Journal of Anatomical Sciences
Email: anatomicaljournal@gmail.com

J. Anat Sci 13(2)

Histological and Hormonal Response of Hypothalamus and Pituitary Gland to *Alchornea cordifolia* in male Wistar Rats

[1]Fischer CE, [2]Agaba EA, [3] Ujah W, [4]Fischer VA*

[1,2,] Department **of** Anatomical Sciences**,** Faculty of Basic Medical Sciences, University of Calabar, Cross River State, Nigeria.
[3]Department of Anatomy, Faculty of Basic Medical Sciences, Bingham University, Karu, Nigeria
[4]Department of Anatomy, Faculty of Biomedical Sciences, Kampala International University WC, Uganda
Corresponding author: Fischer VA

Email: victorfischer@kiu.ac.ug,

ABSTRACT

With the high rate of infertility within our society, most men have resolve into using herbs as first treatment. *Alchornea cordifolia* is one of the plants used as performance enhancers in livestock and poultry industry therefore, this research proposes to find out the effect of this extract on the hypothalamic-pituitary response to hormone profile in male wistar rats. Group A animals served as normal control and were given normal saline of 0.5ml, Group B, C and D animals received daily administration of 500mg/kgw, 1000mg/kgw and 1500mg/kgw of the extract respectively for a period of 28 days. Results from this study showed no significant differences in the serum concentrations of hormones studied, cytoarchitecture of hypothalamus and pituitary gland. Extract of *Alchornea cordifolia* leaves may not to have positive effects on the hypothalamic-pituitary response to the hormone profile in male wistar rats and as such not enhance effect on fertility.

Key words: Alchornea cordifolia, Hormones, infertility

INTRODUCTION

Hypothalamic-pituitary gonadal axis allows the brain communicate with the gonads using molecules called hormones. The male HPG axis is a system that direct and also helps to develop the process of spermatogenesis, androgen biosynthesis and their functions[1]. The system works together to regulate development, reproduction, aging and many other body processes. Its regulation relies upon a number of complex negative feedback loops which when lost result in disease. When a shift in hormones occurs, it causes a change to that particular hormone and a great negative impact on the body[2]. The pathway for the axis begins by the hypothalamus secreting gonadotropin releasing hormone (GnRH)-expressing neurons which travels to the anterior pituitary and bind to the receptors present. The anterior portion of the pituitary gland produces luteinizing hormone (LH) and follicle-stimulating hormone (FSH), and the gonads produce estrogen and testosterone[3]. Luteinizing and follicle stimulating hormones move through the blood to the testicles. The LH activates the leydig cells to release testosterone while the FSH activates the sertoli cells to release androgen binding globulin (ABG) inhibin[3]. Increase in testosterone and inhibin cause a depressing response on the pituitary and hypothalamus. This result in decreased secretion of LH and FSH, as a result, production of testosterone and inhibin is also decreased[4]. FSH and LH play an important role in communicating to the gonads. In females FSH and LH act primarily to activate the ovaries to produce estrogen and inhibin and to regulate the menstrual and ovarian cycle. Estrogen forms the negative feedback loop by inhibiting the production of GnRH in the hypothalamus. In males, LH stimulates the interstitial cells located in the testis to produce testosterone, and FSH plays a role in spermatogenesis[4]. There are a lot of factors affecting male fertility hormone which leads to infertility, some of which include fast carb (carbohydrate), obesity, cell phones, stress and diets[3]. According to World Health Organization, infertility is a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse. Infertility is one major problem in the society among couples. *Lophira lanceolata* and *Alchornea cordifolia* are plants used for the treatment and management of perimenopausal, menopausal and menstrual cycle disorders, with claims of high efficacies by users. These plants have been reported to be effective in treating infertility and other conditions[5,6,7]. *Alchornea cordifolia* (Christmas bush or Dove-wood) is a medium size shrubby tree that is widely distributed throughout Africa where it is used extensively in traditional medicine. *Alchornea cordifolia* is one of the plants that grows ubiquitously (everywhere) in the southern Nigeria and used as performance enhancers in livestock and poultry industry[8]. Investigations have been reported that the phytochemical components of A. cordifolia include Tannins, Flavonoids, Saponnis, Alkaloid, Phenols, Steroid, Anthraquinone, Cardiac glycosides, Resin, Carbohydrates, Terpenoids. It is of great value locally and has been found to have anti-inflammatory, anti-bacterial and analgesic properties[9]. *Alchornea cordifolia* is also known to have flavonoids and reports on the biological activity of *Alchornea* suggest that it is spasmolytic[10], hepato-protective[11], anti-diarrhoeal[12], and anti-oxidant[13]. With the high rate of infertility within our society, and the high cost of conventional treatment, most men in the quest for solution, have resolve into using herbs, as first treatment. Studies have shown that despite the high prevalence and psychological consequences of infertility, relatively few men had sought for orthodox treatment, therefore, this research proposes to find out the effect of *Alchornea cordifolia* on the hypothalamic-pituitary response to hormone profile in male wistar rats.

