**Scholars Journal of Applied Medical Sciences (SJAMS)** **ISSN 2320-6691 (Online)**
**Sch. J. App. Med. Sci., 2017; 5(9D):3748-3754** **ISSN 2347-954X (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublishers.com DOI:10.36347/sjams.2017.v05i09.049

# Effects of the Aqueous Extract of *Evolvulus alsinoides* Linn on Sperm Parameters and the Histology of the Testis in Adult Wistar Albino Rats

**Isa ZA*, Garba SH.**
Department of Human Anatomy, College of Medical Sciences, University of Maiduguri, Maiduguri.

***Corresponding author**
*Isa ZA*

**Article History**
*Received: 10.09.2017*
*Accepted: 16.09.2017*
*Published: 30.09.2017*

**Abstract:** *Evolvulus alsinoides* Linn. Is a medicinal plant that belongs to the family convolvulaceae. This study seeks to evaluate the effects of the aqueous extract of *Evolvulus alsinoides* Linn. on sperm parameters and the testis. A total of fifty (50) Wistar albino rats weighing 135-150g were used in this research. The rats were divided into five (I-V) Groups of ten (10) rats each. Group I was designated as the control group and was administered with distilled water, while groups II, III and IV were administered with 150 $mgkg^{-1}$, 250 $mgkg^{-1}$ and 350 $mgkg^{-1}$ of the extract respectively. Group V received 350 $mgkg^{-1}$ and was allowed for 14-day recovery period. The rats in the treated groups were administered with the extract by gavage and approximately the same time daily for 28 days. Sperm parameters (sperm concentration, morphology and motility) were estimated using standard established methods. Administration of 250 $mgkg^{-1}$ and 350 $mgkg^{-1}$ of the extract caused a significant ($P$<0.005) decrease in body weight gain. While the post recovery group revealed a significant ($P$<0.005) increase in body weight gain. Absolute testicular weight showed a significant decrease at a dose of 350 $mgkg^{-1}$. Ratio of testis-body weight did not show any significant variation in all the treated groups. Sperm parameters in all the treated groups as well the post recovery group did not show any significant difference. Histological sections from the control group and the group administered with 150 $mgkg^{-1}$ were normal. While the groups administered with 250 $mgkg^{-1}$, 350 $mgkg^{-1}$ and the post recovery group showed interstitial vascular congestions. In conclusion, no obvious effects were observed on sperm parameters. However, the vascular congestions observed at histological level may hinder blood and nutrient supply to the testicular tissues and hence affect fertility.
**Keywords:** *Evolvulus alsinoides*, Testis, Epididymis, Sperm, Morphology, Motility, Vascular congestion

## INTRODUCTION

Many medicinal plants are used in Africa and elsewhere in the world with the notion that they can cure diseases, boost immunity, enhance fertility or improve the functions performed by the various systems of the body. Such medicinal plants are usually consumed by many with little or no caution as to the side effects they may produce.

*Evolvulus alsinoides* is one of such medicinal plants commonly used worldwide. It is a small perennial herb with a small woody and branched rootstock; it belongs to the family convolvulaceae. It is called dwarf morning glory in English [1]. In Nigeria, *Evolvulus alsinoides* Linn. Is called kaafi malam (Hausa), ndottiyel (Fulfulde) and efunle in Yoruba [2]. Its medicinal uses include use as adaptogenic, antiphlogistic, antipyretic, antiseptic, aphrodisiac, febrifuge, and against asthma, bronchitis, syphilis, epilepsy, insanity, nervous debility and loss of memory [3-5]. In Africa, *Evolvulus alsinoides* is used for the treatment of low spirit and depression [6]. In Nigeria, *Evolvulus alsinoides* is used as stomachic, and against asthma and bronchitis [7, 8].

## MATERIALS AND METHODS

### Collection and Identification of Plant Materials

The whole plant (*Evolvulus alsinoides* Linn.) used in this study was obtained from an herb seller at Monday market, Maiduguri, Borno State. The plant was identified and authenticated by a plant taxonomist, Department of Biological Sciences, University of Maiduguri. A specimen voucher (EA.01) of the plant was prepared and deposited at the herbarium.

