G3 (PF+Low Dose LGLEs) at magnification (X400)

Showing normal arrangement of germ cells at different stages of spermatogenesis. The tubule contains spermatogonia (Sg), Sertoli cell (s), however, with reduced spermatozoa in the lumen L

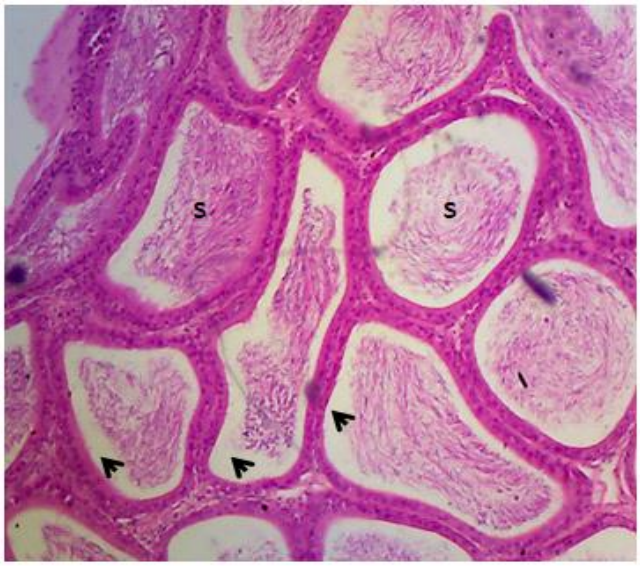


Fig 6B: Histological Section of Epididymis (PE+Low Dose LGLEs) H & E Stain)

Showing normal epididymal cytoarchitecture (normal epithelial cell arrangement and the presence of spermatozoa (S) in the lumen (L)) at magnification (X400). There is average volume of spermatozoa within the lumen but with little space (arrow ahead) that separates the spermatozoa from the inner wall of the ductus epididymis.

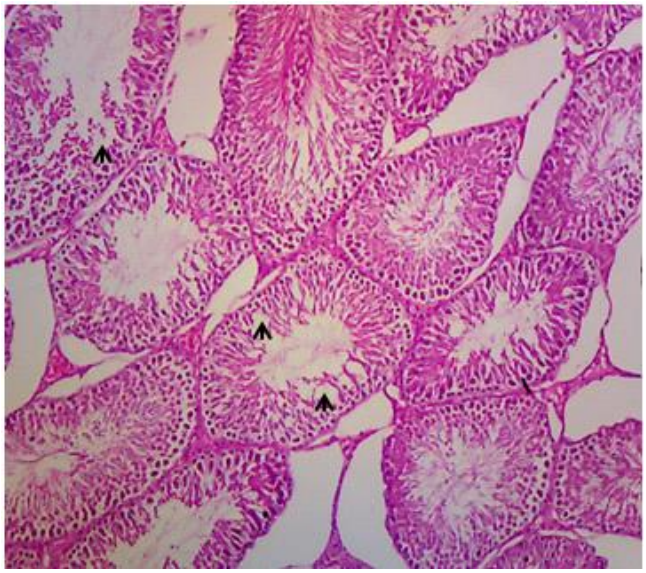


Fig 7A: Histological Section of Testis of animals in group 4 (PF+Medium Dose LGLEs) (H & E Stain)

Showing several seminiferous tubules with focal points of germ cells depletion (arrowheads), and normal seminiferous tubules within the lumen at magnification (X400).

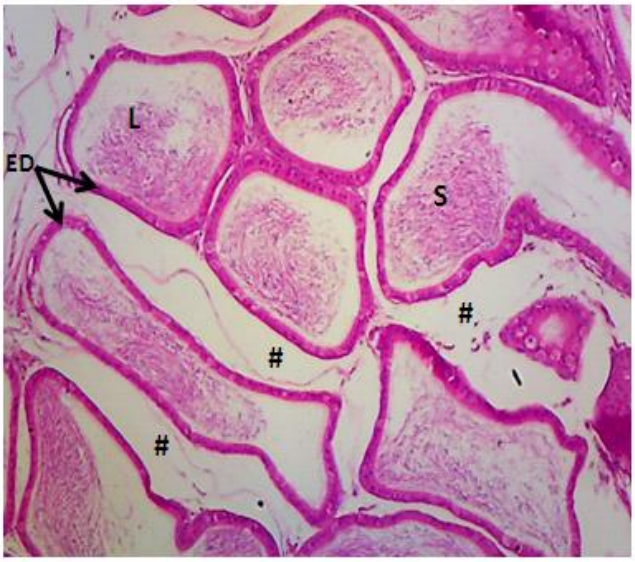


Fig 7B: Histological Section of Epididymis (H & E Stain) of animals in G4 (PF+Medium Dose LGEs)

Showing ductus epididymis (ED) at magnification (X400) with a reduction in mature sperm cell (S) volume within the lumen (L) and a complete loss of connective tissues in the spaces (#) between the ductus epididymis.

