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Effect of Ricin on Some Biochemical, Haematological, and Histopathological Variables in Mice

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ABSTRACT

Acute toxicity studies of ricin were carried out in Swiss albino male mice. The median lethal concentration (LD_{50}) values were determined for mice through intraperitoneal and oral routes and were found to be 1.01 µg/kg and 28.29 mg/kg, respectively. The ricin (1.0 LD_{50}) was administered in mice through intraperitoneal route and various toxicity-related clinical variables were studied on the 1st, 3rd, and the 7th day of post-exposure. The prominent symptoms before death, were diarrhoea with black sticky vent and piloerection. The body weight decreased significantly in a dose-dependent manner. No significant change was observed in organ-to-body weight ratio on the 1st, 3rd, and the 7th day of post-exposure except kidney weight. On the 7th day, kidney weight increased significantly. The levels of blood urea, uric acid, and glucose increased, while total protein level decreased. However, activities of transaminase and phosphatases were not altered. Leukocytosis was also observed. The ricin also affected blood coagulation parameters. There was a significant increase in the clotting time. However, prothrombin time, bleeding time, and erythrocyte sedimentation rate were not altered. Histopathological studies showed degenerative changes in various visceral organs, viz, lungs, liver, spleen, kidney, and testis. Acute toxicity studies of ricin revealed that it is a highly toxic toxin. The ricin intoxication caused alterations in biochemical, haematological variables, and degenerative changes in various visceral organs.

Keywords: Ricin, intraperitoneal route, oral route, toxicity, leukocytosis, median lethal concentration, uric acid, piloerection, mice, proteineous toxin

1. INTRODUCTION

The ricin is a proteineous toxin found in the seeds of castor plant, *Ricinus communis*. The toxicity of ricinus seeds has been recognised since ancient times. More than a century ago, Stillmark isolated toxic protein from the seeds, which he termed ricin[1]. The ricin is a small dipeptide molecule (molecular weight approx. 65 kDa) containing an A-chain (~30 kDa) and a B-chain (~31-32 kDa) coupled with a disulfide bond[2]. The A-chain is an *n*-glycosidase and contains the physiologically active site of the molecule. The B-chain is a galactose-specific lectin and is essential for binding the toxin to the cell surface

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and for the entry into the cell. The ricin inhibits protein synthesis by interfering with *r*RNA of protein synthesis apparatus. It prevents the binding of elongation factor (EF-2), and thus, the formation of the initiation complex by cleaving a specific adenine residue at 4324 position in mammalian[3,4] 28S *r*RNA. As a result, protein synthesis is arrested and the cell dies.

The ricin is one of the most toxic materials known. Toxicity of ricin varies with caster plant species, route of administration, and purity of the toxin used. The median lethal dose of ricin has been reported from 0.10 ng to 9.8 μg/kg body weight through the intraperitoneal route. Orally, the ricin is less potent. Ingestion of three to five seeds may be lethal. The severity of seed toxicity depends upon mastication of seeds. Oral exposure results in nausea, vomiting, and diarrhea, hours after the exposure[5]. Toxicity causes swollen lymph nodes with haemorrhage; spleen, liver, lungs, and heart are affected; body cavities contain clear fluid; hypothermia is observed; and death is accompanied by convulsions. Tissues with high turnover, eg, intestine, immune system are more severely affected than the other tissues. The ricin also potentiates the release of inflammatory cytokines, TNF-alpha and IL-1 beta, which may cause pyrexia and contribute to the formation of edema[6].

The ricin causes myocardial haemorrhage, decreased blood pressure, vasodilatation, decreased vascular contraction, and increased endothelial-dependent vascular relaxation when administered through intravenous route[7,8]. The ricin administered intravenously also disturbs calcium homeostasis in rabbit heart that is partially responsible for altering cardiac function and myocardial cell death[9]. The pulmonary edema accompanied by acute destructive alveolitis and necrosis/apoptosis of the lower respiratory tract epithelium is described[10]. Following ricin inhalation, multifocal-to-coalescing fibrinopurulant pneumonia, diffuse necrosis, acute inflammation of airways, diffuse alveolar flooding with peribronchovascular edema is also reported[11] in *Rhesus* monkeys. Mass casualties from aerosol exposure are possible and would be expected to have local pulmonary as well as systemic effects.

