International Journal of Biochemistry Research & Review
7(3): 139-147, 2015, Article no.IJBcRR.2015.063
ISSN: 2231-086X

SCIENCEDOMAIN *international*
www.sciencedomain.org

Toxicity Study of *Ricinus cummunis* Lnn Seed Suspension in Female Wister Albino Rats

B. Y. Muhammad [1*], A. J. Alhassan [2] and I. J. Jaafaru[3]

[1]Department of Biochemistry and Molecular Biology, Nasarawa State University Keffi, Nigeria.
[2]Department of Biochemistry, Bayero University Kano, Nigeria.
[3]Department of Veterinary Biochemistry and Physiology, Faculty of Veterinary Medicine, Uthman dan Fodiyo University, Sokoto, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author BYM designed the study, carried out laboratory assays and wrote the first draft. Author AJA wrote the protocol. Supervised the work and edited the first draft. Author IJJ carried out animal feeding participated in histological study and performed the statistical analysis. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJBcRR/2015/17511
Editor(s):
(1) Chunying Li, Department of Biochemistry and Molecular Biology, Wayne State University, School of Medicine, Detroit, USA.
Reviewers:
(1) Abioye Oluwatayo Emmanuel, Department of Microbiology, Obafemi Awolowo University, Il-Ife, Osun state, Nigeria.
(2) Papiya Bigoniya, Department of PG Pharmacology, Radharaman College of Pharmacy, Ratibad, Bhopal, M.P, India.
(3) Anonymous, University of Karachi, Pakistan.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=1038&id=3&aid=9110

Original Research Article

Received 17th March 2015
Accepted 15th April 2015
Published 4th May 2015

ABSTRACT

Ricinus communis seed has been proven effective to prevent conception since time immemorial. This research focuses on the toxicity study of the seed suspension in Wister albino rats. Twenty four (24) rats were used for the sub-chronic toxicity study, while 13 mice for the acute toxicity study. The seed suspension of *Ricinus communis* seed at three graded concentrations (3.80, 7.60 and 11.40 mg/kg body weight) was administered orally; to Groups I, II and III respectively once every day for the period of one month. The liver and kidney functions were determined after the last administration. Serum alanine amino transferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), urea and creatinine were determined. The activity of ALT, AST, ALP and the concentrations of urea and creatinine at 3.80 mg/kg body weight showed no significant difference ($p>0.05$) compared to the control. However, a significant increase ($p<0.05$) in these parameters was observed in rats given 7.60 and 11.40 mg/kg body weight. Similarly a significant increase ($p<0.05$)

**Corresponding author: E-mail: rabbanimuhammad@yahoo.com;*

in lipid profile was observed in rats given 11.40 mg/kg body weight. Acute toxicity revealed the median lethal dose (LD_{50}) of 1587 mg. Histological analysis of the liver and kidneys of the rats after three months revealed no cellular death, necrosis or inflammation. This indicates that consecutive use of the seed at the traditional dose (3.80 mg/kg in rat; equivalent to 3 seed/70Kg in human) for long period of time is neither hepatotoxic nor nephrotoxic. *Ricinus communis* seed is therefore safe in rats at the concentrations administered.

Keywords: Wister albino rat; LD_{50}; sub-chronic toxicity; lipid profile and histology.

1. INTRODUCTION

The castor oil plant, *Ricinus communis*, is a species of flowering plant in the spurge family, Euphorbiaceae. It belongs to a Monotypic genus, Ricinus, and sub tribe, Ricininae. The evolution of castor plant and its relation to other species is currently being studied [1]. Its seed is the castor bean which despite its name is not a true bean. Castor plant is indigenous to the south eastern Mediterranean Basin, Eastern Africa and India, but is widespread throughout tropical regions and widely grown elsewhere as an ornamental plant [1].

