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PHYSIOLOGICAL ROLE OF HUNTERIA UMBELLATA FRUIT EXTRACT ON HORMONAL AND RENAL PROFILE IN CADMIUM INDUCED TOXICITY MALE WISTAR RATS

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ABSTRACT

The present study was design with an objective to investigate the toxicity level of cadmium chloride on hormonal and biochemical markers and the restoration strength of Hunteria umbellata aqueous fruit extract administered at various dosage level of treatment in induced male wistar rats. The parameters investigated includes LH,FSH, Testosterone, Prolactin, Urea, Creatinine, and Uric acid following 0.07ml and 0.06ml single dose $cdcl_2$ induction of group2 and group3.However group4 was administered 0.03ml $cdcl_2$ daily while group1 serve as the control.Group2 was treated at 400mg/kg and group3 at 200mg/kg extract daily while group4 received no treatment but feed and water ad libidum. Results from this study shows higher FSH (0.52 m/u/ml), LH (1.32 m/u/ml) and Prolactin (1.17ng/ml) among group3 treated at 200mg/kg extract of the Hunteria umbellata fruit. This was closely followed by group2 treated at 400mg/kg body weight ie LH (0.74 m/u/ml), Prolactin (1.04ng/ml) compared with group4 FSH of (0.25 m/u/ml), LH (0.37 m/u/ml) and control. The testosterone level was higher in group2 (4.65 ng/ml) compared with other groups. The study shows higher renal markers in group4 administered $cdcl_2$ without treatment. However in group2 treated at 400mg/kg extract had decreased renal markers followed by group3 treated at 200mg/kg extract.ie results from this study further indicate higher renal indices among group4 administered oral $cdcl_2$ daily with urea having (10.70mmol/l), Creatinine (178.5µmol/l) and uric acid (430.0µmol/l) compared with the control group of (9.6mmol/l),(165µmol/l) and (375µmol/l) respectively.

This study have shown clearly the physio-pharmacological effect of Hunteria umbellata fruit extract in increasing glomerullar filtration rate to clear off these parameters from the blood and restore normal renal function.

KEYWORDS: Cadmium, Creatinine, Hunteria umbellata, Hormones, Male, Testosterone.

INTRODUCTION

The reproductive hormones are essential for normal development of the male organs and continuity of the human species. Cadmium is a heavy metal that causes severe structural damages to male testis ie sertoli cells of leydig, seminiferous tubules leading to the reduction in sperm count. The rate of infertility among males has been on the rise over the century accounting for about 40–50% globally (Kilchevsky and Honig 2012) (Ojqi *et al.,* 2020). Increase in environmental pollution is a predisposing factor leading to male infertility consistency due to incessant exposure during their life expectancy including embryonic developmental period. Environmental pollution on humans and animals can be associated with infertility due to the release of different forms of anthropogenic activities in the environment. The use of cadmium produces a form of anthropogenic pollution and its exposure to humans is becoming a course for public health concern with a half life of almost forty years. Cadmium depresses mitochondrial function and generates free radicals that induce apoptosis. Exposure to cadmium disorganizes the normal coordination of the hypothalamic–hypophysis–testis axis resulting in decrease testicular function and spermatogenesis (Marcela *et al.,* 2021).

Significant quantities of cadmium are channel into the soil, rivers where they become accumulated in water with sub lethal effect and death to both humans and aquatic population (Uluturban *et al.,* 2007) and (Nadmitov *et al.,* 2015). Fabio *et al,* (2016) in his study observed the distribution of heavy metal in the internal organs of fishes such as the spleen and liver which should not be considered for human consumption due to their detrimental effect on human health. The severity of effect depends on the level of concentration in the tissues. The major routes of cadmium into the circulatory system is either through ingestion or inhalation (tobacco) and accumulate in the epithelial cells of proximal tubule in the kidney resulting in a generalized reabsorption dysfunction through necrotic mechanism of renal epithelia cell death.

