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RESEARCH PAPER

**THE HISTOLOGICAL EFFECT OF CNIDOSCOLUS ACONITIFOLIUS
AQUEOUS LEAF EXTRACTS ON THE ARCHITECTURE OF THE OVARY,
TESTIS AND SPERM CELLS OF ADULT WISTAR RATS.**

¹EBEYE O.A ²EKUNDINA V.O. ¹EKELE C.M¹EBOH D.E.O

¹Department of Anatomy, Delta State University Abraka, Nigeria

²Department of Medical Laboratory Science, Afe Babalola University, Ado-Ekiti, Nigeria

*Corresponding author: princessebeye@gmail.com

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ABSTRACT

This study accessed the effects of *Cnidoscopus aconitifolius* on some reproductive organs (testis and ovary) and semen analysis cells of adult wistar rats. Twenty four (24) adult wistar rats weighing 170g-215g were used for this study; the animals were weighed and sorted into control and three treatment groups of six rats each. The control received feed mash and water liberally, while the treated groups 2-4 were given 200mg, 400mg and 600mg of *Cnidoscopus aconitifolius* aqueous extract respectively, also they received feed mash and water liberally. At the end of the four weeks experiment animals were sacrificed, organs harvested and fixed in 10% formal saline for histological studies and sperm cells were placed in normal saline for semen analysis. The extract has no effect on body weight as gradual increase in body weight was observed in all the groups. Microscopic examination of the testis and ovary showed a dose dependent effect, for treated groups; testis revealed spermatogenesis arrest, the ovary revealed luteinization of the ovarian stroma and semen analysis for motility, morphology, viability and sperm count showed significant differences when compared to control group. Therefore caution should be taken in the use of *Cnidoscopus aconitifolius*.

Keywords: *Cnidoscopus aconitifolius*, medicinal plant, phytochemical screening, anti-fertility agents, reproductive organs,

INTRODUCTION

Herbal medicines are the sum total of all knowledge and practices of herbs, whether explicable or not, used in diagnosis, prevention and elimination of physical, mental or social imbalance and relying exclusively on practical experience and observations handed down from generation to generation whether verbally or in writing (Ernst, 2007). Herbal medicine is one of the most popular, if not the most popular form of traditional treatments.

In response to health problems, many people have for centuries, developed various herbal medicines using locally available plants as answers to their health problems. In Nigeria, a generally used plant for the above purpose is "*Cnidoscopus aconitifolius*" from the family "Euphorbiaceae" which is believed to have originated from Mexico. It is often referred to as Chaya, EfolyanaIpaja and Efo Jerusalem in the western part of Nigeria; Obarandu or Akwukwonriohurun in the eastern part of Nigeria; "Hospital Too Far" in the Southern part of Nigeria -because they believe it gives blood almost immediately even before one can rush to get from the hospital; and "Catholic

vegetable” because it was commonly cultivated and used as vegetables in convents (Donkoh *et al.*, 1990; Iwalewa *et al.*, 2005).

Cnidoscopus aconitifolius is a large, fast growing leafy perennial shrub, it is evergreen or drought deciduous shrubs up to 6 meters in height with alternate palmate lobed leaves, it has a succulent stem which releases a milky sap when cut and small white flowers or dichotomously branched cymes. Leaves are large and chartaceous or sometimes succulent, up to 32cm long and 30cm wide on petioles up to 28cm in length (Ross-Ibarra and Molina-cruz, 2002). In Nigeria, over 60% of rural populace depends solely on traditional medicines from herbs for their health problems (Ghani *et al.*, 1989; Senjobi, 1999). Herbal medicines and treatments is also known in urban areas because people tend to try out herbs with the believe that herbal plants are natural and therefore contains no side effect, this is very untrue as plants contains hundreds of constituents and some of them may elicit toxic side effects (Mordi and Akanji 2012). *Cnidoscopusaconitifolius* is believed to increase blood level, strengthen fingernails, darken gray hair and act as an antioxidant (Jensen, 1997; Atuahene *et al.*, 1999). It is also known for its great nutritional values; it is rich in protein, vitamins (A, B and C), calcium, and iron, potassium, carotene (Kuti *et al.*, 1996; Kuti and Konuru, 2004)

However raw chaya leaves contains toxic cyanide compound, cooking for 20minutes or more is essential prior to consumption, to inactivate the toxic components. People cook for 2 to 3 minutes prior to consumption or consume it raw (Mordi and Akanji 2012). It is also known to contain phenol, tannin etc. A number of studies exist reporting the toxic effect of herbal medicines (Shaw *et al.*, 1997; Kaplowitz, 1997; Calixto, 2000).

This present study however, attempts to determine the histological effect of aqueous extract of *Cnidoscopus aconitifolius* on some reproductive organs (ovary and testis) of adult wistar rats and on sperm cells.

MATERIALS AND METHODS

Study Location: This research was conducted in the Department of Anatomy, Delta State University, Abraka, Delta state, Nigeria within a period of 10 weeks.