### Extraction Procedures

Extraction of the plant material was conducted as described by the World Health Organization [9]. In order to prevent ultra-violet rays from sunlight to affect the phytochemical constituents present in the plant

material, the plant material was air-dried under shade for one week. Air-dried plant material was pulverized using mortar and pestle. One hundred grams (100g) of the pulverized plant material was subjected to exhaustive soxhlet extraction in distilled water (500ml) for 72h at 60 ˚C. The extract was further concentrated in a water bath at temperature of 40-60˚C until a dark sticky residue with a constant weight was obtained. A mean extract yield of 12.3g w/w was obtained. The extract was stored in stoppered container in a refrigerator at -4 ˚C until required and stock solution (50 mg/ml) was prepared by dissolving 1g of the extract in 20 ml of normal saline.

**Animal and Husbandry**

A total of fifty (50) young adult male Wistar rats weighing (134-150g) were obtained from National Veterinary Research Institute (NVRI), Vom, and Plateau State, Nigeria. The rats were weighed and individually identified by colour tattoo. They were acclimatized for two weeks after which they were screened for body weight gain and any signs of diseases. The rats were kept in plastic cages at room temperature of 32 $\pm$ 4 ˚C and < 30% relative humidity with a 12 hours light/dark cycle. Standard laboratory diet (pelletized grower feed from Grand Cereals and Oil Mills Ltd, Jos, Nigeria) and water was provided to the rats ad libitum.

**Experimental Design**

Fifty (50) young adult male Wistar rats were used for this study. The rats were divided into five (5) Groups (I-V) of 10 rats each. Group I was designated as the control group. Groups II, III and IV were administered with 150 mg/kg, 250 mg/kg and 350 mg/kg respectively. While Group V was administered with 350 mg/kg and allowed a post recovery period of 14 days to observe for reversibility or delayed effect. The extract was administered to the treatment groups daily by gavage for 28 days. At the end of the administration period, sperm parameters were determined and the testes were harvested for histopathological analyses.

**Epididymal Sperm Concentration**

Fresh epididymis was trimmed of fats and cut into smaller pieces and ground in a mortar containing 1ml of normal saline and homogenized. Further 1.5ml of normal saline was added to the homogenate and filtered using a nylon mesh. The homogenate was then stained with 2% Eosin in normal saline. Haemocytometer (AC1000-Hawsky Medical and Laboratory Equipment, UK) was covered with a coverslip and charged with 10µl of the homogenate. The spermatozoa in the eight haemocytometer chambers (except the central erythrocyte chamber) were counted [10, 11]. The count was performed under the microscope (Olympus OLCHBS/R Microscope, Japan) at a magnification of ×400.

**Sperm Motility**

The determination of sperm motility was carried out according to WHO [9]. Ten microlitres (10µl) of the homogenate from the cauda epididymis was deposited onto a clean microscope slide, coversliped and viewed under the light microscope. At least 200 sperm cells under a minimum of five (5) microscopic fields were assessed and evaluated for motility. The motility of each spermatozoon was graded as: progressive motility (PR), meaning spermatozoon moving actively, either linearly or in a large circle, regardless of speed; non-progressive motility (NP), meaning all other patterns of motility with an absence of progression, e.g. swimming in small circles, the flagellar force hardly displacing the head, or when only a flagellar beat can be observed; and immotility (IM), meaning no movement at all.

**Sperm Morphology**

In this study, the method used by Garba and others [11] for the evaluation of sperm morphology was employed. A homogenate prepared from the cauda epididymis previously used for sperm count and sperm motility was used. To a clean glass slide placed on flat surface, a drop of the sperm suspension was released on to a point approximately one centimeter (1cm) from the edge of the slide along its long axis. Another slide was held at an angle of approximately 45˚ to the first slide, making sure that the tip of the second slide was fully in contact with the sperm suspension and end-to-end in contact with the first slide. The second slide was then pushed down the full length of the first slide containing the sperm suspension. Each slide containing the smear was identified by writing, in pencil, the identification number of the rat used. The slides were allowed to dry overnight in a dust-free atmosphere, and viewed under the microscope at a magnification of ×1000. From each rat, 200 spermatozoa were screened for morphological abnormalities [9].

