

## Testicular effect of Dr. Aladdin's seven keys herbal mixture in male Wistar rats

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### Abstract

**Background:** Dr. Aladdin's seven keys herbal mixture is widely used to relieve feverish conditions, and skin rashes especially in measles, chickenpox, viral infections, malaria, diarrhoea, ulcers, bacterial infections and other conditions.

**Objective:** The present study examined the testicular effect of Dr Aladdin's seven keys herbal mixture on male Wistar rats.

**Materials and methods:** A total of 20 adult male. Wistar rats weighing between 140– 240 grams were divided into five groups (1-5) of 4 rats each. Group 1 served as control, while Groups 2, 3, 4, and 5 received via oral route 250 mg/kg, 500 mg/kg, 1000 mg/kg, and 2000 mg/kg of the herbal mixtures respectively for 28 days. At the end of the drug administration, the rats were sacrificed and blood samples were collected to determine serum levels of testosterone and follicle-stimulating hormone (FSH). The testes were harvested and prepared for semen analysis and light microscopic study.

**Results:** The sperm count and sperm motility were significantly reduced with marked alteration in morphology in a dose-dependent manner. The hormonal assay showed testosterone levels were significantly decreased in a dose-dependent manner. Histological findings also showed mild focal areas of haemorrhage, mild infiltration of inflammatory cells, moderate significant harmful effect on the seminiferous tubules/testicular functions, and clumping of cells in a dose-dependent manner.

**Conclusion:** This herbal mixture has shown to cause moderate to severe effects on the testes, serum level of testosterone and alteration in semen parameters, thus may predispose addicted consumers to infertility. The consumption should strictly be based on the prescription of qualified medical personnel.

**Keywords:** Aladdin's seven keys; Testicular; Wistar rats; Testosterone; Histology

### 1. Introduction

The inability of the testes to produce viable sperm cells could be genetic or acquired in aetiology [1]. Failure to produce viable sperm as a result of genetic factors arises from inherited characteristics which could lead to abnormalities that are structural or numerical in nature, while acquired factors could be a result of deliberate or accidental exposures to teratogens (especially chemicals such as formalin, atrazine and paraquat) that affect the general architecture and

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functions of the testis, and alter various activities in the reproduction system that ultimately leads to oligospermia and in severe cases of azoospermia [1,2]. Often, semen with a decreased sperm concentration has significant abnormalities in sperm morphology and motility, a condition termed oligoasthenoteratozoospermia [3].

Dr Aladdin's herbal mixture is one of the major herbal mixtures in Nigeria with wide usage for so many decades. It is produced by F.A Ike and Sons LTD at plot 1N/29 Harbour Independence Layout, Onitsha, Nigeria and used for the relief of feverish conditions and skin rashes especially, measles and chickenpox [4]. For adults, two tablespoonfuls are usually taken three times daily in feverish conditions. It is also applied topically in conditions of skin rashes measles and chickenpox. In children, one tablespoonful is taken three times daily for feverish conditions and also applied topically in conditions of rashes and allowed to dry on the skin [4]. There is no stipulated duration of usage for this herbal remedy. It is usually administered till symptoms of the diseases disappear [4]. There is no recorded side effect of the administration of this drug inadvisable for general and individual uses [4].

Dr Aladdin's Seven Keys Herbal Mixture is a widely used herbal drug valued for the management of skin diseases, viral infections, liver inflammation, malaria, inhibition of platelet aggregation, stomach cancer and other conditions. Dr Aladdin's seven keys herbal mixture contains *Allium sativum*, *Combretum misrantom*, *Ficus carica*, *Nauclea latifolia*, *Sterculia urens*, *Tetrapleura tetraptera*, and *Xylopi aromaticas* its active ingredients in water base [5]. Dr Aladdin's seven keys herbal mixture act by slowing down blood clotting, improving glucose intolerance, dyslipidemia, lowering levels of tumour necrosis factor-alpha and interleukin-6 in the liver, treating hepatic steatosis, inflammation, exhibiting antioxidant activity, decreasing blood sugar levels, decreasing prostaglandin levels, possessing hypoglycemic activity, possessing hypolipidemic activity, and exhibiting suppressing effects on proliferation of various cancer cell lines [6]. It is said to contain polyphenols, flavonoids, and anthocyanins [7]. Its activities include possessing antibacterial and antiviral activities; and inhibiting the various cellular processes of microbes [5]. This could pose a health risk as overdose and or prolonged consumption may be harmful to several organs in the body leading to clinical conditions that could be potentially life-threatening. This study examined the testicular effect of Dr Aladdin's seven keys herbal mixture in male Wistar rats.

