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Comparative Effects of *Cissus Populnea* Extract and *Jatropha Curcas* on *Propoxur*-Induced Testicular Toxicity of Adult Male Wistar Rat.

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ABSTRACT

This study is aimed to compare the ameliorative properties of *Cissus populinea* and *Jatropha curcas* on testicular damage caused by propoxur. Eighteen adult male Wistar rats were used in this study and were divided into six groups A, B, C, D, E, F consisting three rats in each group. Group A received 1ml distilled water and served as the control group. Group B received 0.72 mg/liter/kg body weight of *Propoxur.* Group C received 200 mg/kg body weight of *Jathropha curcas.* Group D rats received 200 mg/kg body weight of *Cissus populnea.* Group E rats received 0.72 mg/liter/kg of *propoxur* and 200 mg/kg body weight of *Jathropha curcas.* Group F received 0.72 mg/liter/kg of *Propoxur* and 200 mg/kg body weight of *Cissus populnea* extract. The administration was done once in a day for 14 days. After which the rats were sacrificed and the testes were removed, weighed and fixed in 10% buffered formo-saline and processed using the Haematoxylin and Eosin staining technique for histological observations, sperm analysis were also carried out on the testes. Blood sample were also collected through cardiac puncture to evaluate for serum level of follicle stimulating hormone (FSH), leutenizing hormone (LH) and testosterone (TT). There was significant ($p<0.05$) decrease in body weight and testicular weight of animals in group B, however, no significant difference was observed in sperm count, but sperm motility and morphology was significantly increased in group B and F animals. FSH, LH and testosterone was significantly $p<0.05$ decrease in group B and C animals. There was mild destruction of testes in group B and C as evidence from histology sections while group D shows some regenerative effects. In conclusion, *propoxur* causes moderate destruction of the testes which is evident by progressive tissue damage while *Cissus populnea* had some regenerative effect and improve the testes however *Jathropha curcas* had mild destructive effect on the testes.

Keywords: *Propoxur*, *Cissus populnea*, *Jathropha crucas*, sperm parameters

INTRODUCTION

There has been a considerable increase in fertility in Nigeria over the past many years though it is likely that claims that up to 30-40% of women in Sub-Saharan Africa will spend their productive life without being able to achieve pregnancy may present an over estimation. Increasing prevalence of infertility among the human population has raised interest in agents such as drugs, herbs, chemicals and various forms of environmental insults that may impair testicular function. Many of such substances have been identified through animal experiments [1] .

Male infertility refers to the inability of a male to achieve a pregnancy in a fertile female [2]. In humans it accounts for 40-50% of infertility [3]. Male infertility is commonly due to deficiencies in the semen, and semen quality is used as a surrogate measure of male fecundity [4]. Pre-testicular factors refer to conditions that impede adequate support of the testes and include situations of poor hormonal support and poor general health including: hypogonadotropic hypogonadism due to various causes, obesity increases the risk of hypogonadotropic hypogonadism, drugs and alcohol, Strenuous riding e.g bicycle riding and horseback riding [5].

It has long been demonstrated that semen quality and sperm output are frequently lower than is generally accepted among fertile men. A malfunction/arrest of spermatogenesis, ductal obstruction, hypogonadotropic hypogonadism, among others is some of the causative factors of male infertility. About 20% of childless marriages result from fertility problems/sterility on the part of the male partners, while contributing to about 30–50% of others [6].

In Nigeria today, even though statistics are not accurate, clinical experience and focal reports dating back about a decade suggest a high male contribution, ranging between 40–51%, in childless marriages. The rise in childlessness among married couples in Nigeria is further compounded by cultural, religious, and traditional beliefs [7].

The diagnosis of infertility begins with a medical history and physical examination by a physician, preferably a specialist with experience or who specializes in male infertility. Typically two separate semen analyses will be required. The provider may order blood tests to look for hormone imbalances, medical conditions, or genetic issues. For sperm sample, the volume of the semen sample, approximate number of total sperm cells, sperm motility, and percentage of sperm with normal morphology are measured. This is the most common type of fertility testing [8]. Semen deficiencies are often labeled as follows: **Oligo**spermia or **Oligozoo**spermia - decreased number of spermatozoa in semen, **A**spermia - complete lack of semen, **Hypo**spermia - reduced seminal volume, **Azoo**spermia - absence of sperm cells in semen, **Terato**spermia - increase in sperm with abnormal morphology and **Asthenozoo**spermia - reduced sperm motility [9].