Preparation of the Aqueous Plant Extract: Fresh leave samples of *Cnidoscopus aconitifolius* were collected from a farmland at the site II of Delta State University, Abraka, Delta State, Nigeria. The leaves were authenticated at the Botany Department of Delta State University, Abraka. The fresh leaf (*Cnidoscopusaconitifolius*) were washed in clean water and pounded into a paste form, which was transferred into a soxhlet apparatus (Surya, 2012).The mixture (active agent of *Cnidoscopus aconitifolius* + water) was about 800ml using the soxlet apparatus. The mixture was concentrated with hot air oven at a temperature of about 80C. The yield of the crude aqueous plant extract was 74.9g. The paste – like extract was stored in the refrigerator until required for use. 8g of extract was dissolved in 80ml of water (desired concentration).

Experimental Animals and Design: Twenty four (24) adult wistar rats (comprising of twelve 12 males and 12 females) were purchased from animal unit, Faculty of Basic Medical Science, Delta State University, Abraka, Delta State, Nigeria. The 24 rats were divided into sets, set A consisting of all males (12 animals) and set B consisting of all females (12 animals), each animal weighing 170g-215g were used for this study, the animals were acclimatized for two weeks, thereafter were weighed and sorted into four groups (1- 4) with respect to their weight (with 3rats in each group), this applies to rats in set A and B. Each group was housed in a well-ventilated cage at room temperature.

TreatmentProcedure: Aqueous extract of *Cnidoscopus aconitifolius* was administered orally using an orogastric tube for twenty eight (28) days; LD 50 which is 5.0g/kg (Lorke, 1983)administration is as follows; group one served as control and given feed (Mayer’s mash) and water, group 2, 3 and 4 were administered 200mg, 400mg and 600mg per kg of body weight respectively of aqueous extract of *Cnidoscopus aconitifolus*, this was done once a day plus feed (Mayer’s mash) + water. The animals were observed daily for any sign of morbidity and mortality and their body weight was measured every week during the experimental period.

Sample Collection: At the end of experimental period animals were sacrificed with the aid of chloroform and ovaries and testis were quickly harvested and fixed using 10% formal saline. Also sperm cells were collected from the epididymis (known as epididymal extraction) for semen analysis and were immediately place in formal saline. Semen analysis was carried out immediately to acquire the best result possible, for sperm count; 19 drops of seminal fluid to 1 drop of sample (semen + normal saline) was used for sperm count, also few drops of samples were stained

in 0.5% of eosin for other semen analysis such as morphology, viability, motility, etc. selected piece of tissues were processed into paraffin wax, sectioned and stained by Haematoxylin-Eosin method to show the general structures (Avwioro, 2014)

RESULT

Table 1 below shows the weekly total body weight differences in animal models treated with different doses *Cnidoscoulous aconitifolius* aqueous leaf extracts with that of the control. Comparatively, there was no statistically significant difference between the values obtained from the control and treated groups ($p>0.05$).

Table 2 below shows the effect of *CnidoscoulousAconitifolius* aqueous leaf extracts on sperm motility, morphology and viability in animal models treated with different doses of *Cnidoscoulous aconitifolius* aqueous leaf extracts and the control. Comparatively, there was a statistically significant difference between the values obtained from the control and treated groups ($p<0.05$).

Table 3 below shows the effect of *Cnidoscoulousaconitifolius*aqueous leaf extract on sperm count in animal models treated with different doses of *Cnidoscoulous aconitifolius* aqueous leaf extracts with that of the control. Comparatively, there was a statistically significant difference between the values obtained from the control and treated groups ($p<0.05$).

Table 1: Total body weight differences in animal models

GROUPS	WEEK1	WEEK2	WEEK3	WEEK4
Group 1 (ctrl)	152.3±13.7	160.8±12.4	179.5±24.7	197.5±36.2
Group 2	164.2±10.7	165.8±8.6	181.7±17.8	194.7±25.0
Group 3	187.5±16.1	190.8±13.6	198.3±23.2	196.7±29.6
Group 4	202.5±12.9	203.3±14.0	214.0±20.9	219.0±32.4

Values were expressed as mean ± SD; n =6; $p>0.05$

Table 2: Effect of *CnidoscoulousAconitifolius* aqueous leaf extracts on sperm motility, morphology and viability

Groups	Group 1	Group 2	Group 3	Group 4
Motility (% with forward progression)	81.7±2.1	59.0±3.0	36.7±4.7	31.7±6.2
Morphology (% with normal form)	45.0±4.1	43.3±2.4	36.7±4.7	18.3±2.4
Viability (% alive)	86.7±4.7	68.3±4.1	42.7±9.0	26.7±6.2

Values were expressed as mean ± SD; n =6; $p<0.05$

Table 3: Effect of *Cnidoscoulousaconitifolius*aqueous leaf extract on sperm count in animal models

Groups	Group1	Group2	Group 3	Group 4
Sperm count (x10 ⁶)	67.0±6.2	55.3±9.7	56.3±4.5	47.3±11.8

