Global Scientific JOURNALS

GSJ: Volume 11, Issue 8, August 2023, Online: ISSN 2320-9186

www.globalscientificjournal.com

THE EFFECT OF AQUEOUS LEAF EXTRACT OF *ANNONA SENEGALENSIS* PERS ON MALE FERTILITY IN ALBINO RATS

Yalma P. MBAYA[1*], Dauda YAHI[2], Fartisincha P. ANDREW[3,4*], Arastus WILLIAM[2]

[1]Department of Animal Science and Range Management, Modibbo Adama University, Yola, Nigeria.

[2]Department of Veterinary Physiology and Biochemistry, University of Maiduguri, Nigeria.

[3]Department Science Laboratory Technology, Modibbo Adama University, Nigeria.

[4]School of Chemistry and Physics, University of KwaZulu Natal, South Africa.

Abstract

In this study, the effect of effect of aqueous leaf extract of *Annona senegalensis* was evaluated *in vivo* on male fertility using albino rats. The effect of the extract on sperm count, sperm morphology, was determined. The testosterone enzyme immunoassay was also carried out from the serum of the sacrificed rats using test kit. The results indicated a significant decrease in week 2 in the 100, 200 and 400 mg/kgbwt and in week 4 in the 100 and 200 mg/kgbwt. There were significant increases at weeks 2 in the 300 (7.740 ± 0.8414 mmol/L) and in week 3 in the 100 mg/kgbwt; 200 mg/kgbwt (1.240 ± 0.2191; 3.800 ± 0.7483 mmol/L) respectively and in week 4 in the 400 mg/kgbwt (1.780 ± 0.3564). There was an

increase in testicular sperm count and head of epididymis but there was decrease in the tail. Similarly, there were significant increases in total sperm count in the testis at 100 mg/kgbwt ($2.702 \pm 0.13 \times 10^{6}$) in week 2 while in week 3 at 300 mg/kgbwt ($3.650 \pm 0.23 \times 10^{6}$) and in week 4 at all the doses respectively. In head epididymis significantly increases in week 2 at 100 mg/kgbwt ($0.668 \pm 0.06 \times 10^{6}$) and in week 4 at 100 mg/kgbwt and 400 mg/kgbwt (0.790 ± 0.05 and $0.620 \pm 0.02 \times 10^{6}$). The sperm morphology revealed more than 80 % for normal head and abnormalities constitute 8-9 %. The study suggests that the aqueous extract of *Annona senegalensis* leaf might improve male fertility with reasonable increase of sperm count in both testicular and head of epididymis and high percentage of normal head sperm morphology.

Key words: *Annona senegalensis* Extract, Sperm Count, Sperm Morphology, Testosterone, Epididymis

*Corresponding author.

E-mail addresses: AndrewF@ukzn.ac.za

1. Introduction

Plants have been useful to human beings and animals in many respects. Humans use plants in many ways, among which are: food, clothing, shelter, fuel, and management of various diseases/ailments. Plants are important gifts of nature, ever provided to mankind with various benefits through their uses, especially medicinal purposes. There are many ingredients in plants from which pharmaceutical industries derive their drugs from and so do folkloric medical practitioners [1, 2].

Annona senegalensis Pers belongs to the family Annonaceae, genus *Annona* and species *senegalensis* [3, 4]. The name *Annona* is from the Latin word ‘anon’, a genus that produce

its fruits of the various species annually [5], commonly referred to as African custard apple and Wild Soursop [6]. The plant grows wild in tropical Africa, often, but not exclusively, on coral-based rocks with mostly sandy and loamy soils [7, 8]

Apart from its used locally as food and food additives, Annona senegalensis possesses a wide spectrum of biological activities. The leaves, root, fruits, flowers, bark etc. have been used for the treatment of various diseases such convulsion, cancer, diarrhea, eye problems, respiratory problems, stomach and intestinal problems, the plant extract also act as antioxidant [6, 9-12]. The leaves contain essential oil used as parasiticide [13, 14]. The aqueous leave extract of the plant is locally believed to enhance sexual performance and boost male fertility. The plant extracts are reported to contain some bioactive compounds such as flavonoids, alkaloids, tannins and saponins [15, 16] which may be responsible for its therapeutic activities [17]. Similarly, Annona senegalensis has been reported to have anti-spasmodic and stomach muscular relaxant activities, anti-ulcer activity against indomethacin-induced ulcer and reduces the effect of stress on ulcer induction [17-19].