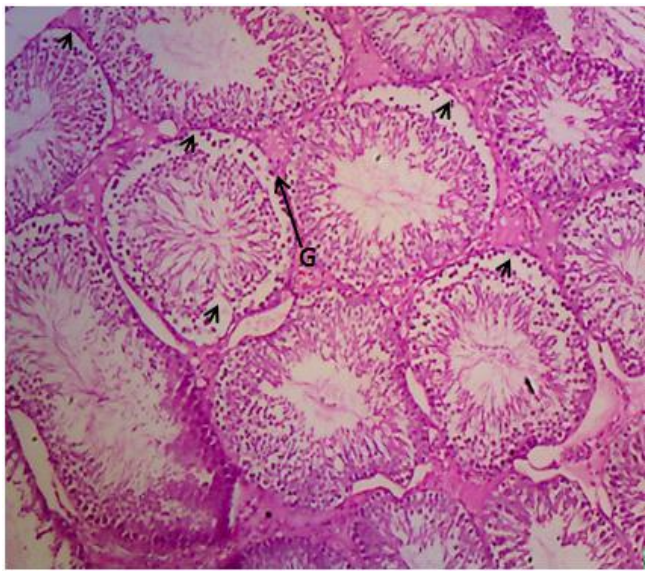


Fig 8A: Histological Section of Testis (H & E Stain) of animals in G5 (PF + High Dose LGLEs)

Showing seminiferous tubules at magnification (X400). Some of the seminiferous tubules showed degenerative changes in the connective tissues (arrowheads) with germ cells separating the basal germinal layer (G)

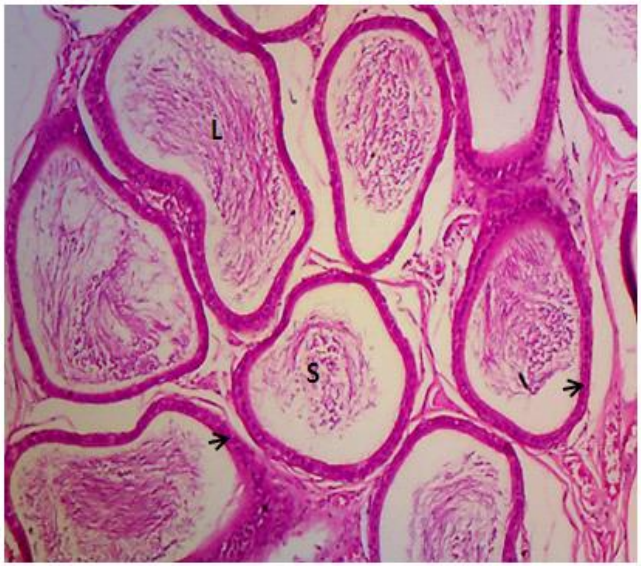


Fig 8B: Histological Section of Epididymis (H & E Stain) of animals in G5 (PF+High Dose LGLEs)

Showing normal architecture of the ductus epididymis lumen (L) at magnification (X400). Some of the ductus epididymis are partly filled with mature sperm cells (S) with typical epididymal epithelium (arrow head).

6. Discussion

Results of this study showed that exposure to PF could be associated with a significant risk of male reproductive system dysfunction mediated through oxidative damage to male reproductive organs (testis and epididymis). Likewise the use of *LGLEs in an optimal concentration* could reverse these disorders. These findings support previous studies that showed that optimal use of vegetable rich diets and other plants derived foods including fruits and unprocessed foods with a mixture of antioxidants could be beneficial in attenuating adverse environmental exposures [12]. The variation in results from different groups are also in line with previous studies that showed that compounds with diverse antioxidant constituents might produce diverse antioxidant effects, which could be synergistic, additive and/or antagonistic) [13].

The findings of significant alterations in OS markers (e.g., significant decrease and increase in CAT and MDA levels respectively) clearly demonstrate a state of heightened oxidative abuse on testicular and epididymal tissues in animals exposed to PF alone.