The name Ricinus is a Latin word for tick; the seed is so named because it has markings and a bump at the end which resembles certain ticks. The common name "Castor oil" probably comes from its use as a replacement for castoreum; a perfume base made from dried perineal glands of the beaver (Castor in Latin). It has another common name, palm of Christ or *Palma Christi* that derives from Castor oil's ability to heal wounds and cure ailments. Other common names include: Castor plant, castor oil plant, castor bean plant, wonder boom, dhatura, "Kharwa" (Arabic), "Retsinoladia" (Greece), "Kikayon" (Hebrew) "Erando" (Bangla) "Arandi" (Hindi) "Gulo" (Ethiopia) etc. Locally the plants are known in Nigeria by such names as "Zurman" (Hausa) "Laraa" (Yoruba). "Ogilisi" (Igbo), "Kpamfinigulu" (Nupe), "Jongo" (Tiv), and "Era ogi" (Bini) [2].

Although castor is indigenous to the South-Eastern Mediterranean Basin, Eastern Africa, and India, today it is widespread throughout tropical regions [3]. In areas prone to frost it is usually shorter, and grown as if it were an annual [3]. However, it can grow well out doors in cooler climates, at least in Southern England and the leaves do not appear to frost damage in sheltered spots, where it remains evergreen. It was used in Edwardian times in the parks of Toronto, Ontario and Canada.

The toxicity of raw castor beans due to the presence of ricin is well-known [4]. Although the lethal dose in adults is considered to be 4 to 8 seeds, reports of the actual poisoning are relatively rare [4]. If castor bean is ingested, symptoms may be delayed by up to 36 hours but commonly begin within 2–4 hours. These include a burning sensation in mouth and throat, abdominal pain, purging and bloody diarrhea. Within several days there is dehydration, a drop in blood pressure and a decrease in urine. Unless treated, death can be expected to occur within 3–5 days, however, in most cases, a full recovery can be made [5].

In different societies, the use of the seeds of *R. communis* as oral contraceptive has been documented [6]. Although the exact variety of the seeds used is not always reported, it is known that 4–5 seeds of the plant in India are orally taken with water during menstrual period to prevent conception for a period of one [7]. In Saudi Arabia, three intact seeds are used on the first day of menstruation as oral contraceptive [6].

It has also been the practice that in the Middle Belt of Nigeria, traditional healers administer to women three seeds once of the variety Minor as contraceptive for duration of 12 month [8,9].

The aim of this research was to study the acute and sub-chronic toxicity of castor bean suspension on Wister albino rats through the observation of biochemical and histological parameters.

2. MATERIALS AND METHODS

2.1 Collection of Samples

The seeds of the castor plant (*Ricinus communis*) were collected in November 2011 from Kano along "Jan Bulo" (about 0.5 km west of Bayero University Kano) and then authenticated at the Botany Unit of Biological Sciences Department, Bayero University Kano and correlate to Voucher NO. BUKHAN No: 225

2.2 Preparation of Castor Seed Suspension

The outer coating (husk) of the seeds were manually removed and the residual wet flesh ground into pulp. 6grams of the pulp was weighed and transferred into a clean, dry and tightly closed glass jar and stored at 4°C. A required mg of the ground pulp was usually weighed and stirred gradually with a spatula and distilled water gradually added until the pulp form an oily suspension yellowish in color. This is done every day before administration. The formulae used to convert mg/kg B.W to mg/g B.W weight of the rat was as below;

Mg required = wt. (mg) of known X wt. (g) B.W/1000 g

2.3 Experimental Animals

Twenty four adult non pregnant female Wister albino rats (100-120 g) were purchased and kept in the Department of Biological Sciences, Bayero University, Kano; under laboratory condition of 28°C and supplied food (vital feed; growers palletized feeds) and water for the period of research. Thirteen albino mice, 20.40±3.6 g weights were used for the acute toxicity tests.

2.4 Experimental Design

An acute toxicity study of the aqueous suspension of castor seed was carried out by the method of Lorke [10]. In the initial phase female albino mice were divided into three groups (Groups I, II, and III) of three mice each. These groups were administered orally, doses of 10, 100 and 1000 mg/kg body weight of the aqueous suspension of the seed respectively. Animals were observed for 24 hours and the number of death recorded, if any .They were also observed for 72 hours for any sign of delayed toxicity as described by Lorke [10]. In the second phase, mice were grouped into four (4) of one rat each and administered extract orally at doses of 1400, 1800, 2200 and 2600 mg/kg. Animals were observed for 24 hours and final LD_{50} value was calculated by using geometric mean of 1400 and 1800 mg/kg for which 0/1 and 1/1 death occurred respectively.

