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EVALUATION OF HORMONAL LEVEL AND HISTOMORPHOLOGICAL CHANGES IN LEAD ACETATE INDUCED MALE ALBINO RATS TREATED WITH *ALLIUM CEPA* AND *CURCUMA LONGA* ETHANOIC EXTRACTS IN MANAGEMENT OF TESTICULAR DAMAGE

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ABSTRACT
Lead poisoning has been implicated in the pathogenesis of several diseases. This study evaluated hormonal levels and histomorphological changes in male albino rats induced with lead acetate and treated with *Allium cepa* and *Curcuma longa* ethanoic extracts in management of testicular damage. This study was conducted on 35 male albino rats divided into 5 treatment groups; Group 1 (Negative control), Group 2 (positive control), Group 3 (treated with *Allium cepa* only), Group 4 (treated with *Curcuma longa* only) Group 5(treated with combination of *Allium cepa* and *Curcuma longa* extracts) for 4 weeks before sacrificing and harvesting the testes for histological analysis, and blood sample obtained through cardiac puncture for hormonal analysis. Group 1-5 were induced with 100mg/kg body weight of lead acetate. Using the Layman L.C method, Follicle Stimulating Hormone and Luteinizing hormone levels were estimated. There were significant increases in the levels of FSH (P=<0.001) and LH (P=0.010) in the treatment groups compared to Group 2 (positive control). This indicates the ability of the extracts to attenuate hormonal imbalance. Thus, the *Allium cepa* and *Curcuma longa* ethanoic extracts can be used as a safe therapy for the management of hormonal imbalance resulting from testicular damage.

KEYWORDS: Hormonal Level, Histomorphology, Lead Acetate, Allium cepa, Curcuma longa, Testicular damage.

INTRODUCTION

1.1. Background of Study

The world of natural remedies and traditional medicine is filled with a vast array of plants and herbs that have been used for centuries to address various health concerns.[1,2] Among these, *Curcuma longa* (Turmeric) and *Allium cepa* (onion) are two well-known botanicals that have gained significant attention for their potential health benefits.[1,2] While these plants are often discussed for their anti-inflammatory and antioxidant properties, their effects on reproductive health, particularly on the male reproductive system, remain an intriguing area of study.

Curcuma longa, commonly known as Turmeric, is a flowering plant native to South Asia. The rhizome of this plant is the primary source of the spice and medicinal compound known as curcumin, which has garnered significant scientific interest due to its myriad health benefits.[1] Curcumin has long been recognized for its potent anti-inflammatory properties, and inflammation plays a crucial role in various diseases, including those affecting the male reproductive system.[3] Chronic inflammation in the testes can lead to conditions like orchitis, which may result in infertility. Research has shown that curcumin's anti-inflammatory effects may help alleviate testicular inflammation and protect against testicular damage.[1] Oxidative stress is another factor that can negatively impact testicular health, leading to sperm damage and reduced sperm quality.[4] Curcumin's antioxidant properties have been demonstrated to combat oxidative stress, potentially improving sperm quality and overall reproductive health.[1] Additionally, maintaining hormonal balance is crucial for male reproductive health, and some studies suggest that curcumin may help regulate hormone levels, particularly testosterone.[5] While more research is needed to establish the extent of its impact, the potential hormonal benefits of curcumin on the male reproductive system are promising.[5]

Allium cepa, commonly known as onion, is a staple ingredient in cuisines worldwide and is appreciated not only for its culinary uses but also for its potential health benefits.[2] *Allium cepa* are rich in various bioactive compounds, including quercetin, sulfur compounds, and

flavonoids, which contribute to their medicinal properties.[2] Similar to curcumin, *Allium cepa* possess potent antioxidant and anti-inflammatory properties.[1] These properties can help reduce inflammation in the testes and protect against oxidative stress, potentially improving overall testicular health.[1]

Allium cepa have also been linked to cardiovascular health due to their ability to lower blood pressure and reduce the risk of heart disease.[2] A healthy cardiovascular system is essential for adequate blood flow to the testes, ensuring the delivery of nutrients and hormones necessary for sperm production.[3] Furthermore, *Allium cepa* are particularly rich in sulfur compounds, such as allicin, which play a role in detoxification and may help remove harmful substances from the body, potentially benefiting the reproductive system.[2]

The testes, often referred to as the testicles, are a pair of male reproductive organs responsible for producing sperm and testosterone.[3] They are vital for fertility and male sexual development, and maintaining the health and proper function of the testes is essential for overall reproductive health.[3] Sperm production, known as spermatogenesis, occurs within the seminiferous tubules of the testes, and proper nutrition, antioxidant support, and the reduction of inflammation are essential for optimal sperm production.[3] Both *Curcuma longa* and *Allium cepa* have properties that may positively influence these factors.[1, 2]

MATERIALS AND METHODS

Experimental Animal

A total of thirty-five (35) healthy male Albino rats weighing 118-160g used in this study were purchased from the animal holdings of the department of Pharmacology, Rivers State University, Port Harcourt, Nigeria.

