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Original Research Article

Low Dose Earth Ball (*Icacina mannii*) may be Beneficial to Reproductive Health in Males

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Abstract

This study investigated the effects of ethanolic extract of Earth ball (*Icacina mannii)* tuber on the testes. *Icacina mannii* is a well-known plant in Africa due to its unique ball-shaped underground tuber. The extract was prepared by washing the tuber, cutting it into pieces, sun drying it, and extracting it with 80% ethanol. Four-week-old male Wistar rats were used in the experiment and were randomly assigned to four groups. Group A served as the control and received distilled water, while groups B, C, and D received low, intermediate, and high doses of the extract, respectively. The rats were fed for 28 days. On the twenty-eighth day, the animals' final weight was taken, and blood samples were obtained by cardiac puncture for testosterone level analysis. The testes were recovered for histology. The results showed a significant increase in testosterone level in the low and medium dose groups and a significant decrease in the high dose group when compared with the control. The histologic section of the testes of rats in groups B and C revealed hypochromic sections of seminiferous tubules with swollen germinal cells and spermatogenic lining cells and irregular alignment of myoid cells when compared to the control group. The testicular histologic section of rats in group D showed atrophied and necrotic testicular cells. This study shows that *Icacina mannii* tuber could be a potential plant for research in the management of infertility in men due to testosterone deficiency, but high doses could be necrotic and atrophic to the testes.

Keywords: Earth Ball, *Icacina mannii*, testosterone, testes, male infertility.

1. INTRODUCTION

Infertility is a reproductive health issue characterized by the inability to achieve a successful pregnancy after one year of regular unprotected sexual intercourse among individuals aged 15-49 (World Health Organization (WHO), 2023). It affects more than 10% of the global population and around 30% of the population in Sub-Saharan Africa (Menashe-Oren and Sánchez-Páez, 2023). WHO (2023) reports that a significant number of people experience infertility at some point in their lives, with approximately 17.5% of the global adult population, or roughly 1 in 6 individuals, facing fertility challenges. This underscores the urgent need to enhance access to affordable and high-quality fertility care for those in need and there has been a rising exploration into the potential of herbal medicine (Diame, 2010; Menashe-Oren and Sánchez-Páez, 2023). Herbal medicine focuses on the study and utilization of plants for medicinal purposes. Petrovska (2012) opined that healing with medicinal plants is as old as mankind itself. Plants contain phytochemicals, including antioxidants, which play a vital role in their normal functioning, adaptation to the environment, and potential health benefits for humans. Nonetheless, studies have noted deleterious effects when medicinal plants are consumed in high doses. For example, Rojas-Armas *et al., (*2019) reported that in a 28-day period of treatment with repeated doses of *Thymus vulgaris* (a medicinal plant), toxicity was observed. The investigators thus concluded that high doses are deleterious to health and should be avoided. Anywar *et al.,* (2021) added that despite the benefits derived from medicinal plants, some may be a threat to the health of the users, due to potential harmful effects or side effects which may be related to overdoses or toxic principles. Therefore, it is crucial to explore the dosage that is optimal for health.

Icacina mannii is a shrub found in scandent forests, forest regrowth areas, and swamp forests. It typically reaches a height of 0.8–3.4 meters and has alternate leaves with leaf stalks measuring 0.4–1.2 centimeters in length. The leaves are broadly obovateelliptic in shape, abruptly acuminate at the apex, and either cuneate or acute at the base. They range from 11.3–22.5 centimeters in length and 5.4–12.0 centimeters in width. The leaves are sparsely hairy beneath or glabrescent. The tuber (corm) of *Icacina mannii*, which is the focus of this study, is large, cylindrical, and weighs between 0.2–0.4 kilograms (Ekeke *et al.*, 2021).

Ekeke *et al.,* (2021) identified the presence of starch and tannin in the corm of *Icacina mannii*. Previous phytochemical screenings conducted by Antai and Obong in 1992 also revealed the existence of oxalates, alkaloids, hydrogen cyanide, phytic acid, and tannins in the plant. Phytochemicals found in the tuber of *Icacina mannii* have been reported to exert both positive and negative effects on the body's systems. Tannins, for example, have been shown to have various physiological effects, such as promoting blood clotting, reducing blood pressure, lowering serum lipid levels, inducing liver necrosis, and modulating immune responses (Zhou and Jin, 2021).