For subchronic toxicity study, twenty four (24) female Wister albino rats were grouped into six(6) rats each, randomly assigned to four cages labeled I, II, III, and IV respectively and kept at room temperature (28°C). All the rats were allowed free access to water and feed for a week to acclimatize them to laboratory conditions. After this period, the control animals (group I) were given distilled water orally, with the aid of a syringe. While groups II, III, and IV were given orally 3.80 mg/kg, 7.60 mg/kg and 11.40 mg/kg respectively of the suspension; equivalent to three(3), six(6) and nine(9) seeds respectively, for twelve(12) weeks.

At the end of the twelve weeks of oral administration of aqueous seed suspension of *Ricinus communis*, the rats were sacrificed. Blood samples were immediately collected in clean and dried Wassermann tubes from the portal vein and then centrifuged at 3000 rpm for 15 minutes. Serum samples were separated and frozen at -10°C until further determination of the tested parameters.

A clean Pasteur pipette was used to carefully collect the serum and dispensed into a clean-labeled specimen bottle. Sera samples collected were analyzed for liver function (AST, ALT, ALP) and kidney function (urea and creatinine).The liver and kidneys were removed by careful dissection and blotted free of adhering blood immediately after sacrificing the rats. The organs were preserved in 10% neutral buffered formalin prior to the histological analysis.

2.5 Biochemical Studies

Serum aspartate amino transferase (AST) activity was determined by the method of Reitman and Frankel [11] serum alkaline phosphatase (ALP) by the method of King and Kings [12], serum urea concentration by the method of Weather burn [13], serum creatinine concentration by Jaffe`s method [14]. The determination of serum total cholesterol and HDL-cholesterol was done by the method described by Richmond [15], LDL-cholesterol and VLDL-cholesterol by that of Freidwald et al. [16] while triglyceride by the method of Trinder [17].

3. RESULTS AND DISCUSSIONS

Many researches were carried out on the contraceptive effect of this seed ethanoic extract but no research however, to the best of our knowledge that studied its sub chronic toxicity in rats. Nigerian village women usually swallow 3 seed to prevent conception. Our research is unique therefore, because we seek to find out

the toxicity of the seed as a whole (by making suspension instead of extract) in rats by mimicking the exact route of administration in humans.

3.1 Acute Toxicity

The result of the acute toxicity studies revealed no mortality at the concentration of 1400 mg/kg body weight in rats; even though the animals were a little bit depressed, sluggish and had a mild diarrhea. Decreased appetite, increased water consumption by the rats and frequent urination were also observed, especially in those given the highest dose of the suspension (1800 mg/kg); at which full death occurred. From Table 1. The median lethal dose (LD_{50}) of the aqueous seed extract was calculated to be 1587 mg/kg body weight in rats, which is far higher than the therapeutic/effective dose (3.80 mg/kg), this however gave rise to therapeutic index (therapeutic index = LD_{50}/ED_{50}) of 417.63 which is high enough and therefore safe [18].

3.2 Sub Chronic Toxicity Studies

For sub chronic toxicity studies (Fig. 1), the AST, ALT and ALP activities as well as the urea and creatinine concentrations of group I treated rats (given 3.80 mg/kg) revealed no significant difference ($p>0.05$) compared to the untreated control group. This might be due to the fact that the dose administered was far below the LD_{50} (1587 mg/Kg). Given that high levels of AST, ALT and ALP are indicators of liver damage or disorders [19] and that abnormally high concentrations of urea and creatinine indicate renal dysfunction; it can therefore be deduced that the castor seed suspension at the traditional concentration (therapeutic dose; 3.80 mg/Kg) in rats is neither hepatotoxic nor nephrotoxic. Both urea and creatinine are by-products of metabolism and are excreted by the kidneys through glomerular filtration. Impairment of kidney function results in alteration of its glomerular or tubular function, leading to accumulation of metabolites that are mainly excreted through the kidneys such as urea, uric acid and creatinine [20].

