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MITIGATING EFFECTS OF COMBINED ADMINISTRATION OF TURMERIC (CURCUMA LONGA) AND CUCUMBER (CUCUMIS SATIVUS) JUICE ON LEAD ACETATE INDUCED TESTICULAR DYSFUNCTION IN WISTAR RATS

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ABSTRACT
The present study investigated the mitigating effects of combined administration of Turmeric (C*urcuma longa*) and Cucumber (C*ucumis sativus*) juice on Lead induced testicular dysfunction in Wistar Rats. Twenty-five (25) male rats were randomly distributed into 5 groups (n=5). Testicular toxicity was induced by administering a daily oral dose of 2.25mg/kg bw of Lead acetate in all rat groups except group 1, and the rats were later grouped as follows: Group 1 (control); Group 2: (Lead (Pb) only); Group 3: Lead with 1ml Turmeric juice (Pb + TUM); Group 4: Lead with 1 ml Cucumber juice (Pb + CUC); Group 5: Lead with 1ml Turmeric juice and 1ml Cucumber juice (Pb + TUM + CUC) respectively. All interventions were orally administered with the aid of an oral cannula for 28days. On day 29, the rats were anesthetized with diethyl ether and caudal epididymides were separated and segmented for sperm analysis (Sperm count, viable sperm, non-viable sperm and Motility), also blood samples were collected by direct cardiac puncture for the determination of serum male reproductive hormones (Testosterone, Follicle Stimulating Hormone and Luteinizing Hormone) using ELISA kits. Results of sperm analysis revealed significant decrease ($p < 0.05$) in sperm count, viable sperm cells and sperm motility among Group 2 (Pb Only) rats while significant increase ($p < 0.05$) was observed in the number of non- viable sperm cells when compared to Group 1 (Control) rats. Significant ($p < 0.05$) improvements were observed amongst groups 3 (Pb + TUR) and 4 Pb + CUC) rats administered single daily dose of Turmeric and Cucumber juice individually when compared to Group 2 (Pb Only) rats, with Cucumber (Pb + CUC) juice showing a better outcome compared to Turmeric (Pb + TUR). The number of non- viable sperm cells significantly ($p < 0.05$) decreased in the two treatment Groups. In Group 5 (Pb + TUR + CUC) rats, significant ($p < 0.05$) increase was observed in sperm count, viable sperm cells and

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motility when compared to Groups 2, 3 and 4 rats, suggesting synergism of action. Significant reduction ($p < 0.05$) was observed in non-viable sperm cells among Group 5 (Pb + TUR + CUC) rats when compared to Group 2 (Pb Only) rats. Furthermore, administration of Lead acetate caused a significant ($p < 0.05$) decrease in Testosterone, FSH and LH serum concentration among Group 2 (Pb Only) rats when compared to Group 1 (Control) rats, indicating a possible negative effect. Significant increase ($p < 0.05$) was observed in Testosterone, FSH and luteinizing hormone levels among Groups 3(Pb + TUR) and 4 (Pb + CUC) rats when compared to Group 2 (Pb Only) rats, suggesting a possible reversibility effect, with Cucumber juice demonstrating a greater potency. Significantly ($p < 0.05$) higher values of Testosterone, FSH and LH were observed, amongst group 5 (Pb + TUR + CUC) rats co-administered 1ml each of Turmeric and Cucumber juice compared to other treatment groups, indicating a possible synergistic effect. This study showed that combined administration of Turmeric and Cucumber juice had a better mitigating effect on lead induced testicular toxicity in Wistar rats than singular administration of each juice. This could be due to their synergistic effects. Further investigation should be conducted to ascertain the actual mechanisms of action of the combined juice.

Keywords: Turmeric (C*urcuma longa*), Cucumber (C*ucumis sativus*), Toxicity, Sperm analysis, Reproductive hormones

INTRODUCTION

The prevalence of infertility in a population has a range of important demographic and serious health implications. The incidence of infertility varies considerably: it is less in developed countries and in a higher proportion in developing countries where only limited resource for research and treatment of identified associated ailments are available (Okwu, 2001). Infertility is a serious global medical and social challenge as approximately 15% of married couples are infertile. About forty percent of infertility cases are attributable to male factors alone, whereas the remaining 60% is shared between both couples and unknown causes (Aliu and Nwude, 1982). The reproductive system is controlled by hormones in both male and female. Hence hormonal disturbances account majorly for infertility (Aliu and Nwude, 1982). Determination of sperm characteristics and serum male reproductive hormones is therefore very important in evaluating the reproductive efficacy in male and female animals including humans. Disturbances traceable to all or any of the above male reproductive hormones may cause deranged or problematic reproductive functions (Saronee *et al.*, 2024).