The toxicity of cadmium at various levels has been previously studied. However a detailed account of its basis of toxicity on hormonal profile, biochemical parameters and the ameliorating effect of aqueous Hunteria umbellata fruit extract is yet to be fully unearthed. Due to rapid industralization, air pollution has emerged in an alarming rate in the Niger Delta region of Nigeria. The cadmium and lead level among consuming vegetables and other food stuffs harvested from oil and gas flares polluted environment have been reported in an alarming level by Solomon *et al.*,2021; Sumera *et al.,* 2014). Hunteria umbellata fruit has been used for the treatment of so many illnesses traditionally. Simple sugars, alkaloids, saponins presence have have been revealealed phytochemically from the fruit. The tree is mostly found in the rain forest zone in the sourthern part of Nigeria. The fruit ranges between 5.25cm in lenghth and despite the wide range of the fruit for variuos ailment, there has been no scientific basis to back up its used therapeutically (Igbe *et al.,*2009).

MATERIALS AND METHODS

This experimental study was aim to determine the Physiological effect of Hunteria umbellata treatment on cadmium chloride induced wistar rats. The rationale behind the use of albino wistar rats for research studies is their physiological similarities to humans and is small, sociable and easy to handle, (Barnett, 1963).

STUDY DESIGN

TABLE1: EXPERIMENTAL ANIMAL GROUPINGS AND DOSAGE ADMINISTERED

Groups	Route of Administration– orally	Daily Dosage administered : aqueous fruit extract of Hunteria Umbellata (mg/kg) and $cdcl_2$ (ml)
1	Control: Fed with normal feeds and Water ad libitum.	Nil
2	Induced with $cdcl_2$	400mg/kg
3	Induced with $cdcl_2$	200mg/kg
4	Administered $cdcl_2$ daily	0.03 $cdcl_2$/kg body weight

The Administration was done daily for one month ie 30 days. The 40 wistar rats were randomly divided into four (4) groups of ten (10) rats each. Six deaths were reported in group 4 while there were four deaths in group2 and five in group3 during the time of study respectively.

SAMPLE COLLECTION AND ANALYSIS

The animals were anesthetized using 0.5ml ketamin intramuscularly; thereafter they were layed in a supine position, on a dissecting board and with limbs fastened to the board with dissecting pins under this condition for harvesting of organs while cardiac puncture of sample blood from the left chamber of the heart was performed.

A total of 5ml blood was collected into a 5ml syringe needle and then introduced into plain bottles. The serum was separated and collected for the analysis of hormonal profile such as testosterone, Prolactin, luteinizing hormone, follicle stimulating hormone, and biochemical parameters ie urea, Creatinine, and uric acid. Details of methods of analysis are outline in subsequent pages below.

Figure 1: Some laboratory animals used for this study

Figure 2: Collection of blood samples by the Researchers through cardiac puncture in the BMS University of port Harcourt.

Collection of fruits: Fresh fruits of *Hunteria Umbellata w*ere purchased from Swali market, Bayelsa State Nigeria. The fruits were identified by the department of plant Science, University of Science and Technology, Rivers state Nigeria.

WEIGHT MEASUREMENT

The animal's weight was obtained weekly using a weighing scale (Golden Meter USA) calibrated in grammes during the thirty (30) days during the period of the study.

STATISTICAL ANALYSIS

The data were analysis with SPSS version 23.0. The results are presented as mean± standard deviation and p-value of 0.05 and below considered significant.

Fruit Extraction

The extraction and photochemical analysis was done in the Central Laboratory for Phytomedicines, Department of Pharmacognosy and Phytotherapy, Faculty of Pharmaceutical Sciences, University of Port Harcourt by a Principal investigator (Natural products, Analytical and Functional Food Chemistry Research) Nuclei for Phytomedicines and chemical Ecology Research Group Nigeria.