The albino rats were randomly grouped into five (5) groups of seven rats each and maintained under normal laboratory conditions (temperature of 25ºc, relative humidity of 60-70%). They were fed with vita feed (manufactured by Top Feeds Nigeria Limited) and water ad libitum. The beddings were changed and cages cleaned daily. The rats were acclimatized to laboratory conditions for one week with 12hours light prior to the experiments. Body weight of each animal was recorded at the beginning and at the end of the experiment. All rats were handled in accordance with the standard guide for the care and use of laboratory animals.

Extract Preparation

Curcuma longa were bought from mile 3 market in Port Harcourt. It was washed, peeled, oven dried, and grounded into powder. 100g of the powdered *Curcuma longa* was weighed into a clean labeled jar, and extracted using soxhlet extractor and maceration method[6] with 500ml of absolute ethanol used as the solvent for 72hours. The resultant extract was filtered using Whatman filter paper 125mm and evaporated to dryness on a low heat to concentrate it.

Allium cepa bulbs werealso bought from mile 3 market in Port Harcourt. The *Allium cepa* bulbs were grated after they had been carefully cleaned, rinsed, and peeled. 250g of the grated *Allium cepa* was weighed into a clean labeled jar, and extracted using soxhlet extractor and maceration method[6], with 1000ml of absolute ethanol used as the extracting solvent, for 72hours. The resultant extract was filtered using Whatman filter paper 125mm and evaporated to dryness on a low heat to concentrate it.

Acute Toxicity Testing of *Allium cepa*

The acute toxicity study is done to ascertain the toxicity level of *Allium cepa*, utilizing Up and Down method.[7] In this study Five (5) rats were used. The first rat was given 500mg/kg of *Allium cepa* extracts after fasting for the entire night, and was monitored for 48 hours for any sign of toxicity. Following survival, the dosage was adjusted to 1000mg/kg for the next rat, and toxicity indicators were monitored. The dosages were subsequently raised for the third, fourth and fifth albino rats to 2000mg/kg, 3000mg/kg, and 5000mg/kg. For a total of 14days, Signs of toxicity and mortality were noted every 8 hours.

Acute Toxicity Testing of *Curcuma longa*

The acute toxicity study is done to ascertain the toxicity level of *Curcuma longa*, utilizing Up and Down method. In this study five (5) rats were used. The first rat was given 500mg/kg of *Curcuma longa* extracts after fasting for the whole night, and was monitored for signs of toxicity for 48 hours. The second rat's dosage was increased to 1000mg/kg after it survived. It was monitored to check for signs of toxicity. The doses were increased subsequently for the third, fourth and fifth albino rats to 2000mg/kg, 3000mg/kg and 5000mg/kg. For a total of 14 days signs of toxicity and mortality were noted every 8 hours.

Acute Toxicity Testing of Lead Acetate

The purpose of this acute toxicity test was to use the Up and Down method to ascertain the toxicity level of lead acetate. The albino rat was given 100mg/kg of lead acetate intraperitoneally after an overnight fast. At eight hourly and 24 hours intervals, signs of toxicity and mortality were noted. The dosage of lead acetate utilized in this investigation was taken from similar study.[8]

Dosage Calculation for Extracts

For this study, 1000mg/kg of *Allium cepa* and *Curcumin longa* extracts were use.

An albino rat weighing 1kg would get 1000mg of ethanoic extracts

Average weight of rat =100g

$$\text{Dose} = \frac{\text{weight of rat}}{1000} \times \text{dosage}$$

Dose = 100g× 1000mg = 100mg
1000

In accordance with the OECD Guidelines (2001), this was dissolved in water based on the weight of animals and administered as follows: 1ml was given to 100g rat, 1.6ml to 160g rat, and so on.