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## 2. Material and methods

### 2.1. Animal Procurement, Care, and Treatment

Twenty (20) adult male Wistar rats weighing between 140–240 grams were purchased from the best farm in Okofia, Nnewi South L.G.A Anambra State. The rats were allowed to acclimatise for two weeks before the commencement of the study. They were weighed and randomly assigned to metallic cages of 5 groups (1-5) of 4 rats each at the animal house of the Department of Anatomy Nnamdi Azikiwe University, Nnewi campus. The animals were placed at 12h light/dark cycle, and at 27°C for 28 days. They were allowed free access to livestock feed (Top Feeds, Nigeria Ltd), and tap water throughout the duration of the experiment. The institution's guide for the care and use of laboratory animals is strictly adhered to.

### 2.2. Procurement of Drug used

Eighteen (18) bottles (200 ml each) of Dr Aladdin's seven keys herbal mixture were obtained from F.A Ike and Sons Ltd, Plot 1n/29b Harbour Industry Layout Onitsha, Nigeria.

### 2.3. Toxicity test for Dr. Aladdin's seven keys herbal mixture

The LD<sub>50</sub> of the Dr Aladdin's seven keys herbal mixture in Wistar rats was determined orally using the method of [8]. In the first phase, rats were divided into three groups of three rats each and administered with the drug solution at the doses of 10 mg/kg, 100 mg/kg, and 1000 mg/kg body weight orally. They were observed for signs of toxicity for 24 hours. Four adult male Wistar rats were obtained and placed in phase two. One of the animals received 1200 mg/kg of the mixture, the second animal received 1600 mg/kg of the mixture, and the third animal received 2900 mg/kg of the mixture while the fourth animal received 5000 mg/kg of the mixture. The median lethal dose (LD<sub>50</sub>) of Dr Aladdin's seven Keys Herbal Mixture was calculated. The acute toxicity (LD<sub>50</sub>) test for Dr Aladdin's 7 Keys Herbal Mixture was calculated as;

$$LD_{50} = \sqrt{a \times b}$$

Where;

a = the maximum dose with 0% mortality = 1600 mg/kg

b = minimum dose with 100% mortality = 2900 mg/kg.

$$LD_{50} = \sqrt{1600 \times 2900} = 4640000$$

$$= \sqrt{4640000} = 2154.06 \text{ mg/kg.}$$

Therefore, the LD<sub>50</sub> of Dr. Aladdin's Seven Keys Herbal Mixture via oral route was found out to be 2154.06 mg/kg.

## 2.4. Experimental Design and Protocol

The weight of the male Wistar rats was measured by using an electronic weighing balance. After acclimatization and acute toxicity tests, twenty (20) animals were randomly divided into five (5) groups (1-5) of 4 rats each. Four groups (2, 3, 4, and 5) were treated while group 1 served as control. The groups were treated as shown in table 1 below. The test substance was administered orally to the rats by carefully inserting the syringe with cannula affix into the oral cavity of the rats.

**Table 1** Experimental Design and Protocol

Group	Materials administered (feed and water (all groups))	Duration
1	Distilled water + feed only	28 days
2	250mg/kg of Dr. Aladdin's seven keys herbal mixture	28 days
3	500mg/kg of Dr. Aladdin's seven keys herbal mixture.	28 days
4	1000mg/kg of Dr. Aladdin's seven keys herbal mixture.	28 days
5	2000mg/kg of Dr. Aladdin's seven keys herbal mixture.	28 days

## 2.5. Blood Sample Collection

Blood samples were collected at the end of the third week (where applicable) and the sixth week (at the end of the experiment) by cardiac puncture after anaesthetizing with chloroform. Two (2) milliliters of blood were collected from three rats in each group including the control group. The sera were separated after centrifuging the blood sample using the centrifuging machine, collected, refrigerated at -20°C and assayed for the following sex hormones, testosterone and follicle-stimulating hormone (FSH).

## 2.6. Organ collection

While still under the influence of anaesthesia, the testes from each rat were quickly and carefully exposed, retrieved and weighed following the incision of the scrotum. The epididymis was then separated and the epididymal fluid collected from the caudal part for the assessment of sperm count, motility and morphology were assessed. The testis was then fixed in freshly prepared Bouin's fluid.

### 2.6.1. Sperm count

Sperm count was carried out using the method described by [9]. A graduated cylinder was used to dilute the semen in sodium bicarbonate formalin diluting fluid in the proportion of 1 in 20. A Pasteur pipette was then used to an improved Neubauer ruled chamber with well-mixed diluted semen and allowed to stand for 3-5 minutes and allowed to settle. The 10X objective was used to view, with the condenser iris closed sufficiently to give good contrast, and the number of spermatozoa in 2 large squares (2 sq mm) counted. The number counted was multiplied by 105.

### 2.6.2. Sperm motility

Sperm motility was carried out using the method described by [9]. A well-mixed drop of liquefied semen was placed on a slide, evenly distributed and covered with a cover slide. The 40X objective was used to observe and count. A total of 100 spermatozoa were counted out of which the number of motile ones was recorded in percentage.