Previous studies investigating different forms of *Icacina mannii* extracts have demonstrated both positive and negative effects on bodily functions. Essien and Sam (2018) and Essien (2021) did not observe any hematological alterations following the administration of *Icacina mannii* extract, while Solomon *et al.,* (2011) observed an increase in total white blood cell count and a decrease in packed cell volume after consumption of the extract. Other known effects of *Icacina mannii* include increased weight in dressed organs such as the lungs, gizzard, liver, neck, shank, and intestine (Umoren *et al.,* 2007). Male infertility is a growing concern and accounts for a significant percentage of total infertility cases and can be caused by a myriad of reasons. One notable one is prolonged stress as observed by Rehman *et al., (*2020). They found that long-term stress produces elevated cortisol levels which are found to be associated with male infertility owing to decreased conversion of androstenedione to testosterone, thereby reducing sperm volume and concentration. Plants have a long folklore of use in aiding fertility (Ebiyemzi *et al.,* 2021). A study by Abarikwu *et al.,* (2020) revealed some plants used in the management of infertility with possible mechanisms of action including spermatogenesis-inducing and antioxidative potency with the primary target organ as the testis. These plants share some phytochemicals as contained in *Icacinia mannii* tuber. *Icacinia mannii* tuber has been reported to be used in the treatment of some diseases but there is very limited evidence on its use for the treatment of male infertility.

The purpose of this study is therefore to evaluate the dose of ethanolic extract of *Icacina mannii* tuber that can potentially favour male fertility with specific focus on the testis of male rats using the following criteria: testosterone levels and testicular histology.

2. METHODOLOGY

The study involved the collection and identification of *Icacinia manni*, a plant found in Uyo, Akwa Ibom state of Nigeria. The tuber of the plant was washed, cut into pieces, and sun-dried before being sent to the Department of Pharmacognosy, University of Uyo, Nigeria for extraction with 80% ethanol. The extraction process involved macerating the dried tuber in 80% ethanol for 72 hours to obtain the crude ethanolic extract, while the other part was successively macerated for 72 hours in n-hexane and ethanol to obtain the corresponding gradient fraction of these solvents. The pure extract was then concentrated and evaporated to dryness using a rotary evaporator before being stored in a refrigerator at 4°C.

Forty (40) Four-week-old male Wistar rats with weights between 60gm and 100gm were used in the experiment, and they were obtained from the animal house of the Department of Physiology, University of Calabar. The animals were kept in a well-ventilated experimental section of the animal house of the faculty of Pharmacy, University of Uyo, Uyo and acclimatized for seven days before the experiment began. The rats were randomly assigned to four groups, and the control group (Group A (was) given distilled water at 5ml/kg, while the other groups (Groups B, C, and D respectively) were given low, intermediate, and high doses of the extract. Group B received 1/10 of LD_{50} by feeding tube i.e. 1/10 x 894.43 mg/kg = 89.44mg/kg. Group C received 2/10 of LD_{50} by mouth through a feeding tube. Group D received 3/10 of LD_{50} by mouth through a feeding tube i.e. 268.32mg/kg. Stock concentration was 50mg/ml. Due to small variation in body weight of the animals, the average dose per group was used for all the animals in that group.

The median lethal dose (LD_{50}) of the extract was estimated using albino Wistar mice by intra peritoneal (ip) route using the method of Lorke (1983). The animals' final weight was taken on the 28th day, and they were anaesthetized with chloroform. Blood samples were obtained by cardiac puncture, and serum was obtained for biochemical analysis. Abdominal dissection was extended to recover the testis and epididymis for histological analysis.

The study involved biochemical and histological analysis of the testes. Testosterone levels were analyzed using the Enzyme Linked Immunosorbent Assay (ELISA) method, while histological analysis involved fixing the testes in Bouin's solution, processing them until embedded in paraffin for histological analysis, preparing five-micron thick sections using a microtome, and staining them using Hematoxylin and Eosin (H&E) method. The specimens were examined under Olympus/3H light microscope-Japan.

