EFFECT OF AQUEOUS EXTRACT OF ANNONASENEGALENSIS LEAVES ON THE SPERMIOGRAM OF MALE ALBINO RATS.

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ABSTRACT

The aqueous leaf extract of the plant Annonasenegalensis (Pers) commonly known as African custard apple, at different doses of 200, 300, and 500mg/kg b.w was tested for its spermatogenic effect by studying its activities on the spermiogram of male albino rats. The animals were administered with the aqueous extract of the plant for 14 consecutive days through gastric intubation. The result on the 15th day of the study shows the weight of the testes and epididymis increased significantly for the 300 and 500mg/kg doses. The sperm concentration of the 200, 300 and 500mg/kg doses also significantly increased and the sperm motility of 300 and 500mg/kg also increased significantly, however, the decrease in abnormal sperm morphology is not significant for any of the doses. It was therefore concluded that the plant Annonasenegalensis is capable of enhancing the male fertility especially in rats.

Keywords: Fertility, Spermiogram, Male rats, Annonasenegalensis, Leaf extract.

INTRODUCTION

Infertility is a major problem of human and animal populations, it is a common clinical problem affecting 13-15% of couples worldwide¹ and of great economic importance in domestic and zoological animals. Infertility could be from male or female counterpart but reports have shown male problems as the commonest single defined causes of infertility².
More than 90% of male infertility cases can be attributed to low sperm counts, poor sperm quality or both and these may be as a result of congenital or acquired urinogenital abnormalities, infection of the genital tract, increase scrotal temperature, endocrine disturbances, genetic abnormalities and immunological factors\(^3\). The total sperm count, sperm motility and the morphological features of the sperm have been reported as the indices of fertility in males\(^4\). The use of plant extracts to enhance fertility in animals is now in the increase because of the shift of attention from synthetic drugs to natural products from plants\(^5\) due to their little or no side effect when used\(^6\).

*Annona Senegalensis* (Pers) otherwise known as African custard apple, wild custard-apple or wild sour sopp\(^7,8\) belong to the family Annonaceae. It is a small tree of about 2-6m tall which grows wild in tropical Africa. The flower is used to flavour food due to its aromatic nature. The ripped fruit is yellow in colour with sweet edible jelly. *Annonasenegalensis* has been reported for its medicinal purposes. The antibacterial activities was reported by Apak and Olila 2006\(^9\), antidiarrhoea by Suleiman et al 2008\(^10\), anticonvulsant by Ezugwu and Odoh, 2003\(^11\), and its analgesic and anti-inflammatory effects have also been reported\(^12,13\).

This plant has been shown to be reputed in the treatment of fever, intestinal troubles, stomach aches, gonorrhoea, syphilis, rheumatism and central disorders\(^14,15,16\).

Earlier studies on the plant showed its activities against cancer\(^17\), chest pain, cough, anaemia and urinary tract infection\(^18,19\), arthritis and rheumatism\(^20\), head and body ache\(^21,22\) and treatment of snake bite\(^23,24\) among its several documented uses.

The phytochemical screening of *Annonasenegalensis* showed it to contain (-)-roemerine\(^25,26\) and ent-kaurenediterpenoids\(^27\), Wax and alkaloids\(^25,28\) proteins, amino acids, anthraquinones\(^29,18,30\), Sterols, glycosides, flavonalterpenoids\(^31,32,33,27,34\) and terpenes\(^35\). In this study, we investigated the ethnomedicinal use of the leaves of *Annonasenegalensis* in the treatment of male infertility as it has been used by the traditional physicians, by studying the effects of the aqueous leaf extract of the plant on the spermogram of the male albino rats.

**MATERIALS AND METHODS**

**Collection and identification of the plant:** *Annonasenegalensis* was identified by a herbalist from its natural habitual in Ogbooro, in Saki- East Local Government area of Oyo State.
Nigeria. The plant was collected and further authenticated by botanist at Forestry Research Institute of Nigeria (FRIN) Ibadan, Nigeria.

**Preparation of the extract:** The plant leaves were washed and air-dried to a constant weight. The dried material was pulverized into a dry powder using a mortar and pestle. 300g of the pulverized leaves was extracted with 4.0 litres of distilled water obtained from Department of Chemistry (Natural product laboratory) University of Ibadan, Ibadan. The filtrate was concentrated with vacuum rotary evaporator maintained at 40°C from where a greenish-brown semisolid was obtained. The percentage yield of the extract was 5.56% w/w. A fresh 10% solution of the extract was prepared with distilled water to obtain appropriate dosages required for the studies.

