

EFFECT OF *CORCHORUS OLITORIUS* EXTRACT ON REPRODUCTIVE FUNCTIONS IN MALE ALBINO RATS

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Received: 31 Mar 2013, Revised and Accepted: 10 May 2013

ABSTRACT

Objective: This study was designed to evaluate the reproductive effect of aqueous extract of *Corchorus olitorius* on reproductive parameters in male albino rats.

Methods: The extract (250 mg/kg BW, 500 mg/kg BW, 750 mg/kg) was administered on daily basis for 30 days and blood samples were collected for hormonal assay; semen analysis was also carried out. Data were analysed using ANOVA at $p < 0.05$.

Results: Treatment of rats with all the treatment doses (250 mg/kg BW, 500 mg/kg BW, 750 mg/kg BW) caused significant ($p < 0.05$) decrease in testosterone levels, sperm motility, sperm count, sperm viability as well as significant ($p < 0.05$) increase in the percentage of abnormal sperm cells (with disorganization of testicular structure) relative to their respective controls.

Conclusion: These findings on the reproductive parameters suggest that aqueous extract of *Corchorus olitorius* has deleterious effect on reproductive functions in male albino rats.

Keywords: *Corchorus olitorius*, Sperm motility, Sperm count, Testosterone, Albino rats.

INTRODUCTION

Corchorus olitorius belongs to the family of *Tiliaceae*. It is commonly called jute in English language, "Moroheiya" by the Japanese and "Ewedu" by the Yoruba tribe of Nigeria.

It is an excellent source of vitamin A and C, fiber, minerals including calcium and iron and other micronutrients [1].

The plant has been reported to be demulcent, deobstruent, diuretic, lactagogue, purgative and tonic, as well as a folk remedy for aches and pains, dysentery, enteritis, fever, dysentery and tumors [2].

The methanolic extract of its seeds has been reported to show a broad spectrum of antibacterial activity [3]. Its aqueous extract has been reported to possess opioid-mediated anti-nociceptive activity [4]. The anticonvulsive property of its methanolic extract in mice has also been reported [5]. Its leaf extract has been reported to reduce elevation of postprandial blood glucose levels in rats as well as humans [6].

However, due to dearth of information from literature on the effect *Corchorus olitorius* on reproductive parameters in male albino rats, this study therefore aims at investigating the effect of aqueous extract *Corchorus olitorius* on these aforementioned 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, Ogbomosho 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 *Corchorus olitorius* harvested from a local farm in Ogbomosho, Nigeria, were authenticated in the taxonomy unit of the department of pure and Applied Biology, LAUTECH, Ogbomosho.

Preparation of Aqueous Extract of *Corchorus olitorius* (AECO)

Large quantities (2.00 kg) of the fresh specimens of *Corchorus olitorius* 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 (590 g) of the pulverized specimens were macerated with distilled water (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 distilled water was later evaporated using steam bath to give a percentage yield of 12.5% of the starting material.

Ten grams of the aqueous extract of *Corchorus olitorius* (AECO) was dissolved in 100 ml of distilled water to give a concentration of 0.1g/mL.

Acute Toxicity Test

Acute toxicity test was carried out as described by [7]. Adult albino mice of either sex were divided into four groups with each group consisting of five animals. The mice were fasted for 18 hours with water *ad libitum*.

The extract was administered by gavage at doses of 2500 mg/kg BW, 5000 mg/kg BW and 7500 mg/kg BW to the first three groups. The fourth group was given 1.0 ml of distilled water as the control group. The animals were observed for 72 hours for behavioural changes and mortality.

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 250 mg/kg BW of AECO

Group II rats received 500 mg/kg BW of AECO

Group III rats receive 750 mg/kg BW of AECO

Group IV rats received 0.5 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 euthanised

by cervical dislocation for semen analysis. Histological preparation of the testes was also carried out.

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) [8].

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 [9].

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 [9]. 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 (5 µm). 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

Acute Toxicity Test

No mortality and changes in behavior were observed in all the treated and control groups of mice up to 7500 mg/kg BW. Hence, one-tenth of the doses were used for this study.

Effect on Hormonal Levels

The effect of AECO at various doses on testosterone levels of albino rats after treatment of rats for 30 days is shown in Figure 1.

Treatment of rats with all the treatment doses (250 mg/kg BW, 500 mg/kg BW, 750 mg/kg BW) caused significant ($p < 0.05$) decrease in testosterone levels relative to the control.

