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Effect Of Ethanolic Leaf Extracts Of Rhizophora Mangle On Some Reproductive Parameters Of Male Wistar Rats.

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ABSTRACT
The process of producing offsprings have been threatened by various health challenges; and the search for affordable and effective therapy have led to the use of plant extracts as valuable substitutes to synthetic drugs. This study was carried out to investigate the effect of ethanolic leaf extract of Rhizophora mangle on some reproductive parameters of male Wistar rats. Adult male Wistar rats weighing 150–200g used for the study were randomly placed into 4 groups (n = 5 per group) and allowed 2 weeks for acclimatization. Group I – served as control and received distilled water and normal feed ad libitum. Groups II, III and IV – served as test groups and received 200mg/kg bw; 400mg/kg bw; 600mg/kg bw of ethanolic leaf extract of Rhizophora mangle orally once daily consecutively for 30 days. At the end of the administration, the animals were anesthetized using chloroform and blood sample collected by cardiac puncture into lithium heparin bottles for estimation of some male reproductive hormones such as follicle stimulating hormone, luteinizing hormone and testosterone. The epididymis was dissected and semen collected for analysis of sperm parameters. The data was analysed using the SPSS version 23 software.
Results showed significant ($p<0.05$) increases in luteinizing hormone (LH), follicle stimulating hormone (FSH) and testosterone. However, there were significant ($p<0.05$) reductions in sperm parameters including, percentages of viable sperm cells; sperm cells with normal morphology; actively motile sperm cells and sperm count. The results suggest that Rhizophora mangle leaf extract possess possible antifertility potentials.We recommend further studies using isolated compounds of the extract to identify the active agent with its exact mechanism of action.
Keywords: Rhizophora mangle, antifertility, sperm parameters, Wistar rats, ethanolic leaf extract.

I. INTRODUCTION

Since ancient times, plant or plant products has been used as a source of therapeutic medicines. At the times before the development of orthodox medicine, medicinal plants also known as medicinal herbs, were discovered and employed in traditional medicine practices (Andrade-cetto & Heinrich, 2011). In non-industrialized communities especially in third world countries, medicinal plants are popular and frequently employed for therapeutic purposes mostly because they are more accessible and affordable than contemporary medications (Ahn, 2017). Rhizophora mangle also known as red mangrove is a medicinal plant found in estuary habitats all over the tropics. (Ellison et al, 2015). The tree grows on aerial prop roots arching over the surface of the water and giving its stands the unique "mangrove" appearance. It belongs to the Rhizophoraceae family, which is a group of tropical or subtropical flowering plants (Ellison et al, 2015; Guo et al, 2017), that contains a wide range of active compounds, including tannins, phenols, hydrolyzable tannins, saponins, alkaloids, and flavonoids . Other well-known and beneficial secondary products, including,glycosides, essential oils, and other organic compounds have all been derived from Rhizophora.. Plant phenolics, which can be found in all plant components including leaves, roots, bark, fruits, vegetables, nuts, and seeds, are primarily sources of natural antioxidants. The leaves which reportedly contain high flavonoid concentration, together with other plant phenolics also found in other parts of the plants are associated with its antioxidant action. Tannin-rich aqueous extracts have been demonstrated in studies to have antibacterial, wound-healing, and anti-ulcerogenic properties (Berenguer et al, 2006). Under in vitro conditions, ethanolic extracts of Rhizophora mangle exhibited antibacterial effect against staphylococus aureus, a gram-positive bacterium and also demonstrated a cytotoxic activity against human fibro-sarcoma HT1080 cells (Hicks et al, 2011). Furthermore, the plant has been used as an aphrodisiac as it has been shown to cause increased arousal and increased virility in addition to its haemostatic and antifungal effects (Galvez et al, 2005). However, there is dearth of literature on the

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effect of Rhizophora mangle on the male reproductive functions; hence this study is designed to investigate the effect of ethanolic leaf extract of Rhizophora mangle on some male reproductive parameters in Wistar rats.

