Effects Of Methanolic Root Extract Of*Holarrhena floribunda* OnLiver Enzymes and Histopathology of the Ovaries and testes Tissues In Wistar Rats.

ABSTRACT

Aim:This study was conducted with the aim of investigating the effects of methanolic root extract of *Holarrehna floribunda*T.Durand & Schinz onserum activities of some liver enzymes and histopathology of the ovaries and testes tissues of rats .
Methods: Twenty- four male and female Wistar rats (150-250g body weight) were randomly assigned into 4 groups of 6 rats each. Group 1 (control male) took normal rat chow and drinking water. Group 2(control female) took normal rat chow and drinking water, Group 3(Male test group), was administered with 200mg/kg of ~~*Holarrehna*~~ *H.floribunda* extract, Group 4(Female test group), was administered with 200mg/kg of ~~*Holarrehna*~~ *H.floribunda*,Group The feeding regimens lasted for 5weeks.
Results: The effects of administrating the extract on serum enzymes, shows the activity of ALTin Group 1 is 28.60±2.71U/L and Group 3 is 29.20±1.43U/L while for Groups 2 and 4 were 34.00±0.00U/L and 32.86±2.87U/L,there was significant decrease($p<0.05$) in ALT between group 2 and 4 and no significant increase($p<0.05$) between Group 1 and Group 3.The values of ASTobtained in Group1 is 53.00±1.87U/L and Group 3 is 33.40±2.60U/L,Group 2 and 4 were 38.00±2.50U/L and 35.43±1.91U/L. There was significantdecrease($p<0.05$) between Group 2 and Group 4 and a increase between Group 1and Group 3.
The histological integrity of the testes and ovary tissues were examined. Results revealed that for the tissues of the testes, there was no evidence of histological distortion; the oocytes and the follicles were normal. While in the extract administered group, there were large number of Leydig cells present in between the seminiferous tubules.

Conclusion: This shows that the extract has no toxic effect on the liver .Histological results showed no disorganization and degeneration in the ovary and testes. These results can help to explain why *H. floribunda* might be used in solving some women with sterility/infertility problems.

Key words: Histopathology,liver enzymes, histological distortion, oocytes,ovaries and testes tissues

Introduction

Globally, infertility affects about 50 to 80 million couples at some point of their reproductive lives with a variety of biological and behavioral determinants. A variety of plants are claimed to have fertility regulating properties and a few have been tested for such effect[1~~,2,3,~~ 4]~~*Holarrhena*~~ *H.floribunda* is one of these plants.Various medicinal plants ranging

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[12] Pehlivan M, Sevindik M. Antioxidant and antimicrobial activities of Salvia multicaulis. Turkish Journal of Agriculture-Food Science and Technology, 2018; 6(5): 628-631.

[13] Mohammed FS, Akgul H, Sevindik M, Khaled BMT. Phenolic content and biological activities of Rhus coriaria var. zebaria. Fresen Environ Bull, 2018;27(8): 5694-5702.

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from Quassiaamara, [5,6,7,8], *Garcinia kola*, [9] and *Vernonia amygdalina*, [10] have been implicated in male infertility. Fortunately, several countries in the world are endoured with plant biodiversity, and there is currently an awareness about the significance of plant remedies in health care delivery system. In many parts of the world, efforts are now being aimed at investigating therapeutic efficacy of locally available medicinal herbal plants[11,12]. The beneficial role of medicinal plants in the treatment of female infertility has been numerously indicated.Phytochemicals are biochemical compounds formed during the plants normal metabolic processes[13,14]. These substances are often referred to as "secondary metabolities". There are several classes of phytochemicals including; alkaloids, flavonoids, coumarins, glycosides, gums, polysaccharides, phenols, tannins, terpenes and terpenoids [11,15]. Phytochemicals can act as agents that prevent undesirable side effects of the main active substances or assist in the assimilation of the main substances[12,16].