Values are expressed as mean ± SD, n =6. $p<0.05$:

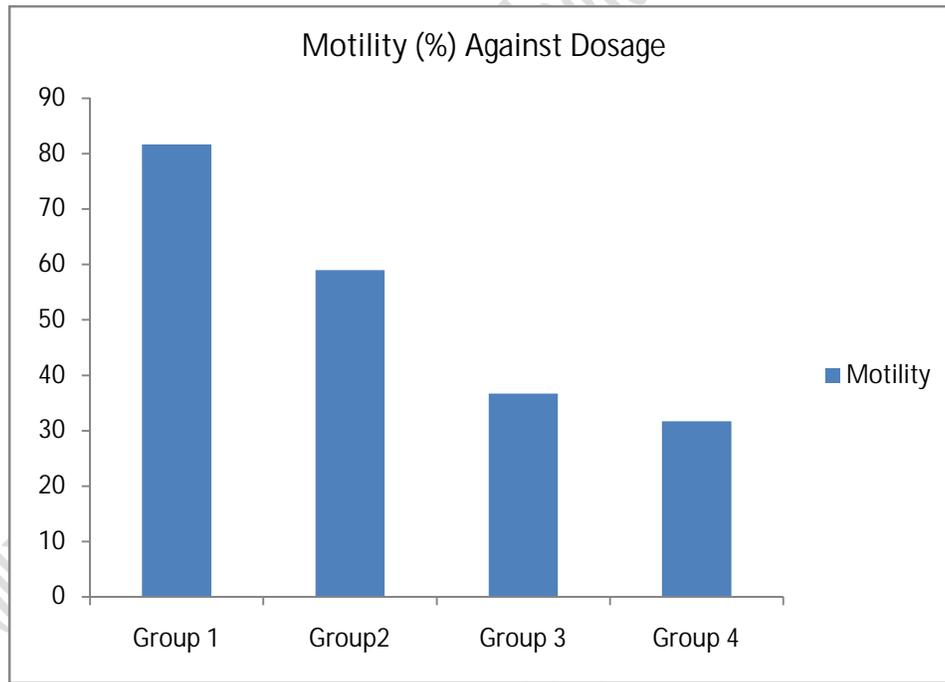


Fig 1: A graph showing the changes in wistar rat average motility in percentage during 28 days of administration.

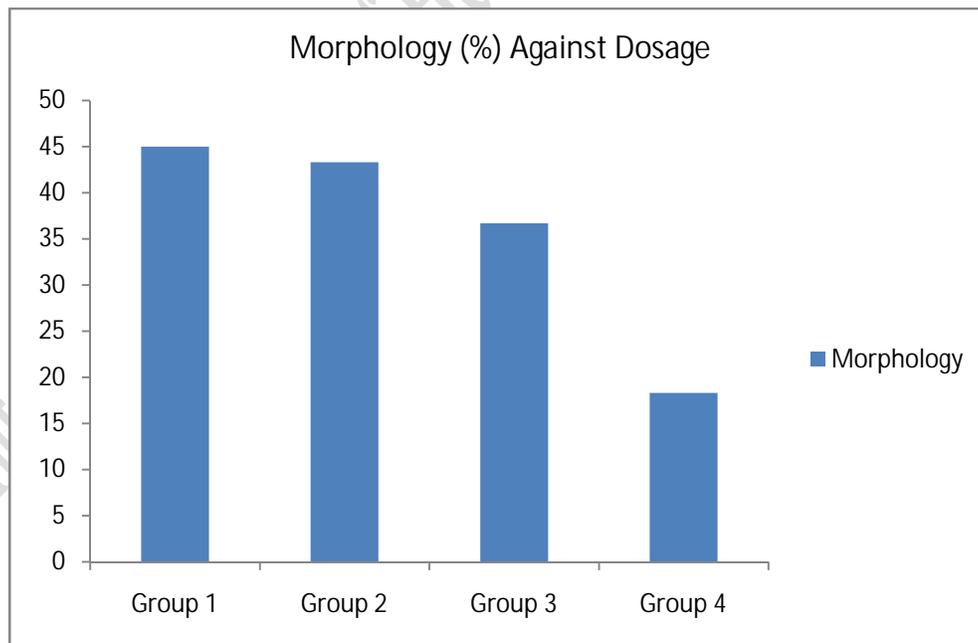


Fig 2: A graph showing sperm cell morphology (%) in animal models Treated with different doses *Cnidoscoulous aconitifolius* aqueous leaf extracts with that of the control during 28 days of administration.