Turmeric is a rhizomatous medicinal plant that belongs to the *Zingiberaceae* family, it is cultivated widely in different countries of the world like India, China, Nigeria etc. (Akter *et al.*, 2019). It grows between 3 to 5 feet tall, with oblong leaves with funnel-shaped, yellowish rhizome growing below the soil (Iweala *et al.*, 2023). Turmeric is used in most Asian countries as a medicinal herb, it is a well sought after Phyto-medicinal plant and has been in use in folklore medicine in most African countries (Okwu, 2001). The rhizome is mostly used and has been confirmed therapeutic in the management of various ailments like oxidative stress, gonorrhea, inflammation, syphilis, stomach aches, blood cell disorder, bloody diarrhea, diabetes, reproductive abnormalities, toothaches and poisoning (Okwu,2001; Fortune *et al*., 2019 and Saronee *et al*., 2019). Cucumber (*Cucumis sativus*) belongs to the *Cucurbitaceae* family and is distributed mostly as a culinary vegetable (McLean *et al.*, 2013). It is cultivated in major countries of the world. Cucumber possesses in-vitro anti-inflammatory, reproductive

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functions, antioxidant, antifungal, antibacterial, and anti-diabetic effects and has been reported to be rich in several bioactive compounds like cardiac glycosides, flavonoids, alkaloids, terpenoids, saponins, coumarins, tannins, sterols and terpenes (McLean *et al.*, 2013; Dan-Jumbo *et al*., 2024). Application of turmeric and cucumber juice in traditional medicine is common in our environment. However, scientific reports validating the above-described anecdotal applications of the juice of Turmeric and Cucumber are scanty in our environment. Hence, this study, aimed to determine the possible mitigating effects of combined administration of Turmeric (C*urcuma longa*) and Cucumber (C*ucumis sativus*) juice on Lead Acetate induced testicular dysfunction in Wistar Rats.

MATERIALS AND METHODS

Procurement of Wistar Rats, Lead Acetate and Induction of Lead Toxicity

Twenty -five (25) male Wistar rats (170 to 250g) were purchased from PAMO University of Medical Sciences animal house. They were housed in transparent polycarbonate cages with wired top covers, with 12 hours light/dark cycle and were fed with normal rat chow with unhindered access to clean water. Procured experimental animals were acclimatized for 14 days and were later grouped for the experiment. Lead acetate was obtained from Eddy Chemicals and Safety Supply Co. Port Harcourt, Rivers State, Nigeria.

Toxicity was induced with an oral daily administration of 2.25mg/kg bwt of Lead acetate in the morning hours (between 8- 9am daily) in line with Saronee *et al.,* (2024).

Purchase and Preparation of Turmeric and Cucumber Juice

Turmeric and Cucumber used for this study were bought from a local fruit market in Port Harcourt, Nigeria and were duly identified and authenticated. Turmeric juice was prepared according to the method of Gul and Bakht (2015). After collection, a known quantity of Turmeric rhizomes was washed thoroughly and grinded using an electronic blender with 400ml of distilled water. The solution was later filtered using a sieve and a mesh cloth, and the juice obtained was put in a universal bottle and stored in the refrigerator for preservation prior administration. The process was done in the Department of Physiology Laboratory, PAMO University of Medical Sciences.

Cucumber juice was prepared according to the method of Aderinola and Abaire, (2019). Fresh Cucumbers were washed thoroughly and grinded using a blender. The solution was later filtered using a sieve and a mesh cloth, and the juice obtained was also put into a universal bottle and stored in the refrigerator for preservation. This study was conducted in line with the guidelines set by the United States Institute for Laboratory and Animal Research (1996).

Ethical Approval

The study was approved by the Research Ethics Committee of PAMO University of Medical Sciences and the ethical approval code of PUMS/REC/2024022 was issued.

Acute Toxicity Study

Acute toxicity (LD_{50}) of Turmeric was estimated as reported by Amadi *et al.,* 2025, ≥3000mg/kg bw. Similarly, the LD_{50} of Cucumber was as previously reported by Saronee *et al.,* 2024 ≥1000mg/kg bw.