Phytochemicals are found in a variety of plants which constitutes an important component of either human or animal diets. Such plants may include fruits, seeds, herbs and vegetables [13,17]. Contrary to synthetic pharmaceuticals based upon single chemicals, many medicinal and aromatic plants exert their health beneficial effects through the synergistic action of several chemical compounds acting at single or multiple target sites associated with a biochemical or physiological process. These synergistic pharmacological effects can be beneficial by eliminating the problematic side effects associated with the predominance of a single xenobiotic compound in the body[14,18].

The additive interactions that underlie the effectiveness of a number of phytomedicines have been extensively documented[15,19]. Most of these phytochemical constituents are potent bioactive compounds found in medicinal plant parts which are precursors for the synthesis of

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useful drugs[~~16~~20] and in-depth knowledge of such composition in a plant can aid in its wider and efficient exploitation for medicinal purposes.

Traditional medicine of this same Africa region indicates that the root extract of ~~*Holarrhena*~~ *H.floribunda* is used to stimulate fertility. Although there is no scientific evidence to support the ethnopharmacological efficacy of ~~*Holarrhena*~~ *H.floribunda* on female reproduction, tribes continue to popularlise the use in the management of cases of sterility/infertility in women. In Nigeria the root of ~~*Holarrhena H.Floribunda*~~ *floribunda* is employed traditionally in the treatment of Malaria, diarrhea, dysentery, fever, pains, female sterility, skin infections, veneral diseases, cancer and snake bite[~~17~~21]. The use of the root of this plant in the treatment of the above named diseases have not been studied scientifically hence the need for this research.

Methods

Identification and preparation of plant materials

Fresh roots of *Holarrhena floribunda* was collected from local garden at the University of Uyo, Uyo, Akwa Ibom State,Nigeria. The sample of the plant specimen was identified and authenticated by a Botanist from the botanical garden, and the voucher specimen with identification number (PES/Herb/uc/129) was deposited in the Herbarium of the University of Calabar. The roots were sorted to eliminate any dead matter and other unwanted particlesThe roots of ~~*Holarrhena*~~ *H.floribunda* were washed with clean water, cut into pieces and dried under shade at a temperature of 25±0.5^{0}C.Mortar and pestle were used to pulverize the root until it formed a coarse powder.~~m~~Methanol extracts were obtained by soxhlet extractionof the powder. The extracts were concentrated to dryness in vacuo at 40^{0}C.The dried extracts were weighed, stored in specimen bottles and kept in the refrigerator at -4^{0}C until used Appropriate

concentration of the extract will be subsequently made by dilution with distilled water and administered to the animals.

Handling and treatment of animals

Twenty four (24) adult male and female albino rats weighing between 150-250g obtained from the disease free stock of the animal house, Biochemistry Department, College of Medical Sciences University of Calabar, Calabar Nigeria were used for the study. The rats were divided into four (4) groups of six (6) rats each as shown in Table 1. The rats were acclimatized in the experimental Animal House for one week before the commencement of the experiment. The animals were housed in stainless steel cages under standard conditions (ambient temperature, 28.0±2.0º C and humidity,46%, with a 12 hr light/dark cycle), fed with the normal rat pellets. All the rats in both test and control groups are allowed free access to feed and water *ad libitum*, throughout the experimental period. Good hygiene maintained by constant cleaning and removal of faeces and spilled feed from cages daily.The extract was administered for 40 days.

TABLE 1

Animal groupings

Groups	Number of animals	Treatment

1. (Normal Control Male)	6	Distilled Water
2.(Normal Control Female)	6	Distilled Water
3. (Male 200mg/kg)	6	Root extract of HF
4. (Female 200mg/kg)	6	Root extract of HF

Group 1 (normalcontrol male group received distilled water as placebo),

Group 2 (normal control female group received distilled water as placebo),

Group 3 (test group male received oral dose of ~~*Holarrhena*~~ *H. floribunda*).

Group 4(test group female received oral dose of ~~*Holarrhena*~~ *H. floribunda* root extract).

Collection of blood and tissues for analyses

All the animals were anaesthetized in chloroform vapour, twenty-four (24) hours after the last day of extract administration, and dissected for blood collection. Blood was collected by cardiac puncture into a set of plain sample bottles, allowed to clot for 2 hours after which serum was obtained by centrifugation at 3000rpm. Serum was used for biochemical estimations. The testes and ovaries were obtained and fixed in 10% formaldehyde solution for further processing and histological examination.

