

Original Research Article

TOXICITY STUDY OF THE OF *RICINUS CUMMUNIS* LNN SEED SUSPENSION IN FEMALE WISTER ALBINO RATS.

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 11.40mg/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 3.80mg/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 11.40mg/kg body weight. Similarly a significant increase ($p < 0.05$) in lipid profile was observed in rats given 11.40mg/kg body weight. Acute toxicity revealed the median lethal dose (LD_{50}) of 1587. . 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 (3.80mg/kg in rat; equivalent to 3 seed/70Kg in human) for long period of time is not harmful to the liver is neither hepatotoxic nor nephrotoxic. *Ricinus communis* seed is therefore safe in rats at the concentrations administered.

Key words: Wister albino rat, LD_{50} , 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

30 Mediterranean Basin, Eastern Africa and India, but is widespread throughout tropical regions
31 (and widely grown elsewhere as an ornamental plant [1].

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

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

48 The toxicity of raw castor beans due to the presence of ricin is well-known [4]. Although the
49 lethal dose in adults is considered to be 4 to 8 seeds, reports of the actual poisoning are relatively
50 rare [4]. If castor bean is ingested, symptoms may be delayed by up to 36 hours but commonly
51 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 of the variety Minor as contraceptive for duration of 12 month [8],[9].

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

2.0 MATERIALS AND METHODS

2.1 Collection of samples:

The seeds of the castor plant (*Ricinus communis*) were collected from Kano along “Jan Bulo” (about 0.5km west of Bayero University Kano) and then authenticated at the Botany Unit of Biological Sciences Department, Bayero University Kano and a voucher specimen kept.

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^{oc}.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.

2.3 Experimental animals: twenty four adult non pregnant female Wister albino rats(100-120g) 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±3.6g 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 1000mg/kg body weight of the aqueous suspension of the seed respectively. Animals were observedfor 24hours and the number of death recorded, if any .They were alsoobserved for 72hours 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 2600mg/kg. Animals were observed for 24hours and final LD₅₀ value was calculated by using geometric mean of 1400 and 1800mg/kg for which 0/1 and 1/1 death occurred respectively.

For subchronic toxicity study, twenty four (24) femaleWister albino rats were grouped into four(4) rats each, randomly assigned to four cages labeled I,II,III,and IV respectively and kept at

room temperature(25^oc). 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 3.80mg/kg, 7.60mg/kg and 11.40mg/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^oC 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]

115 3.RESULTS AND DISCUSSIONS:

116 Many researches were carried out on the contraceptive effect of this seed ethanoic extract but no
 117 research however, to the best of our knowledge that studied its sub chronic toxicity in
 118 rats.Nigerian village women usually swallow 3 seed to prevent conception. Our research is
 119 unique therefore, because we seek to find out the toxicity of the seed as a whole (by making
 120 suspension instead of extract) in rats by mimicking the exact route of administration in humans.

121 3.1 Acute toxicity

122 The result of the acute toxicity studies revealed no mortality at the concentration of 1400mg/kg
 123 body weight in rats; even though the animals were a little bit depressed, sluggish and had a mild
 124 diarrhea. Decreased appetite, increased water consumption by the rats and frequent urination
 125 were also observed, especially in those given the highest dose of the suspension (1800mg/kg); at
 126 which full death occurred. The median lethal dose(LD₅₀) of the aqueous seed extract was
 127 calculated to be 1587mg/kg body weight in rats, which is far higher than the therapeutic/effective
 128 dose (3.80mg/kg), this however gave rise to therapeutic index(therapeutic index=LD₅₀/ED₅₀)of
 129 432.37 which is high enough and therefore safe[18].

