

Effect of powdered castor oil seed (*Ricinus communis* L.) on some internal organs of albino rat

Olayioye, A., Olaniran*, O. A. and Olaifa, J.I.

Department of Crop and Environmental Protection, Ladoké Akintola University of Technology Ogbomoso, Nigeria

*Corresponding author's E-mail: oolaniran@lautech.edu.ng

Abstract

A study was carried out at the Toxicology laboratory of the Department of Crop and Environmental protection, Ladoké Akintola University of Technology Ogbomoso, to determine the effects of powdered castor oil seed (*Ricinus communis* L. Euphorbiaceae) on kidney, liver, spleen of albino rats. The rats were in five groups, which were replicated three (3) times. The castor oil seed was turned to powdery form using pestle and mortar. Four feed formulations were used; powdered castor oil seed and commercial rat feed mixed in ratio 1:1, 1:2, 1:5, 1:10 and ordinary commercial rat feed, which serves as the control. These formulations were given to four separate groups of rats for a period of three days. The behaviour of the rats was monitored over the three day period. The histology of the kidney, liver and spleen which was initially preserved in formalin was later analysed. Compared with the control the hemorrhagic and necrotic tissues in rats administered with ratio 1:10 and ratio 1:5 showed mild disruption. In ratio 1:2 fed group, hemorrhagic and necrotic tissues showed complete disruption while group fed with 1:1 were extensively disrupted. The results also relayed changes in the body weight and the spleen weight where significant highest, spleen weight were recorded for the rats fed with commercial feed (control) than the rats fed with the treated feeds. The rats fed with ratio 1:1 (treated feed) had the least spleen weight compared to the animals fed with treated feed of ratio 1:2 and 1:5 respectively. No significant difference was observed in the kidney fresh weight for all the treatments. Also, there is significant highest liver weight in rats fed with control feed when compared with rat fed with treated feed, followed least concentration (1:10) of the treated feed. The observation revealed that a powdered castor oil seed to commercial rat feed can be effectively used as rodenticide and it is clearly seen that the function of a powdered castor oil seed affected the internal organ tested.

Key words: castor oil seed, albino rat, kidney, liver, spleen, hemorrhagic and necrotic tissues.

Introduction

The Castor oil seed is the only member of the genus *Ricinus*, which has no immediate relatives. The castor oil seed plant, *Ricinus communis*, is a native of tropical Africa cultivated in several varieties

for the oil found in its leaves and for its bold foliage (Alber and Alber 1993).

The stalked leaves of castor oil seed plant consist of usually eight radiating, pointed leaflets with slightly serrated edges and prominent central veins. Many of the castor bean plant varieties are green, but

some are reddish brown (Cooper and Johnson 1994). The flowers are green and inconspicuous, but pink or red in the pigmented varieties. Many stamens are near the base and branching pistils are near the top of the flower. The soft-spine fruits contain an attractively mottled seeds which are distinctive features of the plant.

The plant growing well in tropical area as a shrub or a tree that can reach 15 metres in height along streams and rivers and on bottom lands with well-drained, nutrient rich soils (Ombrello, Noteworthy 1999). Castor oil seed are grown annually in temperate areas. Castor oil seed contains approximately 50% oil which is composed of 80% to 90% ricinoleic acid (12-hydroxyl-cis-9-octadecenoic acid) (Robbelen et al. 1989). This unique hydroxy fatty acid is used in a number of processes to create unique chemicals and polymers.

The seeds from the castor oil plant, *Ricinus communis*, are poisonous to people, animals and insects. One of the main toxic proteins is "ricin", named by Stillmark in 1888 when he tested the beans extract on red blood cells and saw them agglutinate. Now we know that the agglutination was due to another toxin that was also present, called RCA (*Ricinus communis* agglutinin). Ricin is a potent cytotoxin but a weak hemagglutinin, whereas RCA is a weak cytotoxin and a powerful hemagglutinin.

Poisoning by exposure to castor oil seed is due to ricin, not RCA, because RCA does not penetrate the intestinal wall, and does not affect red blood cells unless given intravenously. If RCA is injected into the blood, it will cause the red blood cells to agglutinate and burst by haemolysis.

Each fruit contains three mottled seeds, 5-15 mm long. They are often used to make necklaces in Mexico, because of their

attractive appearance. The plant also contains compounds which may elicit anaphylactic reactions and these have been recorded in people handling these necklaces, crushing them or putting them in their mouths, as well as in agricultural workers. Because of this, ingestion of any dose of ricin can be fatal within minutes, due to the anaphylactic reaction. (Bernard Knight 1979).

Persons exposed to ricin exhibit different symptoms depending on the route of exposure. Ingestion of ricin causes nausea, vomiting, diarrhea, gastric hemorrhaging and shock. With a sufficient dose, death occurs within 3 to 5 days. Injection of ricin produces severe internal bleeding and tissue death, which can result in the collapse of major organ systems. Death often follows such a collapse. Ricin inhalation irritates the lung linings and airways, leading to weakness and fever. Following this stage, pulmonary lesions may occur causing tissue swelling and further pulmonary damage.

Ricin from castor oil seed has been known as a poison for years, usually through livestock deaths. In Nigeria, the beans/seed are eaten as a food after careful preparation, as heat detoxify or inactivates the toxin, but unwanted children in the past have been murdered by adding castor beans to their food in East Africa.

The lethal dosage of ricin depends on the mode of exposure. Inhaled or injected doses as low as 3 to 5 micrograms per kilogram body weight may be lethal. This dose equals 240 to 400 micrograms for a 80 Kg individual. Because ricin is less well absorbed in the gastrointestinal tract, the lethal dosage for ingestion is higher and it is not active upon skin contact.

A lot of preliminary test has been done using different African plant extract which are known to be toxic, therefore this study is carried out to determine the effect of castor oil seed on the internal organs of albino rats.

Materials and methods

Site of Study

The experiment was carried out at the Toxicology Laboratory in the Department of Crop and Environmental Protection, Faculty of Agricultural Sciences, Ladoko Akintola University of Technology, Ogbomoso.