**Absolute Testicular Weight**

From each rat, the left and right testes were removed, weighed and mean testicular weight was calculated as the absolute weight.

**Relative Testicular Weight**

Testicular weight relative to body weight was evaluated as was performed previously by de Sousa and others [12] with little modifications. For each rat, the relative testicular weight was obtained by evaluating the ratio of absolute testicular weight to body weight.

**Histopathological Analyses**

At the end of the administration period, the testes were processed histologically [13, 14]. The

tissues were fixed in Bouin's fluid, dehydrated using graded series of alcohol and then embedded in paraffin wax. Tissues were sectioned at 5µm, cleared in xylene and then stained with haematoxylin and eosin. Microscopic examinations of the tissue slides were then made.

**Statistical Analysis**

Statistical analysis was performed as was previously employed by [15]. Data obtained were analysed using statistical software (SPSS) version 16.0 by one way analysis of variance (ANOVA) and were expressed as the mean ± standard error of mean (SEM). Differences between means of the various groups were determined; *p*-values less than 0.05 were considered statistically significant.

**RESULTS**

**Effect of the Extract on Mean Body Weight and Mean Testicular Weight of Male Rats**

The results of the effect of the extract on the mean body weight and mean testicular weight are shown in Table 1. The mean body weight in the group administered with 150 mg $kg^{-1}$ did not show significant difference from the mean body weight of the control group. The group administered with 250 $mgkg^{-1}$ showed a significant ($P$<0.05) decrease in the mean body weight gain. The group administered with 350 $mgkg^{-1}$ also showed a significant ($P$<0.05) decrease in the mean body weight gain when compared with the control group. However, the post recovery group showed a significant increase in the mean body weight gain when compared with the group administered with 350 mg $kg^{-1}$. The mean absolute testicular weight showed significant ($P$<0.05) decrease when compared with the control group. The post recovery group showed a significant ($P$<0.05) increase in testicular weight. However, the mean relative testicular weight of all the treated groups including the post recovery did not show any significant change.

**Effect of the Extract on Sperm Parameters**

Sperm concentrations in all the treated groups (except the post recovery group) were compared with the sperm concentration in the control group. No significant difference was observed when all the groups were compared with the control group. The sperm concentration in the post recovery group was compared with the concentration in the group administered with 350 $mgkg^{-1}$. No significant difference was also observed (Table 2). Sperm morphology in all the treated groups did not result into any significant change. Also, the result of sperm morphology in the post recovery group did not significantly differ from the result of the group administered with 350 $mgkg^{-1}$ (Table 2). Sperm motility in all the treated groups did not differ significantly from the control group. Also, no significant difference was observed when the post recovery group was compared with the group administered with 350 $mgkg^{-1}$ (Table 2).

**Table−1: Effect of 28-Day Oral Administration of the Aqueous Extract of *Evolvulus alsinoides* Linn. On Mean Body Weight and Mean Testicular Weight in Male Rats**

| | Dosages administered ($mgkg^{-1}$) | | | | |
|---|---|---|---|---|---|
| **Parameter Control** | **150** | **250** | **350** | **350PRG** | **P-values** |
| **Body Weight** | | | | | |
| Day 0 (g) 0.0822 | 138.70 ± 1.90 | 139.56 ± 1.07 | 140.00 ± 1.17 | 145.00 ± 0.60 | 142.00 ± 0.71 |
| Day 28 (g) 0.0642 | 175.30 ± 1.18 | 175.00 ± 0.95 | 173.70 ± 0.92 | 172.10 ± 0.60 | 173. 20 ± 0.63 |
| Weight difference (g) <0.0001 | 36.60 ± 0.58 | 35.44 ± 0.44 | 33.70 ± 0.68 | 27.10 ± 1.02 | 31.20 ± 0.68 |
| ***Absolute Testicular Weight (g)** 0.0007 | 1.36±0.01 | 1.35±0.02 | 1.33±0.01 | 1.28±0.01 | 1.30±0.01 |
| ****Relative Testicular Weight ($\times10^{-3}$)** 0.783 | 7.7 ± 0.20 | 7.0 ± 0.20 | 7.6 ± 0.20 | 7.5 ± 0.20 | 7.5 ± 0.20 |