The castor plant is distributed widely and is grown in various geographical locations. In spite of the Chemical Warfare Convention, the ricin can be misused by any country and also by the terrorists. In contrast to global scenario, very less work has been done in India on the toxin, ricin, even though, India is a big producer of castor seeds. This study aims to investigate systemic acute toxicity of the ricin isolated from the Indian castor seeds in animal model with special reference to biochemical, haematological, and histopathological variables following ricin intoxication.

2. MATERIALS & METHODS

The castor seeds were purchased from the local market. Bio-gel A-0.5 m gel was obtained from the Bio-Rad, Laboratories, USA. The sepharose 4B, and all electrophoresis chemicals were purchased from the Sigma Chemicals Co (St.Louis, MO). All other chemicals used were of analytical grade from SRL/Himedia/Merck.

The Swiss albino male mice weighing between 24 g to 28 g, bred at the Animal Facilities, Defence Research and Development Establishment (DRDE), Gwalior, were used in this study. The animals were kept at room temperature with 12 h light/dark cycle. The animals were fed on standard pellet diet (Amrut Laboratory Feeds Pvt Ltd, Maharashtra) and maintained on dust-free rice husk as bedding in polypropylene cages. The food and water were given *ad libitum*.

The ricin was purified in the Laboratory[12]. The affinity chromatography was performed[10] on acid-treated sepharose 4B. Under these conditions, lectins bind to the gel matrix (to galactose residues available on the partially acid-hydrolsed matrix). The matrix-bound proteins were eluted with β-D-galactose. These lectins were then separated on the basis of their size, using Bio-gel A-0.5 m gel. The protein containing fractions were pooled separately, concentrated, and used for all the experiments. The polyacrylamide gel electrophoresis under reduced and non-reduced conditions were performed each time to assess purity of the ricin.

The median lethal concentration (LD_{50}) of ricin with 95 per cent confidence limits for intraperitoneal and oral routes was determined by the Gad and Weil method[13]. For each dose (log dose), four mice were used and three to four doses were administered. After administration of ricin, the animals were observed for toxicity-related symptoms and mortality till 7th day of post-exposure. The LD_{50} of ricin with 95 per cent confidence limit was calculated from the table values[13].

The effect of ricin intoxication on body weight was studied. For this study, 20 male mice were divided into four groups of five animals each. The groups 1, 2, and 3 were injected 0.25 LD_{50}, 0.5 LD_{50}, and 1.0 LD_{50} of ricin intraperitoneally. The group 4 (control) was injected with equal volume of normal saline. The body weight and mortality were recorded up to 14th day.

For the study of acute toxicity, 40 male mice were divided into four groups of ten animals each. The groups 1, 2, and 3 were given 1.0 LD_{50} (1.01 μg/kg) of ricin, while group 4 was given normal saline. The animals were lightly anaesthetised with ether and blood was collected in heparinised vials from the orbital sinus after the 1st, 3rd and the 7th day. The animals were then sacrificed by cervical dislocation and visceral organs, such as lungs, liver, spleen, kidney, heart, and testis were dissected out. Blood and visceral organs were also collected from two control animal groups on the 1st, 3rd and the 7th day. The data of control animals were pooled later. The tissues were blotted, freed from blood and adhering tissues, weighed, and preserved in buffered formalin for histopathological studies, using standard procedures[14].