The albino rats were obtained from the Virology Department, University College Hospital (U.C.H) Ibadan, Oyo State. Thirty (30) experimental rats were divided into 3 replicates and each replicate had 5 cages. Each cage had 2 rats, both male and female. The age of the rats used for this experiment was 9 to 10 weeks.

The rats were fed ad libitum with commercial feed and they were given fresh water once daily from a suspended water feeder.

The castor oil seeds used for this experiment were obtained from the Botanical Garden Ladoko Akintola University of Technology, Ogbomoso. The seeds were weighed (gm) and ground into powder using pestle and

mortar and mixed thoroughly with commercial feed in ratio 1:1, 1:2, 1:5, 1:10 w/w respectively. Commercial rats feed only served as control. The treatments were administered to the test animals.

All the treatments were replicated three (3) times. The experiment lasted for 3 days

Probit analysis was used to determine the LD₅₀.

Statistical Analysis

Genstat statistical package was used to analyse the data collected on the following parameters: body weight, Kidney weight, Liver weight, Spleen weight, and expiration time.

Histopathological Studies

The rats were dissected immediately after the death of each rat and fresh part of the kidney, liver and spleen were removed and preserved in formalin for preservation. The specimens were then taken for histopathological studies at the Histopathology Laboratory, University of Ilorin Teaching Hospital Ilorin, Kwara state. Photomicrograph of tissues were taken and recorded.

Table 1: The effect of powdered castor seed on the body weight, kidney weight, liver weight, spleen weight and time of death of albino rats

Treatment	Body weight after treatment (g)	Kidney weight (g)	Liver weight (g)	Spleen weight (g)	Expiration Time (Hr)
1:1	175.0 ^b	1.0000 ^a	3.83 ^d	0.833 ^b	33.7 ^{bc}
1:2	182.2 ^b	0.9967 ^a	5.00 ^{cd}	0.960 ^{ab}	24.7 ^c
1:5	204.4 ^{ab}	0.9833 ^a	5.00 ^{cd}	0.973 ^{ab}	36.7 ^b
1:10	210.9 ^{ab}	0.9767 ^a	6.83 ^b	0.993 ^a	52.0 ^a
Control	241.1 ^a	1.0000 ^a	9.00 ^a	1.0000 ^a	

Means followed by the same letters within the same column are not significantly different at $p \leq 0.05$, using DMRT.

Results

The effects of the powdered castor seed on the body weight and some internal organ weights of albino rat.

The least body weight was recorded on the rats fed with the highest concentration of castor oil seed 1:1 (Table 1). No significant difference was observed among the rats that were fed with the treated feed irrespective of the concentration but there was a decrease in body weight from the lowest concentration 1:10 to the highest concentration 1:1.

No significant difference was observed in the kidney fresh weight for all the treatments. The fresh weight of kidney in rats fed with various feed treated with castor oil seed powder were not significantly different from those of control (Table 1).

The fresh liver weight, rats that were fed with control feed had highest liver weight followed by the rats fed with the least concentration (1:10) of the treated feed. The same liver weight was recorded for the rats fed with the treated feed of ratio 1:2, and 1:5 while treatment ratio 1:1 recorded the least liver weight (Table 1).

With reference to weight of the spleen, the rats fed with the normal feed (control) had significant higher weight than the rats fed with the treated feed. The rats fed with the highest concentration of ratio 1:1 (treated feed) had the least weight compared to the rats fed with the treated feed of ratio 1:2 – 1:5 (Table 1).

It took higher number of hours (52hrs) before the rats fed with the least concentration (1:10) of the treated feed could die, followed by the rats fed with the treated feed of ratio 1:5 (36.7hrs). No significant difference was observed in the expiration time among the rats fed with the treated feeds.

Ricin is toxic when introduced orally, this has been studied in relation to castor oil seed seeds, The LD 50 (or lethal dose responsible for killing 50 percent of the test population) is 15.49g ricin.

Histopathological studies

The histology Sections of the kidney in the control treatment show glomeruli, proximal and distal tubules as well as the interstitium. Both the cortical and medullary glomeruli appear normal. There is normal mesangial cellularity, i.e. there is normal renal histology. The kidney section of the Albino Rats fed with treatment at ratio 1:1 show distorted histological architecture as evidenced by numerous foci of haemorrhage within the interstitium, more severe within the medullary interstitium. There is extensive necrosis of the tubular structures. The glomeruli are reduced in number and of poor outline which shows that features are in keeping with acute tubulo-interstitial necrosis of the kidneys. The histology Section of the albino rat kidney treated at ratio 1:2 Section shows incomplete loss of renal histological architecture. Features agree with partially necrotic tissue. Treated feed at ratio 1:5 and 1:10 Section shows faint outline of tubules and glomeruli. There are also foci of haemorrhages.

The histology sections of the albino rat liver fed with the normal feed show plates of hepatocytes arranged as anastomosing cords and plates in radial orientation around terminal hepatic venules. Portal tracts are disposed peripherally within each lobules of liver. Within the portal tracts are seen terminal portal venule, bile ductules and branches of the portal artery which shows that there is normal liver histology. The histology sections of the albino rat liver fed with treated feed at ratio 1:1 show severely

distorted histological architecture as evidenced by loss of hepatocytes outline and extensive intraparenchymal haemorrhage. Features are in keeping with severe hepatocellular necrosis. Section of the albino rat liver fed with treated feed at ratio 1:2 shows ghost appearance of hepatocytes and foci of haemorrhages. Features are in keeping with ongoing hepatocellular death. Both treatment 1:5 and 1:10 section shows haemorrhagic and necrotic tissue.

