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Evaluation of the Ethanolic Extract of the Fruit of *Dennettia tripetala* on Fertility Indices of Male Wistar Rats

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Abstract: ***A major burden of infertility exist in Africa, where it presents an enormous challenge due to social and cultural demands associated with the condition, thereby causing severe psychological and emotional distress to infertile couples. Male factor infertility also contribute tremendously to the incidence of infertility among couples. This study was done to investigate the effects of the fruit of Dennettia tripetala on fertility of male wistar rats. Male wistar rats were divided into three (3) groups of six (6) rats each. Group one (1) which served as control received distilled water. Group two (2) and group three (3) were treated with 100mg/kg bw and 200mg/kg bw of the ethanolic extract of the fruit of Dennettia tripetala respectively. Hormone and sperm quality studies lasted for 30 and 58 days respectively. The results obtained showed that the extract caused significant increase in level of serum testosterone, testicular and epididymal weights as well as the percentage of actively motile cells and sperm count . It however, did not significantly alter the serum levels of luteinizing hormone and follicle stimulating hormone. This study proved that, the fruit of Dennettia tripetala possesses fertility enhancing effects in male wistar rats.***

Keywords: Dennettia tripetala, fruit, ethanolic, wistar rats, fertility

1. Introduction

Infertility is a common problem associated with reproductive health in Africa; where an "infertility belt" spreading through West Africa, Central Africa upto East Africa was described [1-3]. Though, the differences in sources of data and it's analysis has made it difficult to accurately measure or compare infertility rates, it is evident that the level or prevalence vary widely between and within countries, regions and groups. In a report, the Demographic health survey (DHS) and world fertility survey (WFS) showed that the prevalence of infertility in sub-Saharan Africa ranges from 11 to 20 percent (11-20%); which was demonstrated in a survey of 27 countries [4-6].

Globally, the problem of infertility presents a significant social and public health concern [7-8]. Couples who suffer from infertility go through profound psychological and social issues due to cultural demands and societal pressures in Nigeria and many other developing countries [9-10].

Infertility in couples was also reported to be a major cause of marital disharmony among couples in Nigeria, because of the high premium placed on child bearing [9]. The inability of a couple to reproduce may be due to a problem of the male. Male factor infertility accounted for 25% of infertility cases in a study in south eastern Nigeria [9], and 26.8% in another study in south western Nigeria [11]. Problems associated with sperm production like Oligospermia, Azoospermia, Asthenozoospermia and Teratospermia has been implicated as a cause of male infertility [9]. Sperm production and sperm count is reportedly influenced by the serum levels of luteinizing hormone, follicle stimulating hormone and testosterone [12]. There are various treatment options for male infertility; however, a significant proportion of African population still rely on the use of plant materials for their health needs. *Dennettia tripetala* is a medicinal plant which grow along the coastal regions predominantly in the rain forests of West Africa. It is applied in folklore remedies in the treatment of cough, toothache, fever and nausea occurring due to pregnancy [13-14]. It has also been shown to possess some bioactive compounds such as flavonoids and steroids [15]. Extracts of the plant has been reported to show anti oxidants effects [16], as well as analgesic and anti-inflammatory effects [17]. It is difficult to find studies done to assess the effects of *Dennettia tripetala* on fertility. This study was done with the objective to assess the effects of the fruits of *Dennettia tripetala* on male reproductive functions.

2. Materials and Methods

2.1 Animal models

Male wistar rats weighing between 130g-150g, bred in the animal house of Faculty of Basic Medical Sciences, University of Port Harcourt, were used in this study. They were randomly selected and allowed two (2) weeks to acclimatize. They were given free access to standard rat feeds/chows (Top feeds Nigeria Limited) and water. While accommodated in clean cages, they were exposed to standard conditions and temperature of $25^{o}C - 30^{o}C$; 12 hour light and 12 hour dark cycles. Cleaning of cages including change of water, feeds and beddings were a daily routine throughout the period of the experiment. In general, animal handling in this study was done in accordance with the recommendations in the care and use of laboratory animals by the American Physiological Society[18].

