Effect Of Two Detoxifing Agents (Thermal, Irradiation) On *Ricinus Communis* Seed Toxicity In Rats

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ABSTRACT

Castor bean seeds *(Ricinus Communis)* is mainly a source of oil, used for technical purposes and as a purgative. Pressed cake is used as feed and fertilizer after detoxification of seeds. Castor bean contains up to 25% protein, among the protein is the highly toxic lectin ricin. Destruction of ricin has been attempted by heat,certain chemoprotectants, irradiation and others.

The present work provides a comparative study on the changes of chemical constituents resulting from gamma irradiation of *Ricinus Communis* seed at dose levels of 10, 20, 30, 40, 50 and 60 kGy and heat (Microwave Siting Power 50°C) for 2, 4, 6 and 8 min.. Ultra vilot spectra of seed powder and hemagglutination activity were carried out (as indicator of ricin levels). Also a study on the effects of diets contain irradiated (50 kGy) or heat treated (siting power 50°C for 8 min.) and raw castor bean after feeding Albino rats wasdone. Rats fed diet contained irradiated or heated castor bean showed a decrease in body weight, hemoglobin content, packed cell volume. Serum alanine and aspartate aminotransferase activities and uric acid level were elevated. Decrease of hemagglutination activity of liver and kidney extracts were recorded. Patholgoic examination revealed destruction of epithelium cells of small intestine of the group fed diet contains irradiated castor bean. Liver showed degenerative changes and spleen showed erosion and hemorrage. Kidney congestion was seen in groups fed diets contain irradiated or heated castor bean.

INTRODUCTION

Ricnus Communis seeds have been found in Egyptian graves dating around 400 B.C. The seeds contain 1 mg / g ricin *(1)* and up to 25% protein which can be pressed as pressed cake used as food and fertilizer after detoxification by heat *(2)* . Ricin is an extremely toxic phytotoxin formed from two polypeptide (A and B chains) linked together by disulfure bound; tryptic hydrolysis gives several peptides *(3)*. Two agglutinins have hemagglutinating property and different molecular weights were isolated and purified from *Ricinus Communis* (RCAI , RCAII) *(4)*. The specificities of RCAI and RCAII were determined by inhibition of quantitative precipitation of blood *(5)* .

The lethal dose of purified ricin was 1 ug/kg body weight for mouse , rat and dog, while in rabbits it was 10 times more sensitive *(1)* .

Ricin caused weakness, apathy, dullness, poor growth , dysmenorrhoa, hypertension and locomotor and gastrointestinal disturbances, *(6-9)* . Urine showed protein casts, R. B. C. , and haemoglobin; also an increase in blood urea; non protein nitrogen and increase in serum transaminases were recorded *(7,10)*. Animals died from dramatic hepatonephritis *(3)* . *Ricinus Communis* caused no change in blood urea and transaminases but caused a decrease in body weight *(11)* .

Ricin caused liver necrosis , damage in sinusoidal cells, severe necrosis of red pulp of spleen, sinusoidal haemorrhage; severe gastro-enteritis with erosion *(12-14)*. Atrophy of villus, elongation of crypt, degeneration of epithelium, decrease of goblet cells, infiltration of neutrophils and eosinophils between eptiehlium and lamina propria and hydropic change of small intestine of rat *(15,16)*. Glomerular thrombotic microangiopathy was also recorded *(17)*.

Certain chemoprotectants inhibit cytotoxicity of ricin *(18)* . *Ricinus Communis* agglutinin dissociated to a lower molecular weight when heated in sodium dodecyl sulfate in the absence of reducing agents *(19)*. Toxicity can be blocked by diethyl maleate *(20)* and alpha mannosidase and endoglycosidase H *(21)*. Detoxication of ricin could be achieved by cross linking of A and B chains by 1,6 bismale imidohexane or N-bromosuccinimide and the detoxicated ricin caused no appreciable damage in the small intestine of rat *(22)*. Triaceylated galacto and glucoderivatives of 2-deoxy -2-fluoro- D-pyranosyl fluoride as well as alpha and beta N bromoacetuyle D-galactopyranos- ylamine can also inhibit the cytotoxicity of ricin *(23)* . Rats immunised against ricin by formaldehyde toxids protect against lethality by inhalation *(24)* .

Heat stress on small intestinal mucosa of rats receiving ricin intraluminally caused protection against intestinal inflammation *(25)*.

World Health Organization (WHO), Food and Agriculture Organization (FAO) and International Atomic Energy Agency (IAEA), have jointly confirmed that irradiation is safe for a wide range of food when the absorbed dose does not exceed 10 KGy *(26)*.

Irradiation of food and feeds with gamma rays , x rays (up to 5 Mev.) beta rays and high energy electrons (up to 10 Mev.) does not render the foods and feed radioactive *(27)*.

This work was aimed to study the effect of different doses of radiation and heat on the chemical composition and ricin level in *Ricinus Communis* seeds. In addition toxicological study was carried out on rat fed raw , heat treated or irradiated seeds.

MATERIAL AND METHODS

Castor oil seeds *(Ricinus Communis)* were obtained from Agricultural Research Center, Ministry of Agriculture, Giza, Egypt.

Seed treatment

Radiation treatment

Castor bean seeds were free from husk, stone, etc. packed in polyethelene bags and sealed. Seeds were irradiated by Gamma-cell 220, Candian facility (in National Center for Radiation Research and Technology at Nacer City, Cairo Egypt). The applied doses were 10, 20, 30, 40, 50 and 60 KGy, calibrated using small pieces of radiochromic film *(28)*.

Thermal treatment

Seeds were treated by heat using Microwave (Power siting 50°C) for different time (2, 4, 6 and 8 minutes).

Chemical analysis of *Ricinus communis* seeds

Crude protein and fat content of treated (Radiation, heat) and non treated (control) seeds were determined *(29)* . Ultraviolet spectra of ricin *(Ricinus Communis)* were determined*(30)* and the ricin levels were qualitatively determined using agglutination test *(31)*.

Toxicologic study

Thirty rats (weighted 90.2-94.1 g) were divided into three groups, each of 10 rats of both sexes. The first group was fed control diet, the second and third groups were fed diets contain irradiated (50 KGy) and heated castor bean (power siting 50°C for 8Min.) respectively. A fourth group fed diet contain 35% untreated castor seed and died after 4 days. The diets composition (Table 1) was rat standard diet *(32)*. Body weight were calculated weekly.

Sampling

At the end of experiment (4 weeks) rats were sacrified and blood samples were collected for determination of haemoglobin and packed cell volume *(33)*. Serum samples were stored at -20 until used for determination of transaminases activities (ALT and AST) *(34)*. Serum urea *(35)* ; creatinine *(36)* and uric acid were determined *(37)*.

Table (1):Control and experimental diets using ingredents irradited and heated caster bean .

Ingredient	Control diet %	Experimental deits *
Castor bean	-	35
Corn oil	51	47.5
Soy bean meal	30	10
Fish powder	16	5
Sodium chloride	0.5	0.5
Smooth wood powder	2	1.5
Vitamins	0.5	0.5

*Irradited and heated castor bean (full fat) were used by same levels in diets fed to the second and the third groups.

Samples from liver and kidney were obtained for extraction and determination of ricin by detection of haemaglutination activity as indication of ricin residue *(31)*.