The histology of the splenic tissue in control cage showed splenic pulps which are arranged in nodular pattern with partial trabecular partitions. Both red and the white pulps are distinguishable and numerous small vascular channels are also seen which showed the normal spleen. Whereas the histology of the animal spleen in the first cage treated with a powdered castor seed at ratio 1:1 showed an extensive necrosis and haemorrhage, that is, features are that of necrotic splenic tissue. Derenzini et al (1976) quoted that a severe necrosis of the red pulp of the spleen was observed in rats poisoned with this dose of ricin. The histology of the spleen of the animal in 2nd treatment which was given a powdered castor seed and a commercial rat feed at ratio 1:2 shows completely necrotic tissue while 3rd and 4th treatments which were given a powdered castor seed and a commercial rat feed at ratio 1:5 and 1:10 shows haemorrhagic and necrotic tissue.

In short, all test groups show histological features of lethal tissue damage in the organ examined (kidney, liver, spleen). The control group shows normal tissue.

Discussion

The observation revealed that the castor oil seed caused decrease in body weight and there is a negative effect on both the liver weight and spleen weight of test rats compared to the control rats which were fed with normal feed ration. The negative effect depends on the concentrations applied that is why the highest concentration has the least weight. The internal photomicrograph of the organ of the control rats shows normal tissue compared with tested rat organ fed with treated feed which shows a lethal tissue damage. Ricin is quite stable and extremely toxic to the cells of different organs such as the liver, kidney, lung, pancreas, intestine, and thyroid (Sadani, 1997; Franz, 1997; Greenfield, 2002; DaSilva, 2003). Ingestion of ricin results in gastrointestinal hemorrhage, necrosis of the liver, spleen and kidneys; severe localized muscle pain; regional lymph node necrosis, and moderate involvement of visceral organs. Intravenously administered ricin is found in the spleen followed by kidneys, heart, liver, and thymus (Fodstad, 1976, 1979; Ramsden, 1989; Franz, 1997; Stirpe, 2004; Bismuth, 2004).

This study revealed that the histology of the kidney, liver, spleen showed an extensive necrosis and haemorrhage. Again, mixture of powdered castor oil seed to commercial rat feed fed to rats can be effectively used as rodenticide instead of synthetic rodenticides and it is clearly seen that the function of a powdered castor oil seed affected the internal organ tested.

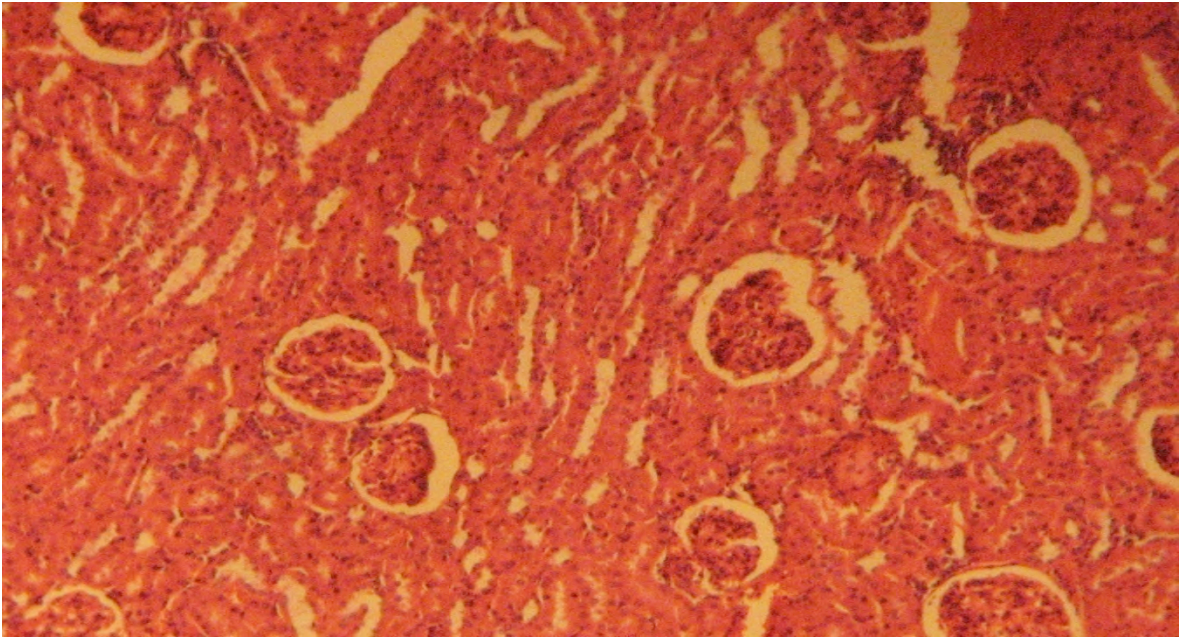
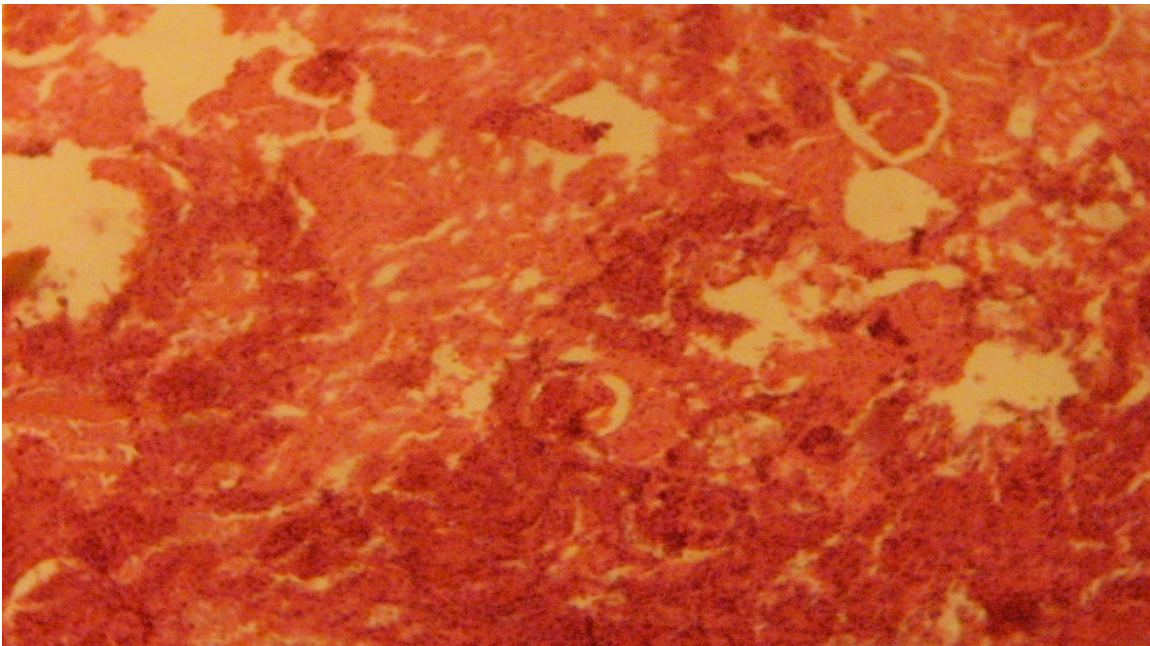