N=10, Results are presented as Mean±SEM. PRG=Post Recovery Group: Sacrificed 14 days after the last administration of the extract.

***Absolute Testicular Weight= (Left Testis + Right Testis)/2

****Relative Testicular Weight= Absolute Testicular Weight /Body Weight

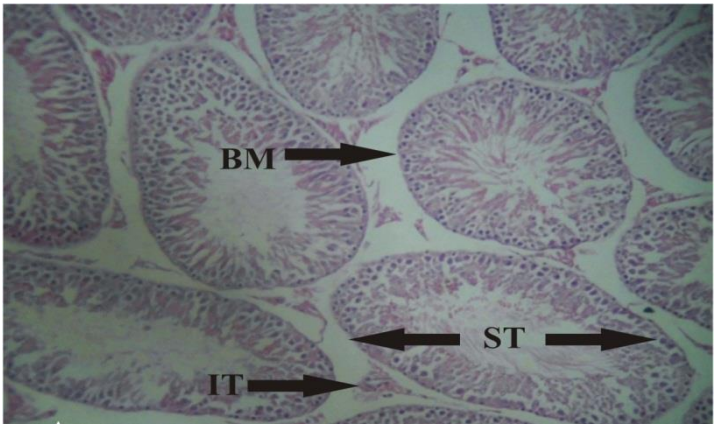
**Table−2: Effect of 28-Day Oral Administration of the Aqueous Extract of *Evolvulus alsinoides* Linn. On Sperm Parameters in Rats**

| | Dosages administered (mgkg$^{-1}$) | | | | |
|---|---|---|---|---|---|
| Parameter Control | 150 | 250 | 350 | 350PRG | P-values |
| **Sperm Concentration** (millions/ml) | 51.20 ± 0.49 | 51.2 ± 0.44 | 51.40 ± 0.37 | 52.40 ± 0.56 | 52.6 ± 0.56 0.356 |
| **Sperm Morphology (%)** | 93.20 ± 0.08 | 92.80 ± 0.63 | 94.00 ± 0.94 | 94.70 ± 0.79 | 94.50 ± 0.89 0.352 |
| **Sperm Motility (%)** | 83.50 ± 1.16 | 83.50 ± 0.86 | 85.70 ± 0.99 | 86.10 ± 0.88 | 85.40 ± 0.70 0.126 |

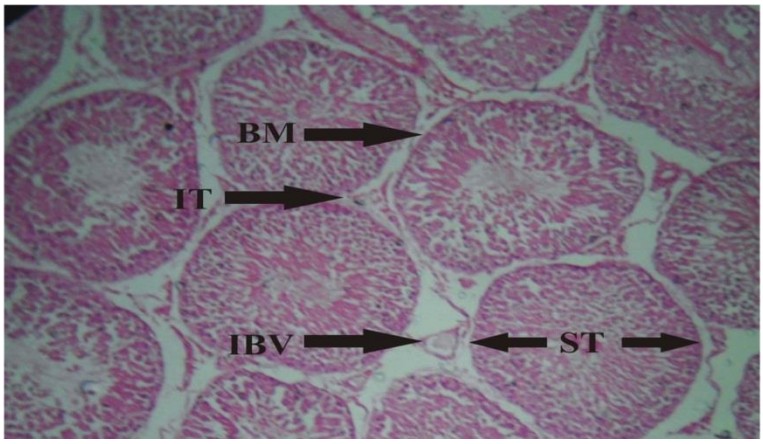
N=10, Results are presented as Mean±SEM. PRG=Post Recovery Group: Sacrificed 14 days after the last administration of the extract