Generally, annonas have potential for agroforestry, although this is rarely exploited. The presence of annonaine in the leaves, stems and other parts makes the plant bitter to goats or cattle [20]. Aiyelaagbe [21] reported on a system that improved the productivity in a cashew-coconut system in Kenya and which could also be adopted for Annona production. However, care should be taken in annona production as the plant does not perform well under low light intensity conditions, which may be created with combined planting [20].

In view of the above uses and activities of this plant and in our continuous effort to explore the pharmaceutical potentials of this plant [22], this study attempt to scientifically evaluate the enhance male fertility potential of the plant as claimed by local traditional medicine for

possible modern pharmaceutical integration. Therefore, we report here the effect of the aqueous leave extract in vivo on male sperm count, testosterone, and sperm morphology.

2. Materials and Methods

2.1. Collection and Identification of Plant Sample

The leaves of *Annona senegalensis* were collected from Girei Local Government Area of Adamawa State which lies between 90 º – 14 ºN and longitude 12 º – 38 ºE. The maps (**Figure. 1**) below shows the location of the sample areas. The leaves were identified and authenticated by a Taxonomist in the Department of Forestry and Wildlife Management of the Modibbo Adama University of Technology, Yola Nigeria. Voucher specimen (PG/15/CHM/008) was deposited in the Departmental herbarium. The leaves of the plant were dried under shade, ground into powder using pestle and mortar and kept until required.

2.2. Experimental Animals

Male albino rats were obtained from the Laboratory Animal House of the Biochemistry Department, University of Maiduguri and kept in the Postgraduate Veterinary Anatomy Research Laboratory, University of Maiduguri. The rats were given pelletized growers mash 120 g daily (Vital feeds Nigeria Ltd) and water *ad libitum*. A total of 30 male rats weighing between 175 – 200 g were used for the male fertility study.

2.3. Plant Extraction

The leaf powder of *Annona senegalensis* weighing 200 g was used. The *Annona senegalensis* aqueous leaf extract was obtained using Soxhlet extractor and distilled water as the solvent at about 90°C. The extract was evaporated to near dryness on a water bath at 40°C which was allowed to dry under room temperature, then ground into powder using pestle and mortar, weighed, and kept until required for use [23].

2.4. Acute Toxicity

This study on the same plant part was studied by Mbaya *et al*. [24] in the same region. The calculated LD_{50} was 2400 mg/kg and is adopted.

2.5. Male Reproductive Study

Method described by Das and Karmakar, 2015 was adopted with little modification. Thirty (30) male albino rats weighing between 175 – 200 g were used for the study. The rats were divided into 5 groups containing six rats each. Group 1 served as the control while groups 2-5 were treated with varying doses of 100, 200, 300, and 400 mg/kgbwt of the extract daily for 28 days.

2.6. Determination of Sperm Count and Morphology

a. Sperm Count

The tests were obtained by humanely sacrificing the rats every week and the scrotum was carefully opened and the testes removed surgically. Using one of the testes, sperm count was performed as reported by Narayana *et al*. [25] with minor modifications. Also, cauda, caput and corpus epididymis were carefully separated from the testis and minced in 2.5 ml normal saline followed by filtration through a nylon mesh. The suspension was then stained with 2% eosin in normal saline and the sperm heads were counted using a Neubauer haemocytometer chamber (except the central erythrocyte chamber) and were averaged and expressed as the number of sperms per cauda, caput, corpus, and epididymis.

b. Sperm Morphology

A drop of stained sperm suspension (which was prepared for sperm count) was smeared on a glass slide, air-dried, and visualized microscopically at a magnification of X 40. For each rat, sperms were screened, and the percentage of total sperm abnormalities were determined [25].

2.7. Testosterone Assay

Every week, serum was obtained for testosterone assay using standard protocol according to the manufacturer's instruction. The Testosterone Enzyme Immunoassay (EIA) Test Kit (Perfemed Group, Inc. Beijing China) [26].

2.8. Statistical Analysis

Data obtained during this study were presented as Mean ± standard Deviation (S.D). The differences in values among groups were analyzed by one-way analysis of variance (ANOVA) using GraphPad InStat version 3.0 computer software (GraphPad, 2003). Values were considered significant at $P<0.05$.

3. Results

3.1. Effects of Prolonged Administration of A. senegalensis on Testosterone and some Semen Characteristics of Male Albino Rats

The effects of aqueous leaf extract of *A. senegalensis* on testosterone is shown in **Figure 2**. The mean values of testosterone in week 1 did not produce any significant change in all the treatment groups as compared to the control. In week 2, there were significant ($P<0.05$) decreases in the treatment groups at 100, 200 and 400 mg/kgbwt (0.7400±0.1517, 0.4000±0.1000 and 0.2600±0.05477 mmol/L respectively) in a dose dependent manner except at 300 mg/kgbwt (7.740±0.8414 mmol/L), where there was significant ($P<0.05$) increase as compared to the control.