Data obtained from the experiment were analyzed using Mean, Standard Error of Mean, and Analysis of Variance followed by Duncan's test to determine the direction of significance. The levels of testosterone were reported in the form mean ± SEM, and statistical significance was established at the 0.05 level of significance with $p<0.05$ signifying significance. The Statistical Package for Social Sciences (SPSS version 22.0) and GraphPad Prism 5.0 were used for data analysis. The Local Research Ethical Committee of the University of Uyo, Uyo, Akwa Ibom State, Nigeria approved the study.

3. RESULTS

3.1. Effect of the Ethanolic Extract of Icacinia mannii on Testosterone Levels

In the low dose group, we observed a significant increase in testosterone levels ($P<0.05$), with an average concentration of 1.93 ± 0.86 ng/ml, compared to the control group's average of 1.08 ± 0.49 ng/ml. Similarly, the medium dose group demonstrated a significant increase in testosterone levels ($P<0.05$), with an average concentration of 1.62 ± 0.73 ng/ml. This indicates a notable rise compared to the control group. Conversely, in the high dose group, we observed a significant decrease in testosterone levels ($P<0.05$), with an average concentration of 0.94 ± 0.42 ng/ml. This decrease was found to be statistically significant when compared to the control group.

These findings underscore the importance of carefully considering dosage when examining testosterone-related effects.

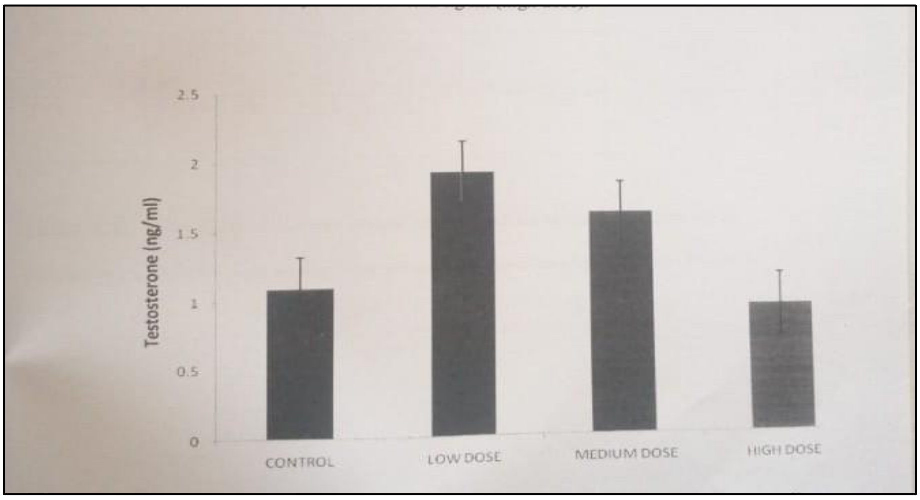

Figure 1: A bar chart showing the effect of ethanolic extract of *Icacina mannii* tuber on testosterone levels in male albino wistar rats