**Effect of the Extract on the Histology of the Testis**

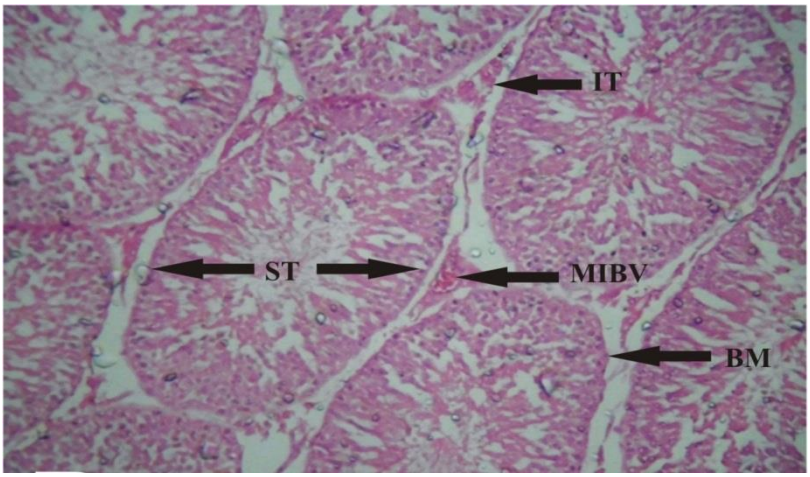
The effects of the extract on the histology of the testes were evaluated. Sections from the control group showed normal seminiferous tubule (ST), basement membrane (BM) and interstitial tissue (Fig. 1). Sections of the testes from the group administered with 150 mgkg$^{-1}$ did not result into apparent histological changes (Fig. 2), dose of 250 mgkg$^{-1}$ resulted into unremarkable interstitial tissue (IT), seminiferous tubule (ST) and mild congestion of interstitial blood vessel (Fig. 3). Figure 4 shows a histological section from the testis of rat administered with 350 mgkg$^{-1}$. The section showed conspicuous congestion of interstitial blood vessel (CIBV) and unremarkable seminiferous tubule (Fig. 4). Sections from the post recovery group (Fig. 5) also showed conspicuous congestion of interstitial blood vessel (CIBV). All slides were photomicrographed at magnification of ×100



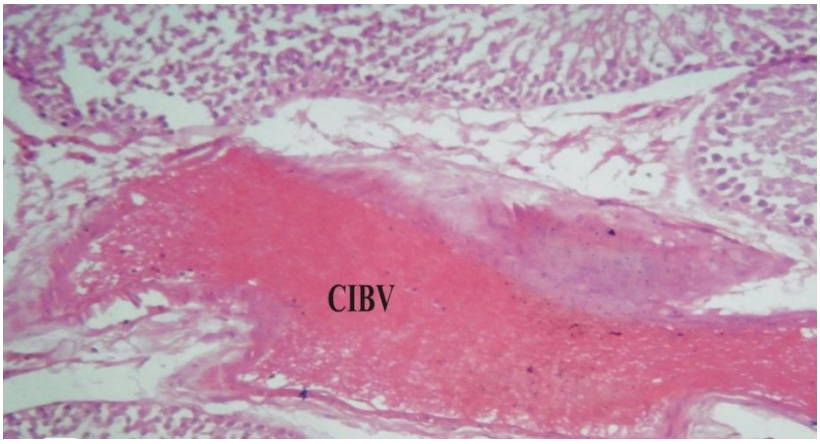

**Fig–1: Photomicrograph of transverse section of testis of rat from the control group (IT: interstitial tissue; ST: seminiferous tubule; BM: basement membrane) H and E ×100.**



