ISSN 2349-7823

International Journal of Recent Research in Life Sciences (IJRRLS)
Vol. 10, Issue 1, pp: (33-40), Month: January - March 2023, Available at: www.paperpublications.org

REPRODUCTIVE HORMONE CHANGES IN MALE WISTAR RATS ADMINISTERED GRADED DOSES OF *Chromolaena odorata* LEAF EXTRACT

Okwakpam, FN[1], Omeodu SI, Odinga, T[1], Ijeoma, SN[3]

[1]Department of Biochemistry, Faculty of Science, Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, Nigeria.

[2]Department of Biochemistry, Faculty of Science, University of Port Harcourt, Choba, Nigeria.

[3]Department of Zoology and Environmental Biology, College of Natural Sciences, Michael Okpara University, Agriculture Umudike, Abia State, Nigeria.

Corresponding Author: Okwakpam FN, Department of Biochemistry, Faculty of Science, Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, Nigeria.

DOI: https://doi.org/10.5281/zenodo.7701708

Published Date: 06-March-2023

Abstract: **The effect of ethanol extract of *Chromolaena odorata* on reproductive hormones and histology of the testes in adult male albino rats was investigated in this study. Twenty male albino rats weighing between 120-130g were used for this study. They were randomly divided into four (4) groups, n=5. Group 1 served as control group and administered with distilled water only. Groups 2, 3, and 4 were orally administered with 250mg/kg, 500mg/kg and 100mg/kg body weight (b.w) of the extract respectively. At the end of experimental period of 14 days, the animals were fasted overnight and sacrificed by cervical dislocation. Testis tissues and blood samples were collected and taken to the laboratory for biochemical analysis using standard procedures. The result showed that 250mg/kg b.w of *Chromolaena odorata* administered to group 2 rats had no significant difference ($p>0.05$) in Luteinizing hormone (LH), follicle stimulating hormone (FSH) , prolactin and testosterone serum concentration when compared to the control group while groups administered with 500mg/kg and 1000 mg/kg b.w of *Chromolaena odorata* extract respectively, had significant ($p<0.05$) decrease in concentration of LH, FSH, testosterone and a significant increase in serum prolactin concentration. There was normal testicular architecture in groups 2 and 3 administered with 250mg/kg and 500mg/kg b.w. However, testicular atrophy was observed in group 4 administered with 1000mg/kg b.w. from the histology of the testes. From this study, it can be deduced that the extract exhibited potential to induce spermatogenic arrest in male albino rats.**

Keywords: **Reproductive hormones, Testes, Histology, Graded Doses and *Chromolaena odorata.***

1. INTRODUCTION

The treatment of ailments with medicinal plants is an age long practice by which man have depended on as a reliable means of healing and minimizing disease infections (Ayuk *et al*., 2017). A medicinal plant is any plant which, in one or more of its parts, contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs (Sofowora *et al*., 2013). Although modern medicine has been greatly developed, many rural people especially in African developing countries still depend on medicinal plants for treatment of diseases. This may be because of poverty and high cost of modern health care services. It has been estimated that 80% of the world's population rely on herbal medicine for their primary health care (Chit *et al*., 2012). *Chromolaena odorata* commonly known as Siam weed, Awolowo Leaf, Bitter bush, Jack in the bush, Independent weed, Elizabeth weed, Christmas bush, devil's weed is an example of

ISSN 2349-7823

International Journal of Recent Research in Life Sciences (IJRRLS)
Vol. 10, Issue 1, pp: (33-40), Month: January - March 2023, Available at: www.paperpublications.org

medicinal plant that is commonly used in Nigeria for treating ailments. It is a perennial scrambling flowering shrub that is mainly a weed in plantation crops. It is used mainly in the rural areas singularly or in combination for the treatment and management of several ailments. The leaves of the plant has been shown by several researchers to possess antibacterial effect, antidiarrheal effect, anti-diabetic effect, anthelmintic properties, anticonvulsant effect, analgesic effect, antihypertensive effect, anti-inflammatory effect, blood clotting and wound healing effect (Anyanwu *et al*., 2018). A formulation prepared from the aqueous extract of the leaves has been licensed for clinical use in Vietnam (Vaisakh *et al*., 2012).

