

## PHYTOCHEMISTRY OF METHANOL SEED EXTRACT OF *ABRUS PRECATORIUS* AND ITS EFFECT ON SPERMATOGENESIS IN RATS

Mahre\*<sup>1</sup> M.B., Umaru<sup>2</sup>, B., Ojo<sup>1</sup>, N.A., Yahi, D<sup>1</sup>., Sa'idu<sup>3</sup>, A.S., Musa<sup>1</sup>, A.S., and Bukola<sup>1</sup> O. O.

<sup>1</sup>Department of Veterinary Physiology and Biochemistry, Faculty of Veterinary Medicine, University of Maiduguri, P.M.B. 1069, Maiduguri, Nigeria.

<sup>2</sup>Department of Veterinary Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Maiduguri, P.M.B. 1069, Maiduguri, Nigeria.

<sup>3</sup>Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, University of Maiduguri, Nigeria

\*Corresponding author: [mbmahre@gmail.com](mailto:mbmahre@gmail.com)

### ABSTRACT

The methanol seed extract of *Abrus precatorius* was studied for its acute toxicity and its effect on spermatogenesis in rats as well as its phytochemical constituents. The results of this investigation showed that the LD<sub>50</sub> of the methanol seed extract following oral administration was above 5000 mg/kg showing low toxicity. Histological studies of the liver, kidneys and testes of the rats treated with the various oral doses (10 - 5000 mg/kg body weight) showed no remarkable changes in the hepatocytes, kidney cells and testes compared to the control. The effects on sperm cells did not show any significant increase in total sperm head counts. The Phytochemical analysis revealed the presence of pharmacologically active compounds such as reducing sugars, tannins, cardiac glycosides, terpenoids, saponins and flavonoids. In conclusion, the methanol seed extract of *Abrus precatorius* contain important phytochemical constituents possessing pharmacological activities and it is relatively safe but has no effects on sperm cell production.

**Keywords:** *Abrus precatorius*, acute toxicity, phytochemical constituents, spermatogenesis

### INTRODUCTION

*Abrus precatorius* has been described as a deadly plant with the most beautiful seeds (Acharya *et al.*, 2010). In Nigeria, it is locally known as Idon zakara in Hausa; Ewe ire yeye in Yoruba and Otoberebere in Igbo. According to ethno botanical literature the genus *Abrus* is used widely for a variety of conditions in African traditional medicine. Various African tribes use the powdered seeds as oral contraceptives (Chopra *et al.*, 1956; Chopra, 1958) and in the treatment of watery sperm (Sinha and Mathur, 1990). In veterinary medicine, it is used in the treatment of fractures (Acharya *et al.*, 2010). It is

an ingredient of the product “Tranquil” used in the treatment of stress and anxiety (Members rediff, 2004).

Many herbs including the decoction from seeds of *Abrus precatorius* have been used in traditional medicine for the treatment of watery sperm. *Abrus precatorius*, although widely used in Africa for treatment purposes, it has not been scientifically evaluated for its efficacy and acute toxicity. Therefore, this study was designed to determine its acute toxicity and its effect on spermatogenesis in rats as well as its phytochemical constituents.

## MATERIALS AND METHODS

### Seed Collection, Identification and extract preparation:

Fresh seeds of *Abrus precatorius* were collected from Ngoshe montane forest in Gwoza Local Government Area of Borno State. The seeds were identified and authenticated by a Botanist in the Department of Biological Sciences, University of Maiduguri, Nigeria and a set of voucher herbarium (Species Vet. 208 A) was deposited in the Department of Veterinary Physiology, Pharmacology and Biochemistry, University of Maiduguri, Maiduguri, Nigeria. The air - dried seeds were crushed into fine powder. Two hundred and seventy grams (270 g) of powdered seed was extracted using 2 litres of methanol in a soxhlet apparatus at 65<sup>o</sup>c for 6 hours. The extract obtained was concentrated by simple distillation and evaporation. The yield of the extract was determined and the dried extract was stored at 4<sup>o</sup>C until used.

### Experimental animals

Fifteen males adult albino rats (Wistar strain) weighing between 150 grams to 260 grams were obtained from the Animal House of Ahmadu Bello University Zaria, Nigeria. The animals were housed under standard environmental conditions, and feed and water were provided *ad libitum*. Proper handling and used of the animals were in accordance with the guidelines and regulations of Council for International Organizations of Medical Science (1985).