Fig 1: Photomicrograph of the Albino rat kidney treated without castor oil seed powder



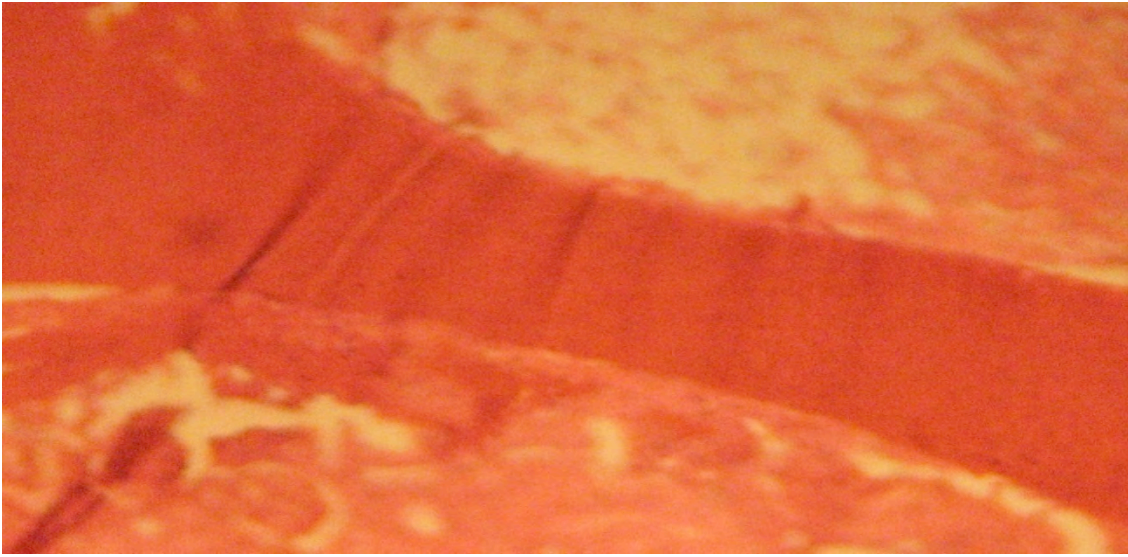


Fig 3: Photomicrograph of the Albino rat kidney treated with powdered castor oil seed and a commercial rat feed at ratio 1:2

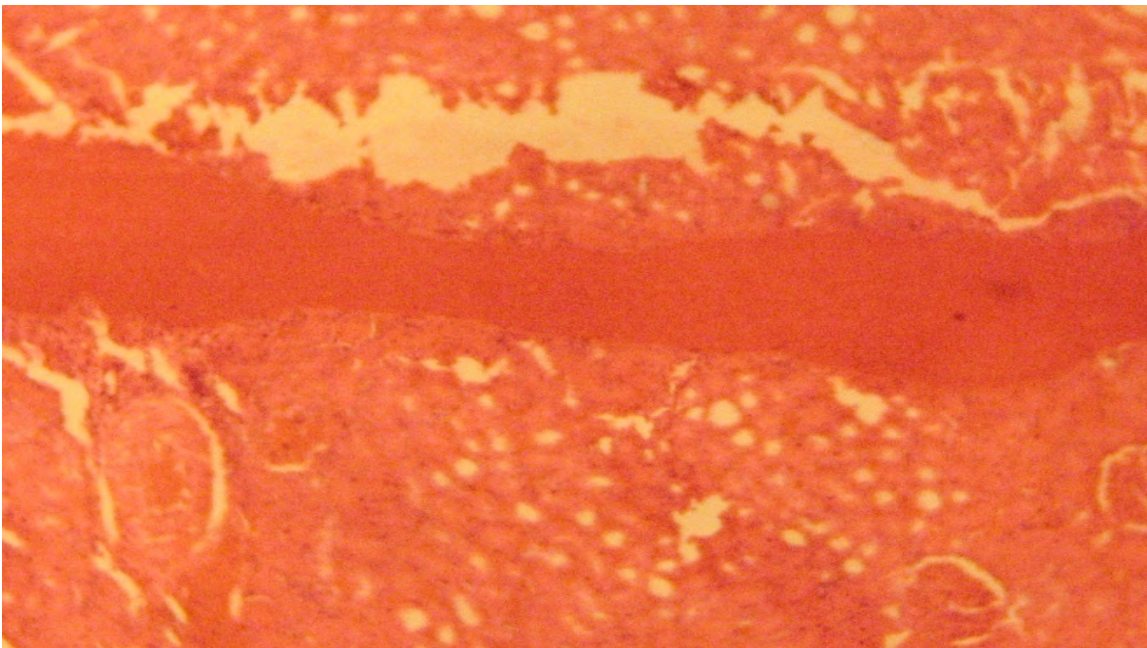


Fig 4: Photomicrograph of the Albino rat kidney treated with powdered castor oil seed and a commercial rat feed at ratio 1:5

