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SENNA ALATA (L.) ROXB. LEGUMINOSAE LEAF ETHANOL EXTRACT HAS SPERMICIDAL EFFECT IN MALE WISTAR RATS: A POTENTIAL CONTRACEPTIVE AGENT?

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ABSTRACT

Contraceptive options for birth control in males are limited. This has given rise to the surge of botanicals as an alternative. Several works on medicinal plants have established that botanicals can effectively induce sterility in laboratory animals. *Senna alata* (L.) Roxb (Leguminosae) is a popular plant whose traditional claim as an abortifacient has been validated. Its flower has also been shown to reduce sperm count and motility. The study was aimed at exploring the impact of the ethanol extract of *S. alata* leaf on the histology of the testes and sperm parameters in male Wistar rats by employing standard procedure. Twenty four (24) adult male rats which were randomly assigned into four groups; control group (I) and three test groups (II - IV) (n=6/group). Rats in group I were given distilled water while those in Groups II - IV received 250, 500 and 1000mg/kg of the *S. alata* leaf extract, respectively for a period of 20 days. They were thereafter sacrificed and the epididymis sperm count, motility and morphology were done while the histology of the testes was also evaluated. Assayed sperm parameters indicated a significant decrease in sperm concentration [44.6 (500mg/kg) and 89.3% (1000mg/kg)] and sperm motility (33%, 1000mg/kg) and a remarkable increased % abnormal sperms in a dose dependent manner. Histo-architectural evaluation of the testes indicated a depletion of spermatogonia and sertoli fibrinous necrosis and a deprivation of Leydig cells, also in a dose dependent fashion. It can therefore be posited that ethanol leaf extract of *S. alata* is spermatotoxic at the tested doses.

INTRODUCTION

Senna alata (L.) Roxb (Leguminosae), generally recognized as 'Ringworm' or 'Craw-Craw' plant, and 'Asunwon oyinbo', 'Nelkhi' and 'Filisko' among the Yoruba, Ibo and Hausa speaking tribes of Nigeria, respectively[1] (Muhammad et al., 2021) is widely found growing on continents like Asia, Australia, and Africa. Nigeria, Somalia and Egypt are examples of African countries which have it in abundance. It is erect and can be annual or biannual, usually growing in the tropics (Muhammad et al., 2021, Oladeji et al., 2020).[1-2] Dermatitis, athlete's foot, skin rash, mycosis, eczema, hepatitis, medical abortion, jaundice, constipation, and diarrhoea are among the globally acclaimed traditional uses (Muhammad et al., 2021, Ugbogu et al., 2016; Kazeem et al., 2015; Yakubu & Musa, 2012)[1,3-5]. It has been proven to exhibit antioxidant, antitumour (Sugumar et al., 2019; Pieme et al,. 2008),[6-7] abortifacient (Yakubu et al., 2010),[8] antibacterial, antifungal, antiviral, anti-inflammatory, hepatoprotective[1,9] (Muhammad et al., 2021; Adedayo et al., 2001) and hypoglycaemic[4] (Kazeem et al., 2015) activities. Kaempferol, chrysoeriol, quercetin, 5,7,4'-trihydroflavanone (Kudatarkar, 2018),[10] rhein, aloe emodin, alatinone and alatonal (Adedayo 2001),[9] kaempferol 3-O-gentiobioside (Dewi et al., 2019).[11] kaempferol-3-*O*-β-D-glucopyranoside, 3,5,7,4′-tetrahydroxy flavone and 2,5,7,4′-tetrahydroxy isoflavones[12] (Fatmawati et al., 2020) are some of the phytoconstituents that are present in the plant. Alkaloids from the leaf exhibited anti-gonadotropic, anti-implantation and fetal toxic activities (Yakubu & Musa, 2012).[5] The ethanol extract of the flower indicated a reduction in sperm count and motility[13] (Jain & Ali, 2007) while the aqueous extract of the leaf exhibited abortifacient effect (Yakubu et al., 2010).[8] This work is aimed to evaluate the leaf ethanol extract of *S. alata* for probable spermicidal activity in male Wistar rats.