In week 3, there were significant ($P<0.05$) increases at 100 mg/kgbwt and 200 mg/kgbwt (1.240±0.2191 and 3.800±0.7483 mmol/L) while at 300 and 400 mg/kgbwt, there were no significant difference as compared to the control. There were significant ($P<0.05$) decrease at 100 and 200 mg/kg (0.4600±0.1673 and 0.6800±0.1789 mmol/L) and significant increase

at 400 mg/kgbwt (1.780±0.3564 mmol/L) and no significant difference in the 300 mg/kgbwt as compared to the control in week 4.

3.2. Effects of Prolonged Administration of Aqueous Leaf Extract of A. senegalensis on Sperm Counts in Albino Rats

The effects of the aqueous leaf extract of *A. senegalensis* on sperm counts in albino rats are shown in **Figure 3**. The mean sperm count values of testis in week 1 significantly ($P<0.05$) decreased at 200, 300 and 400 mg/kgbwt (2.352±0.13, 2.258±0.14 and 2.014±0.11 X 10^6) extract treatment and while it was not significant at 100 mg/kgbwt dose as compared to the control. At week 2, the mean values significantly increased for the 100 mg/kg (2.702±0.13 X 10^6) dose and significantly decreased for 400 mg/kgbwt (1.496±0.09 X 10^6) dose as compared to the control. The extract did not produce any statistically significant change at 200 and 300 mg/kgbwt as compared to the control. In week 3, there was significant decrease at the doses of 100, 200 and 400 mg/kgbwt (2.800±0.05, 2.530±0.02 and 0.932±0.06 X 10^6) except for the 300 mg/kgbwt (3.650±0.23 X 10^6) dose which was significantly increased as compared to the control.

The mean sperm count in week 4 significantly increased with all the doses (100, 200, 300 and 400 mg/kgbwt) as compared to the control.

The mean values of sperm count in the head of epididymis significantly decreased at 300 and 400 mg/kgbwt (0.222±0.04 and 0.310±0.03 X 10^6) and did not produce any significant change at 100 and 200 mg/kgbwt as compared to the control in week 1. In week 2, it did not show any significant change in the mean sperm count in all the treatment groups except at 100 mg/kgbwt (0.668±0.06 X 10^6) which produced significant increase as compared to the control. The mean values in week 3 significantly decreased at the dose of 400 mg/kgbwt (0.172±0.01 X 10^6) and did not statistically show any significant change in all the remaining

treatment groups as compared to the control. In week 4, there was significant increase in the mean values at 100 and 400 mg/kgbwt (0.790±0.05 and 0.620±0.02 X 10^6) and did not show significant difference at 200 and 300 mg/kgbwt respectively as compared to the control.

The mean values of sperm count in the body of epididymis in weeks 1, 2 and 3 did not show statistically significant changes in all the treatment groups as compared to the control. Whereas in week 4, the mean values significantly decreased with the doses of 100, 200, 300 and 400 mg/kg (**Figure 3**).

The mean values of sperm count in the tail of the epididymis significantly decreased at 200 and 400 mg/kgbwt but was not significant at 100 and 300 mg/kgbwt as compared to the control in week 1. In week 2, none of the treatment groups showed any statistically significant changes as compared to the control. The mean values in week 3 significantly ($P<0.05$) decreased in the doses of 100, 200 and 400 mg/kgbwt except at 300 mg/kgbwt dose which was not significant as compared to the control. In week 4, all the treated groups were not significant except at 100 mg/kgbwt which significantly increased as compared to the control.

3.3. The Effect of Aqueous Leaf Extract of A. senegalensis on Testicular Sperm Morphology in Albino Rats

The effect of aqueous leaf extract of *A. senegalensis* on testicular sperm morphology in albino rats is shown in **Figure 4**. There was significant ($P<0.05$) decrease in the mean values of normal head (TSMNH) in all the treatment doses at week 1, whereas in week 2 at the doses of 100, 200 and 300 mg/kgbwt (80.00±1.58, 79.00±4.30 and 79.60±2.61 %) and week 3 at the doses of 100, 200 and 300 mg/kgbwt (80.000±1.58, 77.800±4.49 and 79.600±3.21 %) there were significant decreases respectively and no significant difference at 400

mg/kgbwt respectively as compared to the control. At week 4 no statistically significant change was observed in all the treatment groups as compared to the control.