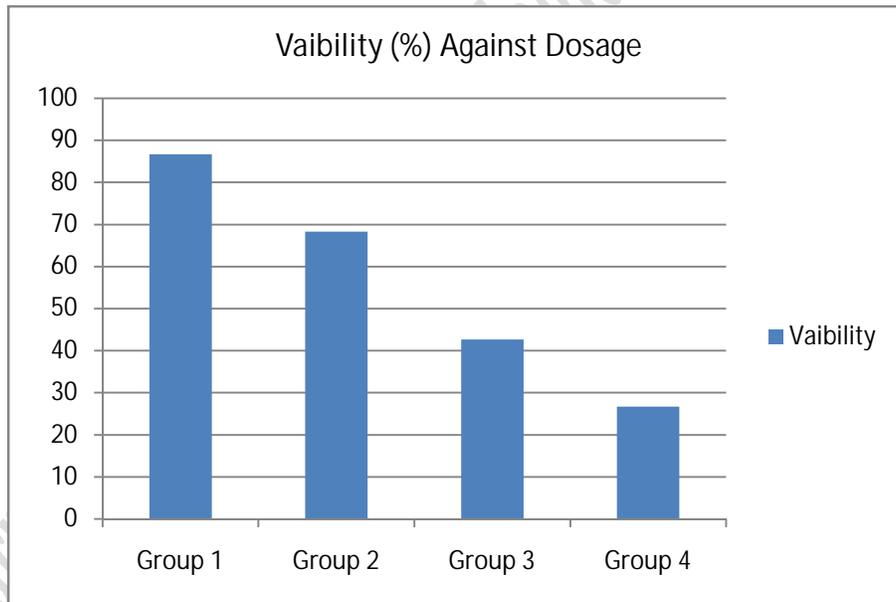


Fig 3: A graph showing changes average viability (%) in animal models Treated with different doses *Cnidocolous aconitifolius* aqueous leaf extracts with that of the control during 28 days of administration

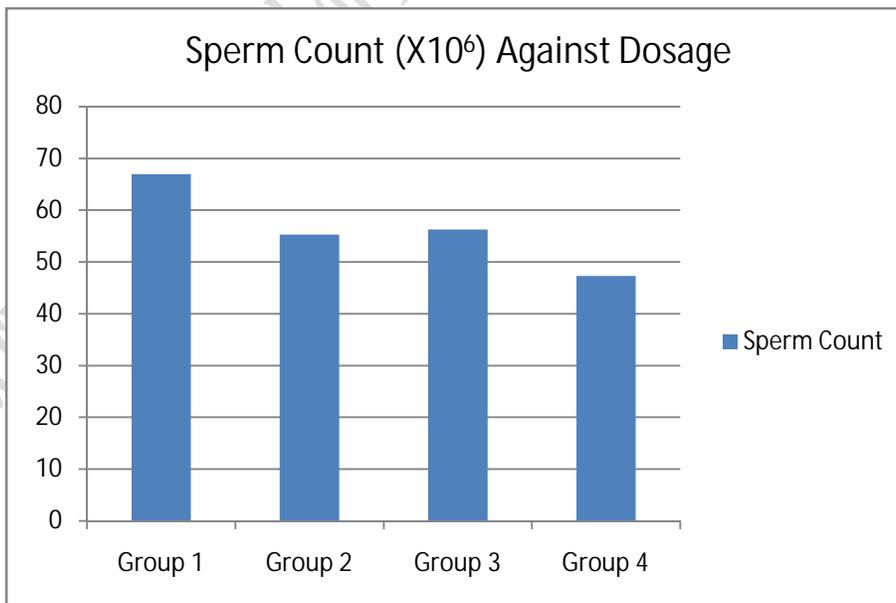


Fig 4: A graph showing the average sperm count (x10⁶) in animal models Treated with different doses *Cnidocolous aconitifolius* aqueous leaf extracts with that of the control during 28 days of administration