MATERIALS AND METHODS

Ethical approval was obtained for all experimental procedures from the Faculty Animal Research Ethics Committee of the Faculty of Basic Medical Sciences, University of Calabar, Calabar, Cross River State, Nigeria with Approval number 08ANA2321. Fresh leaves of *Alchornea cordifolia* were obtained, authenticated by a Botanist in the Botany Department of the University of Calabar, Calabar and air dried. The powdered plant material (330g) was extracted with ethanol to yield 21g of darken semi-solid extract. Twenty male albino Wistar rats weighing between 140-200g were divided into four groups (designated as A, B, C and D). Group A animals served as normal control and were given normal saline of 0.5ml, Group B, C and D animals received daily administration of 500mg/kgw, 1000mg/kgw and 1500mg/kgw of the extract respectively by the use of metal oropharyngeal cannula for a period of 28 days. All groups were fed with growers mesh along with water *ad libitum*. At the end of the administration, the animals were anaesthetized by chloroform inhalation method. Blood was collected from the left ventricle for haematological, biochemical, and hormonal profile studies. The anterior pituitary gland and hypothalamus were obtained and preserved using 10% formalin for tissue processing. The tissues were processed according to method described by Zuhair[14]. Haematoxylin and eosin (H & E)[15] and PAS Orange G technique[16] was used for staining sections. ELISA (enzyme linked immunosorbent assay) technique for hormonal assay. Students T-test was used for data analysis of variance at $P<0.05$.

RESULTS

There were no significant differences in the serum concentrations of the sex hormone concentrations among the different experimental groups (Fig. 1-3). The mean serum concentrations of testosterone in the Control (Group A), High Dose (Group D), Medium Dose (Group C) and Low Dose (Group B) groups were 0.287 ±0.018, 0.189 ±0.005, 0.220 ±0.056 and 0.237 ±0.023 respectively (Fig. 1). Their FSH concentrations were 2.716 ±0.340, 2.468 ±0.101, 2.644 ±0.139 and 2.617 ±0.076 respectively (Fig. 2), while their LH concentrations were 1.673 ±0.066, 1.622 ±0.064, 1.671 ±0.070 and 1.674 ±0.035 respectively, (Fig. 3).

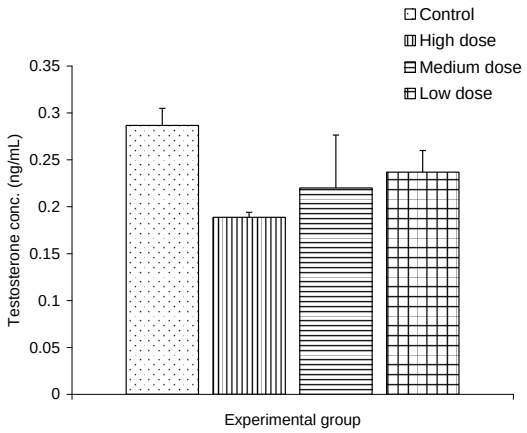


Figure 1: Comparison of testosterone concentration in the different experimental groups
Values are expressed as mean +SEM, n=4
No significant difference among groups

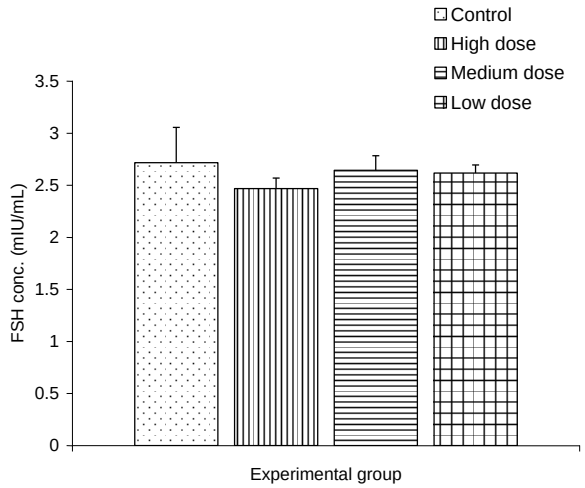


Figure 2: Comparison of follicle stimulating hormone concentration in the different experimental groups