130 **Table: 1 (a-b) LD₅₀ Determination of aqueous suspension of *Ricinus communis* (castor**
 131 **bean)seed.**

132 (a)

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

1000 0/3

133 (b)

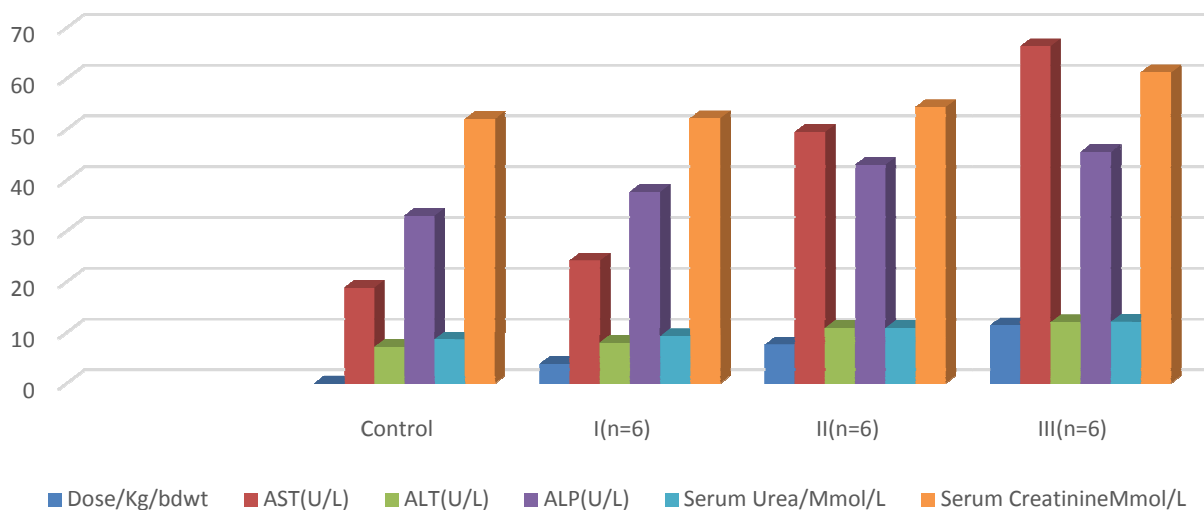
Dose (mg/kg)	Result of second phase
1400	0/1
1800	1/1
2200	1/1
2600	1/1

134 $LD_{50} = \sqrt{(\text{minimum conc. with full death} \times \text{maximum conc. with no death})}$

135 $LD_{50} = \sqrt{(1400 \times 1800)} = 1587 \text{mg/kg}$

136 3.2 Subchronic toxicity studies

Figure1: Effect of oral administration of *Ricinus communis* aqueous seed suspension for four (4) weeks on Liver and Kidney functions of Albino rats



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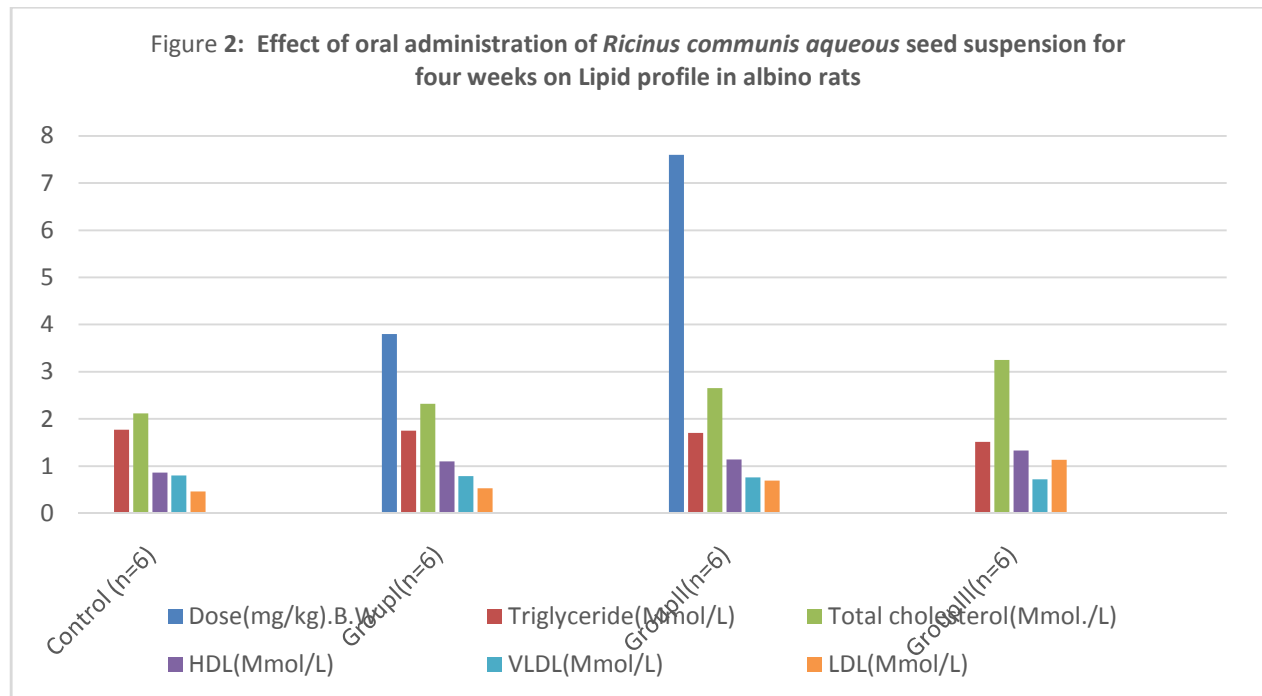
138 For sub chronic toxicity studies, the AST, ALT and ALP activities as well the urea and
 139 creatinine concentrations of group I treated rats (given 3.80mg/kg) revealed no significant