Conversely, the AST, ALT and ALP levels in the treated Group 2 (7.60 mg/Kg) were significantly different ($p<0.05$) compared to control untreated Group; with AST, ALT and ALP Furthermore, the level of urea (10.83±0.56) but not creatinine (54.33±7.28) was significantly different ($p<0.05$), compared to the control untreated Group with urea (10.83±0.56) and Creatinine (54.30±7.28). Similarly, the same significant difference ($p<0.05$) was observed between the control Group and treated Group 3 with respect to AST, ALT, ALP, urea and creatinine.

These results show that excessive consumption of the *Ricinus communis* seed above the traditional antifertility dose / effective dose (3.80 mg/kg; equivalent to 3 seed in human) could impair liver and renal functions. Thus, the seed should be used with caution in individuals with renal and /or liver disorder as it could further exacerbate their condition(s).

Table 1 (a-b). LD_{50} Determination of aqueous suspension of *Ricinus communis* (castor bean) seed

(a)

Dose(mg/kg)	Result of first phase
10	0/3
100	0/3
1000	0/3

(b)

Dose (mg/kg)	Result of second phase
1400	0/1
1800	1/1
2200	1/1
2600	1/1

LD_{50} =√ (minimum conc. with full death ×maximum conc. with no death); LD_{50} = √ (1400×1800) = 1587 mg/kg

3.3 Lipid Profile

The result of the lipid profile (Fig. 2) revealed no significant difference ($p>0.05$) between the treated groups I, II and the untreated (control) group. On the contrary, there was significant ($p<0.05$) increase in the levels of total-cholesterol, HDL-cholesterol, LDL-cholesterol and decreased levels of triglyceride and VLDL-cholesterol in treated Group III compared to untreated control Group. In view of the fact that increase in LDL from(0.46±0.3) to (1.13±o.25) is accompanied by corresponding increase in HDL from (0.86±0.12) to(1.33±0.12): the risk of cardiovascular diseases posed by high level of plasma LDL-cholesterol could be minimal due to the role HDL-cholesterol plays in the reverse cholesterol transport. Even though, traditionally only three seed of this castor bean is being consumed to prevent conception for a complete year, from the result of this research over dosage or excessive ingestion of the seed could pre

dispose one to diseases associated with high serum LDL-cholesterol.

3.4 Histological Analysis

Histological analysis tallied closely with the biochemical analysis on liver and kidney functions in rats. That is no pathological changes were observed: normal macrostructure of the organs with no inflammation, congestion or necrosis of the Liver (plate 1-4); normal glomeruli and tubules of the kidneys (plate 5-8). This further confirmed the safety of using *Ricinus communis* seed as an oral contraceptive in rats.

