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Research Article

Reproductive Toxicological Evaluation of *Ficus exasperata* Ethanolic Extract in Male Albino Rats

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ABSTRACT
Ficus exasperata popularly known as sand paper tree (due to its rough surfaces) is an important medicinal plant in Africa used traditionally for treating asthma, dyspnea, high blood pressure, rheumatoid, arthritis, ulcer and diabetes. Due to its wide application as a medicinal herb, there is a special need to evaluate the safety and probable toxicological effects of the plant. Hence, this study was aimed at investigating the possible reproductive toxicological effects of ethanolic extract of *F. exasperata* on male albino rats. Phytochemical screening was done to analyse the active constituents in the extract (Alkaloids, flavonoids, tannins, phlobatannins, saponins, anthraquinones, glycosides and phenols). Three concentrations: 50, 100, 150 and control (0.0) mg/kg body weight of the extract were utilized and administered orally to the test animals for 3 days. The levels of a major reproductive androgen hormone (testosterone) were measured with the enzyme immune assay (EIA) and changes in reproductive organ weights were evaluated. Ethanolic extract of *Ficus exasperata* significantly decreased ($p<0.05$) serum testosterone levels which paralleled changes in gonadal growth and development and this decrease were concentration dependent. Our results suggest that ethanolic extract of *F. exasperata* contain some bioactive constituents that may have reproductive toxicological effects which inhibit testosterone synthesis and reduce reproductive organ development and consequently may result in infertility. The mechanism of action of *F. exasperata* inducing reproductive toxicological effects may have resulted from the potential ability of some phytochemicals to interact with steroid hormone synthesis and therefore inhibiting testosterone biosynthesis.

Keywords: *F. exasperata*, Reproductive effects, Testosterone, Phytochemical screening, Male albino rats.

INTRODUCTION

It is common practice that many herbs (plants) and their derivatives are employed in the treatment of numerous diseases due to the increasing awareness that several plant species are known to have active constituents that maybe of medicinal advantage[1-3]. To this effect, the World Health Organization (WHO) reported that about 25 % of modern medicines are developed from over 21,000 plant species which are used traditionally[4] and research on traditional medicinal herbal plants led to discovery of 75% of herbal drugs[5]. While this advantage is often widely explored, a major limitation is that conventional traditional medicine has always ignored the side effects and safety evaluation of long term exposure of most of these active chemical constituents of plants. Recognising the possibilities of toxicological effects of most bioactive compounds will help provide information on setting safety limits and dosage for most plants. *Ficus exasperata* vahl belongs to the family moraceae and it is a terrestrial afro-tropical scabrous, ovate tree popularly referred to as sand paper tree in Nigeria because of the rough surface of the leaves[6]. In Nigeria, *F. exasperata* is called Kawusa, Ameme, Erepin, Anwerenwa in Nupe, Edo, Yoruba and Igbo languages respectively. *F. exasperata* roots, stems and leaves are traditionally employed to manage asthma, dyspnea and venereal diseases, treat high blood pressure, rheumatoid arthritis, intestinal pains and colic, epilepsy, bleeding and wounds and also for the treatment of cough and hemorrhoids[7]. Their young leaves have been prescribed as a common anti-ulcer remedy and also found to have anti-diabetic (lipid lowering) and antifungal activities[8]. A recent study demonstrated that the aqueous extract of leaves of this plant reduces blood pressure in male rabbits due to its cholinomimetic effect[9]. It has been demonstrated that *F. exasperata* has anti-inflammatory, glycaemic[10], antipyretic and antinociceptive properties[11] as well as stimulatory effect on uterine smooth muscles[6]. According to Nimenibo-Uadia[12], the administration of aqueous extracts of *F. exasperata* resulted in decreased plasma triacyl-glycerol and β-hydroxybutyrate levels in alloxan treated rats. *F. exasperata* is a very important plant in herbal medicine and considered as one of the most important plants in Africa as it is employed as a remedy for a variety of diseases in the continent. In