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} (1587mg/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; 3.80mg/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 (3.80mg/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).

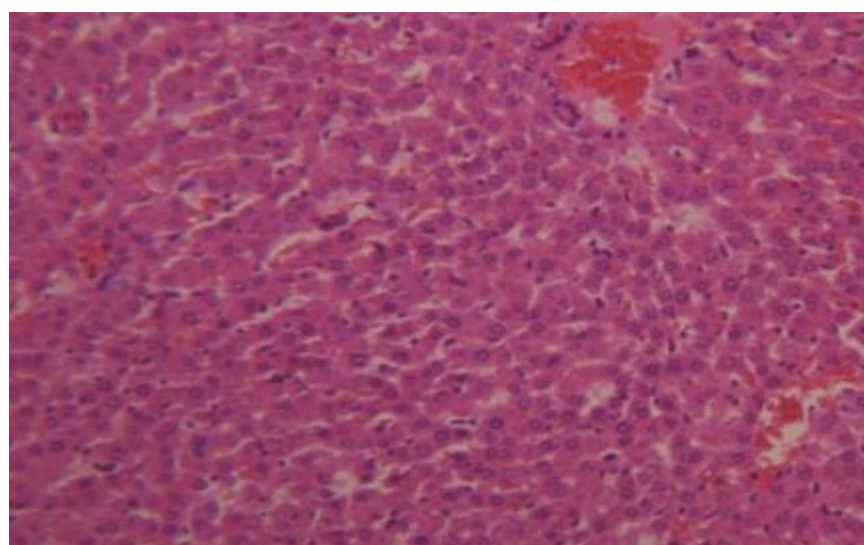
3.3 Lipid profile



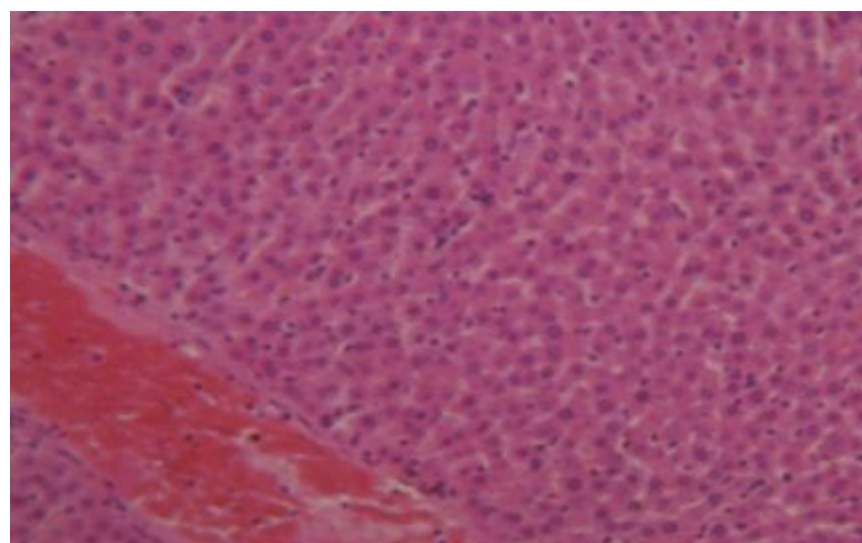
The result of the lipid profile revealed no significant difference ($p>0.05$) between the treated groups I, II and the untreated (control) group. On the contrary, there was significant ($p<0.05$) increase in the levels of total-cholesterol, HDL-cholesterol, LDL-cholesterol and decreased levels of triglyceride 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 ± 0.25 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 predispose one to diseases associated with high serum LDL-cholesterol.