Values are expressed as mean +SEM, n=4
No significant difference among groups

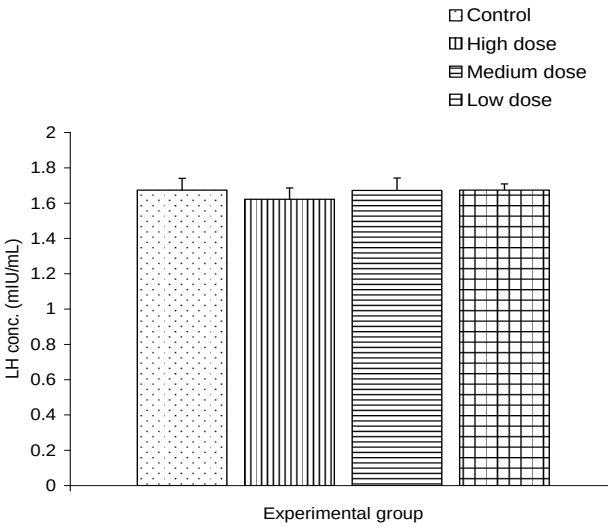
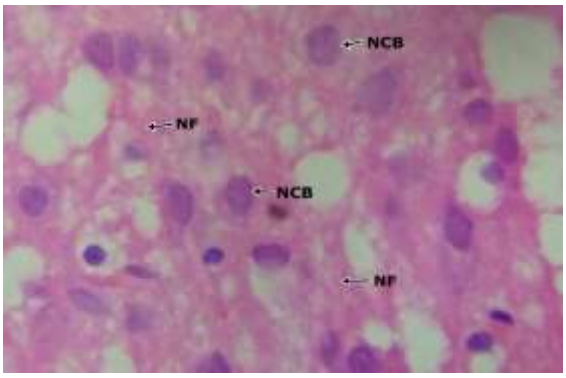
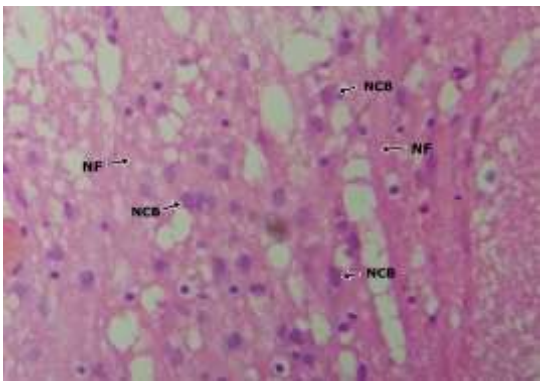


Figure 3: Comparison of luteinizing hormone concentration in the different experimental groups
Values are expressed as mean +SEM, n=4
No significant difference among groups

Sections of the hypothalamus observed from the control animals showed even distribution of neuronal cell bodies which were seen deeply stained with abundant neuronal fibers and neuroglia cells. The experimental animals showed moderate cytoplasm which are separated by bundles of neuronal fibers and glia cells.