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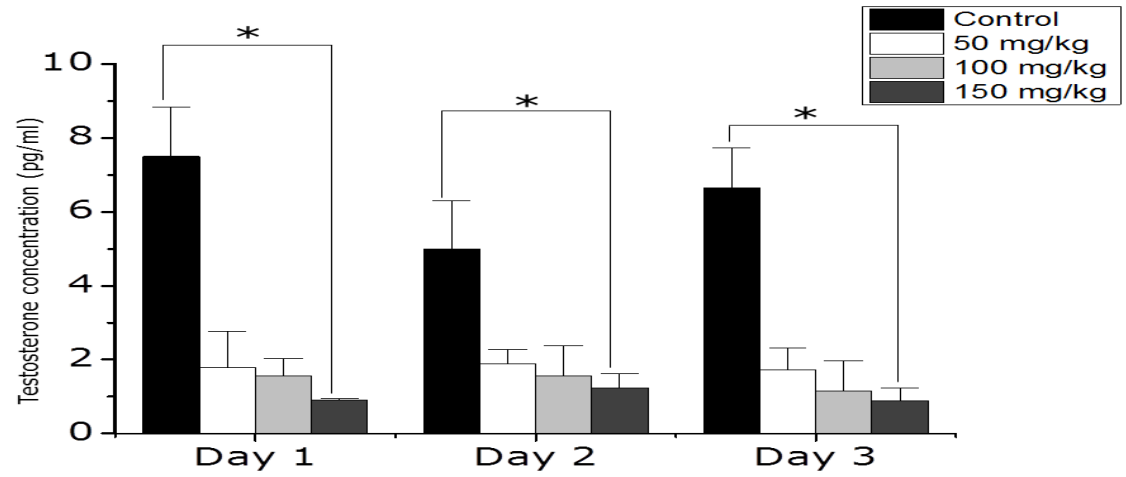

Figure 1: Plasma levels of testosterone hormone in male albino rats treated with ethanolic extract of *Fiscus exasperata*. Asterisk (*) represents daily significant difference ($p<0.05$) between control and exposure groups.

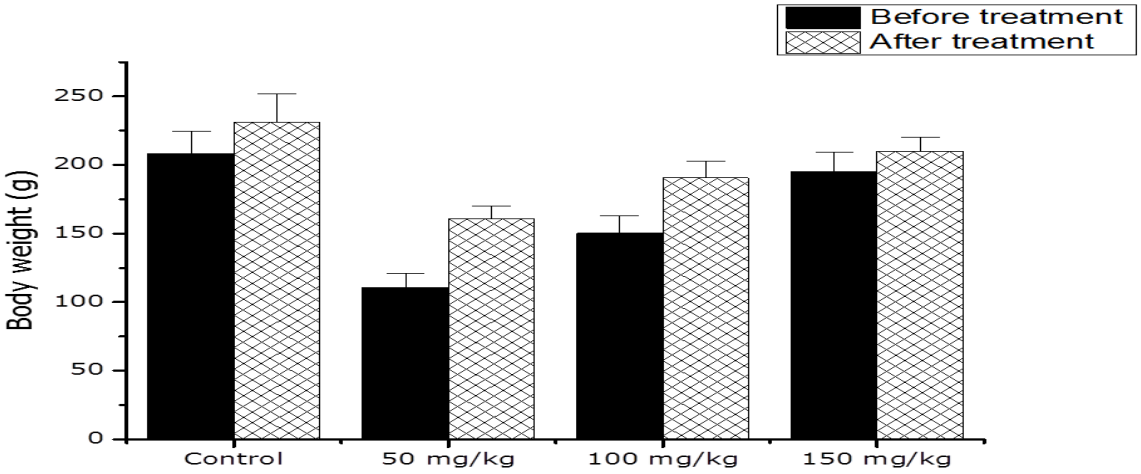

Figure 2a: Changes in body weight of male albino rats treated with ethanolic extract of *Ficus exasperata*.