There are various combinations of these as well, e.g. *Teratoasthenozoospermia*, which is reduced sperm morphology and motility. Low sperm counts are often associated with decreased sperm motility and increased abnormal morphology, thus the terms "oligoasthenoteratozoospermia" or "oligospermia" can be used as a catch-all. In the blood, Common hormonal test include determination of FSH and testosterone levels. A blood sample can also reveal genetic causes of infertility [8].

Propoxur (*PPX*) locally called Baygon (2-isopropoxy-phenyl-N-methylcar-bamate) is a non-systemic insecticide which was introduced in 1959 [10]. It is used against mosquitoes in outdoor areas, against flies in agricultural settings, against fleas and ticks on pets, as an acaricide, on lawns and turf for ants, on flowering plants, and in private dwellings and public buildings. It is also used as a molluscicide. It is effective against cockroaches, aphids and leafhoppers.

Propoxur is one of the chemicals that have, to a large extent, replaced DDT in the control of black flies and mosquitoes [11]. Like all N-methyl carbamates and organophosphorous insecticides, *Propoxur* is believed to act by inhibiting acetylcholinesterase activity [12]. This results in accumulation of acetylcholine in the synapse, which in turn can stimulate cholinergic receptors producing both nicotinic and muscarinic effects in the organism, such as muscle contractions and secretion in many glands [13].

Because of its toxicity and widespread use, the possibility that *Propoxur* can affect humans and wildlife in their natural habitats is of great concern [14]. There is abundant information about the neurotoxic effects of *Propoxur*, but less is known about its impact on reproduction. US Environmental Protection Agency (1999) reported decreased numbers of pups, and depressed fetal weight in rats orally exposed to *Propoxur* [15]. Other studies on rats showed the reduction of the weight of pups born from mothers treated with *Propoxur* at the dose of 5 mg/kg/day [16].

The aqueous extract of its stem bark of *Cissus populnea* is associated with aphrodisiac and fertility potentials among the Yoruba-speaking people of South West Nigeria, where it is observed that men consume the aqueous and ethanolic extracts copiously and consistently for long periods of time either in mono or polyherbal formulations [17]. This use of various herbal remedies, including *C. populnea,* as an aphrodisiac and fertility enhancer amongst the males has been attributed to the declining fertility trend that has been established in this population over the years coupled with the attendant increasing levels of erectile dysfunction [18].

Jathropha curcas and *Cissus populnea* have been analysed to contain similar compounds such as flavonoid, saponin and alkaloid compounds [19]. The extracts were subjected to both qualitative and quantitative phytochemical tests for plant secondary metatobolites; tannins, phylobatannins, saponins, flavonoids, steroids, terpenoids, cardiac glycosides, alkaloids, anthraquinones and phenols according to the method described by [20]. *J. curcas* extracts are of importance and interest due to their relationship with various anabolic hormones including sex hormones [21].

Flavonoid is one of the active components present in stem of *Cissus populnea* and *Jatropha curcas* [22], which is known to have a very potent fertility effect [23]. The significance of this study is thus to evaluate the effect of flavonoids on *Propoxur* induced damage on the testis of adult Wistar rat.

MATERIALS AND METHODS

Plant Materials

The plant stem bark of *Cissus populnea* and *Jathropha crucas* were purchased from herbal medicinal practitioner at Oja tuntun market, Ilorin, Kwara State and was authenticated at the Department of Plant Biology, Faculty of Science, University of Ilorin, Nigeria by Mr Ajayi, Boluwatife and were deposited at their herbarium with Voucher number U.I.V 23 and U.I.V 24 for *Jathropha crucas* and *Cissus populnea* respectively.

Extract Preparation for *Jathropha crucas* and *Cissus populnea.*

600 g of stem bark of *J. curcas* was air-dried until a constant weight was obtained, and ground using a

locally made blender at Oja Tuntun market. 500 g of the sample was percolated in distilled water for 72 hours and then decanted and poured into six conical flasks (250 ml each). The filtrate was concentrated using Oven (Gallenkamp oven BS-064744) at 35^{0}C for 120 hours. After complete drying of the sample, 250 g of sample residue was reconstituted in distilled water to give an equivalent dose of 200 mg/kg body weigh which was used in the study.