Haematological variables like total red blood cell count (TRBC), total leukocyte count (TLC), differential leukocyte count (DLC), packed cell volume (PCV), erythrocyte sedimentation rate (ESR) and haemoglobin (Hb) were carried out using standard procedures. The bleeding time (BT), clotting time (CT), and prothrombin time (PT) were determined as described by Biggs and McFarlance[15]. Plasma glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT), alkaline phosphatase (ALP) and acid phosphatase (ACP) were carried out according to Wotton[16]. Estimation of blood glucose was carried out using diagnostic kits from Ranbaxy, India. The blood uric acid was estimated by the phosphotungatic method[17]. The total protein and urea were estimated by the Folin's[18] and diacetyl monooxime methods[19], respectively. The statistical analysis of the data was done by Student's t-test and the level of significance was kept at $P < 0.05$.

3. RESULTS

Table 1 summarises the LD_{50} values with confidence limits for mice through intraperitoneal and oral routes. The LD_{50} for male mice was 1.01 μg/kg and 28.29 mg/kg for intraperitoneal and oral routes, respectively. All the LD_{50} values were determined for a 7-day observation period. The prominent symptoms observed were piloerection and diarrhoea with black sticky vent, extension of the hind legs after death of the mice. At higher doses, mortality occurred even within 20-24 h of intraperitoneal injection. Death probably resulted from multi-organ malfunction and shock.

Table 1. Estimated LD_{50} values of ricin administered in mice through different routes*

Animal	Route	LD_{50}	Confidence limit
Mice	Intraperitoneal	1.01 μg/kg	0.82-1.47 μg/kg
Mice	Oral	28.29 mg/kg	16.25-49.24 mg/kg

*LD_{50} values were determined for 7 days.

The effect of ricin on body weight of the mice has been presented in the Fig. 1. There was a significant change in body weight on 2nd day of ricin administration in all the doses in comparison to control mice. At 0.25 LD_{50} dose of ricin, decrease in the body weight was seen up to 6th day of intoxication, while at 0.50 LD_{50} dose, decrease in the body weight was seen up to 10th day. Later on, body weight recovered. At 1.0 LD_{50} dose of ricin, sharp decrease in body weight was observed, which remained constant up to 14th day.

Table 2 indicates organ-to-body weight ratio in mice following ricin intoxication. No significant

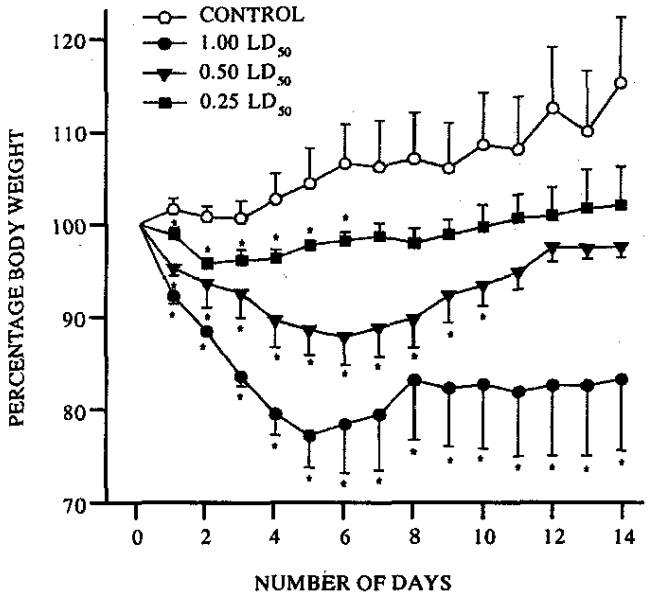

Figure 1. Effect of ricin on percentage body weight. The values are mean ± SE; $n = 5$ ($n = 3$ on 7th day). $P < 0.05$; *significant from control.

change was observed in organ-to-body weight ratio of mice at 1.0 LD_{50} dose of ricin on the 1st, 3rd, and the 7th day of post-exposure, except in kidney. On the 7th day, kidney weight increased significantly.