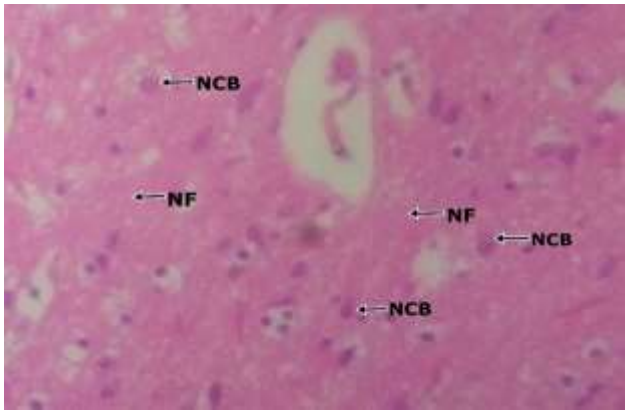


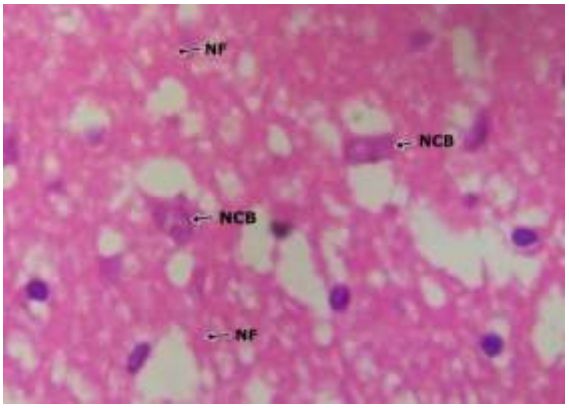


GROUP A (H & E) X400

X1000

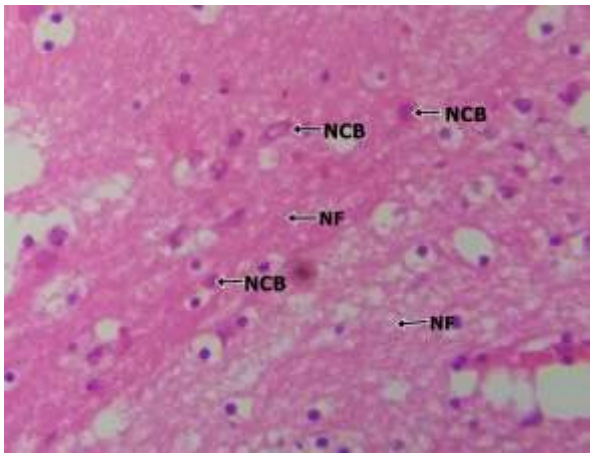
Figure 4: Photomicrograph of the hypothalamus shows evenly distributed neuronal cell bodies with deeply stained nuclei separated by abundant neuronal fibres and neuroglia cells. NF= Neuronal fibres, NCB= Neuronal cell bodies.

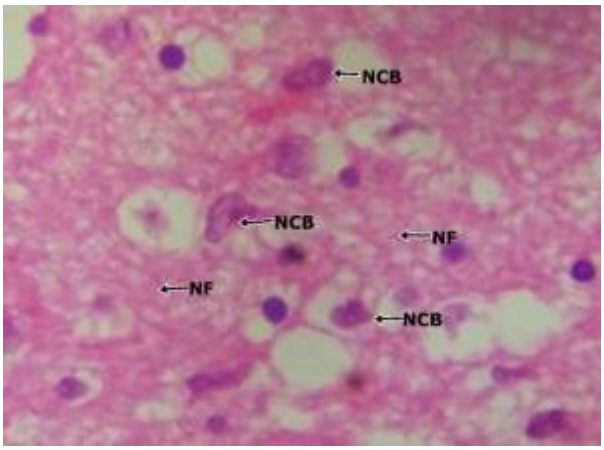




GROUP B (H &E) X400 X1000

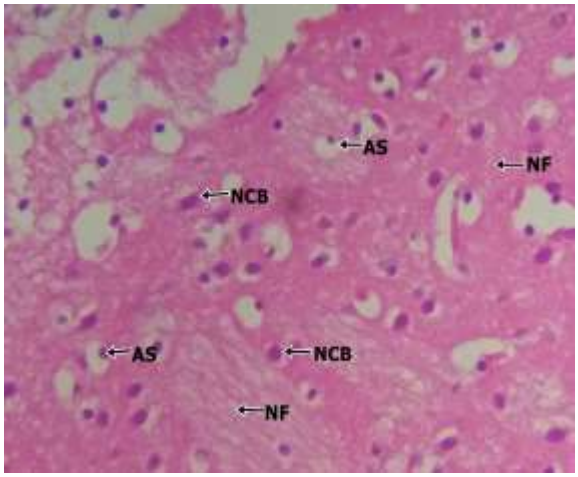
Figure 5: Photomicrograph of the hypothalamus, shows moderately distributed neuronal bodies, deeply stained nuclei which are separated from each other by neuronal fibres. The neuronal cell bodies have oval to round cell bodies with moderate amount of cytoplasm. NF= Neuronal fibres, NCB= Neuronal cell bodies.

