T-2 toxin induced hepatotoxicity in rats: Serum enzyme, oxidative stress and patho-morphological alterations

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Abstract

The present experiment was designed to elucidate the serum biochemical, oxidative stress and patho-morphological changes in liver induced by feeding of graded doses of T-2 toxin in Wistar rats. A total of 192 male Wistar rats, 4 weeks of age were divided into four groups of 48 rats each receiving different levels of T-2 toxin in feed i.e. groups I- 0.5, II- 0.75 and III- 1.0 parts per million (ppm) and group IV (control) toxin free diet. The duration of the experiment was 12 weeks and 8 animals each were sacrificed at 2, 4, 6, 8, 10 and 12 weeks intervals. Biochemically, hypoproteinemia, hypoalbuminemia, hypoglobulinemia, decreased albumin: globulin ratios (A:G), and increased serum aspartate amino transferase (AST), alanine amino transferase (ALT) and lactate dehydrogenase (LDH) activities were observed in T-2 treated group rats in dose and duration dependent manner. Significant ($P<0.05$) changes in oxidative stress enzymes like Malondialdehyde, Superoxide dismutase, Catalase and Reduced glutathione levels in liver were observed indicating potential role of free radicals in causing hepatic injury due to T-2 toxicity. Pathomorphological studies revealed appreciable gross changes in liver viz, mottling of surface, enlargement and paleness in the highest dose group. The relative weights of liver were increased in T-2 treated rats as compared with those in control group rats. The most consistent histopathological lesions in the liver included swollen hepatocytes with granular or vacuolated cytoplasm, karyomegaly, double nuclei and anisokaryosis, proliferation of bile ductules and mononuclear cell infiltration in the portal areas in a dose and duration dependent manner. It was concluded that oxidative stress played a major role in T-2 toxin induced hepatotoxicity.

Keywords: Biochemical changes; hepatotoxicity; oxidative stress; patho-morphological observation; rat;, T-2 toxin

Introduction

Mycotoxins are low molecular weight secondary metabolites predominantly produced by molds that act as plant pathogens or saprophytes on stored plants and grains, including wheat, maize, oats, barley, rice, beans, and soybeans, as well as certain cereal-based products. The Food and Agricultural Organization estimates that approximately 25% of global agricultural commodities are contaminated with mycotoxins, highlighting the persistent concern they pose (Chatterjee et al., 2023). Consumption of food and feed contaminated by mycotoxins presents a potential health risk to both humans and animals resulting in teratogenic, carcinogenic, mutagenic, and immunosuppressive effects (Chandratre et al., 2014).

Trichothecene mycotoxins are a family of structurally similar secondary metabolites produced by a variety of Fusarium species, with define characteristic of 12,13-epoxytrichothec-9-ene ring structure, which is responsible for their toxicological effects (Chandratre et al., 2014). While over 200 trichothecenes have been identified, but T-2 toxin stands out as a highly potent cytotoxic mycotoxin, capable of inducing severe toxic reactions in both humans and animals. In India, T-2 toxin has become one of the most prevalent mycotoxin including aflatoxins, fuminisins and ochratoxin A contaminating a variety of agricultural products, posing serious risks to livestock and general public (Chatterjee et al., 2023).

The occurrence of T-2 toxin has been reported from different parts of the world, and its incidences are increasing since the first report of contamination. However, data on the overall occurrence of T-2 toxin in India are still limited (Chatterjee et al., 2023). Several studies have reported contamination in feed and fodder samples from different Indian states viz. Andhra Pradesh (Reddy & Reddy, 1994), Karnataka (Janardhana et al., 1999), Punjab (Rana & Singh, 1991), Maharashtra (Kurkure & Pande, 2008) and Uttar Pradesh (Rai et al., 2011). These findings highlight the importance of regular monitoring and surveillance of animal and poultry feeds to minimize potential risks to the dairy and poultry industries.

Toxicodynamic studies have explained the mechanism of action of T-2 toxin which has been shown to cause oxidative stress, lipid peroxidation, mitochondrial dysfunction, apoptosis and inhibition of DNA, RNA and protein synthesis in eukaryotic cells (Chandratre et al., 2014).

Although T-2 toxin is known to induce hepatotoxicity, comprehensive investigations into chronic, low-dose exposure-induced hepatotoxicity are limited. Therefore, the present study employed a planned experimental design and standard protocol to investigate the effect of T-2 toxin on serum enzyme levels, oxidative stress, and patho-morphological alterations in hepatic injury.