In normal testicular physiological processes, a balance in the OS status is maintained by the interplay of the ROS from normal activities of the spermatozoa and the antioxidants from testicular antioxidant systems [14] leaving a small physiological level of ROS which is necessary for normal sperm physiological processes (e.g., capacitation, hyper-activation of acrosome reactions and signal transduction) that ultimately leads to fertilization. Empirical evidences indicate that this balance could be altered following exposure to adverse environmental conditions (e.g., exposure to environmental toxins such as PF) associated with abnormal cellular metabolism and consequently generate additional significant amount of ROS.

The synergistic/or additve oxidative activities (physiologically mediated ROS and the pathologically generate ROS) will typically result in an enhanced high levels of ROS and OS in the reproductive tract and consequently undermine the antioxidant defense mechanisms of spermatozoa and seminal plasma with a resultant increase in OS. OS is a known cause of peroxidative damage to sperm plasma membrane and genetic components such as damage to sperm deoxyribonucleic acid(DNA) (DNA strand breakage and chromatin cross-linking) and several other processes that render the sperm incapable of participating in the fertilization processes such as ATP depletion and loss of sperm viability and motility [15]. In fact, OS has the potential to cause damage to all major classes of biomolecules.

Therefore, the pathological changes in biochemical markers of reproductive function such as significant decrease in testosterone, LH and FSH concentrations and the histo-pathological changes in the testicular and epididymal structures of animals (degenerative changes in seminiferous tubules, depletion of germ cells, congested blood vessels and low luminal sperm cell volume) exposed to PF alone were pathognomonic for OS and are in tandem with previous studies that implicated OS as one of the major mechanisms of xenobiotic-induced male infertility [16, 17, 14].

According to Burmistror [18], oxidative cell injury is the major pathophysiological mechanism in the reproductive toxicity of some gasoline constituents. Owagboriaya *et al.* [19] found significant alterations in serum levels of OS markers, testosterone, FSH, LH, prolactin in animals exposed to PFs. Ugwoke *et al.* [20] reported significant reduction in levels of male reproductive hormone (testosterone) and spermatocytic arrest in male rats acutely exposed to PFs. Many studies indicate that high seminal level of ROS is potentially toxic to sperm quality and functioning [21] including testicular degeneration [22], poor sperm motility and morphology [23], defective acrosome reaction and loss of fertility [24]. These findings suggest the inclusion of antioxidant (synthetic or natural) source in the therapeutic cocktail for male infertility. Although synthetic sources of antioxidant such as vitamins A, C and E have been found beneficial in restoring the balance between ROS generation and scavenging activities, recent studies have shown that some patient groups that used synthetic antioxidant sources failed to achieve optimum treatment level due to several constraints including cost, low compliance, unavailability and the monovalent mode of action/ effect. Therefore natural sources of antioxidant are highly recommended [25], affordable, devoid of expert consultation, contain multiple antioxidants, have huge compliance level, reduce side effects, produce synergistic action and pleiotropic effects.

Current evidence suggests that plants and plant products such as *LGLEs* are rich sources of bioactive constituents with significant antioxidant properties and could provide a broad range of antioxidant activity which could be used in the management of some cases of OS-related male infertility [26]. In the present study, animals treated with different concentrations of *LGLE* had significant, but dose dependent changes (decrease or increase) in biochemical (CAT, MDA, LH, FSH and testosterone) and histo-pathological features of the testis and epididymis of animals in PF exposed groups. In our previous representation [4, 27] we had shown that similar species of *C. citratus plant* obtained from the same environment, at the same age of maturity and subjected to similar thermal processing and extraction methods contained various bioactive constituents including the phyto-compounds (e.g., saponins, tannins, flavonoids, phenols and alkaloids), vitamins (vitamins A, C, E, folate, thiamine, niacin, pyridoxine and riboflavin) electrolytes (potassium (K^+), calcium (Ca^{2+}), copper (Cu^{2+}), magnesium (Mg^{2+}), manganese

(Mn), selenium (Se), phosphorus(PO4), iron (Fe^{2+}) and zinc (Zn^{2+}). Others include the essential oil constituents (α-citral, β-citral, mycene and eugenol) [28].