500 g of *C. populnea* stem bark was, chopped, dried and then subjected to Soxlet extraction using 600 ml of n-hexane as the solvent. All active components in C*issus populnea* were extracted using Soxhlet extractor and were done continuously using n-hexane as solvent (50-65^{o}C). The homogenate was freeze dried and yielded 100.5g

Experimental Animals

Experimental procedure involving the animals and their care was conducted in conformity with International, National and institutional guidelines for the care of laboratory animals in Biomedical Research and the use of laboratory Animals in Biomedical research as promulgated by the Canadian Council of Animal care (1985) [24].

Eighteen (18) adult male albino adult Wistar rats weighing between 150-220 g with an average age of 3 months were obtained from the small Animal Holding unit at Zobi Barracks, Ilorin Kwara State and were later transferred from there to the Animal Holding Unit of the Department of Anatomy, University of Ilorin where they were kept in well ventilated house with conditions (temperature 28-310C; photoperiod of 12 hour natural light and 12 hour dark; humidity of 50-55%) with free access to rat pellets (Feed-Master growers marsh) and distilled water.

Animal grouping and treatment

The animals were randomly distributed into six groups of A – F. Each rat in the group has a weight between 150 to 220 g which gives an average weight of 185 g.

Group A animals were given distilled water only for 14 days. Group B animals were exposed to 0.72 mg/liter/ kg body weight of *Propoxur* by inhalation for 20 minutes for 14 days. Group C animals were given a dose of 200 mg of aqueous extract of *Jathropha crucas* per kg body weight of the animal for 14 days. Group D animals were given 200 mg of the Soxhlet extract of *Cissus polpulnea* per kg body weight of the animal for 14 days. Group E animals were given 0.72 mg/liter of *Propoxur* for 1 week then 200 mg of crude extract of *Jathropha crucas* per kg body weight of the animal for another 1 week, Group F animals were given 0.72 mg/liter of *Propoxur* for 1 week then 200 mg of Soxhlet extract of *Cissus populnea* per kg body weight of the animal for another 7 days.

Determination of epididymal sperm parameter.

After the animal sacrifice, the lower abdominal wall was dissected and right caudal epididymis was excised. The spermogram was obtained using a modification of the method described by ([25]Yokoi *et al* . 2010). Briefly, the caudal epididymis was dissected and minced in 2ml (19 drops) of freshly constituted Ham F 10 solution micro-filtered (Milli-pore) to internationally accepted In-Vitro Fertilization (IVF) grade and kept at 250C.

After allowing it to stand for 10 min, with periodic rocking of the tube, one drop of this suspension was placed on a clean slide and examined at x400 magnification for motility score. A 1:1 preparation from this suspension was then made with water to immobilize spermatozoa.

The improved haemocytometer (Marienfeld-Germany) was charged from this final preparation and the total counts were done x400 magnification using a light microscope. Abnormal features of sperm morphology were observed and categorize as tail defects, neck and middle piece defects, and head defects; and the findings were expressed as percentage (%) of morphological abnormal sperm. The Progressivity was graded as follows:

A – Excellent forward directional movement (EFDM)
B – Good forward directional movement (GFDM)
C – Fair forward directional movement (FFDM)
D – Poor forward directional movement (PFDM) [1] .

Histology of testes

The testes were fixed in 10% formol saline. After 72 hours they were dehydrated in graded alcohol, cleared in xylene, and embedded in paraffin. The resulting blocks were sectioned. The sections were randomized and selected sections stained in Haematoxylin and Eosin. They were then examined at x400 magnification.

RESULTS

Morphological Observation

The administration of *Propoxur* only to animals in Group B show significant decrease ($p<0.05$)in weight and *Cissus populnea* only to Group D has a significant increase in the weight of the Wistar rat ($p>0.05$) when compared with the Group A (control) while the administration of *Jathropha crucas* only to animals in Group C, *Propoxur* and *Jathropha crucas* to animals in Group E, *Propoxur* and *Cissus populnea* to animals in Group F has no significant effect on the weight change of the male Wistar rat ($p<0.05$) when compared with the control Group A. Therefore group B showed a significant decrease in the weight of the rat.

Table 1: Effects of *Cissus populnea*, *Jatropha curcas* and propoxur on body weight of rats

Groups	Initial Weight(g)	Final Weight(g)	Weight Difference(g)
Group A	116.33±1.8 6	160.00±10.00	43.70
Group B	214.67±5. 33	201.33±5.92*	-13.34*
Group C	188. 33±19.64	210.00±29.29	21.67
Group D	148. 33±7.26	150.00 ± 5.77	1.67
Group E	188. 33±14.24	208.33 ± 24.88	20.00
Group F	166.67±4.40	186.67 ± 6.01	20.00

Value expressed as mean ±SEM *p< 0.05, SEM – Standard Error Mean.