Experimental Design

Laboratory animals were acclimatized for 2 weeks and were later randomly distributed into 5 groups (5 rats per group). Toxicity was induced through a single daily oral administration of 2.25mg/kg of lead in all rat groups except group 1 rats for 28 days and were treated as follows:

Group 1: Control; rats in this group had free access to tap water and normal rat chow.

Group 2: Pb Only; rats in this group received 2.25mg/kg bw of Lead acetate only

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Group 3: Pb + TUM; rats in this group were given 1ml of Turmeric juice only following induction of Lead toxicity.
Group 4: Pb + CUC; rats in this group were given 1ml of Cucumber juice only following induction of Lead toxicity.
Group 5: Pb + TUM + CUC; rats in this group received 1ml each of Turmeric and Cucumber juice following induction of Lead toxicity.
All administrations were done orally using oral cannula for 28 days.

Blood Samples Collection for Hormonal Assay
On day 29, all the rats were anesthetized using diethyl ether and blood samples were collected by cardiac puncture into plain sample bottles for reproductive hormonal assays and epididymides harvested for sperm parameters analysis.
Sperm parameters
Sperm count
As descried by Raji *et al*. (2006). Cauda epididymis was collected and dipped in 2 ml normal saline before being pre-warmed to 37^{o} C. Tiny incisions were made in the cauda epididymis to retrieve the spermatozoa contained inside. We suspended the spermatozoa in normal saline and transferred 200 L of the sperm suspension to both chambers of the modified Neubauer hemocytometer using a Pasteur pipette. Using a microscope (Leica DM 750), the spermatozoa were counted in five big Thoma squares, and the volume of normal saline injected was adjusted.
Sperm motility
It was as earlier explained by Atashfaraz *et al.* (2013). A pre warmed glass slide containing 10 µl of sperm suspension was covered with a cover slip and viewed at 1000 × magnification using a light microscope (Leica DM 750) with a heated (37^{o}C) stage.
Sperm Viability
The viability of spermatozoa was determined using Wyrobek *et al*. (1983) techniques. To an identical volume of sperm suspension, 20 µl of 0.05% eosin Y-nigrosine was added briefly. At room temperature, the mixture was incubated for 2 minutes. Following that, all slides were examined using a microscope (LeicaDM 750) at 100× and 400× magnifications. The living spermatozoa were not discoloured, but the dead ones stained pink. For each assay, 400 spermatozoa were counted and percentage viability computed (Wyrobek *et a*l., 1983).
Serum levels of FSH, LH and testosterone
Blood samples were centrifuged, and serum obtained following a 3000 rpm for 15 min oscillation. Testosterone, follicle stimulating hormone and luteinizing hormone levels in the serum were determined with ELISA kits (BioAssay Systems, Hayward, CA, USA) as instructed by the manufacturer.
Statistical Analysis
Obtained data were analyzed with one-way ANOVA followed by a post hoc (LSD) test with using SPSS 23.0Version. Results were presented in table and figures. A p value < 0.05 was considered statistically significant.