Biochemical estimations

Biochemical analysis which included estimation of serum activities of liver enzymes-alanine aminotransferase and aspartate aminotransferase were carried out according to the method described by Reithman and Frankel [22] ~~(1957).~~

Statistical analysis

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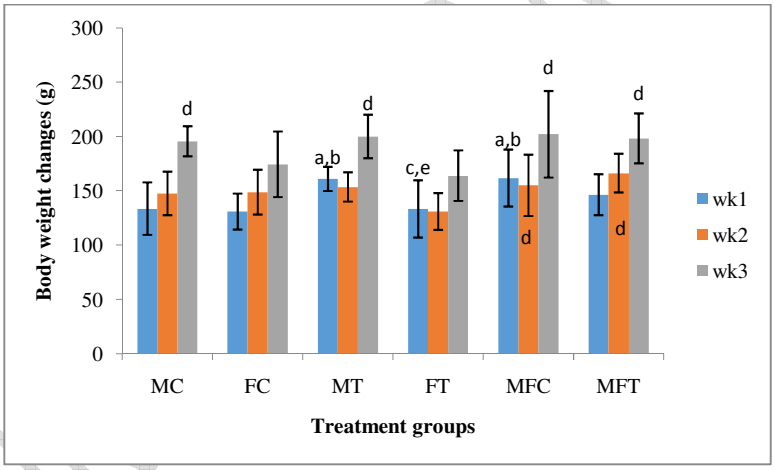
Results obtained from this study was analyzed by one-way analysis of variance (ANOVA), followed by Student's t-test to evaluate the significance of the difference between the mean value of the measured parameters in the respective test and control groups using SPSS windows. A significant change was considered acceptable at $P < 0.05$.

Results

Result showing comparison of weekly body weight changes of animals in the respective groups.



Comparison of weekly Body weight changes of animals in the respective groups. Values are presented as mean ± SEM; n = 6; a = P>0.05 vs C; b = P>0.05 vs FC; c = P>0.05 vs MT; d = P>0.05 vs FT; e = P>0.05 vs MFC

Effects of administration of *H.~~olarrhena~~ floribunda* root extract on liver enzymes

Result of the effect of administration of ~~*Holarrhena*~~ *H.floribunda* root extract on liver enzymes in the respective groups is shown on Table 2. Values obtained shows the activity of ALT(U/L) in Groups 1 and 3 were 28.60±2.71U/L and 29.20±1.43U/L while for group 2 and 4 were34.00±0.00U/L and 32.86±2.87U/L respectively,There was nosignificance difference($p>0.05$) in ALT between Groups 2 and 4 and no significant increase($p>0.05$) between 1 and 3. The values of AST (U/L) obtained from Table 2 shows group1 and 3 were 53.00±1.87U/Land 33.40±2.60U/L respectively, group 2 and 4 were 38.00±2.50U/L and 35.43±1.91U/L difference respectively. There was no significant ($p<0.05$) decrease between group 2 and 4 and a slight increase between 1and 3.

Results of the effect of administration of ~~*Holarrhena*~~ *H.floribunda* root extract on liver enzymes in the respective groups

Table 2

	ALT (U/L)	AST (U/L)
Group 1 MC	28.60±2.71	35.00±1.87
Group 2 FC	34.00±0.00	38.50±2.50
Group 3 MT	29.20±1.43[a]	33.40±2.60[a]
Group 4 FT	32.86±2.87[b]	35.43±1.91[b]

Values expressed as mean ± SEM, significant at p<.05. [a] insignificant at p<.05compared with group 1 (Male control). [b] insignificant at p<.05 compared with Group 2 (Female control). MC = male control group; FC = female control group; MT = male test group; FT = female test group.

Effect of administration of ~~*Holarrhena*~~ *H.floribunda* extract on the histology of testes and ovarian tissues

Plate 1: Photomicrographs of testis of Male control (MC) group surrounded by a thick capsule called tunica albuginea (Basement membrane).Normal appearance of the spermatozoa with no visible histological distortion seen.