According to Usunobun (2016), the phytochemicals found in aqueous and ethanolic leaf extract of *Chromolaena odorata* includes Alkaloids, cardiac glycosides, tannins, saponins, steroids, flavonoids and the mineral compositions includes calcium (Ca), sodium (Na), copper (Cu), iron (Fe), potassium (K), and zinc (Zn).

Although *Chromolaena odorata* was used traditionally for its healing properties, it never enjoyed the status of a medicinal herb. Instead, efforts were always made to eradicate the so-called weed (Vaisakh *et al*., 2012) but recently the use of *Chromolaena odorata* as herbal medicine has greatly increased to an alarming level with users not concerned about the safety of the plant. Although medicinal plants are useful therapeutically, but the unregulated usage of these plants has brought about a number of health-related problems like infertility (Oigbochie *et al*., 2019). Though infertility is not life threatening, it has been described as a radical life changing problem because it carries with it significant psychological trauma (Uadia *et al*., 2015). It affects an estimate of 60-80 million couples globally (Bhamani *et al*., 2020). In Africa, it is estimated that infertility affects 20–35 million couples (Uadia *et al*., 2015). It has contributed to many broken homes. In Nigeria, it is common for women to be blamed for the childlessness of their marriage because the average Nigerian man believes that he is fertile as long as he can maintain an erection and have sex. This belief, associated with shame has made it difficult for most men to visit fertility clinics with their wives. But studies have proven that male fertility is not just dependent on a man's ability to have sex, and scientific surveys has reported that 40-50% of all infertility cases in Nigeria is due to "male factor" infertility (Uadia *et al*., 2015).

This study is therefore aimed at evaluating the effect of ethanolic leaf extract of *Chromolaena odorata* on reproductive hormones and the histology of the testes in adult male albino rats.

Figure 1: Leaves and young flower- heads of *Chromolaena odorata* (Kent *et al*., 2001).

2. MATERIALS AND METHODS

Collection of Plant Material

Leaves of *C. odorata* were collected from around Rivers State University campus and were authenticated by the Department of Plant Science, Rivers State University, Port Harcourt.

Preparation of Plant Extract

The leaves of the plant were thoroughly washed under running water to remove contaminants and were air dried at room temperature in the laboratory for 7 days. The dried leaves were then grinded into fine powder using electric grinder and were dissolved in 1L of absolute ethanol and allowed to stand for 48 hours. The extract was filtered into a clean beaker

using Whitman No. 1 filter paper, the filtrate was concentrated by heating at 60^{o}C using a water bath to get the extract that was used for the study. Then the extract was reconstituted in distilled water at different concentrations using Tween 80 as an emulsifier.

Preparation of Sample Stock

3g of the extract was dissolved in 10ml of distilled water to make a concentration of 300mg/ml. The stock solution was prepared daily throughout the period of administration. The extract was administered to the experimental rats with their respective dosages base on their body weight.

Experimental Animals

Twenty (20) appreciably healthy adult male albino rats weighing between 120-130g used for this study were obtained and housed in the animal house of the Department of Biochemistry, Rivers State University, Port Harcourt. The rats were housed in ventilated cages containing saw dust (for water absorption) with wire mesh top and were allowed to acclimatize to the animal house for one week and they had free access to standard pellet feed and water *ad libitum*. After acclimatization, the rats were randomly divided into four groups of five (5) rats each. Group 1 that served as the control received distilled water, groups 2, 3 & 4 that served as the treatment groups received 250, 500, 1000 mg/kg body weight of *Chromolaena odorata* respectively orally by gastric gavages for 14 days. The choice of these doses was based on rational decision and to maintain a non-lethal dose based on findings from acute toxicity evaluation of the extract as reported by Ijeoma *et al*. (2014).

Collection of Samples for Biochemical Analysis

On the last day of the dosage administration, the animals were fasted overnight, weighed and three (3) animals from each group were sacrificed via cervical dislocation and their blood were collected in plain bottles and the testes were collected in culture bottles containing 10% formaldehyde. The blood was allowed to clot and the serum was collected for biochemical analysis to determine the levels of the reproductive hormones in the serum.