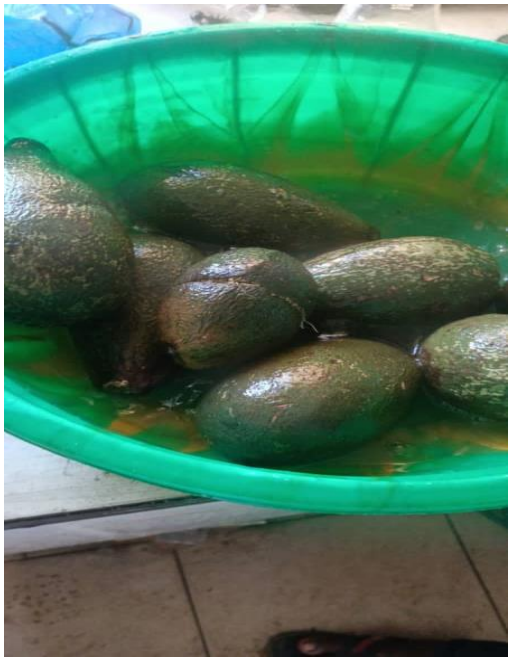

Figure 3: Hunteria umbellata fruits

Testosterone (Tiets Niu method) unit ng/ml

- Procedure: format the micro plate's wells for each test and standard.
- Pipette 10ul of the standard & sample into appropriate wells.
- Add 50ul of the testosterone enzyme reagent into all the wells
- Swirl the micro plate gently for 20 – 30 seconds to mix
- Add 50ul of testosterone biotin reagent to all well.
- Swirl the micro plate gently for 20-30 seed to mix
- Cover and incubate for 60mins at RT

- Add 350ul of water buffer and decant. Repeat two additional times for a total of three washes.
- Add 0.lml of working substrate solution to all wells.
- Incubate at reagent for15mins
- Add 50ul of stop solution to each well and gently mix for 15-20 seconds
- Read the absorbance in each well at450nm

Progesterone (Tiets NW Method) Unit, ng/ml

- Procedure format each micro plate wells for std and test
- Pipette 25ul of sample and std into appropriate wells
- Add 50ul of progesterone enzyme reagent to all wells
- Swirl the micro plate gently for 10-20 seconds to mix.
- Add 50ul progesterone biotin reagent to all the wells.
- Swirl the micro plate gently for 20 - 30seconds t0mix
- Cover and incubate for 60mins at R.T
- Discard the content add 350ul of wash buffer and decant.

Repeat two additional times for a total of three washes

- Add 0.1ml of substrate solution to all wells
- Incubate at R.T for20mins
- Add50ulofstopsolutiontoeachwellandgentlymixfor15-

20 seconds

- Ready the absorbance in each well at450nm
  Estradiol (E_2) (Tiets NW method) Unit pg/ml
- Procedure: Format micro plate well for each std &test
- Pipette 25ul of sample & std into appropriate wells
- Add 50ul of Estradiol biotin reagent to all wells.
- Swirl the micro plate gently for 20-30 seconds to mix
- Cover & incubate for 30mins at R.T
- Add 50ul of Estradiol enzyme reagent to all wells
- Swirl the micro plate gently for 20-30 seconds to mix
- Cover & incubate for 90mms at RT
- Discard the content and add 350ul of wash buffer and discard the content and repeat two times to make a total of three times, blot the plate dry with absorbent paper.
- Add 0.1ml of substrate solution to all wells
- incubate at R.T for 20mins
- Add 50ul of stop solution to each well and gently mix for 15-20 seconds
- Read the absorbance in each well at450nm.

Prolactin (Tiets N Method) Unit ng/ml

- Procedure: Format micro plate wells for std and test.
- Pipette 25ul of sample & std into appropriate wells
- Add 0.1ml of prl enzyme reagent into all wells.
- Swirl the micro plate gently for 20 – 30 seconds to mix.
- Cover and incubate for 60mins at R.T
- Discard the contents of the micro plate, Add 350ul of wash buffer and decant. Repeat two times to make a total of three times. Blot the plate dry with absorbent paper.
- Add 0.1ml of working substrate solution to all wells.
- Incubate for 20mins at R.T
- Add 50ul of stop solution to each well and gently mix for 15-20 seconds
- Read the absorbance in each well at450nm.