Plant collection

Fresh leaves of *S. alata* were harvested from the Medicinal plant Garden of the Department of Pharmacognosy &Herbal Medicine, Faculty of Pharmacy, Niger Delta University, Bayelsa State, Nigeria on the 10th of February, 2022. The identification of *S. alata* and its authentication were executed at the Department of Pharmacognosy & Herbal Medicine Herbarium, Niger Delta University where a voucher

specimen with reference number NDUP237 was thereafter deposited. The plant leaf sample was blended into coarse powder. The sample (800g) was extracted cold in 50% ethanol for 72 h. with occassional agitations. The filtrate was concentrated *in vacuo* at 30℃ to obtain a dry extract (13.7% yield). The extract was stored in the refrigerator for bioassay.

Animals

Healthy male Wistar rats weighing 148-155 kg were procured from the Animal house of the Department of Pharmacology, Faculty of Pharmacy, Niger Delta University, Bayelsa State, Nigeria. The rats were sheltered under controlled conditions of light (12-h light-dark cycle) and room temperature with free access to animal feeds and water *ad libitum*. Ethical approval was secured from the same department before the commencement of the study and animal care guideline of NIH was strictly adhered to (Ikokide et al., 2019).[14]

Experimental Design

Rats were randomly distributed into four groups of six each. Group I (Control) was maintained on distilled water only while Group II - IV (Test) were administered with the ethanol extract of *S. alata* suspended in distilled water at 250, 500 and 1000 mg/kg *p.o* at 9.00 am daily for 20 days. After an overnight fasting, on termination of the extract administration, animals were euthanized employing ketamine i.p. at a concentration of 20 mg/kg. The epididymis was removed for sperm concentration evaluation in all animals. The testicles were also carefully removed for histology.

Evaluation of sperm count, motility and morphology

The epididymis was snipped off the testicles, inserted into a beaker containing normal saline, and the spermatozoa samples were obtained from a needle incised cauda epididymis with the aid of a Pasteur pipette. Analysis of the semen for estimation of properties of spermatozoa (sperm concentration, sperm motility and morphology) was performed using standard method as earlier described (Alade et al., 2011)[15]. In brevity, sperm motility and morphology were determined with a mixture of a drop each of semen buffer and semen sample on a microscope slide and examined under x 40 microscope. The sperm count was estimated with the use of improved Neubauer Haemocytometer (Emmanuel et al., 2018, Sethi et al., 2010).[16-17]

Histological study

Buffered formalin ($10\%^{v}/_{v}$) was employed to fix the testes that were obtained from the animals in labelled glass containers. Routine processing was executed on them by embedding the tissues in paraffin wax. Sections (5 µm thick) were carefully lacerated, stained with haematoxylin and eosin (H&E) after which they were observed using a microscope at x 100 and 400. An attached digital camera was utilized to take the photomicrographs (Ikokide et al., 2019).[14]

Data analysis

Obtained data were analysed statistically by utilising GraphPad Prism version 6.0.0 for Windows (GraphPad Software, www.graphpad.com). They were analyzed by using one-way analysis of variance (ANOVA) and Tukey's multiple comparison test. They were recorded as mean ± standard error of the mean, and a value of $P < 0.05$ was taken to be significant.

RESULTS AND DISCUSSION

The reduction in the motility of sperm is not unconnected to an increased percentage of morphologically abnormal and dead spermatozoa. The number of abnormal, sluggish and non-motile ones increased in a dose-dependent fashion. The reduction in the caudal epididymal sperm concentration in the treated rats when juxtaposed with the control rats showed that the *S. alata* leaf ethanol extract had the ability of affecting one or more steps of spermatogenesis negatively (Table 1).

Table 1: Effect of ethanol extract of *S. alata* on sperm parameters in Wistar rats.