Determination of Serum level of reproductive hormone profile

Serum FSH, LH, Testosterone, and Prolactin was determined using enzyme immunosorbent assay (ELISA) kit (Diagnostic Automation/Cortes Diagnostic Inc. Immuno Diagnostics CA, Woodland Hills, Califonia) according to the manufacturer's instructions.

Histological Examination

For Histological examination, the testis were dissected out, blotted free of blood and weighed with the help of a digital weighing balance and were fixed in alcoholic fixative and embedded in paraffin. Transverse sections of the organs were cut at 5 µm and stained with hematoxylin and eosin and were studied under light microscope (Nikon) at 100 and 400 magnifications. Slides of all the groups were studied and photographed by Cannon digital camera.

Statistical Analysis

Differences between the groups were determined by one-way analysis of variance (ANOVA) and post hoc testing was performed for intergroup comparisons using Tukey's test using SPSS software version 20. Values were regarded as significantly different at $p<0.05$.

3. RESULTS

Effect of *Chromolaena odorata* leaf extract on serum concentration of reproductive hormones of adult male albino rats

From Figure 2-5, after 14 days of administration of *Chromolaena odorata* leaf extract , there were no significant ($p>0.05$) difference in the serum concentrations of the luteinizing hormone, follicle stimulating hormone, testosterone and prolactin in group 2 animals who received 250mg/kg body weight of the extract as compared to that of control group. However, administration of the extract for 2 weeks at higher doses of 500mg/kg and 1000mg/kg resulted in significant increase in serum concentration of prolactin and a significant ($p<0.05$) decrease in the serum concentrations of luteinizing hormone, follicle stimulating hormone and testosterone of the treated groups (group 3 and 4 respectively) as compared to the control group**.**

ISSN 2349-7823

International Journal of Recent Research in Life Sciences (IJRRLS)
Vol. 10, Issue 1, pp: (33-40), Month: January - March 2023, Available at: www.paperpublications.org

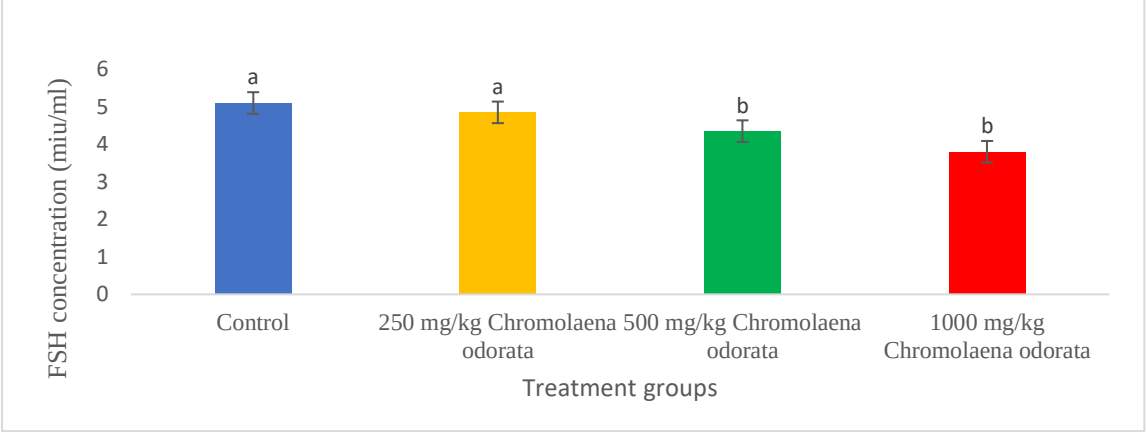

Figure 2: Effect of *Chromolaena odorata* leaf extract on serum concentration of follicle stimulating hormone of adult male albino rats.