The mean values of the two heads (TH) and amorphous head (AH) did not show any change in all the treatment groups throughout the experiment as compared to the control.

There was significant ($P<0.05$) increase in the mean values of amorphous body (AB) in week 1 in the doses of 200, 300 and 400 mg/kgbwt (3.200±0.84, 2.800±0.84 and 1.600±0.55 %) except for 100 mg/kgbwt dose as compared to the control. In weeks 2, 3 and 4, there was no statistically significant change in all the treatment groups as compared to the control.

The mean values of coiled tail (CT) in week 1 did not show any statistically significant change in all the treatment groups except for 100 mg/kgbwt dose (4.000±0.71 %) which had significant ($P<0.05$) increase when compared to the control. In weeks 2, 3 and 4, there were no significant changes compared to the control.

The mean values of short tail (ST) significantly increased at week 1 in all the treatment doses when compared to the control. In week 2 at the doses of 100, 200 and 300mg/kgbwt (3.200±0.84, 2.600±0.89 and 1.800±1.30 % respectively) and week 3 in the doses of 200 and 300 mg/kgbwt (2.800±0.84 and 2.600±0.55 %), there were significant decreases except at 400 mg/kgbwt dose which was not significant as compared to the control. At week 4, no significant change was observed in all the treatment groups compared to control.

The mean values of two tails (TT) did not show any statistically significant change in all the treatment groups in all the weeks of the experiment as compared to the control.

The mean values of the immature (I) at week 1 were not different significantly in all the treated groups as compared to control. Whereas at week 2 in the doses of 100, 200 and 300 mg/kgbwt (11.00±1.23, 12.40±2.51 and 13.00±2.12 %) and week 3 in the doses of 100, 200

and 300 mg/kgbwt (10.800±1.48, 12.400±2.51 and 12.200±3.11 %), there were significant increases in all the treatment groups except the 400 mg/kgbwt dose as compared to the control. In week 4, there was significant increase at 200 mg/kgbwt dose (13.000±2.45 %) and decrease at 400 mg/kgbwt dose (7.600±1.34 %) however, no statistically significant change was observed at 100 and 300 mg/kgbwt as compared to the control.

3.4. The Effect of Aqueous Leaf Extract of A. senegalensis on Head Epididymal Sperm Morphology in Albino Rats

The effect of aqueous leaf extract of *A. senegalensis* on head epididymal sperm morphology in albino rats is shown in **Figure 5**. The mean values of normal head (NH) significantly increased at 100 mg/kgbwt at week 1 (88.400±1.67 %) and week 2 (88.000±1.23) while at week 3 in the doses of 100 and 400 mg/kgbwt (87.800±1.92 and 87.200±1.64 %) and week 4 in the 100 and 400 mg/kgbwt (88.400±1.14 and 86.400±1.67 %) groups respectively as compared to the control. All other groups did not show any significant change as compared to the control.

The mean values of two heads (TH), amorphous head (AH), amorphous body (AB), short tail (ST), two tails (TT) and immature (I) were not statistically significant in all the treatment groups as compared to the control throughout the weeks of treatment.

The mean values of coiled tail (CT) at week 1 significantly decreased in the 100 and 200 mg/kgbwt (3.000±1.00 and 3.800±1.30 %) groups, but not significant in the 300 and 400 mg/kgbwt as compared to the control. There were significant decreases at weeks 2 and 4 in the 100 mg/kgbwt (3.000±1.00 and 3.000±1.00 %) group but not with the other as in the other groups respectively as compared to the control. At week 3 there was no statistically significant change observed in all the groups as compared to control.

3.5. The Effect of Aqueous Leaf Extract of A. senegalensis on Body Epididymal Sperm Morphology in Albino Rats

The effect of aqueous leaf extract of *A. senegalensis* on body epididymal sperm morphology in albino rats is shown in **Figure 6**. The mean values of the normal head (NH) significantly increased at the doses of 100, 200 and 300 mg/kgbwt (87.800±1.30, 89.800±0.84 and 90.800±0.84 %) in week 1, week 2 at the doses 100, 200 and 300 mg/kgbwt (87.200±1.79, 90.000±1.58 and 90.000±1.10 %), week 3 at the doses 100, 200 and 300 mg/kgbwt (86.600±1.14, 89.800±1.92 and 90.800±1.30 %) and week 4 in the 100, 200 and 300 mg/kgbwt (87.600±1.52, 89.000±2.74 and 89.800±1.92 %) and significantly decreased at 400 mg/kgbwt in weeks 1, 2, 3 and 4 (81.800±0.84, 82.400±1.67, 83.200±1.79 and 82.000±1.00 %) compared to control.

