# Methanol Root Extract of *Rhaphiostylis beninensis* Planch Ex Benth (Icacinaceae) Reverses Alcohol-induced Changes in Sex Hormones and Semen Quality in Male Rats

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**The methanol root extract of *Rhaphiostylis beninensis* was investigated for its ability to reverse alcohol-induced changes in sex hormones, semen quality and histo-architecture of testes in male rats. Rats were divided into 5 groups, namely, normal control (1), negative control (2), extract treated (3 and 4) and vitamin E treated (5). The rats were fed 20% v/v alcohol except for those in group 1. In addition, groups 3 and 4 rats received the extract at concentrations of 200 and 400 mg/kg, respectively while group 5 received 300 mg/kg of vitamin E. After 30 days, serum concentrations of testosterone, estrogen, progesterone, prolactin, follicle stimulating hormone, and luteinizing hormone were determined. Sperm count, motility, morphology and histo-architecture of testes were also evaluated. Significant increase ($p < 0.01$) in serum concentration of testosterone, sperm count, and progressive motility was observed in extract-treated groups. Increase in spermatogenic activity of testes of extract-treated rats was also observed, suggesting that the plant extract may be useful in ameliorating alcohol-induced male reproductive toxicity.**

**Keywords:** *Rhaphiostylis beninensis*, Icacinaceae, semen quality, testes-histology, male hormones, vitamin E

## INTRODUCTION

Infertility, typically defined as "inability to achieve pregnancy within 1 year of regular, unprotected intercourse", is reported to affect approximately 15% of all couples, with no identifiable cause in 25% of cases and an identifiable male causative factor in about 40% of couples [1]. Male factor infertility (MFI), where present, is almost always defined by findings of abnormal semen analysis, evidenced by alteration in sperm concentration, motility, and/or morphology [2]. Chronic alcohol use is associated with a damaging effect on the male reproductive system and by extension impairment of male fertility [3]. Evidence abounds to support a steady increase in the use of herbal medicine to manage various adverse health conditions, including MFI [4].

*Rhaphiotylis beninensis*, a liana native to tropical Africa, grows well in the West and South African regions. In Nigeria, it is found mainly in the southern part of the country and known by local names such as; "Usuende/Usumede" (Edo), "Kpolokoto/Ike/Ikpokrikpo" (Igbo), "Kumeri" (Itsekhiri), "Umeni" (Urhobo) and "Idiapata/Itapara" (Yoruba) [5]. Different parts of the plant are used in the treatment of arthritis, inflammation, hemorrhoids, respiratory complaints, insanity and improvement of male fertility [5, 6-7]. Locally the root of the plant is used as a spice and condiment in making soups. Anti-inflammatory, analgesic, antibacterial, cytotoxic, growth inhibition, anti-sickling, anti-oxidant, and aphrodisiac activities have been reported for the plant [5, 8-14].

A previous study documented the aphrodisiac activity of the root of the plant [14]. This study aimed at investigating the possible ameliorative effects of the methanol root extract of the plant on reproductive damage induced by chronic alcohol consumption in male rats.

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## EXPERIMENTAL

### Plant collection and identification

The root of *R. beninensis* was collected in Iguiye community, Ovia South East Local Government of Edo State, in February 2016. It was identified and authenticated by Dr. Olufemi Shasanya of Forest Research Institute of Nigeria, Ibadan, and issued with herbarium number FHI 100680.

### Plant preparation and extraction

The plant material was washed free of adhering debris, chopped into smaller pieces, shade dried for two weeks and milled to a powder. The powdered plant material (2.1 kg) was defatted with petroleum ether (40-60ºC) in a Soxhlet apparatus and re-extracted with methanol. The methanol extract (RBE) was concentrated *in vacuo* and stored in a glass bottle at 4ºC until required.