Values are means ± Standard Error Mean (SEM) n=5. Values with different superscript are statistically different at ($p<0.05$). Superscript (a, b) compares group two to four (250mg/kg, 500mg/kg and 1000mg/kg) to group one (control Group)

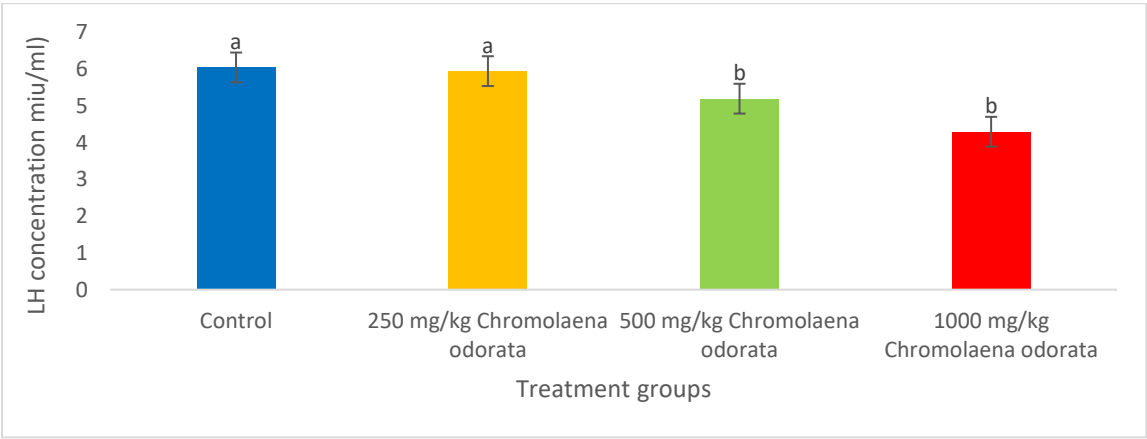

Figure 3: Effect of *Chromolaena odorata* leaf extract on serum concentration of luteinizing hormone of adult male albino rats.

Values are means ± Standard Error Mean (SEM) n=5. Values with different superscript are statistically different at ($p<0.05$). Superscript (a, b) compares group two to four (250mg/kg, 500mg/kg and 1000mg/kg) to group one (control Group)

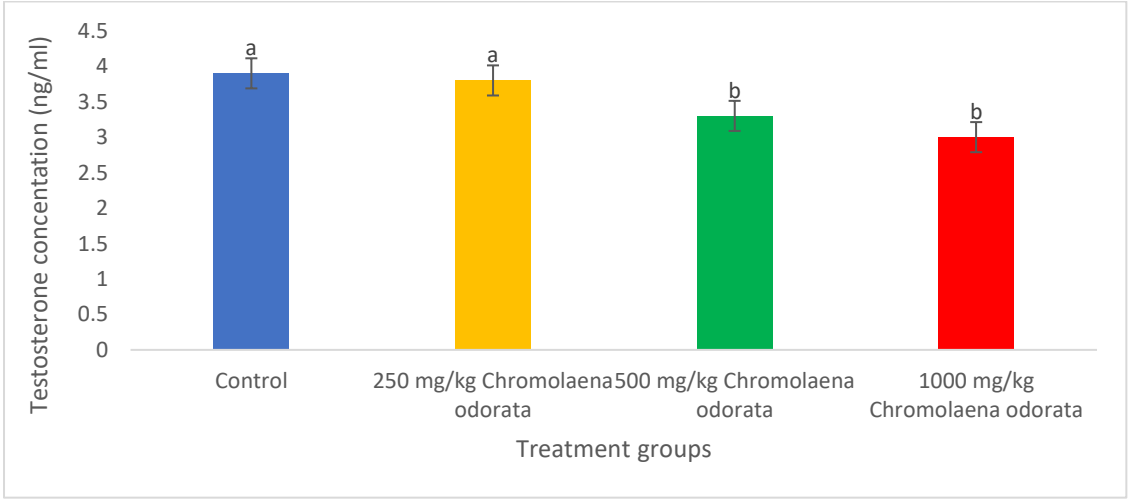

Figure 4: Effect of *Chromolaena odorata* leaf extract on serum concentration of testosterone of adult male albino rats

ISSN 2349-7823

International Journal of Recent Research in Life Sciences (IJRRLS)
Vol. 10, Issue 1, pp: (33-40), Month: January - March 2023, Available at: www.paperpublications.org

Values are means ± Standard Error Mean (SEM) n=5. Values with different superscript are statistically different at ($p<0.05$). Superscript (a, b) compares group two to four (250mg/kg, 500mg/kg and 1000mg/kg) to group one (control Group)