GROUP A: Control
GROUP B: *Propoxur*
GROUP C: *Jathropha curcas* only
GROUP D: *Cissus polpulnea* only
GROUP E: *Jathropha curcas* and *Propoxur*
GROUP F: *Cissus polpulnea* and *Propoxur*

Table 2: Effects of *Cissus populnea*, *Jatropha curcas* and propoxur on testicular weight of rats

Groups	Mean Weight (g)
Group A	1.3613±0.29
Group B	0.8401±0.73*
Group C	1.4578±0.21
Group D	1.5392±0.83
Group E	1.3082±0.05
Group F	1.2618±0.29

Value expressed as mean ± SEM * p<0.05.

GROUP A: Control
GROUP B: *Propoxur*
GROUP C: *Jathropha curcas* only
GROUP D: *Cissus polpulnea* only.
GROUP E: *Jathropha curcas* and *Propoxur*.
GROUP F: *Cissus polpulnea* and *Propoxur*.

From t-test, it was observed that the administration of *Propoxur* has a significant effect (p<0.05) on the testicular weight while other groups showed no significant response in term of testicular weight (p>0.05). Weights of testes were obtained as the mean of the left and right organs.

Table 3: Effects of *Cissus populnea*, *Jatropha curcas* and propoxur on Sperm Parameters

Groups	Sperm Count ($\times 10^6$/ml)	Sperm Motility (%)	Sperm Morphology (%) Abnormal	Sperm Progessivity (Grade)
A	40	77.5	22.50	A
B	52	46.15	53.85*	D*
C	34	64.70	35.30	B
D	42	64.29	35.71	A
E	46	60.87	39.13	C
F	56	42.86	57.14*	C*

Value expressed as mean ± SEM * p<0.05.

GROUP A: Control
GROUP B: *Propoxur*
GROUP C: *Jathropha curcas* only
GROUP D: *Cissus polpulnea* only.
GROUP E: *Jathropha curcas* and *Propoxur*.
GROUP F: *Cissus polpulnea* and *Propoxur*.

From t-test, it was observed that Group B and F has a significant decrease ($p<0.05$) on the sperm motility and normal morphology when compared to groups A,

Table 4: Effects of *Cissus populnea*, *Jatropha curcas* and propoxur on hormones

Groups	**FSH (mIU/ml)**	**LH (mIU/ml)**	**TT (ng/ml)**
Group A	0.80	2.60	5.20
Group B	0.20*	1.20*	2.75*
Group C	0.60*	1.82*	3.48*
Group D	1.50	3.00	6.20
Group E	1.30	2.80	5.10
Group F	1.70	3.17	6.00

Value expressed as mean ± SEM * $p<0.05$

GROUP A: Control
GROUP B: *Propoxur*
GROUP C: *Jathropha curcas* only
GROUP D: *Cissus polpulnea* only.
GROUP E: *Jathropha curcas* and *Propoxur*.
GROUP F: *Cissus polpulnea* and *Propoxur*.

From t-test, it was observed that Group B and C has a significant decrease ($p<0.05$) on FSH, LH and TT level when compared to Group A (control).

HISTOLOGY OF TESTES

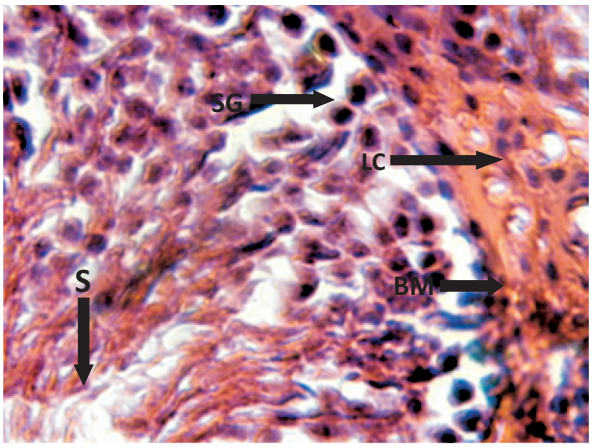

Plate 1: Photomicrograph showing a section of the testis of control rat using Haematoxylin and Eosin (H&E) stain Mag. (x400).LC= Leydig cell, BM= basement membrane, S= Spermatid, SG= Spermatogonium.