### Acute toxicity Studies

The method of Lorke (1983) was used to investigate the oral dose of methanol seed extract of *Abrus precatorius* that produced immediate or acute toxicity in rats. The animals were divided into three groups of three rats each and treated with respective doses of 10, 100 and 1000 mg/kg body weight of the seed extract of *Abrus precatorius* by the oral route and were labelled as groups A, B, and C respectively. In the second investigation (after 24 hours), three groups were used with one animal per group. The following doses were used: 1500, 3000 and 5000 mg/kg and labelled as groups D, E, and F respectively. Group G was kept as control. Animals were observed on hourly basis for the first day and afterwards, daily for four more days. Mortality

or any visible sign of injury were recorded. The dried extract was reconstituted to make a solution of 500 mg/ml which was used for the experiment. This concentration was maintained throughout the experiment.

### Phytochemical analysis

The methanol seed extract was freshly prepared and divided into different test tubes and the various phytochemical constituents were analysed according to the methods described by Allen (1974) and Harbone (1976). The various chemical constituents tested for included reducing sugars, tannins, free anthraquinones, combined anthraquinones, cardiac glycosides, saponins, flavonoids and alkaloids.

### Histology

From each group, a rat was randomly picked and humanely sacrificed. The liver and kidneys were harvested and fixed in 10% formalin for 24 hours and further processed for paraffin sectioning. Samples (1 cm<sup>3</sup>) of the right testes from each rat in each group were fixed in Bouin's solution for 24 hours and also processed for paraffin sectioning. The paraffin sections were cut at 4µm using the rotary microtome and stained with Haematoxylin and Eosin (H & E) as described by Drury and Wallington (1979).

### Sperm head counts

The left testes and epididymis from the aforementioned rats were dissected out. The *tunica albuginea* was removed from each left testes before its homogenization in 5 mls of normal saline. The head, body and tail of the left epididymis were separately homogenized in 2 mls of normal saline. The sperm head count per millilitre of the homogenate was done using a haemocytometer (Almquist and Amann, 1961; Amann and Lambaise, 1969). The total sperm head count per homogenate was determined using the formula; (volume of homogenate) × (count in 5 squares) × (0.05 × 10<sup>6</sup>).

### Data analysis

Data were analyzed using the statistical software IBM SPSS (2013). Results were expressed as mean ± standard error of the mean. One way analysis of variance was used to

compare differences between the means obtained from the control and tested rats. Differences were considered statistically significant at  $P < 0.05$ .

## RESULTS

Table 1 shows the phytochemical constituents of methanol seed extract of *Abrus precatorius*. The results of this investigation showed that the LD<sub>50</sub> of the methanol seed extract following oral administration was above 5000 mg/kg. There was no significant ( $P > 0.05$ ) effect on the testicular and epididymal sperm head count (Tables 2 and 3). Histological studies of the liver, kidneys and testes of the rats treated with the various oral doses (10 - 5000 mg/kg body weight) showed no remarkable changes in the hepatocytes (Figs. 1 and 2), kidney cells (Figs. 3 and 4) and testes (Figs. 5 and 6) compared to the control.

## DISCUSSION

Phytochemical analysis of the methanol seed extract of *Abrus precatorius* revealed the presence of reducing sugars, tannins, cardiac glycosides, terpenoids, saponins and flavonoids. This is in contrast with the findings of Prathyusha *et al.*, (2010) who reported that saponins and tannins were absent in the seed extract of *Abrus precatorius*. These differences may be due to geographical variation which has been shown to affect the phytochemistry of plant materials (Addae-Mensah *et al.*, 1997).

Although the seeds of *Abrus precatorius* have been reported in the literature to be highly toxic, the result of the present study showed that albino rats survived the acute effect of the extract even at the dose rate of 5000 mg/kg body weight. This showed that the methanol seed extract used was relatively safe. The present study showed that the methanol seed extract of *Abrus precatorius* is not toxic when given orally to albino rats at the doses investigated. This may be as a result of heat denaturation of the active

principle (abrin) that would have caused the toxicity. Abrin is heat stable at 60°C for 30 minutes. At 80°C, most of the toxicity is lost in 30 minutes (Budavari, 1989). This supports its use in folk medicine by the Indians when they boil it to denature the abrin before eating it or using the hot water extract as remedy for eye diseases (Rajaram & Janardhanan, 1992).