II. MATERIALS AND METHODS

Collection and Extraction of Plant Material

Fresh leaves of Rhizophora mangle were collected from its natural habitat at Eagle Island, an area located in Port Harcourt, Rivers State, Nigeria, and were authenticated by the taxonomist in the herbarium unit, Department of Plant Science and Biotechnology, Rivers State University, Port Harcourt, Nigeria. It was given an identification code (RSU PB 097). The leaves were washed in tap water to remove dirt, and dried at room temperature (26^{o}C) over a period of 3 weeks. The dried leaves were pulverized using a blender and 500g of the leaves in powder form was obtained. The weighed quantity of the plant was dissolved in 400ml of ethanol for 48 hours in an extraction jar. The mixture was filtered after 48 hours using a Whattman filter paper to separate the filtrate from the residue. The filtrate was poured into a beaker and concentrated using a heating mantle at a temperature of 50^{o}C until a paste (jelly) form was obtained. The dry weight was obtained after heating in an ovum. The yield of the crude ethanolic extract of Rhizophora mangle leaves obtained weighed 127g. The extract was stored in a refrigerator at 4^{o}C until it was reconstituted and used for the study.

Experimental Animals and Protocols

Thirty adult male rats weighing 150–200g were purchased from the experimental animal centre of Faculty of Basic Medical Sciences, Rivers State University, Port Harcourt, Nigeria. The rats were acclimatized for two weeks, and randomly assigned into 4 groups (n = 5 per group) according to their body weight, as follows: Group I – served as control and received distilled water. Groups II , III and IV – served as test groups and received 200mg/kg bw, 400mg/kg bw and 600mg/kg bw of ethanolic leaf extract of Rhizophora mangle orally, once daily for 30 days. They were allowed access to water and feeds ad libitum. Animal handling was in accordance with the National Institutes of Health's guide for the care and use of laboratory animals [National Institute of Health, USA. 1985]. The animals were anesthetized using chloroform and sacrificed at the end of the administration. Blood was collected by cardiac puncture into lithium heparin bottles for estimation of some male reproductive hormones such as Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH) and Testosterone.

Sperm parameters

The caudal epididymis was dissected; an incision (about 1 mm) was made in the caudal epididymis. Semen was then squeezed onto the microscope slide and observed under the light electron microscope. Epididymal sperm motility was assessed by calculating motile spermatozoa per unit area and was expressed as percent motility. Epididymal sperm counts were made using the improved Neubauer cytometer and was expressed as million/ml of suspension. The procedures were done in accordance with standard protocols described by the World Health Organization [WHO, 2010].

Statistical Analysis

The data was analyzed using Statistical Package for Social Sciences (SPSS) version 23 and the results expressed as Mean ± SEM. The significant differences between means was determined by Least Significance Difference (LSD) and the results were regarded as significant at $p<0.05$.

III. RESULTS

The results for the study are presented in tables 1-4

Table 1: Mean serum levels of luteinizing hormone and follicle stimulating hormone

Groups (mg/kg)	LH (m/µ/ml)	Sig.	FSH (m/µ/ml)	Sig.
Control	0.29±0.02		0.25±0.01	
200	0.44±0.02*****	0.00	0.55±0.06*****	0.00
400	0.31±0.01	0.51	0.35±0.07	0.19
600	0.26±0.01	0.12	0.44±0.06*****	0.02

Values presented as Mean±SEM. n=5. *****Significant at P<0.05 when compared to control

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Table 2: Mean levels of serum testosterone

Groups (mg/kg)	Testosterone (ng/ml)	Sig.
Control	0.53±0.02	
200	0.75±0.03*****	0.01
400	0.69±0.03	0.05
600	0.88±0.10*****	0.00

Values presented as Mean±SEM. n=5. *****Significant at P<0.05 when compared to control