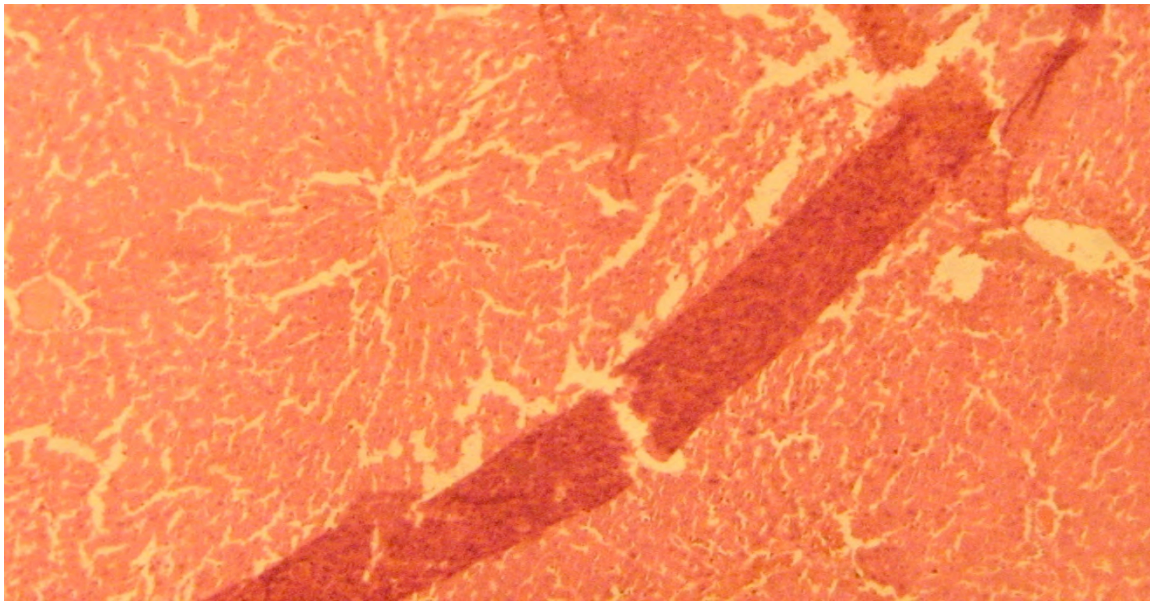


Fig 5: Photomicrograph of the Albino rat kidney treated with powdered Castor oil seed and a commercial rat feed at ratio 1:10

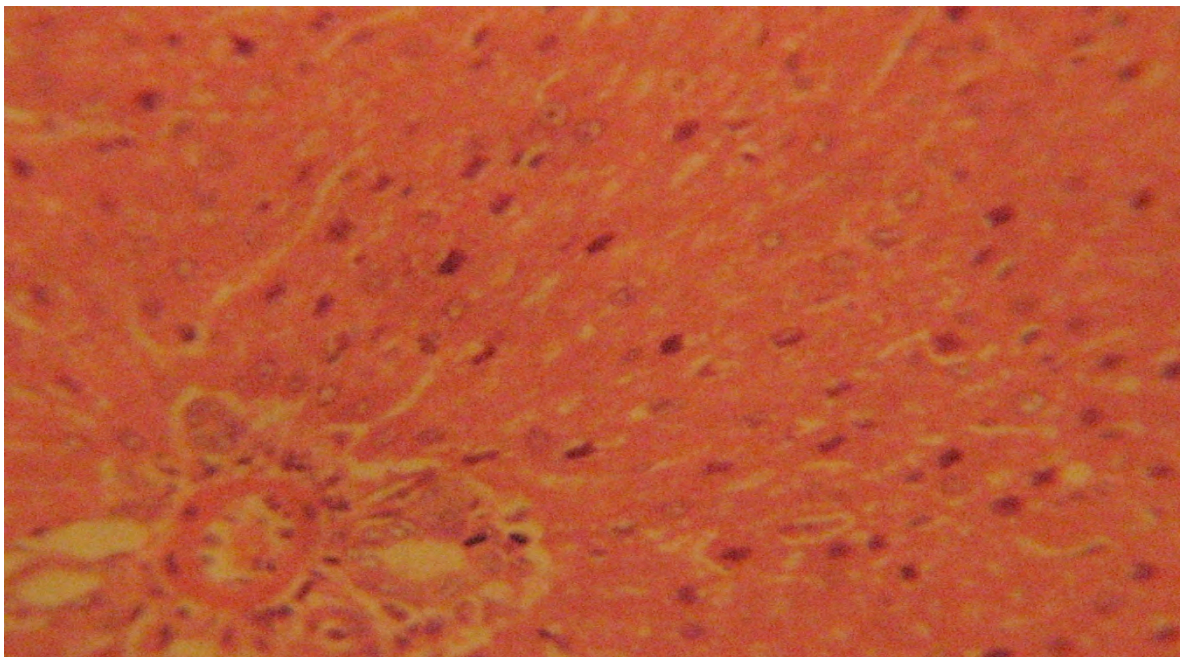




Fig 7: Photomicrograph of the Albino rat liver treated with powdered castor oil seed and a commercial rat feed at ratio 1:1