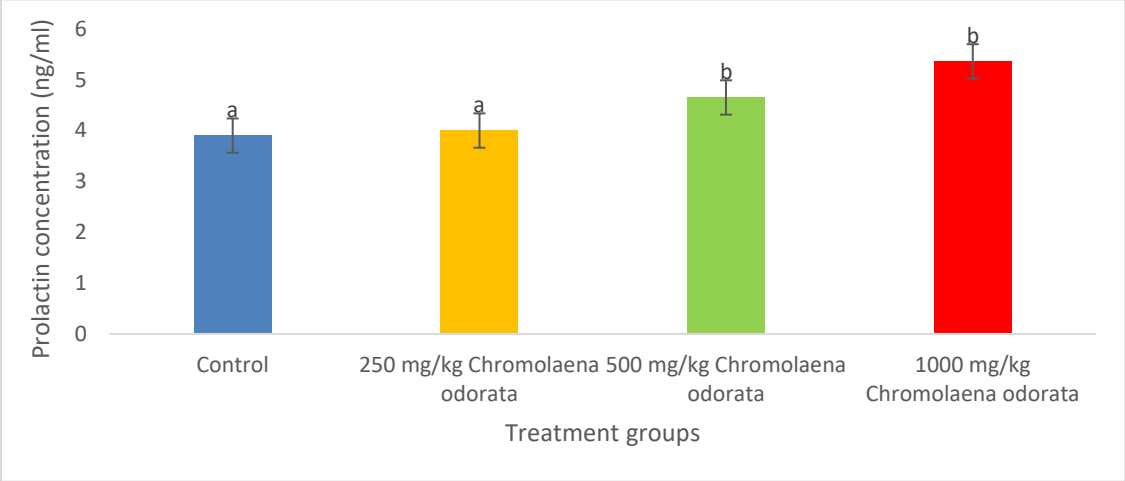

Figure 5: Effect of *Chromolaena odorata* leaf extract on serum concentration of prolactin of adult male albino rats.

Values are means ± Standard Error Mean (SEM) n=5. Values with different superscript are statistically different at ($p<0.05$). Superscript (a,b) compares group two to four (250mg/kg, 500mg/kg and 1000mg/kg) to group one (control Group).

Effect of *Chromolaena odorata* leaf extract on the Histology of the Testis of male Wistar rats

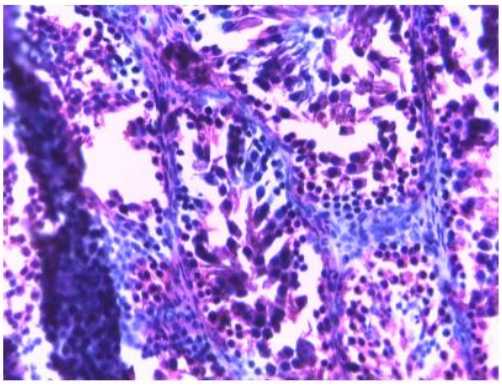

Plate 1: Photomicrograph of histology of testis of control rat (H&E mag. X 100) shows normal testicular architecture which consist of seminiferous tubules lined by stratified germinal epithelium containing proliferating germ cells (spermatids) and non-proliferating sertoli cells. The basement membrane is normal. The intervening stroma is normal.

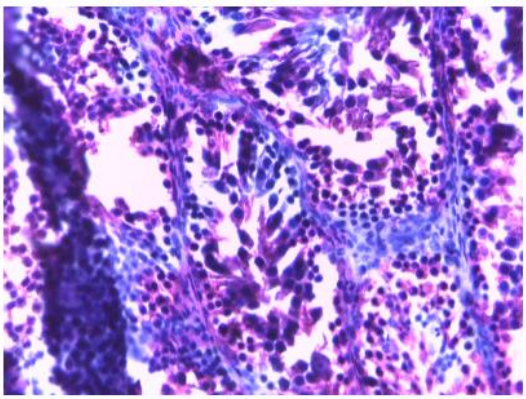

Plate 2: Photomicrograph of histology of testis of group 2 rat (H&E mag. X 100) after 14-day oral administration of 250mg/kg/*Chromolaena odorata* shows normal testicular architecture which consist of seminiferous tubules lined by

ISSN 2349-7823

International Journal of Recent Research in Life Sciences (IJRRLS)
Vol. 10, Issue 1, pp: (33-40), Month: January - March 2023, Available at: www.paperpublications.org

stratified germinal epithelium containing proliferating germ cells (spermatids) and non-proliferating sertoli cells. The basement membrane is normal. The intervening stroma is normal.