Table 3: Mean value of viable sperm cells and sperm cells with normal morphology

Groups (mg/kg)	Viable sperm cells (%)	Sig.	Normal morphology (%)	Sig.
Control	85.00±2.24		80.00±2.24	
200	55.00±2.24*****	0.00	59.00±2.91*****	0.00
400	68.00±3.39*****	0.01	66.00±4.58*****	0.01
600	80.00±2.24	0.19	78.00±1.22	0.64

Values presented as Mean±SEM. n=5. *****Significant at P<0.05 when compared to control

Table 4: Effect of extract on actively motile sperm cells and sperm count

Groups (mg/kg)	Actively motile sperm cells (%)	Sig.	Sperm count ($\times 10^6$/ml)	Sig.
Control	78.00±1.22		76.00±6.78	
200	54.00±2.45*****	0.00	24.80±2.94*****	0.00
400	61.00±5.58*****	0.00	36.00±6.78*****	0.01
600	75.00±2.24	0.47	64.00±6.78	0.18

Values presented as Mean±SEM. n=5. *****Significant at P<0.05 when compared to control

IV. DISCUSSION

The present study assessed effects of ethanolic extract of Rhizophora mangle on some reproductive parameters of male Wistar rats. Male reproductive process is regulated by intricately balanced mechanisms that involve the hypothalamus-pituitary-testicular axis and accessory sex organs. After 30 days of oral administration of the extract to male rats, the results revealed significant (P<0.05) increase in serum concentration of follicle stimulating hormone (FSH) and luteinizing hormone (LH) when test groups were compared to control group rats. These hormones referred to as gonadotropins are glycoprotein hormones secreted by the anterior pituitary gland that act directly on the testes to stimulate somatic cell function in support of spermatogenesis (Pierce and Parsons, 1981). Testicular secretion of testosterone is regulated by LH which together with FSH play important roles in the initiation and maintenance of spermatogenesis (Johnston, et al, 2001). The serum level of testosterone was significantly (P<0.05) increased in the test groups when compared to control. Male fertility is hinged on the production of matured normal sperm cells. The growth of primordial germ cells and maturation of spermatozoa are mainly controlled by FSH and LH. FSH control spermatogenesis through its receptor (FSH – R), expressed in the sertoli cell, while the LH stimulate testosterone synthesis in the leydig cells of testis (Eblen et al., 2001; Lei et al., 2001).

However, the sperm parameters were significantly (P<0.05) reduced in the test groups when they were compared to the control. The percentages of viable sperm cells, sperm cells with normal morphology, actively motile sperm cells were significantly (P<0.05) decreased The sperm count was also reduced significantly(P<0.05) when the test groups were compared to control. The total sperm count, actively motile spermatozoa and normal morphological features has been reported as indices of fertility in males (Oyeyemi et al.,2000). These significant changes suggest that the extract may have the potential to permeate the blood-testis barrier. The observed effects on the testicular function clearly shows that the increased testosterone concentrations in the test groups was inconsequential to improving spermatogenesis. The extract was observed to have acted primarily on the

testis where it exhibited suppressive effects in regards to spermatogenesis. The decrease in the density of spermatozoa has been shown to be the possible mechanism in which natural substances used in the form of plant based contraceptive, inhibits male fertility (Sharma and Jocob.,2001). Male infertility may occur as a result of dysfunction in sperm production. It has been shown that pathological transformations in seminiferous tubules and epididymis cause distortions in testicular and epididymal functions giving rise to reduction in quality and number of spermatozoa, including percentage of motile sperm, live sperm and normal sperm morphology; all of which has the tendency to cause infertility. Infertility has been related to reduction in quantity and quality of spermatozoa occurring as a result of testicular and epididymal damages. The estimation of number of sperm is an important test of spermatogenesis which has a direct relationship to fertility (Nwoke etal.,2015).

The possible mechanism in which natural substances can be used to inhibit male fertility acting as a form of plant based contraceptive is by causing a decrease in the density of spermatozoa (Sharma and Jocob., 2001). The result has revealed that extract of Rhizophora mangle adversely affected testicular function thereby, suppressing spermatogenesis and may ultimately reduce male reproductive capabilities.

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