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Research Article

Comparative effect of Flavonoid Rich Fraction and Whole Leaves Extract of *Vernonia amygdalina* on Male and Female Sex Hormones in Streptozotocin Induced Diabetic Albino Wistar Rats

Odama Richard Ikani[1]*, Ujong Peter Ujong[2], Ezeh Charles Ogbonna[1], Kenechukwu Chibuike Onyekwelu[1], Akighir John[3]

[1]*Department of Medical Biochemistry, College of Medicine, University of Nigeria, Enugu Campus, Enugu.*
[2]*Department of Medical Biochemistry, Faculty of Basic Medical Science, Cross River University of Technology, Calabar.*
[3]*Department of Medical Laboratory Science, College of Health Science, Benue State University, Makurdi, Nigeria.*

*Corresponding author's e-mail: ikanirichard@yahoo.com

Abstract

Vernonia amygdalina is a shrub that grows throughout the tropical Africa. Several studies have reported the nutritional, anti-malaria, anti-diabetic, anti-cholesterol and anti-helminthic effects of its extracts, but there is scanty information on its effect on reproduction. Against this background, this study was design to investigate the comparative effect of methanolic 30% flavonoid rich fraction and whole leave extract of *Vernonia amygdalina* on reproductive hormones profile of treated diabetic albino Wistar rats. After 28 days of treatment for gonadal studies. The animals were anaesthetized at the end of the treatment period using chloroform in an enclosed chamber prior to dissection in conformity with University of Calabar animal handling ethical standards. Blood was collected by cardiac puncture into labeled sterilized sample bottles and allowed to clot at room temperature for one hour and then centrifuged at 3000 rpm for 10minute to recover serum. Sera was isolated and stored at -30°C for hormonal assay. Plasma testosterone, luteinizing hormone, follicle stimulating hormones, and estradiol levels were assayed using Enzyme-link Immuno-absorbent Assay (ELISA). Data were analyzed using ANOVA and post hoc test at $p<0.05$. Treatment of rats with all the doses caused significant decrease in serum testosterone, follicle stimulating hormone levels in group treated with crude extract and flavonoid rich fraction when compared to normal control group while serum estradiol level only has a significant increase in 30% flavonoid treated group. Luteinizing hormone has no significant increase or decrease in crude treated group but has a significant increase in 30% flavonoid treated group in relative to normal control group. It can therefore be concluded that 30% flavonoid rich fraction of *Vernonia amygdalina* induces increase in reproductive hormones when compared to the crude extract in both sexes of albino Wistar rats.

Keywords: *Vernonia amygdalina;* Male and Female Sex Hormones; Luteinizing hormone; Diabetes.

Introduction

Medicinal plants are plants in which one or more organs, contain substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi-synthesis. When a plant is designated as medicinal, it implies that the said plant is useful as drug or is a therapeutic agent or an active ingredient of a medicinal preparation. Medicinal plants may therefore be defined as group of plants that possess some special properties or virtues that qualify them as article of drugs and therapeutic agents, and are used for medicinal purpose [1].

Medicinal plants have provided humans with many of their essential needs including life-saving pharmaceutical agents. Recently, the World Health Organization (WHO) estimated that 80% people worldwide rely on herbal medicine for some aspect. Many developing countries have intensified their efforts in documenting the ethnomedical data and scientific research in medicinal plants [2].

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Natural products or natural plant derivatives comprise of 14 out of the 35 drugs in 2000 based on the worldwide sales. There are more than 270,000 higher plants existing on the planet but only a small portion has been explored. As a vast proportion of the available higher plant species have not yet been screened for biologically active compounds, drug discovery from plants remain an essential component in the search for new medicine and the scientific study of traditional medicine. Concerned medicinal plants are thus of great importance [3].