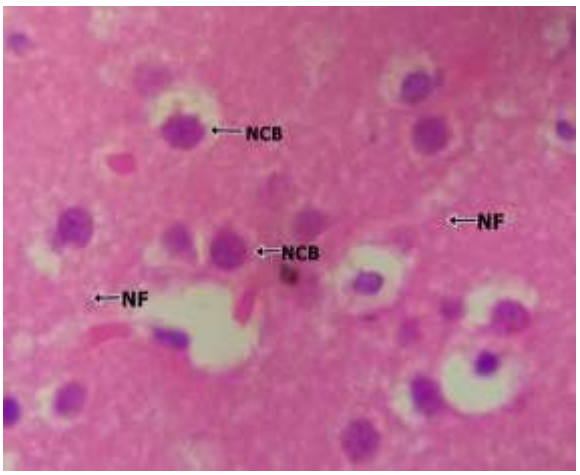




GROUP C (H &E) X400 X1000

Figure 6: Photomicrograph of the hypothalamus shows neuronal cell bodies with deeply stained nuclei and moderate cytoplasm separated from each other by wavy bands of neuronal fibres. NF= Neuronal fibres, NCB= Neuronal cells bodies

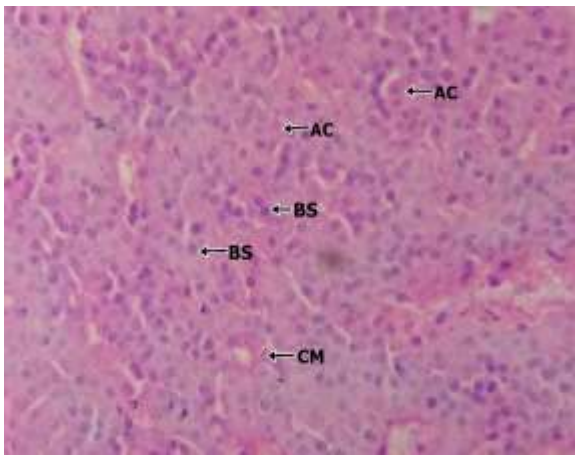


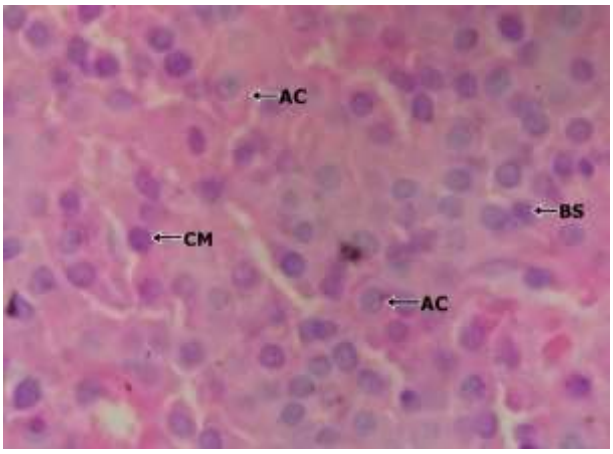


GROUP D (H&E) X100 X1000

Figure 7: Photomicrograph of the hypothalamus shows evenly distributed neuronal cell bodies with deeply stained nuclei and moderate cytoplasm separated by bundles of neuronal fibres and glia cells. Neuroglia cell is seen with vacuolated cytoplasm. NF= Neuronal fibres, NCB= Neuronal cell bodies, AS = Astrocytes

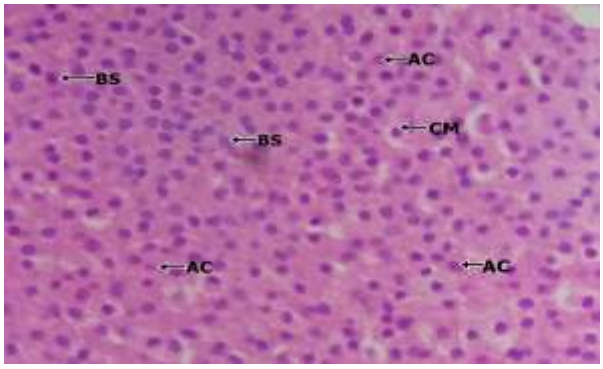
Sections of the anterior pituitary gland observed from the control animals using Haematoxylin and eosin (H&E) staining method showed oval to round nuclei having abundant cytoplasm, predominant cells of the basophilic cells with deeply stain nuclei. Cells of the anterior pituitary gland had no distortion of any form, the cells appeared normal, including the ones stained with PAS Orange G staining method, when compared with the control animals. The experimental animals showed clusters and cords of oval to round nuclei having abundant cytoplasm.