### Experimental animals

Thirty male Wistar rats weighing 200-220 g housed in the Animal House of the Department of Pharmacology, Faculty of Pharmacy, University of Benin, were utilized in this study. The housing environment was clean, well ventilated with a relative humidity of 67% ± 3% and a normal day/night cycle of 12 hr. Animals were allowed to acclimatize for two weeks and had unrestricted access to clean drinking water and animal chew. Ethical approval for the use of laboratory animals in the study was obtained from the Faculty of Pharmacy, University of Benin Ethics Committee. The animals were handled as much as possible in accordance with standards of the Public Health Service policy on Humane Care and Use of Laboratory Animals [15].

Animals were divided into five groups of six rats each. Animals in group 1(normal control) had access to food and water only. Group 2 animals (negative control group) were fed with 5 ml/kg of 20% v/v alcohol orally. Groups 3 and 4 animals received 200 and 400 mg/kg of RBE, respectively, while group 5 animals received 300 mg/kg of vitamin E orally. Additionally, animals in groups 3, 4, and 5 received 5 ml/kg of 20% v/v alcohol. Treatment lasted for 30 days.

### Morphological, biochemical and histological tests

At end of the treatment period, animals were anesthetized by chloroform inhalation and blood was collected by cardiac puncture into lithium-free bottles, allowed to clot, and centrifuged for 10 minutes at 3000 rpm. Serum collected was assayed for testosterone, progesterone, estrogen, luteinizing hormone, prolactin, and follicle stimulating hormone levels using the enzyme-linked immune-sorbent assay (ELISA) hormone test kits. The sperm count, motility, and morphology were evaluated according to the WHO method [2], following excision of the caudal epididymis to obtain caudal epididymal sperm. Histological evaluation of the testis was carried out as described by Drury and Wellington [16]. Harvested testes from animals were preserved in 1% formaldehyde and thereafter fixed in 4% paraformaldehyde in 0.1M phosphate buffer solution. Tissue was dehydrated with ethanol, cleared with xylene, and embedded in paraffin. Sectioned tissue (5 µm) was counterstained with hematoxylin in eosin, examined under a microscope at ×400, and photographed.

### Statistical analysis

Results are presented as means ± standard error of mean (S.E.M). Statistical analysis was performed using IBM SPSS Statistics for Windows, version 20 (IBM Corp., Armonk, N.Y., USA) and analyzed by one-way analysis of variance (ANOVA) followed by Tukey's test. Differences were considered significant at $p < 0.05$ and $p < 0.01$.

## RESULTS AND DISCUSSION

### Effect of methanol root extract of *R. beninensis* on sex hormones

Compared to the negative control rats, RBE at both concentrations occasioned a decrease in circulating serum concentration of estrogen, progesterone and prolactin while a significant ($p < 0.01$) increase in testosterone was observed as shown in table 1. Serum concentration of LH and FSH were relatively unaffected in 200 mg/kg treated animals. An increase in LH and FSH was recorded in the 400 mg/kg RBE-treated rats. Vitamin E elicited a significant reduction in serum concentration of prolactin ($p < 0.05$) and increase in testosterone ($p < 0.01$). Decrease in serum concentration of estrogen and progesterone, and increase in LH and FSH was equally observed in Vitamin E treated rats compared to the negative control rats.