Medicinal plants have formed the basis for health care throughout the world since the earliest days of humanity and have remained relevant in both developing and the developed nations of the world for various chemotherapeutic purposes. The use of plant derived natural compounds as part of herbal preparations for alternate source of medicament continues to play major roles in chemotherapy especially in third world countries [4]. Several studies carried out have shown that traditional medicines could provide better glycaemic control than currently used conventional drugs [5-6]. Plants by means of secondary metabolism contain a variety of herbal and non-herbal ingredients that can ameliorate a disease condition by acting on a variety of targets (various modes and mechanisms) in the host organism. On the basis of the above, polyherbal therapy is considered the preferred therapeutic approach to management of diabetes mellitus given its multi-factorial pathogenicity [7-8].

Polyherbal therapy which is the use of a combination of various agents from different plant sources for therapeutic purposes is a current pharmacological principle and has the advantage of producing maximum therapeutic efficacy with minimum side effects [8]. This enhanced efficacy is thought to be derived from phytochemicals endowed in traditional medicinal plants, since they present exciting opportunities for the development of new types of therapeutics for the management of diabetes mellitus. Such phytochemicals include tea polyphenols which suppress Post-Prandial Hyperglycaemia and glucose transport across the small intestine [9] and saponins which delay glucose transfer from the stomach to the small intestine [10-11]. Epicatechin has a restorative effect on pancreatic β-cells against alloxan damage [11] and plant flavonoids which exert their antidiabetic activity via antioxidant properties [12]. These reports have accelerated the global efforts to harness and harvest those medicinal plants that bear substantial amount of potential phytochemicals showing multiple beneficial effects in combating diabetes and diabetes related complications [7].

In our laboratory, the antidiabetic activities of *Vernonia amygdalina* (VA) have been reported. In a recent report, the chemical components thought to exert the antidiabetic action were compared [13]. Although extracts from these plants have individually demonstrated antidiabetic action, recent evidence from our laboratory show that antidiabetic efficacy of the extracts is enhanced when given in combination [8,14-15]. Accordingly, the present study was set up to investigate the comparative effect of flavonoid extract and whole leave extracts of *Vernonia Amygdalina* on sex hormones and the histology of the gonads, with a view to ascertaining whether or not flavonoid is the active component of *vernonia amygdalina* which increases or decreases the sex hormone level in diabetic patients treated with *Vernonia amygdalina.*

Materials and methods

Fresh mature leaves of *Vernonia amygdalina* were harvested from the Endocrine Laboratory farm, University of Calabar, Nigeria. The leaves were authenticated at the department of Botany, University of Calabar, Nigeria and Voucher specimen deposited in the department's herbarium. The leaves were rinsed severally with clean tap water to remove dust particles and debris then allowed to drain, air dried, pulverized using a manual blender and kept in cellophane bags before extraction. 5 kg of homogenized *Vernonia amygdalina* was subjected to extraction, in 10 liters of 80 % (v/v) methanol and allowed to stand for 48hrs at a temperature of 4°C. This was then filtered using a cheese cloth and filter paper (Whatman No. 1) and the methanol evaporated using a rotary evaporator.

The concentrate was allowed to dry completely in a water bath at 40°C. This yielded 119g of crude extract. The methanolic crude extract was subjected to serial solvent fractionation using solvents in increasing order of polarity. The crude extract was chromatographed on silica gel (60-120 mesh size) and eluted in succession using methanol in

different percentages (30%). Phytochemical screening was carried out on the methanolic extracts for the qualitative determination of phytochemical constituents as described by Trease and Evans 1989 [16].

Thirty male and female albino Wistar rats weighing between 150-200g were obtained from the animal house of the department of biochemistry, University of Calabar, Calabar. The animals were allowed one week acclimatization and housed in cages under room temperature (25±2°C), relative humidity (50±5%) and a 12 hr light / dark cycle in animal house of the department of biochemistry. The animals were allowed free access to rat chow and tap water *ad libitum* with all experimental procedure approved by the university research and ethics committee. Oral acute toxicity of the *Vernonia amygdalina* fractions was determined in mice as described by Lorke 1983 [17].