Table 3 shows how ricin intoxication affected biochemical variables at various time intervals in mice. The level of total protein decreased on the 7th day of administration of ricin. The blood glucose increased significantly on 1st day and returned to normal level later on. The blood urea was increased significantly after the 1st day of ricin administration and remained elevated till the 7th day. The level of blood uric acid was also increased on the 7th day following ricin intoxication. No significant changes were observed in the activity of glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, alkaline phosphatase, and acid phosphatase in ricin-exposed mice at various time intervals (Table 4).

Table 5 presents the effect of ricin on various haematological parameters. Total leukocyte count was increased. The number of polymorphs were also increased significantly. The effect of ricin on prothrombin time, clotting time, bleeding time, and erythrocyte sedimentation rate are presented in the Table 6. The clotting time increased significantly following ricin toxicity. However, prothrombin time, bleeding time, and erythrocyte sedimentation rate were not altered when compared with the control group.

A comprehensive account of various histopathological lesions caused by ricin in various visceral organs are depicted in the Table 7 and the Fig 2. The histopathological changes caused by ricin administration are haemorrhage, congestion, and mild atrophy of various cellular components of the visceral organs.

4. DISCUSSIONS

Till date, no medications, vaccines, or specific antidotes are available to counteract the toxicity of the ricin. Only supportive treatment helps. The ricin is one of the most toxic substances of plant origin. The LD_{50} value of ricin varies from 100 ng to 9.8 μg/kg body weight[12, 20]. The LD_{50} values depends on various factors like variety of the castor seed, location, and purity of the toxin. The LD_{50} values determined in the present study for mice for different routes of administration are within the

Table 2. Effect of ricin (1.0 LD_{50}) on organ-to-body weight ratio following intraperitoneal route in mice

Group	Lung (%)	Liver (%)	Spleen (%)	Kidney (%)	Heart (%)	Testis (%)
Control	0.70 ± 0.42	5.29 ± 0.42	0.42 ± 0.06	1.12 ± 0.04	0.48 ± 0.01	0.40 ± 0.08
1st day	0.644 ± 0.08	3.81 ± 0.68	0.33 ± 0.03	1.20 ± 0.07	0.49 ± 0.02	0.45 ± 0.07
3rd day	0.798 ± 0.06	4.58 ± 0.14	0.30 ± 0.03	1.27 ± 0.11	0.51 ± 0.03	0.40 ± 0.09
7th day	0.855 ± 0.07	5.41 ± 0.35	0.54 ± 0.10	1.46* ± 0.11	0.48 ± 0.05	0.63 ± 0.07

Values are mean ± SE; $n = 5$ ($n = 3$ on the 7th day); $P < 0.05$; *significant from control.

Table 3. Effect of ricin (1.0 LD_{50}) on biochemical parameters following intraperitoneal route in mice

Group	Protein (mg/dl)	Glucose (mg/dl)	Urea (mg/dl)	Uric acid (mg/dl)
Control	87.07 ± 2.79	99.40 ± 2.18	41.38 ± 0.48	2.48 ± 0.42
1st day	91.56 ± 2.98	123.92 ± 6.96*	49.03 ± 1.13*	2.95 ± 0.79
3rd day	97.72 ± 5.93	105.02 ± 3.18	44.94 ± 0.60*	4.13 ± 1.13
7th day	77.17 ± 1.50*	95.95 ± 1.17	55.99 ± 12.83*	4.46 ± 0.18*

Values are mean ± SE; $n = 5$ ($n = 3$ on the 7th day); $P < 0.05$; * significant from control.