Parameters	Control	250 mg/kg	500 mg/kg	1000 mg/kg
Actively motile (%)	12.0 ± 1.23	10.0 ± 1.58 (16.7	8.0 ± 1.22 (33.3)	8.0 ± 1.22 (33.3)
Sluggishly motile (%)	19.0 ± 2.46	17.0 ±2.55 (10.5)	13.0 ± 1.224 (31.6)	15.0 ± 0.00 (21.1)
Non motile (%)	69.0 ± 3.67	73.0 ± 4.06 (-5.5)	79.0 ± 2.45 (12.7)	77.4 ± 1.12 (-10.4)
Normal morphology (%)	64.0 ± 2.45	58.0 ± 4.90 (9.4)	52.0 ± 4.90 (18.8)	52.0 ± 4.90 (18.8)
Sperm count (x 106 cells/mL)	2.8 ± 0.07	2.2 ± 0.45 (21.4)	1.50 ± 0.66 (46.4*)	0.3 ±0.07 (89.3)*

Values are mean ± S.E.M., $p < 0.05$ as compared to control, n = 6 animals per group
Key: ( ) – percentage reduction.

There was a 33% reduction in the quantity of active sperms at 1000mg/kg, the number of sluggish ones increased by almost 32% at 500mg/kg while the concentration of non-motile ones increased by 10.4%. There was a depletion of approximately 19% of those with normal forms while a significant ($p < 0.001$) reduction of 46.4 and 89.3% sperm concentration was observed for the extract at 500 and 1000 mg/kg, respectively.

Decrease in sperm motility has been correlated with alteration of the accessory organs' functions (Kushwaha & Preeti, 2017).[18] Any modification of sperm concentration, morphology and motility can make the subject infertile. Studies on botanical based

contraceptives have revealed that halting fertility in male, administered with plant based substances has a direct correlation with decreased spermatozoa density (Mostafa et al., 2021; Kushwaha & Preeti, 2017).[18-19] Sometimes, spermatogenesis is not halted but the morphology of the sperms may change, thereby affecting their physiological functions. The ethanol leaf extract of *S. alata* showed atypical seminiferous tubules with reduced spermatogonia and sertolic cells population. The interstitium showed an extensive fibrinoid necrosis with loss of Leydig cells (Plates 1-3). The testis is the sperm producing organ in animals and it does so through spermatogenesis whose process commences with the production of spermatogonia which develop through primary spermatocytes, secondary spermatocytes, spermatids to mature spermatozoa (Griswold, 1998).[20] The existence of Sertoli cells in the testis is vital to spermatogenesis by facilitating germ cells progression to spermatozoa. Hormones which regulate the process of spermatogenesis reside on the Sertoli cells. The numbers of Sertoli cells, spermatogonia were significantly reduced in patients diagnosed with maturation arrest at the level of primary spermatocytes. In the same report, patients with hypospermatogenesis exhibited a significant reduction in the quantity of their spermatogonial cells (Hentrich et al., 2011).[21] The primary source of testosterone is the Leydig cells and it is crucial to spermatogenesis (Aladamat & Tadi, 1998).[22] Loss of Leydig cell is implicative of testicular failure in man (Gnessi et al., 2000).[23] Emphasis has been laid on the direct proportional relationship between spermatogenic dysfunction and Leydig cell impairment (de Kretser, 2004).[24] Both laboratory studies on animals and clinical ones have also revealed that seminiferous tubular impairment is frequently accompanied by Leydig cell deficit (Winters et al., 2018).[25] This plant will therefore adversely affect production of spermatozoa and so should not be used by a young adult male of child bearing age. The plant can be explored as a male contraceptive.

