

EFFECT OF METHANOLIC EXTRACT OF *VERNONIA AMYGDALINA* ON REPRODUCTIVE PARAMETERS IN MALE ALBINO RATS

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ABSTRACT

Vernonia amygdalina is a shrub that grows throughout the tropical Africa. Several studies have reported the nutritional, anti-malaria and anti-helminthic effects of its extracts, but there is scanty information on its effect on reproduction. This study was designed to investigate the effect of its methanolic extract on reproductive parameters in male albino rats.

Methanolic extract of *Vernonia amygdalina* is designated as MEVA. MEVA was prepared using cold maceration. The extract was administered for 30 days for andrological studies. Distilled water (0.5 ml) served as the control. Plasma testosterone levels were assayed using Enzyme-link Immunosorbent Assay (ELISA) and semen analysis was done microscopically, histology of the testes was also done. Data were analyzed using ANOVA at $p < 0.05$.

Treatment of rats with all the doses of MEVA caused significant decrease in testosterone levels, sperm motility and sperm count relative to control, while there was no significant change in sperm viability. MEVA also caused severe germinal erosion and necrosis in the seminiferous tubules in the testes.

It can therefore be concluded that *Vernonia amygdalina* has deleterious effect on the reproductive functions in male albino rats.

Keywords: *Vernonia amygdalina*, Sperm count, Albino rats, Testosterone, Sperm motility

INTRODUCTION

Vernonia amygdalina belongs to the family of *Asteraceae*. It is commonly called "Bitter leaf" in English language, "Shuwaka" in Hausa language, "Onugbu" in Igbo language and "Ewuro" in Yoruba language.

It is a highly appreciated vegetable in West and Central Africa and can be consumed in various dishes (Bosni *et al.*, 1995). Medicinally, the leaves are widely used for fevers and are known as quinine substitute (Challand and Willcox, 2009). It is used to prepare cough medicine in Ghana (Akinpelu, 1991) and the root infusion is taken in Nigeria as an antihelminthic as well as for enteritis and rheumatism (Ainslie, 1937).

Pharmacological studies have shown that the leaf extract of *V. amygdalina* has both hypoglycemic and hypolipidemic properties in experimental animals and so could be used in the management of diabetes, hypertension etc (Akah and Okafor, 1992). The extracts of *V. amygdalina* have been reported to have analgesic and antipyretic effects (Tekoba *et al.*, 2002). The aqueous extract of *V. amygdalina* has also been reported to have anti-oxidant property (Nwajo and Nwokoro, 2004).

However, due to paucity of information from literature on the effect of *V. amygdalina* on reproductive parameters in male albino rats, this study therefore aims at investigating the effect of methanolic extract of *V. amygdalina* on these reproductive parameters.

MATERIALS AND METHODS

Experimental Animals

Adult male albino rats weighing between 160 g and 180 g bred in the Animal House of Physiology Department, LAUTECH, Ogbomoso were used. They were housed under standard laboratory conditions with a 12 hours daylight cycle and had free access to feed and water; they were acclimatized to laboratory conditions for two weeks before the commencement of the experiments. All experiments were carried out in compliance with the recommendations of Helsinki's declaration on guiding principles on care and use of animals.

Plant Material

Fresh specimens of *V. amygdalina* harvested from a local farm in Ogbomoso, Nigeria, were authenticated in the taxonomy unit of the department of pure and Applied Biology, LAUTECH, Ogbomoso.

Preparation of Methanolic Extract of *Vernonia Amygdalina* (MEVA)

Large quantities (1.56 kg) of the fresh specimens of *V. amygdalina* were washed free of soil and debris, and the roots were separated from the leaves and stems. The leaves and stems were air-dried for four weeks, and the dried specimens were pulverized using laboratory mortar and pestle.

Weighed portion (556 g) of the pulverized specimens were macerated with 70% methanol (1:2 wt./vol.) for 72 hours at room temperature (26 - 28° C). The resulting solution was then filtered using a wire-gauze and a sieve with tiny pores (0.25 mm). The 70% methanol was later evaporated using steam bath to give a percentage yield of 10.24% of the starting material.

Ten grams of the methanolic extract of *V. amygdalina* (MEVA) was dissolved in 100 ml of distilled water to give a concentration of 0.1 g/mL. The dosages of the extract administered in this study were in accordance with those reported by Challand and Willcox (2009).