Experimental design

The design consisted of twenty (20) male and female Wistar rats divided into five (5) groups of four (4) animals each. The groups were a diabetic, non-diabetic group and the treated groups. The doses used were based on the LD50 determined for the fractions and the predetermined LD50 for the crude extract obtained from preliminary studies. The groups are: Normal Control (NC) group which receives 0.5 ml DMSO, Diabetic Control (DC) group which receives 0.5 ml DMSO, Insulin treated group (Insulin) which receives 5 iu/kg bw of the standard drug (Insulin), Crude group (Crude) which receives 400 mg/kg bw of the crude extract and group treated with 75 mg/kg bw of 30% of flavonoid rich fraction (30%) twice daily for the experimental period of 28 days.

Induction of diabetes mellitus

The rats were subjected to 12 hr fast before induction of diabetes. Diabetes was induced intraperitoneally using 40 mg/kg bw of Streptozotocin (STZ) reconstituted in sodium citrate 0.5 M. Control animals received 0.5 ml Dimethyl sulfuroxide (DMSO) only. Diabetes was confirmed three days after STZ treatment in rats with a fasting blood sugar concentration ≥120 mg/dl. This was estimated using Accu-Check glucometer (Roche diagnostics, Germany) with blood obtained from the tail vein of the rats.

Collection of blood samples for analysis

At the end of the 28 days experimental period, the animals were fasted for 12 hr but had free access to water, anaesthetized under chloroform vapor and dissected. Whole blood was collected from the heart by cardiac puncture using sterile syringes and needles, put into sterile plain tubes and allowed tom clot for two hours for biochemical assays. The clotted blood was centrifuged at 3000 rpm for 10minutes to recover serum from the clotted cells. Serum was separated with sterile syringes and needles then stored frozen for biochemical analysis. Data were presented as mean ±standard error of mean, computed and analyzed using one way ANOVA and unpaired Student's t-test. The data were analyzed with the help of a statistical package, SPSS version 18.0 for Windows, considered significant at $p<0.05$.

Results and discussion

Despite the rapid expansion in conventional medicine, majority of people still utilizes medicinal plants because available evidence which shows that these traditional regiments are efficient and affordable and their uses in management of ailments have gain several preference over orthodox/western medicine on the account of relative accessibility & availability, low incidence of drug toxicity, enhancing maximum therapeutic efficacy as well as having a synergistic, potentiative, gonistic/antagonistic pharmacological property (polyherbal therapies) and these works in a combined dynamic way to produce maximum efficacy with minimum side effects [7].

Therefore, this work is carried out to evaluate the comparative effects of flavonoid rich fraction and whole leaves extracts of *Vernonia amygdalina* on reproductive hormones of streptozotocin induced diabetic albino Wistar rats. The experiment comprises of twenty (20) males and females albino Wistar rats grouped into five (5) groups; Normal Control (NC) group which receives 0.5 ml DMSO, Diabetic Control (DC) group which receives 0.5 ml DMSO, Insulin treated group (Insulin) which receives 5 iu/kg bw of the standard drug, Crude group (Crude) which receives 400 mg/kg bw of the crude extract and group treated with 75 mg/kg bw of 30% of flavonoid rich fraction (30%) twice daily for the experimental period of 28 days. The results *Values are expressed as ±SEM,*

** =Significant different from Normal Control (NC) at p<0.05 a=p<0.05 vs DC. b=p<0.05 vs INS.*

In figure 1, the extract caused a significant decrease (p<0.05) in serum testosterone level of group treated with the extract and group treated with 30% of the flavonoid rich fraction when compared with the normal control group, diabetic control group and group treated with insulin. This is in agreement with report given by Das *et al.,* 2009 [18] in rats treated with *Aegle mermelos* extract. The decrease in serum testosterone level indicates that extracts of *Vernonia amygdalina* inhibits the mechanism intervening the process of hormones synthesis in the leydig cells, this is also shown in a similar report given by Krishnamoorthy *et al.*, 2007 [19] in *Terminalia chibula* extract treated rats. Treatment with extract caused severe germinal erosion and necrosis, this is due to insufficient amount of testosterone, since it has been reported that testosterone is essential for the growth and division of the germinal cells of the seminiferous tubules [20] as shown in result obtained from rats treated with *Colebrookra opositifolia* [21].