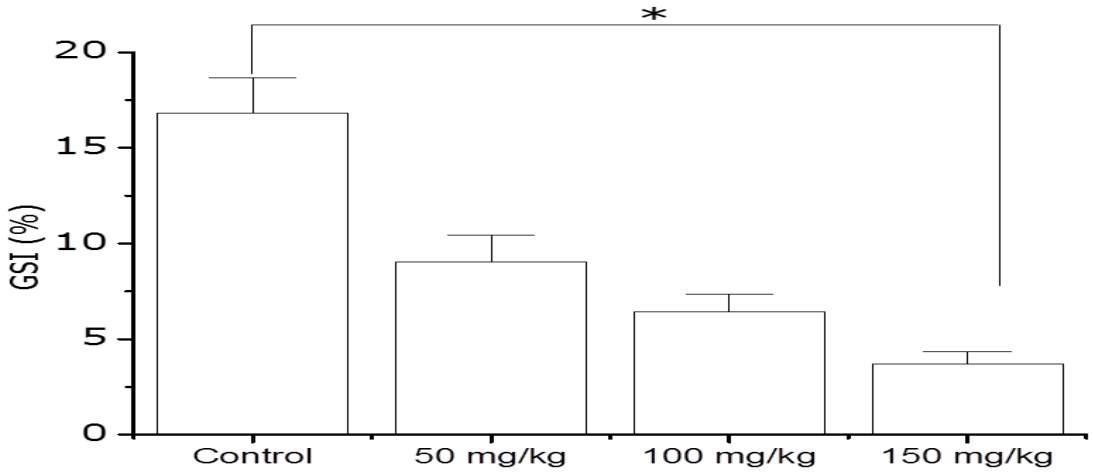

Figure 2b: Changes in gonado somatic index (GSI) of male albino rats treated with ethanolic extract of *Ficus exasperata*.

Congo it is used by traditional birth attendants to ease childbirth and applied to leprous sores in Ivory Coast[13]. In Southern Africa, scrapings of the bark is used as a stimulant and as a medication for ring worm in Zaire[14]. In Gambia, chest complications are treated by steam inhalation of the *F. exasperata* leaves boiled in water. In Ghana, it was reported that the leaves were toxic to goats and sheep as such *F. exasperata* leaves are used in

Table 1: Phytochemical constituents of leaves and stem bark of *Ficus exasperata*

Phytochemical	Leaves	Stem bark
Alkaloids	+	+
Flavonoids	+	+
Tannins	+	+
Phlobatannins	+	+
Saponins	+	+
Anthraquinones	-	-
Glycosides	+	+
Phenols	+	+

+ = Present, - = Absent

poisoning arrows for hunting[15]. Despite its potential benefits, *F. exasperata* has been shown to have negative effects on kidney function in albino rats[7,16]. Information on the potential reproductive effects of *F. exasperata* is limited or almost non-existent, this necessitated the need to evaluate the potential toxicological effects of *F. exasperata*. Therefore this study was aimed at investigating the reproductive effects of the ethanolic extract of *F. exasperata* in male albino rats.

MATERIALS AND METHODS

Plant collection, extraction and phytochemical screening

Fresh stem and leaves of *F. exasperata* were collected from the premises of Calabar preparatory primary school in Calabar, Nigeria. It was identified and authenticated at the University of Calabar Herbarium, Department of Botany. Leaves of the plants were air dried and ground into coarse powder. Two hundred grams (200 g) of the grinded plant material was extracted from 500 ml of absolute ethanol and filtered with Whatman® (11 µm) filter paper (Maistone, UK). The filtrate was then concentrated under reduced pressure using a rotary evaporator to obtain a gelatinous semi-solid extract which was preserved at 4° C until use. Preliminary phytochemical screening was performed for the presence of Alkaloids, flavonoids, tannins, phlobatannins, saponins, anthraquinones, glycosides and phenols in triplates using standard procedures[17-18].

Experimental Animals

Sixty (60) sexually mature male albino rats (Wistar strain) weighing between 110 – 250 g were obtained from the animal breeding unit of the Department of Zoology and Environmental Biology, University of Calabar, Nigeria and allowed to acclimatize to laboratory conditions for three (3) weeks. The animals were housed and maintained in the experimental animal house (with 12 hours light and 12 hours darkness). Animals were fed with Ladokun pelleted feed and drinking water supplied ad libitum. Animals were cared for according to the standard guidelines[19].

Experimental procedure

After a three (3) week acclimation period, animals were weighed and divided into three (3) treatment groups and a control group; with each animal cage accommodating five (5) rats. The control group received distilled water while the other 3 groups received 50, 100, 150 mg/kg body weight of extract respectively. Each albino rat was orally administered with the extract for 3 consecutive days. After the 3 day exposure period, blood was collected using a 2 mL syringe and animals were sacrificed and the gonads extracted.