The sperm head count and the histological assessment of the testes was used in this study to evaluate the effect of acute oral administration of *Abrus precatorius* on male reproductive system using the albino rats as animal model. These parameters are usually evaluated to determine the fertility of a male subject (Garner and Hafez, 1993). In the current study, histological studies showed that there were no remarkable changes in the testes as compared to the control. The effects on sperm cells did not show any significant difference ( $P > 0.05$ ). This is contrary to the findings of Sinha and Mathur (1990), who reported a decrease in sperm head count by treatment with steroidal fraction of seeds of *Abrus precatorius* in male rats. This may be due to variation in bioactive compound of the same plant found in different environment (Elujoba, 1989). Saponins have been reported to have positive effects on the viability of human sperm cells *in vitro* (Francis *et al.*, 2002), but the presence of saponins in the extract of the present study did not cause any increase in sperm cells. Thus, this finding will expand the scope of phytochemistry evaluation.

## CONCLUSION

The results of this study showed that *Abrus precatorius* methanol seed extract was relatively safe and contain important phytochemical constituents such as reducing sugars, tannins, cardiac glycosides, terpenoids, saponins and flavonoids but has no effect on the spermatogenesis of albino rats.

## REFERENCES

- Acharya, D.; Shrivastava, A.; Pawar, S.; Sancheti, G. (2010). The Medicinal Value of Indian Herb *Abrus precatorius* used in Herbal Medicines in India.
- <http://www.disabled-world.com/medical/alternative/herbal/abrus-precatorius.php>
- Addae-Mensah, I.; Demoyeka, F. G.; Buxter, C. L.; Sandlis, K. M. (1977). A Novel:

- Amide Alkaloid from the root of *Piper guinense*; *Phytochemistry*, 16:757.
- Allen, S.T. (1974). *Chemical Analysis of ecological material*. Blackwell Scientific Publication, New York; 313.
- Almquist, J.O and Amann, R.P (1961). Reproductive capacity of dairy bulls II. Gonadal and extragonadal sperm reserves as determined by direct counts and depletion trials, dimension and weight of genitalia. *Journal of Animal Science*, 44: 1668 – 1678.
- Amann R.P and Lambiase J.T (1969). The male rabbit III. Determination of daily sperm production by means of testicular homogenates. *Journal of Animal Science*, 28: 369 – 374.
- Budavari, S. ed. (1989). *The Merck Index: An Encyclopedia of Chemicals, Drugs and Biologicals*, 10<sup>th</sup> Ed. Rahway, New Jersey, Merck and Co., Inc.
- Chopra, R. N. (1958). *Indigenous Drugs of India*. UN Dhar and Sons Pvt. Ltd, Calcutta.
- Chopra, R. N.; Nayar, S. L.; Chopra, I. C. (1956). *Glossary of Indian Medicinal Plants*, New Delhi: Council of Scientific & Industrial Research, 1956-92.
- Council for International Organization of Medical Science (1985). *International Guiding Principles for Biomedical Research Involving Animals*.
- Drury, R.A.B and Wallington, E.A (1979). *Carlington's histological technique*. 4<sup>th</sup> ed. London, U.K.: Oxford University Press.
- Elujoba A (1989). Chemical and biological analysis of Nigerian Cassia species for laxative activity. *Journal of Pharmacological and Biomedical Analysis*, 712: 1453 – 1457.
- Francis G., Zohar K., Harinder P.S., and Becker K (2002). The biological action of saponins in animal system: a review. *British Journal of nutrition* 88: 587 – 605.
- Garner, D.L and Hafez E.S.E (1993). *Spermatozoa and seminal plasma. Reproduction in farm animals (6<sup>th</sup> Ed.)*. Lea and Febiger, Philadelphia. USA. Pp. 165 – 187.
- Harbone, J.R. (1976). *Phytochemical Methods. A guide to modern techniques of plant analysis*. Charpan and Hal, London; 78.
- Members.rediff.(2004).<http://members.rediff.com/excelser/tranquil.html>. Accessed December 22, 2012.
- Lorke, D. (1983). A New Approach to Practical Acute Toxicity Testing, *Archives of Toxicology*, 54: 275-287.
- Prathyusha, P.; Subramanian, M. S.; Sivakumar, R. (2010). Pharmacological Studies on White and Red Forms of *Abrus precatorius* Linn. *Indian Journal of Natural Product Resources*. 1:476-480.
- Rajaram, M., Janardhanan, K. (1992). The Chemical Composition and Nutritional Potential of the Tribal Pulse, *Abrus precatorius* L. *Plant Foods Hum Nutr*, 42 (4):285-290.
- Sinha, S and Mathur R.S (1990). Effect of steroidal fraction of seeds of *Abrus precatorius* Linn. on rat testis. *Indian Journal of Experimental Biology*. 28 (8): 752 – 756.