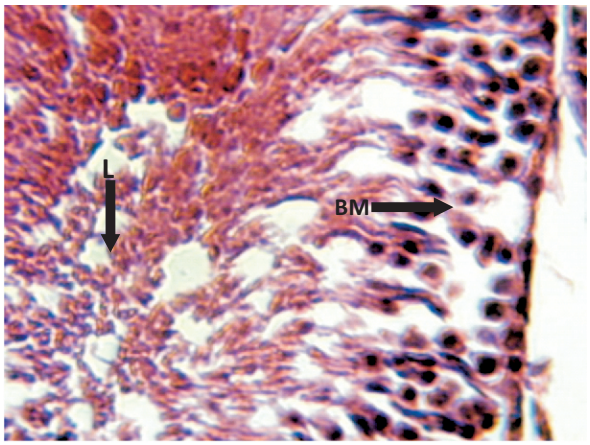

Plate 2: Photomicrograph showing a section of the testis treated with *Jatropha crucas* only rat using H&E stain Mag. (x400) L= Lumen, BM= Basal Membrane

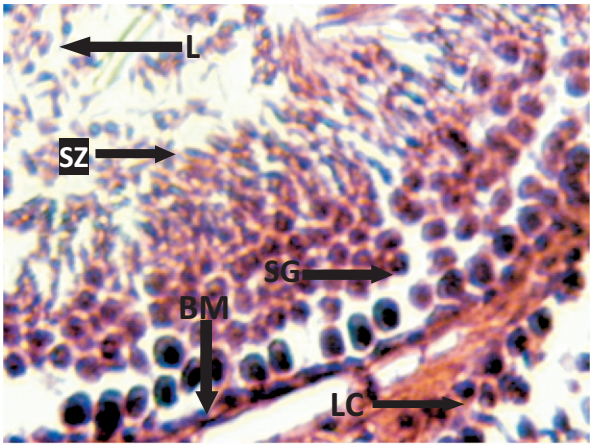

Plate 3: Photomicrograph showing a section of the testis treated with *Cissus populnea* only rat using H&E stain Mag. (×400). LC= Leydig Cell, L= Lumen, SZ= Spermatozoa, SG= Spermatogonium, BM= Basal membrane.

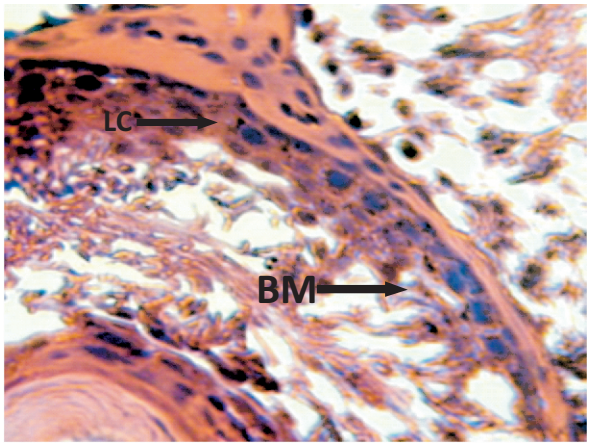

Plate 4: Photomicrograph showing a section of the testis treated with *Propoxur* only rat using H&E stain Mag. (×400).LC= Leydig Cell, BM= Basal membrane.

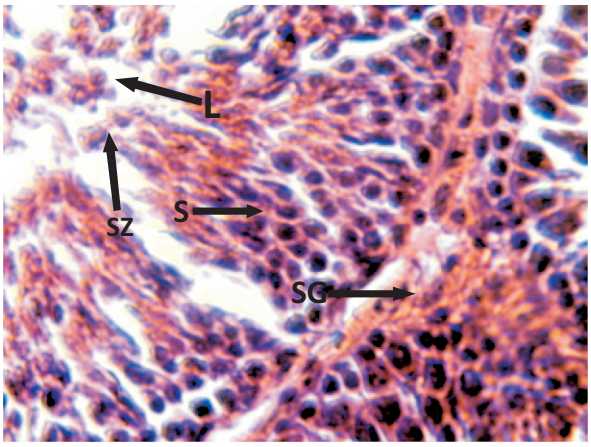

Plate 5: Photomicrograph showing a section of the testis treated with *Cissus populnea* and *Propoxur* only rat using H&E stain Mag. (×400). L= Lumen, S= Spermatid, SG= Spermatogonium, SZ= Spermatozoa.