Experimental Design

Twenty-four animals were randomly divided into four groups with each group consisting of six rats. The four groups of rats were subjected to the following oral treatments once a day for 30 days:

Group I rats received 50 mg/kg BW of MEVA

Group II rats received 100 mg/kg BW of MEVA

Group III rats receive 150 mg/kg BW of MEVA

Group IV rats received 1.0 ml of distilled water as the control group.

Twenty-four hours (day 31) after the last dosing of the four groups, blood samples were collected and the animals were then euthenised by cervical dislocation for semen analysis. Histological preparation of the testes was also carried out.

Body Weight

Body weight of each rat was monitored on weekly basis.

Collection of Blood Sample

Blood samples were collected through the medial cantus into EDTA bottles for hormonal assay.

Hormonal Assay

Plasma samples were assayed for testosterone using the enzyme-linked immunosorbent assay (ELISA) technique using the Randox kit.

Semen Collection

The testes were removed along with the epididymides. The caudal epididymides were separated from the testes, blotted with filter papers and lacerated to collect the semen.

Semen Analysis

Progressive Sperm Motility

This was done immediately after the semen collection. Semen was squeezed from the caudal epididymis onto a pre-warmed microscope slide (27°C) and two drops of warm 2.9% sodium citrate was added, the slide was then covered with a warm cover slip and examined under the microscope using X400 magnification. Ten fields of the microscope were randomly selected and the sperm motility of 10 sperms was assessed on each field. Therefore, the motility of 100 sperms was assessed randomly. Sperms were labelled as motile, sluggish, or immotile. The percentage of motile sperms was defined as the number of motile sperms divided by the total number of counted sperms (i.e. 100) (Mohammad-Reza *et al.*, 2005).

Sperm viability (Life/dead ratio)

This was done by adding two drops of warm Eosin/Nigrosin stain to the semen on a pre-warmed slide, a uniform smear was then made and dried with air; the stained slide was immediately examined under the microscope using x400 magnification. The live sperm cells were unstained while the dead sperm cells absorbed the stain. The stained and unstained sperm were counted and the percentage was calculated (Laing, 1979).

Sperm morphology

This was done by adding two drops of warm Walls and Ewas stain (Eosin/Nigrosin stain can also be used) to the semen on a prewarmed slide, a uniform smear was then made and air-dried; the stained slide was immediately examined under the microscope using x400 magnification (Laing, 1979). Five fields of the microscope were randomly selected and the types and number of abnormal spermatozoa were evaluated from the total number of spermatozoa in the five fields; the number of abnormal spermatozoa were expressed as a percentage of the total number of spermatozoa.

Sperm count

This was done by removing the caudal epididymis from the right testes and blotted with filter paper. The caudal epididymis was immersed in 5 ml formol-saline in a graduated test-tube and the volume of fluid displaced was taken as the volume of the epididymis. The caudal epididymis and the 5 ml formol-saline were then poured into a mortar and homogenized into a suspension from which the sperm count was carried out using the improved Neubauer haemocytometer under the microscope.

Testicular Histology

After weighing the testes, they were immediately fixed in Bouin's fluid for 12 hours and the Bouin's fixative was washed from the samples with 70% alcohol. The tissues were then cut in slabs of about 0.5 cm transversely and the tissues were dehydrated by passing through different grades of alcohol: 70% alcohol for 2 hours, 95% alcohol for 2 hours, 100% alcohol for 2 hours, 100% alcohol for 2 hours and finally 100% alcohol for 2 hours. The tissues were then

cleared to remove the alcohol, the clearing was done for 6 hours using xylene. The tissues were then infiltrated in molten Paraffin wax for 2 hours in an oven at 57°C, thereafter the tissues were embedded. Serial sections were cut using rotary microtome at 5 microns (5µm). The satisfactory ribbons were picked up from a water bath (50°-55°C) with microscope slides that had been coated on one side with egg albumin as an adhesive and the slides were dried in an oven. Each section was deparaffinized in xylene for 1 minute before immersed in absolute alcohol for 1 minute and later in descending grades of alcohol for about 30 seconds each to hydrate it. The slides were then rinsed in water and immersed in alcoholic solution of hematoxylin for about 18 minutes. The slides were rinsed in water, then differentiated in 1% acid alcohol and then put inside a running tap water to blue and then counterstained in alcoholic eosin for 30 seconds and rinsed in water for a few seconds, before being immersed in 70%, 90% and twice in absolute alcohol for 30 seconds each to dehydrate the preparations. The preparations were cleared of alcohol by dipping them in xylene for 1 minute. Each slide was then cleaned, blotted and mounted with DPX and cover slip, and examined under the microscope. Photomicrographs were taken at x40, x100 and x400 magnifications