Hormones play a vital role in the endocrine regulation of male reproductive system activities and functions. In particular, the development of male secondary sexual characteristics and spermatogenesis is stimulated by testosterone [17]. The production of testosterone is controlled by LH, released from the anterior pituitary to act on the Leydig cells of the testes. Testosterone is released from the testes into the peripheral circulation via a concentration gradient. Under normal physiological conditions, the amount of testosterone in circulation is regulated through a negative feedback mechanism that controls the release of LH and FSH from the pituitary gland and their action on the testes. Decrease in serum concentration of testosterone may be due to impaired production and/or increased metabolism as a result of an up-regulation in the activity of the aromatase enzyme [17]. Induction of oxidative stress is a recognized mechanism by which alcohol; a gonado-toxin induces low levels of testosterone and high levels of estrogen [18]. In the present study, rats in the negative control group had the lowest concentration of testosterone; a finding supported by previous studies [19, 20]. Although the exact mechanism by which RBE and vitamin E spur increase in the serum concentration of testosterone as well as a decrease in estrogen, progesterone and prolactin, when co-administered with alcohol is not clear, it may not be unconnected to their antioxidant activities. Natural antioxidants have been reported to attenuate damaging changes in the reproductive system of man and rodents due to oxidant molecules [21]. Previous studies have reported the *in vivo* and *in vitro* antioxidant activities of RBE [12]. Asuquo et al [22] reported increase in serum concentration of testosterone, LH and FSH in male rats treated concomitantly with alcohol and the ripe fruit extract of *Carica papaya*. The mechanism of action, though not specific, was linked to the antioxidant activities of secondary metabolites such as flavonoids, saponins and alkaloids present in the extract. These same secondary metabolites have previously been identified in RBE [8,10]. Vitamin E is a potent antioxidant molecule that scavenges free radicles and, prevents the formation of new ones. It is reported to reverse alcohol-induced decline in serum concentration of testosterone, a finding supported by this study [21]. However, reports of its effect on alcohol-induced changes in LH, FSH, estrogen prolactin and progesterone could not be substantiated at this time.

**Table 1: Effect of methanol root extract of *R. beninensis* on sex hormones**

| Groups/hormone | Estrogen (pg/ml) | Progesterone (ng/mL) | Prolactin (mIU/mL) | Testosterone (ng/mL) | LH (mIU/mL) | FSH (mIU/mL) |
|---|---|---|---|---|---|---|
| **Normal control** | 23.70±2.18 | 0.38±0.07 | 6.48±1.11 | 0.48±0.04 | 1.72±0.25 | 4.24±0.41 |
| **Negative control** | 35.40±4.55 | 0.68±0.09 | 12.52±1.14 | 0.08±0.04 | 1.24±0.24 | 4.28±1.29 |
| **200 mg/kg RBE** | 27.20±3.49 | 0.56±0.12 | 8.82±1.26 | 0.28±0.04* | 1.44±0.01 | 4.10±0.61 |
| **400 mg/kg RBE** | 26.85±2.08 | 0.50±0.30 | 7.98±1.31* | 0.30±0.07** | 1.46±0.04 | 4.62±0.84 |
| **Vit. E (300 mg)** | 25.48±1.48 | 0.47±0.22 | 7.03±0.94* | 0.30±0.04** | 1.51±0.02 | 4.92±0.75 |

n = 6, *Statistically significant ($p < 0.05$),**Statistically significant ($p < 0.01$); (LH = Luteinizing hormone; FSH = Follicle stimulating hormone).

**Effect of methanol root extract of *R. beninensis* on semen quality**

Male fertility is always almost determined from data obtained from a sperm analysis [2]. The number of sperm cells per millimeter of ejaculate is known as sperm count, while the ability of sperm cells to move properly is referred to as sperm motility. Sperm cells that move in a straight, forward and propulsive manner are referred to as progressive motile sperm, while those that show some form of movement, but in a non-propulsive and forward pattern are described as non-progressive motile sperm. Immotile sperm refers to sperm cells with no movement. Deformity in the morphology of sperm cells manifest in several ways which may lead to the term normal/abnormal sperm cells [23]. As shown in table 2, in comparison to the normal control rats, the negative control rats recorded a decrease in sperm count, PMS and the percentage of normal sperm cells, while there was an increase in NPMS, IMS and abnormal sperm cells. Rats that received 200 mg/kg of RBE showed an increase in sperm count, PMS, normal sperm and a decrease in NPMS, and abnormal sperm compared to the negative control rats. An increase in sperm count, PMS, normal sperm and a significant decrease in NPMS, IMS and abnormal sperm was evoked by 400 mg/kg of RBE, compared to negative control rats. Vitamin E evoked an increase in sperm count, PMS, normal sperm and a decrease in NPM, IMS and abnormal sperm in comparison to the negative control rats.