Experimental Design

Table 1

GROUP	TREATMENT DOSE	DURATION
Group 1 (Negative Control)	Feed and water only	4 weeks
Group 2 (Positive Control)	100mg/kgbwt of lead acetate	24 hours
Group 3	100mg/kgbwt of lead acetate + 1000mg/kgbwt of *Allium cepa*	4 weeks
Group 4	100mg/kgbwt of lead acetate + 1000mg/kgbwt of *Curcuma longa*	4 weeks
Group 5	100mg/kgbwt of lead acetate + 1000mg/kgbwt of *Curcuma longa* and *Allium cepa*	4 weeks

Allium cepa and *Curcuma longa* extracts were administered orally across the groups.

Sample Collection

The final body weights of the rats were obtained at the end of the experiment. After an overnight fast the rats were sacrificed on the 29th day. They were anesthetized using chloroform. After a cardiac puncture, blood samples were taken and placed in plain bottles for hormone analysis. For histopathological examination, the testis of each albino rats was removed and stored in 10% formol-saline.

MATERIALS

Distilled water, vita feed, *Curcuma longa*, *Allium cepa*, iron cages, absolute ethanol, cardboard paper, nose mask, face mask, carpenter bag, chloroform, universal container, 10% formal-saline, plain hematology bottle, lead acetate, cotton wool, Whatman filter paper 125mm, conical flask, clean bottle, paraffin wax, markers, digital weighing balance(A123), measuring cylinders, glass slides, tissue embedder (LEICAEG 1160), rotary microtome(LEICARM 2125RTS), foil, plastic funnel, heating mantle, glass beakers, hot plate, brush and forceps, digital camera(ICC50 E), ascending grades of alcohol, xylene, hematoxylin, eosin, 1% acid-alcohol, tap water, dpx mountant, coverslip, microscope, water bath, oven, knife, grater, 5ml syringes, needles, microplate well, automatic micropipette, FSH and LH enzyme reagent, wash buffer, absorbent paper, substrate solution, stop solution, spectrophotometer.

Laboratory Analysis

Tissue Processing

The tissues were properly fixed in 10% formal-saline, they were properly dehydrated by passing through ascending grades of alcohol: 70%, 75%, 90%, 95%, Absolute I, II and III. Each change of alcohol was carried out for 2hours. The tissues were cleared by further passing them through two changes of xylene (I and II), for 2 hours each. They were infiltrated and embedded into molten paraffin wax present in the hot air oven, so as to provide internal support, using two changes of molten paraffin wax for 2hours each.

The tissues were further sectioned into thin uniform slices of tissue sections, with the use of a mechanical device called microtome. The tissue sections were stained using Hematoxylin and Eosin stain. They were mounted using Dibutylphthalate Polysterene Xylene (DPX) mountant. Sections on slides were examined and photomicrographs captured with Leica DM 750, Camera ICC50 E.

Estimation of Follicle Stimulating Hormone

Using Layman L.C Method (unit m/u/ml)

Principle

FSH assay is based on the microparticle enzyme immunoassay technology, anti-β FSH- coated microparticle is delivered into the incubation well of the reaction cell containing the sample and standard each. The FSH from the sample and standard binds to the anti-β FSH- coated microparticle forming an antibody-antigen complex, the complex is transferred to the glass-fiber matrix, where it binds irreversibly. The matrix is washed with the wash buffer to remove unbound materials. The enzyme substrate is added, the enzymatic reaction is terminated by addition of the stop solution. The absorbance is measured on a microtiter plate reader at 450nm. The intensity of the color formed by the enzymatic reaction is directly proportional to the concentration of FSH in the sample.

Procedure

Each standard and test microplate well was formatted, and 50ul of each was pipetted into the corresponding wells. 0.1ml of the FSH enzyme reagent solution was added into each wells. The microplates were swirled gently for 20-30secs to mix properly. They were covered and incubated for 60mins at room temperature; the well contents were further discarded. 350ul of wash buffer was added, discarded and this step was repeated two times to make a total of three washes. The plates were blotted dry with absorbent paper, and 0.1ml of working substrate solution was added into all wells, they were incubated for 20mins at room temperature. 50ul of stop solution was added to each well and gently mixed for 15-20 sec. The absorbance reading in each well was taken at 450nm.