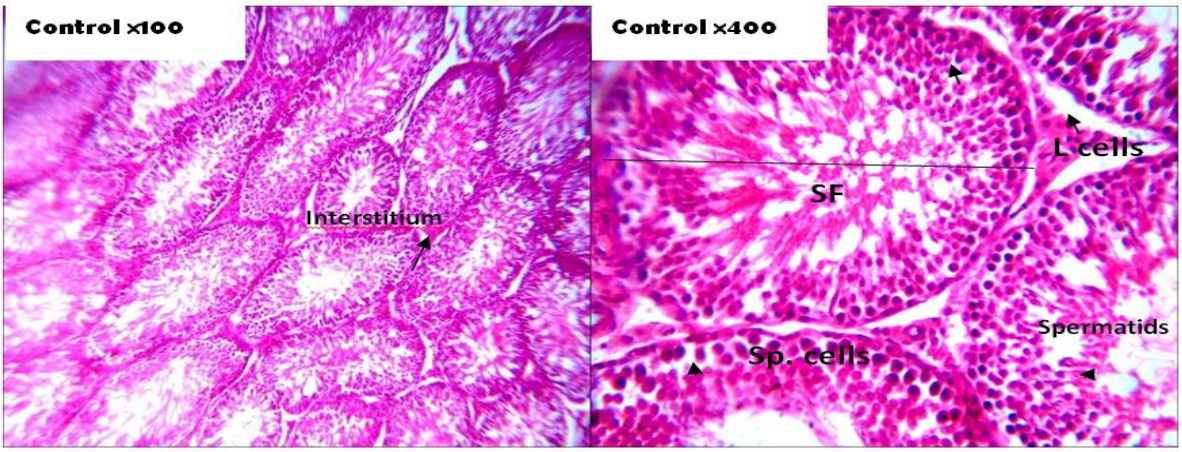

Plate 1a: Transverse section of rat testis showing control group with seminiferous tubules epithelium containing spermatogonia and sertolic cells, and lumen filled with flagella (sperm cells). The interstitial cells show normal histology displaying leydig cells.
Features are consistent with abnormal histology of the testis.

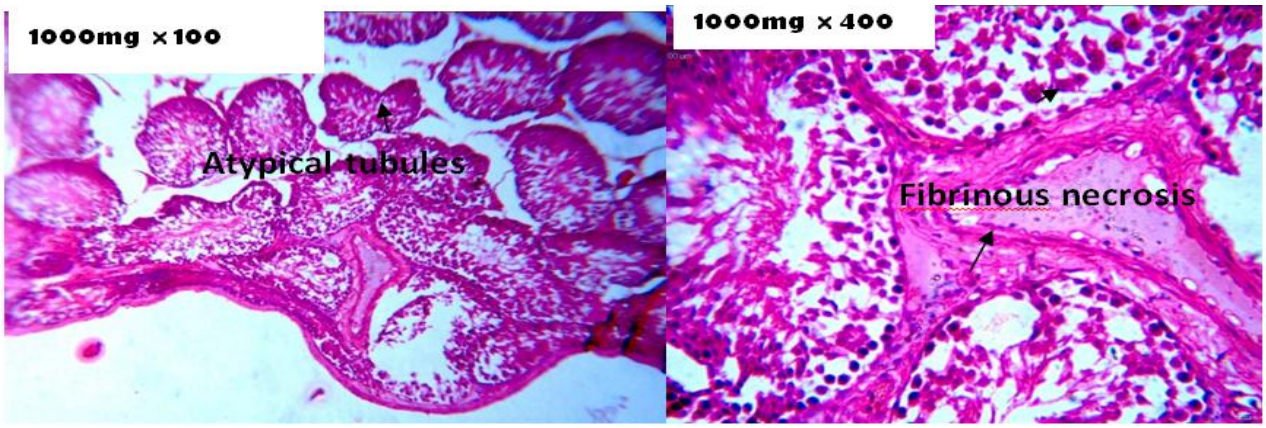

PLATE 1b: Transverse section of rat testis showing group administered with 1000mg/kg *S. alata* leaf ethanol extract showing atypical seminiferous tubules with reduced spermatogonia and sertolic cells population. The interstitium shows extensive Fibrinoid necrosis with loss of Leydig cells.
Features are consistent with abnormal histology of the testis.

Extract administered is injurious to the testis and can cause reduction in testosterone production.