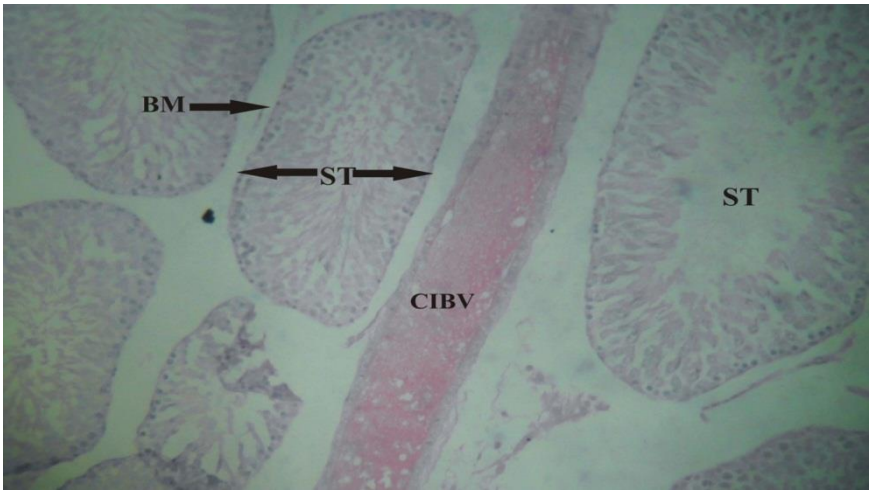

**Fig–2: Photomicrograph of transverse section of testis of rat from the group administered with 150 mgkg$^{-1}$ (IBV: interstitial blood vessel; IT: interstitial tissue; ST: seminiferous tubule; BM: basement membrane) H and E×100.**




**Fig–3**: **Photomicrograph of transverse section of testis of rat from the group administered with 250 mgkg$^{-1}$ (BM: basement membrane; IT: interstitial tissue; ST: seminiferous tubule; MIBV: mild congestion of interstitial blood vessel) H and E×100.**




**Fig–4**: **Photomicrograph of transverse section of testis of rat from the group administered with 350 mgkg$^{-1}$ (CIBV: conspicuous congestion of interstitial blood vessel) H and E ×100.**




**Fig–5**: **Photomicrograph of transverse section of testis of rat from the post recovery group administered with 350 mgkg$^{-1}$ (BM: basement membrane; ST: seminiferous tubule; CIBV: conspicuous congestion of interstitial blood vessel) H and E ×100.**

## DISCUSSION

Dose-dependent decrease in body weight gain observed could be attributed to dose difference. There was significantly lowest weight gain ($P<0.001$) among the group that received 350 $mgkg^{-1}$. However, the weight improved following the two weeks recovery period. The increase in body weight gain suggests that there was recovery after withdrawing the extract. The reduced body weight gain might be attributed to the presence of various phytochemicals present in the extract. Although, no research was previously conducted on the effect of *Evolvulus alsinoides* Linn. On body weight but other plant extracts containing phytochemicals similar to those present in the extract of *Evolvulus alsinoides* Linn. Were shown to cause decrease in body weight. For example, terpenoids from Glycyrrhiza glabra and Coleus forskohlii promote weight loss. Tannins from Allium sativum, Garcina cambognia, Habiscus sabdariffa and Panex ginseng have all being found to cause weight loss [16]. Tannins have the effects of reducing body weights in rats, rabbits, pigs and poultry. They do so by binding to food proteins and carbohydrates, or by binding to digestive enzymes [17, 18]. Roots of Withania somnifera promote weight loss without negative side effects. In humans, it caused significant reductions in serum total cholesterol, triglycerides and low density lipoproteins [19]. Saponins reduce protein digestibility probably by the formation of sparingly digestible saponins-protein complexes. Saponins from different sources have been shown to lower serum cholesterol in animals and humans [20]. Significant decrease in the mean absolute testicular weight corresponds to decrease in body weight.