The mean values of two heads (TH), amorphous head (AH), two tails (TT) and immature (I) were all not significant in all the treatment groups throughout the weeks of the experiment as compared to control.

There was significant increase in the mean values of the amorphous body (AB) in week 1 in the 300 and 400 mg/kgbwt (3.400±0.55 and 3.400±1.14) but no significant difference at 100 and 200 mg/kgbwt as compared to control. There was also no statistically significant change in weeks 2, 3 and 4 in all the treatment groups as compared to the control.

The coiled tail (CT) mean values significantly decreased in week 1 at the doses of 100, 200, 300 and 400 mg/kgbwt (3.400±0.55, 3.800±0.45, 5.200±1.30 and 7.000±0.71 %), week 2 at the doses of 100, 200, 300 and 400 mg/kgbwt (1.400±0.55, 3.400±0.55, 3.800±0.45 and 4.800±0.84 %), week 3 at the doses of 100, 200 and 300 mg/kgbwt (1.400±0.55, 3.200±0.45 and 3.400±0.89 %) and week 4 in the doses of 100, 200, 300 and 400 mg/kgbwt

(2.200±1.64, 3.400±0.55, 4.000±0.71 and 4.800±0.84 %) in all the treatment groups throughout the experimental period except at week 3 in the 400 mg/kgbwt dose where there was no significant change as compared to the control.

The short tail (ST) mean values significantly decreased in the 100, 200, 300 and 400 mg/kgbwt (4.400±1.14, 4.400±0.55, 7.400±1.34 and 6.000±0.71 % respectively) groups at week 1, whereas it significantly increased in the 100 mg/kgbwt at weeks 2, 3 and 4 (9.600±2.10, 9.600±2.07 and 7.800±1.30 %), but showed no significant change in the doses 200, 300 and 400 mg/kgbwt groups respectively as compared to the control.

3.6. The Effect of Aqueous Leaf Extract of A. senegalensis on Tail Epididymal Sperm Morphology in Albino Rats

The effect of aqueous leaf extract of *A. senegalensis* on tail epididymal sperm morphology in albino rats is shown in **Figure 7**. The mean values of the normal head (NH) significantly increased in the 100 mg/kgbwt (87.200±1.64 %) group at week 1 but there were no significant changes observed in the 200, 300 and 400 mg/kgbwt groups as compared to the control. At week 2, there was significant increase in the 100 mg/kgbwt (88.200±2.28 %) group and a significant decrease in the 400 mg/kgbwt (82.200±0.84 %) group as compared to the control.

The mean values at week 3 significantly increased in the 100, 200 and 300 mg/kgbwt (87.600±1.95, 87.000±1.23 and 86.200±1.30 %) groups and decreased in the 400 mg/kgbwt (83.000±1.00 %) group. Whereas at week 4, there was a significant increase in the 100 mg/kgbwt (88.200±2.59 %) group and not in the 200, 300 and 400 mg/kgbwt groups as compared to the control.

The mean values of the two heads (TH), amorphous head (AH), amorphous body (AB), coiled tail (CT), two tails (TT) and immature (I) did not show any significant changes in all the treatments groups throughout the weeks of treatment as compared to the control.

The mean values of the short tail (ST) significantly decreased at 100 mg/kgbwt in weeks 1, 2, 3 and 4 (5.800±1.64, 4.800±2.59, 5.200±2.59 and 5.200±3.11) throughout the experiment and increased in the 400 mg/kgbwt group at weeks 1, 2, 3 and 4 (8.200±0.84, 10.600±1.14, 9.800±1.30 and 9.800±1.30) respectively. There were no significant differences in 200 and 300 mg/kgbwt in weeks 1, 2, 3 and 4 compared to the control.

The study revealed a decrease in the serum level of testosterone. The reduction in the serum level of testosterone could probably be due to the decrease of serum levels of LH. Leydig cells secrete testosterone by the stimulatory effect of LH [27-30], have shown that, in males, reduction of testosterone level may impair spermatogenesis and cause male infertility.