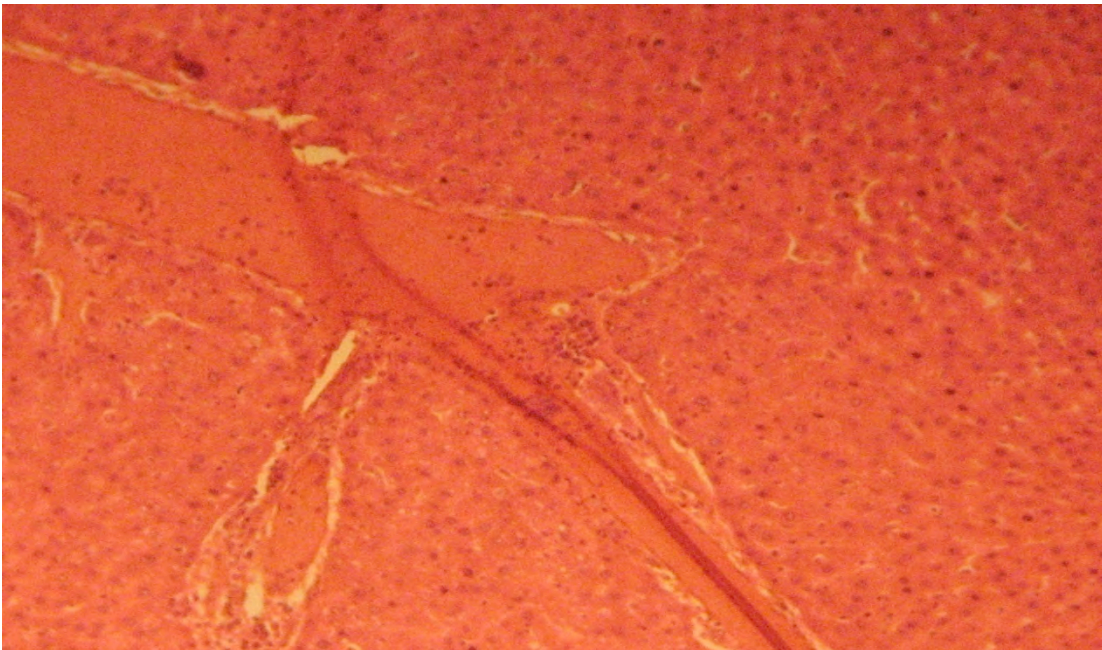


Fig 8: Photomicrograph of the Albino rat liver treated with powdered castor oil seed and a commercial rat feed at ratio 1:2



Fig 9: Photomicrograph of the Albino rat liver treated with powdered castor oil seed and a commercial rat feed at ratio 1:5

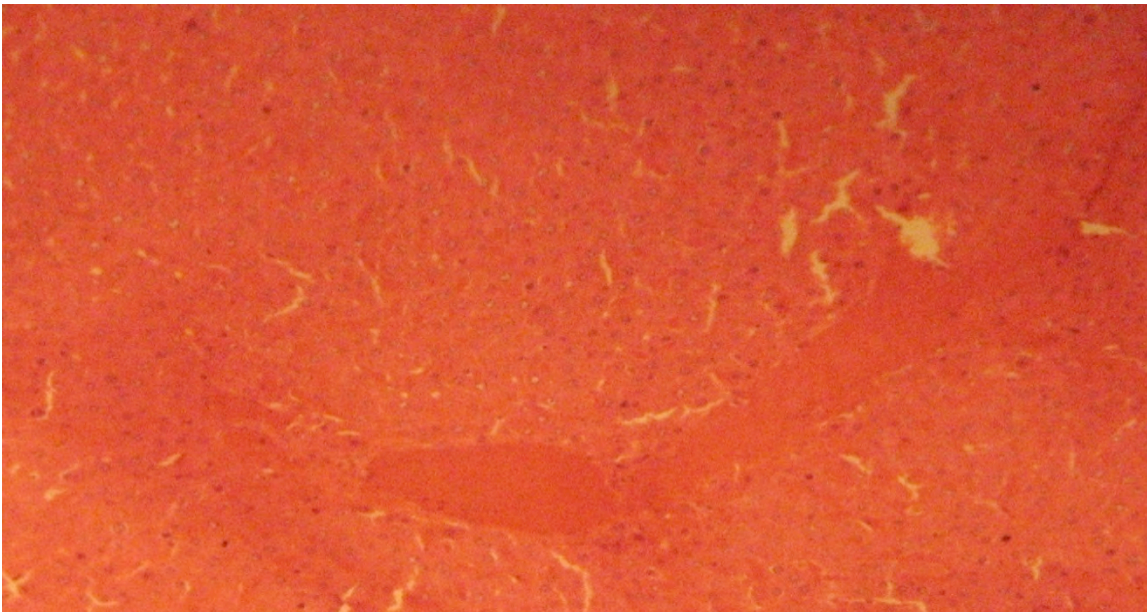


Fig10: Photomicrograph of the Albinorat liver treated with powdered castor oil seed and a commercial rat feed at ratio 1:10