Estimation of Luteinizing Hormone

Using Layman L, C Method (unit m/u/ml)

Principle

LH assay is based on the microparticle enzyme immunoassay technology, anti-β LH- coated microparticle is delivered into the incubation well of the reaction cell containing the sample and standard each. The LH from the sample and standard binds to the anti-β LH- coated microparticle forming an antibody-antigen complex, the complex is transferred to the glass-fiber matrix, where it binds irreversibly. The matrix is washed with the wash buffer to remove unbound materials. The enzyme substrate is added, the enzymatic reaction is terminated by addition of the stop solution. The absorbance is measured on a microtiter plate reader at 450nm. The intensity of the color formed by the enzymatic reaction is directly proportional to the concentration of LH in the sample.

Procedure

Each standard and test microplate well was formatted, and 50ul of each was pipetted into the corresponding wells. 0.1ml of the LH enzyme reagent solution was added into each well. The microplates were swirled gently for 20-30secs to mix properly. They were covered and incubated for 60mins at room temperature; the well contents were further discarded. 350ul of wash buffer was added, discarded and this step was repeated two times to make a total of three washes. The plates were blotted dry with absorbent paper, and 0.1ml of working substrate solution was added into all wells, they were incubated for 20mins at room temperature. 50ul of stop solution was added to each well and gently mixed for 15-20 sec. The absorbance reading in each well was taken at 450nm.

Data Analysis

SPSS version 23 was used for the analysis of the data generated from this study. Results were expressed as mean SD with p-values less than 0.05 being considered statistically significant.

RESULTS

Toxicity Study of *Allium cepa* and *Curcuma longa*

There was no mortality or any significance of toxicity after 14 days of observation upon administration of 500mg, 1000mg, 2000mg, 3000mg, and 5000mg 0f *Allium cepa* and *Curcuma longa* extracts. The extracts were therefore considered safe and non-toxic upto 5000mg/kg body weight.

Table 1: Result of Toxicity Study of *Allium cepa.*

PLANT EXTRACT	DOSAGE(mg/kg)	OBSERVATION
Allium cepa	500	No mortality
Allium cepa	1000	No mortality
Allium cepa	2000	No mortality
Allium cepa	3000	No mortality
Allium cepa	5000	No mortality

Table 2: Result of Toxicity Study of *Curcuma longa.*

PLANT EXTRACT	DOSAGE(mg/kg)	OBSERVATION
Curcumalonga	500	No mortality
Curcumalonga	1000	No mortality
Curcumalonga	2000	No mortality
Curcumalonga	3000	No mortality
Curcumalonga	5000	No mortality

Hormonal RESULT

Effect of *Allium cepa* and *Curcuma longa* Ethanoic Extracts on Follicle Stimulating Hormone

Table 3; shows the effect of Allium cepa and Curcuma longa ethanoic extracts on follicle stimulating hormone in lead acetate induced albino rats.

The follicle stimulating hormone level for group 1,2,3,4 and 5 were 1.43±0.07a, 0.25±0.02b, 0.93±0.08c, 0.99±0.05c, and 0.67±0.06d. Group 2(positive control) had significantly lower follicle stimulating hormone than the other groups (F=118.838, P= < 0.001).

Table 3: Effect of *Allium cepa* and *Curcuma longa* Ethanoic Extracts on Follicle Stimulating Hormone.

FOLLICLE STIMULATING HORMONE (FSH)	
Group 1 (Negative control)	1.43 ± 0.07^{a}
Group 2 (Positive control)	0.25 ± 0.02^{b}
Group 3 (*Allium cepa* only)	0.93 ± 0.08^{c}
Group 4 (*Curcuma longa* only)	0.99 ± 0.05^{c}
Group 5 (*Allium cepa + Curcuma longa)*	0.67 ± 0.06^{d}
P-value	<0.001
F-value	118.838

Values with different superscripts are significantly different from each other ($p\leq0.05$)

Effect of *Allium cepa* and *Curcuma longa* Ethanoic Extracts on Luteinizing Hormone

Table 4; shows the effect of Allium cepa and Curcuma longa ethanoic extracts on Luteinizing hormone in lead acetate induced albino rats.

The luteinizing hormone level for group 1,2,3,4 and 5 were 1.55±0.11a, 1.01±0.06b, 1.67±010c, 1.30±0.11d and 1.91±0.08e. Group 2 (positive control) had significantly lower luteinizing hormone level than the other groups (F= 26.819, P= 0.010).

Table 4: Effect of *Allium cepa* and *Curcuma longa* Ethanoic Extracts on Luteinizing Hormone.