174 3.4 Histological analysis

175 Histological analysis tallied closely with the biochemical analysis on liver and kidney functions
 176 in rats. ~~That is no pathological changes were observed;~~ normal macrostructure of the organs
 177 with no inflammation, congestion or necrosis of the Liver; normal glomeruli and tubules of the
 178 kidneys. This further confirmed the safety of using *Ricinus communis* seed as an oral
 179 contraceptive in rats.

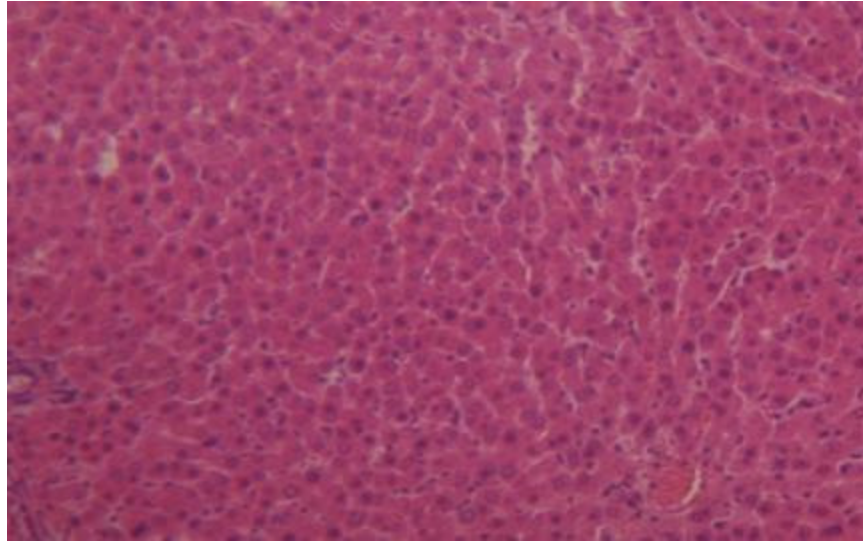


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 181 Plate LC: Liver stained section of control (untreated) rats showing the portal tract area, with no
 182 pathological changes (HandE $\times 100$)



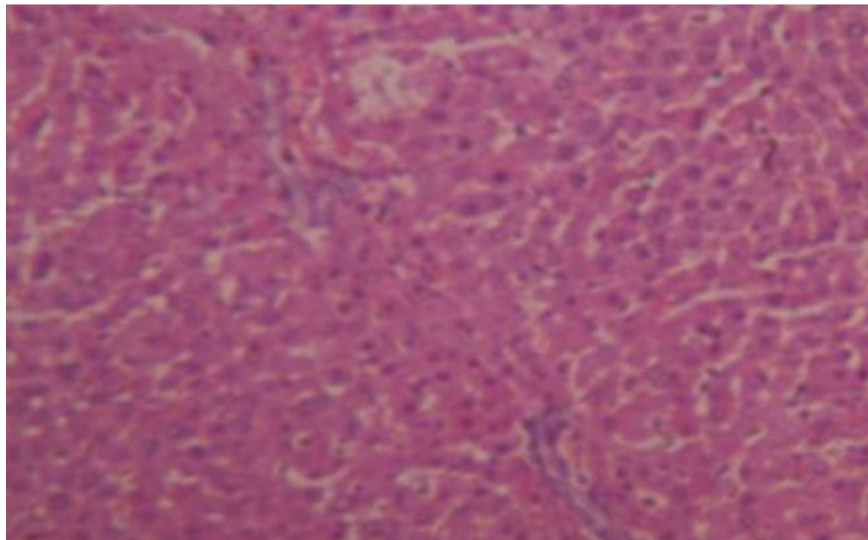
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187 Plate L1: Liver stained section of group1 treated rats showing the portal tract area, with no
188 pathological changes (HandE $\times 100$)