Table 1: Phytochemical constituents of methanol seed extract of *Abrus precatorius*

Phytochemical Tests	Results
Test for Carbohydrates	++
Test for Free Reducing Sugars	++
Test for Tannins	++
Test for Anthraquinones	-
Test for Cardiac Glycosides	+
Test for Terpenoids	+++
Test for Saponins	++
Test for Flavonoids	+++
Test for Alkaloids	-

(+) = Present in low concentration, (++) = Present in moderate concentration, (+++) = Present in high concentration, (-) = absent.

Table 2: Acute effects of oral administration of the methanol seed extract of *Abrus precatorius* on mean testicular sperm head count ( $\times 10^6$ ) of albino rats

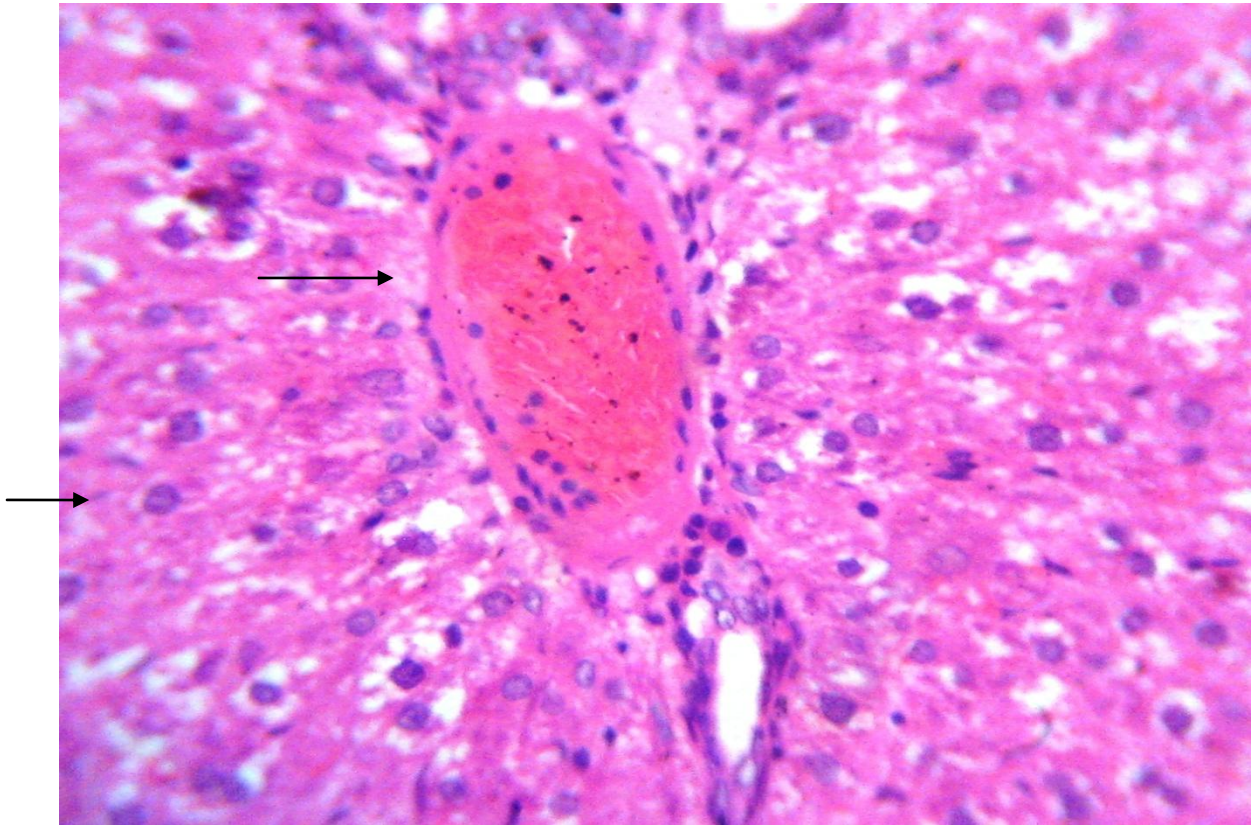
		Dose (mg/kg)			
	Control	10	100	1000	5000
	23.4 $\pm$ 1.14	24.7 $\pm$ 1.32	23.8 $\pm$ 0.35	25.5 $\pm$ 0.43	26.6 $\pm$ 1.56

P>0.05 compared with control.