3.2. Testicular Histology

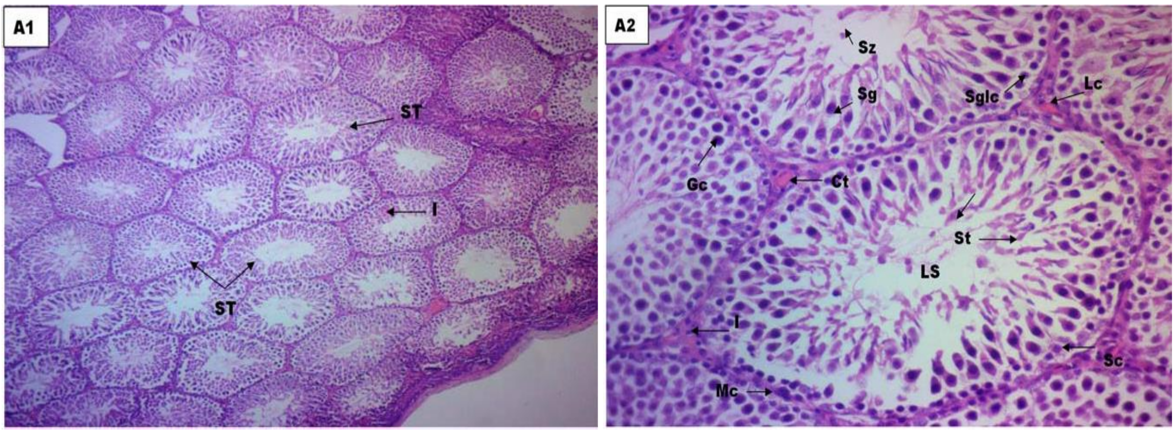

Figure 2: Photomicrographs of Testes of Rat without treatment (group A, control) stained with H & E method at Mag. A1(X100) & A2(X400).

Keys: Seminiferous Luminal Tubules (ST), Germinal cells (Gc), Interstitium (I), Spermatogenic lining cells (Sglc), Myoid cells (Mc), Spermatocytes (Sp), Spermatids (St), Sertolic cells (Sc), Leydig cells (LC) and Connective Tissue

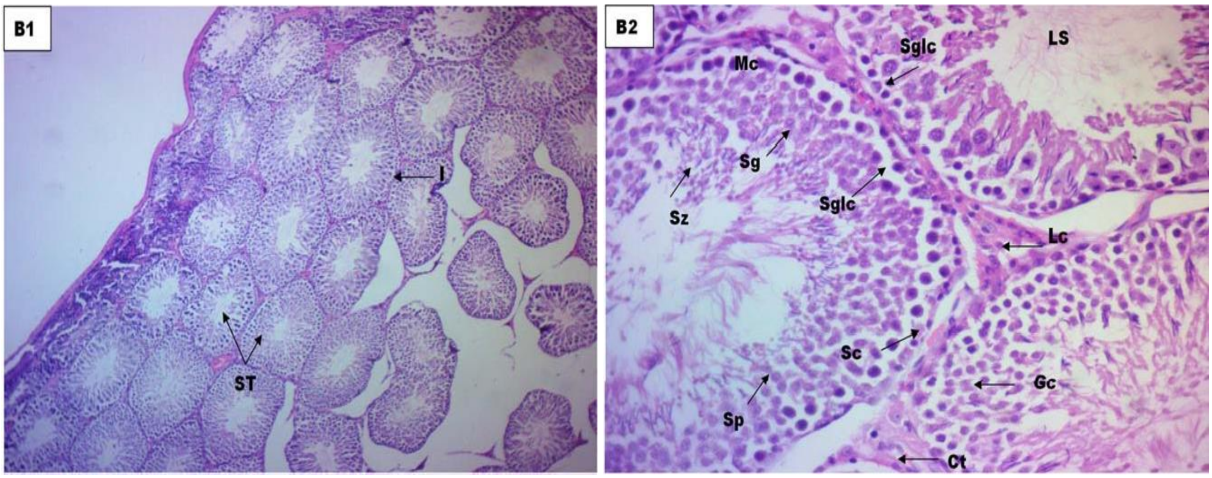

Figure 3: Photomicrographs of testes of rats treated with 89.44mg/kg ethanolic extract of Icacina mannii tuber (group B) stained with H & E method at Mag. B1(X100) & B2(X400).

Keys: Seminiferous Luminal Tubules (ST), Germinal cells (Gc), Interstitium (I), Spermatogenic lining cells (Sglc), Myoid cells (Mc), Spermatocytes (Sp), Spermatids (St), Spertolic cells (Sc), Leydig cells (LC) and Connective Tissue (Ct)