Plate 2: Photomicrographs of the ovary of Female control(NC) group shows normal appearance of the oocytes surrounded by epithelial cells.The section shows an outer region called cortex(top arrow head) and an inner portion called medulla(big arrow).The portion of these two components varies according to age. No pathological changes seen.

Plate 3: Photomicrographs of Male testes of ~~*Holarrhena*~~ *H.floribunda* (HF)-treated group with 200mg/kg b.w extract showed normal appearance of testes surrounded by a basement membrane which is still intact.There were large numbers of Leydig cells present in between the seminiferous tubules.No visible histological abnormality seen.

Plate 4: photomicrographs of female ovary of ~~*Holarrhena*~~ *H.floribunda* (HF)-treated group, treated with 200mg/kg b.w extract shows ovarian section with follicles seen in the cortex.Equally seen are oocytes that are surrounded by epithelial cells that form the follicles.No disorganization or degeneration was seen.

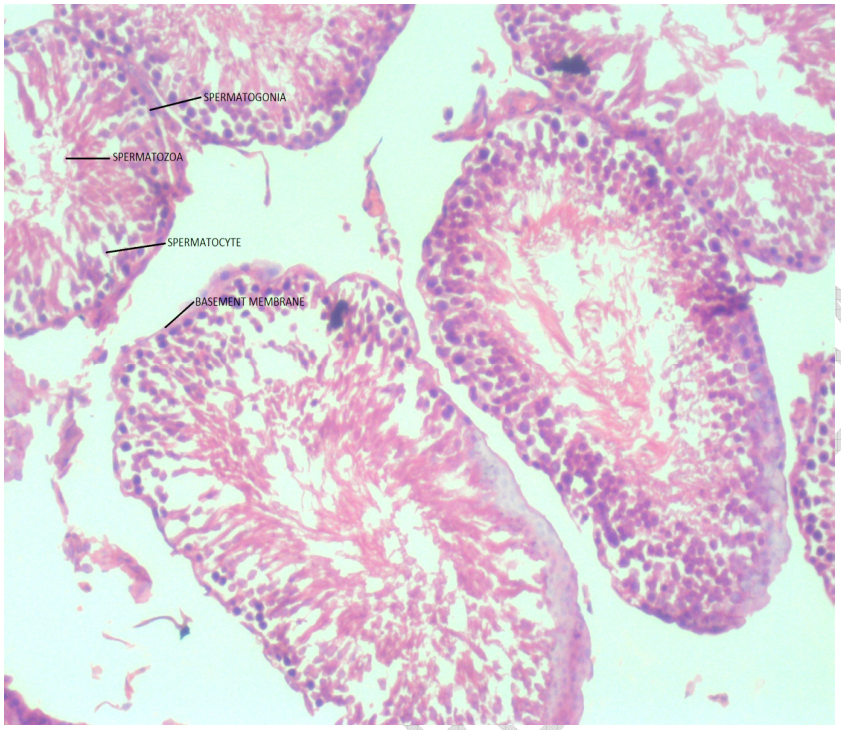


PLATE 1: Photomicrographs of testis of male control (MC) group. Magnification × 100.