Hormone analysis

Plasma hormone levels of testosterone were measured daily for the 3 days exposure period using the enzyme imune assay (EIA) kits purchased from Synton Bioresearch. Inc. Catalog #44 10-96) according to the manufacturer's protocol. The cross-reactivity between the antibodies of each testosterone hormone against other hormones was below 0.5 %, as reported by the kits manufacturer.

Morphometric indices

The weights before and after exposure were taken with a digital weighing balance, while gonado somatic index (GSI) was estimated according to the equation: (gonad weight / total body weight) x 100.

Statistical Analysis

Data were expressed as mean ± standard deviation and analyzed using one-way ANOVA with Origin 8 software, (Originlab software, USA). Significant difference between treatment groups were considered at $p<0.05$.

RESULTS

Phytochemical screening

Phytochemical analysis of the ethanolic extract of leaves and stem bark of *F. exasperata* showed the presence of alkaloid, flavonoids, saponins, tannins, phlobatanins, phenols, glycolyside, while anthraquinone was the only bioactive compound absent among all the active compound screened in the leaves and stem bark of *F. exasperata* (Table 1).

Plasma hormonal assay

Exposure of male albino rats to ethanolic extracts of *F. exasperata* significantly decreased ($p<0.05$) plasma testosterone levels in a dose dependent manner with the highest exposure concentration 150 mg/kg having the lowest testosterone concentration (0.6 ± 0.08 pg/mL) compared with the control (6.33 ± 1.03 pg/mL) (Table 2). The decrease is plasma testosterone levels were consistent across the three days exposure period with the highest exposure concentration having the lowest level of plasma testosterone levels compared with the control. A significant increase ($p<0.05$) in testosterone levels was observed in control group compared with exposure concentrations across the 3 days exposure period (Figure 1).

Body weight and gonado somatic index (GSI)

The body weights of both control and treated albino rats increased throughout the duration of the experiment when compared with the weight before treatment. Although, we observed an increase in body weight of organisms in the highest exposure concentration (150 mg/kg: 210.02 ± 10.28 g) compared with the lowest exposure concentration (50 mg/kg: 160.81 ± 9.47 g), control group had a higher body weight (231.18 ± 20.93 g) (Fig. 2a). Gonado somatic index significantly decreased ($p<0.05$) in exposure groups compared with control and this decrease was dose dependent (Fig. 2b).

Table 3: Effects of increasing doses of *Ficus exasperata* leaf extract on plasma testosterone levels in male albino rats.

Treatment doses	No. of test organisms	Plasma testosterone levels (pg/mL)
Control (0.0)	5	6.33 ± 1.03^{a}
50 mg/kg	5	1.53 ± 0.06^{b}
100 mg/kg	5	1.27 ± 0.39^{b}
150 mg/kg	5	0.6 ± 0.08^{c}

Different letters denote significant difference ($p<0.05$) across exposure concentrations.