2.2 Preparation of plant materials

Dennettia tripetala fruits were obtained from local dealers in Rivers state, Nigeria.The identification was subsequently done at the herbarium of the Department of Plant Science and Biotechnology of the University of Port Harcourt, Nigeria.

The fruits of *Dennettia tripetala* were first dried at room temperature for three (3) weeks and later blended to fine powder. Extraction was done at 60-70^{o}c using ethanol with the aid of the soxhlet apparatus. A solution containing the extracts was obtained and filtered after 24 hours. The rotary evaporator was used to concentrate the filtrate under reduced pressure of 60^{o}c to a semi-solid form. The extract yield was weighed and preserved in a refrigerator .Measured quantity of extract was later dissolved to obtain 100mg/ml and 200mg/ml of solution which was orally administered to the rats.

2.3 Experimental Design

This study was carried out to investigate the effects of the fruits of *Dennettia tripetala*. In this study, male wistar rats were randomly divided into three (3) groups containing six (6) rats each for sperm quality and hormonal studies. Group one (1) which served as control received distilled water. Group two (2) and group three (3) were treated with 100mg/kg bw and 200mg/kg bw of the ethanol extract of the fruits of *Dennettia tripetala* respectively. While the treatments for the hormonal studies lasted for 30 days; that of sperm quality studies lasted for 58 days . Extracts were administered as single oral doses with animal feeding hypothermic syringes. The animals were sacrificed under chloroform induced anaesthesia on days 31 and 59, which was 24hours after administration of last dose.

2.4 Collection of blood

Blood samples were collected through cardiac puncture into dry sample tubes and centrifuged at 3000 rev/min for 10-15 minutes. The serum obtained was initially stored in the refrigerator and later used for analysis of hormone.

2.5 Collection of semen / sperm analysis

A small cut was made at the inguinal region to allow access to the caudal epididymis . Another incision of about 1mm was made on the caudal epidydimis to make possible the collection of semen. Semen was gently squeezed through the vas deferens. The improved Neubauer cytometer was used to determine the epidydimal sperm count through the method of cytometry. The sperm count was expressed as million/ml [19,20]. Sperm parameters were analysed through standard methods which has been documented [21].

2.6 Hormone assay

The assay for luteinizing hormone, follicle stimulating hormone and testesterone, was done in accordance with established methods [22] ; with the use of appropriate hormonal kit. The hormone which competitively binds on immobilized antibody formed the basis for testosterone assay. The principle involved in assay of all these hormones was based on the use of a solid phase enzyme linked immunosorbent assay (ELISA) kit. This system incorporated the mouse monoclonal anti - α - hormone antibody for solid phase (microwells) immobilization with another mouse monoclonal anti-β-hormone antibody in solution of the antibody enzyme conjugate.

2.7 Statistical analysis

Statistical analysis of data was carried out with the use of statistical package for social sciences (SPSS) version 20. The results obtained were expressed as mean ± SEM. The one way analysis of variance (ANOVA) was used to determine the difference between the mean(s). In all statistical tests, a value of $p<0.05$ was considered significant.

3. Result

3.1 Result presentation

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

Table 1: Effect of ethanolic extract of *Dennettia tripetala* on some hormones

Groups	Serum hormones		
	LH	FSH	TET
Group 1 (Control)	4.27±0.40	4.73±0.61	1.60±0.25
Group 2 (100mg/kg)	4.80±0.56	5.30±0.57	2.15±0.20
Group 3 (200mg/kg)	5.87±0.76	5.87±0.76	2.64±0.20*

Values expressed as Mean ± SEM. n=6. Significant at [*(P<0.05)] when compared with control group.