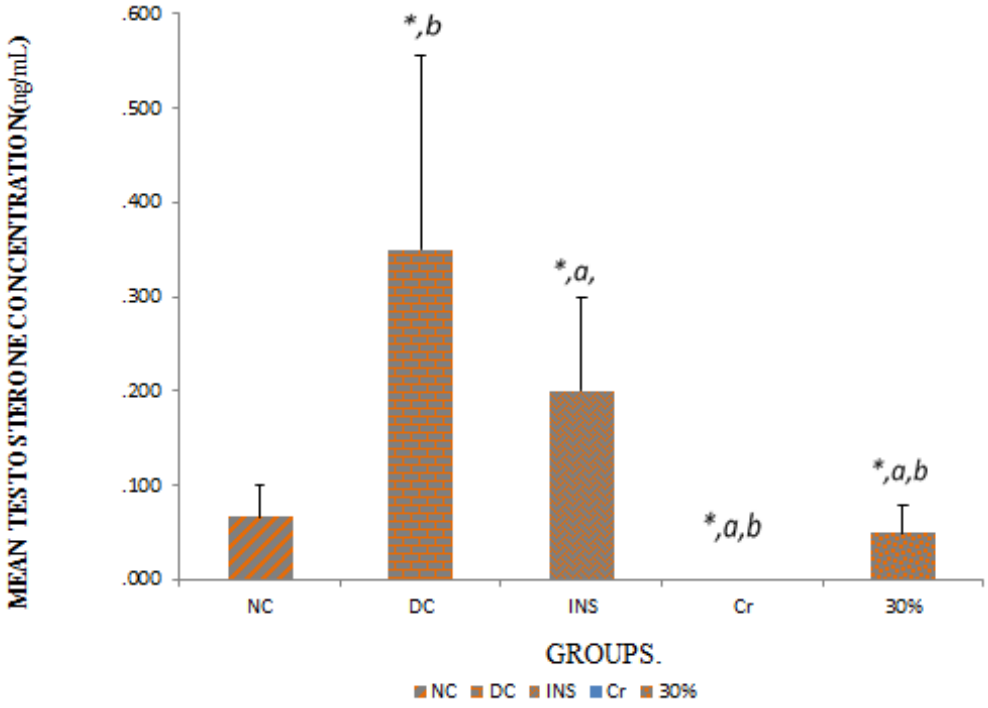

Figure 1. A graph of testosterone concentrations of the different experimental groups

Figure 2 shows a significant decrease (p<0.05) in serum estradiol level in group treated with the extract, diabetic control group and insulin treated group when compared to the normal control group while a significant increase (p<0.05) was observed in group treated with 30% of flavonoid rich fraction when compared to normal control group, diabetic control group and group treated with insulin, which is in agreement with the report given by Yakubu *et al.*,2008 [22] in female rats treated with *Cnidoscolous aconitifolius* which shows decrease in serum estradiol level and by Aziz *et al.*,2009 [23] which show a significant increase in serum estradiol level in rats treated with *Khat extract microcapsules* as against *Khat extract* treated rats due to the release of dried *Khat* alkaloids which was said to enhance libido and increasing serum estradiol level in female rats.

Figure 3 shows no significant increase (p<0.05) in serum luteinizing hormone level in group treated with the crude extracts when compared with normal control group while a significant increase (p<0.05) was observed in group treated with 30% flavonoid rich fraction, group treated with insulin and diabetic control group when compared to normal control group. This is in agreement with the finding reported by Yakubu *et al.,* 2008 [22] in female rats treated with *Cnidoscolous aconitifolius* which shows decrease in serum luteinizing hormone level and in a similar report given by Saalu *et al.*, 2013, [24] which suggest that as antioxidant, flavonoid in *Vernonia amygdalina* produce a stimulatory effect in the hypothalamus thereby increasing serum luteinizing hormone level and serum testosterone level as well as maintaining sperm morphology, sperm survival and function.