Specimens from liver, spleen, kidney and small intestine were fixed in 10% buffered neutral formalin solution and used for histopathologic examination, using hematoxylin and eosin *(38)*.

Statistical analysis of data was carried out *(39)*.

RESULTS

Protein and fat percentages of raw, irradiated and heated castor bean *(Ricinus Communis)* are shown in table (2). Crude protein content of raw castor bean was 34.5 ±0.62%. Crude protein content in irradiated castor bean were 28.08 ± 0.47, 27.82 ± 0.74, 32.5± 1.13, 33.15 ± 1.01, 30.75 ± 0.41 and 30.3 ± 0.32 % for irradiated doses of 10, 20, 30, 40, 50 and 60 kGy respectively. Seeds of

Table (2) : Chemical composition of Raw , irradiated and heated castor bean (Mean ± S.E).

Treatment	Protein %	Fat %
Raw caster bean (control)	34.5±0.62	35.047 ± 0.82
10KGy	28.08±0.47**	24.8±1.1**
20KGy	27.82±0.735**	33.09±1.25
30KGy	32.5±1.13	36.37±0.184
40KGy	33.15±1.01	32.63±1.085
50KGy	30.75±0.414**	29.73±1.680
60KGy	30.36±0.32	33.29±0.614
Microwave (power siting 50 for 2 min)	33.99±1.26	33.98±1.16
Microwave (power siting 50 for 4 min)	30.98±0.59*	32.6±1.08
Microwave (power siting 50 for 6 min)	32.88±0.919	36.14±0.741
Microwave (power siting 50 for 8 min)	30.3±0.389*	30.07±0.21

* Significant at P≤ 0.05 ** High significant at P≤ 0.01

castor bean irradiated by 10, 20 and 50 KGy showed a significant decrease in protein %. Castor bean irradiated by 60 KGy showed a significant decrease as compared with control (raw seeds). Heated castor bean (sitting power 50 microwave) showed a significant decrease in crude protein for 4 and 8min exposure time. Crude protein content at 4 and 8 minute were 30.98 ± 0.59 and 30.3 ± 0.389% compared to 34.5 ±0.62% for raw seeds. Fat % for all groups (irradiated or heated) showed no change as compared with fat % in raw seed. Only at 10 KGy high significant decrease in fat content was detected.

Ultravilot absorption spectra of *Ricinus Communious* seed powder extract (raw, irradiated and heated) were shown in Fig. (1). Samples irradiated by 50 and 60 KGy showed the lowest ultraviolt absorption as compared with raw seeds. Castor bean heated in microwave (Siting power 50) for 8 min. showed low ultraviolt absorption as compared with raw castor bean (control), but still higher than samples irradiated by 50 and 60 KGy.

Qualitative determination of ricin residue in raw, irradiated and heated castor bean *(Ricinus Communis)* using hemagglutination test is shown in table (3). End point of hemagglutinating activity was detected at 1:32, 1:16, 1:16, 1 :16, 1:4 and 1:4 dilutions for samples irradiated by 10, 20, 30, 40, 50 and 60

Table (3):Hemagglutinating activity of irradiated and heated castor bean in comparison to raw seeds as an indicator for ricin level.

Castor bean samples	Dilutions										Hemag glutating activity Hu/g**	Destruction rate of hemag-glutination
	Non	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:265	1:512		
Raw castor bean (Control)	4+	3+	3+	3+	2+	2+	2+	1+	-	-	2560	100%
10 kGy	3+	3+	3+	2+	2+	1+	-	-	-	-	640	25%
20kGy	3+	3+	3+	2+	1+	-	-	-	-	-	320	12.5%
30kGy	3+	3+	3+	2+	1+	-	-	-	-	-	320	12.5%
40kGy	3+	3+	2+	2+	1+	-	-	-	-	-	320	12.5%
50kGy	2+	2+	1+	-	-	-	-	-	-	-	80	3.13%
60kGy	2+	2+	1+	-	-	-	-	-	-	-	80	3.13%
Microsave* bawer (2min)	3+	3+	3+	2+	1+	-	-	-	-	-	320	12.5%
Microwave * (4min.)	3+	3+	2+	2+	1+	-	-	-	-	-	320	12.5%
Microwave *(6 min)	3+	3+	2+	1+	-	-	-	-	-	-	160	6.26%
Microwave (8 min)	2+	2+	2+	1+	-	-	-	-	-	-	160	6.26%

* Sitting power 50°C

** Hemagglutinating activity (Hu/gm) = 20 x Da x Db

Where Da = dilution factor of extract in tube 1 (= 1 unless original extract was diluted)

Db = dilution factor of tube containing lhu.

KGy respectively in comparison with dilution 1:128 for raw sample. In heated samples (Siting power 50) end point for hemagglutination activity was detected at dilution 1:16, 1:16 1:8 and 1:8 for heated time 2,4,6 and 8 min. respectively in comparison to 1:128 for raw seeds. The lowest hemagglutination destruction rate was 3.3% for irradiated dose 50 and 60 KGy and 6.26% for heated samples (siting power 50) for 6 and 8 minutes.

Body weight of albino rats fed on control diet (Free from castor bean) and diets contain irradiated or heated castor bean are shown in table (4). Significant decrease in body weight was detected in the third group, (fed diet contains heated castor bean) after two weeks as compared to control group. Body weights were 90.9± 2.04g and 98 ± 1.8g for the third and the control groups respectively. Decrease in body weight of second and third groups were recorded at third and fourth weeks of study in comparison to control group.

Haemoglobin content and packed cell volume are shown in table (5). Second and third groups showed a decrease in haemoglobin content in comparison to control group. Haemoglobin content were 9.38 ± 0.35, 8.45 ± 0.61 and 12.37 ± 0.49 g/dl for second, third and control groups respeclively. Decrease in packed cell volume were detected in second and third groups as compared with the control.

Packed cell volume were 24.16 ± 1.72, 25.67 ± 0.96 and 34 ± 1.69% for second, third and control groups respectively.

Serum biochemical parameters are shown in table (6). Rats fed on diets contain irradiated or heated castor bean for 4 weeks showed an increase in serum alanine amino (ALT) and aspartate (AST) transferases in comparison to control group. Serum ALT levels were 130.25 ± 2.69, 166.67 ± 7.5 and 155.6 ± 4.71 U/L for control, second and third groups respectively. Serum AST levels were 71.56 ± 2.89, 93.94 ± 2.81 and 90.26 ± 2.06 U/L for control, second and third groups respectively. Serum urea and creatinine showed no significant change, while uric acid showed an increase in group fed a diet contains irradiated castor bean and group fed on diet contains heated castor bean.

Hemagglutinating activity of liver and kidney extracts are shown in table (7). Hemagglutinating end point was detected at 1:4 and 1:8 dilution for liver extract of second group (fed diet contains irradiated castor bean) and third group (fed diet contains heated costor bean) respectively. End point of hemagglutinating activity of kidney extracts was detected at 1:2 dilutions for second and third groups. Hemagglutinating activity were 80, 160, 40 and 40 Hu/g for liver extract of second and third groups and kidney of second and third groups respectively.