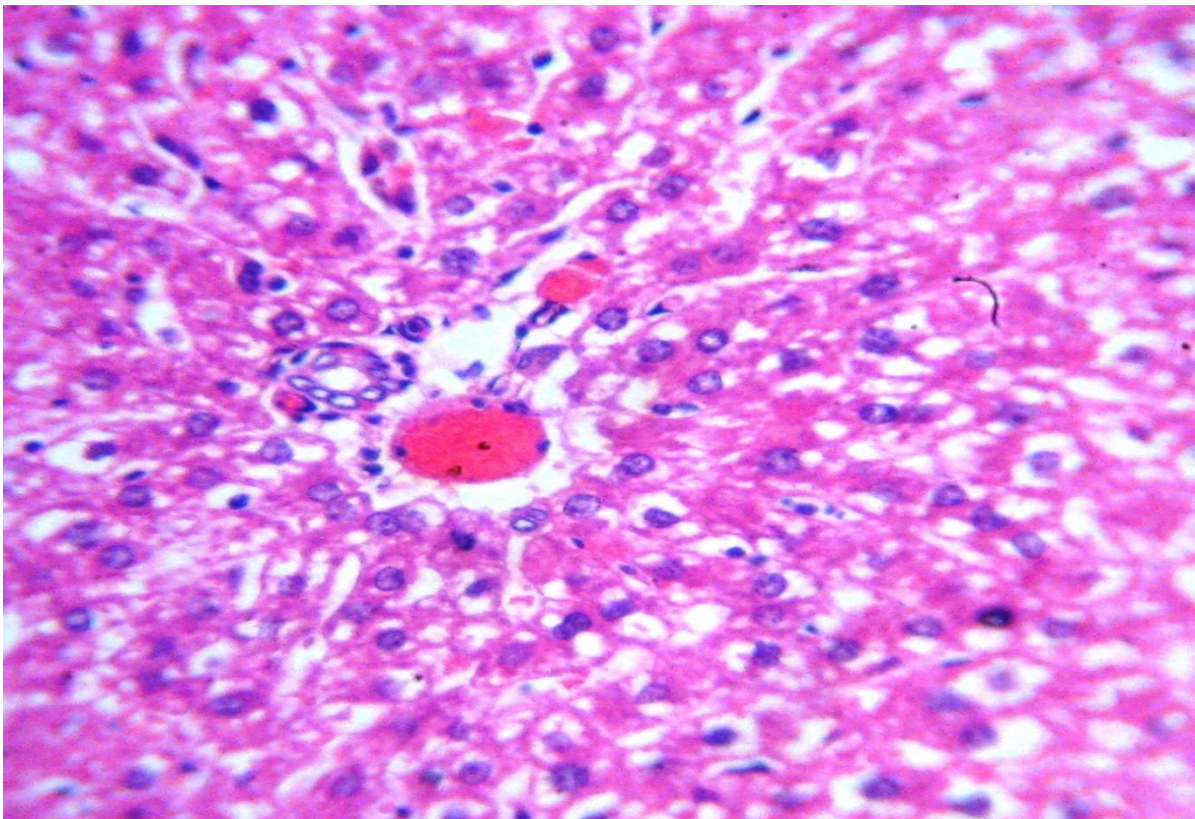
Table 3: Acute effects of oral administration of the methanol seed extract of *Abrus precatorius* on epididymal sperm count ( $\times 10^6$ ) of albino rats

		Dose (mg/kg)			
Epididymal tissues	Control	10	100	1000	5000
Head	3.1 $\pm$ 2.3	3.0 $\pm$ 2.0	2.2 $\pm$ 0.4	2.8 $\pm$ 2.4	2.3 $\pm$ 0.4
Body	1.2 $\pm$ 0.4	1.4 $\pm$ 1.1	1.3 $\pm$ 0.2	1.0 $\pm$ 0.2	1.2 $\pm$ 0.2
Tail	7.0 $\pm$ 2.7	4.2 $\pm$ 3.8	6.0 $\pm$ 1.5	4.9 $\pm$ 3.1	6.0 $\pm$ 1.7