The present study revealed that the aqueous extract of *Evolvulus alsinoides* Linn. is one of the plant extracts that have no significant effect on sperm parameters. However, other plants that have shown no effect on sperm parameters include Licorice extract [21], Ruta graveolens L. extract [22] and Moringa oleifera seed extract [23]. The histopathological analysis of the testes from the group administered with 150 $mgkg^{-1}$ did not show any difference from the control group. However, mild vascular congestion was observed in the interstitial blood vessels in the group administered with 250 $mgkg^{-1}$. Such a vascular congestion was very conspicuous in the group administered with 350 $mgkg^{-1}$ and the post recovery group. It can be concluded that vascular congestions seen in the group administered with 350 $mgkg^{-1}$ and the post recovery group might be as a result of the dose level. Vascular congestion is a result of impairment of venous outflow [24] which itself may be caused by varying endogenous compounds. For instance, inhibition of prostaglandins synthesis, since these compounds are involved in the regulation of blood flow in the testes [25], or could be a consequence of inhibition of vasodilators e.g., serotonin and nitric oxide [26]. Though, the present study did not look into the above mentioned causes of vascular congestion, they should not be excluded or neglected as the possible causative factors of vascular congestion observed in this study. The above mentioned fact is not enough as a reason to declare that the extract has no negative effect on male rat fertility. The vascular congestions noticed from sections of the testes, may disrupt the nutrient supply to testicular tissues which depends on diffusion through blood-testis barrier. Reduction or total cessation of nutrient supply to living tissues results in reduced function or necrosis. Reduced androgenizes or death of germ cells is a negative effect on fertility. Therefore, the extract may have negative effect on male fertility including sperm parameters when administered beyond the study period of 28 days, or at doses higher than used in the present study.

## CONCLUSION

Oral administration of *Evolvulus alsinoides* Linn. Was found to cause no effect on sperm parameters, Leydig cells, Sertoli cells and spermatogenic cells. However, a dose-dependent significant decrease in the mean testicular weight and interstitial vascular congestions were observed. These may affect male fertility.

## REFERENCES

1. NRCS U. The PLANTS Database. Baton Rouge, LA: National Plant Data Center. Online: http://plants. usda. gov. 2007.
2. Austin DF. *Evolvulus alsinoides* (Convolvulaceae): an American herb in the old world. Journal of ethnopharmacology. 2008 May 8;117(2):185-98.
3. Anis M, Sharma MP, Iqbal M. Herbal ethnomedicine of the Gwalior forest division in Madhya Pradesh, India. Pharmaceutical Biology. 2000 Oct 1;38(4):241-53.
4. Auddy B, Ferreira M, Blasina F, Lafon L, Arredondo F, Dajas F, Tripathi PC, Seal T, Mukherjee B. Screening of antioxidant activity of three Indian medicinal plants, traditionally used for the management of neurodegenerative diseases. Journal of Ethnopharmacology. 2003 Feb 28;84(2):131-8.
5. Allimuthu M, Vennila M. Catalogue of Siddha anti-malarial herbs. 2005
6. Bussmann RW, Gilbreath GG, Solio J, Lutura M, Lutuluo R, Kunguru K, Wood N, Mathenge SG. Plant use of the Maasai of Sekenani Valley, Maasai Mara, Kenya. Journal of ethnobiology and ethnomedicine. 2006 May 5;2(1):22.
7. Singh A. Review of Ethnomedicinal Uses and Pharmacology of *Evolvulus alsinoides* Linn. Ethnobotanical leaflets. 2008;2008(1):100.
8. Indhumol VG, Pradeep HR, Sushrutha CK, Jyothi T, Shavas MM. Ethnomedicinal, Phytochemical,