Table 2: Effect of ethanolic extract of *Dennettia tripetala* on some sperm parameters

Groups	Sperm parameters		
	Viable sperm cells (%)	Normal morphology (%)	Actively motile (%)
Group 1 (Control)	85.33±2.51	77.67±4.60	59.67±2.70
Group 2 (100mg/kg)	86.67±3.13	79.00±4.31	64.67±4.61
Group 3 (200mg/kg)	88.67±2.29	68.75±1.57	75.33±5.00*

Values expressed as Mean ± SEM. n=6. Significant at *(P<0.05) when compared with control group.

Table 3: Effect of ethanolic extract of *Dennettia tripetala* on tissue/organ weights

Groups	Tissue/organ weights (g)	
	Testis	Epidydimis
Group 1 (Control)	1.17±0.05	0.30±0.06
Group 2 (100mg/kg)	1.21±0.04	0.34±0.03
Group 3 (200mg/kg)	1.31±0.04*	0.48±0.04*^{a}

Values expressed as Mean ± SEM. n=6. Significant at $^{*/a}$(P<0.05) when compared with control and between test groups respectively.

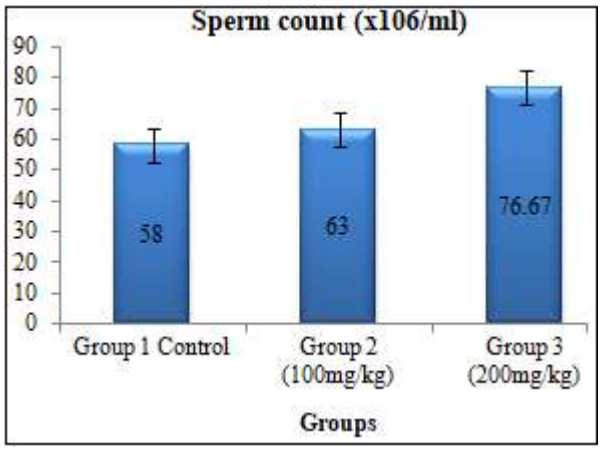

Figure 1: Effect of ethanolic extract of *Dennettia tripetala* on sperm count

3.2 Result analysis

In the result presented, the extract was administered at low dose of 100mg/kg bw to group 2 and higher dose of 200mg/kg bw to group 3. Group 1 served as control and received no extract.

In table 1, the luteinizing hormone and follicle stimulating hormone levels were not significantly ($p<0.05$) increased in groups 2 and 3. Serum testosterone level increased significantly ($p<0.05$) at the higher dose of the extract when test groups were compared to control group.

The effect of the extract on some sperm parameters were highlighted in table 2. The parameters includes the viable sperm cells, sperm cells with normal morphology and actively motile sperm cells. The percentage viable cells and percentage cells with normal morphology were not significantly ($p<0.05$) altered when the test groups were compared to control.

However, the percentage of actively motile cells were significantly increased ($p<0.05$) in group 3 when compared to control.
On the effect of the extract on the weights of the testis and epididymis as shown in table 3, both wights (testis and epididymis) were significantly ($p<0.05$) increased in group 3 when compared to control. In addition, the weight of the epididymis was significantly ($p<0.05$) increased in group 3 when compared to group 2.

Figure 1 show the changes in sperm count in the different groups. A significant increase ($p<0.05$) occurred in sperm count in group 3 when compared to group 1(control) as well as group 2.

4. Discussion

Over the years, the nutritional and medicinal value attached to plants has provoked an increase in the investigations of various parts of plants either as crude extracts or in the form of bioactive constituents. This study was carried out to investigate the effects of the extract of *Dennettia tripetala* on the reproductive functions of male wistar rats.

The results obtained in this study showed that the extract of *Dennettia tripetala* caused a non significant change in the levels of serum luteinizing hormone and follicle stimulating hormone, but, the level of serum testosterone was significantly increased with the higher dose of the extract.