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RESULTS

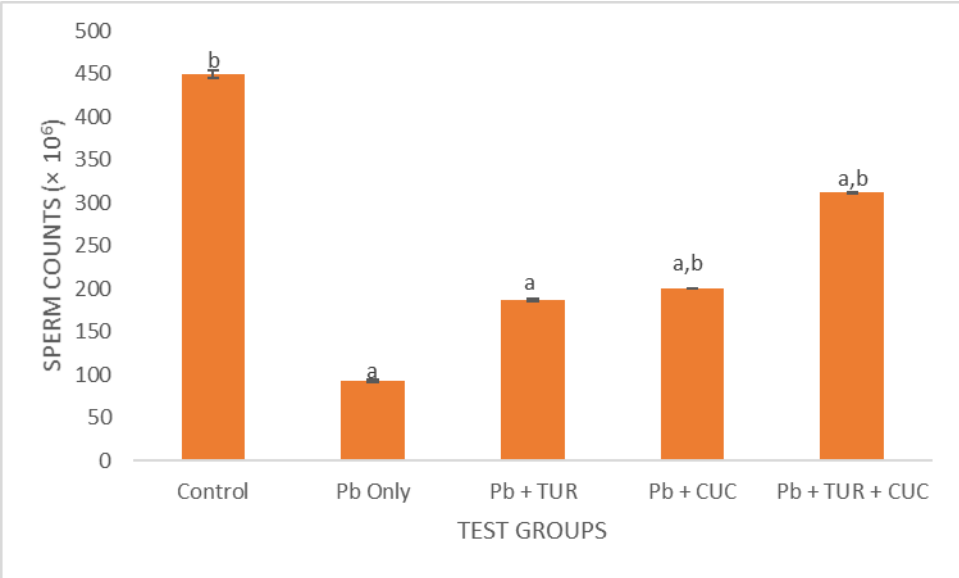

Figure 1: Effect of Turmeric (C*urcuma longa)* and Cucumber (C*ucumis sativus)* juice on sperm count of lead induced testicular toxicity in rats

All values are expressed as mean ± standard error of mean (n = 5). [a] $p < 0.05$ was significant when compared to the control (Group 1) group while [b] $p < 0.05$ was significant when compared to Pb Only (Group 2) .

Figure 1 showed a significant ($p < 0.05$) reduction in sperm count in group 2(Pb Only) rats when compared to group 1 (Control) rats. Significant reduction ($p < 0.05$) was observed in group 3 (Pb +TUR) rats when compared to group 1(Control) rats. Significant ($p < 0.05$) increase was observed in group 4 (Pb + CUC) rats when compared to group 2 (Pb Only) rats. Significant ($p < 0.05$) increase was also observed in group 5 (Pb + TUR + CUC) rats when compared to group 2 (Pb Only) rats but a significant ($p < 0.05$) decrease was observed when compared to group 1 (Control) rats.

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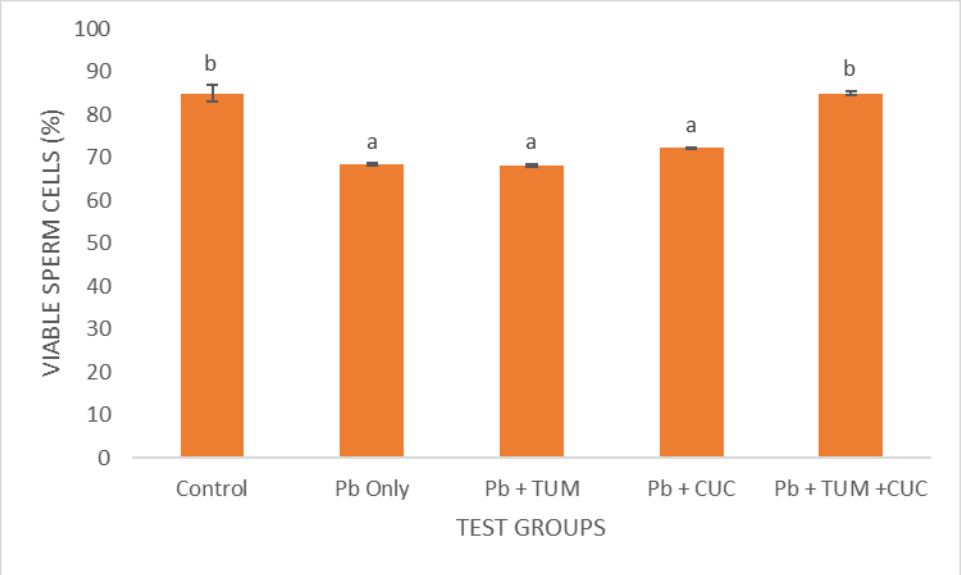

Figure 2: Effect of Turmeric (C*urcuma longa)* and Cucumber (C*ucumis sativus)* juice on viable sperm cells of lead induced testicular toxicity in rats

All values are expressed as mean ± standard error of mean (n = 5) [a] $p < 0.05$ was significant when compared to the Group 1(Control) while [b] $p < 0.05$ was significant when compared to Group 2 (Pb Only).

Figure 2 showed a significant ($p < 0.05$) reduction in viable sperm cells in groups 2 (Pb Only), 3 (Pb + TUR) and 4 (Pb + CUC) rats when compared to Group 1(Control) rats. Significant increase ($p < 0.05$) was observed in Group 5 (Pb + TUR + CUC) rats when compared to Groups 1 (Control) and 2 (Pb Only) rats.