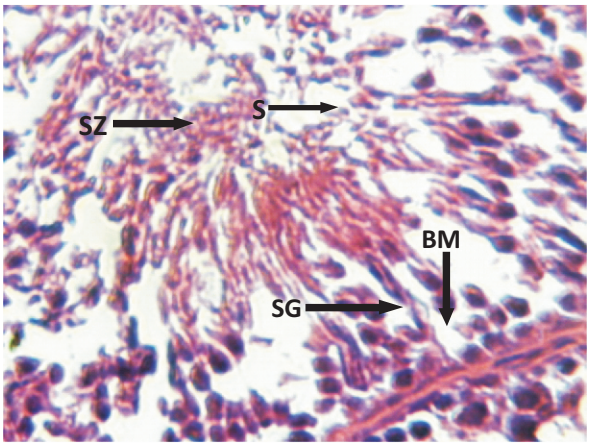

Plate 6: Photomicrograph showing a section of the testis treated with *Jathropha crucas* and *Propoxur* only rat using H&E stain Mag. (×400). S= Spermatid, SG= Spermatogonium, SZ= Spermatozoa, BM= Basal Membrane.

DISCUSSION

The present study was carried out in order to determine the ameliorative properties of *Cissus populinea* and *Jatropha curcas* on testicular damage caused by propoxur. Propoxur is a carbamate insecticide whose active principle is propoxur (2-isopropoxy-phenyl -N-methylcarba-mate), a compound well known for its neurotoxic effects [16]. From the results obtained, it appears that during the last fourteen days of treatment, the body and testicular weight significantly $p<0.05$ decreased in rats treated with propoxur as compared to control.

The photomicrograph of Plate 1 (Control) administered with 0.5ml distilled water only, the sections of the testes revealed normal histological features of the seminiferous tubules showing clearly organized spermatogonia, spermatid and normal Leydig cells. The photomicrograph for plate 2 of the animal administered with 200 mg/kg body weight of *Jathropha crucas* shows a moderate exfoliation of the basement membrane. The photomicrograph for plate 3 of the rats administered with 200 mg/kg body weight of *Cissus populnea* shows that the spermatogonia were prominently stained, suggesting cellular and tubular improvement, there were also increase in number of spermatogonia and spermatids. The basal membrane remains intact so as to give support to the spermatogenic cells. The photomicrograph for plate 4 of the rats administered with 0.72mg/liter/kg body weight of *Propoxur* resulted to a severe exfoliation of basal membrane and cellular degeneration. This was in agreement with the report of many investigators, that PPX-induced testicular toxicity is due mainly to oxidative stress [25.26]. Decline in epididymal sperm count, sperm motility, an increase in sperm with abnormal morphology has also been reported in male rats fed with *Propoxur*.

The photomicrograph for plate 5 of the animal administered with 0.72mg/liter/kg body weight of *Propoxur* and post administration of 200 mg/kg body weight of *Cissus populnea,* 7 days after, showed increase in spermatogonia and improved Leydig cells. The photomicrograph for plate 6 of the animal administered with 0.72mg/liter of *Propoxur* and thereafter with 200 mg/kg body weight of *Jathropha crucas* showed mild cellular degeneration.

It was obsereved from the above analyzed plates that groups treated with *Propoxur, Propoxur* and *Jathropha crucas* only i.e Group B and Group C respectively show foci vacuolation in the seminiferous tubules when compared with the control Group A (the control), Group D that was treated with *Cissus populnea* only has an improved cytoarchitecture when compared with the control group in term of number of cells and the improved state of the cell structure, in Group E where animals were treated with both *Propoxur* and *Cissus populnea* show that *Cissus populnea* reversed the toxicity induced by *Propoxur* as compared to Group B where *Propoxur* was observed to have induced cellular toxicity, in Group F where animal treated with both *Propoxur* and *Jathropha crucas* showed alteration in the process of spermatogenesis by the deletion of the spermatogonia.

It is well established that carbamate and organophosphorous pesticides reduce acetylcholinesterase activity and block nerve impulses [10.27.28]. This effect may altered and decreased the release of pituitary hormones, namely FSH and LH, leading to the reduction of sperm production in the testes. This hypothesis could explain the significant decrease in testicular weight, body weight, normal sperm morphology and motility recorded in rats treated with the dose of propoxur (0.72 mg/kg body weight).

In conclusion, *propoxur* at 0.72 mg/kg body weight has established in this research to induce toxicity in the testes characterized to reduce testicular weight, body weight, normal sperm morphology and motility which also have decrease effect on the reproductive hormone parameters (LH, FSH, and TT). However, administration of 200 mg/kg body weight of *Cissus populnea* was discovered to have improved semen parameters.

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