In this study, in week 1, the aqueous leaf extract of *A. senegalensis* did not produce any significant ($P<0.05$) difference in testosterone in all the treated rats. This means at those levels in the treated groups there was no interference with the testosterone producing cells similarly at week 3 in the 300 and 400 mg/kgbwt groups and in week 4 in the 300 mg/kgbwt group. This agrees with the findings of Muthulakshmi *et al*. [31] who reported that the decrease may be because of interference with testosterone producing cells or decreased serum level of LH. Also, there were significant increases in the 300 mg/kgbwt group at week 2 and in week 3 in the 100 and 200 mg/kgbwt groups and in week 4 in the 400 mg/kgbwt group, which may suggest noninterference with testosterone producing cells or LH. There were also significant interferences with the testosterone producing cells due to significant decreases in week 2 in the 100, 200 and 400 mg/kgbwt and in week 4 in the 100 and 200 mg/kgbwt respectively. This means that the extract has a dose dependent selective toxicity when administered for 2-4 weeks since there was no significant change observed in week 1.

The result of the study suggests that the aqueous leaf extract of *A. senegalensis* might have interfered with spermatogenesis following oral administration. Thus, among the aqueous leaf extract treated rats, most of the groups produced a significant ($P<0.05$) reduction in total sperm count. For example, there was a significant decrease during the weeks except in week 4 where the testicular sperm counts were significantly increased in all the treated rats. The decrease was also observed in epididymal head, body, and tail. This may be because of the ability of the extract at the given doses, to either interfere with spermatogenetic process in the seminiferous tubules, epididymal functions or activities of testosterone (decreased testosterone) on hypothalamic releasing factor. The extract also might have interfered with anterior pituitary secretion of gonadotropins which result in alteration of spermatogenesis. It might also be due to the impairment of the hormonal mechanisms concerned with the regulation of spermatogenesis. This agreed with the finding of [32-34] who showed that, decrease in sperm counts may be due to interference with spermatogenetic process in the seminiferous tubules, epididymal functions or activities of testosterone (decreased testosterone) on hypothalamic releasing factor. It may also be a selective toxicity since there was increase in the sperm counts in some of the doses at 100 mg/kg and 300 mg/kg (testis) and 100 mg/kg (epididymis).

The normal sperm cells constitute about 80 – 88 % and the rest are the abnormal morphologies like the TH, AH, AB, CT, ST, TT and immature sperms (I). The abnormal morphologies were not significant compared to the normal ones and they might have been observed due to the ability of the aqueous leaf extract to exert toxicity on both testis and epididymis. The coiling of the sperm tail is usually due to abnormal axoneme and outer dense fibril as reported by [31].

The testis did not show any lesion suggesting that the extract did not cause any damage nor elicited toxicity in the cells of the testis.

4. Conclusions

In conclusion, the crude aqueous leaf extract of *Annona senegalensis* was evaluated *in vivo* using albino rats for its male fertility potentials. The result indicated an improved male fertility in the albino rats studied, as shown by the significant increases in testosterone, increase in total sperm count in the testis, head epididymis. There was also significant improvement in the percent normal head and significant decreases in some abnormal sperm morphology as observed by the decreased in the number of coiled and short tails at various doses. The testicular histological study did not show any lesions or toxicity because of the extract administered.

Author contributions:

Yalma Philip Mbaya **(YPM)**, Dauda Yahi (**DY**) Fartisincha P. Andrew **(FPA)**, Arastus William (**AW**).

YPM and **AW** were responsible for conceptualization, supervision, funding acquisition, data collection, review, and final editing. **DY** was responsible for some data collection. **FPA** was responsible for data analysis typesetting, editing and correspondence.

Conflict of interest: The authors declare that there is not any conflict of interest.

Ethical Consideration

This study was approved by the Faculty of Veterinary Medicine, University of Maiduguri, Nigeria, Faculty Postgraduate Board Ethics Committee. The ethical permit number is PG/15/CHM/008.

Acknowledgements

This research work was funded by the authors, they however, wish to acknowledge Modibbo Adama University, Yola, Nigeria and University of Maiduguri, Nigeria where the research was conducted and Mr. Ibrahim Wiam for the Technical Assistance.