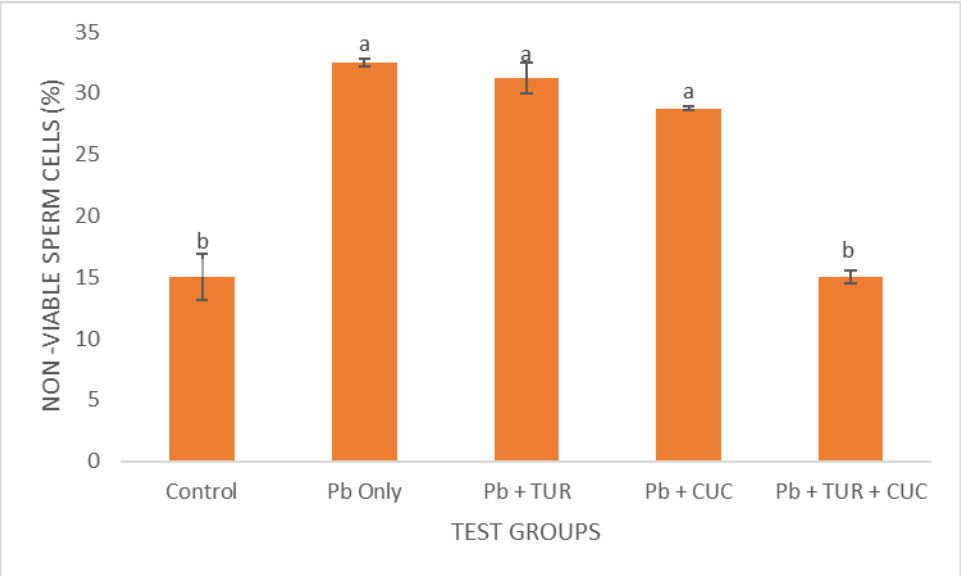

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Figure 3: Effect of Turmeric (C*urcuma longa)* and Cucumber (C*ucumis sativus)* juice on non -viable sperm cells of lead induced testicular toxicity in rats

All values are expressed as mean ± standard error of mean (n = 5). [a] $p < 0.05$ when compared to
the Group 1 (Control) while [b] $p < 0.05$ was significant when compared to Group 2 (Pb Only).
In figure 3, a significant ($p < 0.05$) increase in non -viable sperm cells were observed in Groups 2 (Pb Only), 3(Pb + TUR)) and 4 (Pb + TUR + CUC) rats when compared to Group 1(Control) rats. Significant reduction ($p < 0.05$) was observed in group 5 (Pb + TUR + CUC) rats when compared to Group 2 (Pb Only) rats.

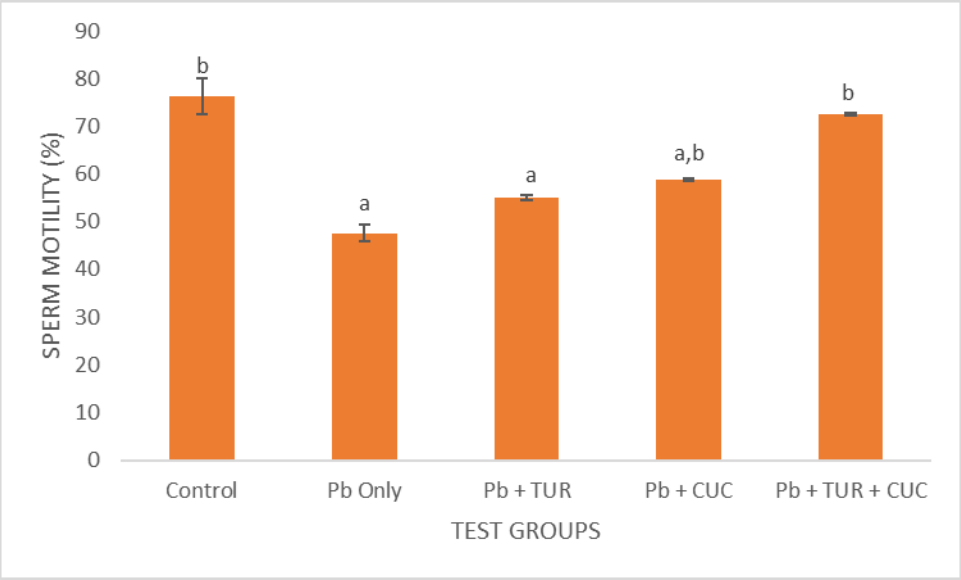

Figure 4: Effect of Turmeric (C*urcuma longa)* and Cucumber (C*ucumis sativus)* juice on sperm motility of lead induced testicular toxicity rats