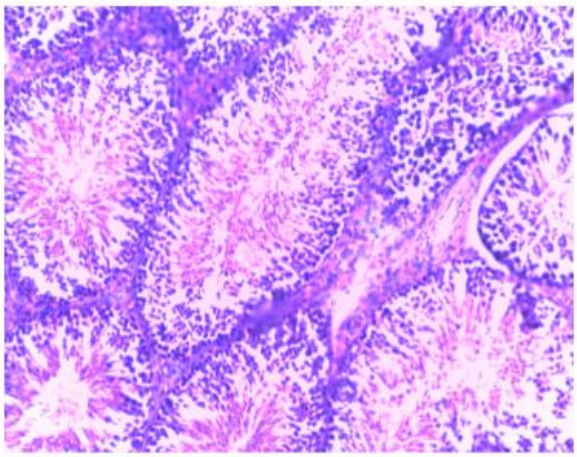

Plate 3: Photomicrograph of histology of testis of group 3 rat (H&E mag. X 100) after 14 day oral administration of 500mg/kg/*Chromolaena odorata* shows normal testicular architecture which consist of seminiferous tubules lined by stratified germinal epithelium containing proliferating germ cells (spermatids) and non-proliferating sertoli cells. The basement membrane is normal. The intervening stroma is normal.

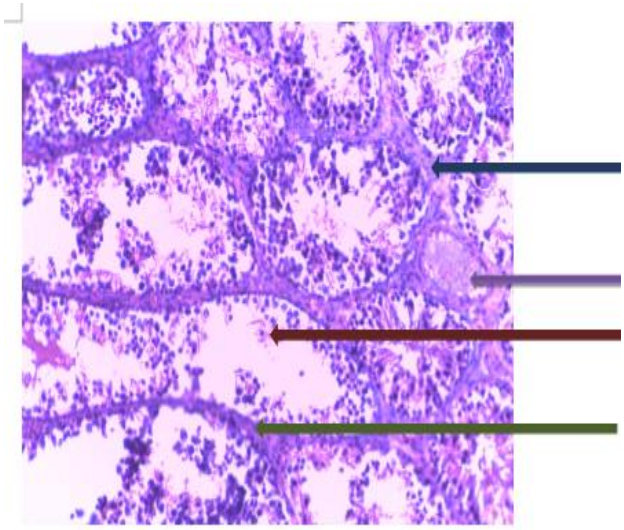

Plate 4: Photomicrograph of histology of testis of group 4 rat (H&E mag. X 100) after 14day oral administration of 1000mg/kg/*Chromolaena odorata* shows seminiferous tubules most of which have few to absent spermatids and prominent sertoli cells (red). The basement membranes are thickened (green). There is widening of the interstitium with Leydig cell hyperplasia (blue). Few congested vessels are seen within the interstitium (purple).

4. DISCUSSION

Plants have been used since human existence not only for their nutritional value but also for their therapeutic potential. Therefore, the increasing consumption of crude extracts or active constituents could warrant investigation into their safety or toxicologic implications on fertility and reproduction (Yakubu *et al*., 2012). Male fertility is dependent upon the successful perpetuation of spermatogenesis in adequate quantity (Smith *et al*., 2014). It is well known that the regulation of spermatogenesis is under the control of testosterone and follicle stimulating hormone (Alphonse *et al*., 2017). Testosterone deprivation studies performed in rodents have established that testosterone is required for germ cells to progress beyond meiosis and that testosterone is required for the release of mature spermatids during spermiogenesis. Then, the withdrawal of testosterone in Sertoli cells results in three major impairments to fertility. First, it exposes post meiotic germ cells to

autoimmune attack and cytotoxic factors. Secondly, it causes the premature detachment of round spermatids from Sertoli cells and thirdly, fully mature spermatozoa cannot be released from Sertoli cells and the germ cells are phagocytized by the Sertoli cells (Alphonse *et al*., 2017). The amount of testosterone synthesized is regulated by the hypothalamic-pituitary-testicular axis (Yakubu *et al*., 2012). When testosterone levels are low, gonadotropin-releasing hormone is released by the hypothalamus, which in turn stimulates the pituitary gland to release FSH and LH. These gonadotropins stimulate the testis to synthesize testosterone. Thus, increased levels of testosterone act on the hypothalamus and pituitary gland through a negative feedback loop to inhibit the release of gonadotropin-releasing hormone and FSH/LH, respectively (Yakubu *et al*., 2012).