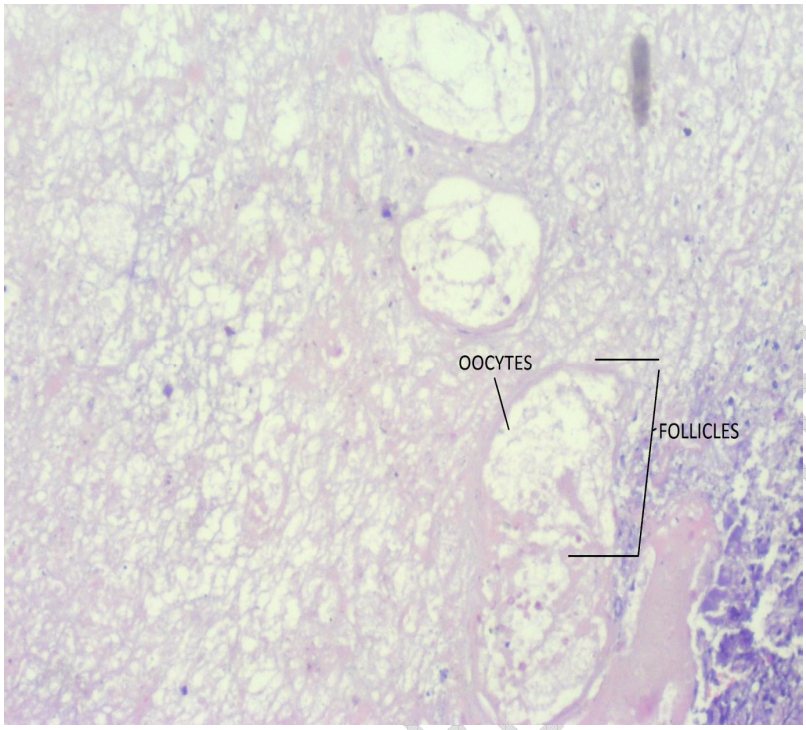


PLATE 2: Photomicrographs of the ovary of Female control(NC) group.Magnification x 100

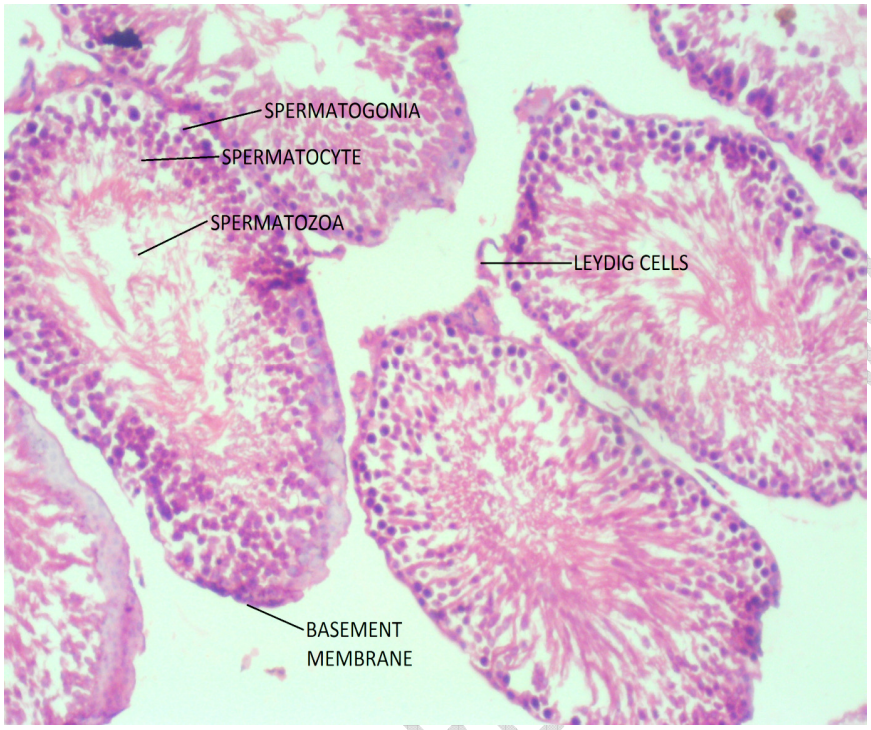


PLATE 3: Photomicrographs of Male testes of ~~*Holarrhena*~~ *H.floribunda* (HF)-treated group with 200mg/kg b.w extract. Magnification x 100