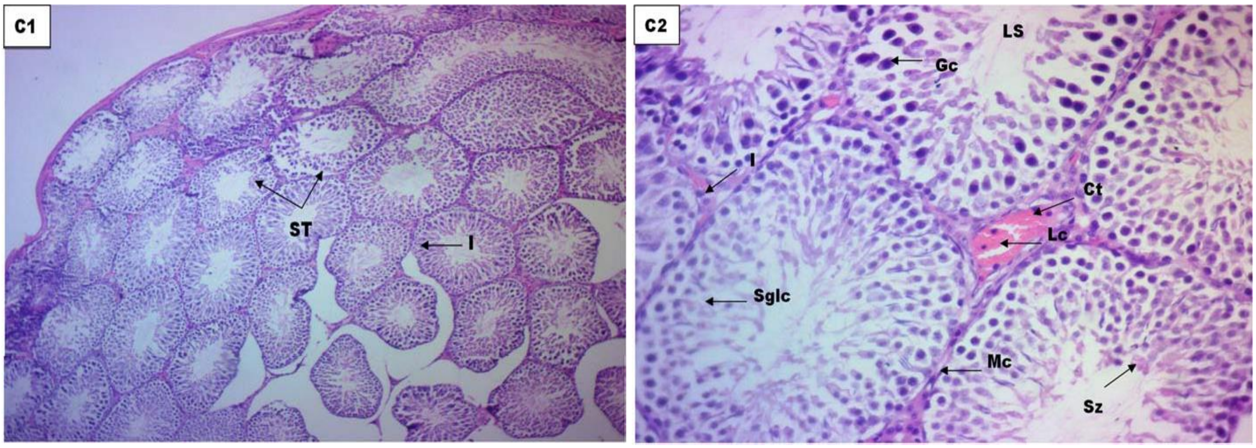

Figure 4: Photomicrographs of testes of rats treated with 178.88mg/kg ethanolic extract of Icacina mannii tuber (group C) stained with H & E method at Mag. C1(X100) & C2(X400).

Keys: Seminiferous Luminal Tubules (ST), Germinal cells (Gc), Interstitium (I), Spermatogenic lining cells (Sglc), Myoid cells (Mc), Spermatocytes (Sp), Spermatids (St), Spertolic cells (Sc), Leydig cells (LC) and Connective Tissue (Ct)

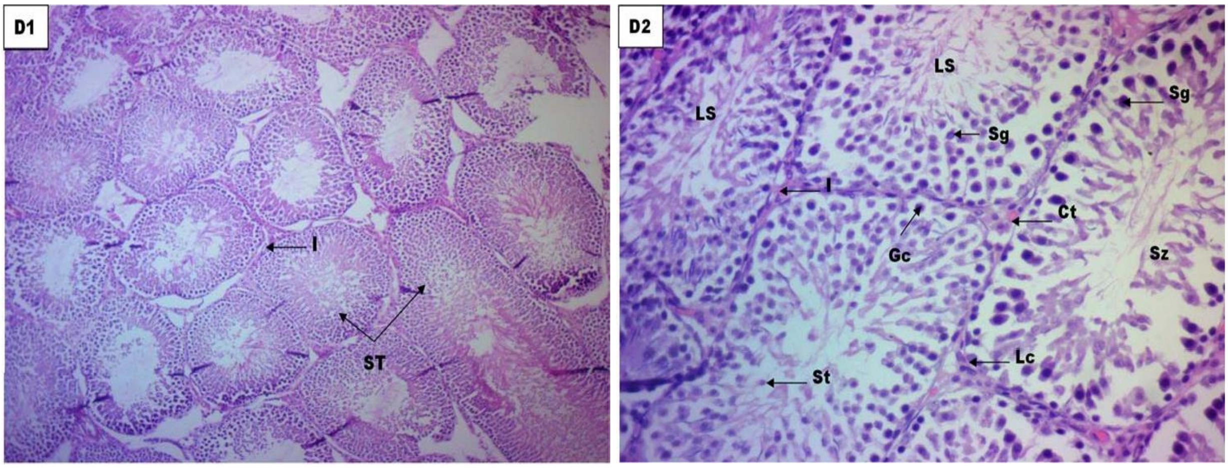

Figure 5: Photomicrographs of Testes of Rat treated with 268.32mg/kg ethanolic extract of Icacina mannii tuber (group D) stained with H & E method at Mag. D1(X100) & D2(X400).

Keys: Seminiferous Luminal Tubules (ST), Germinal cells (Gc), Interstitium (I), Spermatogenic lining cells (Sglc), Myoid cells (Mc), Spermatocytes (Sp), Spermatids (St), Spertolic cells (Sc), Leydig cells (LC) and Connective Tissue (Ct)

Group A, Control (Figure 1): Histologic section of the testes without treatment at magnification A1(x100) and A2(x400) revealed normal cellular profile of seminiferous tubules, Sertoli cells, spermatogenic lining cells, Leydig cells, germ cells, lining myoid cells, spermatogonia, spermatocytes and spermatids all within normal cellular architecture without any form of abnormality.