This present study displays in the experimental rats an overall decrease in the testes-body weight ratio after 14-day oral administration of the ethanol extract of *Chromolaena odorata* leaves at higher doses of 500mg/kg and 1000mg/kg body weight. This significant decrease observed in the testes-body weight may be as a result of decreased synthesis of testosterone since testes is an androgen-dependent organ. The study also reveals a direct relationship between testosterone level and testes-body weight. This finding agrees with the observation of Yakubu *et al*. (2012).

It was observed that the administration of the extract at lower dose of 250mg/kg body weight did not significantly alter the reproductive hormones, testes weight and the histology of the testes. This finding refutes that of Yakubu *et al*. (2012), who reported that the 60-days oral administration of crude alkaloid extracted from the leaves of *Chromolaena odorata* at dosage of 250mg/kg body weight significantly reduced FSH, LH and Testosterone.

Also, it was observed that the administration of the extract at doses of 500mg/kg and 1000mg/kg body weight to experimental animals for 14 days decreased significantly the serum testosterone concentration compared with the control. Testosterone secretion is stimulated by LH and its role is to enhance growth and secretory activity of the testes. The decrease in testosterone level observed in this study corroborates those of Yakubu *et al*. (2012) where he worked on the effect of a 60-Day Oral Gavage of a Crude Alkaloid Extract from *Chromolaena odorata* Leaves on Hormonal and Spermatogenic Indices of Male Rats.

Administration of the ethanolic extract at doses of 500 and 1000mg/kg body weight in this study to experimental animals decreased significantly the level of serum LH. LH is responsible for stimulating testosterone production in leydig cells which subsequently stimulates spermatogenesis by acting on the sertoli cells and peritubular cells of the seminiferous tubules (Olaolu, 2018). The result observed in this current study agrees with those of Yakubu *et al.* (2012).

In addition, the FSH level was observed in this study to decrease significantly in the experimental animals administered with the extract at doses of 500 and 1000mg/kg body weight. FSH facilitates the testosterone passage via sertoli-sertoli junctional complexes by acting on sertoli cells (Olaolu 2018). This result is in agreement with the findings of Yakubu *et al*. (2012).

Following the oral administration of the extract for 14 days at doses of 500mg/kg and 1000mg/kg body weight to the experimental animals, serum concentration of prolactin increased significantly in group 3 and 4 respectively. This observation is in agreement with the work of Dabbous *et al*. (2018) who reviewed that elevated levels of serum Prolactin inhibits the pulsatile release of follicle stimulating hormone (FSH), luteinizing hormone (LH) and testosterone.

Histological examination of testes tissues after oral administration of varying concentrations of ethanolic extract of *Chromolaena odorata* showed evidence of dosage related testis toxicity which is remarkable in test group 4 administered with 1000mg/kg body weight of the extract. The extract at doses of 250 and 500mg/kg body weight did not significantly alter the structure of the testes as compared to the control group but the highest dosage (1000mg/kg) significantly causes impairment in the structure of the testes as compared to the control group. The ethanolic leaf extract of *Chromolaena odorata* suppressed the release of the gonadotropic hormones from the pituitary gland as evidenced by the drop in the FSH and LH levels; Therefore, *Chromolaena odorata* might have exerted a direct and/ or indirect effect on the hypothalamic-pituitary-gonadal axis because the FSH and LH levels did not increase to compensate for the reduced testosterone concentration in the present study, this could be because of the elevated serum concentration of prolactin and this could lead to reduced spermatogenesis and complete spermatogenic arrest (Dabbous *et al*., 2018). Therefore, *Chromolaena odorata* even though have been reported to have nutritional, as well as medicinal benefits may have adverse effect on the reproductive processes in male especially when taken at a higher dosage.