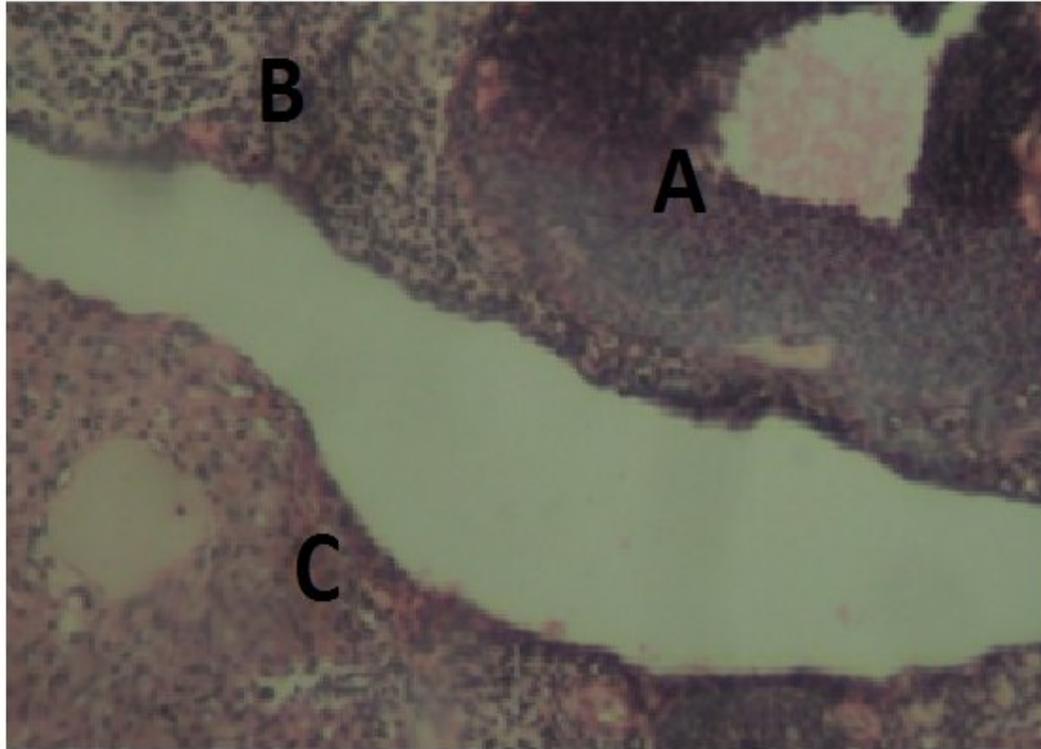


Fig 5: Photomicrograph of Control Rat Ovary (H&E x 10) composed of mature A and maturing follicles B, supported by dense stroma C

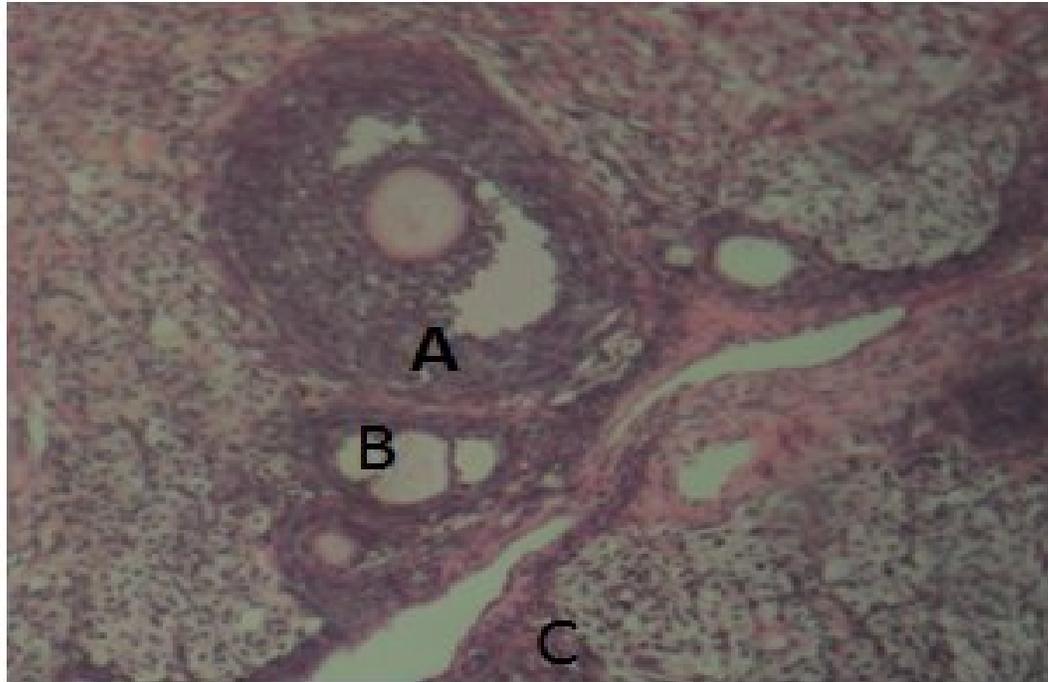


Fig 6: Photomicrograph of Rat Ovary (H&E x 10) treated with 600mg/kg *C. acontifolius* for 28 days showing mature nucleated follicle A and vacuolated B, supported by luteinized stroma C (H&E x 10)