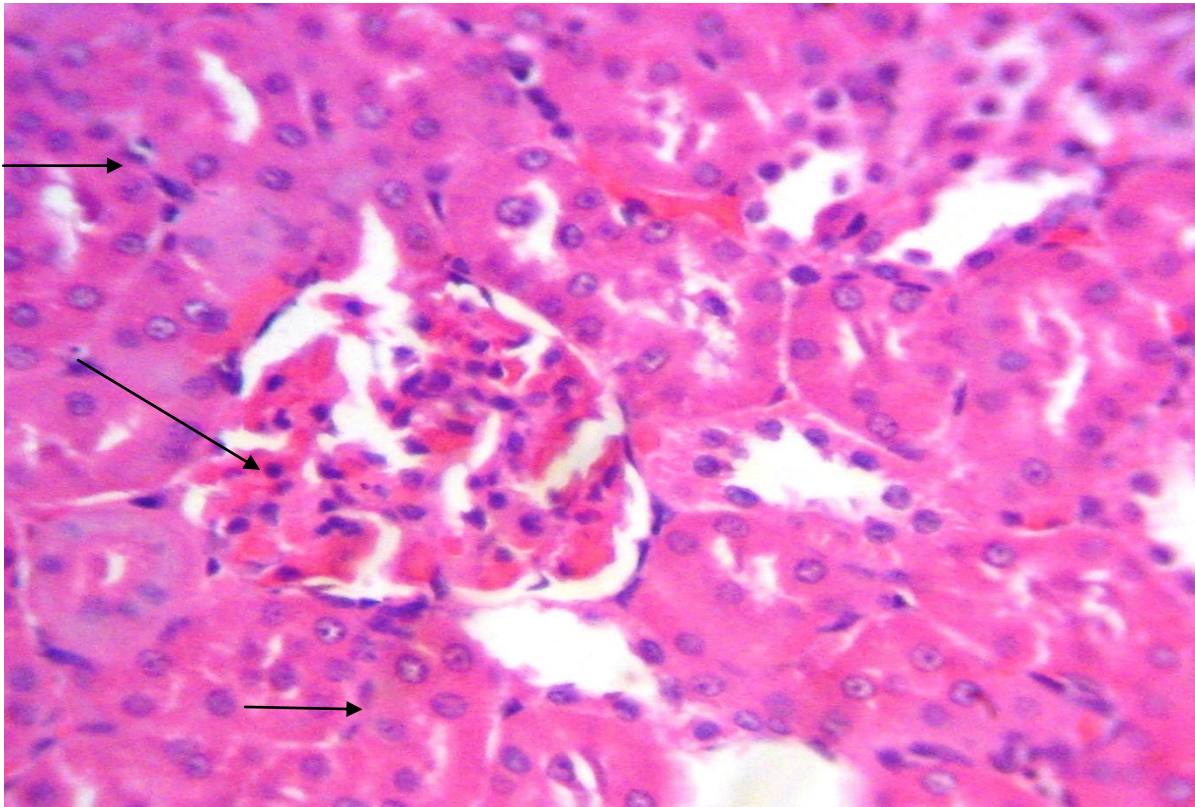
P>0.05 compared with control.