References

1. Tull, D., 2013. *Edible and useful plants of the southwest: Texas, New Mexico, and Arizona*. University of Texas Press 144-150.
2. Elkordy, A.A., Haj-Ahmad, R.R., Awaad, A.S. and Zaki, R.M., 2021. An overview on natural product drug formulations from conventional medicines to nanomedicines: Past, present and future. *Journal of Drug Delivery Science and Technology*, *63*, p.102459.
3. Ziyaev, R., Mukhidova, Z.S., Zakirov, S.H. and Shayzakova, D.A., 2021. Quality analysis of alkaloids of some plants growing in the republic of Guinea. *Academic research in educational sciences*, *2*(6), pp.15-20.
4. Orwa, C., Mutua, A., Kindt, R., Jamnadass, R., and A., Simons, 2012. Agroforestry Database: a tree reference and selection guide version4. 2009.
5. Mainasara, M. M., Bakar, M. F. A., Mohamed, M., Linatoc, A. C., and F., Sabran, 2018. Sugar Apple—Annona squamosa Linn. In *Exotic fruits* (pp. 397-402). Academic Press.
6. Okhale, S.E., Akpan, E., Fatokun, O.T., Esievo, K.B. and Kunle, O.F., 2016. Annona senegalensis Persoon (Annonaceae): A review of its ethnomedicinal uses, biological activities and phytocompounds. *Journal of Pharmacognosy and Phytochemistry*, *5*(2), pp.211-219.

7. Ba, O., Diémé, A., and M. O., Sy, 2021. In Vitro Clonal Propagation from Adult Material of a Savannah Species of Socio-Economic Importance: Annona senegalensis Pers. Agricultural Science 12:370.
8. Adzu, B., Abubakar, M.S., Izebe, K.S., Akumka, D.D. and Gamaniel, K.S., 2005. Effect of Annona senegalensis rootbark extracts on Naja nigricotlis nigricotlis venom in rats. *Journal of ethnopharmacology*, *96*(3), pp.507-513.
9. Okoye, T.C., Akah, P.A. and Omeke, C.P., 2010. Evaluation of the anticonvulsant and muscle relaxant effects of the methanol root bark extracts of Annona senegalensis. *Asian Pacific Journal of Tropical Medicine*, *3*(1), pp.25-28.
10. Suleiman, M.M., Dzenda, T. and Sani, C.A., 2008. Antidiarrhoeal activity of the methanol stem-bark extract of Annona senegalensis Pers. (Annonaceae). *Journal of ethnopharmacology*, *116*(1), pp.125-130.
11. Ajboye, T.O., Yakubu, M.T., Salau, A.K., Oladiji, A.T., Akanji, M.A. and Okogun, J.I., 2010. Antioxidant and drug detoxification potential of aqueous extract of Annona senegalensis leaves in carbon tetrachloride-induced hepatocellular damage. *Pharmaceutical Biology*, *48*(12), pp.1361-1370.
12. Srinivasa, C., Shivamallu, C., Santhosh Kumar, S.R., Sushma, P., Kollur, S.P., Patel, S.J., Patil, S.S., Suhas, R., Egbuna, C. and Tung, B.T., 2021. Medicinal Potentials of Phytochemicals as a Source of Drugs for the Treatment of Onchocerciasis (River Blindness). *Neglected Tropical Diseases and Phytochemicals in Drug Discovery*, pp.283-298.
13. Sahpaz, S., Carmen, M., Hocquemiller, R., Zafra-Polo, M.C. and Cortes, D., 1996. Annosenegalin and annogalene: Two cytotoxic mono-tetrahydrofuran acetogenins from Annona senegalensis and Annona cherimolia. *Phytochemistry*, *42*(1), pp.103-107.

14. Boeke, S.J., Baumgart, I.R., Van Loon, J.J.A., Van Huis, A., Dicke, M. and Kossou, D.K., 2004. Toxicity and repellence of African plants traditionally used for the protection of stored cowpea against Callosobruchus maculatus. *Journal of Stored Products Research*, *40*(4), pp.423-438.
15. Ahmed, M.U., Arise, R.O. and Umaru, I.J., 2022. Identification and biochemical characterization of anti-enteropooling compounds from Annona senegalensis root bark. *Scientific African*, *15*, p.e01128.
16. Kacholi, D.S. and Amir, H.M., 2022. Ethnobotanical survey of medicinal plants used by traditional healers in managing Gonorrhoea and Syphilis in Urambo District, Tabora Region, Tanzania. *Journal of Herbs, Spices & Medicinal Plants*, *28*(2), pp.179-192.
17. Quílez, A.M., Fernández-Arche, M.A., García-Giménez, M.D. and De la Puerta, R., 2018. Potential therapeutic applications of the genus Annona: Local and traditional uses and pharmacology. *Journal of ethnopharmacology*, *225*, pp.244-270.
18. Ojewole, J.A., Awe, E.O. and Chiwororo, W.D., 2008. Antidiarrhoeal activity of Psidium guajava Linn. (Myrtaceae) leaf aqueous extract in rodents. *Journal of Smooth Muscle Research*, *44*(6), pp.195-207.
19. Konaté, K., Sanou, A., Aworet-Samseny, R.R., Benkhalti, F., Sytar, O., Brestic, M., Souza, A., and Dicko, M.H., 2021. Safety profile, in vitro anti-inflammatory activity, and in vivo antiulcerogenic potential of root barks from Annona senegalensis Pers. (Annonaceae). *Evidence-based Complementary and Alternative Medicine*, *2021*.
20. Pinto, A.D.Q., Cordeiro, M.C.R., De Andrade, S.R.M., Ferreira, F.R., Filgueiras, H.D., Alves, R.E. and Kinpara, D.I., 2005. Annona species. embrapa cerrados-livro científico (ALICE). *Southampton: ICUC*.