### 2.6.3. Sperm morphology

Sperm morphology was carried out using the method described by [9]. A thin smear of liquefied well-mixed semen was made on a slide. While still wet, the smear was fixed with diluted 1:20 in 10% neutral buffered formalin and with the aid of a light microscope at x 400 magnification. The smear was counterstained with dilute (1 in 20) Loeffler's methylene blue for 2 minutes and washed off with water. The smear was drained and allowed to air-dry. The smear was examined

for normal and abnormal spermatozoa using the 40X objective. The 100X objective was used to confirm abnormalities. A count of 100 spermatozoa was made and the percentage showing normal and abnormal morphology was recorded.

## **2.7. Tissue Processing and Photomicrography**

The fixed testis was processed at the Histology Laboratory, International Centre for Research and Cancer Diagnosis, Abakiliki, Ebonyi State. The following procedures were used for tissue processing and Photomicrography:

### *2.7.1. Tissue Fixation*

The testes from each rat were fixed in containers (labelled 1, 2, 3, 4, 5) containing freshly prepared Bouin's solution immediately after they were retrieved.

### *2.7.2. Dehydration of the Tissue*

The fixed tissue was first immersed in a bath containing 70% alcohol and left overnight followed by transferring tissues into different ascending baths of alcohol ranging from 50%, 70%, 90%, 95%, and 100% (absolute) alcohol for 30 minutes each.

### *2.7.3. Clearing / Dealcoholisation of the Tissue*

On removing the tissue from absolute alcohol, the tissue was passed through 3 changes of xylene for 30 minutes each.

### *2.7.4. Wax Impregnation/ Infiltration of the Tissue*

The cleared tissue was impregnated and infiltrated with molten paraffin wax to remove the clearing agent (xylene) in a hot oven at 60 °C by passing it through three changes of molten paraffin in a hot air oven for 30 minutes.

### *2.7.5. Embedding and Mounting of the Tissue*

Molten paraffin wax was poured into the embedding mould. With the aid of warm forceps, the paraffin-impregnated tissue was transferred from the paraffin wax bath to the mould. Using the heated forceps, the tissue was oriented until it lay in the desired plane. The corresponding label was removed from the paraffin wax bath and placed against the side of the mould adjacent to the tissue. The mould container was transferred to an ice block to solidify. On solidification, the tissue block was removed from the mould and attached to a wooden block using a heated knife.

### *2.7.6. Sectioning of the Tissue*

After embedding, the wooden blocks which had the attached tissue blocks were placed in the block holder in the microtome parallel to the microtome knife. Prior to sectioning, the tissues were first trimmed so as to expose the surface of the tissue by adjusting the microtome knife to 15 µm. Sections were cut with the rotary microtome and the ribbons were placed onto 20% alcohol on a large slide (5cm x 7.5cm slide) to remove minor folds and creases from sections. The ribbon was gently placed on a water bath preheated to about 45 °C so as to float out the tissues. The tissues were collected with clean slides, allowed to dry, and labelled, using a diamond pencil. The slides were further dried on a hot plate at 5 °C for the tissues to adhere to the slide.

### *2.7.7. Staining of the Tissue Sections*

The sectioned tissue slide was stained using Ehrlich's haematoxylin and Eosin staining technique for general tissue structure.

### *2.7.8. Light Microscopy and Photomicrography*

The stained sections were examined under the light microscope with magnification of X150 and X600 for histological changes. The photomicrograph of each slide was taken using a coloured film with an Olympus photomicroscope (BX 51).

## **2.8. Statistical Analysis**

Data analyses were done with SPSS 16.0 software and the results obtained were represented as Mean±SD. Statistical analysis included analysis of variance, the Duncan test for multiple comparisons, and the Mann-Whitney analysis. Significance was defined as a value of less than 0.05.

### 3. Results

#### 3.1. Physical and Behavioural Observations

The experimental rats were observed for response with respect to the overall appearance, food intake, water intake, animal behaviour and coordination, urination and respiration in control and treated animals. All the rats in the group (1) that served as control were fed with feed and distilled water. They were apparently normal and had no unusual behaviours (circling, head flicking, head searching, biting, licking, self-mutilation and walking backwards). Animals treated with 250 mg/kg (Group 2) and 500 mg/kg body weight (Group 3) of Dr Aladdin’s seven keys herbal mixture were restless, aggressive towards each other, disorganization with characteristic piloerection (erection of the hairs on the surface of the skin to conserve heat) after which such signs subsided and later stopped by the 2nd week. Rats in group 4 reduced food and water intake, and respiration difficulty (including shortness of breath and rattling noise with breathing) for the second week with mortality recorded in the third week of the experiment. Animals in group 5 which received 2000 mg/kg body weight of Dr Aladdin’s seven keys herbal mixture were restless, aggressive, showed disorganization, reduced food and water intake, and respiration difficulty for 4 weeks, and one mortality on 3rd day of the 4th week.