Statistical Analysis

The mean and standard error of mean (S.E.M.) were calculated for all values. Comparison between the control and experimental groups was done using one-way analysis of variance (ANOVA) with Duncan's Multiple Range Test. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Effect on Body Weight

The administration of all treatment dose of MEVA to the rats for 30 days caused non-significant ($p > 0.05$) changes in body weight relative to their control.

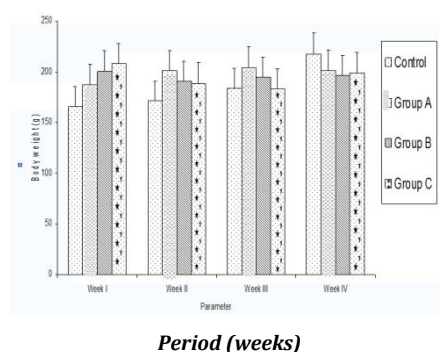


Figure 1: Body weight changes in control rats and rats treated with MEVA for 30 days.

Effect on Hormonal Levels

Treatment of rats for 30 days with all the treatment doses of MEVA caused significant ($P < 0.05$) decrease in testosterone levels relative to the control

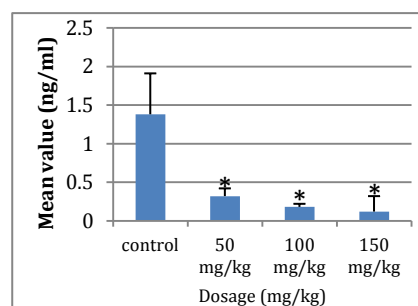


Figure 2: Effect of treatment of rats for 30 days with MEVA on plasma testosterone levels.

Effect on Sperm Characteristics

Treatment of rats for 30 days with all the various doses of MEVA (except 100 mg/kg) caused significant ($p < 0.05$) decrease in sperm motility relative to the control. All the treatment doses of MEVA caused non-significant ($p > 0.05$) decrease in sperm viability (life/dead). MEVA (except 50 mg/kg BW) caused non-significant ($p > 0.05$) increase in the percentage of abnormal sperm cells, while all the treatment doses of MEVA (except 150 mg/kg BW) caused significant ($p < 0.05$) decrease in sperm count.

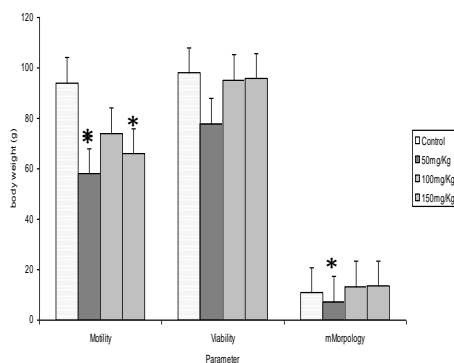


Figure 3: Spermogram showing the effect of MEVA on sperm characteristics after treatment of rats for 30 days (N=6*P<0.05)

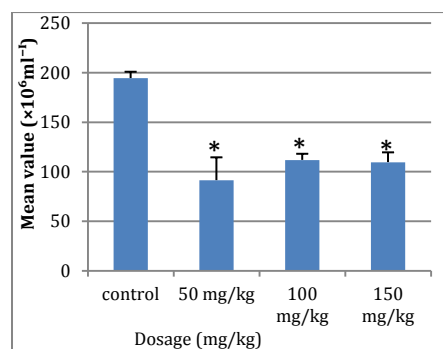


Figure 4: Spermogram showing the effect of MEVA on sperm count after the treatment of rats for 30 days (n=6,*p<0.05)

Histopathological Findings

Treatment of rats for 30 days with various doses of MEVA caused severe germinal erosion and necrosis in the seminiferous tubules while the control rats presented with normal germinal epithelium in the seminiferous tubules.