Histopathologic examination of the small intestine of the second group showed destruction and necrosis in comparison to

Table (4) : Body weight (g) of Albino rats fed on control diet and experimental diet contain irradiated (50 KGy) and heated (siting power 50°C in microwave for 8 min.) bean (Mean ±S.E.)

Groups	Diet	Zero weak	First weak	Second weak	Third weak	Fourth weak
Group (I)	Free from castor bean	90.2± 1.8	92.6± 1.88	98± 1.8	105.3± 1.65	112.3± 1.61
Group (II)	Irradiated (50 kGy) castor bean	94.1± 2.51	92.4± 2.47	93.5± 3.16	94.7**± 2.6	98.2**± 1.94
Group (III)	Heated (for 8 min) castor bean.	92.2 ± 2.67	89.9 ± 3.16	90.9* ± 2.04	96.8** ± 2.49	103.1** ± 2.31

* Significant P ≤ 0.05 ** High significant P ≤ 0.01

Table (5) : Haemooglobin content (g/dl) and packed cell volume % of rats fed on control diet and diets contain irradiated (50 kGy) and heated (mierowave siting power 50 for 8 min) castor bean diet (Mean ± S.E.).

Groups	Diet	Hb (g/dL)	P.C.V.%
I	Free from castor bean	12.37 ±0.49	34 ±1.69
II	Irradiated castor bean (50 kg Gy)	9.38* ±0.35	24.16* ±1.72
III	Heated castor bean by (Microwave siting power 50 for 8 min)	8.45* ±0.61	25.67* ±0.96

* High significant $P \leq 0.01$

Table (6): Effect of ration contains irradiated or heated castor bean fed to Albino rats on Serum AST, ALT, urea, creatinine and uric acid in comparison to control group (Mean (S.E).

Groups	Diet	ALT U/L	AST U/L	Urea gm/L	Creatinin mg/100ml	Uric acid mg/L
I (Control)	Free from castor bean	130.25± 2.69	71.56 ±2.89	0.46 ±0.038	1.79 ±0.05	67.5 ±0.22
II	Irradiated castor bean	166.67* ±7.5	93.94* ±2.81	0.417 ±0.02	1.53 ±0.13	79.32* ±3.11
III	Heated castor bean	155.6* ±4.71	90.26* ±2.06	0.58 ±0.05	1.55 ±0.28	77.9* ±3.13

* High significant $P \leq 0.01$

Table (7): Hemagglutinating activity of ricin extracted from liver and kidney of Albino rats fed on control and experimental diets contain irradiated or heated castor bean for 4 weeks.

Dilution	Liver			Kidney		
	Group I Control	Group II	Group III	Group I Control	Group II	Group III
Non	-	3+	3+	-	2+	2+
1 : 2	-	2+	2+	-	1+	1+
1 : 4	-	1+	2+	-	-	-
1 : 8	-	-	1+	-	-	-
1 : 16	-	-	-	-	-	-
1 : 32	-	-	-	-	-	-
1 : 64	-	-	-	-	-	-
1 : 128	-	-	-	-	-	-
1 : 265	-	-	-	-	-	-
1 : 512	-	-	-	-	-	-
Hemagglutinting activity Hu/gm	-	80	160	40	40	40

control group (Fig 2a and b). Liver of second group showed necrosis, (Fig 3b); liver of third group showed congestion and necrosis (Fig. 3c) in comparison to control group (Fig. 3a). Spleen of second group showed heamorrage and necrosis (Fig. 4b). Spleen of third group showed necrosis and erosion (Fig. 4c) in comparison to control group (Fig. 4a). The kidney of second group showed congestion and necrosis (Fig. 5b), while kidney of third group showed necrosis, degeneration and haemorrhage (Fig. 5c) in comparison to control group (Fig. 5a).

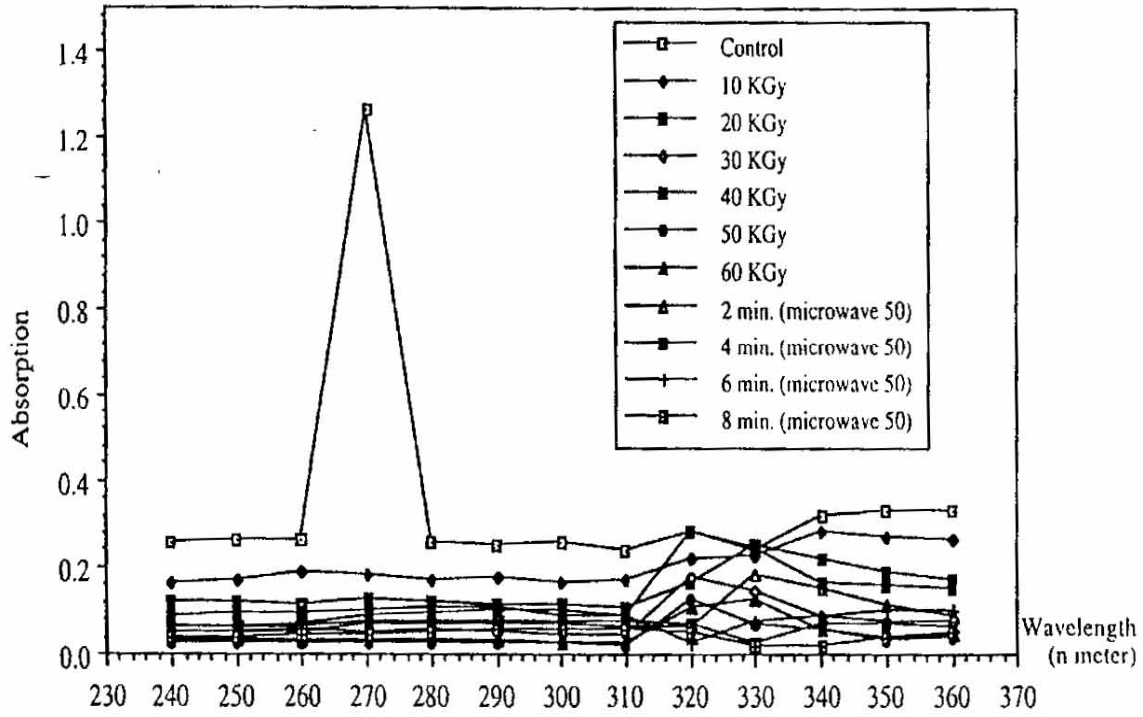

Fig. (1) Ultraviolt absorption spectra of the *Ricinus Communious* seeds powder extract (conc. 120 ug/ml)

Fig. (2a , b) : Small intestine from rats fed on diet contained castor bean irradiated with 50 KGy showed destruction and necrosis of epithelial cells (2 a) in comparison to small intestine of control group (From left to right x 100) .