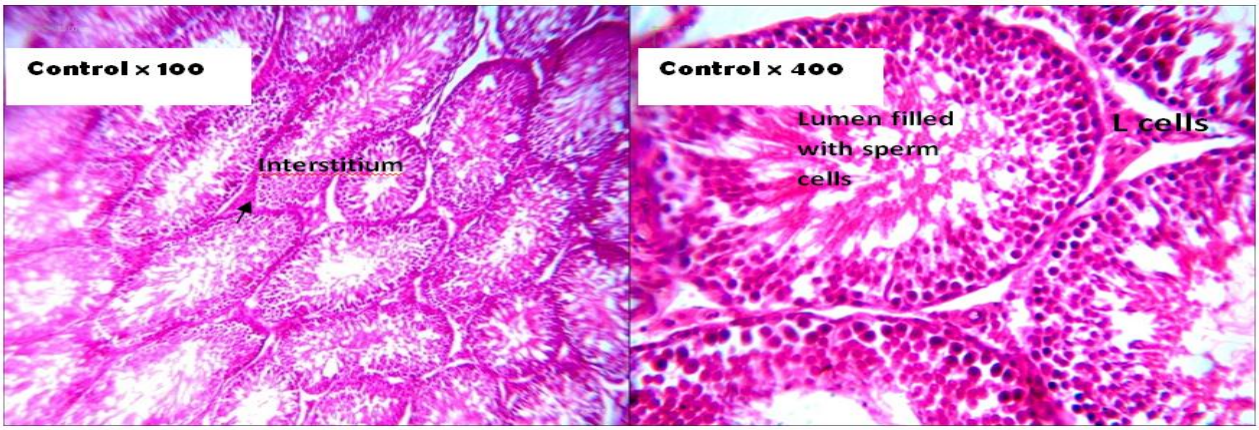

PLATE 2a: Transverse section of rat testis showing control group with seminiferous tubules epithelium containing spermatogonia and sertolic cells, and lumen filled with flagella (sperm cells). The interstitial cells show normal histology displaying leydig cells.

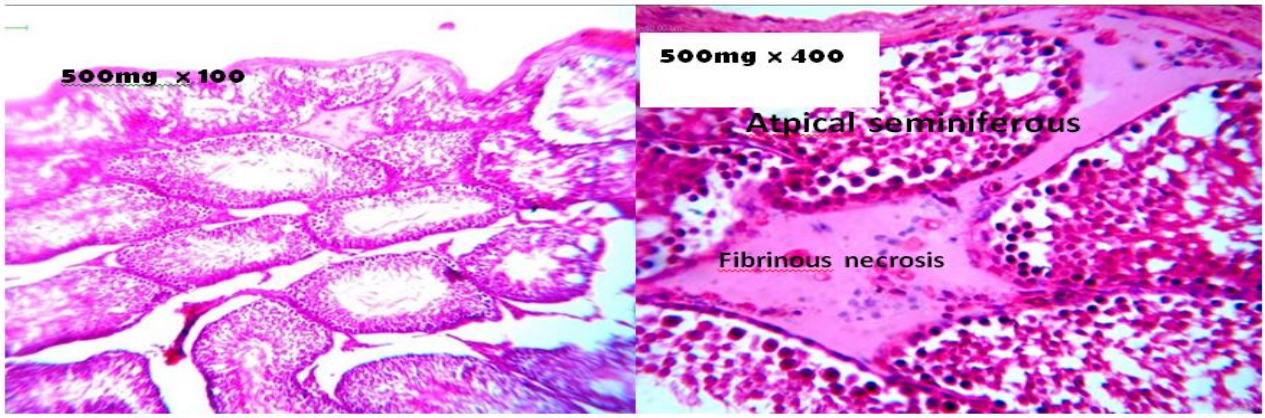

PLATE 2b: Transverse section of rat testis showing group administered with 500mg *S. alata* leaf ethanol extract showing atypical seminiferous tubules with reduced spermatogonia and sertolic cells population. The interstitium shows extensive Fibrinoid necrosis with loss of Leydig cells.
Features are consistent with abnormal histology of the testis. Extract administered is injurious to the testis and can cause reduction in testosterone production.