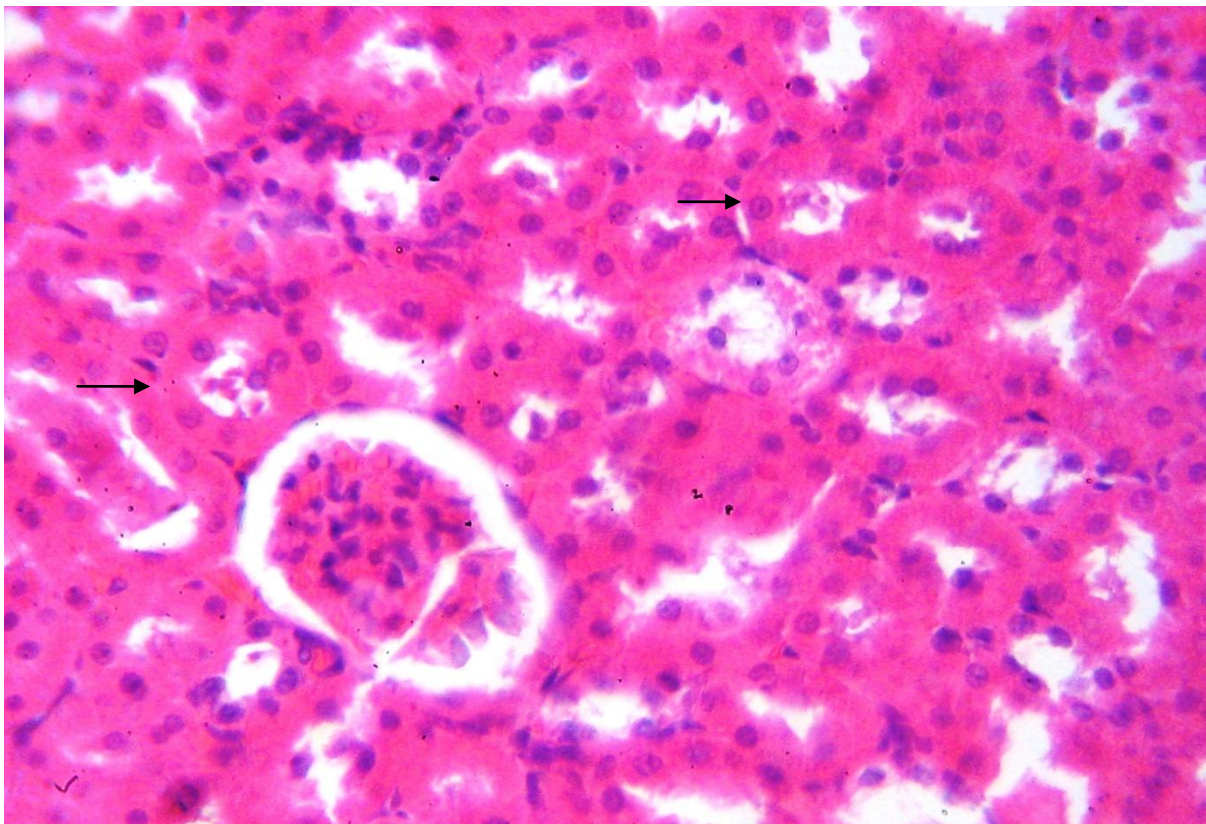
**Fig.1:** Section of the liver of rats (Control). The arrows are showing a congested central vein and hepatocyte (arrows) (H and E  $\times$  760).



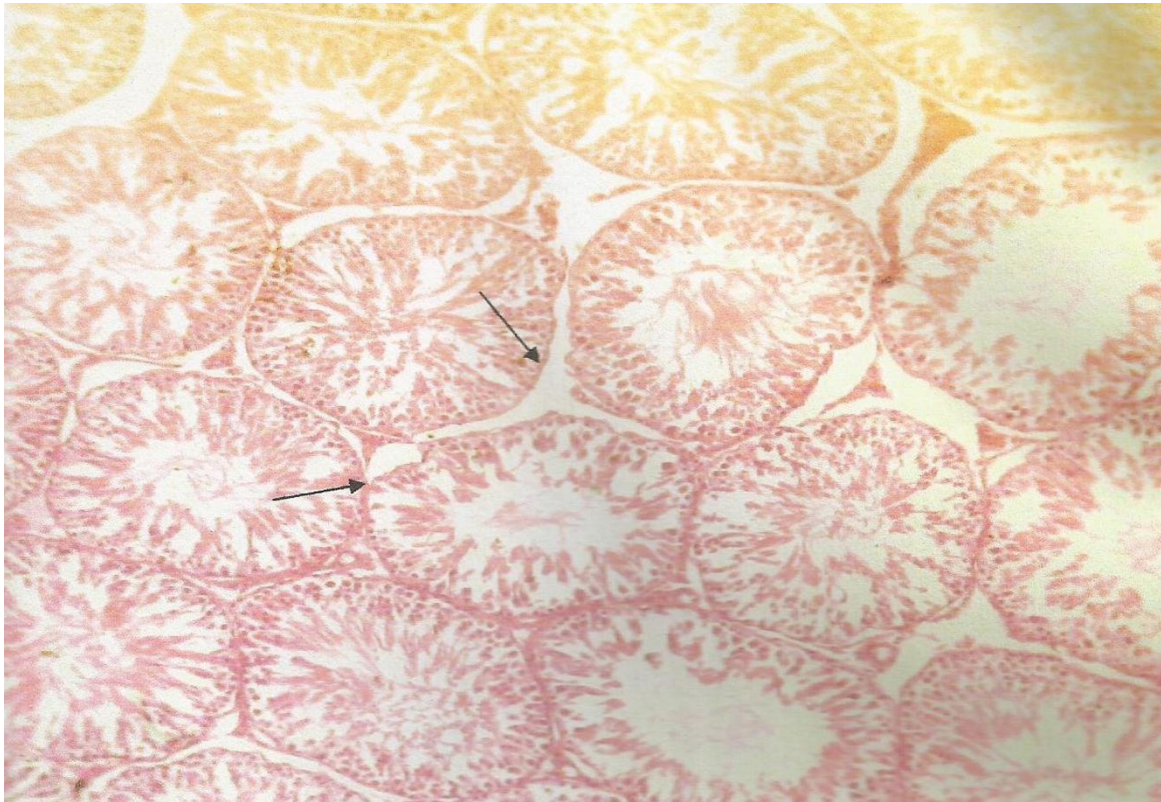
**Fig. 2:** Section of the liver of rats (5000mg/kg) (H and E  $\times$  760).