The same mechanisms responsible for normal endocrine and hormonal regulation of male fertility are associated with the attainment of normal and healthy spermiogram in men. Increased alcohol use is closely linked with decreased sperm count, increase in the percentage of non-progressive motile, immotile and morphologically abnormal sperm cells [18], a finding corroborated in this study. It has been hypothesized that these effects may be due to an alteration of the endocrine system controlling the hypothalamic pituitary testicular axis functions and/or the male reproductive accessory glands through increased production and activity of reactive oxygen species [23]. Ability of plants such as *Zingiber officinale, Vitis vinefera, Tetracarpidum conophorum* and Vitamin E to ameliorate the damaging effect of alcohol on sperm count, motility and morphology is closely linked with their free radical scavenging and quenching properties [24-26, 21].

**Table 2: Effect of methanol root extract of *Rhaphiostylis beninensis* on semen characteristics**

| Group/Par-ameter | Sperm count ($\times 10^6$/mm) | PMS (%) | NPMS (%) | IMS (%) | Normal sperm (%) | Abnormal sperm (%) |
|---|---|---|---|---|---|---|
| **Normal control** | 32.10±4.46 | 89.00±2.60 | 6.30±0.15 | 4.70±1.13 | 81.00±3.50 | 19.00±1.50 |
| **Negative control** | 20.67±5.07 | 51.30±1.15 | 29.20±2.53 | 19.50±3.60 | 56.00±2.60 | 44.00±3.10 |
| **200 mg/kg** | 23.22±3.39 | 59.50±1.68 | 20.80±3.01 | 19.70±2.80 | 62.20±3.10 | 37.80±2.20 |
| **400 mg/kg** | 26.15±3.81 | 80.10±3.40 | 8.40±1.01** | 11.50±1.83* | 78.00±1.80 | 22.00±1.90* |
| **Vitamin E** | 28.78±2.93* | 75.50±3.80 | 15.30±2.40 | 9.20±1.21** | 75.30±2.10 | 24.70±1.70 |

n = 6, *Statistically significant ($p < 0.05$);**Statistically significant ($P < 0.01$): PMS -Progressively motile sperm; NPMS - Non-progressively motile sperm; IMS-Immotile sperm; N. Control- Normal control.

**Effects of the methanol extract of the root of *R. beninensis* on the histology of rat testis**

The testes serve as the primary organs for spermatogenesis and testosterone production in males. The Leydig cells under the influence of LH, control the production of testosterone, while the Sertoli cells are involved in spermatogenesis under the influence of FSH. Both functions are regulated by the hypothalamic-pituitary gonadal systems [27]. Acute and chronic consumption of alcohol is reported to induce testicular atrophy in man and experimental animals, where it acts as a Leydig and Sertoli cell toxin impacting the production of testosterone negatively and impairing spermatogenesis [28]. In our present study, normal control rats displayed normal morphology of seminiferous tubules with thick epithelium (Figure 1(I)). Focal arrest in spermatogenesis, decreased number and activity of the Sertoli cells were observed in the negative control animals (figure 1(II)). Animals treated with 200 and 400 mg/kg of RBE were observed to present with interstitial congestion of the seminiferous tubules, tightly packed tubules, Leydig cell hyperplasia and an increase in number and activity of the Sertoli cells (seen by the presence of a dark vacuole when viewed under the microscope) showing that RBE was able to ameliorate the effect of alcohol on the testis of experimental animals and impact positively on spermatogenesis (Figure 1(III) and 1(IV)). Testes of rats treated with Vitamin E (figure 1(V)) were equally observed to present with tightly packed tubules and Leydig cell hyperplasia signifying increased activity. The observed effects of RBE and vitamin E may be due to their countering effects on the ability of alcohol to reduce the concentration of testicular and/or blood levels of testosterone which in turn led to increased levels of serum testosterone and spermatogenesis.