Table 4. Effect of ricin (1.0 LD_{50}) on transaminase and phosphatase following intraperitoneal route in mice

Group	GOT (IU/L)	GPT (IU/L)	ALP (IU/L)	ACP (IU/L)
Control	55.24 ± 3.42	51.30 ± 4.12	90.19 ± 15.49	59.32 ± 22.10
1st day	60.88 ± 4.28	47.45 ± 3.16	79.95 ± 1.91	44.17 ± 11.09
3rd day	70.12 ± 5.67	52.68 ± 3.80	62.01 ± 9.12	90.30 ± 31.52
7th day	62.36 ± 3.86	42.92 ± 2.86	85.39 ± 5.56	67.47 ± 4.81

Values are mean ± SE; $n = 5$ ($n = 3$ on the 7th day); $P < 0.05$; glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), alkaline phosphatase (ALP), and acid phosphatase (ACP).

Table 5. Effect of ricin (1.0 LD_{50}) on haematological parameters following intraperitoneal route in mice

Group	Haemoglobin (%)	Packed cell volume (%)	Red blood carpulse ($\times 10^6$/mm^3)	Total leukocyte count (/mm^3)	Differential leukocyte count		
					Polymorphs (%)	Lymphocytes (%)	Eosinophils (%)
Control	12.62 ± 0.32	40.33 ± 0.88	4.20 ± 0.06	9333 ± 240	61.00 ± 1.53	38.00 ± 1.53	1.0 ± 0.20
1st day	13.79 ± 0.79	42.80 ± 0.58	4.54 ± 0.07*	9100 ± 141	58.86 ± 1.16	40.40 ± 1.21	0.60 ± 0.24
3rd day	13.39 ± 0.20	41.80 ± 0.80	4.26 ± 0.06	11880* ± 106	67.80* ± 0.86	31.61 ± 1.08	0.60 ± 0.24
7th day	12.50 ± 0.23	41.33 ± 0.67	4.43 ± 0.13	12583* ± 116	67.00* ± 2.75	32.50 ± 2.91	0.60 ± 0.24

Values are mean ± SE; $n = 5$ ($n = 3$ on the 7th day); $P < 0.05$; *significant from control.

range of reported values[12, 20]. The signs and symptoms of ricin intoxication vary. A series of symptoms were observed in the experimental animals after a lethal dose of ricin given parenterally. The first sign noticed about 12 h later was the loss of body weight. After 24 h of lethal dose of ricin, animal lies on its side, with its head bent down, and sometimes, clonic convulsions are noticed. The corneal reflex weakens, the tendon reflexes are much weakened or are absent. The pulse and respiration are still normal. Later, clonic convulsions become regular, and between two attacks of convulsion, the animal lies on its side. The convulsions become more frequent and intense, then dyspnoea and opisthotonus develop. About 30 min after the first convulsion, the animal dies of paralysis of the respiratory centre, in inspiration[21].

Table 6. Effect of ricin (1.0 LD_{50}) on blood coagulation parameters and ESR following intraperitoneal route in mice

Parameter	Control group	Ricin-treated group
Prothrombin time (s)	13.20 ± 0.86	15.20 ± 0.37
Clotting time (min)	2.22 ± 0.05	6.51 ± 0.52*
Bleeding time (min)	2.17 ± 0.08	2.06 ± 0.15
ESR (fall in mm)	2.17 ± 0.08	2.06 ± 0.15

Values are mean ± SE; $n = 5$ ($n = 3$ on the 7th day); $P < 0.05$;
* significant from control.

The decrease in body weight following ricin intoxication is a consistent finding and is documented[21]. In the present study, no change was noticed in organ-to-body weight ratio except an increase in the kidney weight. However, reports are available that ricin affects most, if not all the organs and the tissues of the body. A significant decreased in liver and intestinal weight following ricin intoxication was reported[22]. However, these investigators[21-22] could not observe changes in spleen and kidney weight.

The ricin intoxication affects various biochemical variables. Following ricin toxicity, decreased levels of blood glucose, total protein, and an increased level of blood urea were reported[21]. In the present study, decreased level of total protein was observed. The decreased protein concentration may be associated with disturbed liver function due to inhibition of protein synthesis. The levels of urea and uric acid were increased. Initial increase in glucose level may be due to shock and body fluid loss.