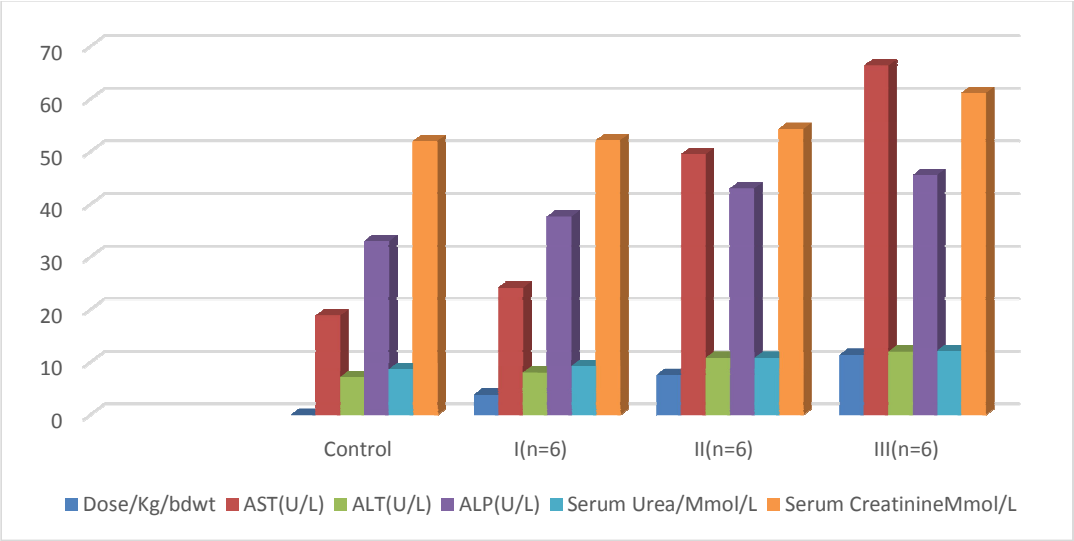

Fig. 1. Effect of oral administration of *Ricinus communis aqueous seed* suspension for four (4) weeks on liver and kidney functions of albino rats

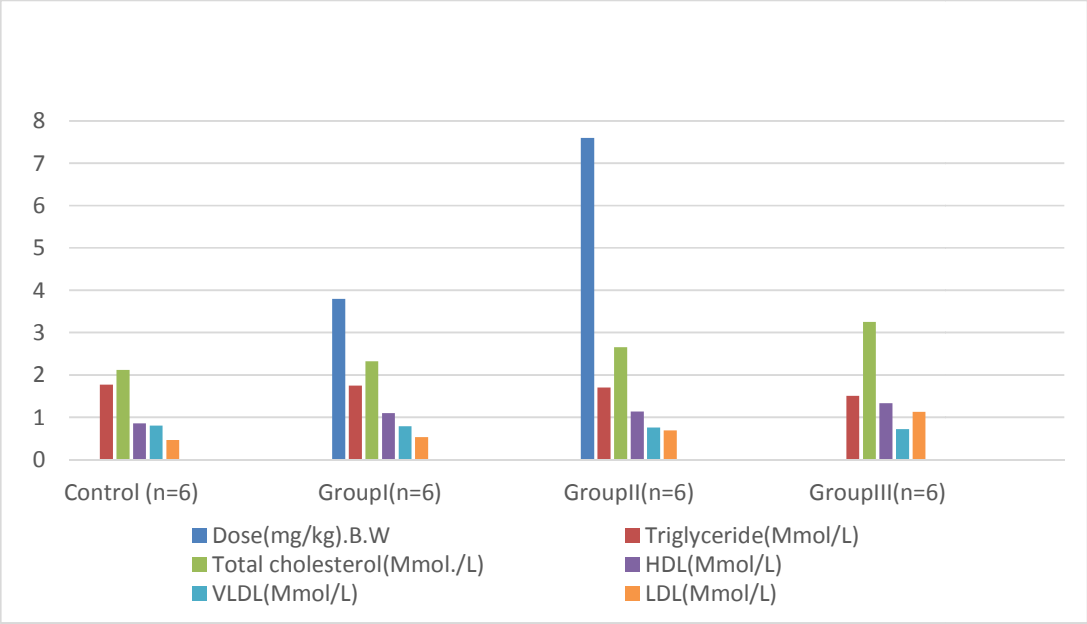

Fig. 2. Effect of oral administration of *Ricinus communis* aqueous seed suspension for four weeks on lipid profile in albino rats

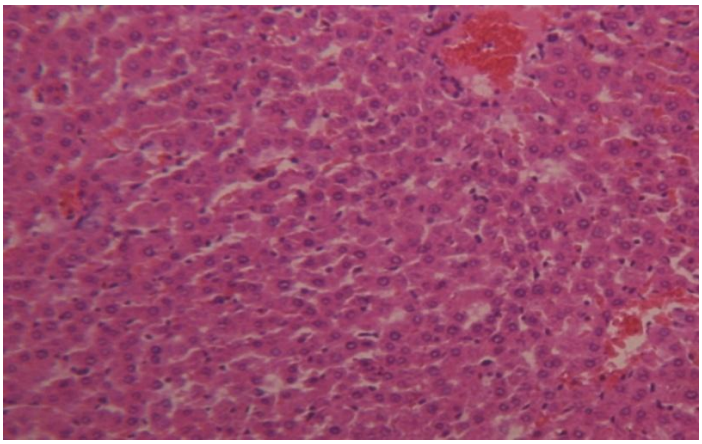

Plate 1. Liver stained section of control (untreated) rats showing the portal tract area, with no pathological changes (Hand E×100)