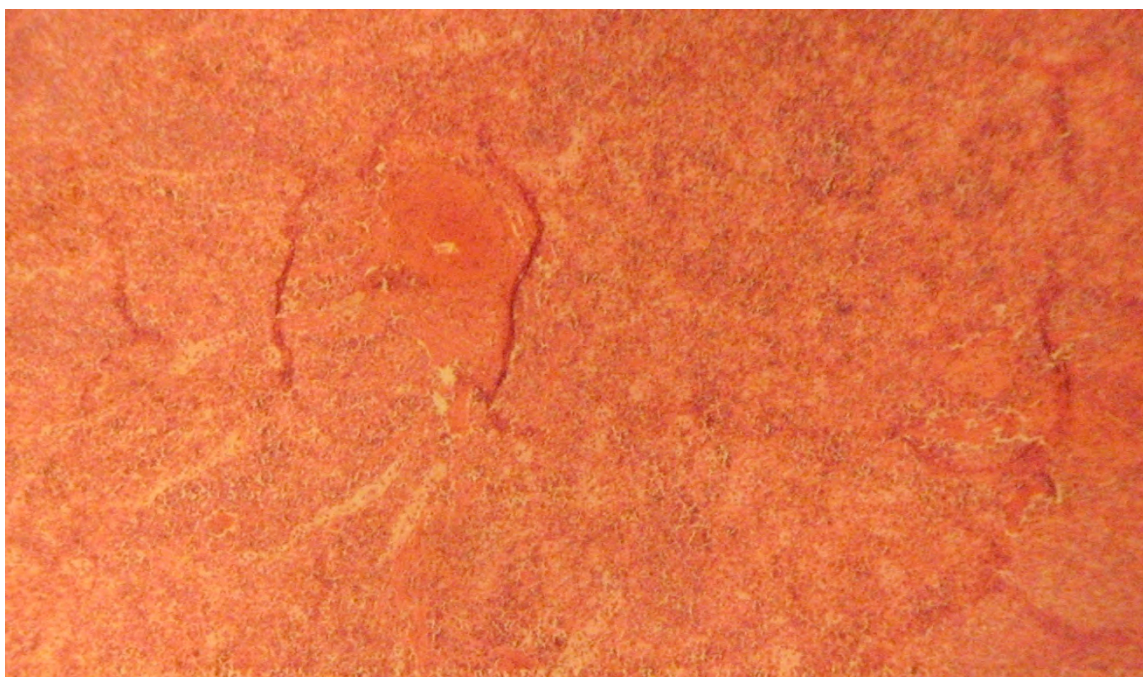


Fig 11: Photomicrograph of the Albino rat spleen treated without castor oil seed powder



Fig 12: Photomicrograph of the Albino rat spleen treated with powdered castor oil seed and a commercial rat feed at ratio 1:1

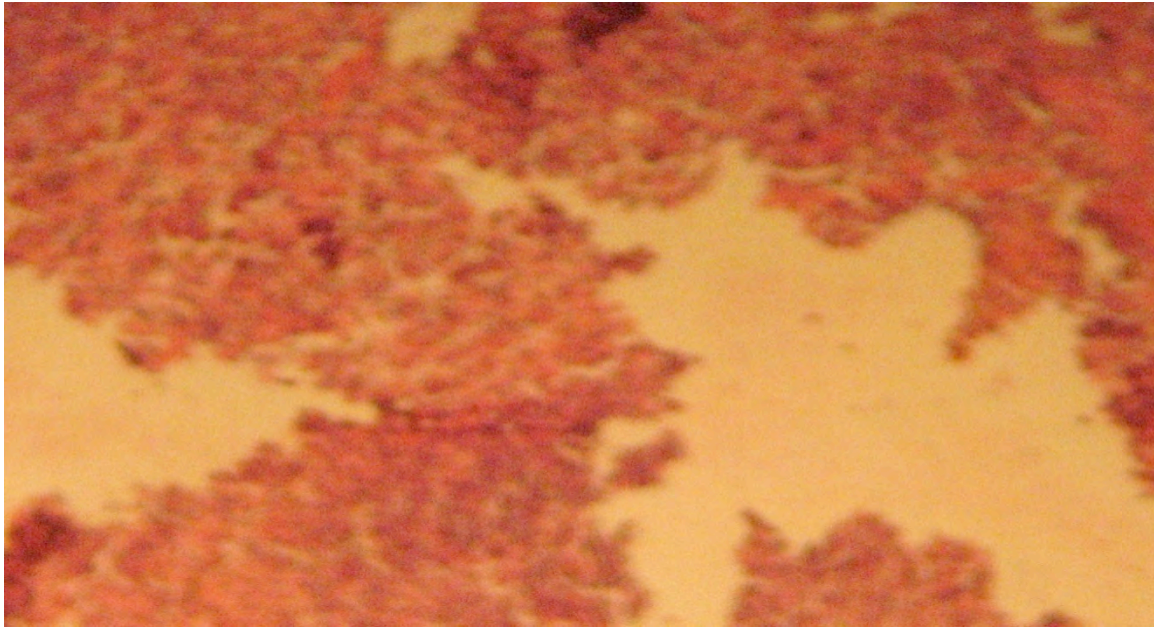


Fig 13: Photomicrograph of the Albino rat spleen treated with powdered castor oil seed and a commercial rat feed at ratio 1:2

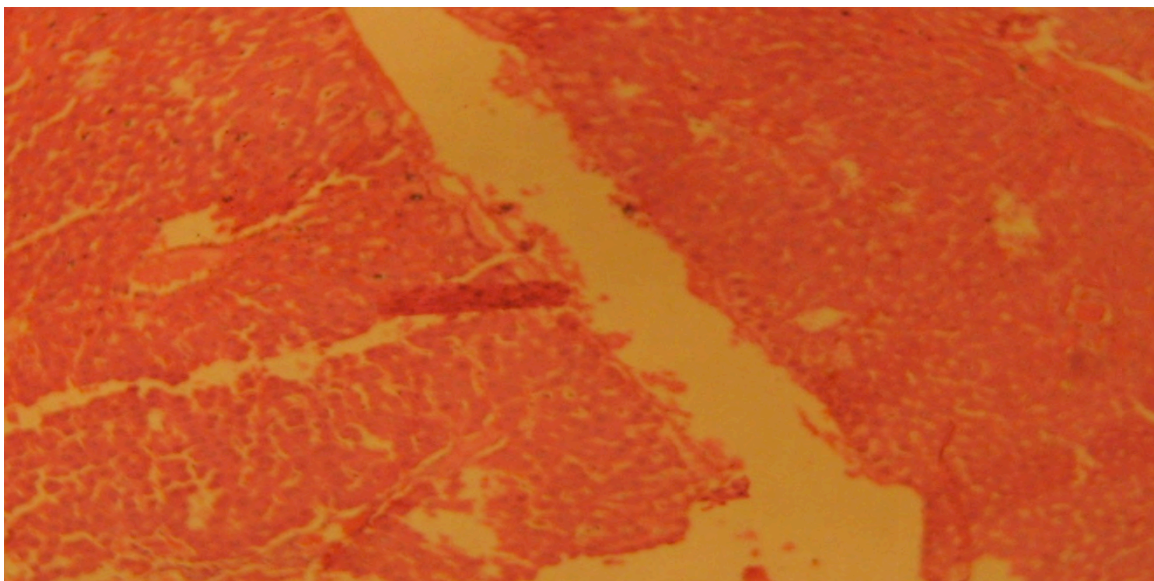


Fig 14: Photomicrograph of the Albino rat spleen treated with powdered castor oil seed and a commercial rat feed at ratio 1:5