All values are expressed as mean ± standard error of mean (n = 5). [a]$p < 0.05$ was significant when compared to the Group 1 (Control) while [b] $p < 0.05$ was significant when compared to Group 2 (Pb Only).
Figure 4 showed that a significant ($p < 0.05$) reduction in sperm motility in Groups 2 (Pb Only) and 3 (Pb + TUR) rats when compared to Group 1 (Control) rats. Significant ($p < 0.05$) increase was observed in Group 4 (Pb + CUC) rats when compared to Group 2 (Pb Only) rats and significant ($p < 0.05$) reduction when compared to Group 1(Control) rats. Significant ($p < 0.05$) increase was observed in Group 5 (Pb + TUR + CUC) rats when compared to Group 2 (Pb only) rats.

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TABLE 1: Effects of Turmeric (C*urcuma longa)* and Cucumber (C*ucumis sativus)* juice on Testosterone, FSH and LH of lead induced testicular toxicity rats

Groups	Testosterone (ng/dl)	Follicle Stimulating Hormone (m/u/ml)	Luteinizing Hormone (m/u/ml)
1: Control	4.70 ± 0.65^{b}	2.10 ± 0.35^{b}	2.60 ± 0.30^{b}
2: Pb Only	4.10 ± 0.13^{a}	1.80 ± 0.08^{a}	1.70 ± 0.07^{a}
3: Pb + TUM	$6.10\pm0.81^{a,b}$	2.00 ± 0.05^{b}	1.90 ± 0.14^{a}
4: Pb + CUC	$6.90\pm0.38^{a,b}$	2.30 ± 0.04^{b}	$2.10\pm0.32^{a,b}$
5: Pb + TUM + CUC	$7.40\pm0.37^{a,b}$	2.40 ± 0.11^{b}	2.50 ± 0.07^{b}

All values are expressed as mean ± standard error of mean (n = 5). ^{a} $p < 0.05$ was significant when compared to the Group 1 (Control) while ^{b} $p < 0.05$ was significant when compared to Pb Only (Group 2).

Table 1 shows changes in male reproductive hormone levels across all treatment groups in male Wistar rats. Compared to Group 1 (Control) rats, administration of 2.25mg/kg body weight of lead acetate amongst Group 2 (Pb Only) rats significantly reduced serum testosterone, follicle stimulating hormone and luteinizing hormone concentration ($p< 0.05$). Perhaps suggesting a potential harmful effect of lead acetate. Administration of turmeric (C*urcuma longa)* juice significantly increased serum testosterone concentration but only marginally elevated luteinizing hormone concentration and follicle stimulating hormone levels amongst Group 3 rats ($p< 0.05$) when compared to Group 2 (Pb Only) rats. Indicating a possible ameliorative and reversibility effects. Significantly higher values of testosterone and luteinizing hormone with a marginal increase in follicle stimulating hormone concentration were observed amongst group 4 (Pb + CUC) rats administered 1ml of Cucumber juice when compared to both groups 1 (Control) and 2 (Pb Only) rats ($p< 0.05$), this is suggestive of a possible ameliorative effect. This effect of Cucumber juice is comparable to the effect of administration of Turmeric (C*urcuma longa)* juice observed amongst group 3 rats. Cucumber juice however, demonstrated a greater potency compared to Turmeric (C*urcuma longa).* The combined administration of both juices amongst Groups 5 (Pb + TUM + CUC) rats caused a significant improvement in assayed male reproductive hormones compared to Group 2 (Lead acetate treated) rats ($p< 0.05$). Noteworthy, the observed increase in testosterone, follicle stimulating hormone and luteinizing hormone concentrations following the combined administration of both juices is greater than the increase observed following single administration of either juice: apparently suggesting either a possible additive or indeed a synergistic effect of both juices on male reproductive hormones concentration.