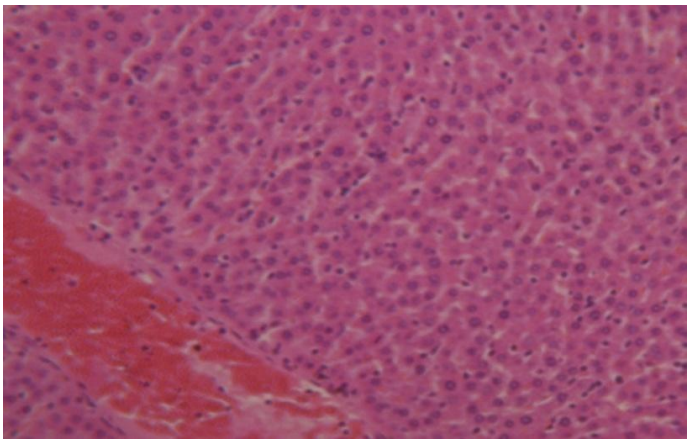

Plate 2. Liver stained section of group1 treated rats showing the portal tract area, with no pathological changes (Hand E×100)

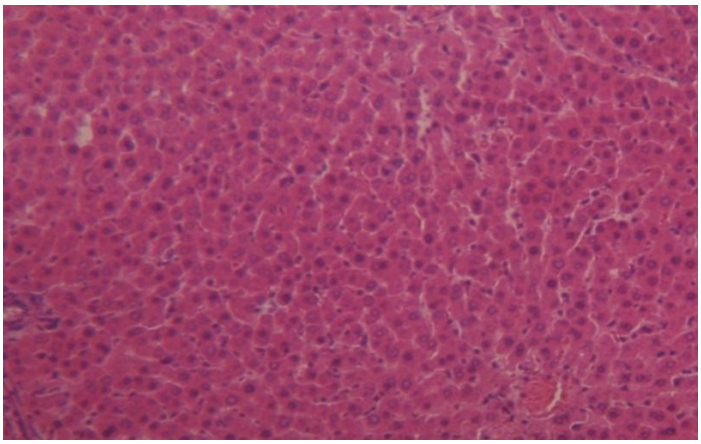

Plate 3. Liver stained section of group II treated rats showing the portal tract area, with no pathological changes (Hand E×100)

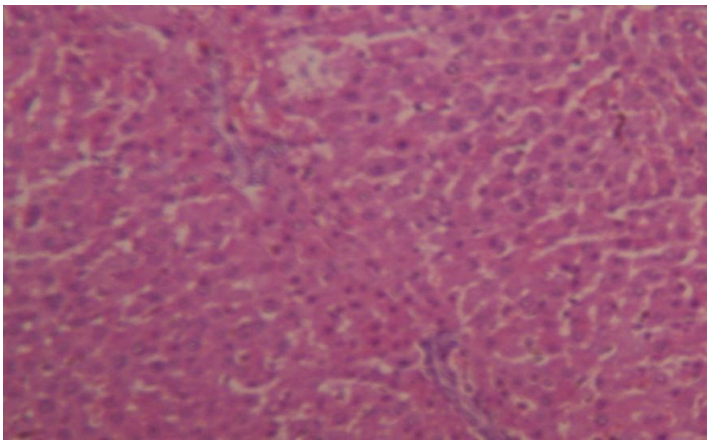

Plate 4. Liver stained section of group lll treated rats showing the portal tract area, with no pathological changes (Hand E×100)