Materials and Methods

Production and analysis of T-2 mycotoxin

Cultures of *Fusarium sporotrichioides* var. *sporotrichioides*, obtained from the Institute of Microbial Technology, Chandigarh, India, was utilized to produce T-2 mycotoxin on partially ground maize and intact wheat grains. The stock culture was subcultured on Sabouraud's dextrose agar and maintained at 16 ± 1°C in a B.O.D incubator for 5-7 days. To prepare the substrate, maize was partially ground and soaked in 40% broth for 2 hours, while intact wheat grains were soaked in 60% distilled water overnight. The grain mixtures were then placed in flasks and sterilized via autoclaving at 15 lb pressure for 15 minutes. T-2 mycotoxin production was carried out on sterile maize and wheat following the method described by AOAC (1995).

T-2 toxin quantification

T-2 toxin was extracted from both maize and wheat cultures and then quantified using thin-layer chromatography (TLC) and spectrophotometry (Chandratre et al., 2014). Enzyme-linked immunosorbent assay (ELISA) was used for further confirmation, adhering to the manufacturer's instructions (Agra-quant T-2 Toxin Test kit; Romer Labs, USA). To validate the T-2 toxin concentration results obtained in our laboratory, maize and wheat samples were sent to the Referral Mycotoxin Laboratory at the Animal Feed Analytical and Quality Control Laboratory (AFAQCL), Veterinary College and Research Institute, Namakkal, Tamil Nadu.

Preparation of experimental feed and diet

Cultured maize and wheat powder, containing known concentrations of T-2 toxin, were separately added to the basal ration and thoroughly mixed to achieve the desired concentrations of 0.5, 0.75, and 1.0 ppm. Aliquots from the mixed diet were then subjected to further quantification for T-2 toxin concentration using TLC and ELISA to ensure accurate toxin levels in the experimental diet.

Experimental animals

A total of 192 male Wistar rats (3-4 weeks of age), with individual weights ranging from 80 to 100 g, were obtained from the Laboratory Animal Resources section of the Indian Veterinary Research Institute, Izatnagar, India, and were examined for any signs of abnormality or overt illness. The rats were housed in a room under

controlled laboratory conditions. Throughout the experiment, the rats were provided with standard feed and water *ad libitum*. The feed was pre-tested to ensure it was free from commonly occurring mycotoxins, and only toxin-free feed was used to formulate the experimental diets. All animal experiments were conducted with the approval of the Institutional Animal Ethics Committee (IAEC) (vide No. IVRI/PATH/09-12/002), adhering to the regulations set forth by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Experimental design

Following a 7-day acclimatization period, all animals underwent weighing and were subsequently and randomly divided into four groups, each comprising 48 rats, to ensure approximately equal initial mean body weights across groups. The experimental animals were administered varying concentrations of T-2 toxin in their feed, with groups receiving 0.5 ppm (I), 0.75 ppm (II), and 1.0 ppm (III), while group IV (Control) received a toxin-free diet. The experiment spanned 12 weeks, during which 8 animals from each group were sacrificed at intervals of 2, 4, 6, 8, 10, and 12 weeks.

Parameters studied

Serum biochemical studies

For serum biochemistry, blood samples (2-3ml) were collected via cardiac puncture from each sacrificed animal into sterile test tubes and allowed to clot. Sera were preserved at -20°C until analysis. Total protein, albumin, aspartate amino transferase, alanine amino transferase, and lactate dehydrogenase levels were analyzed using standard kits (Span Diagnostics Ltd., Surat, India). Globulin levels were calculated by subtracting albumin values from total protein values.

Oxidative stress studies

The levels of various oxidative stress markers in liver tissue were assessed using spectrophotometer. Fresh tissue samples (200 mg) were homogenized in ice-cold PBS (pH 7.4) at a ratio of 1:10 (w/v). The homogenate was then centrifuged at 3000 rpm for 10 minutes. The resulting supernatants were used to determine Lipid peroxidation (Shafiq-Ur-Rehman 1984), Catalase (Aebi 1983), and Superoxide dismutase activity (Madesh & Balasubramanian 1998). For Reduced glutathione determination, a 10% homogenate was prepared in 0.02 M EDTA and was determined by method described by (Sedlak & Lindsay 1968).

Patho-morphological observations

Gross Pathology

At predetermined intervals of 2, 4, 6, 8, 10, and 12 weeks, eight rats from each experimental group were euthanized via deep inhalation anesthesia. Immediately following euthanasia, a comprehensive necropsy was performed. The livers were meticulously examined *in situ*, and any observable patho-morphological changes were documented based on the organ's location, shape, size, color, and consistency.

Hepatosomatic Index (HSI)

The livers were weighed individually at every sacrifice interval and their relative eights were calculated as the per cent body weight.