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190 Plate L 2: Liver stained section of group II treated rats showing the portal tract area, with no
191 pathological changes (HandE $\times 100$)

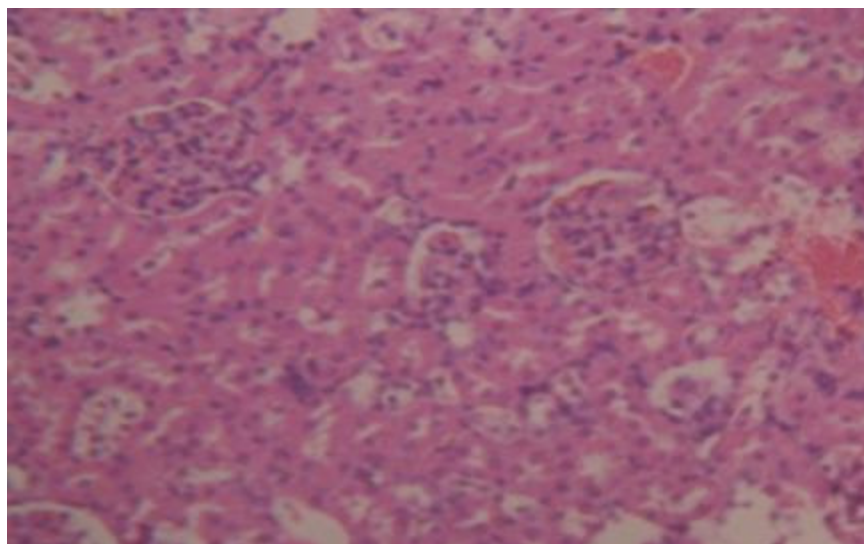


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193 Plate L 3: Liver stained section of group III treated rats showing the portal tractarea, with no
194 pathological changes (HandE $\times 100$).

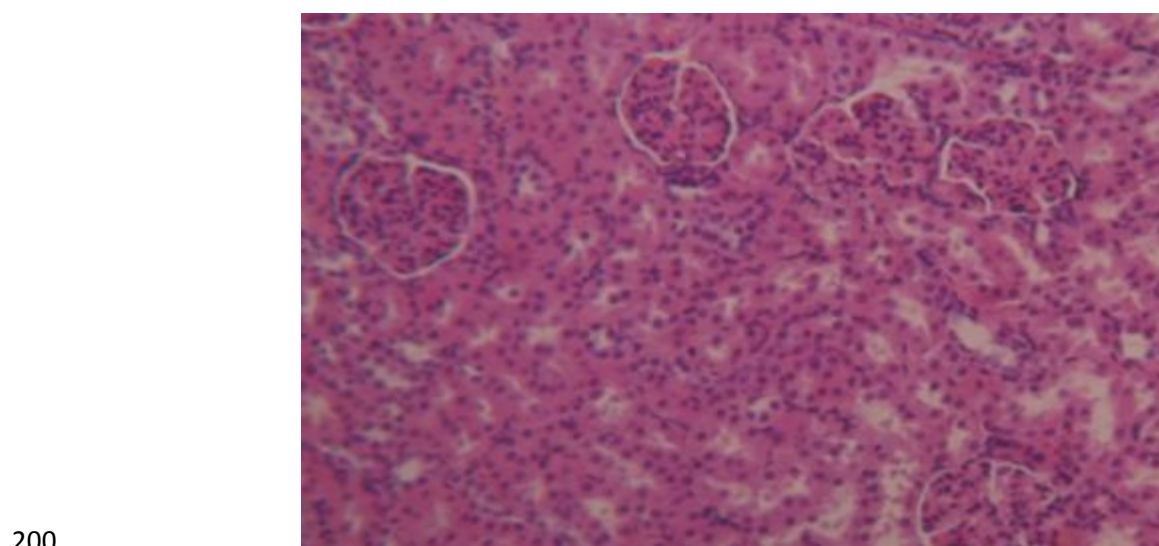
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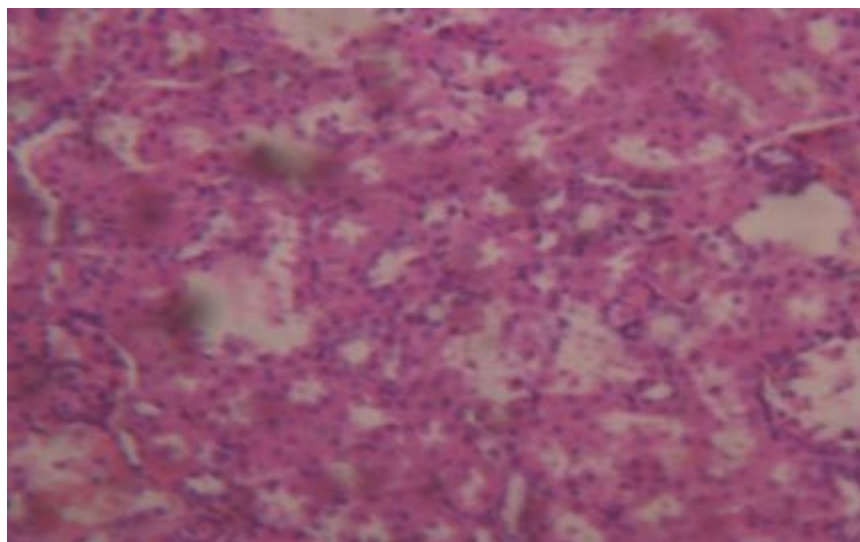
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198 Plate KC: Kidney stained section of control (untreated) rats showing the glomeruli and tubules
199 with no pathological changes (Hand E ($\times 100$))