Group B, treated with 89.44mg/Kg (Figure 2): Histologic section of the testes at magnification B1(x100) and B2(x400) revealed hypochromic section of seminiferous tubules with swollen germinal cells and spermatogenic lining cell and irregular alignment of myoid cells when compared to control group.

Group C, treated with 178.88mg/kg (Figure 3): Histologic section of the Testes at magnification C1 (x100) and C2 (x400) revealed hypochromic section seminiferous tubules with swollen germinal cells and spermatogenic lining cell and irregular alignment of myoid cells when compared to control group

Group D, treated with 268.32mg/Kg (Figure 4): Histologic section of testes at Magnification D1(X100) & D2(X400) show numerous atrophied and damaged seminiferous tubules, degenerated myoid cells, spermatogenic lining cells, spermatogonia, spermatocytes, spermatids, spermatozoa and lumen filled with semen, degenerated interstitial cells of leydig and interstitium against background of connective tissues with marked area of necrosis as compared to the control group.

4. DISCUSSION

The therapeutic properties of plants have long been established (Petrovska, 2012) and have been found to aid infertility management (Ebiyemzi *et al.*, 2021; Rehman *et al.*, 2020) through spermatogenesis-inducing and antioxidative mechanisms with the primary target organ being the testes (Abarikwu *et al.*, 2020). They do so via the phytochemicals they contain. *Icacinia mannii* tuber has been implicated in the management of certain diseases.

The testis is well-equipped with powerful intrinsic defense systems that protect the spermatozoa during its spermatogenic/post-spermatogenic journey and from the injuries caused by other intrinsic or extrinsic factors. The current study showed that *Icacina mannii* tuber extract was able to penetrate the testis (revealing its ability to improve testicular processes like spermatogenesis). This penetration resulted in the stimulation of the testis. The stimulation is revealed in the swollen of germinal cells and spermatogenic lining cell in the histologic section of the testes of the rats treated with low and medium dose *Icacinia mannii* tuber at magnification B1(x100) and B2(x400) when compared to control group (figures 3 and 4).

Therapeutically useful plants could also be dangerous at high doses. The stimulation of testicular cells was revealed in the testicular histology of rats treated with low and medium dose of the extract while at high doses, numerous atrophies and damaged testicular cells with marked area of necrosis were observed as compared to the control group (figure 5). Most medicinal plants are believed to be safe, therefore dosages are rarely taken into consideration. Some phytochemicals in medical plants have been reported to be toxic and mostly manifest at high doses. However, high doses have been reported to be toxic by Rojas-Armas *et al., (*2019) and Anywar *et al.,* (2021) and hence pose as a threat to the health of the users. In the same manner, *Icacina mannii* tuber extract has shown damaging effects at a high dose in this current study, suggesting that it is therapeutic at low and medium doses but detrimental at high doses.

The result in this current study also revealed an increased level of testosterone in the low and medium dose groups treated rats, with the highest level being observed in the low group. The elevated level of testosterone supports the suggestion of the stimulation of testicular cells which is revealed in the swollen cells by the histology. This stimulation must have affected the Leydig cells as well, leading to the increased production of testosterone.

A reduction in the level of testosterone was also in the high dose group in this study. The testicular histologic section of rats in the high dose group showed atrophied and necrotic cells. It is therefore possible that the reduced testosterone level resulted from the reduced number of cells available for the production of testosterone.

Testosterone is the major androgen in the test is that regulates spermatogenesis. An increase in the level of testosterone probably suggests *Icacina mannii* tuber's ability to influence and increase the process of spermatogenesis. This finding could suggest that *Icacina manni* tuber would be an option in the management of infertility cases in men due to low sperm count.

CONCLUSION

From this current study, it is shown that *Icacina mannii* tuber could be necrotic and atrophic to the testes at high doses but at low and intermediate doses, it could as well be a plant for consideration in the research for herbs in the management of infertility in men due to testosterone deficiency.

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