LUTEINIZING HORMONE (LH)	
Group 1 (Negative control)	1.55±0.11[a]
Group 2 (Positive control)	1.01±0.06[b]
Group 3 (*Allium cepa* only)	1.67±010[c]
Group 4 (*Curcuma longa* only)	1.30±0.11[d]
Group 5 (*Allium cepa* + *Curcuma longa)*	1.91±0.08[e]
P-value	0.010
F-value	26.819

Values with different superscripts are significantly different from each other (p≤0.05).

Histological Examination of Testes

Histological examination of the testes treated with *Allium cepa* and *Curcuma longa* ethanoic extract for 4 weeks with their respective controls.

GROUP	PHOTOMICROGRAPH OBSERVATION	H & E
Group 1 (Negative Control)	Seminiferous tubules with sertoli cells (SC), spermatogonia (SG), primary spermatocytes (PS) and spermatids. The interstitium (IT) showed Leydig cells (LC). Tubular Germ cell maturation is variable.	X100
Group 2 (Positive Control)	Sloughing of germ cell (SGC) elements into tubular lumen and with hypolastic (GCH) and degenerative changes in germ lesions. Interstitial tissues degeneration (ITD) with Leydig cells lesions (LCL).	X100
Group 3 (*Allium cepa)*	Displayed recovered germ cells (RGC) with less sloughing of sertoli cells (SC), germ cells (RGC) and interstitium (RIT) with Leydig cells (RLC).	X100
Group 4 (*Curcuma longa)*	Some recovered seminiferous tubules with sertoli cells (SC), spermatogonia, primary spermatocytes (PSC) and spermatids. There are interstitial tissues lesions with Leydig cells (LCD) degenerations.	X100
Group 5 (*Allium cepa* and *Curcuma longa)*	Seminiferous tubules with sertoli cells (SC), Recovered germ cells (RGC), spermatogonia, primary spermatocytes (SPC) and spermatids. The interstitium connective tissues are regenerated (RIC) with Leydig cells (RLC).	X100

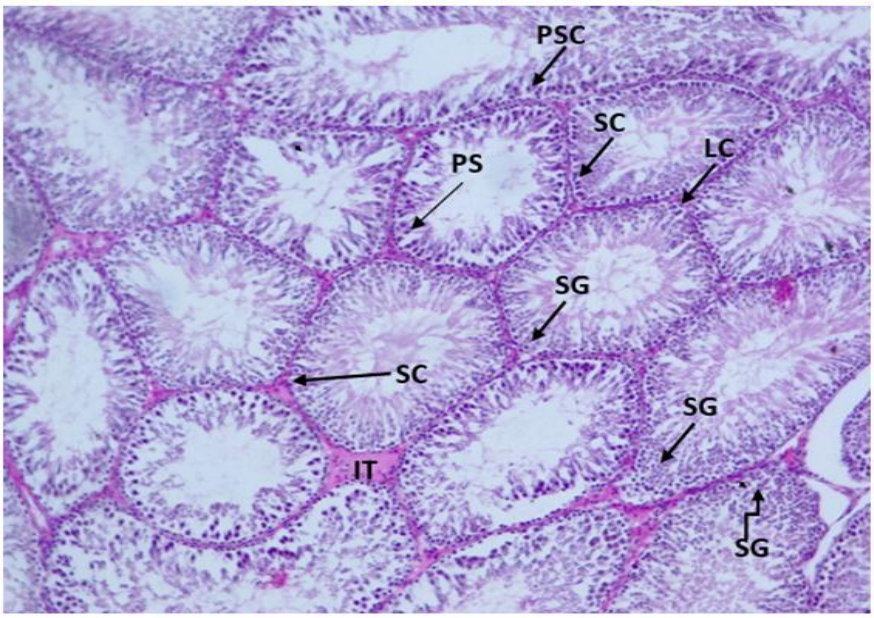

Fig 1: Photomicrograph section of testes of Wistar rat from control group 1 administered distilled water. Section showed seminiferous tubules with sertoli cells (SC), spermatogonia (SG), primary spermatocytes (PS) and

spermatids. The interstitium (IT) showed Leydig cells (LC). Tubular Germ cell maturation is variable. H&E X100.