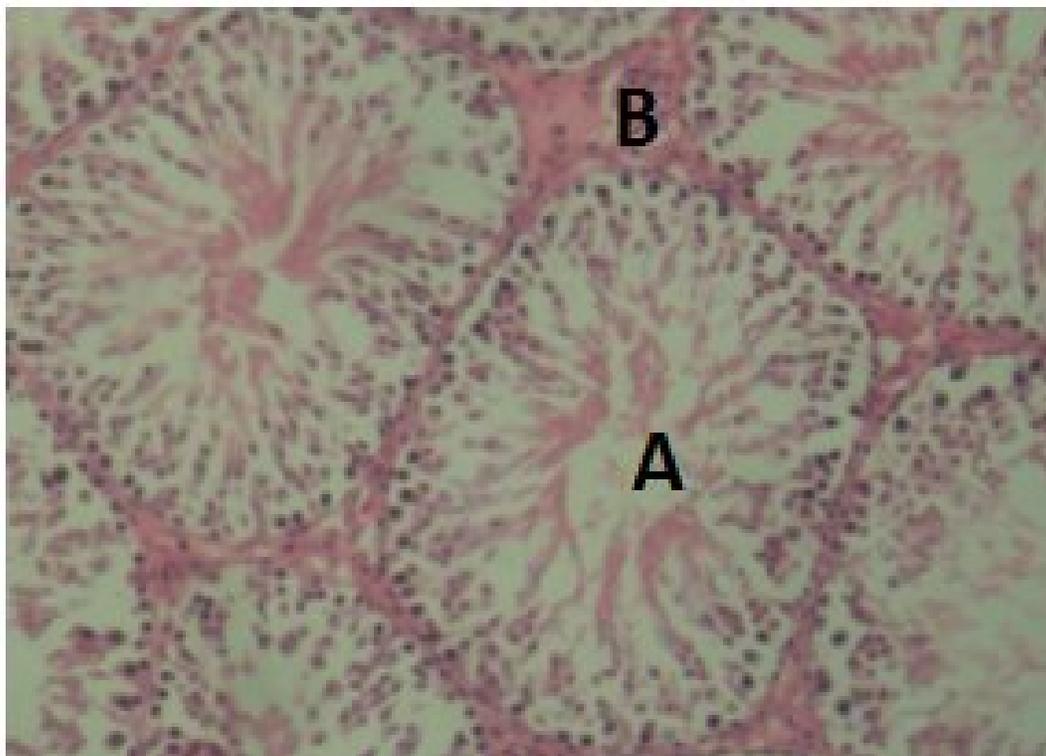


Fig 7: Photomicrograph of Control Rat Testis (H&E x 10) composed of seminiferous tubules A, separated by interstitial space B (H&E x 10).

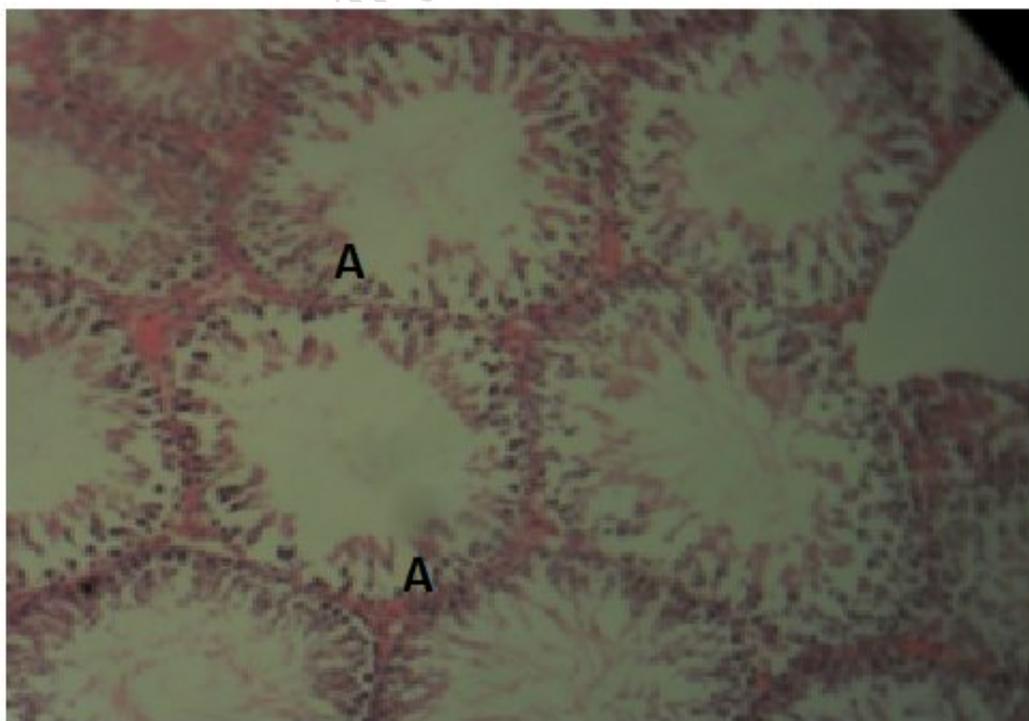


Fig 8: Photomicrograph of Rat Testis (H&E x 10) treated with 600mg/kg of *Cnidoscclusa contifolius* for 28 days showing areas of spermatogenic arrest A (H&E x 10)