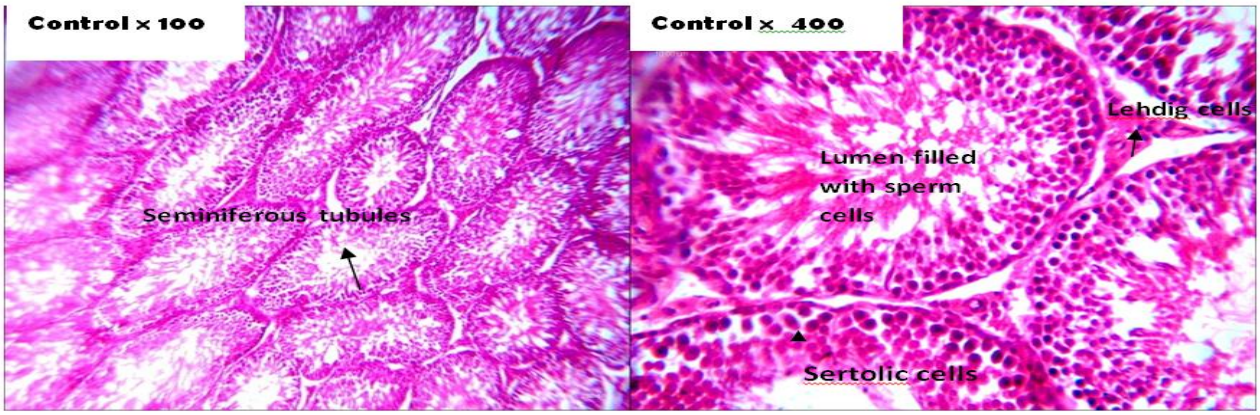

PLATE 3a: Transverse section of rat testis showing control group with seminiferous tubules epithelium containing spermatogonia and sertolic cells , and lumen filled with flagella (sperm cells). The interstitial cells shows normal histology displaying leydig cells.
Features are consistent with normal histology of the testis – Normal Testis