and Therapeutic applications of *Evolvulus alsinoides* Linn.–A Review. 2013; 1(2): 1-9.
9. Press WH. Laboratory manual for the examination and processing of human semen. World Health Organization, Geneva. 2010:7-113.
10. Narayana K, Prashanthi N, Nayanatara A, Kumar HH, Abhilash K, Bairy KL. Effects of methyl parathion (o, o-dimethyl o-4-nitrophenyl phosphorothioate) on rat sperm morphology and sperm count, but not fertility, are associated with decreased ascorbic acid level in the testis. Mutation Research/Genetic Toxicology and Environmental Mutagenesis. 2005 Dec 7;588(1):28-34.
11. Garba SH, Jacks TW, Onyeyili PA Nggada HA. Testicular and andrological effects of the methanol extract of the root of *Cissampelos mucronata* (A. Rich) in Rats. J Biol Sci Bioconserv. 2014; 6(2): 18 – 30.
12. de Souza Predes F, Diamante MA, Dolder H. Testis response to low doses of cadmium in Wistar rats. International journal of experimental pathology. 2010 Apr 1;91(2):125-31.
13. Carleton HM, Drury RA. Histological technique for normal and pathological tissues and the identification of parasites. Histological technique for normal and pathological tissues and the identification of parasites.. 1957(3rd edit).
14. Seif MM, Khalil FA, Abou Arab AAK, Abdel-Aziz AS, Abou Donia MA, Mohammed ShR. Protective effect of *Melissa officianalis L*. against malathion toxicity and reproductive impairment in male rats. Internatiol Scholarly and Scientific Research and Innovation. 2014; 8(8): 902-907.
15. Balogun ST, Jibrin J, Tahir R, Bassi PU, Balogun FA, Fehintola, FA. Perceptions of artemisinin-based combination therapies among Nigerian women seven years after adopting as first line drugs. American Journal of Research Communication. 2015; 3(4): 107-126.
16. Verma RK, Paraidathathu T. Herbal medicines used in the traditional Indian medicinal system as a therapeutic treatment option for overweight and obesity management: A review. Int J Pharm Pharm Sci. 2014;6:40e7.
17. Al-Mamary M, Molham AH, Abdulwali AA, Al-Obeidi A. In vivo effects of dietary sorghum tannins on rabbit digestive enzymes and mineral absorption. Nutrition Research. 2001 Oct 31;21(10):1393-401.
18. Muriu JI, Njoka-Njiru EN, Tuitoek JK, Nanua JN. Evaluation of sorghum (Sorghum bicolor) as replacent for maize in the diet of growing rabbits (Oryctolagus cuniculus). ASIAN AUSTRALASIAN JOURNAL OF ANIMAL SCIENCES. 2002 Apr 1;15(4):565-9.
19. Andallu B, Radhika B. Hypoglycemic, diuretic and hypocholesterolemic effect of winter cherry (Withania somnifera, Dunal) root.
20. Matsuura H. Saponins in garlic as modifiers of the risk of cardiovascular disease. The Journal of nutrition. 2001 Mar 1;131(3):1000S-5S.
21. Shin S, Jang JY, Choi BI, Baek IJ, Yon JM, Hwang BY, Park D, Jeon JH, Nam SY, Yun YW, Kim YB. Licorice extract does not impair the male reproductive function of rats. Experimental animals. 2008;57(1):11-7.
22. Halvaei I, Roodsari HR, Harat ZN. Acute effects of Ruta graveolens L. on sperm parameters and DNA integrity in rats. Journal of reproduction & infertility. 2012 Jan;13(1):33.
23. Obembe AO, Urom SE, Ofutet EO, Ikpi DE, Okpo-Ene AI. Morphology and motility were not affected when the aqueous extract of *Moringa oleifera* seed was administered to albino Wistar rats. Der Pharmacia Lettre. 2015; 7(3): 129-133.
24. Maina MB, Garba SH, Jacks TW. Histological evaluation of the rats' testes following administration of an herbal tea mixture. Journal of Pharmacology and Toxicology. 2008; 3(6): 464-470.
25. Balasubramanian A, Manimekalai S, Singh AG, Ramakrishnan S. Short and long term effect of Aspirin on testes of albino rats: A histological and biochemical study. Indian Journal of Experimental Biology. 1980; 18(12): 408-410.
26. Mirhoseini M, Mohamadpour M, Khorsandi L. Toxic effects *Carthamus tinctorius* on mouse spermstogenesis. J. Assist. Reprod Genet. 2012; 29(5): 457-461.