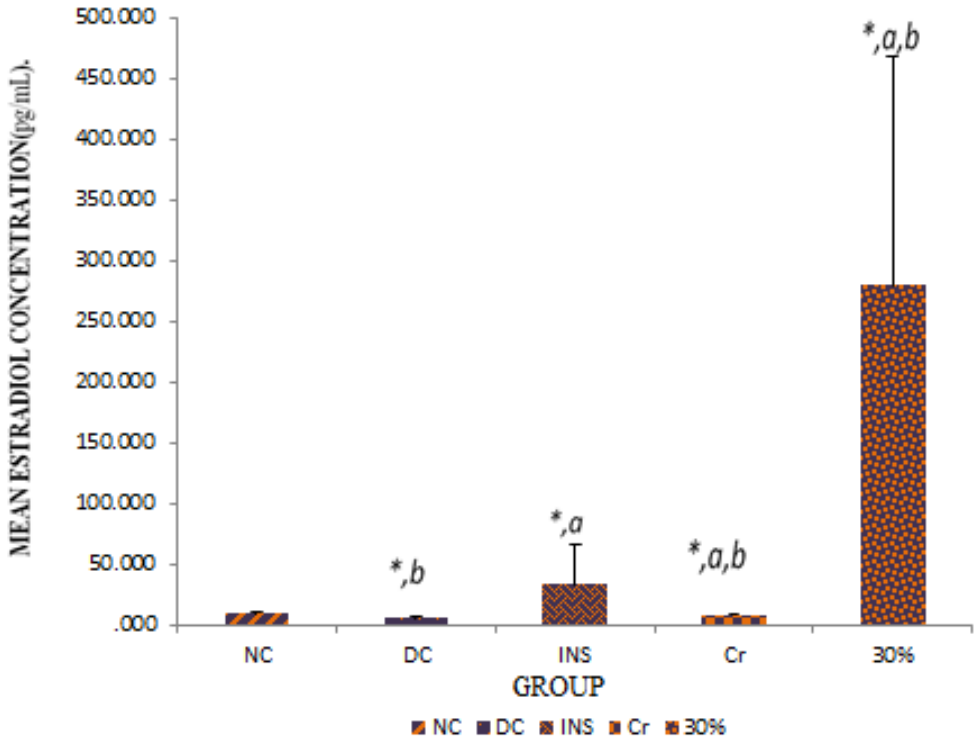

Figure 2. A graph of estradiol concentrations of the different experimental groups

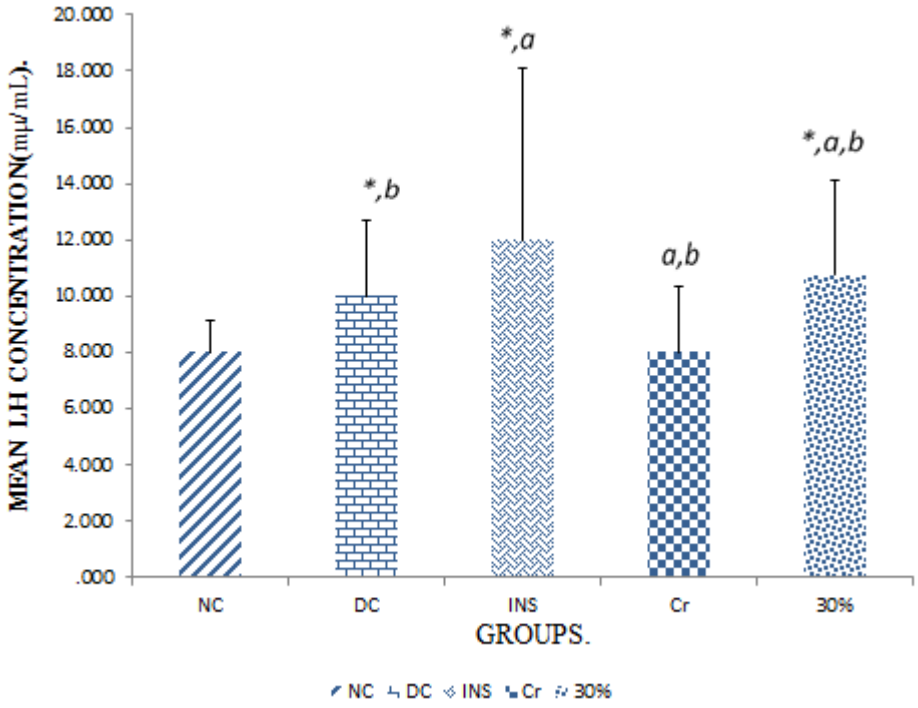

Figure 3. A graph of luteinizing hormone concentrations of the different experimental groups