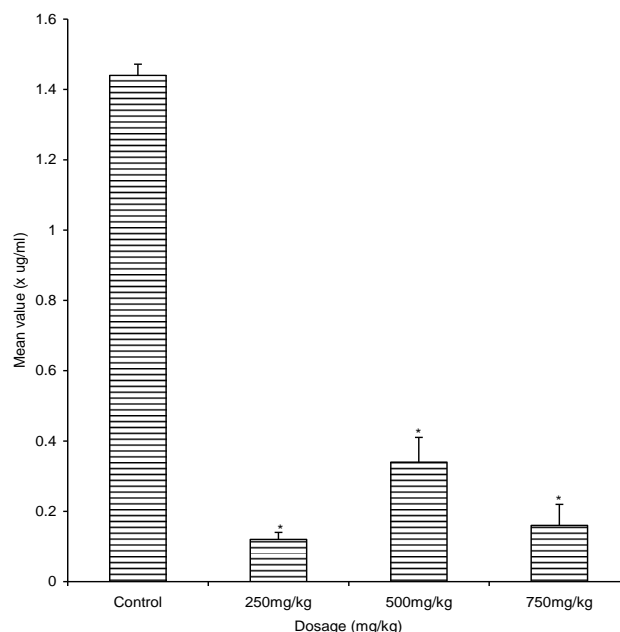


Fig. 1: Effect of treatment of rats for 30 days with AECO on plasma testosterone levels (n=6, * $p < 0.05$)

Effect on Sperm Characteristics

The effect of AECO at various doses on sperm characteristics and sperm count of albino rates after treatment of rats for 30 days shown respectively in spermograms of Figures 2 and 3.

Treatment of rats with all the treatment doses (250 mg/kg BW, 500 mg/kg BW, 750 mg/kg BW) caused significant ($p < 0.05$) decrease in sperm motility, sperm count, sperm viability as well as significant ($p < 0.05$) increase in the percentage of abnormal sperm cells relative to their respective controls.

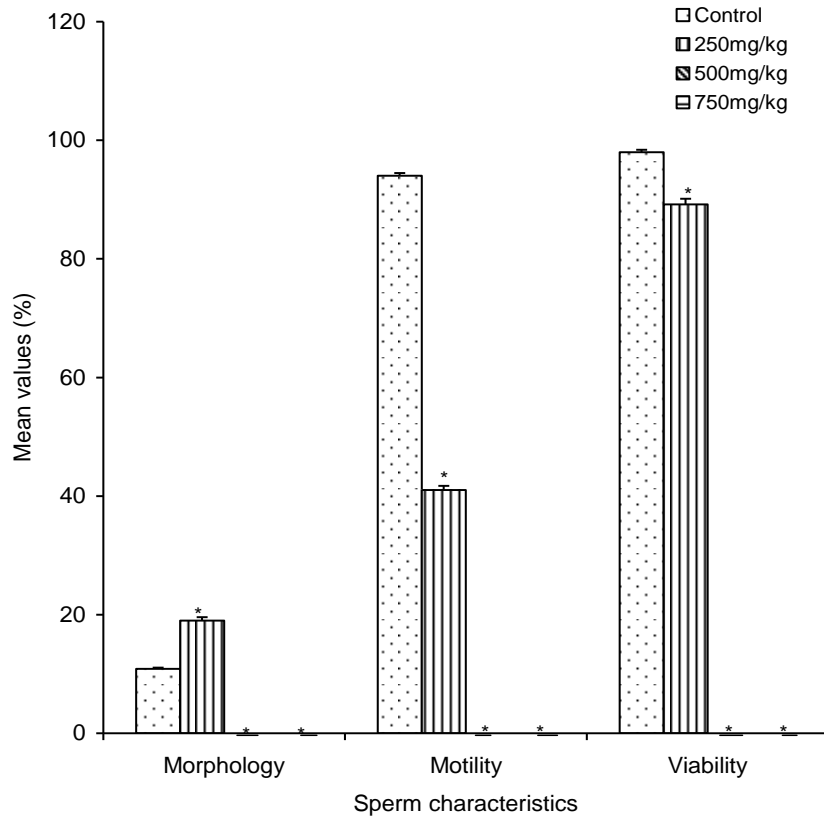


Fig. 2: Spermogram showing the effect of AECO on sperm characteristics after treatment of rats for 30 days (n=6, *p<0.05)