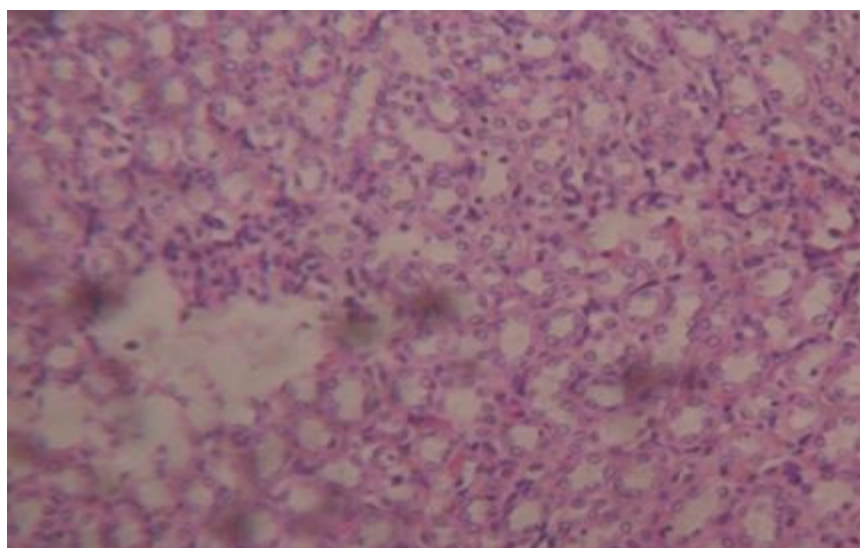
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201 Plate K1: Kidney stained section of group I treated rats showing the glomeruli and tubules with
202 no pathological changes (Hand E ($\times 100$))



203

204 Plate K2: Kidney stained section of group II treated rats showing the glomeruli and tubules with
 205 no pathological changes (Hand E (×100))



206

207 Plate K3: Kidney stained section of group III treated rats showing the glomeruli and tubules with
 208 no pathological changes (Hand E (×100))

209 **Conclusion:** The research revealed that the castor bean seed (*Ricinus communis*) suspension has
 210 no toxicity at 3.80mg/kg body weight/four weeks/day. However higher dosage slightly **increases**

211 liver and kidney functions without visible pathological changes in the organs. Moreover,
 212 excessive consumption of the seed as could lead to weight gain due increase lipid profile
 213 parameters.

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