FSH & LH (Layman L,C Method) unit m/u/ml

- Procedure: Format micro plate well for each standard and test.
- Pipette: 50ul of sample and standard into appropriate wells.
- Add 0.1ml of the FSH & LH enzyme reagent solution to all wells
- Swirl the micro plate gently for 20-30secs to mix.
- cover and incubate for 60mins and RT
- Discard the well content
- Add 350ul of wash buffer, discard and repeat two times to make a total of three washes. Blot the plate dry with absorbent paper.
- Add 0.1ml of working substrate solution to all wells.
- Incubate for 20mins at RT.
- Add 50ul of stop solution to each well and gently mix for 15-20 seconds.
- Read the absorbance in each well at450nm

Renal functions analysis

Creatinine (µmol/L): Agappe Diagnostics (Switzerland GMBH) was used to determine the serum Creatinine using modified jaffe's methods and linear up to 24mg/dl with no interference with Bilirubin of up to 10mg/dl.Principle: Creatinine reaction with picric acid lead to the production of a colored compound, Creatinine alkaline picrate. The change in absorbance is proportional to the Creatinine concentration. Other materials used for the Creatinine test includes pipettes, tips, test tubs, racks and analyser with product numbers: 51009001

- Creatinine concentration= (T2-T1 of sample x 2T2-T1 of standard

Urea (mmol/l): (Urease-Berthelot Method) mmol/L

- Principle: Urea in serum is hydrolyzes to ammonia in the presence of urease. The ammonia is then measure photometrically by Berthelot's reaction.
  Procedure: Label the tubes as test, standard and blank.

- Pipette 0.1ml of the reagent (R_1) into all the tubes
- Add 10µl of the samples, standard and d/win to appropriate tubes- mix and incubate at 37℃ for10mms.
  Pipette 2.5ml of R_2& R_3 to all the tubes.
- Mix and incubate at 25°c for15mins
- Read and record the absorbance at 546nm.
- Randox urea test kit (United Kingdom) was used to analyze the serum urea of the samples.

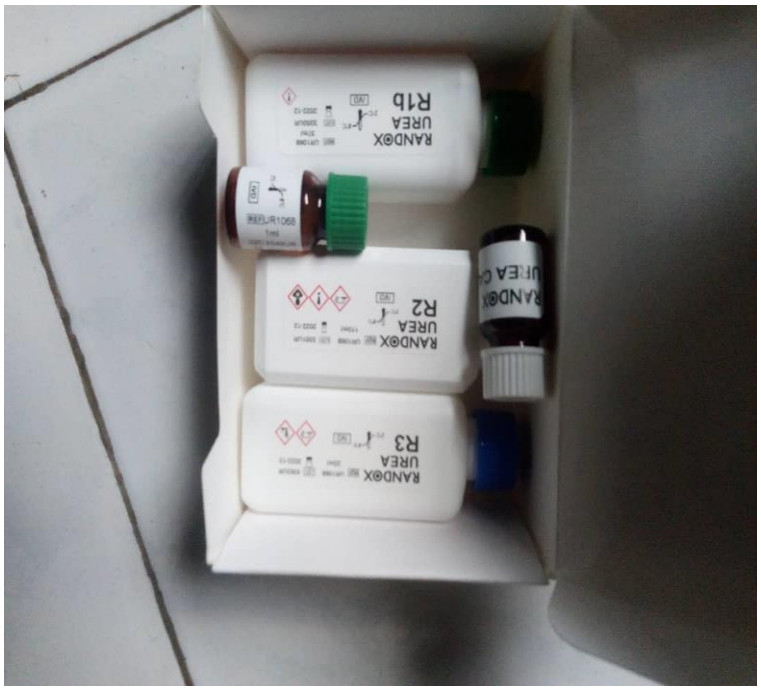

Figure 4: Urea test kits

Uric Acid (µmol/L); Randox (United Kingdom) uric acid test kits, using enzymatic colorimetric method.