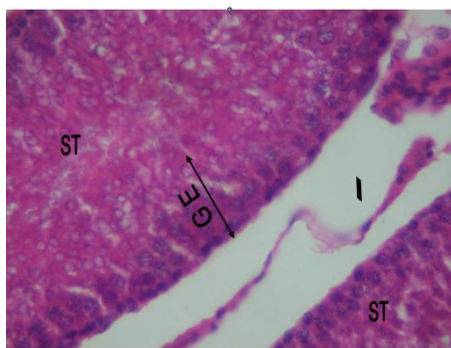


Plate 1: Effect of 0.5 ml of distilled water (control) on the testis after treatment of rats for 30 days at x 400. Photomicrograph showing the seminiferous tubules (ST) with normal germinal epithelium (GE).

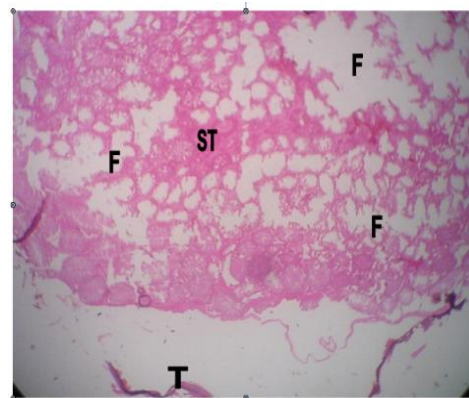


Plate 2: Effect of 100 mg/kg BW of MEVA on the testes at x 40. Photomicrograph showing severe germinal erosion and necrosis (F) in most of the seminiferous tubules (ST).

DISCUSSION

The extract caused non-significant changes in body weight of rats after treatment for 30 day, this suggests the extract was not toxic as well as non-androgenic in nature, since androgens are known to possess anabolic activities. Similar report was given by Gonzales *et al.* (2006) in rats treated with *Lepidium meyenii* extracts.

The extract caused significant decrease in testosterone levels. Similar report was given by Das *et al.* (2009) in rats treated with *Aegle mermelos* extract. This decrease in testosterone levels could indicate that the extract inhibit the mechanism intervening in the process of hormone synthesis in the Leydig cells.

The andrological results show that treatment of rats for 30 days with the extract significant decrease in sperm motility. Similar report was given by Verma *et al.* (2002) in rats treated with *Sarcotemma acidum* extract. This suggests that the extract was able to permeate the blood-testis barrier with a resultant alteration in the microenvironment of the seminiferous tubules, since it has been reported that the decrease in sperm motility caused by chemical agents was due to their ability to permeate the blood-testis barrier (Baldessarini, 1980) and thus, creating a different microenvironment in the inner part of the wall of the seminiferous tubules from that in the outer part (Bloom and Fawcett, 1975).

There was a statistically non-significant decrease in sperm viability as well as a non-significant increase in the percentage of morphologically abnormal sperm cells induced after treatment of rats with the extract. This could be due to the ability of the extract to either interfere with the spermatogenic processes in the seminiferous tubules, epididymal functions or activities of testosterone on hypothalamic release factor and anterior pituitary secretion of gonadotropins which may result in alteration of spermatogenesis (William, 2000; Bowman and Rand, 1985).

Sperm count is considered to be an important parameter with which to assess the effects of chemicals on spermatogenesis (Reddy *et al.*, 2006). Spermatogenesis is influenced by the hypothalamic-adenohypophysial - Leydig cell system relating gonadotrophin releasing hormone, leutinizing hormone and androgen. This implies that the decrease in sperm count caused by extract in the treated rats might be as a result of decrease in plasma level of testosterone, because this hormone has been reported to be important in the initiation and maintenance of spermatogenesis (Christensen, 1975). Similar report was given by Krishnamoorthy *et al.* (2007) in *Terminalia chebula* extract treated rats.

Treatment of rats with the extract caused severe germinal erosion and necrosis. This could be 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 (Burger and de Kretsner, 1989). Similar result was obtained in rats treated with *Colebrookia opositifolia* (Gupta *et al.*, 2001).

In conclusion, this study has shown that *Vernonia amygdalina* extract could have some toxic potentialities on the reproductive functions of male albino rats. However, its effect on human reproductive functions are unknown; nevertheless, considering these findings in animal model, it is recommended that men with infertility or reproductive problems should abstain from taking *Vernonia amygdalina* during the treatment period.

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