#### 3.2. Body weight of various groups when compared with control

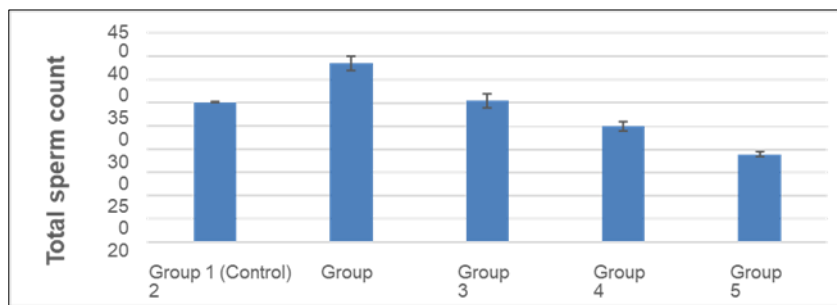
The mean body weight of the Wistar rats showed a significant decrease in group 2 to group 5 that received Dr Aladdin’s seven keys Herbal mixture when compared to group 1 that served as the control. In the Relative testicular weight, there was an insignificant increase in groups 2 and 4, while a significant increase in group 5 when compared to group 1 (control) and group 3 that received the herbal mixture (Table 2).

**Table 2** Effect of Dr. Aladdin’s seven keys herbal mixture on body weight and Relative Testicular Weight of male Wistar rats

Groups	Body weight (g)	Relative Testicular weight (g)
Group 1 (control)	92.50±0.10	1.00±0.00
Group 2 (250 mg/kg)	35.00±2.00	1.50±0.50
Group 3 (500 mg/kg)	23.33±0.50	1.00±0.00
Group 4 (1000 mg/kg)	40.00±1.50	2.00 ±0.00
Group 5 (2000 mg/kg)	20.00±2.00	2.50 ±0.50

p<0.05, means significant when comparing with group (1) that served as control.

#### 3.3. Effect of Dr. Aladdin’s seven keys herbal mixture on sperm count.

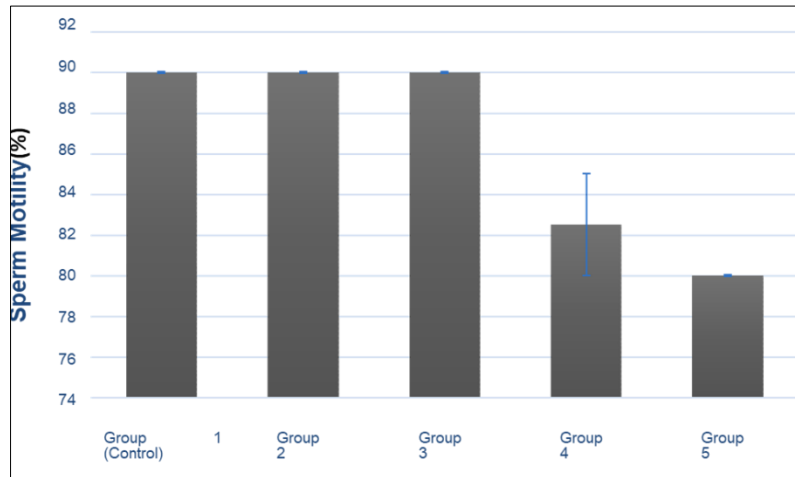


**Figure 1** Effect of Dr. Aladdin's Seven Keys herbal Mixture on Sperm Count

Below shows that there was an increase in sperm count in group 2 (385.00±15.00) which received 250 mg/kg of Dr Aladdin’s seven keys herbal mixture and group 3 (305.00±15.00) which received 500 mg/kg of Dr Aladdin’s seven keys herbal mixture, while a decrease in group 4 (250.00±10.00) and 5 (189.00±5.00) that received 1000 mg/kg and 2000 mg/kg of Dr Aladdin’s seven keys herbal mixture respectively when compared to group 1 (301.50±1.50) that served as control. The increase was significant in group 2, while the decreases were also significant in groups 4 and 5.

### 3.4. Effect of Dr. Aladdin’s seven keys herbal mixture on sperm motility

Below shows that the sperm motility in percentage was significantly decreased in groups 4 (82.50%±2.50) and 5 (80.00%±0.00) that received 1000 mg/kg and 2000 mg/kg of Dr Aladdin’s seven keys herbal mixture respectively, while group 2 (90.00%±0.00) and 3 (90.00%±0.00) that received 250 mg/kg and 500 mg/kg of Dr Aladdin’s seven keys herbal mixture respectively had similar values when compared to group 1 (90.00%±0.00).