DISCUSSION

A common assumption and belief is that traditional medicine is devoid of toxicological effects since the products are of natural origin, as such often times the side effects of most of these bioactive constituents are ignored. While considering the therapeutic importance of herbal medicine, it is important to also evaluate the potential toxicological effects of most of the natural plants because some of their constituents may have additive, synergistic and antagonistic effects in exposed organisms. Herein, we have demonstrated for the first time the reproductive toxicological effects of *F. exasperata* in male albino rats. We demonstrated that ethanolic extract of *F. exasperata* significantly reduced plasma testosterone levels, gonado somatic indices due to the presence of some phytochemical constituents, suggesting that *F. exasperata* may induce infertility in exposed organisms. The preliminary phytochemical screening of the leaves and stem bark indicated the presence of alkaloids, flavonoids, tannins, phlobatannins, saponins, glycosides and phenols) with no traces of anthraquiones. Some reports have shown the presence of similar active constituents in several extracts of *F. exasperata*[16,20,21]. However, the presence of saponins, alkaloids, and phlobatannins in the plant may suggest the pharmacological importance of *F. exasperata*[22]. Our data suggest that exposure to ethanolic extract of *F. exasperata* significantly increased the body weight of male albino rats. The increase in body weight observed in the highest exposure concentration compared with the lowest treated group could probably reflect the ability of some bioactive constituents like tannins present in *F. exasperata* to stimulate increase in body mass. Several reports have demonstrated the ability of tannins to elicit an increase in growth in some experimentally treated animals[23-26]. The presence of phenols, alkaloid and flavonoids may be an early indication of the probable ability of *F. exasperata* to interact with endogenous steroid hormones. Interestingly, we observed that *F. exasperata* significantly decreased plasma testosterone levels in male albino rats and this decrease may have resulted from the ability of some phenolic compounds detected in *F. exasperat*a to interact with steroid hormone synthesis and therefore inhibiting androgen (testosterone) synthesis. Testosterone is the active androgen in males regulating and controlling the development of male sexual characteristics. The inhibition of testosterone biosynthesis by *F. exasperata* may suggest the ability of the plant to generate infertility in exposed organisms. Also, we observed the presence of flavonoids, alkaloids, and phenols which have been classified as endocrine disruptors and have been shown to inhibit testosterone production[27-28]. It has been shown that some flavovoids such as daidzein, genistein and apigenin inhibits testosterone synthesis[29-30]. Probably, it may be suggested that most of the bioactive compounds like phenols reported in *F. exasperata* could inhibit the activity of 17α-hydroxylase enzymes which are involved in testosterone synthesis. Also, the decrease in plasma testosterone levels could have resulted from a decrease in leydig cells which secrete testosterone by stimulatory effects of luteinizing hormone (LH)[31]. This assumption is supported by the knowledge that testicular leydig cells are crucial for the production of testosterone and regulation of spermatogenesis. Hence, a disruption in its functions may adversely affect male reproduction and fertility. Some reports have suggested several mechanisms involved in testosterone inhibition. Opuwari and Monsees[27], demonstrated that *Aspalathus linearis* (rooibos) and *Camellia sinensis* (tea) with flavonoids as active constituents possess anti-androgenic property on TM3 Leydig cells. In isolated Leydig cells, it was shown that the mechanisms underlying the inhibitory effect of green tea extract and epigallocatechin-3-gallate (EGCG) on testosterone production involved the inhibition of the protein kinase A/protein kinase C (PKA/PKC) signalling pathways, as well as the inhibition of P450scc enzyme and 17β-hydroxysteroid dehydrogenase function[32]. Udoh et al[33], suggested that a decrease in testosterone production may reflects the disorganization of the leydig cells, seminiferous tubule, hyperplasia and spermatogenic atrophy and this ultimately will result in a decrease in sperm production, hence infertility problems. In this study, we observed a significant decrease in testosterone production with increasing dosage of *F. exasperata* and we believe these findings could suggest that an increased intake of *F. exasperata* may generate severe fertility problems in males. Gonado somatic index is an indication of gonadal growth, maintenance and development and it is generally believed that organisms with heavier gonads will have good reproductive progeny[34]. Herein, we observed a significant reduction in GSI with increasing dosage of *F. exasperata* and this may correlate with the decreasing level of plasma testosterone in male albino rats. The decrease in GSI observed may further support the hypothesis that *F. exasperata* may be exerting reproductive effects by decreasing gonadal growth and development. Such decreases in gonadal growth and development may have profound consequences on the quality of gametes that may be produced resulting in reproductive failure in exposed organisms. In conclusion, we demonstrated that ethanolic extract of *F. exasperata* possess some phytochemicals (Alkaloids, flavonoids, tannins, phlobatannins, saponins, , glycosides and phenols) that have anti-androgenic potential by decreasing testosterone synthesis in male albino rats. We showed that the decrease in testosterone also paralleled a

significant reduction in gonadal growth and development and this may have reproductive (fertility) consequences in exposed individuals. To the best of our knowledge, this is the first study demonstrating the anti-androgenic effects of *F. exasperata* in experimentally treated organisms. Hence, care should be taken in the application of *F. exasperata* as a conventional herbal medicine. Also, it may be of urgent priority to isolate the bioactive constituents in plants with therapeutic and toxic effects, as well as establishing minimal concentrations that may be beneficial in ethno-medicine.

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