RESULTS

TABLE 2: MEAN VALUES OF HORMONAL PROFILE/RENAL PARAMETERS

Groups	FSH (m/u/ml)	LH (m/u/ml)	TES (ng/ml)	PRL (ng/ml)	UREA (mmol/L)	CREATININE (µmol/L)	U.ACID (µmol/L)	Wt(g)
1 (control)	0.41±0.13	0.56±0.10	2.83±0.11	0.74±0.04	9.6±0.20	165±1.32	375±3.0	141±5.20
2 (400mg/kg)	0.21±0.01	0.74±0.05	4.65±0.28	1.04±0.11	5.40±0.10	159.5±2.50	386.0±6.0	161±7.60
3. (200mg/kg)	0.59±0.04	1.32±0.03	0.74±0.07	1.17±0.13	8.70±0.30	161.0±1.00	392±20	147±5.50
4 (0.05ml $cdcl_2$)	0.25±0.04	0.37±0.03	4.48±0.28	0.86±0.06	10.70±0.10	178.5±3.5	430.0±5.0	135±10.1

TABLE 3: COMPARISON OF FSH SIGNIFICANT P-VALUES OF CONTROL WITH OTHER GROUPS

FSH

FSH	Group 2	Group 3	Group 4
Group 1(control)	0.03	0.04	0.06

Using the analysis of variance (ANOVA) test, the mean FSH levels of groups 2 and 3 were significantly different from the FSH level of the control group (p-value: 0.03 and 0.05 respectively).

Also, there was a statistically significant difference in the mean FSH values of the three test groups (F: 50.5; p-value: 0.00). The mean FSH levels of group 2 significantly differed from the FSH levels of group 3 (p-value: 0.00) and the mean FSH levels of group 3 significantly differed from the FSH levels of group 4 (p-value: 0.01). Mean FSH values of groups 2 and 4 were however not significantly different from one another (p-value: 1.00).

TABLE 4: COMPARISON OF LH SIGNIFICANT P-VALUES OF CONTROL WITH OTHER GROUPS

LH	Group 2	Group 3	Group 4
Group 1 (control)	0.09	<0.00	0.07

Regarding the LH values in this study, it was found that the mean LH levels of group 3 was significantly different from the LH level of the control group (p-value: <0.00).

Also, there was a statistically significant difference in the mean LH values of the three test groups (F: 180.1; p-value: 0.00). The mean LH levels of group 2 significantly differed from the LH levels of groups 3 (p-value: 0.00) and 4 (p-value: 0.01). Also, the mean LH levels of group 3 significantly differed from the mean LH levels of group 4 (p-value: 0.00).

TABLE 5: COMPARISON OF TESTOSTERONE SIGNIFICANT P-VALUES OF CONTROL WITH OTHER GROUPS

TES	Group 2	Group 3	Group 4
Group 1(control)	0.01	0.01	0.02

Regarding the TES values in this study, it was found that the mean TES levels of groups 2, 3 and 4 were significantly different from the TES level of the control group (p-values: 0.017, 0.010 and 0.025 respectively).

Also, there was a statistically significant difference in the mean TES values of the three test groups (F: 93.6; p-value: 0.00). The mean TES levels of group 2 significantly differed from the TES levels of group 3 (p-value: 0.00) and the mean TES levels of group 3 significantly differed from the TES levels of group 4 (p-value: 0.00). Mean TES values of groups 2 and 4 were however not significantly different from one another (p-value: 1.00).