Most of these bio-constituents have potent antioxidant effects for treatment of male reproductive disorders and even a more or less potent antioxidant effect when they coexist as found in LGLEs due to the synergistic or antagonistic antioxidant activities. For instance, phenolic compounds and flavonoids found in LGLEs possess high antioxidant activity [29]. They possess hydroxyl (OH^-) group which function as hydrogen donors and can react with ROS and reactive nitrogen species and inhibit free radical formation. The OH- group can modify benzene ring and inhibit it ability to form ROS [30]. The phenolic compounds in LGLEs can also chelate metals, thereby reducing their ability to form free radicals [31]. Additionally, phenolics can inhibit some enzymes known to function in free radical formation such as cytochrome P450 isoforms, lipo-oxygenases. cyclooxygenase and xanthine oxidase [30]. A previous study [32] demonstrated how flavonoids and flavonoids sub-fractions (Luteolin, catechin and fiestin) caused improvements in OS markers (increased level of superoxide dismutase and decreased level of MDA) and serum level of testosterone and spermatogenesis. Other bio-constituents from LGLEs such as vitamins C and E are reported to cause decrease in lipid peroxidation and improved sperm motility, neutralized hydrogen peroxide, boosted the activities of other scavengers and preserved the motility and morphology of sperm [33]. Se contributes to the development and maturation of the testes, and protects sperm DNA and cell membrane especially when it co-exists with vitamin E as in *LGLEs.*

Zn^{2+} chelates and binds to ROS [34]. Mn^- promotes sperm motility [35], Vitamin C aids in breaking the chain and neutralizes OH^-, superoxide and H_2O_2 radicals and hence protects the life span of sperm and motility [36]. Vitamin A (β-carotene and lycopene) quenches singlet oxygen molecules, and peroxidative damage to seminal plasma membrane [37].

These antioxidants bio-constituents in LGLE are responsible for its ability to reverse OS-related reproductive dysfunction. Accordingly, Rahim *et al*. [38] showed that *LGLEs* and vitamin E caused significant increase in testicular and epididymal weight as well as testosterone and glutathione levels. There were improvements in values of semen characteristics and testicular histomorphological distortions and reduction in testicular and serum MDA levels in H_2O_2-induced reproductive dysfunction. Also, current knowledge indicates that some LGLEs phyto-constituents such as flavoniods through their anti-oxidative actions could interact with Cytochrome P450 enzyme system in the liver and modulate their synthesis and activities and consequently enhance metabolism of gasoline and render them less toxic to humans.

The dose dependent effect observed in the present study is consistent with results of precious studies that showed that when multiple antioxidants are present in a mixture they could interact with each other to produce effect which could be synegestic, antagonist or additive. However, a growing number of evidence indicates that the resultant/net effect of antioxidant mixture varies depending on their concentrations in the mixture, structures, time, and method of determining the antioxidant activity [38, 39]. Synergistic antioxidant effect improves antioxidant activities as evident in the biochemical and histomorphological indices, whereas antagonistic interaction between antioxidants leads to deterioration in the biochemical and histomorphological indices, explaining the observed dose dependent effects of LGLEs in the present study.

In the present study, significant reduction and deterioration in biochemical and histomorphological markers of reproductive function respectively were observed in specific groups following treatment with LGLEs compared with the PF alone group. Serum testosterone levels decreased significantly in PF exposed animals treated with low (G3) and medium doses (G4) of the leaf extracts compared to levels in the PF alone group, but significantly increased to reach the level in the unexposed control group (G1) when treated with high dose of LGLE (G5).

LH concentrations decreased significantly in the low (G3) and high (G5) dose treated groups compared to levels in the PF alone group (G2), but significantly increased to level similar to that found in the unexposed group when fed with medium dose of the extract (G4). Serum levels of FSH significantly decreased in animals exposed to PF alone compared with animals in the unexposed control group, further significant decreases in serum FSH levels were observed in animals fed low and high doses of the extracts. Serum level of FSH was not significantly different from the level in the group exposed to PF only.

Similarly, low dose of LGLE caused a reduction in the number and volume of spermatozoa with a resultant obliteration of the space between the inner walls of the epididymis ducts. Administration of medium dose caused a complete loss of connective tissues in the epididymal ducts. There was no observable improvement in the histomorphology of the testis and epididymis compared with changes in the PF alone group. However, high doses of CCLEs caused progressive restoration of the damaged testicular and epididymal histomorphology. A restoration of the epididymal lumen which was partially filled with matured sperm cells and epididymal epithelium was also observed.

Accordingly, Shi *et al*. [39] reported a synergistic interaction between lycopene and vitamin E at a specific concentration and ratio to cause inhibition of 2, 2-azobis (2, 4-dimethyl valeronitrile)-induced oxidation of linoleic acid methylester, but the interaction between vit E and B-carotene showed no synergistic effect at the same concentrations.

7. Conclusions

The present study findings showed that prolonged exposure to PF is a significant risk factor for male reproductive system dysfunction. Furthermore, that supplementation with LGLEs could have concentration-dependent ameliorative effects.

8. References

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