The ricin intoxication caused alteration in haematological variables. Total leukocytes count increased. An increase in the number of red blood cells and leukocytosis was observed following ricin intoxication[21, 23]. Prolonged clotting time reported in ricin poisoning may be due to liberation of heparin-like substances into the blood[21]. In the present study, prolonged clotting time with no change in the prothrombin and bleeding times was observed as compared with the control groups.

The ricin toxicity produces ultrastructural changes in various tissues following inhalation. Histologically, the lungs showed severe, overwhelming intra-alveolar edema, accompanied by established acute destructive alveolitis with significant apoptosis at the alveolar surface. The epithelium lining of the larger airways was frankly necrotic. However, liver, spleen, and kidney showed severe passive venous congestion with hepatic sinusoidal ectasia[10]. The results obtained in the present study were similar to these reports. The ultrastructural changes in the lungs of the mice-following inhalation of ricin aerosol using transmission electron microscopy (TEM) have been reported[24]. The first signs of change in ultrastructure appeared between 6 h and 12 h of post-exposure in alveolar macrophages and took the form of apoptotic change

Table 7. Histopathological lesions in the visceral organs of mice following intraperitoneal administration of ricin (1.0 LD_{50}) at various time intervals

Ricin-treated group organ	Control group	Ricin post-treatment period	
		Symptoms after 24 h and 72 h	*Symptoms after 7th day*
Lung	Normal	Perivascular edema and severe bronchiolar congestion	Severe haemorrhage and infilteration of RBCs into the lumen of alveoli
Liver	Normal	Congestion in hepatic capillaries and necrosis of hepatocytes in the vicinity of central canal	Condensation of chromatin in the nucleus and degeneration of hepatocytes
Spleen	Normal	Hypocellularity, atrophy of white pulp and formation of megakaryoytes	Hypocellularity and appearance of large number of megakaryocytes
Kidney	Normal	Congestion and obliteration of renal parenchyma	Liquificative degeneration of renal parenchyma and formation of thrombus