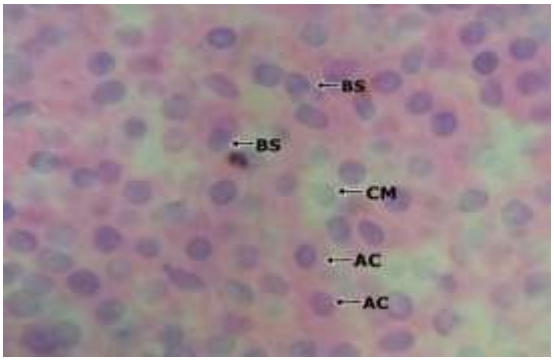




Group A (H & E) X400 X1000

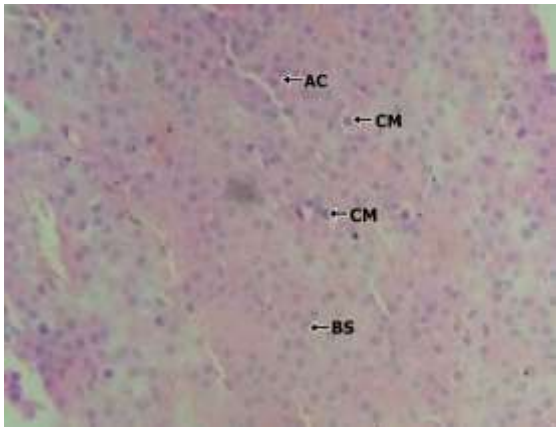
Figure 8: Photomicrograph of pituitary glands shows clusters of oval to round nuclei acidophilic cells having abundant eosinophilic cytoplasm. The clusters are separated by thin dilated and congested capillaries. Sparsely populated basophilic cells are scattered within the field with few cells having clear cytoplasm. AC= Acidophilic cells, BS= Basophilic cells, CM= Chromophils.

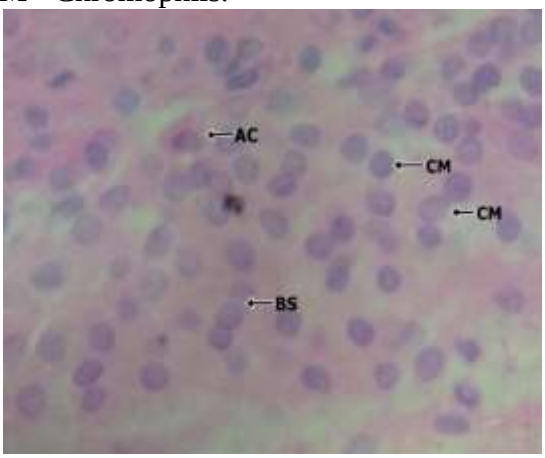




GROUP B (H & E) X400 X1000

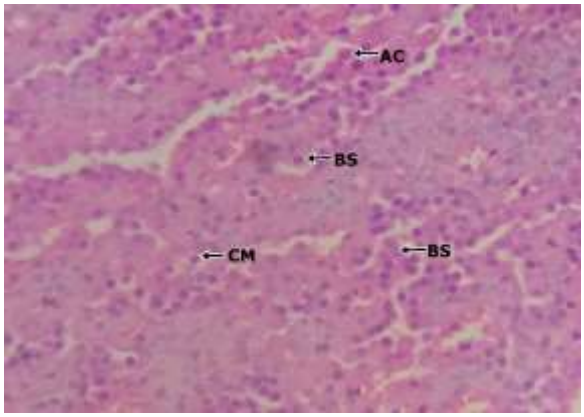
Figure 9: Photomicrograph of the pituitary gland shows clusters of round to oval nuclei having abundant cytoplasm separated by blood vessels. The predominant cells have abundant basophilic cytoplasm with deeply stained nuclei. There are few acidophilic cells with deeply eosinophilic nuclei having fine chromatin patterns. Sparse population of chromophils are seen. AC= Acidophilic cells, BS= Basophilic cells, CM= Chromophils.