The anterior pituitary gland secretes the gonadotrophins which are the follicle stimulating hormone and luteinizing hormone.These hormones are glycoprotein hormones that act directly on the testis to stimulate somatic cell function in support of spermatogenesis [23].

The somatic cells include, the interstitial steroidogenic cells of Leydig, whose function is primarily the production of testosterone [24]; the myoid cells which surround the seminiferous tubules providing physical support as well as contractile motion to the structures [25]; and the Sertoli cells, which are in close contact with proliferating and differentiating germ cells in the seminiferous tubules, thus making them very essential as they provide physical and nutritional support for spermatogenesis [26]. The functions of each of the somatic cell types is influenced by one or more of the hormones involved in regulation and sustenance of male fertility.

In males, FSH receptor is expressed on the testicular Sertoli cells [27], while LH receptors are expressed mainly in the Leydig cells, although receptor staining has been observed in spermatogenic cells [28-29].

Since testosterone is primarily secreted by the leydig cells in males [24,30]; the extract of *Dennettia tripetala* may have exerted its action by causing increased stimulation of the interstitial steroidogenic cells of leydig to release testosterone. Similarly, in a study on the effects of a plant extract [31], on some male related fertility hormones, it was found that the extract caused a non significant change in serum levels of luteinizing hormone and follicle stimulating hormone but a significant increase in serum level of testosterone.

Although it was reported that the amount of testosterone secreted is regulated by the hypothalamic-pituitary-gonadal axis [32]. The relationship existing at these three control levels is such that, a reduction in the level of serum testosterone makes the hypothalamus to release the gonadotropin releasing hormone which in turn, stimulates the release of follicle stimulating hormone and luteinizing hormone from the pituitary gland . The actions of the gonadotropins on the testis leads to increased synthesis of testosterone. But in a negative feedback trend, increased levels of testosterone act on the hypothalamus and pituitary gland to inhibit the release of hypothalamic gonadotropin releasing hormone and pituitary follicle stimulating hormone with the luteinizing hormone respectively. The extract of *Dennettia tripetala* may have influenced the resultant significant increase in testosterone which would have lead to a non significant change in gonadotropin levels.

The extract of *Dennettia tripetala* did not significantly alter the percentages of viable spermatozoa and that of morphologically normal spermatozoa, however, it caused significant increase in the other epididymal sperm

parameters that were assessed in the present study. The percentage of actively motile spermatozoa was significantly increased with the higher dose of the extract. Furthermore, the increase in the sperm cell motility may be connected to an increased testicular glycogen content due to high energy content of *Dennettia tripetala*. *Dennettia tripetala* was reported to possess high energy value with high protein, carbohydrates and lipids content giving a high food value [33]. In another study, a decrease in testicular glycogen content reportedly deprived the sperm cells of much needed energy which may be responsible for the observed reduction in sperm motility and increased sluggishness of sperm cells [30].

Also, the sperm count was increased in a dose dependent manner. The increase in sperm count may not be unrelated to the increase in testosterone level. It has been reported that the amount of spermatozoa in the seminiferous tubules, which shows the degree of spermatogenesis is quantitatively maintained by testosterone and follicle stimulating hormone [30]. Therefore, increase in sperm count observed in this study could be attributed to the increased testosterone level which followed the administration of *Dennettia tripetala* extract.

There was also a significant increase in the weight of the testis with the higher dose of the extract and a dose dependent increase in the weight of the epidydimis. This increase in testicular and epidydimal weight may be due to an increased testosterone which resulted in increase in spermatogenic activities. The testes and epididymis are androgen-dependent organs, that rely on testosterone for their growth and function [34]. The described effects are in keeping with the physiological actions of the male sex hormone.

5. Conclusion

The observed changes in testosterone level and it's effect on testicular function and spermatogenesis showed that the extract of *Dennettia tripetala* enhanced fertility and reproductive parameters of male wistar rats.

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