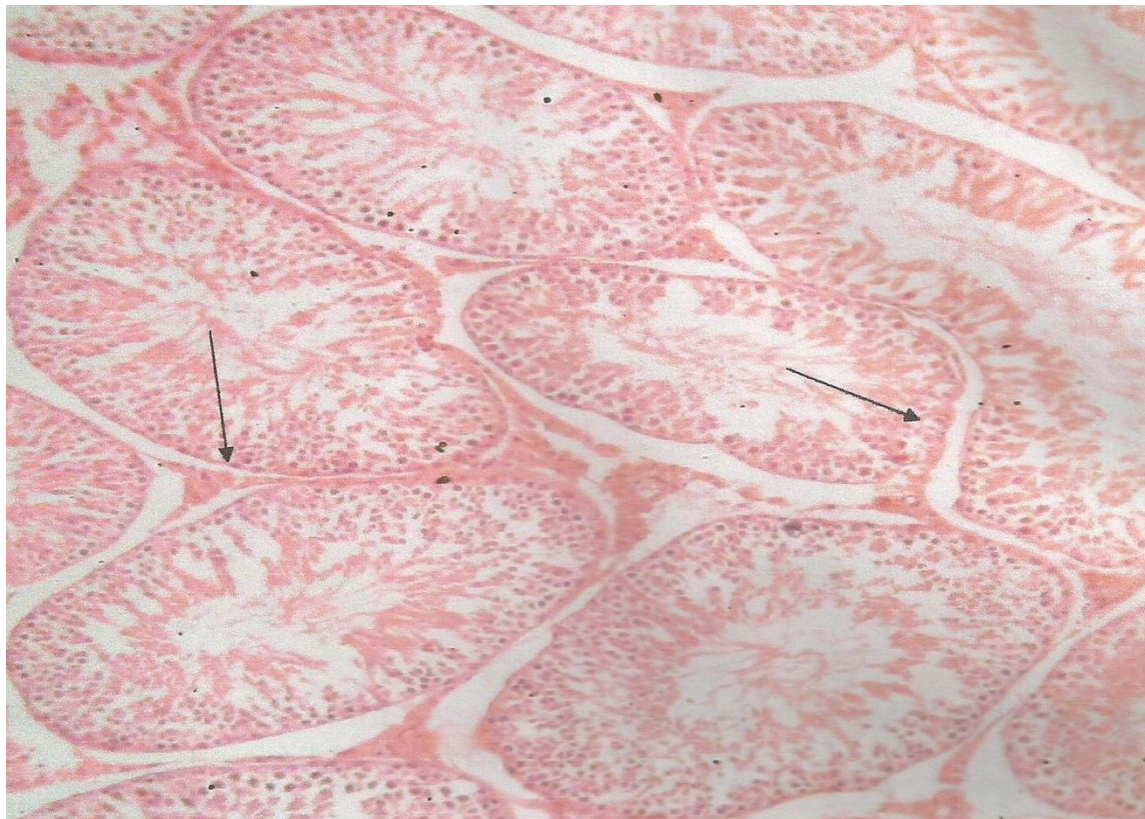
**Fig. 3:** Section of the kidney of rats (control). The arrows are showing the glomerulus and the renal tubular cells (arrows) (H and E  $\times 760$ ).



**Fig. 4:** Section of the kidney of rats treated with 5000 mg/kg of the extract. The arrows are showing basophilic homogenous casts within the renal tubules (arrows) (H and E  $\times 760$ ).



**Fig. 5:** Section of the testes of rats (control). The arrows are showing spermatogenic cells within the seminiferous tubules (H and E  $\times$  190)



**Fig. 6:** Section of the testes of rats treated with 5000 mg/kg of the extract. The arrows are showing spermatogenic cells within the seminiferous tubules (arrows) (H and E  $\times$  190)