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RESEARCH ARTICLE

EVALUATION OF HYDROMETHANOLIC LEAF EXTRACT OF *BRYOPHYLLUM PINNATUM* ON REPRODUCTIVE FUNCTIONS OF MALE WISTAR RATS.

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Abstract

The use of Brophyllum pinnatum in the treatment of several ailments in traditional medicine practice is common. The effect of hydromethanolic leaf extract of Bryophyllum pinnatum on reproductive parameters of male wistar rats was investigated in this study. Male wistar rats were randomly assigned into three (3) groups. Group one which served as control received distilled water. Groups two and three received 100mg/kg body weight and 200mg/kg body weight of the extract . The extracts were administered as single oral doses for 30 and 58 days for hormonal and sperm quality studies respectively. Results obtained showed that there was significant ($p<0.05$) increase in LH and TET at the low dose (100mg/kg) bw. However, there was significant ($p<0.05$) reduction in sperm count, volume and the percentage of morphologically normal spermatozoa. The leaf extract of Brophyllum pinnatum possesses antifertility effects due to its suppressive effects on spermatogenesis in male wistar rats.

Introduction:-

Bryophyllum pinnatum is a perennial herb growing widely and used in folkloric medicine in tropical Africa, tropical America, India, China, and Australia. The plant flourishes throughout the southern part of Nigeria [Gill,1992]. It is commonly known as "Miracle leaf or Resurrection plant", amongst other names.It is called "ewe abamoda or odundun" by the Yoruba tribe of southwestern Nigeria, "odaaopue" among the Igbos. It is referred to as "da busi" in Chinese [Iwu, 1993; Ghasi et al.,2011]. It has been accepted as a herbal remedy in almost all parts of the world [Igwe and Akunyili ,2005]. The plant which is widely known in traditional medicine practice, for its haemostatic and wound healing properties [Anjoo Kamboj and Ajay Kumar Saluja, 2009]; is also applied in the treatment of stomach ulcers, flu and fever [Da Silva et al., 1995]. Although,traditional herbal medicines are gaining importance nowadays, they are also been studied to find the scientific basis of their numerously acclaimed therapeutic actions [Abedi et al.,2013].

However, extracts of *Bryophyllum pinnatum* has been reported to possess antimicrobial [Akinpelu, 2000], antifungal [Misra et al, 1979], antiulcer [Pal and Chaudhuri, 1991], anti-inflammatory and analgesic [Pal and Chaudhuri, 1989; Pal and Chaudhari, 1992], antihypertensive [Ojewole, 2002], anti-histamine and anti-allergic [Pal etal.,1999] as well

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as antioxidant properties [Ojewole, 2002] .The plant contains a wide range of active compounds such as alkaloids, triterpenes,glycosides [Okwu and Josiah, 2006], flavonoids [Cao et al,2005] , as well as steroids and bufadienolides [Yamagishi et al,1989]. These bioactive compounds are considered to be responsible for the various biological actions of *Bryophyllum* pinnatum.

There is dearth of scientific reports on the effect of *Bryophyllum pinnatum* on male reproductive functions.This study was carried out to investigate the effect of hydromethanolic leaf extract of *Bryophyllum pinnatum* on male reproductive functions in wistar rats.

Materials and Methods:-

Collection and extraction of plant material:-

Fresh leaves of *Bryophyllum pinnatum* were collected from Idama/Ekulama community in Akuku-Toru Local Government area of Rivers State, Nigeria and were authenticated in the herbarium unit, Department of Plant Science and Biotechnology, University of Port Harcourt, Nigeria. The leaves were washed with water to remove dirt, and dried at room temperature (26^oC) over a period of 3 weeks. The leaves were dried and grinded using a manual engine grinder to obtain 500g of the fine powder. This quantity was soaked in 400ml of hydromethanol (20%:80%) for 48 hours. The solution obtained was then filtered to separate the filtrate from the residue. The extract was concentrated under reduced pressure in a vacuum at 45^{oC} using a rotary evaporator (Searl Instruments Ltd., England). The yield of the crude extract of *Bryophyllum pinnatum* leaves obtained weighed 63.1g. The extract was stored in a refrigerator at 4^{oC} before use for the study.