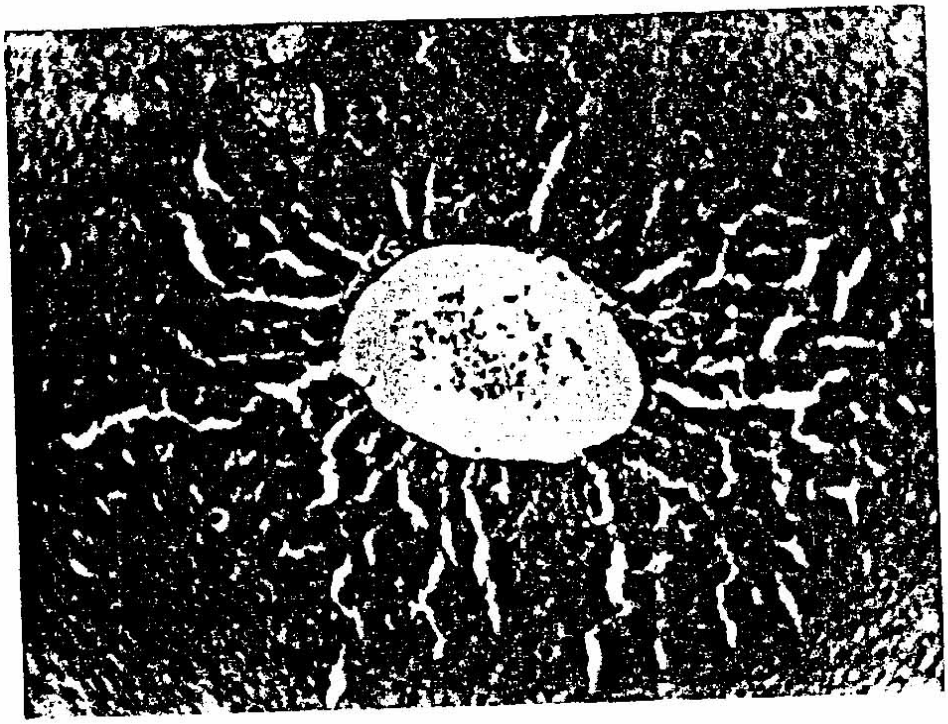

Fig (3a): Liver of control group x 200

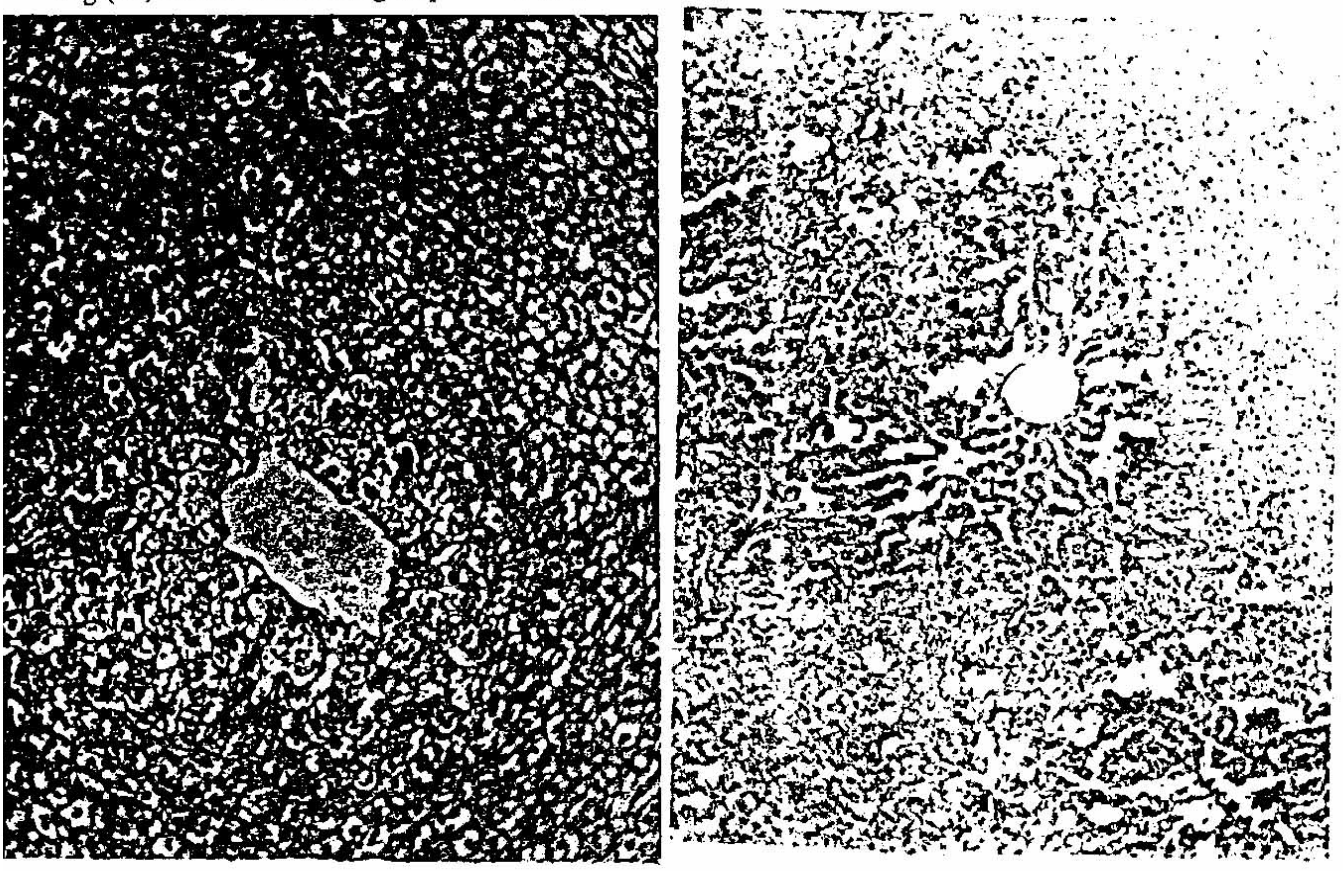

Fig. (3 b, c) : Liver of second group fed on diet contained irrated castor bean showed necrosis of hepatocytes (b) and liver of third group fed on diet contained heated castor bean (c) showed congestion of sinusoid and necrosis of hepatocyte (from left to right).x 200