TABLE 6: COMPARISON OF PROLACTIN SIGNIFICANT P-VALUES OF CONTROL WITH OTHER GROUPS

PRLOLACTIN	Group 2	Group 3	Group 4
Group 1(control)	0.47	0.16	1.00

Regarding the PROL values in this study, it was found that the mean PROL levels of groups 2, 3 and 4 were not significantly different from the PROL level of the control group (p-value: 0.09).
Also, there was no statistically significant difference in the mean PROL values of the three test groups (F: 2.23; p-value: 0.25).

TABLE 7: COMPARISON OF UREA SIGNIFICANT P-VALUES OF CONTROL WITH OTHER GROUPS

UREA	Group 2	Group 3	Group 4
Group 1(control)	<0.00	0.11	0.05

Regarding the UR values in this study, it was found that the mean UR level of group 2 was significantly different from the UR level of the control group (p-values: <0.00).
Also, there was a statistically significant difference in the mean UR values of the three test groups (F: 195.4; p-value: 0.00). The mean UR levels of group 2 significantly differed from the UR levels of group 3 (p-value: 0.00) and group 4 (0.00). Also, the mean UR levels of group 3 significantly differed from the mean UR levels of group 4 (p-value: 0.01).

TABLE 8: COMPARISON OF CREATININE SIGNIFICANT P-VALUES OF CONTROL WITH OTHER GROUPS

CREATININE	Group 2	Group 3	Group 4
Group 1(control)	0.91	1.00	0.07

Regarding the CR values in this study, it was found that the mean CR levels of the 3 test groups were not significantly different from the CR level of the control group (p-values: 0.07).
Also, there was a statistically significant difference in the mean CR values of the three test groups (F: 17.2; p-value: 0.023). The mean CR levels of group 2 significantly differed from the CR levels of group 4 (p-value: 0.04). Also, the mean CR levels of group 3 significantly differed from the mean CR levels of group 4 (p-value: 0.05).

TABLE 9: COMPARISON OF URIC ACID SIGNIFICANT P-VALUES OF CONTROL WITH OTHER GROUPS

URIC ACID	Group 2	Group 3	Group 4
Group 1(control)	1.000	1.000	0.134

Regarding the UA values in this study, it was found that none of the mean UA levels of the test groups were significantly different from the UA level of the control group (p-values: 0.078). To buttress this, there was no statistically significant difference in the mean UA values of the three test groups (F: 3.7; p-value: 0.155).

DISCUSSION

The results from this study indicate that group2 treated at 400mg/kg body weight aqueous extract of the Hunteria umbellata fruit extract daily had a higher weight of 160g; this was followed by group3 treated at 200mg/kg of 147g compared with the control group of 141g body weight. However in group4 administered 0.03ml daily of 2grammes $cdcl_2$ diluted with 200mls of distilled water has the lowest weight value of 135g compared with other groups. This study is in line with Solomon *et al,* 2022 observation of an elevated body mass index of 16.8kg/mg^2 among adolescent school girls in non gas flaring communities compared with adolescent girls exposed to gas flares of 14.6kg/m^2 body

mass index in Yenagoa Bayelsa state, Nigeria. Gas flares contains heavy metals such as cadmium, lead etc which has been observed by Solomon *et al*, 2021among pregnant and non pregnant women in Bayelsa state. Similar weight gain in wistar rats induced with aloxan monohydrate treated with Hunteria umbellata fruit extract was also observed by Uvoh *et al*, in 2023.

The main values of follicle stimulating hormone in group2 treated at 400mg/kg body weight daily was 0.21 m/u/ml while group3 treated at 200mg/kg was 0.59 m/u/ml compared with the control of 0.41 m/u/ml and group4 0.25 m/u/ml. Higher mean values of luteinizing hormone was observed among group3 (1.37 m/u/ml) compared with the control of (0.56 m/u/ml). However the values among group2 treated at 400mg/kg was 0.74 m/u/ml compared with group4 administered cadmium chloride (0.37 m/u/ml) having decrease hormonal level. Follicle stimulating hormone and testosterone act on specific cells in the testes called sertoli cells responsible in the production of spermatogenesis in the seminiferous tubules and stored in both epididymis. Sperm production production is dependent on LH released from pituitary gland which takes about 64days for new sperm to be made with approximately 1000 sperm cells pruduced during each heart beat (Donnell *et al.,* 2019; Corona *et al.,* 2016).