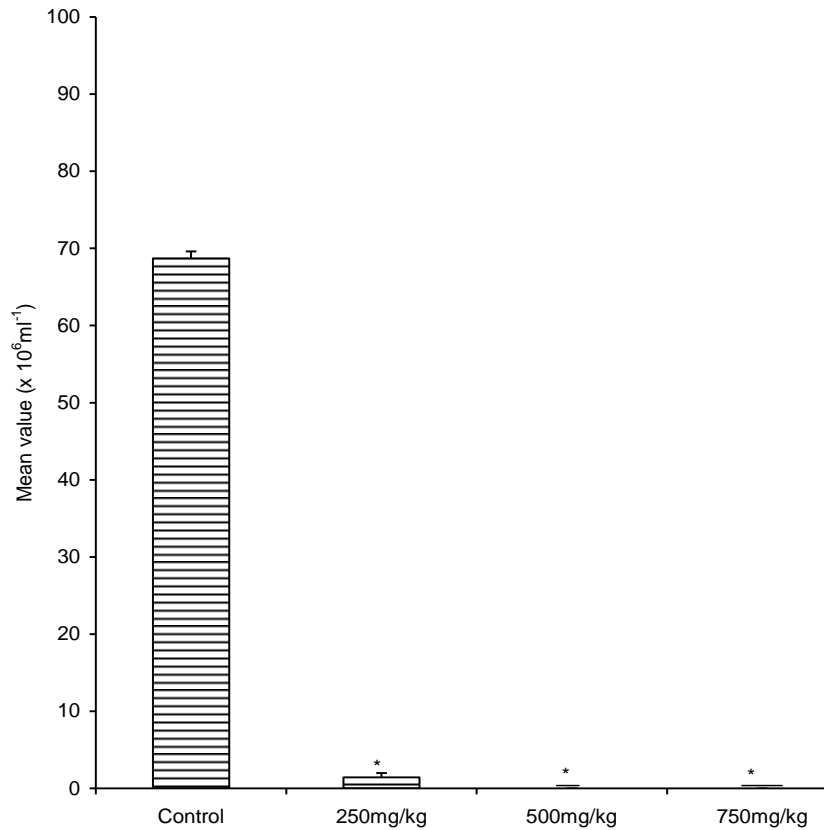


Fig. 3: Spermogram showing the effect of AECO on sperm counts after treatment of rats for 30 days (n=6, *p<0.05)

Histopathological Findings

Treatment of rats with the extract caused disorganization of the testicular structure as well as loss seminiferous tubules; however, the control rats presented with normal germinal epithelium.

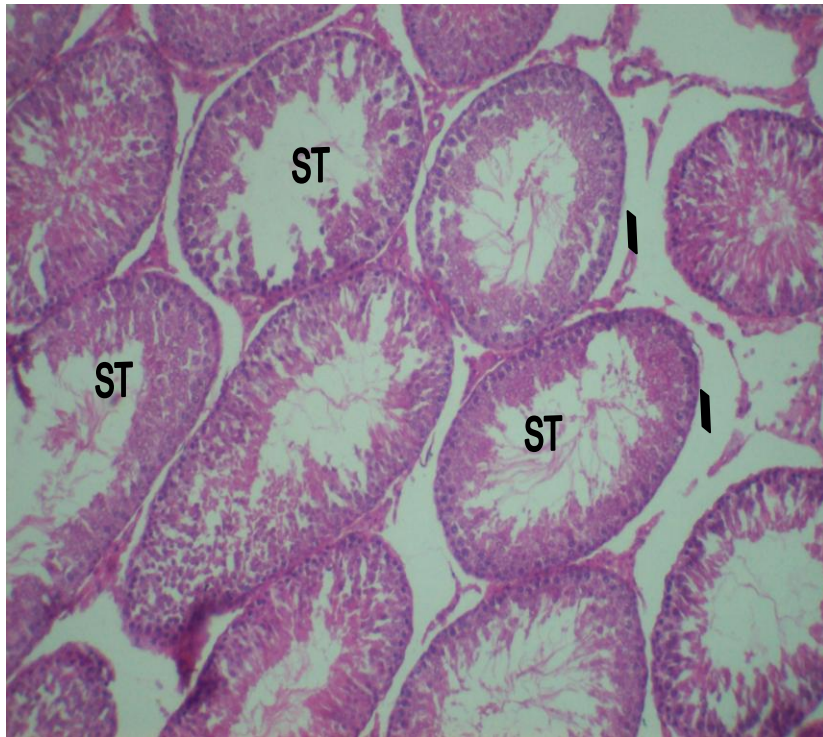


Plate 1: Effect of 0.5ml distilled water (control) on the testes after treatment of rats for 30 days (at x100)