**Figure 2** Effect of Seven keys Herbal mixture on Sperm Motility

### 3.5. Effect of Dr Aladdin’s seven keys herbal mixture on sperm morphology.

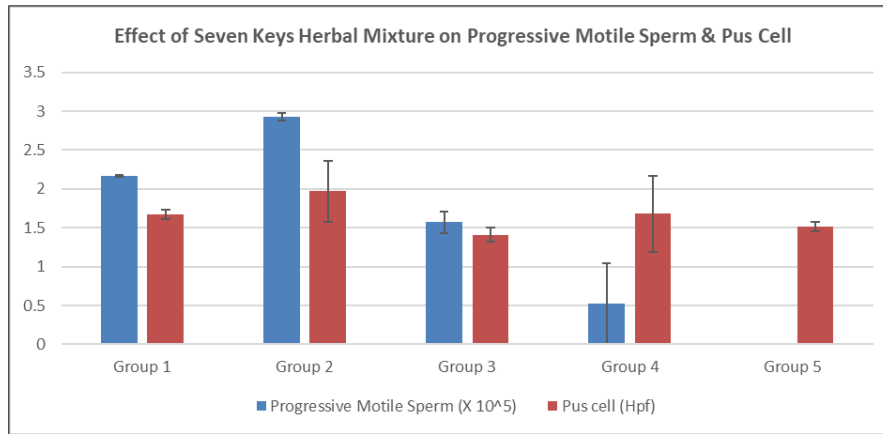
Table 3 shows that there was an insignificant increase in the normal sperm in group 2 (85.00%±5.00) that received 250 mg/kg of herbal mixture, while significant decreases were observed in groups 3 (57.50%±2.50), 4 (47.50%±7.50) and 5 (40.00%±5.00) that received 500 mg/kg, 1000 mg/kg and 2000 mg/kg of Dr. Aladdin’s seven keys herbal mixture respectively, when compared to group 1 (80.00%±0.00) that served as control. However, an increase in the number of abnormal sperm and sperm clumping was noted in groups 3 (27.50%±7.50; 15.00%±5.00), 4 (20.00%±10.00; 32.50%±17.50), and 5 (25.00%±10.00; 35.00%±15.00) that received 500 mg/kg, 1000 mg/kg and 2000 mg/kg of the herbal mixture respectively, when compared with control, although such increase was not statistically significant.

**Table 3** Effect of Dr Aladdin’s seven keys herbal mixture on sperm morphology

Groups	Normal sperm (%)	Abnormal sperm (%)	Sperm clumping (%)
Group 1 (control)	80.00±0.00	10.00±0.00	10.00±0.00
Group 2 (250mg/kg)	85.00±5.00	10.00±0.00	5.00±5.00
Group 3 (500mg/kg)	57.50±2.50	27.50±7.50	15.00±5.00
Group 4 (1000mg/kg)	47.50±7.50	20.00±10.00	32.50±17.50
Group 5 (2000mg/kg)	40.00±5.00	25.00±10.00	35.00±15.00

### 3.6. Effect of Dr Aladdin’s seven keys herbal mixture on the progressive motile sperm and pus cell.

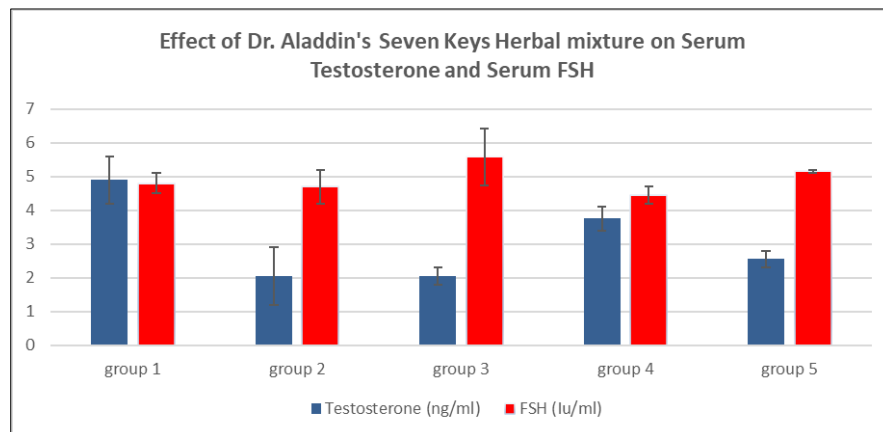
Below shows that there was an insignificant increase in the progressively motile sperm in group 2 (2.93±0.05) that received 250 mg/kg of Dr. Aladdin’s seven keys herbal mixture when compared to control, while group 3 (1.57±0.14), 4 (0.52±0.52) and 5 (0.01±0.00) that received 500 mg/kg, 1000 mg/kg and 2000 mg/kg of Dr Aladdin’s seven keys herbal mixture respectively, showed a decrease when compared to group 1 (2.17±0.01) that served as control. The decrease was significant in groups 4 and 5. The presence of Pus Cell was insignificantly increased in groups 2 (1.97±0.39) and 4 (1.68±0.49) that received 250 mg/kg and 1000 mg/kg of Dr Aladdin’s seven keys herbal mixture respectively, while an insignificant decrease in group 3 (1.41±0.09) and 5 (1.51±0.06) that received 500 mg/kg and 2000 mg/kg of Dr Aladdin’s seven keys herbal mixture respectively was observed, when compared to group 1 (1.67±0.06) that served as control that received only feed and water.