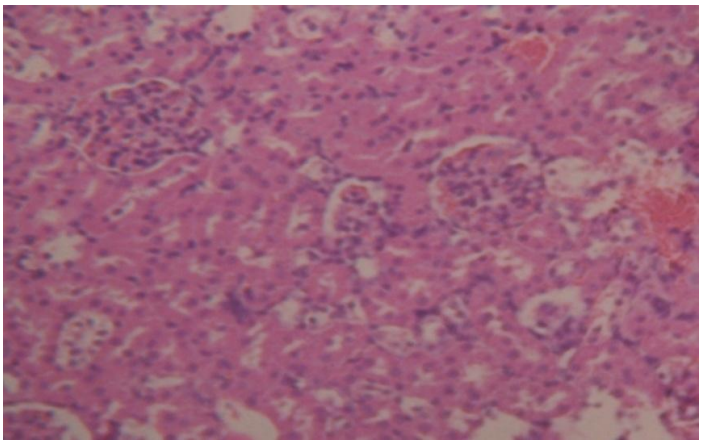

Plate 5. Kidney stained section of control (untreated) rats showing the glomeruli and tubules with no pathological changes (Hand E×100)

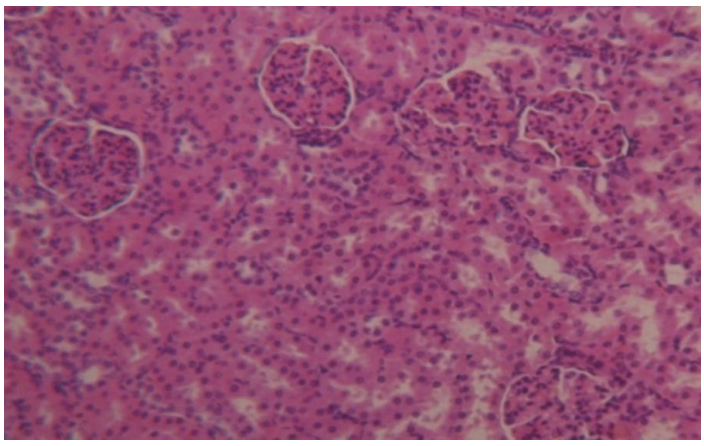

Plate 6. Kidney stained section of group I treated rats showing the glomeruli and tubules with no pathological changes (Hand E×100)

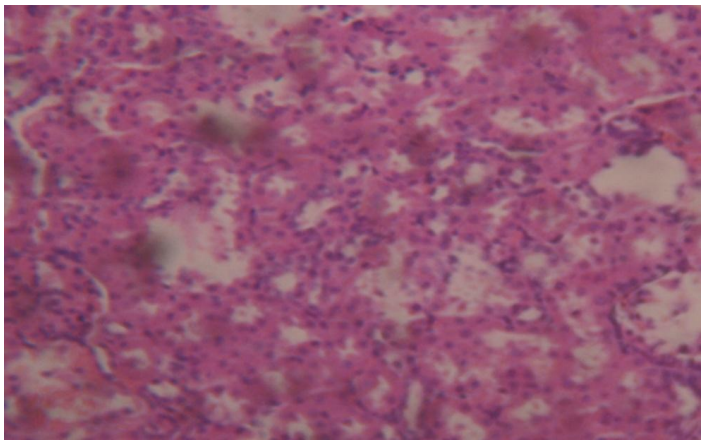

Plate 7. Kidney stained section of group II treated rats showing the glomeruli and tubules with no pathological changes (Hand E ×100)

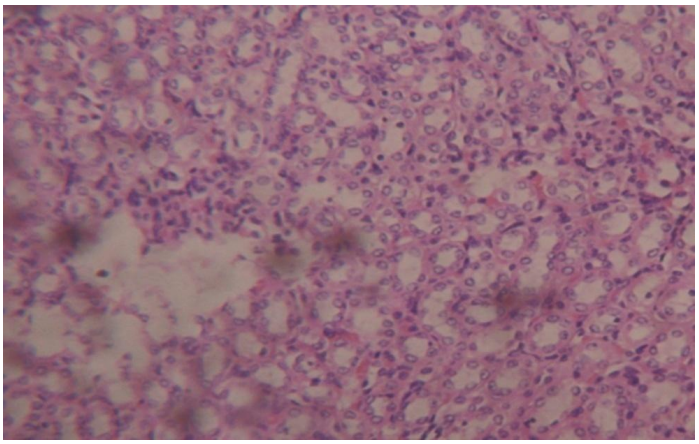

Plate 8. Kidney stained section of group III treated rats showing the glomeruli and tubules with no pathological changes (Hand E ×100)

4. CONCLUSION

The research revealed that the castor bean seed *(Ricinus communis*) suspension has no toxicity at 3.80mg/kg body weight/day for four weeks. However higher dosage slightly increases liver and kidney functions without visible pathological changes in the organs. Moreover, excessive consumption of the seed could lead to weight gain due increase lipid profile parameters.