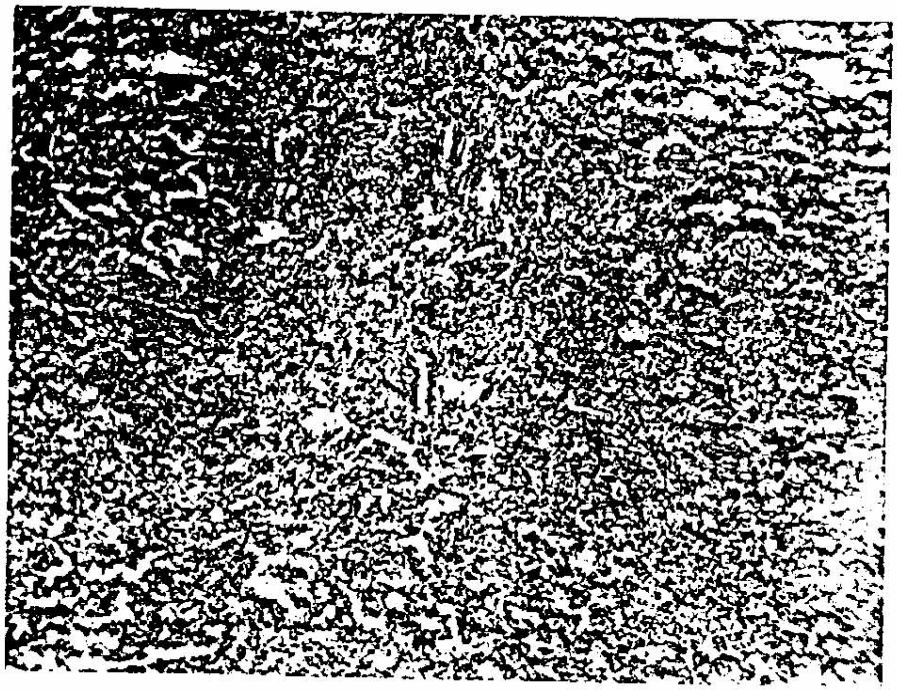

Fig.(4a): Spleen of control group. X 100

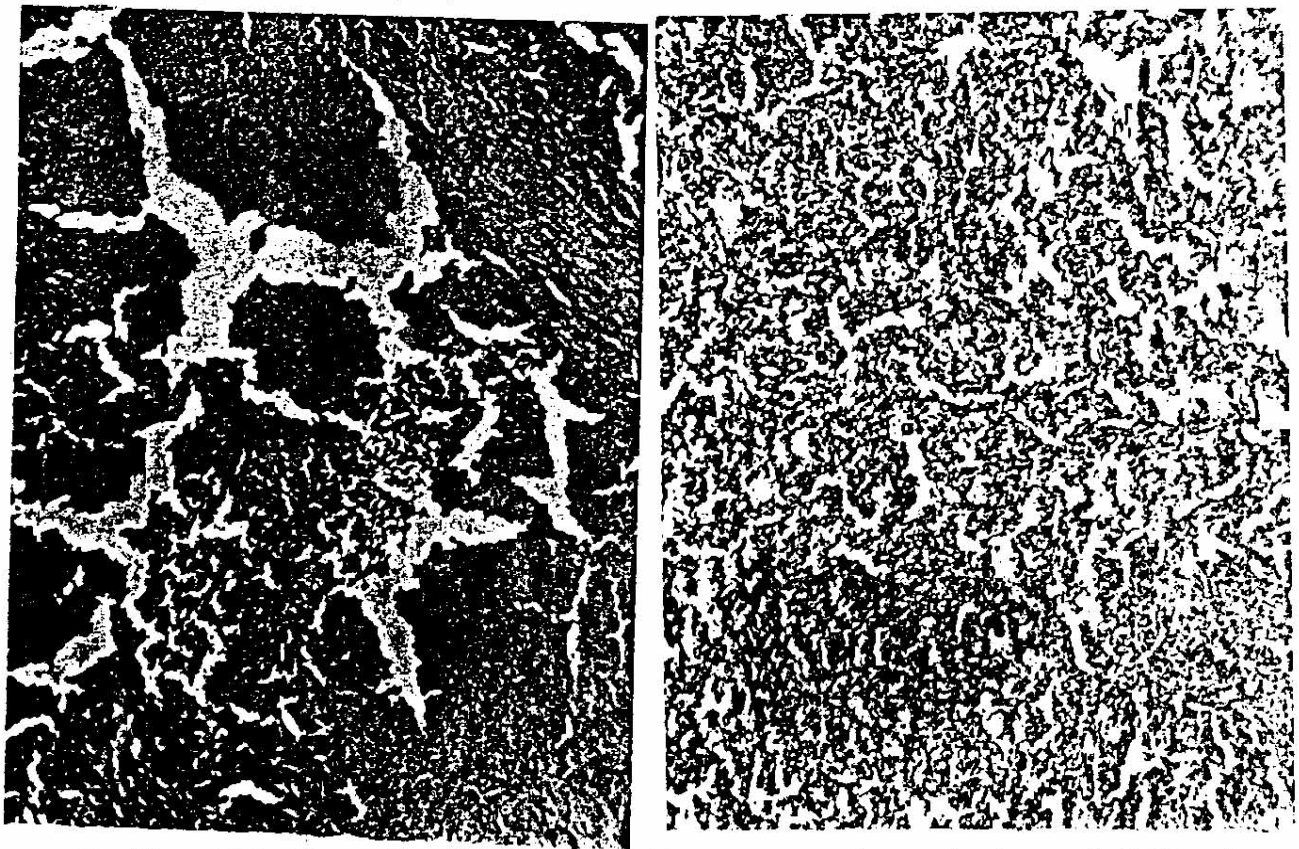

Fig. (4b and 4c): Spleen of second group showed haemorrage and necrosis of red pulb (4 b) and spleen of third group showed necrosis and erosion (4 c) (From left to right). X 100.