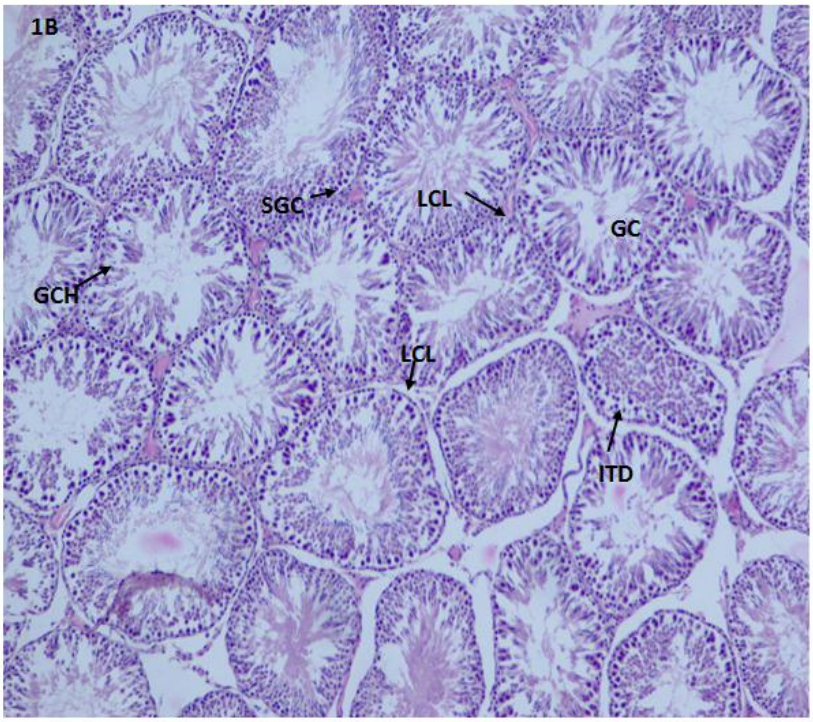

Fig 2: Photomicrograph section of seminiferous tubule of Testes of Wistar rats treated with Lead. Section showed sloughing of germ cell (SGC) elements into tubular lumen and with hypolastic (GCH) and degenerative changes in germ lesions. Interstitial tissues degeneration (ITD) with Leydig cells lesions (LCL). H&E X100.

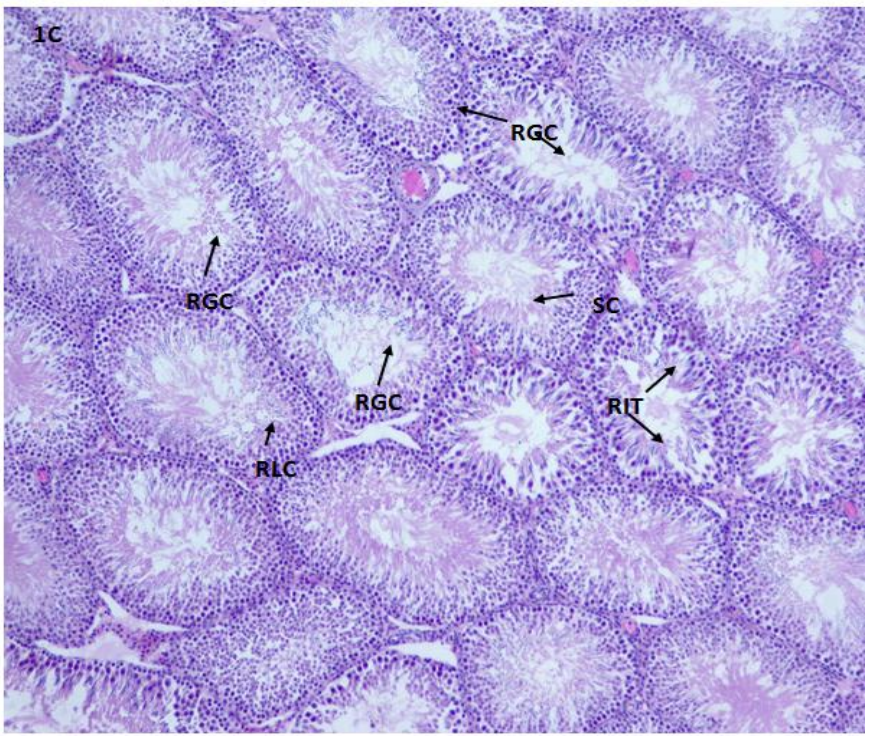

Fig 3: Photomicrograph section of seminiferous tubule of Testes of Wistar rats treated with Lead plus *Alium cepa* ethanol extract Section displayed recovered germ cells (RGC) with less sloughing of sertoli cells (SC), germ cells (RGC) and interstitium (RIT) with Leydig cells (RLC). H&E X100.