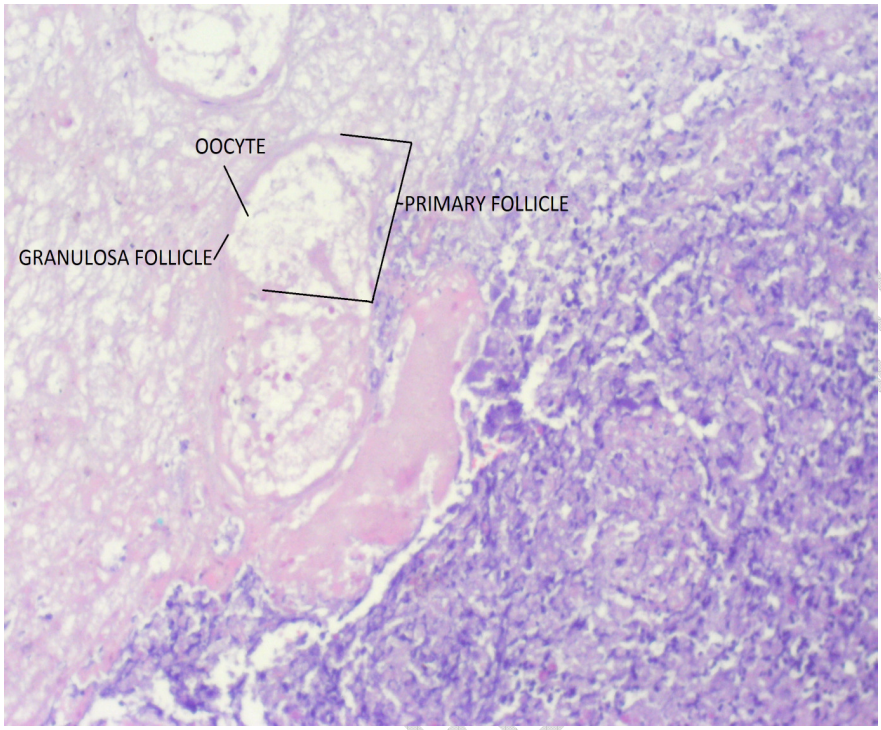


PLATE 4: Photomicrographs of Female ovary of ~~*Holarrhena*~~ *H.floribunda* (HF)-treated group with 200mg/kg b.w extract. Magnification x 100

Discussion

The aim of the present study was evaluating the effects methanolic root extract of ~~*Holarrhena*~~ *H.floribunda* on serum activities of some liver enzymes and histopathology of the ovaries and testes of rats . Serum enzyme levels are the most commonly used biochemical tools for the assessment of hepato-cellular injury whereas increase in amino transferases (ALT and AST) generally reflects liver cell damage. That of ALP is more specific for cholestasis. Free radical induced lipid peroxidation of cellular membrane alters membrane integrity leading to increased membrane permeability and loss of cellular content into the circulation. The extract reduced the levels of AST and ALT in Groups 1 and 3 while for group 2 and 4. The values of

AST shows groups 1 and 3 and groups 2 and 4 were indications of its non toxicity and protective activity of this extract.

Effect of administration of *H. floribunda* extract on the histological architecture of testes and ovary tissues. it showed that the extract improved the level of sex hormones in both the male and female rats[~~18~~23]. The histological and/or architectural integrity of the testes and ovary tissues in the experimental groups were examined and compared with the controls. Results revealed that for testes tissues there was no evidence of histological distortion for the controls, because cross sections of the testes tissues showed a preserved architecture of the seminiferous tubules and basement membrane. For normal control ovary, there was no indication of degeneration as the oocytes and the follicles were normal. In the extract administered groups where the testes showed normal histological appearance of the testes surrounded by basement membrane,indicating that they were intact.There were large number of leydig cells present in between the seminiferous tubules.There was no histological abnormality.In the ovary of the extract treated group.~~t~~There was no degeneration seen on the ovarian section with follicles seen in the cortex,the appearance of the general tissue structures were normal.From the results obtained in this research, It showedthat the extract improved the level of sex hormones in both the male and female rats and the extract treated rats showed non toxicity.

Conclusion

With these findings, it could be concluded that ~~*Holarrhena*~~ *H. floribunda* methanol root showed no toxic effect on the liver. There was increased in body weight changes. Histological results showedno disorganization and degeneration in the ovary and testes. These results can help

to explain why *H. floribunda* might be used in solving some women with sterility/infertility problems.

ETHICAL APPROVAL

Authors received ethical approval according to international /university standard.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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