**Experimental animals:** Adult male albino rats weighing between 190-220g obtained from the experimental animal unit of the Faculty of Veterinary Medicine University of Ibadan, Ibadan were used for the study. The animals were kept in cages, allowed 14 days for acclimatization before the commencement of the study. They were supplied with clean drinking water and fed with Grower’s mash pellets of Vital feeds Nigeria® Limited. The animals were allowed food and water freely and were used in accordance with laboratory practice regulations and principle of laboratory animal care\[36\] and the study was conducted in accordance with the recommendation from the declarations of Helsinki on guiding principles in the care and use of animals.

**Acute toxicity test:** The acute toxicity of aqueous extract of *Annonasenegalensis* leaf was determined using the method described by Locke 1983\[37\]. Thirty matured albino rats were randomly divided into 6 groups of 5 rats each (group I-VI). Group I-V was administered varying doses (100, 500, 1000, 2000 and 3000 mg/kg respectively) of the extract orally by gastric intubation while group VI received 10ml/kg of distilled water. The animals were observed for signs of toxicity for 72 hours post administration, the number of dead animals within this period were also determined.

**Animal treatment:** Twenty-four matured male albino rats were randomly selected and divided into four groups of A-D of 6 rats each. Groups A, B and C which represents the test group were administered with 200, 300 and 500mg/kg body weight of the extract respectively for 14 days, the doses selected were based on the tolerance of the extract by the animals determined during our acute toxicity study. The animals in group D which served as the
control were given 10ml/kg body weight of distilled water also for 14 days and all the treatments were administered orally by gastric intubation. The animals were weighed on the first day before the administration of the extract, and on the fifteenth day of the study. They were then anaesthetized with diethyl ether and sacrificed. The testes and epididymis of the rats were dissected free of fats and weighed independently. The epididymis was carefully opened into 5ml of physiological saline, mixed thoroughly and one drop of the suspension was applied to a Neubauer’s counting chamber with coverslip to determine the sperm motility which is expressed as percentage by counting motile and immotile sperm cells per unit area. Sperm count and morphology were also determined by routine procedure[38,39] from the sperm taken from caudal epididymis of the rats. The sperm cell morphology was expressed as percentage of the total sperm count.

**Statistical evaluation:** All data obtained from this study were analysed statistically using one way analysis of variance (ANOVA). Further comparison between groups was performed using Ducan’s multiple range test and all difference were considered significant at 5% level (P<0.05), all data were expressed as mean ± standard error of mean (mean ± SEM).

**RESULTS**

**Description of the extract:** The aqueous extraction of *Annonasenegalensis* leaves produced a sticky greenish brown colour residue with no peculiar smell. The percentage yield was 5.56% w/w

**Acute toxicity study:** The extract was safe in rats at the tested doses (100 – 3000mg/kg) as no sign of toxicity was observed, there was also no mortality within the studied period.

**Effect of Annonasenegalensis extract on organ weight:** Table 1 shows the effects of aqueous leaf extract of *Annonasenegalensis* on body weight and weight of reproductive organs such as testis and epididymis in intact adult male albino rats. Oral administration of the extract at doses 200, 300 and 500 mg/kg for 14 consecutive days does not show any significant increase in body weight (P>0.05), but the 300 and 500 mg/kg had a significant increase in the weight of testis and epididymis in a dose – dependent manner as shown in table 1.
Table 1: Effect of aqueous extract of *Annonasenegalensis* on body weight (g), weights of testis (mg/100g BW) and epididymis (mg/100g BW) in adult male albino rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>BW on day 1</th>
<th>BW on day 15</th>
<th>Testes</th>
<th>Epididymis</th>
</tr>
</thead>
<tbody>
<tr>
<td>200mg/kg</td>
<td>209±3.4</td>
<td>218.7±5.1</td>
<td>659.2±10.5</td>
<td>178.6±3.5</td>
</tr>
<tr>
<td>300mg/kg</td>
<td>198±2.2</td>
<td>208.3±7.8</td>
<td>640.6±7.9*</td>
<td>174.6±5.9*</td>
</tr>
<tr>
<td>500mg/kg</td>
<td>212±2.7</td>
<td>221.6±6.2</td>
<td>683.7±12.7*</td>
<td>179.5±5.1*</td>
</tr>
<tr>
<td>Distilled water</td>
<td>205±3.1</td>
<td>217.1±8.2</td>
<td>638.5±18.6</td>
<td>145.5±4.9</td>
</tr>
</tbody>
</table>

BW = body weight, each value represents mean ± SEM and * p<0.05 significant difference from control.