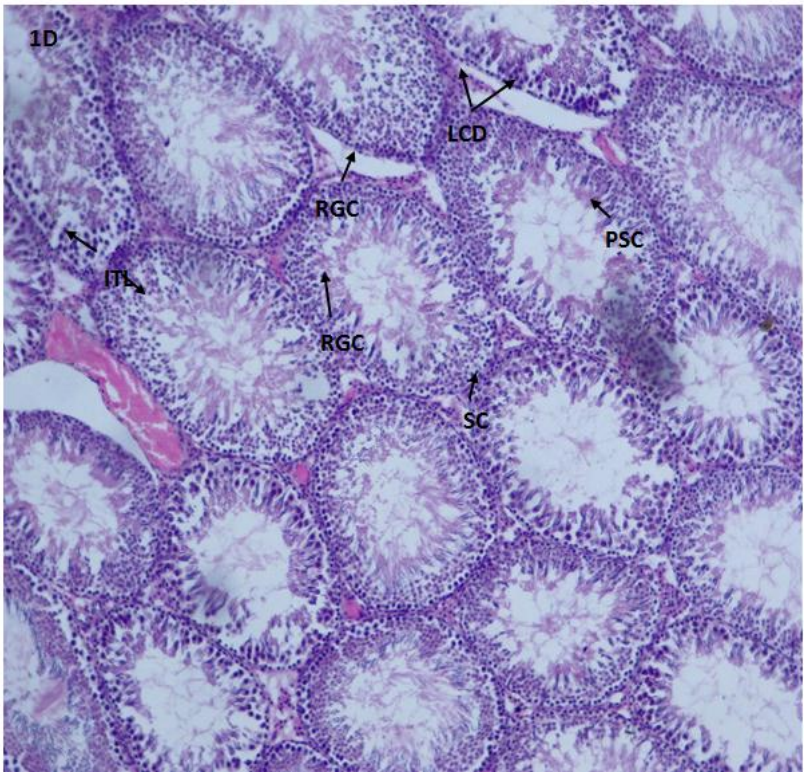

Fig 4: Photomicrograph section of testes of Wistar rat treated with lead plus *Cucumin longa.* Section showed some recovered seminiferous tubules with sertoli cells (SC), spermatogonia, primary spermatocytes (PSC) and spermatids. There are interstitial tissues lesions with Leydig cells (LCD) degenerations. H&E X100.

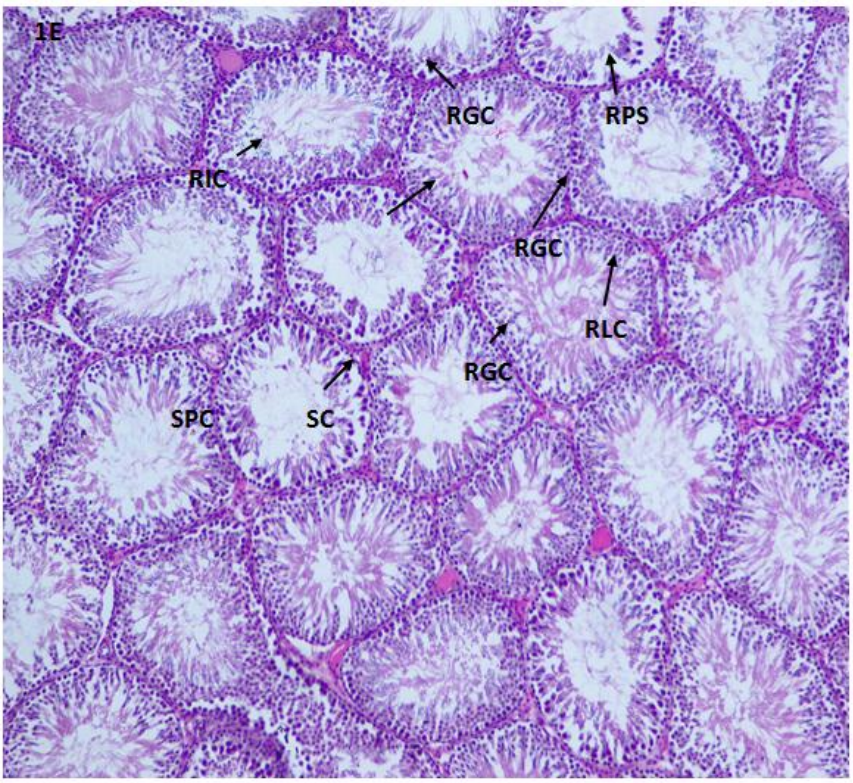

Fig 5: Photomicrograph section of testes of Wistar rat from group 5 given Lead plus *Alium cepa* and *Cucumin longa*. Section showed seminiferous tubules with sertoli cells (SC), Recovered germ cells (RGC), spermatogonia, primary spermatocytes (SPC) and spermatids. The interstitium connective tissues are regenerated (RIC) with Leydig cells (RLC). H&E X100.