Photomicrograph showing normal viable germinal epithelium. No visible lesions were observed at the interstitium (I) and the seminiferous tubules (ST)

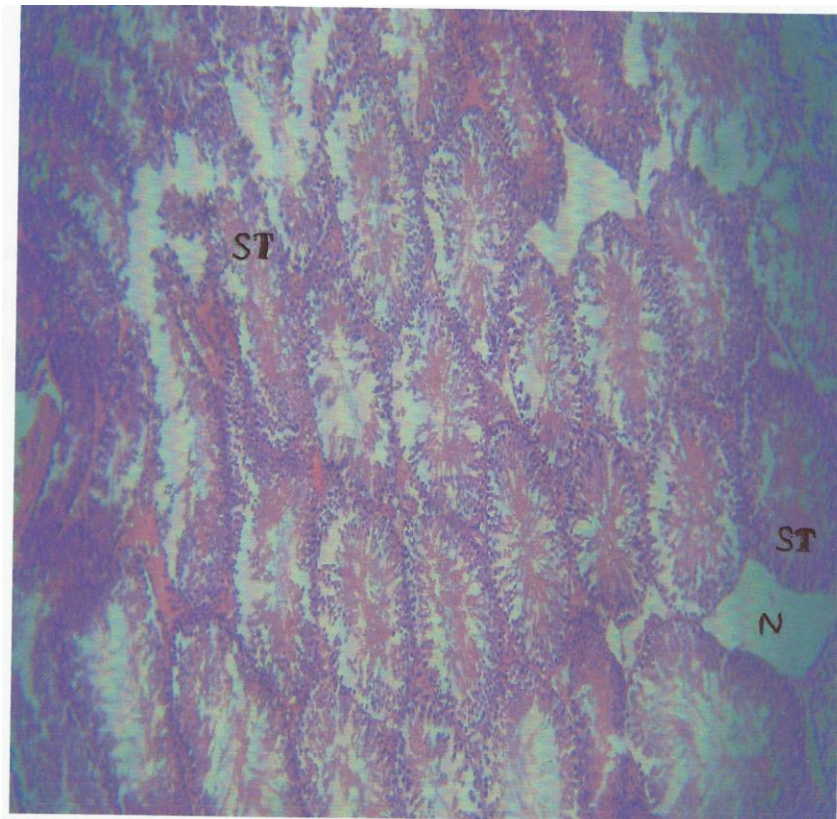


Plate 2: Effect of 250 mg/kg BW of AECO on the testis after treatment of rat for 30 days (X100)

Photomicrograph showing disorganized testicular structure with seminiferous tubules (ST)

DISCUSSION

The LD₅₀ gives a measure of the immediate or acute toxicity of a test substance [10]. This study revealed that AECO at high doses of 2500 mg/kg BW, 500 mg/kg BW and 7500 mg/kg BW had no apparent toxic and lethal effects on the animals which probably indicates that the extract has high safety index. This could be due to hepatic metabolism of the toxic potentialities of the extract, since the extract is administered orally.

The extract caused significant decrease in testosterone levels. Similar report was given by [11] and [12] in rats treated with *Aegle mermelos* and *Carica papaya* extracts respectively. 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 also show that treatment of rats for 30 days with extract caused significant decrease in sperm motility. Similar report was given by [13] 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 [14] and thus, creating a different microenvironment in the inner part of the wall of the seminiferous tubules from that in the outer part [15].

There was a statistically significant decrease in sperm viability and a significant increase in the percentage of morphologically abnormal sperm cells induced after treatment of rats with extract. This could be due to the ability of 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 ([16]; [17]). Contrary result was reported by [18] in *Ficus carica* extract treated rats.

Sperm count is considered to be an important parameter with which to assess the effects of chemicals on spermatogenesis [19]. 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 the 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 [20]. Similar report was given by [21] in *Terminalia chebula* extract treated rats.

The disorganization of the testicular structure and loss of seminiferous tubules induced by the extract could be due to testosterone deficiency, because this hormone has been reported to be important in the initiation and maintenance of spermatogenesis [20].

In conclusion, this study has shown that aqueous extract of *Corchorus olitorius* 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 *Corchorus olitorius* during the treatment period.

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