Study area:-

This experimental study was carried out in the Department of Human Physiology, Faculty of Basic Medical Science, University of Port Harcourt, Nigeria.

Experimental animals and protocols:-

Thirty adult male rats weighing 130–150g at the beginning of experiment, were bred in the experimental animal centre of department of Human Physiology, University of Port Harcourt, Nigeria. The rats were acclimatized for two weeks, and randomly assigned into 3 groups (n = 10). Group 1 served as control and received distilled water. Group 2 and group 3 received 100mg/kg body weight (bw) and 200mg/kg bw of hydromethanolic leaf extract of *Bryophyllum pinnatum* respectively.The extracts were administered orally, once daily for 30 days. The choices of the doses were based on the result of toxicity study of the leaf extract of *Bryophyllum pinnatum* which is 641mg/kg bw [Salahdeen and Yemitan, 2006]. All animals had access to water and feeds *ad libitum.*

This study was conducted 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 25% urethane i.p 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 (TET).

Estimation of sperm motility, volume, counts and morphology:-

An incision (about 1 mm) was made in the caudal epididymis. Semen was then squeezed onto the microscope slide. 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 hemocytometer and were expressed as million/ml of suspension. The appearance, viscosity and pH, volume and morphology were also determined, all in accordance with standard protocols described by the World Health Organization [WHO, 2010].

Statistical analysis:-

The Statistical analysis of data was done using Statistical Package for Social Sciences (SPSS) version 20.0 and expressed as Mean ± SEM. Significant differences between means was determined by Posthoc least significance difference (LSD) and the results were regarded as significant at $p<0.05$.

Result:-

Result presentation:-

The results of this study are presented in tables 1 to 3.

Table 1:- Effect of leaf extract of *Bryophyllum pinnatum* on some hormones.

GROUPS	Parameters		
	FSH (IU/L)	LH (IU/L)	Testosterone (ng/ml)
Group 1 (Control)	3.10 ± 0.45	1.02 ± 0.17	0.38 ± 0.07
Group 2 (100mg/kg)	3.72 ± 0.58	2.14 ± 0.35*	0.88 ± 0.32*
Group 3 (200mg/kg)	2.74 ± 0.29	1.12 ± 0.12	0.44 ± 0.08

Values are expressed as mean ± SEM; n=5; *: Significant at p<0.05 when compared to control

Table 2:- Effect of leaf extract of *Bryophyllum pinnatum* on some sperm parameters

Groups	Group 1 (Control)	Group2 (100mg/kg)	Group3 (200mg/kg)	Test of significance at 95% CI
Sperm count ($x10^6$/ml)	590.0±60.0	420.0±98.2	120.0±20.9	*P=0.02**
Volume (cm^3)	0.14±0.03	0.12±0.02	0.10±0.00	*P=0.025**
Normal Morphology (%)	75.20±0.17	72.00±0.53	57.26±0.34	*P=0.00**
Abnormal Morphology (%)	24.80±0.31	28.00±0.96	42.74±0.65	*P=0.*001*

Values are expressed as mean ± SEM, *=significant at P<0.05 when compared to controls; CI = confidence interval.

Table3:-Effect of leaf extract of *Bryophyllum pinnatum* on sperm motility

Groups	Group1 (Control)	Group2 (100mg/kg)	Group3 (200mg/kg)	Test of significance at 95% CI
Active cell	69.60±3.26	55.00±7.58	43.0±3.39	*P=0.002**
Sluggish cell	11.40±0.98	19.00±1.87	20.40±2.18	*P=0.001**
Dead cell	19.00±3.32	26.00±3.32	36.60±5.79	*P=0.003**

Values are expressed as mean ± SEM, *=significant at P<0.05 when compared to controls; CI = confidence interval.

Result analysis:-

There was a statistically significant (p<0.05) increase in the serum level of LH and TET in group two which received 100mg/kg bw (low dose) of hydromethanolic leaf extract of *Bryophyllum pinnatum* when compared to group one (control). However, a non – significant reduction was observed in the serum level of LH and TET in group three treated with 200mg/kg bw (high dose) of the extract when compared to control. FSH was not significantly altered as shown in table 1.