DISCUSSION

The purpose of this study was to assess the impact of ethanoic extracts of *Curcuma longa* and *Allium cepa* on testicular injury caused by lead acetate in albino rats. Both *Alliumcepa* and *Curcuma longa* have individually demonstrated medicinal properties. The rhizome of the Curcuma longa is the primary source of the medicinal component known as curcumin, its antioxidant effects have been proved to battle oxidative stress and inflammation, potentially enhancing sperm quality and overall reproductive health.[1] Quercetin, sulfur compounds, flavonoids, and other bioactive chemicals are abundant in allium cepa and contribute to its protective effects against various health issues.[2]

The data from this study showed that lead acetate led to degeneration of testicular tissues and decreased level of FSH and LH, as shown by the low levels of Follicle Stimulating Hormone and Luteinizing Hormone in group 2 (positive control) compared to group 1 (negative control). This result is consistent with the work of Oyeyemi and other researchers (2020), who evaluated the hepatic and reproductive toxicity of lead acetate in albino rats, with destruction of the seminiferous tubules and degenerative changes in the spermatogenesis cells in male rats.[9] The findings in this study as it concerns to the decrease of FSH and LH levels in albino rats exposed to lead acetate is also in agreement with similar study which revealed that the levels of Luteinizing Hormone and Follicle Stimulating Hormone decreased in male rats administered with lead.[10]

This study showed the protective effect of *Curcuma longa* and *Allium cepa* extract against the effect of lead induced toxicity of lead acetate on the fertility of male albino rats, where rats administered with lead acetate and treated with *Curcuma longa* and *Allium cepa* extract showed a good recovery in the levels of LH, FSH and the testicular tissues. This result is consistent with a study which evaluated the use of *Curcumin* and *Curcuma longa* to alleviate adverse reproductive outcomes of water nitrate pollution in male rats.[11]

The levels of FSH and LH were significantly increased in the group treated with *Allium cepa* and *Curcuma longa* extract compared to the positive control group. Thus the both extracts has an antagonistic effect on lead acetate induced testicular damage. The protective effect of *Curcuma longa* on the testes is explained by the fact that it prevented cellular damage that occurred as a result of oxidative stress in spermatogenic cells of seminiferous tubules and leydig cells[12], this is in agreement with the present study. *Curcuma longa* also influenced the seminiferous tubules with sertoli cells, promoting spermatogenesis, exhibiting less disruption and better structural integrity which is in agreement with the report from similar study[13], whose studied the protective effect of *Curcuma longa* rhizomes extract against the toxicity of lead acetate that induce infertility in male albino rats. The rats treated with *Allium cepa* extract in this study exhibited signs of maintained seminifereous tubules, decreased degeneration, and minimized disturbance in cellular architecture in group 3 when compared to positive control group 2, and the serum male hormone levels significantly increased, especially LH when compared to the control groups. This finding is in concurrent with the study of Hosseini & Safi (2016), on the effect of *Allium cepa* seed extract on bodyand testicular weight, mature gametes and parietal cells of male rats.[14]

On the other hand, combined extract of *Allium cepa* and *Curcuma longa* helped to ameliorate the lead-related reproductive disorders, as evidenced by the recovered germ cells, seminiferous tubules with sertoli cells, regeneration of the interstitium connective tissues with leydig cells and presence of spermatogonia and primary spermatocyte and also increased serum male hormones, most significantly LH when compared to the control group.

CONCLUSION

This study provided detailed information on the effects of *Allium cepa* and *Curcuma longa* ethanoic extract on testicular damage in albino rats. The result obtained from this study showed the protective effect of *Allium cepa* and *Curcuma longa extracts* against the toxicity effects of lead acetate in the testicular tissues. The ameliorative effects of *Allium cepa* and *Curcuma longa is* evidenced by recovery of the germ cells, spermatogonia, primary spermatocytes and spermatids. Regeneration of the interstitium connective tissues with Leydig cells. It also showed a good recovery of serum level of FSH and LH across the groups treated with plant extracts. *Allium cepa* and *Curcuma longa* with their various rich compounds may provide a viable food based approach for enhancing male fertility.

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