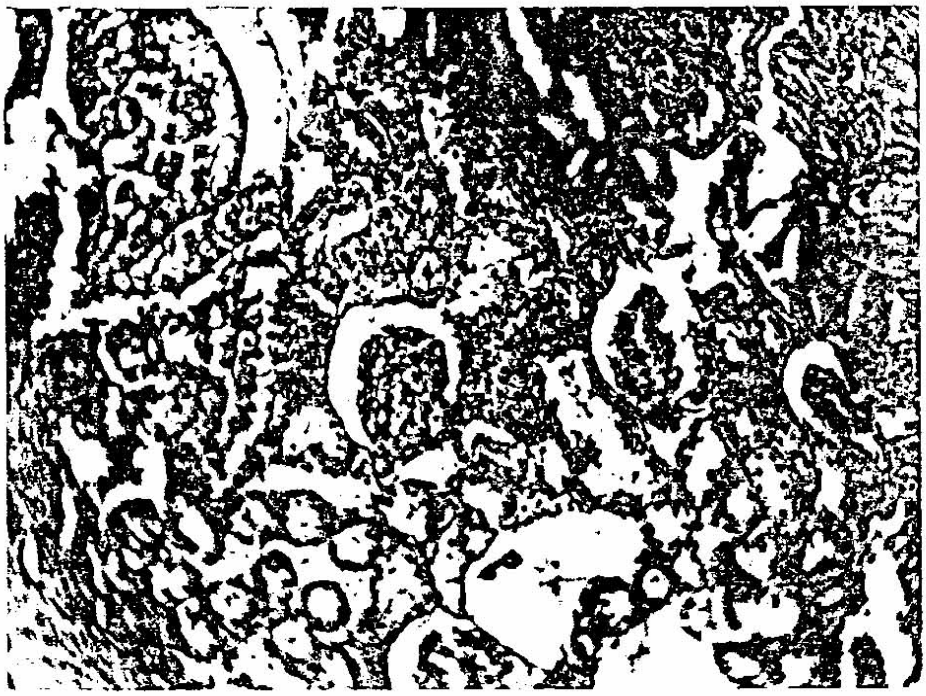

Fig. (5a): Kidney of control group

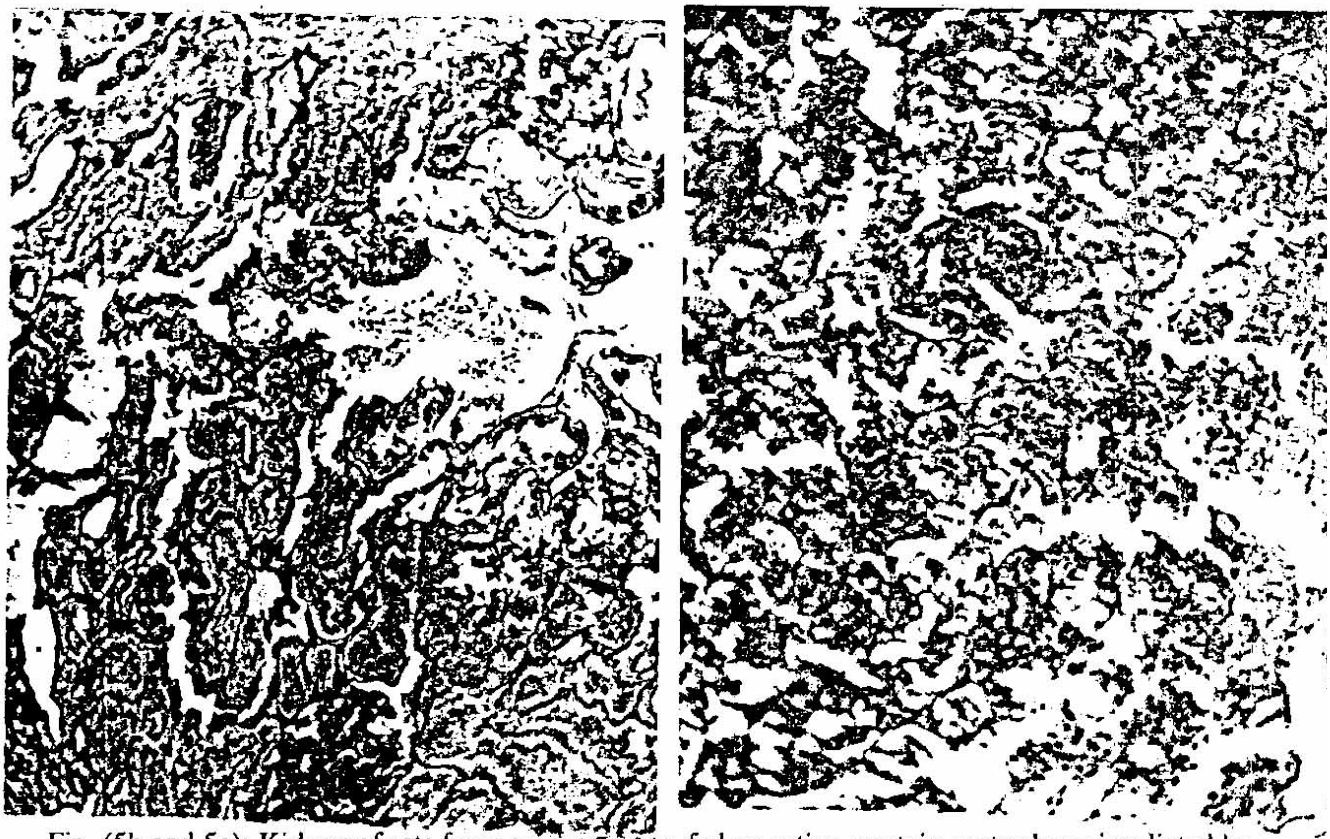

Fig. (5b and 5c): Kidney of rats from second group fed on ration contain castor bean irradiated by 50 KGy showed congestion and necrosis of glomerulai (5b) and kidney of third group fed on ration contained heated castor bean showed necrosis and degeneration of glomerulai (5c). from left to right. x 200

DISCUSSION

This study was undertaken to investigate the effect of different doses of irradiation and heat on detoxification of castor bean, as well as the biological changes in rats fed diets contain treated (irradiated and heated) *Ricinus Communius* seeds.

The results of chemical analysis of castor bean as being affected by gamma irradiation and heat (Microwave) showed a decrease in crude protein level. Effect of irradiation was due to the production of free radicals that attack protein molecules *(40)*.

The lowest ultraviolet spectra for irradiated samples were recorded in seed subjected to 50 kGy and for heated samples after 8 min.

Decrease in ricin level was dependent on irradiated dose and time of exposure to microwave. These results can be explained as ricin is formed of two polypeptides chain (A & B); both chains are glycoproteins linked by disulfide bond*(41-43)* . Gamma irradiation may cause split of the sulfhydryl bond *(40)*. Decrease in Ricin content by heat may be attributed to hydrolysis of protein to several peptides of low molecular weight by heat *(2, 3, 7, 19, 23)*.

The degree at which hemagglutinin was destroyed by irradiation or heat was accompamied by ricin content; where ricin (A120 and A 60) have hemagglutianting property *(4)*.

Examination of the data permits the following: (a) hemagglutinating activity of irradiated or heated samples provides a valid index of ricin content. (b) castor bean samples which have received excessive irradiation (50 and 60 kGy) or heating time (6 and 8 min.) showed decrease in hemagglutinating activity. Hemagglutinating activity can be assumed to be a reflection of the proportion of ricin; which remains in the raw form after irradiation or heat treatment. Decrease in ricin content was previously explained during mentioning the ultraviolt spectra of ricin.

Feeding of rats on diets contain irradiated and heated castor bean *(Ricinus communius)* showed a decrease in body weight especially in last two weeks of expermint in comparison to rats fed on control diet. Similar results were recorded previously *(8, 9, 11,44)*. Decrease in body weight may be attributed to presence of low level of toxic ricin and the decrease in palatability of ration that contain *Ricinus communis*.

Haemoglobin content and packed cell volume showed a significant decrease in groups fed on irradiated and heated castor bean *(7, 8)*. These results may be attributed to the agglutinating effect of ricin previous investigators recorded similar data *(7, 30,45)* and the effect of ricin on haemobiotic system as spleen *(12-14)* as well as effects on circulatory system and haemoglobin precipitation in renal tubule and hemolysis of red cells even with extreme dilutions and haemolytic anaemia *(7, 13, 45)*.

An increase in serum alanine amino and aspertate amino transferases were detected in serum of rats fed diets contain either irradiated or heated castor bean for 4 weeks. Previous record cited similar result *(10)* . While other showed no changes *(11)* . These results attributed to effect of ricin and confirmed by detection of ricin and pathological lesions of the liver.

Urea levels in groups fed on irradiated or heated castor bean showed no change which confirm previous findindg *(11)* and differ from other *(7)*. An increase in serum uric acid was detected in second and third groups. These results attributed to the nephrotic injury of ricin *(3)*. This was confirmed by ricin residue and pathological lesions of the kidney.

Hemagglutinating activity of liver and kidney extract are a reflection of ricin residue, which have agglutination properties *(4)*.

Ricin residue in liver and kidney in groups fed rations contain irradiated and heated castor bean were detected. These results are in accordance with that previously recorded where ricin was detected in liver and kidney when lactose was injected to provid partial protection against ricin *(44)* .

Pathological examination showed destruction and necrosis in small intestine of second group (fed ration contain irradiated castor bean); a finding which was early mentioned *(14-16)*. Liver of second and third groups showed degenerative change, necrosis and lymphocytic infiltration; similar lesions were recorded in previous works *(12,13)* . Spleen showed haemorrage and necrosis of second and third groups. Kidney of second and third groups showed degenerative changes

which confirmed by several investigation *(12, 13, 17)*. This can be attributed to the toxic effect of ricin.

CONCLUSION

From our previous work it can be concluded that complete decomposition of toxic ricin may require higher radiation doses than the dose used. Methods used for destruction of ricin must be carefully controlled since over doses either by irradiation or heat may reduce protein values and some essential amino acids of seeds.