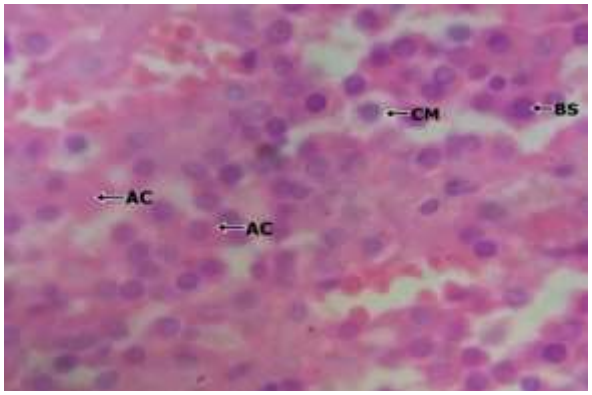




GROUP C (H & E) X400 X1000

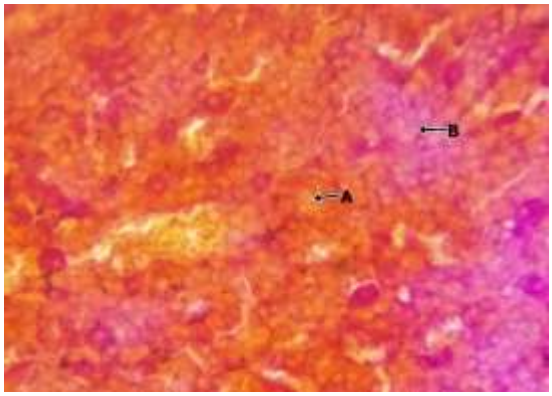
Figure 10: Photomicrograph of sections of pituitary gland showing clusters and cords of oval to round nuclei having abundant cytoplasm separated by dilated and congested blood vessels. The predominant cells are the basophilic cells which are densely populated with abundant cytoplasm having prominent nuclei. The chromophils are sparsely distributed among the fields and the acidophils are scanty. BS= Basophilic cells, AC= Acidophilic cells, CM= Chromophils.





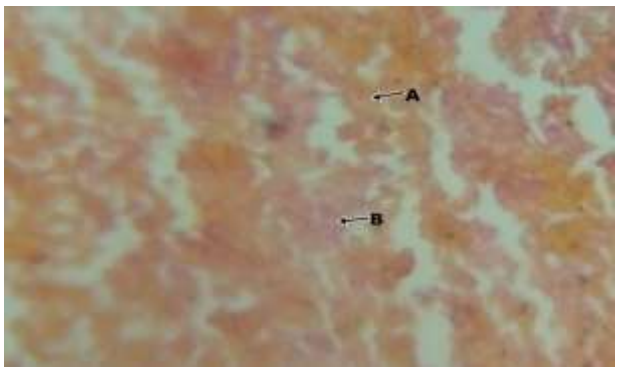
GROUP D (H & E) X400 X1000

Figure 11: Photomicrograph of pituitary glands shows clusters and cords of oval to round nuclei having abundant cytoplasm separated by dilated and congested blood vessels. The basophils are densely populated abundant eosinophilic cytoplasm. The chromophils are also sparsely distributed among the fields and the acidophils are scanty. AC= Acidophilic cells, BS= Basophilic cells, CM= Chromophils.



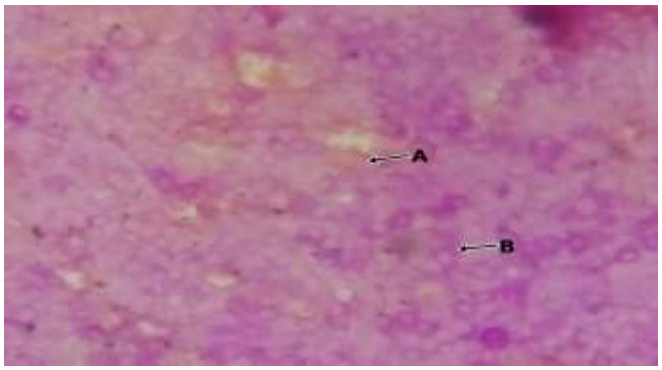
Group A X400

Figure 12: Photomicrograph shows the cytoplasm of the acidophilic cells are deeply stained orange (OG-POSITIVE), the basophilic cells are lightly stained blue (PAS-POSITIVE). The acidophilic cells out numbers the basophilic cells. A= Acidophilic, B=.Basophilic