21. Aiyelaagbe, I.O.O., 1994. Fruitcrops in the cashew-coconut system of Kenya: their use, management and agroforestry potential. *Agroforestry systems*, *27*, pp.1-16.

22. Mbaya, Y.P., William, A., Yahi, D. and Ojo, N.A., 2019. phytochemical, elemental and LD50 screening of aqueous leaf extract of Annona senegalensis in albino wistar rats. FUW Trends in Science & Technology Journal. 4: 596-601

23. Khan, N., Kumar, N., Ballal, A., Datta, D. and Belle, V.S., 2021. Unveiling antioxidant and anti-cancer potentials of characterized Annona reticulata leaf extract in 1, 2-dimethylhydrazine-induced colorectal cancer in Wistar rats. *Journal of Ayurveda and Integrative Medicine*, *12*(4), pp.579-589.

24. Mbaya, Y.P, Yahi, D, & William, A. 2019. Abortifacient Effects of Aqueous Leaf Extract of Annona senegalensis Pers in Albino Rats. Continental J. Applied Sciences, 14(2), 68–77. https://doi.org/10.5281/zenodo.3524825

25. Narayana, K., Prashanthi, N., Nayanatara, A., Kumar, H.H.C., Abhilash, K. and Bairy, K.L., 2005. Effects of methyl parathion (o, o-dimethyl o-4-nitrophenyl phosphorothioate) on rat sperm morphology and sperm count, but not fertility, are associated with decreased ascorbic acid level in the testis. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, *588*(1), pp.28-34.

26. Connell, E., 2012. Tietz textbook of clinical chemistry and molecular diagnostics (5th edn). 615-615.

27. Uboh, F.E., Edet, E.E., Eteng, M.U. and Eyong, E.U., 2010. Comparative effect of aqueous extract of P. guajava leaves and ascorbic acid on serum sex hormones levels in male and female rats. *J. Appl. Sci. Res*, *6*(4), pp.275-279.

28. Payne, K.S., Mazur, D.J., Hotaling, J.M. and Pastuszak, A.W., 2019. Cannabis and male fertility: a systematic review. *The Journal of urology*, *202*(4), pp.674-681.

29. Udoh, P. and Kehinde, A., 1999. Studies on antifertility effect of pawpaw seeds (Carica papaya) on the gonads of male albino rats. *Phytotherapy Research*, *13*(3), pp.226-228.

30. Udoh, F.V., Udoh, P.B. and Umoh, E.E., 2005. Activity of alkaloid extract of Carica papaya. Seeds on reproductive functions in male wistar rats. *Pharmaceutical biology*, *43*(6), pp.563-567.

31. Muthulakshmi, A., Margret, R.J. and Mohan, V.R., 2013. Antifertility effect of ethanol extracts of Feronia elephantum correa leaf and bark on male albino rats. *International Journal of Pharmaceutical Sciences and Drug Research*, *5*(1), pp.23-27.

32. Huleihel, M. and Lunenfeld, E., 2004. Regulation of spermatogenesis by paracrine/autocrine testicular factors. *Asian journal of andrology*, *6*(3), pp.259-268.

33. Kumar, R.V., Reddy, G.V.R., Sathyanarayana, J., Bikshapathi, T. and Reddy, M.K., 2013. Effect of Melia azedarach and Dodonaea viscosa aqueous leaf extracts on fertility in male albino rats. *Indian Journal of Pharmaceutical and Biological Research*, *1*(04), pp.7-12.

34. Das, S.K. and Karmakar, S.N., 2015. Effect of green tea (camellia sinensis l.) leaf extract on reproductive system of adult male albino rats. *International journal of physiology, pathophysiology, and pharmacology*, *7*(4), p.178.