**Figure 3** Effect of Dr Aladdin's Seven Keys Herbal Mixture on Progressive Motile Sperm & Pus Cell

**3.7. Effect of Dr Aladdin's seven keys herbal mixture on serum testosterone and follicle stimulating hormone (FSH) levels.**

Below shows testosterone levels decreased significantly in groups 2 ( $2.05 \pm 0.85$ ), 3 ( $2.05 \pm 0.25$ ) and 5 ( $2.55 \pm 0.25$ ) that received 250 mg/kg, 500 mg/kg and 2000 mg/kg of Dr Aladdin's seven keys herbal mixture respectively when compared to control, while an insignificant decrease in group 4 ( $3.75 \pm 0.35$ ) that received 1000 mg/kg of Dr Aladdin's seven keys herbal mixture was observed when compared to group 1 ( $4.90 \pm 0.70$ ) that served as control and received only feed and water. In the FSH level, there was an insignificant decrease in groups 2 ( $4.70 \pm 0.50$  Iu/ml) and 4 ( $4.45 \pm 0.25$  Iu/ml) that received 250 mg/kg and 1000 mg/kg of Dr Aladdin's seven keys herbal mixture respectively when compared with control, while an insignificant increase in FSH was observed in group 3 ( $5.85 \pm 0.85$  Iu/ml) and 5 ( $5.15 \pm 0.05$  Iu/ml) that received 500 mg/kg and 2000 mg/kg of Dr Aladdin's seven keys herbal mixture respectively when compared to group 1 ( $4.80 \pm 0.30$  Iu/ml) that served as control and received only feed and water.



**Figure 4** Effect of Dr. Aladdin's Seven Keys Herbal mixture on Serum Testosterone and Serum FSH

**3.8. Histological findings**

Photomicrograph of group 1 (control) section of testis show normal testicular architecture with seminiferous tubule that is lined with Sertoli cell (SC) and production of spermatozoa (S) and the overall features appeared normal (figure 1a & 1b).

Photomicrograph of group 2 had the mild arrest of spermatogenesis (MAS) and infiltration of the inflammatory cell (MIIC) (figure 2a & 2b).

Photomicrograph of group 3 showed active seminiferous tubules with well-enhanced spermatogenesis (WES) and mild focal areas of haemorrhage (MFAH) (figure 3a & 3b).

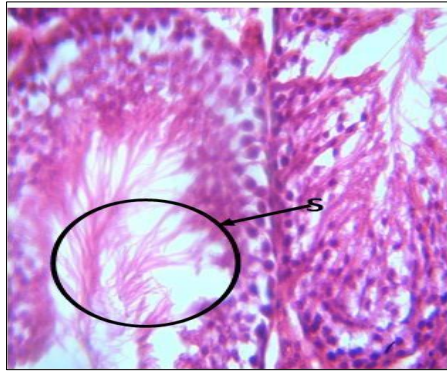


Figure1a (H & E X400).

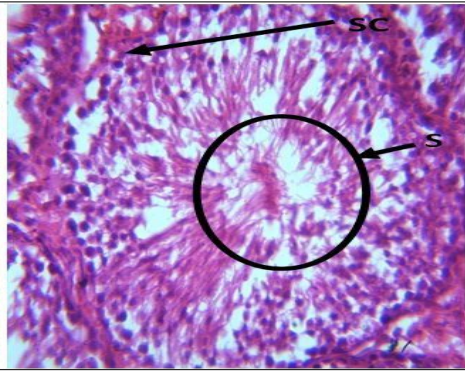


Figure 1b (H & EX400).