5. CONCLUSION

The results from this study has shown that short term administration of *Chromolaena odorata* at low dosages does not cause any significant change in either testicular weight, histology of the testes and serum concentration of reproductive hormones but it may induce severe damage to the testicular tissue and a decrease in the reproductive hormone levels when administered at higher doses as evident in the selected testes functions and the histology of the testes of the rats used in this study.

REFERENCES

[1] Ayuk, E.L., Ambrose, N., Aromino, S., and Oforji, C. (2017). Determination of secondary metabolites and biological potential of *Gnetum africanum* (okazi) leaves. *The Pharmaceutical and Chemical Journal*, 4(4):115-122.

[2] Sofowora, A., Ogunbodede, E. and Onayade, A. (2013). The role and place of medicinal plants in the strategies for disease prevention. *African Journal of Traditional, Complementary, and Alternative Medicines*, 10(5):210–229.

[3] Chit, S.J.W., Jonathan, S.H.L. and Hani, E. (2012). Herbal medicine: Toxicity and recent trends in assessing their potential toxic effects. *Advances in Botanical Research*, 62:365-384.

[4] Anyanwu, S., Inyang, I.J., Asemota, E.A. and Okpokam, D. C. (2018). Evaluation of Hepatotoxicity Associated with Ethanolic Extract of *Chromolaena odorata* in Wistar Rat Model. *Journal of Dental and Medical Sciences*, 17(7):1-8.

[5] Vaisakh, M.N. and Pandey, A. (2012). The invasive weed with healing properties: A review on *Chromolaena odorata*. *International Journal of Pharmaceutical Sciences and Research*, 3(1):80-83.

[6] Usunobun, U. and Ewere, G.E. (2016). Phytochemical analysis, mineral composition and *in vitro* antioxidant activities of *Chromolaena odorata* leaves. *Journal of Pharmaceutical Sciences*, 2(2):16-20.

[7] Oigbochie, E.V., Kingsley, O., and Enoghense, D.O. (2019). Aqueous root extract of *Chrysophyllum albidum* caused dose and duration dependent increases in some reproductive hormones and spermatogenic arrest in the testes of male Wistar rats. Clinical Phytoscience, 5:3

[8] Uadia, P.O. and Emokpae, A.M. (2015). Male infertility in Nigeria: A neglected reproductive health issue requiring attention. *Journal of Basic and Clinical Reproductive Sciences*, 4(2):45-53

[9] Bhamani, S.S., Zahid, N., Farooq, S., and Sachwani, S. (2020). Association of depression and resilience with fertility quality of life among patients presenting to the infertility centre for treatment in Karachi, Pakistan. *Biomedical Central Public Health*, 20(1):1607.

[10] Kent, R.J., Johnson, D.E and Becker, M (2001). The influences of cropping system on weed communities of rice in Côte d'Ivoire, West Africa. Agriculture, Ecosystems and Environment 87, 299-307.

[11] Ijioma, S.N., Okafor, A.I., Ndukuba, P.I., and Nwankwo, A.A. (2014). Hypoglycemic, haematologic and lipid profile effects of *Chromolaena odorata* ethanol leaf extract in alloxan induced diabetic rats. *Annals of Biological Sciences*, 2(3):27-32.

[12] Yakubu, M.T. (2012). Effect of a 60-Day Oral Gavage of a Crude Alkaloid Extract from *Chromolaena odorata* Leaves on Hormonal and Spermatogenic Indices of Male Rats. *Journal of Andrology*, 33(6):1199-1207.

[13] Alphonse, N., Marie, N.N., and Hubert K. (2017). Evaluation of the fertility activity of the aqueous leaves extract of *Zanthoxylum macrophylla* (Rutaceae) on male rats. *The Journal of Phytopharmacology*, 6(5):277-281.

[14] Olaolu, T.D., Rotimi, D.E., and Olaolu, A.P. (2018). Effect of alcohol infusion of *Cissus populnea* root on testicular function and serum hormone of male Wistar rats. *Asian Pacific Journal of Reproduction*, 7(3):117-122.

[15] Dabbous Z. and Atkin S.L. (2018). Hyperprolactinaemia in male infertility: Clinical case scenarios. *Arab Journal of Urology*, 16:44–52