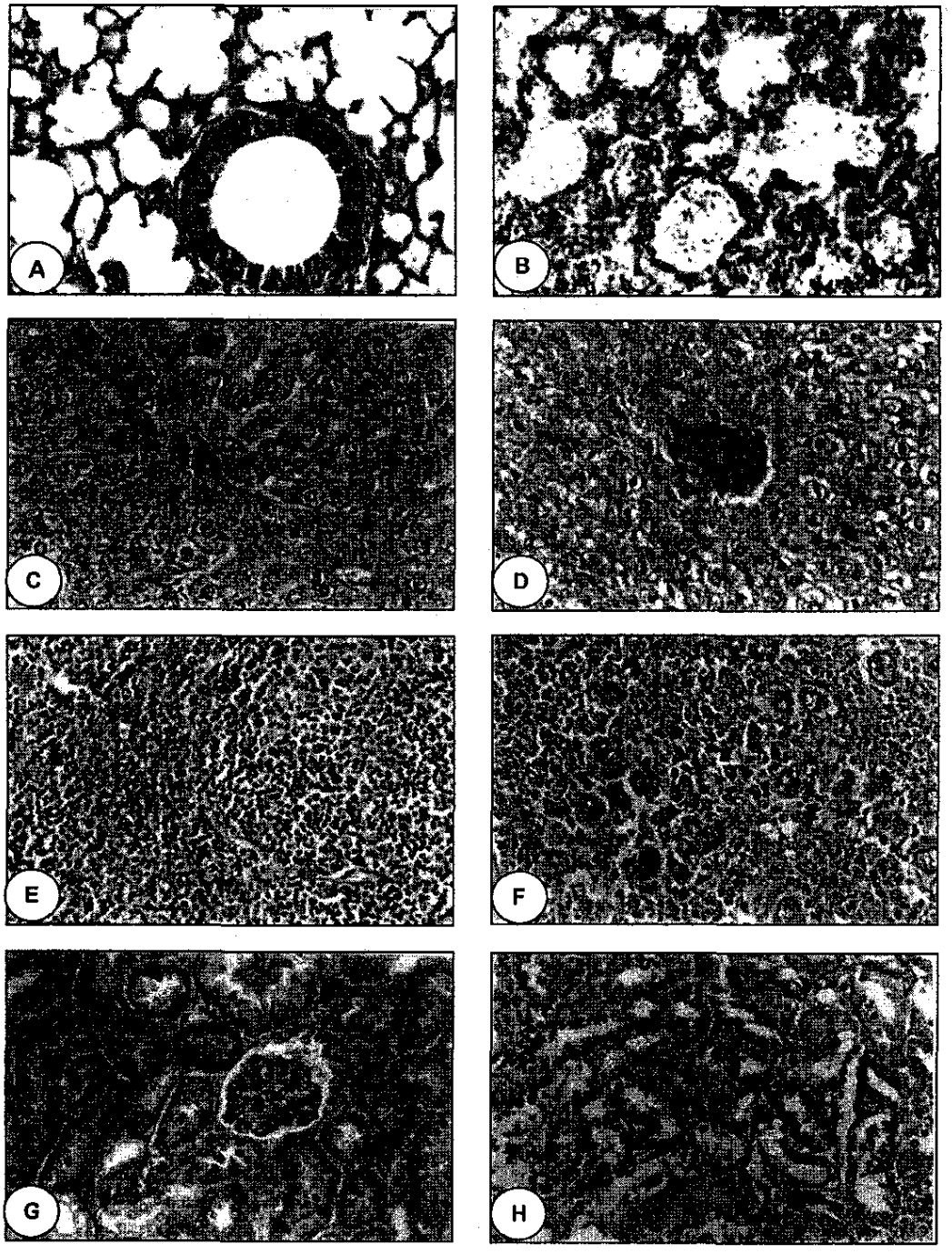

Figure 2. Photomicrographs of control group mice and the ricin-treated mice body organs–lung, liver, spleen and kidney; H & E X 100. (A) Control mice lung showing homogenous alveolar pattern with normal bronchiole, air duct, and alveolar septa. (B) Ricin-exposed mice lung (7th day) showing severe haemorrhage and infiltration of RBCs into the lumen of alveoli. (C) Control mice liver showing normal histoarchitecture with normal hepatic cord lobules, hepatocytes, and central canal. (D) Ricin-treated mice liver (7th day) showing condensation of chromatin in the nucleus and degeneration of hepatocytes by the process of necrosis. (E) Control mice spleen showing normal histoarchitecture with germinal centre, red pulp, marginal zone with white pulp. (F) Ricin-treated mice spleen (7th day) showing hypocellularity and appearance of large number of megakaryocytes. (G) Control mice kidney showing normal glomerulus and renal tubules with connecting renal parenchyma. (H) Ricin-treated mice kidney (7th day) showing liquificative degeneration of renal parenchyma.

primarily in the nucleus[24]. These included heterochromatin condensation at the nuclear periphery and crenulation of the nuclear membrane. These changes appeared to be necrotic rather than apoptotic in nature and suggest that mechanisms other than direct effect of ricin may be involved. Toxicity study of ricin revealed that isolated ricin was highly toxic. The LD_{50} value was determined to be 1.01 μg/kg for intraperitoneal route. The ricin intoxication caused alterations in biochemical, haematological variables, and degenerative changes in various visceral organs in the mice.

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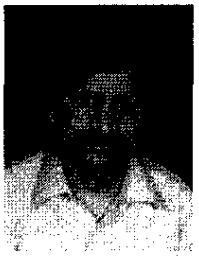
Contributors



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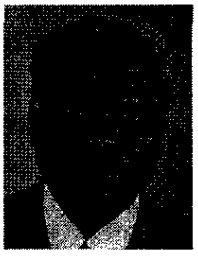

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