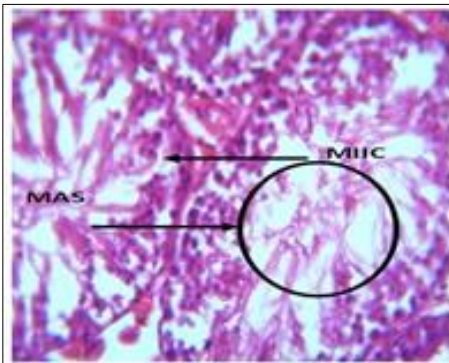


Figure2a(H & E x400)



Figure 2b(H & E x400)

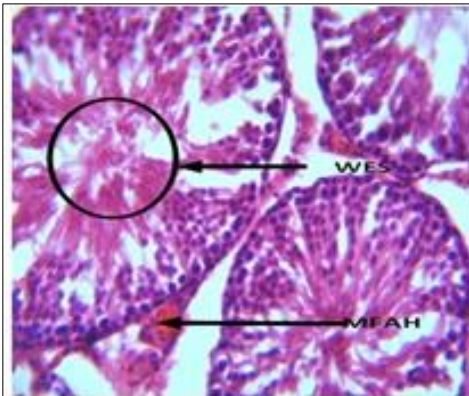


Figure 3a (H & E X400)

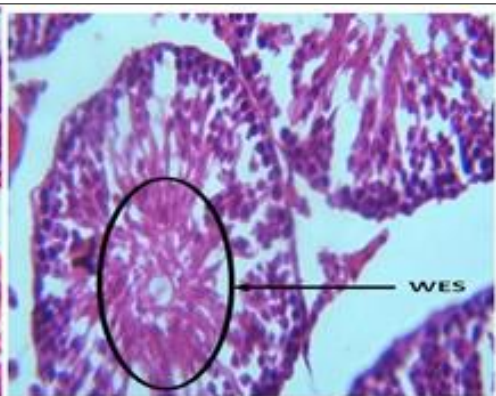


Figure 3b (H & E X400)



Figure 4a(H & E X400)

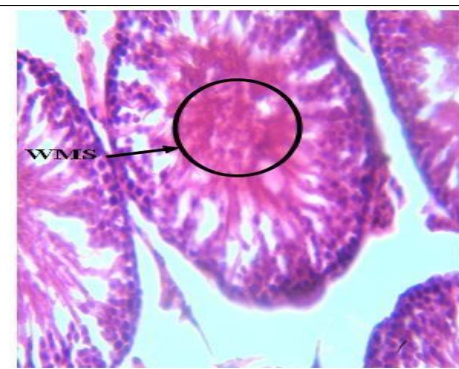


Figure 4b(H & EX400)



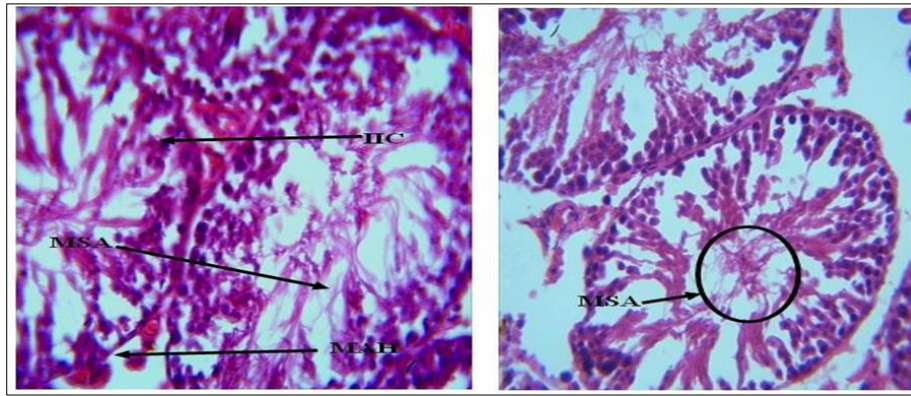


Figure 5a (H & E X400)

Figure 5b (H & EX400)

Photomicrograph of group 4 demonstrated testicular tissue with mature spermatozoa (WMS) (figure 4a & 4b).

Photomicrograph of group 5 showed testicular tissue with moderate spermatogenic arrest (MSA), mild area of haemorrhages MAH) and infiltration of the inflammatory cell (IIC) (figure 5a & 5b).

#### 4. Discussion

This current study showed that oral exposure of the rats to high doses (1000 mg/kg and 2000 mg/kg) of Dr Aladdin's seven keys herbal mixture caused a variety of physical disturbances such as restlessness, aggressiveness towards each other, and disorganization with characteristic piloerection, although the signs subsided and later stopped as the test substance administration progressed. The rats also showed reduced food and water intake with respiration difficulty such as shortness of breath and rattling noise with breathing. The death occurred during the course of administration of this herbal mixture. The findings are in line with that of [10] and [11] who reported cases of restlessness, and aggressiveness, and showed disorganization, and reduced food and water intake in animals treated with high dose (2500 mg/kg) of herbal mixture. The reasons behind these physiological changes and behavioral activities still may be likeable to the high dose of the herbal mixture.