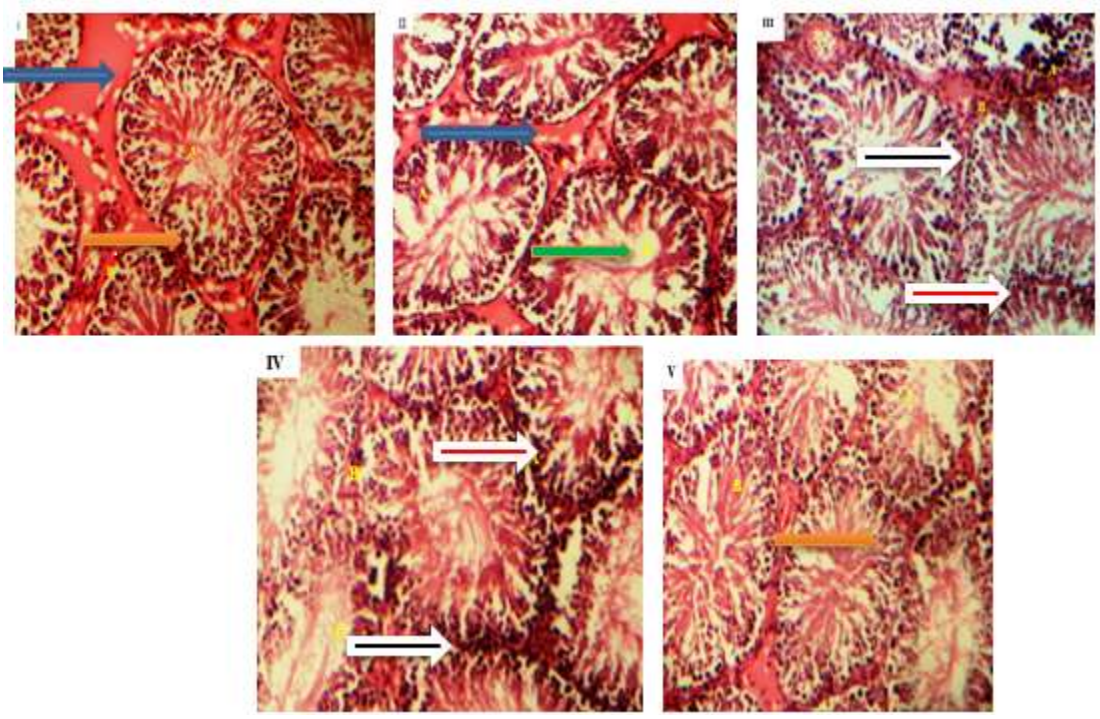


**Figure 1: Effect of methanol root extract of *R. beninensis* on histo-architecture of rat testis. (I- Normal control; II-Negative control; III- 200 mg/kg treated; IV- 400 mg/kg treated; V- Vitamin E treated animals.**

Key:

— Tightly packed interstitial cells;

— Sertoli cells hyperplasia;

Focal spermatogenic arrest

Normal sequential maturation

Increased diameter of tubular lumen

## CONCLUSION

The root extract of *R. beninensis* was shown to ameliorate the damaging effect of alcohol administered to rats for 30 days, by increasing sperm count, motility, and viability. It was also shown to increase the concentration of testosterone, progesterone, luteinizing hormone, and estrogen, while a decrease in prolactin was observed and the concentration of follicle stimulating hormone remained unaffected. Rats that received the extract and vitamin E displayed increased number and activity of Sertoli cells. More studies are advocated to determine the exact constituent/s of the plant and mechanism/s that may be responsible for these actions.

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