**Effect of the extract on sperm concentration motility and morphology:** The concentration of sperm in the caudal epididymis of the control group was 0.48±2.3 \(\times 10^8\)/ml, sperm motility was 4.2±3.7% and abnormal sperm morphology was 3.1±2.5% (Table 2). Treatment with aqueous leaf extract of 200, 300 and 500mg/kg caused a significant increase of sperm concentration to 1.27±3.7, 1.81±5.7 and 1.89±4.1 respectively. Doses of 300 and 500mg/kg increased sperm motility significantly to 57±7.6% and 57± 2.8% respectively. The decrease in abnormal sperm morphology were not significant.

Table 2: Effect of aqueous leaf extract of *Annonasenegalensis* on sperm concentration and motility in adult male albino rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sperm conc.</th>
<th>Sperm motility</th>
<th>Abnormal sperm morphology (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(x10^8/ml)</td>
<td>(%)</td>
<td></td>
</tr>
<tr>
<td>200mg/kg</td>
<td>1.27±3.7*</td>
<td>43±4.8</td>
<td>2.9±1.7</td>
</tr>
<tr>
<td>300mg/kg</td>
<td>1.81±5.7*</td>
<td>57±7.6*</td>
<td>2.6±1.3</td>
</tr>
<tr>
<td>500mg/kg</td>
<td>1.89±4.1*</td>
<td>57±2.8</td>
<td>2.6±1.8</td>
</tr>
<tr>
<td>Distilled H2O</td>
<td>0.48±2.3</td>
<td>42±3.7</td>
<td>3.1±1.5</td>
</tr>
</tbody>
</table>

Conc = concentration, each value represents mean ± SEM and * p<0.05 significant difference from control.

**DISCUSSION**

The present study demonstrated a significant increase in weights of reproductive organ, sperm concentration and sperm motility in rats by *Annonasenegalensis* leaf extract. This study...
corroborates the use of *Annonasenegalensis* in enhancing male fertility by the traditionalists who use the plant in the treatment of various ailments that has to do with male infertility. The total sperm count, motile sperm counts and sperm morphological features as shown in this study have been reported as the indices of fertility in males\(^{[40,4]}\).

In the present study, there is a significant increase in the weight of the reproductive organs such as testis and the epididymis which may suggests an increased functionality of these organs. The extract may act on the pituitary gland and increase main hormones responsible for spermatogenesis. It has been observed that weight and secretary functions of reproductive organs are closely regulated by the hormones and organs\(^{[41,42]}\). The treated animals also showed significant increase in the sperm count and motility. The increased sperm count also suggest the increased functionality of the testes as a result of the extract’s interference with spermatogenesis in the seminiferous tubules, epididymis or activities of testosterone on hypothalamic release factor and anterior pituitary secretion of gonadotropins which may result in alteration of spermatogenesis\(^{[43]}\). The increase in sperm motility observed may be due to the activity of the extract on the enzyme of oxidative phosphorylative. It may also be due to an alteration in the microenvironment in the caudal epididymis which also lead a synergistic action on the spermatozoa\(^{[43]}\). The result of this study therefore, showed an increase in sperm counts which substantiate the spermatogenic effect of this extract thereby enhancing the fertility of the animals.

Plant extracts have been generally reported to be used as an enhancing agent of male fertility. Chemicals found in the leaves of the African khat plant have been shown to enhance male fertility. The cathionine released when leaves of African khatplant were chewed was shown to be an unstable substance which when broken down gives cathine and norephedine which are similar to amphetamine and adrenaline. These chemicals stimulates the final stage of sperm maturation as well as maintained this sperm at a potential fertilizing state for a longer period of time\(^{[44]}\).

**CONCLUSION**

We therefore concluded that the aqueous leaf extract of *Annonasenegalensis* especially in higher concentration of 300 and 500mg/kg body weight will affect fertility in male and this support the use of this plant by the traditionalists as a fertility enhancing agent in male. No toxic effect was observed at even up to 3000mg/kg body weight and this confirmed the plant to be safe for consumption.
REFERENCES