ACKNOWLEDGEMENTS

We acknowledge the assistance of Dr Otanda A. Chemical pathology Unit Aminu Kano Teaching Hospital Nigeria. For her assistance in the histological study. We also thank Mal. Aliyu A. of the animal house Department of Biological Science Bayero University Kano for taking care of the animals.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Oplinger ES, Oelke EA, Kaminski AR, Combs SM, Doll JD, Schuler RT. Castor Beans: Alternative Field Crops Manual, Wisconsin and Minnesota Universities, U.S.A. 1990;93-97.
2. Weiss EA: Castor, Sesame and Safflower, 2nd edition. Leonard Hill, London. 1971;235.
3. Phillips R, Martyn R. A text book of Annuals and Biennials. London: Macmillan. 1999;106.
4. Wedin GP, Neal JS, Everson GW, Krenzelok EP. Castor Bean Poisoning. AMJ Emerg. Medicine. 1986;4(3):259–261.

5. Battelli MG. Cytotoxicity and to Animals and Humans of Ribosome–Inactivating Proteins. Mini Rev Med Chemistry. 2004;4:513–21.
6. Dafallah AA, Al-Mutairy AR. Contraceptive Effect of Castor Beans, Saudi Pharmaceutical Journal. 1994;2:152.
7. Nisteswar K. Review of certain Indigenous Anti-fertility Agents. Deerghayu International Journal. 1988;4(1):4–7.
8. Okwuasaba FL, Das SC, Isichei CO, Ekwenchi MM, Onoruvwe O, Olayinka AO, Uguru VE, Dafur SJ, Ekwere EO, Parry O. The Anti-contraceptive and the Effect on Uterus of Ether Extract of *Ricinus communis*. Journal of Phytotherapy Research. 1997;10:97–100.
9. Kabele-Toge BB. The Seeds of Castor Oil Plant (*Ricinus communis* linn) as an Herbal Oral Contraceptive–A Review Nigerian. Journal of Pharmacy. 2003;34:27–32.
10. Lorke D. A new approach to practical acute toxicity testing. Archives of Toxicology. 1983;54(4):275-87.
11. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxaloacetate and glutamic pyruvate transaminase of serum glutamic oxaloacetate and glutamic pyruvate transaminase. AM. J. Clin. path. 1957;28:56-61.
12. King A, Kings K. Standardization of methods for measurement of enzymatic activities in biological fluids. J. Klin. Chem. Klin. Biochemistry. 1972;10:182-92.
13. Weather burn MW. Photometric method for the determination of urea in serum. J. Anal. Chemistry. 1967;39:182-92.
14. Jaffe MZ. Manual Determination of creatinine J. Physiology. Chemistry. 1996;10:391.
15. Richmond N. Enzymatic Determination of cholesterol in the serum or plasma. J. Clinical Chemistry. 1973;19:1350-1356.
16. Friedewald WT, Kochmar JF, Moss DW. Fundamental of Clinical Chemistry. Tiez NW (Ed). Saunders and Company, Philadelphia. 1972;604.
17. Trinder P. Serum Triglycerides Estimation. Ann. Clin Biochemistry.1999;6:24.
18. Rang H, Dale P, Ritter MM, JM. Pharmacology 4th edition Churchill Livingstone London. 1999;58-9.
19. Kaplan A, Jack R, Opleim KF, Toi Volo B, Lyon AW. Clinical Chemistry Interpretation and Tecniques, 4th ed, Williams and Wilkins. Malvern.USA. 1995;133-151,350-366.
20. Blumenkrantz MJ, Kopple JD, Gutman RA. Methods for assessing nutritional States of patients with renal failure. AMJ. Clin Nutri. 1990;33:1567-1585.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=1038&id=3&aid=9110