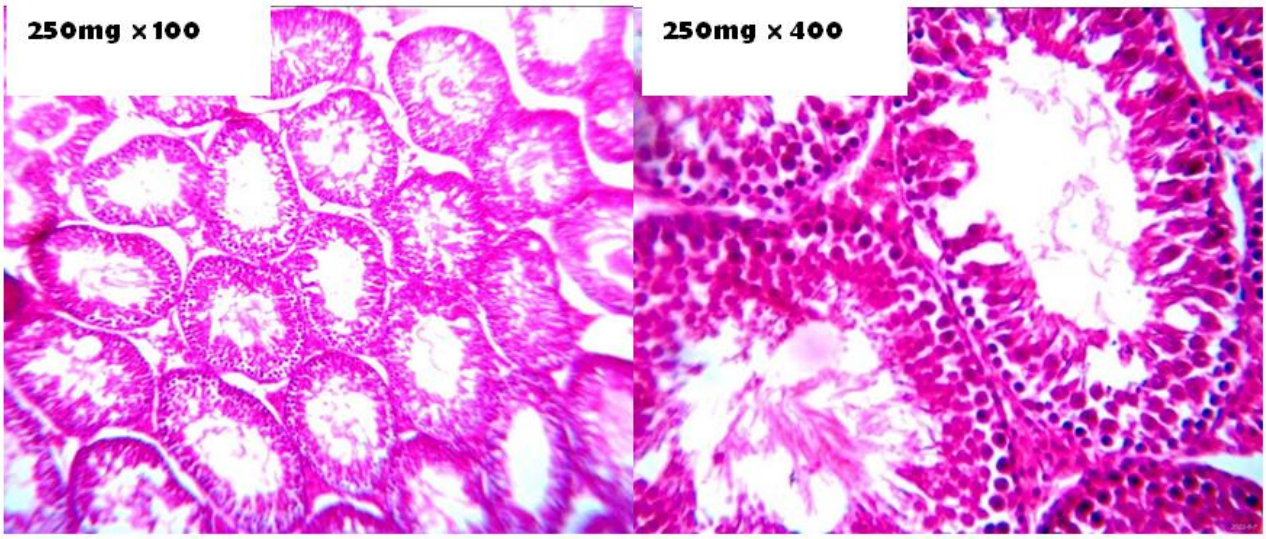

PLATE 3b: Transverse section of rat testis showing group administered with 250mg *S. alata* leaf ethanol extract showing seminiferous tubules with reduced spermatogonia and sertolic cells population. The interstitium shows extensive normal epithelium compared with control.
Features are consistent with normal histology of the testis. Extract administered shows mild toxic effect the testis and can cause reduction in testosterone production.

CONCLUSION

The ethanol extract of *S. alata* leaf possessed a negative fertility effect on treated male Wistar rats since it caused a decrease in sperm count, sperm motility and number of normal sperms. The testes also showed features like reduced spermatogonia and sertoli cells while the interstitium showed extensive fibrinoid necrosis with loss of Leydig cells reflecting abnormal histology of testes in addition to reduction in testosterone production as evident in the loss of the leydig cells. Given the foregoing, the plant can be developed into a potential plant based contraceptive option for male.

REFERENCES

1. Muhammad SL, Wada Y, Mohammed M, Ibrahim S, Musa KY, Olonitola OS, Ahmad MH, Mustapha S, Rahman ZA, Sha'aban A. Bioassay-Guided Identification of Bioactive Compounds from *Senna alata* L. against Methicillin-Resistant *Staphylococcus aureus*. Appl. Microbiol, 2021; 1: 520–536.
2. Oladeji OS, Adelowo FE, Oluyori, AP, Bankole DT. Ethnobotanical description and biological activities of Senna alata, Evidence-Based Complementary and Alternative Medicine, 2020; 2020: Article ID 2580259, 12 pages.
3. Ugbogu EA, Okezie E, Elekwa I, Uhegbu F, Akubugwo E, Chinyere CG, Ewuzie F, Ugorji CJ. Toxicological assessment of the aqueous dried leaf extracts of Senna alata L. in Wistar rats. African Journal of Pharmacy and Pharmacology, 2016; 10(34): 709-717.
4. Kazeem MI, Azeez GA, Ashafa AOT. Effect of *Senna alata* (L) Roxb (Fabaceae) leaf extracts on alpha-Amylase, alpha-Glucosidase and postprandial hyperglycemia in rats. Tropical Journal of Pharmaceutical Research, 2015; 14(10): 1843-1848.
5. Yakubu MT, Musa IF. Effects of Post-coital Administration of Alkaloids from *Senna alata* (Linn. Roxb) leaves on some fetal and maternal outcomes of pregnant rats. J Reprod Infertil, 2012; 13(4): 211-217.
6. Sugumar M, Doss VA, Maddisetty P, Rangaraju M, Kandhasamy S, Munuswamy S. Pharmacological analysis of hydroethanolic extract of *Senna alata* (L.) for *in vitro* free radical scavenging and cytotoxic activities against HepG2. Pak. J. Pharm. Sci, 2019; 32(3): 933-936.
7. Pieme CA, Penlap VN, Nkegoum B, Ngogand J. *In vivo* Antioxidant and potential antitumour activity of aqueous ethanol extract of leaves of *Senna alata* (L.) Roxb (Caesalpinaceae) on Bearing carcinomatous cells. International Journal of Pharmacology, 2008; 4: 245-251.
8. Yakubu MT, Adeshina AO, Oladiji AT, Akanji MA, Oloyede OB, Jimoh GA, Olatinwo AWO, Afolayan AJ. Abortifacient potential of aqueous extract of *Senna alata* leaves in rats. Journal of Reproduction and Contraception, 2010; 21(3): 163-177.
9. Adedayo O, Anderson WA, Moo-Young M, Snieckus V, Patil PA, Kolawole DO. Phytochemistry and antibacterial activity of *Senna alata* flower. Pharmaceutical Biology, 2001; 39(6): 408-412.
10. Kudatarkar NM. Pharmacological screening of *Cassia alata* leaves on Colorectal cancer. Colorec Cancer, 2018; 4(1): 02. doi: 10.21767/2471-9943.100049.
11. Dewi R, Firza Y, Nashiry MA. Al-Suede FSR, Abdul Majid AS. A Review on *Cassia alata*:

Pharmacological, Traditional and Medicinal aspects Australian Herbal insight, 2019; 1(4): 016-0xx.
12. Fatmawati S, Yuliana, Purnomo AS, AbuBakar MF. Chemical constituents, usage and pharmacological activity of *Cassia alata*. Heliyon, 2020; 6(7): e04396. https://doi.org/10.1016/j.heliyon.2020.e04396
13. Jain GC, Ali SM. Effect of ethanolic extract of *Cassia alata* (L.) flowers on reproductive functions of male albino rats.Journal of Experimental Zoology, India, 2007; 10(1): 129-132.
14. Ikokide EJ, Ishmael FJ, Adamu HB, Oyeyemi MO. Comparative effects of methanol and oil extracts of *Ocimum gratissimum* on testicular morphology and epididymal sperm reserve of adult male albino rats (Wistar strain). Toxicology Reports, 2019; 6: 1127-1134.
15. Alade GO, Omobuwajo OR, Moody JO. Contraceptive effect of Ethanolic extract of *Sacoglottis gabonensis* (Bail.) Urb. Humiriaceae Stem Bark on male Wistar Rats. Inventi:Ethnopharmacology, 2011; 1: 64-67.
16. Emmanuel OY, Kingsley K, Lawal I. Spermatogenic and spermatotoxic effects of *Telfairia occidentalis* (Ugu) aqueous leaves extract in adult male Wistar rats (*Rattus novergicus*). Toxicol. Reports, 2018; 5: 954-958.
17. Sethi J, Yadav M, Sood S, Dahiya K, Singh V. Effect of Tulsi (*Ocimum sanctum* Linn.) on sperm count and reproductive hormones in male albino rabbits. International journal of Ayurveda research, 2010; 1(4): 208–210.
18. Kushwaha VB, Preeti S. Effect of Ethanolic Extract of Different Parts of Plant (*Annona squamosa*) on the fertility of male rats. Biomed J Sci & Tech Res, 2017; 1(5) DOI : 10.26717/BJSTR.2017.01.000429
19. Mostafa M, Soliman S, Mohamed RI, El-Sayed WM. Assessment of the antifertility effects of some plants in male albino mice. Scientific Research and Essays, 2021; 16(4): 58-63.
20. Griswold MD. The central role of Sertoli cells in spermatogenesis. Semin Cell Dev Biol, 1998; 9(4): 411-6.
21. Hentrich A, Wolter M, Szardening-Kirchner C. et al. Reduced numbers of Sertoli, germ, and spermatogonial stem cells in impaired spermatogenesis. Mod Pathol, 2011; 24: 1380–1389.
22. Aladamat N, Tadi P. Histology, Leydig Cells. [Updated 2021 Nov 19]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK556007/
23. Gnessi L, Basciani S, Mariani S, Arizzi M, Spera G, Wang C, Bondjers C, Karlsson L, Betsholtz C. Leydig cell loss and spermatogenic arrest in platelet-derived growth factor (PDGF)-A-deficient mice. The Journal of cell biology, 2000; 149(5): 1019–1026.
24. de Kretser DM. Is Spermatogenic Damage associated with Leydig cell Dysfunction?, The Journal of Clinical Endocrinology & Metabolism, 2004; 89(7): 3158–3160.
25. Winters SJ, Moore Jr JP, Clark BJ. Leydig cell insufficiency in hypospermatogenesis: a paracrine effect of activin–inhibin signaling? Andrology, 2018; 6: 262–271.