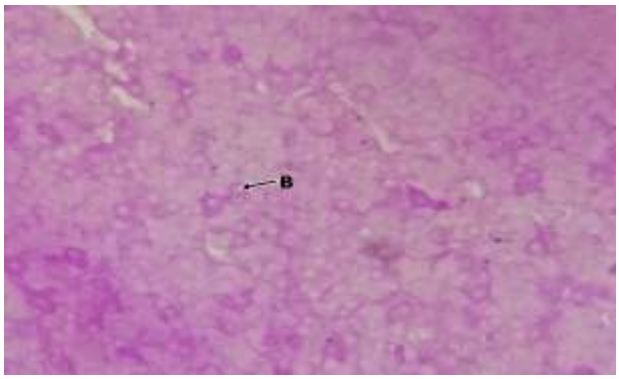
GROUP B X 400

Figure 13: Photomicrograph of the anterior pituitary gland stained with PAS Orange G, shows acidophilic cells are deeply stained orange, the basophilic cells are scanty and lightly stained blue. The acidophilic cells are predominant. A= Acidophilic cell, B= Basophilic cells.



GROUP B X400

Figure 14: Photomicrograph shows the basophilic cells are deeply stained blue, the acidophilic cells are scanty and lightly stained orange. The basophilic cells out numbers the acidophilic cells hence the basophilic cells are predominant. A= Acidophilic cells, B= Basophilic cell.



GROUP C: X400

Figure 15: Photomicrograph of the anterior pituitary gland stained with PAS Orange G, shows the cells are deeply stained blue, with prominent basophilic cells. B= Basophilic cells.

DISCUSSION

The hypothalamus secretes gonadotropin releasing hormone which acts on the anterior pituitary for the release of FSH and LH. Effects of herbs on hypothalamic pituitary gonadal (HPG) axis reported by[17], showed that HPG axis is activated, which leads to the increase in luteinizing hormone (LH), follicle stimulating hormone (FSH) and sex steroid (Testosterone and Estradiol) levels. Results from this study showed no significant differences in the serum concentrations of testosterone, FSH and LH concentrations among the different experimental groups when compared with the control (Fig 1-3). However, the effect of the extract of A. cordifolia on the hormone profile in Group D rats was more when compared to the other treated groups which may be suggestive of a dose related effect. The stained (H&E) histological sections of the hypothalamus and pituitary gland also showed similar cytoarchitecture of cells found in the cytoplasm of the glands in the treated animals when compared to the control (Fig 4-11). This is suggestive of probably no effect of the extract of A. cordifolia on the cells of the hypothalamus and pituitary gland. This is in line with[18], who studied the impact of *Alchornea cordifolia* on the gonads, serum level of testosterone, estrogen, serum enzymes and blood corpuscles of rabbits and reported no significant difference in the mean values. Reports by[19] also observed no negative effect on some visceral organ such as the heart, kidney and liver of rabbits fed with *Alchronea cordifolia.* The tissue sections of the experimental animals in the group B (Fig 13) stained with PAS orange G showed predominantly acidophilic cells with less basophilic cells which is similar to sections obtained from the control animals (Fig 12). However, this was slightly different in the Group C&D animals (Fig 14 &15) which showed predominantly basophilic cells with PAS orange G stain. These results suggest that the properties of the extract probably acting alone may not influence the secretion of gonadotropin releasing hormones (**GnRH)** but could potentiate this action in synergy with other herbs. This is seen in previous study by[20] on Combined leaf extracts of *Lophira lanceolata* and *Alchornea cordifolia on* Hormones and Pituitary gland of menopausal albino Wistar rats. The findings demonstrated that hormone pattern for the treated groups were similar to that of positive control and combined extract was the most potent relative to the individual herbs. Aqueous stem bark extract of *Lophira lanceolata* has been reported[7] to have fertility enhancing effects in male Sprague Dawley rats, sperm count was significantly increased, but motility and morphology was not affected. *Alchornea cordifolia* has also been found to

cause hypertrophy of the gonads in birds, which signifies increased testosterone production[8] contrary to the findings in this study.

CONCLUSION

In conclusion, extract of *Alchornea cordifolia* leaves seems not to have positive effect on the hypothalamic-pituitary response to hormone profile in male wistar rats and as such may not have an enhance effect on fertility.

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