A dose dependent significant (p=0.02) reduction in sperm count was observed in groups two and three when compared to control as shown in table 2. Similarly, a significant reduction (p=0.025) in the volume and percentage of morphologically normal (p=0.00) sperm cells was observed when compared to control as shown in table 2. Also, there was a statistically significant (p=0.001) increase in the percentage of sperm with abnormal morphology in group three. The increase in group two was not statistically significant (p<0.05) when compared to control.

Table 3 highlights the effect of hydromethanolic leaf extract of *Bryophyllum pinnatum* on sperm motility. A dose dependent statistically significant (p=0.002) reduction in the level of actively motile sperm cells was observed in group two and group three when compared to control. However, there was a statistically significant (p=0.001) increase in the number of sluggish sperm cells and number of dead sperm cells (p=0.003) when compared to control.

Discussion:-

The effect of low dose (100 mg/kg) bw and higher dose (200 mg/kg) bw of hydromethanolic leaf extract of *Bryophyllum pinnatum* on male reproductive functions was evaluated using wistar rats as experimental models. *Bryophyllum pinnatum* has been well known for its medicinal properties all over the world [Kirtikar et al, 1975; Da Silva et al, 1995; Akinpelu, 2000].

Male reproductive process is regulated by intricately balanced mechanisms involving the hypothalamus-pituitary-testicular axis and accessory sex organs. It is believed that for initiation as well as maintenance of spermatogenesis in humans, both FSH and TET are needed. The gonadotropin-releasing hormone (GnRH) secreted by the hypothalamus regulates the synthesis and release of FSH and LH from the pituitary. The finding from this study revealed that the low dose (100mg/kg) of the extract caused a significant increase in the level of serum LH and TET. The increase in the level of TET may be as a result of the significant increase in secretion of LH which then stimulated its receptors. LH specific receptors expressed on the surface of Leydig cells controls the production and secretion of TET [deKrester et al, 1971]. The significant increase in serum TET levels did not result in decrease of serum LH and FSH levels in group one [Table 1], as may be expected in a typical negative feedback mechanism. This may be due to the stimulatory action of amino acid compounds and it's derivatives such as Aspartic acid, a bioactive component of *Bryophyllum pinnatum* [Sato and Tsukanmamoto,2000] on hypothalamic pituitary testicular axis. Aspartic acid stimulates secretion of gonadotropin – releasing hormone (GnRH), FSH and LH. Aspartic acid is also converted to nitric oxide, which is one of the most important factors controlling the release of FSH and LH [Pinilla, et al, 2001]. These mechanisms may have contributed to override a negative feedback action of TET on the secretion of FSH and LH. This study revealed that the extract caused a significant reduction in sperm count, volume, percentage sperm cells with normal morphology as well as the actively motile sperm cells. Consequently, there was significant increases in the percentage of sluggish sperm cells as well as dead sperm cells. These significant changes which was more pronounced at the higher dose (200mg/kg) bw may 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 TET was inconsequential to improving spermatogenesis. This may arise because the extract primarily acts on the testis where it exhibits suppressive effects in regards to spermatogenesis.

The decrease in sperm parameters caused by chemical agents was reported to be due to their ability to permeate the blood-testis barrier thus, creating a different microenvironment in the inner part of the wall of the seminiferous tubules from that in its outer part (Bloom and Fawcett, 1975). The sluggish sperm cells may not be effective physiologically, as they are unlikely to penetrate the cervical mucus to fertilize the ova [Chauhan and Agarwal (2008);Abu and Uchendu (2010)]. Also, the enzyme Lactate dehydrogenase , said to be very specialized, catalyzes the reversible reaction between lactate and pyruvate and involved in energy supplying metabolic processes providing for germ cells [Blanco et al, 1975]. Hence, alteration in the production of pyruvate and liberation of energy could alter germinal cells, sperm activity and survival. These alterations may have contributed to the significant increase in sluggish and dead cells observed in the present study.

This study has shown that the hydromethanolic leaf extract of *Brophyllum pinnatum* possess anti fertility potentials evidenced by its suppressive effects on spermatogenesis which resulted in reduced sperm quality of male wistar rats.

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