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REFERENCES

1- Olsnes, S. and Phil, A. (1978): Abrin and ricin two toxic lectins. Trends biochem. Sci. 3, 7-10.

2- Hegi, G. (1966): Illustrierte floravon Mittel curopas, 3rd ed., C. Hanser Munchen, 6 Vols.

3-Lungier, A.A.; Creppy, E.E. and Dirheimer, G. (1980): Ricin, the toxic protein of the castor-oil plant (*Ricinus Communis* L.) Structure and properties. Pathol. Biol. 28 (2): 127-139.

4- Nicolson, G.L. and Blaustein, J. (1972): The interaction of *Ricinus communis* agglutinin with normal and tumor cell surfaces. Biochim. Biophys. Acta, 266 : 543-547.

5- Nicolson, G.L.; Blaustein, J. and Etzler, M.E. (1974): Characterization of two plant lectins from *Ricinus communis* and their quantitative interaction with a murine lymphoma. Biochemistry, 13 (1): 196-204.

6- Fodstad, O.; Johannessen, J.V.; Schjerven, L. and Phil, A. (1979): Toxicity of abrin and ricin in mice and dogs. J. Toxicol Environ. Health 5(6): 1073-1084.

7- Dreisbach, R. H. (1980): Hand book of poisoning: prevention, Diagnosis and treatment. 10 Edition printed in lebanon P. 489-490.

8- El-Badwi, S.M.; Mousa, H.M.; Adam, S.E. and Hapke, H.J. (1992): Response of brown hisex chicks to low levels of jatropha curcas, ricinus communis or their mixture. Vet. Hum. Toxicol. 34 (4): 304-306.

9- Isichei, C.O.; Das, S.C.; Ogunkeye, O.O.; Okwuasaba, F.K.; Uguru, V.E.; Onorurwe, O.; Olayinka, A.O.; Dafur, S.J.; Ekwere, E.O. and Parry, O. (2000): preliminary clinical investigation of contraceptive efficacy and chemical, pathological effects of RICOM- 1013-J. of *Ricinus communis* varminor on women volunteers. Phyto Ther. Res. 14 (1): 40-42.

10- lampe, K.F. (1976): Changes in therapy in Abrus and Ricinus poisoning suggested by recent studies in their mechanism of toxicity. Clin. Toxicol. 9: 21-25.

11-Das, S.C.; Isichei, C.O.; Okwuasaba, F.K.; Uguru, V.E.; Onoruvwe, O.; Olayinka, A.O.; Ekwere, E.O.; Dafur, S.J.and Parry, O. (2000): Chemical, pathological and toxicological studies of the effects of RICOM-1013-J. of *Ricinus communis* var minor on woman volunteers and rodents. Phytother. Res. 14 (1): 15-19.

12- Derenzini, M.; Bonetti, E.; Marionozzi, V.; Stirpe, F. (1976): Toxic effects of ricin: studies on the pathogensis of liver lesions. Virchows Arch cell pathol. 20 (1): 15-28.

13- malizia, E.; Sarcinelli, L. and Andreucci, G. (1977): Ricinus poisoning: a familiar epidemy. Acta Pharmacol. Toxicol. 41 (1): 351-361.

14- Leek, M.D.; Griffiths, G.D.; Green, M.A. (1990): Pathological aspects of ricin toxicity in mammalian lymph node and spleen. Med. Sci. Law. 30 (2): 141-148.

15- Sekine, I.; Kawase, Y.; Nishimori, I.; Mitara, M.; Harada, H.; Ishiguro, M. and Kikutani, M. (1986): Pathological study on mucosal changes in small intestine of rat by oral administration of ricin. I. Microscopical observation. Acta Pathol. J. 36 (8): 1205-1212.

16- Leek, M.D.; Griffiths, G.D. and Green, M.A. (1989): Intestinal pathology following intramuscular ricin poisoning. J. Pathol. 159 (4): 329-334.

17- Taylor, C.M.; Williams, J.M.; lote, C.J.; Howie, A.J.; Thewles, A.; Wood J.A.; Milford, D.V.; Raaft, F.; Chant, I. And

Rose, P.E. (1999): A laboratory model of toxin-induced hemolytic uremic syndrome. Kidney, Int 55 (4): 1367-1374.

18- Hasson, E.A.; Bagchi, D.; Roche, V.L. and Stohs, S.J. (1992): An assessment of potenteal chemoprotectant activity against ricin - toxicity by mechanism based glycosidase inhibitors in macrophage g 744 A.I. Cell cultures. Toxicon, 30 (12): 1545-1554.

19- Cawley, D.B. and Houston, L. L. (1979): Effect of sulfhydryl reagents and prolease inhibitors on sodium dodecyl sulfate heat inducing dissociation of Ricinus communis agglutinin. Biochim. Biophy. Acta. 581(1): 51-62.

20- lewis, M.S. and Youle, R.J. (1986): Ricin subunit association. Thermodynamics and the role of the disulfide bond intoxicity. J. Biol. Chem. 261 (25): 11571-11575.

21- Foxwell, B.M.; Blakey, D.C.; Brown, A.N.; Donovau, T.A. and Thorpe, P.E. (1987): The preparation of deglycosylated ricin by recombinatin of glycosidase-treated A and B chains: effects of degluycosylation on toxicity and in vivo distribution. Biochim. Biphys. Acta. 20, 923(1): 59-65.

22- Ishiguro, M.; Matori, Y.; Tanabe, S.; kawase, Y.; Sekine, I.; Sakakibara, R. (1992): Biochemical Studies on oral toxicity of ricin. V. The role of lection activity in the intestinal absorption of ricin. Chem. Pharm Bull. 40 (5): 1216-1220.

23- Hassoun, E.A.; Bagchi, D.; Roche, V.F. and Stohs, S.J. (1996): Potential chemoprotectant activity of mechanism based glycosidase inhibtors against ricin toxicity in chineses hamster ovary and macrophage J. 774A. I cell culture. J. Appl. Toxicol. 16 (1) 49-54.

24- Griffiths, G.D.; Lindsay, C.D.; Allenby, A.C.; Bailey, S.C.; Scawin, J.W.; Rice, P. and Upshall, D.G. (1995): Protectoin against inhalation toxicity of ricin and abrin by immunisation. Hum. Exp. Toxicol. 14 (2): 155-164.

25- Slojadinovic, A.; Kiang, J.; Goldhill, J.; Matin, D.; Smallridge, R.; Galloway, R. and Shea-Donohue, T. (1997): Induction of heat shock response prevents tissue injury during acute inflammation of rat ileum. Crit: Care. Med., 25 (2) 309-317.

26-W.H.O. (World health Organization) (1981): Wholesomeness of irradiates food tech. Report. Series., 659. Geneva.

27- Farag, M.D.H. (1994): Upgrading wholesomeness of soy beans through radiation deactivation of toxic content. Egypt. J. Ead. Sci. Applic. Vol., 7, No. 1, P. 99-112.

28- McLaughlin, W.L.; Wenxia, C.H.J. and Humphreys, J.S. (1985): Response of radiochromic film Dosimeter Gamma Rays in different atmosphere Radiat. Phys. Chem., 25: 793.