There was a significant decrease ($p<0.05$) in serum follicle stimulating hormone levels in diabetic control group, insulin treated group, crude extract treated group and 30% flavonoid rich fraction treated group respectively when compared to the normal control group (figure 4). This is in agreement with the finding reported by Yakubu *et al.*, 2008, [22] in female rats treated with *Cnidoscolous aconitifolius* which shows decrease in serum follicle stimulating hormones level and is more or less consistent with previous studies on streptozotocin induced diabetic rats treated with combined extract of GL and VA by Asuquo *et al.*,2010, [25] which reported alternations, vaculations and distortion of both seminiferous epithelium and peritubular tissue, these suggested that decline reduction in thickness of seminiferous epithelium is likely to be attributed to follicle stimulating hormone perturbation.

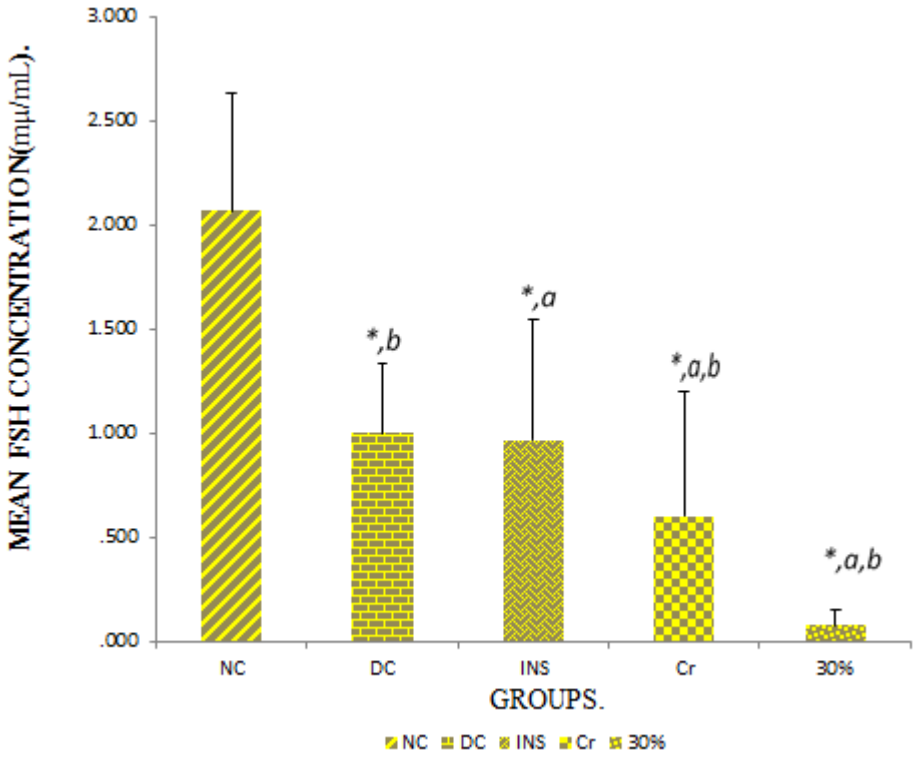

Figure 4. A graph of lollicle stimulating hormone concentrations (FHS) of the different experimental groups

Conclusions

From this work, it can be deduce that 30% flavonoid rich fraction of *Vernonia amygdalina* tend to have positive effect on both male and female sex hormone when compared to the whole leaves extract of *Vernonia amygdalina*, despite having high antidiabetic activities, hyperglycemic activities, hyperlipidemic activities, hypercholesterolemic activities and antioxidant activities.

Conflict of interest

The authors declare that there is no conflict of interest.

Acknowledgement

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