Testosterone level was higher among group2 (4.65 ng/ml) and decrease in group3 (0.74 ng/ml) in comparison with the control (2.83 ng/ml) and group4 (4.48 ng/ml) respectively. Testosterone is a male hormone produced from interstitial cells when stimulated by luteinizing hormone from the anterior pituitary gland. Certain cancers in the testicle may cause an increase testosterone level associated with high blood pressure, increase libido, appetite, excess body hair and liver problems while the decrease in testosterone result in obesity, depression etc. Studies have shown that environmental exposure to cadmium may produce developmental toxicity of the reproductive organs (Peter *et al.,* 2020). The main source of cadmium exposure is through food ingestion and it is effectively absorbed in the kidney in relation to male infertility crisis (peter *et al.,* 2020; Solomon *et al.,* 2021).

Prolactin: Regarding Prolactin, the level was higher among group3 treated at 200mg/kg extract according to body weight (1.17 ng/ml and group2 at 400mg/kg (1.04 ng/ml) following cadmium chloride toxicity induction compared with group4 (0.86 ng/ml) administered $cdcl_2$ daily and the control (0.74 ng/ml). This study shows that an increased in Prolactin among group2 (1.04 ng/ml) affect testosterone level in group2 to (4.65 ng/ml) while increase Prolactin in group3 (1.17 ng/ml) affect the level of LH secretion in group3 to 1.32 m/u/ml above other groups. This effect was also observed in group3 FSH (0.59 m/u/ml).Prolactin is a polypeptide hormone predominantly secreted by lacto tropic cells of the anterior pituitary gland. It has multifaceted roles in growth, endocrine, osmoregulation, metabolism and immunological function. Prolactin act via transmembrane receptors of erythropoietin, interleukin, leptin etc.In males the mechanism by which Prolactin and its receptors remains blurred. (Sanketa *et al.,* 2019). Normal Prolactin level in males ranges below (17ng/ml). Increase in Prolactin level stimulate the secretion of LH from its receptors of leydig which in turn increase testosterone secretion and spermatogenesis. Elevated Prolactin level in males can result in decrease sex appetite, impotence, galactorhea and infertility (ASRM).

Biochemical markers: The results from this study further indicate higher renal indices among group4 administered oral $cdcl_2$ daily with urea having (10.70mmol/l), Creatinine (178.5µmol/l) and uric acid (430.0µmol/l) compared with the control group of (9.6mmol/l),(165µmol/l) and (375µmol/l)

respectively. However the urea and Creatinine levels were lower in group2 (5.40mmol/l; 159µmol/l) treated at 400mg/kg extract daily compared with group3 treated at 200mg/kg (8.70mmol/l; 161µmol/l).Similar increase was also observed in the uric acid level among group2 and group3 compared with group4 though group1 which is the control has the lowest uric acid level in this study. Increase in serum Creatinine clearance leads to a decrease in its serum concentration but a decrease in its clearance thus result in its increase within the blood (Kate *et al.,* 2018). Cadmium and lead components result in body fluid volume depletion and destruction to the macula densa to increase both renal indices and the release of proteolytic enzymes from the specialized cells of the afferent arterioles to induce a cascade of reactions that will lead to the development of high blood pressure (Ovuakporaye, 2016).

Conflict of interest: There is no conflict of interest among the authors.

CONCLUSION

Increases in Prolactin level stimulate the secretion of LH from its receptors of leydig which in turn increase testosterone secretion and spermatogenesis. The results from this study further indicate higher renal indices among group4 administered oral $cdcl_2$ daily without treatment compared with the control group.

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