29- A.O.A.C. (1990): Association of official analytical chemists. Official methods of analysis 13th edition, Washington, D.C., U.S.A.

30- Niyogi, S.K. (1970): The toxicology of Abrus precatorius Linnaeus. J. Foren. Sci. 15 (4): 529-536.

31- Liener, I.E. and Hill, E.G. (1953): The effect of heat treatment on the nutritive value and Heamagglutinating activity of soya bean oil meal. Sci. J. Series, 49: 609-620.

32- N.R.C. (National Research Council) (1979): Requirment of expermental animals ed 2nd Nationial Acad. Press., Washington. D.C.

33- Schalm, O.W. (1961): Veterinary haematology. 1st Ed. Lea and febiger, philadelphia, USA. P. 54.

34- Reitman, S. and Frankel, S. (1957): A colorimetric method for determination of glucamic pyruvic transmainase and glutamic oxaloacetic transaminase.Am. J. clin. Pathol., 28; 56.

35- Patton, C.J. and Crouch, S.R. (1977): Determination of urea. Anal. Chem. 49: 464.

36- Husdan, H. (1968): Chemical determination of creatinine with deproteinization. Clin. Chem., 14 : 222-

37- Caraway, W. (1963): Determination of uric acid with deprotinization. Stand. Meth. Clin. Chem., 4: 239.

38- Drury, R.A.B. and Wallington, A.E. (1980): Carleton's histological technique, 5 th Ed., Oxford. Univ. Press, London.

39- Sendecore, G. (1971): Statistical method.

14th Ed. The lowa state collage press, Amer., Lowa.

40- Karcher, K.H. and Jentzsch, K. (1972): Radiobiology as the basis of radiotherapy. In Ioachim, H.L. (ed.): Pathogioloby Annual, Vol. 2. Appleton-Century-Crofts, Educational Division, Meredith Corp., New York.

41- Olsnes, S. and Phil, A. (1982): Toxic lectins and related proteins. In molecular action of toxins and viruses, P. Cohen and S. Van Heyningen (Eds.). Elsevier/North Holland, Amsterdam, pp. 51-105.

42- Lord, J.M. (1985): Synthesis and intracellular transport of lectin and storage protein precursors in endosperm from castor bean. Eur. J. biochem. 146: 403-409.

43- Harley, S.M. and Lord, J.M. (1985): In vitro endoproteolytic cleavage of castor bean lectin precursors. Plant Sci. 41: 111-116.

44- Fodstad, O.; Olsnes, S. and Pihl, A. (1976): Toxicity, distribution and elimination of the cancerostatic lectins abrin and ricin after parenteral injection into mice. Br. J. Cancer., 34 (4): 418-425.

45- Sidney, K. (1986): Handbook of emergency toxicology: A guide for the identification, Diagnosis, and Treatment of poisoning. P. 469 fifth Edition. Springfield, Illinosi, U.S.A.

الملخص العربى

تأثير الحرارة والأشعاع على سميه بذور نبات الخروع فى الفئران

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** قسم كيمياء التغذية بهيئة الطاقة الذرية بمدينة نصر

يعتبر نبات الخروع أحد محاصيل البذور الزيتية والذى يوجد فى مصر من ٤٠٠٠ سنه قبل الميلاد. تستخدم البذور أساسا كمصدر للزيت ويستخدم الكسب المتخلف كغذاء للحيوان. حيث تحتوى البذور على أكثر من ٢٥٪ بروتين وتحتوى بذور نبات الخروع على المادة السامة الريسين (أحد السموم المعروفة بالفيتوتوكسين) والتى تشكل أعاقة لاستخدام البذور فى تغذية الحيوان. وقد أجريت كثير من المحاولات للتخلص من الريسين عن طريق أستخدام الحرارة أو بعض المواد الكيميائية وبالرغم من نجاح بعض هذة الطرق إلا أنها مكلفة أقتصاديا وقد ينتج عنها أحداث فى حفض القيمة الغذائية. لذلك تم خلال هذة الدراسة توظيف تكنولوجيا التشعيع الجامى لإزالة سمية الريسين وذلك بإستخدام جرعات إشعاعية قدرها ١٠، ٢٠، ٣٠، ٤٠، ٥٠، ٦٠ كيلو جراى على التوالى وكذلك التسخين فى أفران المبكروويف (٥٠ درجة مئوية) لمدة ٢، ٤، ٦، ٨ دقيقة وذلك لدراسة التأثير على التركيب الكيميائى لبذور نبات الخروع ومحتوها من مادة الريسين عن طريق الأمتصاص الضوئى لمستخلص نبات الخروع والتحليل النوعى للريسين باستخدام الفحص الأجلوتينيى لكرات الدم الحمراء كما تم دراسة تأثير تغذية الفئران على علائق تحتوى على بذور الخروع المعاملة أشعاعيا وحراريا على وزن الجسم ونسبة الهيموجلوبين والحجم النسبى لكرات الدم وبعض وظائف الكبد والكلى ومتبقيات الريسين فى كل منهما باستخدام الفحص الاجلوتينى لكرات الدم الحمراء وكذلك الفحص الباثولوجى للكبد والكلى والطحال والامعاء بالمقارنة بالفئران المغذية على العليقة الضابطة.أظهرت النتائج نقص نسبة البروتين فى البذور عند استخدام جرعات ٥٠، ٦٠ كيلو جراى وكذلك عند استخدام الحرارة لمدة ٤، ٨ دقائق . وأظهر الامتصاص الضوئى للريسين والفحص الأجلوتينيى لكرات الدم الحمراء أنخفاض نسبة الريسين فى بذور نبات الخروع عند استخدام جرعات اشعاعية ٥٠، ٦٠ كيلو جراى وكذلك عند استخدام الحرارة بالميكروويف (عند ٥٠ درجة مئوية) لمدة ٨ دقائق. أوضحت الدراسة أنخفاض فى وزن الفئران المغذاة لمدة اربع اسابيع متتالية على عليقة تحتوى على بذور الخروع المشعة بجرعة ٥٠ كيلو جراى وكذلك بذور تم تعريضها لدرجة حرارة ٥٠ درجة مئوية لمدة ٨ دقائق. أنخفاض نسبة الهيموجلوبين فى الدم وكذلك الحجم النسبى لكرات الدم. كما أوضحت الدراسة زيادة أنزيمى الترانس فيريز فى مصل تلك الفئران (المجموعة الثانية والثالثة) بالمقارنة بالمجموعة الضابطة كذلك حدث زيادة فى نسبة حمض اليوريك. كما أظهرت الدراسة وجود متبقيات للريسين فى الكبد والكلى ظهرت فى الفحص الأجلوتينى لكرات الدم الحمراء فى الفئران المغذاه على عليقة بها بذور الخروع المشعة أو المعالجة حراريا. أوضح الفحص الباثولوجى حدوث تأثر خلايا الامعاء والكبد والطحال والكلى نتيجة وجود نسبة من الريسين فى البذور التى تم تغذية الفئران عليها. ويتضح مما سبق أن الجرعات الاشعاعية المستخدمة لا تكفى للتخص من كل المادة السامة (الريسين) الموجودة بالبذور والتى أظهرت سمية للفئران المغذاه على تلك البذور عند مقارنتها بالمجموعة الضابطة.