The body weight in all experimental groups revealed an increased body weight although the degree of weight gain was lesser in treated groups when compared with the control. This could be a result of loss of appetite and little reduction in food intake by the animals in the first week of the substrate administration. Similarly, [12] reported decreased food intake, weight loss, and cell and tissue death following sub-chronic oral toxicity testing of herbal mixture in experimental animals. However, [13] reported weight gains in livestock fed with Dr Aladdin's seven keys herbal mixture and attributed such to its ability to cause changes in general metabolic status, and body and organ weight of the animals. Testicular weight in all experimental groups showed statistically insignificant differences when compared with control (group 1) except for group 5 administered with 2000 mg/kg. The insignificant increases in relative testicular weight could be a result of the presence of haemorrhage and inflammatory cells seen in the histological micrograph of the testes.

Findings from this study also revealed that rats fed with the herbal mixture showed a significant decrease in serum levels of testosterone ( $p < 0.05$ ) in groups treated with 250 mg/kg, 500 mg/kg and 2000 mg/kg respectively, while an insignificant decrease ( $p > 0.05$ ) was observed in the group that was treated with 1000 mg/kg. This could be attributed to the presence of alkaloid which has been found to cause drops in testosterone levels when consumed in excess by inhibiting the production of testosterone [14]. Similarly, [14] reported a significant reduction in serum concentration of testosterone. Likewise, [15] reported that Dr Aladdin's seven keys herbal mixture inhibited the production of testosterone in male Wistar rats. The garlic and other plant constituents present in Dr Aladdin's seven key herbal mixtures have been documented to contain high amounts of alkaloids, saponins, and tannins.

There were insignificant differences in serum levels of follicle-stimulating hormone (FSH) when compared to the control group. This study contradicts the work done by [14] who reported a significant reduction in serum concentration of FSH, as well as work done by [15] reported that Dr Aladdin's seven keys herbal mixture inhibited the production of FSH in male Wistar rats. Total Sperm count in test groups showed a significant decrease ( $p < 0.05$ ) when compared with control. This study is in accordance with the work done by [16] who reported a significant decrease in the total sperm count following administration of Dr Aladdin's seven keys herbal mixture on male Wistar rats. This may be due to the

presence of tannins and alkaloids which when taken in excess causes oxidative stress produced by reactive oxygen species (ROS). These free radicals tend to overwhelm the antioxidant defences, causing damage to DNA and the mitochondria of cells, as well as cell and tissue deaths [16].

The sperm motility in groups that took the highest doses of 1000 mg/kg and 2000 mg/kg of the herbal mixture was significantly decreased ( $p < 0.05$ ) when compared to the control. This agrees with [17] who reported a significant decrease in the total sperm motility following the administration of Dr Aladdin's seven keys herbal mixture on male Wistar rats. This effect occurred maybe a result of the presence of excess amounts of tannins and alkaloids which could have caused oxidative stress. Additionally, [11], reported that Dr Aladdin's seven keys herbal mixture contains chemical constituents such as tannins that when taken at high dose possibly will turn into pro-oxidant, not minding, tannins is categorized as antioxidants. The presence of testicular toxicants are evident in fluid secretion and tubular contraction, this elucidated the association with the observed findings. In many cases, this is a secondary consequence of germ cell loss, but in others, it appears to be an early event and probably represents disturbed Sertoli cell function [17]. Since tubular fluid secretion is an androgen-dependent function, such compounds can cause significant reductions in not only serum testosterone levels but also fluid secretion and reduce tubular diameter in the testis as a secondary effect.

Histological findings showed a dose-dependent alterations in testicular architecture such as the mild arrest of spermatogenesis, mild infiltration of inflammatory cells, and mild focal haemorrhage in the testicular architecture. This could have resulted from oxidative produced by reactive oxygen species (ROS) produced by some constituents of Dr Aladdin's seven keys herbal mixture. These free radicals tend to cause DNA damage, and interruption in important biochemical processes, as well as cell and tissue deaths [16]. This agrees with the reports of [1,18]. They reported a significant decrease in spermatocytes, spermatid and Leydig cells in male Wistar rats.

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## 5. Conclusion

Administration of graded doses of Dr Aladdin's seven keys herbal mixture induced dose- dependent mild to moderate disturbances and alterations in testicular parameters of male Wistar rats such as reduced sperm count, and sperm motility. Serum levels of hormones such as testosterone were also significantly reduced in a dose-dependent manner although serum levels of follicle-stimulating hormone (FSH) were unaltered. Histological findings also revealed the herbal mixture to cause the mild arrest of spermatogenic cells, infiltration of inflammatory cells and mild focal areas of haemorrhage at a high dose.

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## Compliance with ethical standards

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### *Disclosure of conflict of interest*

The authors declare no conflict of interest.

### *Statement of ethical approval*

The institution's guide for the care and use of laboratory animals is strictly adhered to.

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