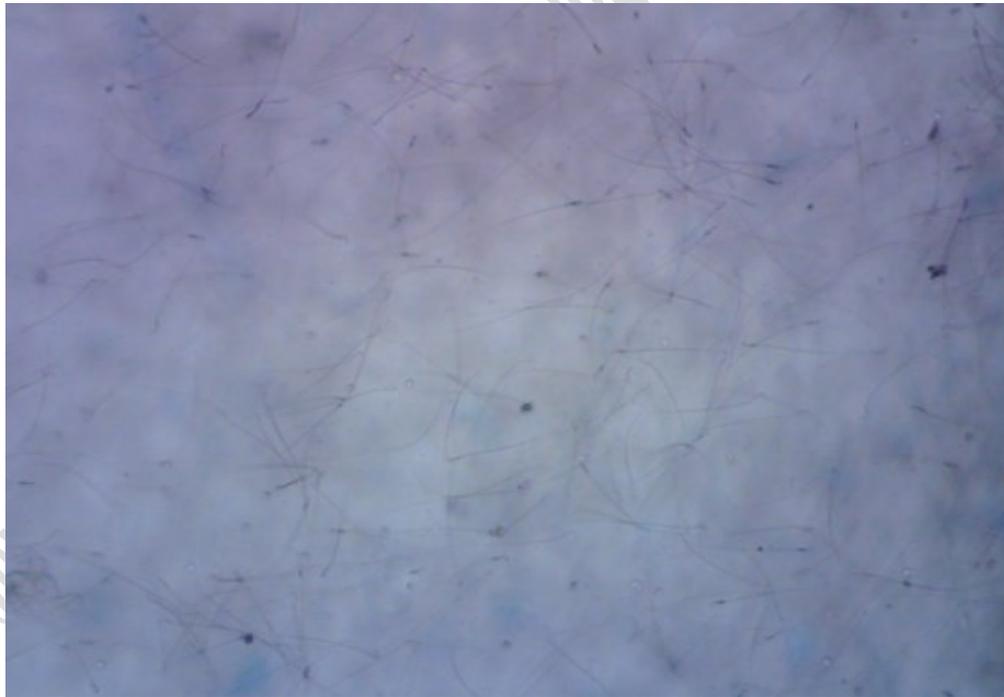


Fig 9: Photomicrograph of Control Rat Semen (0.5% E; x10). Note the sperm cell filled field.

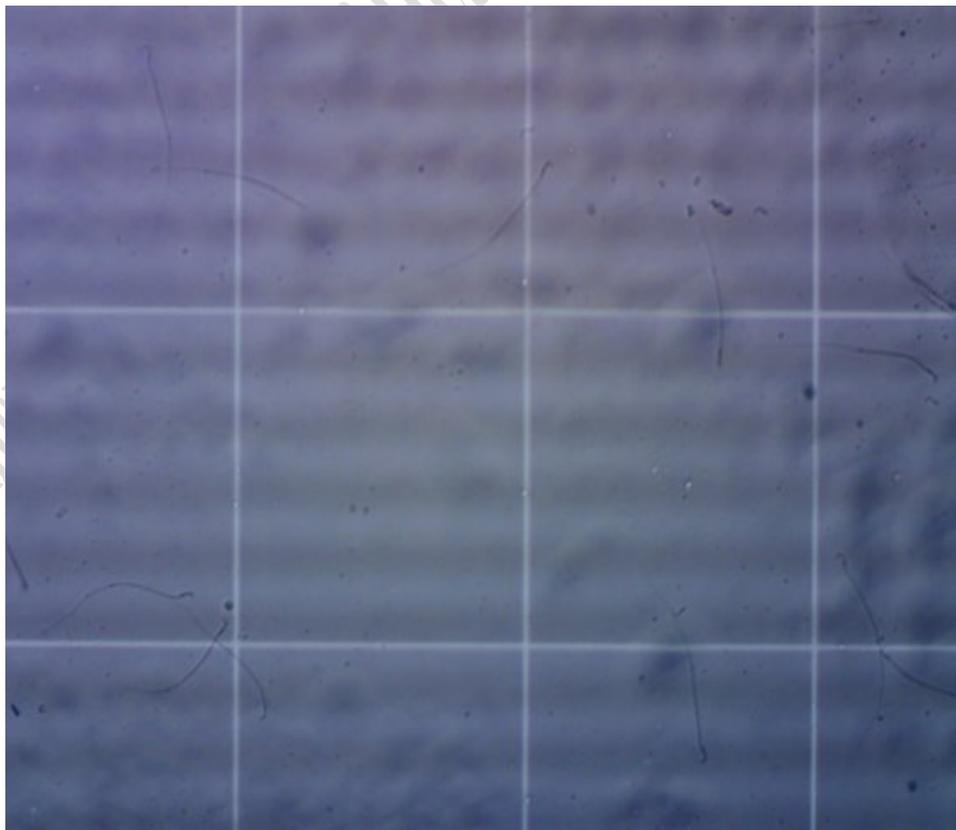


Fig 10: Photomicrograph of Semen/Sperm count of group 4 rats treated with 600mg/kg of *Cnidocolusa contifolius*. Note most of the areas with Stain absorption (x10).