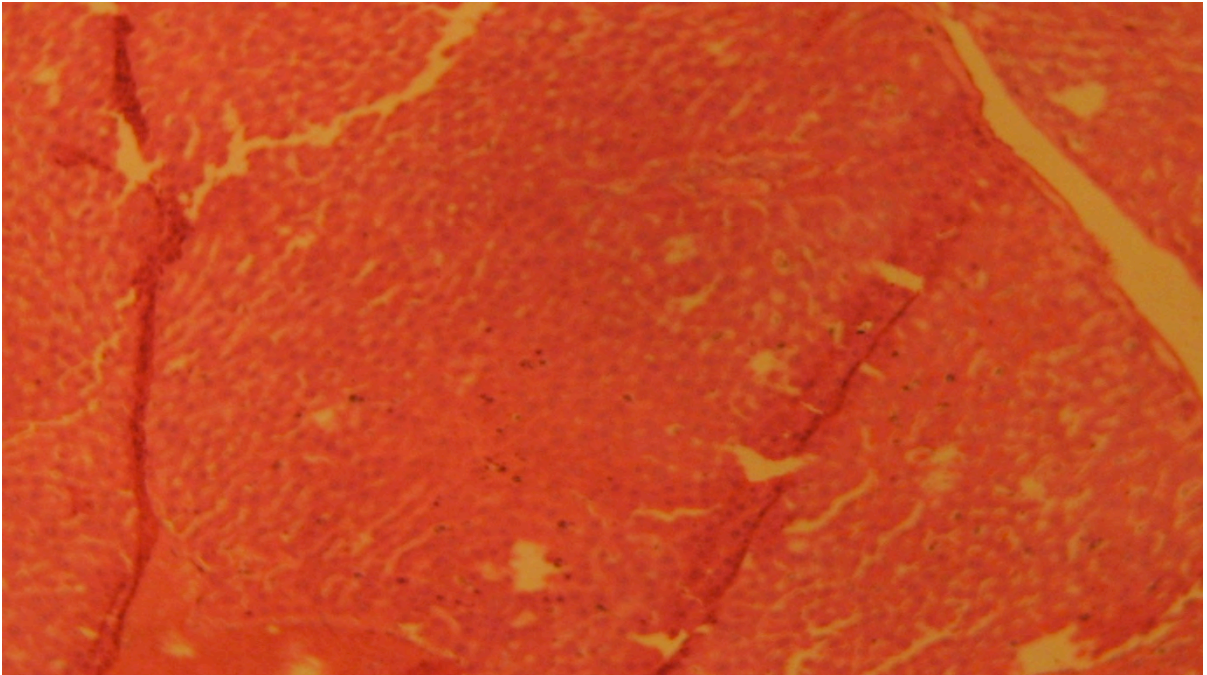


Fig 15: Photomicrograph of the Albin rat spleen treated with powdered castor oil seed and a commercial rat feed at ratio 1:10.

References

- Alber, J.I. and D.M. Alber (1993). *Baby-Safe Houseplants and Cut Flowers: A Guide to Keeping Children and Plants Safely Under the Same Roof*. Story Communications Inc., Pownal, Vermont.
- Cooper, M.R., and A.W. Johnson (1994). *Poisonous Plants and Fungi: An Illustrated Guide*. CAB International Bureau of Animal Health, Weybridge; London.
- Bismuth, C., Borron, S.W., Baud, F. J. and Barriot, P. (2004) Chemical weapons: documented use and compounds on the horizon. *Toxicol.Lett.* 149, 11-18.
- DaSilva, L., Cote, D., Roy, C., Martinez, M., Duniho, S., Pitt, M. L., Downey, T. and Dertzbaugh, M. (2003). Pulmonary gene expression profiling of inhaled ricin. *Toxicol.* 41, 813-822.
- Hartley, M.R.; Lord, J.M. (2004) Cytotoxic ribosome-inactivating lectins from plants. *Biochem. Biophys. Acta* 1701, 1-14.
- Franz, D.R. and Jaax, N.K. (1997) Ricin Toxin. In: *Medical Aspects of Chemical and Biological Warfare*. Borden Institute, Walter Reed Army Medical Center, Washington, DC, Chapter 32, pp. 631-642.
- Fodstad, O., Olsnes, S. and Pihl, A. (1976). Toxicity, distribution and elimination of the cancerostaticlectins abrin and ricin after parenteral injection into mice. *Br. J. Cancer* 34, 418-425.
- Fodstad, O., Johannessen J.V., Schjerven, L. and Pihl, A. (1979) Toxicity of abrin and ricin in mice and dogs. *J.*

- Toxicol Environm Health* 5, 1073-1084.
- Greenfield, R.A., Brown, B.R., Hutchins, J.B., Iandolo, J.J., Jackson, R., Slater, L.N. and Bronze, M.S. (2002). Microbiological, biological, and chemical weapons of warfare and terrorism. *Am. J. Med. Sci.* 323, 326-340.
- Knight B. (1979). Ricin - a potent homicidal poison. *BMJ* 1979 Feb 3: 350.
- Olsnes, S. and Pihl, A. (1972) Ricin-a potent inhibitor of protein synthesis. *FEBS Lett.* 20, 327-329.
- Olsnes, S. (2004) The history of ricin, abrin and related toxins. *Toxicon* 44, 361-370.
- Ramsden, C. S., Drayson, M. T. and Bell, E. B. (1989) The toxicity, distribution and excretion of ricin holotoxin in rats. *Toxicol.* 55, 161-171.
- Sadani, G.R., Soman, C.S., Deodhar, K. K. and Nadkarni, G. D. (1997) Reactive oxygen species involvement in ricin-induced thyroid toxicity in rat. *Hum. Exp. Toxicol.* 16, 254-256.
- Stirpe, F. (2004) Ribosome-inactivating proteins. *Toxicon* 44, 371-383. Dr. T. Ombrello - UCC Biology Department