DISCUSSION

The present study investigated the possible mitigating effects of combined administration of Turmeric (C*urcuma longa*) and Cucumber (C*ucumis sativus*) Juice on Lead induced testicular dysfunction in Wistar Rats. The results of the study showed that administration of lead acetate had significant adverse effects on the sperm parameters investigated vis-a-vis sperm count, viable sperm cells, non-viable sperm cells sperm motility. This observation was found to be consistent with previous works by (Kolawole *et a*l,2022; Hernandez *et al*., 2005; Kolawole *et al*., 2020; Naha *et al*., 2005; Saronee *et al.*, 2024). The deleterious effects of lead have been

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shown to be due to increase in reactive oxygen species (ROS) production especially testicular (cellular) hydrogen peroxide and hydroxyl radicals by lead acetate which impede the synthesis of antioxidants and impair enzyme activities leading to lipid peroxidation in cell membranes (Ni *et al*., 2004; Vaziri and Khan, 2007; Saronee *et al.*, 2024). The harmful consequences of an elevated level of ROS in tissues have been proposed as a fundamental cause of diseases associated with lead exposure (Patrick, 2006). Available reports suggest that increase in free radicals causes the loss of epithelial cells, which can damage cytoplasmic bridges and consequently decrease sperm count and motility levels and increase sperm deformities including dead sperm cells (Aziz *et al*., 2004; Gbaranor *et al.*, 2025; Kolarovic *et al.*, 2010; Saronee *et al.*, 2024). The significant reduction observed in the gonadotropin hormones in the study could be from a possible depressive effect on the hypothalamic neural mechanisms which has been shown to be very important for the release of Gonadotropin Releasing Hormone (GnRH) (Reddy *et al*., 1995; Didia *et. al*., 2000). It could eventually lead to disruption in the production and secretion of pituitary gonadotropins that are essential for spermatogenesis and steroidogenesis (Aydos *et.al*.,2001).This reduction in gonadotropins could also explain the adverse effect of lead on sperm parameters investigated in the study.

On the other hand, it was observed in this study that administration of Turmeric and Cucumber juice either singly or combined mitigated the adverse effects of lead acetate on sperm parameters and reproductive hormones. This mitigating effect of the juice on sperm parameters and hormones could be due to their rich phytochemicals and nutritional contents: electrolytes, sulfates, ascorbic acid, carbohydrate, phenols, caryophyllene, curcumin, flavonoids, tannins, glycosides, among other compounds as shown in previous work (Iwu, 1993; Okwu *et al.*, 2011; Fortune *et al.*, 2019; Saronee *et al.*, 2019; Saronee *et al.*, 2023 and Amadi *et a*l., 2025).

Therefore, it is possible to suggest that the positive effects of the two juices in mitigating the Lead -induced testicular functions in this study could be partly mediated by its counteraction on

oxidative stress within rat reproductive organs via its antioxidant properties ((Al-Waili, 2003; Gheldof *et al.*, 2002; Yao *et al.*, 2004). It has been reported that phenols and flavonoids especially flavones and catechins are perhaps some of the most powerful flavonoids for protecting the body against damage by reactive oxygen species (Sodipo *et a*l., 2000) and also possess anti-radical and antioxidant properties which serve as potent antioxidants (Eruikainure *et al*., 2010). The rich ascorbic content of the juice is also a good source of antioxidant (Edwards *et al*., 2003; Johnson *et al*., 2013).

Phytochemicals, such as quercetin, vitamin C and vitamin E have been reported to improve testosterone concentration and quality of spermatozoa (Ashfaq *et al*., 2020; Nwangwa *et al*., 2020). The increase in the level of testosterone, FSH and LH observed in rats given the juice can comfortably imply that they can operate on hypothalamic -pituitary gonadotropic regulation of secretory granules, resulting in improved testicular function (Kolawole *et al*., 2022)

The positive influence on the hypothalamus causing the gonadotropin releasing hormone (GnRH) stimulation of the anterior pituitary which resulted in the release of follicle stimulating hormone and luteinizing hormone, follicle stimulating hormone and luteinizing hormone stimulate the Leydig cells in the testes leading to the formation of testosterone. This chain of hormonal activities positively improves the rate of spermatogenesis in the testes. These actions could have been made possible because of the stimulatory effects of some of the inherent

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phytochemical compounds present in the juice of both plants on the reproductive organs in male Wistar rats (Yao *et al.*, 2004).

In conclusion, the study showed that combined Turmeric and Cucumber juice significantly mitigated lead acetate-induced testicular toxicity in rats more effectively than either juice alone, improving sperm parameters and hormone levels. Synergistic effects are suspected, and further research is necessary to identify the exact mechanisms responsible for this observed synergy.

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