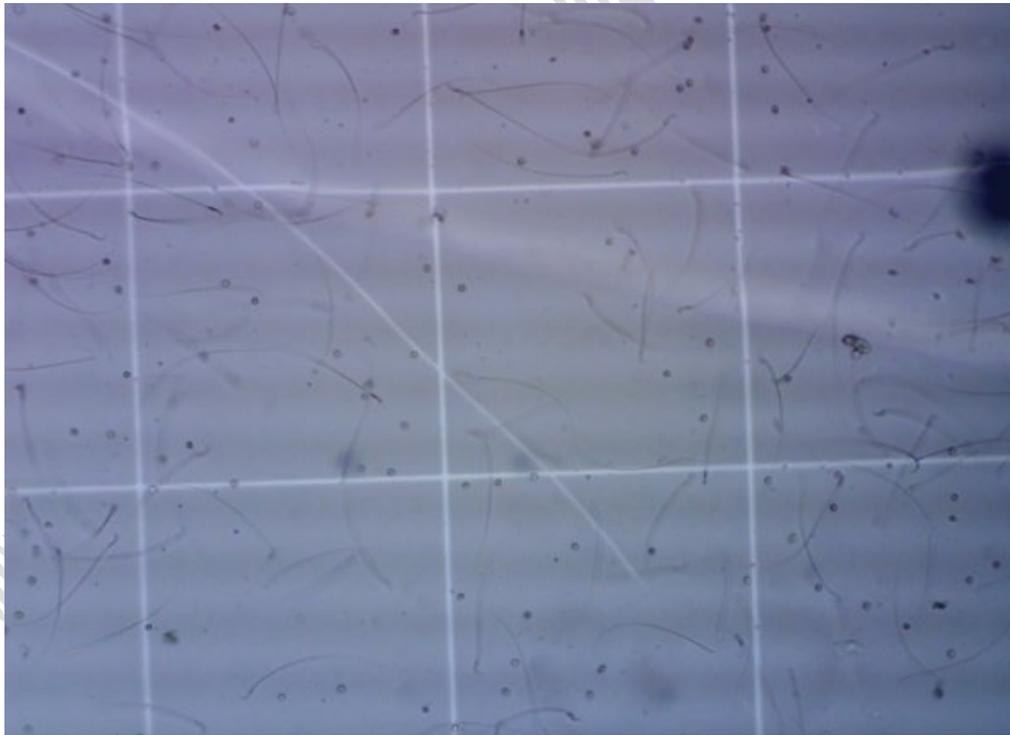


Fig 11: Photomicrograph of Control Semen (Sperm Count; x40)

DISCUSSION:

Herbal medicines are very popular in developing and underdeveloped countries with their indiscriminate use. Therefore, a clear understanding of potential adverse effects of herbs used is necessary for implementing safety measures. In the case of *Cnidocolusaconitifolius*, no systematic safety study had been done so far, hence a study on their toxicity is required. This present study tends to investigate the phytochemical content of the aqueous extract as well as the chronic toxicity of *Cnidocolusaconitifolius*. Toxicity testing in animals is carried out on a new drug to identify potential hazards. It helps in determining the upper limits of administration (Sofowora, 1993; Ebeye et al., 2007). If the toxic effect is low then there is chance of possible introduction of such drugs for therapeutic use.

The experiment revealed food and water intake of the four groups was generally good and similar which account for steady increase in weight of animals in all the groups. Histological examination of the ovary and testis revealed observable changes compared to control as shown in figures 5, 6, 7 and 8. Administration of the aqueous extract of *Cnidocolusaconitifolius* at a dose of 600mg/kg indicates the possibility of the plant aqueous extract containing anti-fertility properties. In females, *Cnidocolusaconitifolius* appears to induce luteinization of the ovarian stroma, while increasing blood flow in the ovary; this effect is dose dependent, the higher the dose the higher the blood flow to the ovaries. The luteinized cells produce androgen, which may lead to hirsutism and virilization (masculinization). However, in males, *Cnidocolusaconitifolius* (aqueous leaf extract) induces arrest of spermatogenesis which becomes more severe as the doses increases (200mg/kg, 400mg/kg to 600mg/kg). From histological studies of the testis; this could be as a result of damage to the testicular cells which aid in spermatogenesis. *Cnidocolusaconitifolius* also exerts a negative effect on the sperm cells; this effect is also dose dependent; noticed was an increased negative effect which occurred as the dose increased from 200mg/kg, 400mg/kg to 600mg/kg (of *Cnidocolusaconitifolius* leaf extract). There was a sharp drop in sperm count as shown in table 3. Previous studies also revealed extract toxicity and destructive effect of *Cnidocolusaconitifolius* on bone marrow at lowest concentration (Senjobi, 1999; Odokuma, 2012).

In this study, *C. aconitifolius* leaf extract administration at dose of 200mg, 400mg and 600mg/kg, may not be safe. It is possible to say that *C. aconitifolius* showed the presence of infertility agents as seen by the significant change in

the parameters studied and from the result of the histological investigation. The observations may be as a result of some of its active constituent like tannin, phenol as well as flavonoid (Mordi and Akanji2012).

Therefore it is recommended that further studies be carried out using this plant (*Cnidoscolumaconitifolius*) to assess its fertility properties as well as its effect on reproductive organs and hormones. If properly investigated can be used locally as a birth control extract.

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AUTHOR'S CONTRIBUTIONS

All the authors involved in this study made significant contributions towards the success of this presentation. Worthy of note, are their contributions during the article's review processes. No conflict of interest is declared.