Histopathology

Following a comprehensive macroscopic evaluation, small tissue samples, not exceeding 0.5cm in thickness, were excised from the liver and preserved in a 10% neutral buffered formalin solution. After a fixation period of 3-4 days, the tissues underwent a washing process. Subsequently, the samples were dehydrated through a series of increasing concentrations of ethyl alcohol, cleared in benzene, and embedded in molten paraffin wax. The resulting paraffin blocks were then sectioned at a thickness of 4-5µm using a hand-operated microtome. The paraffin-embedded sections were processed through a series of steps involving deparaffinization in xylene, rehydration via graded dilutions of ethyl alcohol to running tap water, and finally stained using a standard haematoxylin and eosin staining protocol (Bancroft & Gamble 2002).

Statistical analyses

The data were presented as the Mean $\pm$ Standard Error (S.E.). A two-way analysis of variance (ANOVA) was conducted to assess differences between control and treatment groups, and the means were compared using Duncan's post hoc test with SPSS software (version 11.5). Significance was set at $P < 0.05$.

Results

Animals treated with T-2 toxin showed significant biochemical changes, the severity of which increased in a dose- and duration-dependent manner (Table 1). Serum total proteins (TP) levels was found to be significantly ($P < 0.05$) decreased in all toxin treated groups than that of control animals. Up to 6^{th} week, all toxin treated groups showed comparable TP levels but their levels were significantly ($P < 0.05$) lower than control group. At 12^{th} week, the lowest TP levels were recorded in group III animals followed by group II and I when compared with that of control group IV. Serum albumin levels also decreased

significantly with increasing T-2 toxin dose. In the lowest-dose group I, albumin values were similar to the control group up to the 6th week but a gradual decreasing trend was observed. All treated groups exhibited significant decrease in albumin level from 8th week regarding both dose and duration of toxin exposure. Similarly, the serum globulin levels were lowered in T-2 treated animals than control group animals and the decrease was more marked with prolonged exposure and higher doses of the toxin. There was no significant difference in A:G ratio between the toxin treated groups but a gradual dose and duration dependent reduction was observed. All treated groups had significantly reduced A:G ratios compared with those of control one.

Serum ALT levels showed a continuous increase during the experimental period in all toxin fed groups with the highest values recorded in group III at the 12th week. The control group showed the lowest values. There was no steady progressive increase in the activity of AST but there was significant elevation especially in group III. In the lowest-dose group I, AST values were statistically comparable to controls up to the 8th week. AST levels were similar between groups I and II at weeks 10 and 12, significantly higher than controls but lower than group III. LDH activity was similar to that of AST at all intervals except at 12^{th} week, where values in all treatment groups differed significantly ($P < 0.05$) to each other and also from that of control group.

The oxidative stress parameters showed significant alterations in a dose- and duration-dependent manner following T-2 toxin exposure (Table 2). Liver MDA levels, a marker for lipid peroxidation, increased significantly ($P < 0.05$) in toxin-treated rats compared to controls at almost all intervals. The highest values were consistently observed in group III. From the 6th week onwards, groups I and II had similar levels of MDA, but both were significantly higher than controls. On the other hand, the antioxidant enzymes such as SOD and catalase showed significant decrease levels in T-2 intoxicated animals. Liver SOD activity was decreased progressively with increasing dose and duration of toxin. Catalase activity was comparable among groups during 2nd and 4th week but decreased significantly thereafter in toxin-treated groups. Similarly, liver GSH levels were decreased in a dose-dependent manner. Groups I, II and controls had similar values up to the 4th week, whereas group III showed significantly lower values. After 10th week, all the treated groups showed significant decrease in GSH values with maximum depletion in group III followed by group II and I.

Overall, the results showed that T-2 toxin causes severe oxidative stress in a dose and duration-dependent manner by increasing lipid peroxidation and inhibiting antioxidant defense mechanisms.

The hepatosomatic index showed increasing trend with duration of treatment in all the toxin fed groups, being significantly and consistently higher in group III (Table 3). A statistically significant ($P < 0.05$) increase in HSI was observed as early as on 4 weeks in group II and III. However, HSI in group I and IV remained statistically comparable to each other upto 6^{th} weeks but values were found to differ.

Grossly, the livers of control (Fig. 1a) and lower dose group I animals (Fig. 1b) exhibited normal shape, colour, consistency, size and the cut surfaces were smooth. Group II livers were comparatively enlarged with rounded border and mild pale discoloration (Fig. 1c). As compared to control, the livers in high dose group III were moderately enlarged with rounded borders and pale discoloration (Fig. 1d). The cut surfaces were, however, smooth. The livers with mottled surface showing areas of palor and slight congestion were also observed in few cases at 12^{th} week.

Various histopathological lesions were recorded in liver. Severity, extent and type of lesions varied according to the dose and period of toxin feeding. In group I, changes of lesser intensity were observed at early interval (2 weeks) and included dilated portal vein, mild Mononuclear cells (MNC's) infiltration, engorged sinusoids and swollen hepatocytes with foamy cytoplasm (Fig. 2a). Apart from these changes in more severe form, liver also showed hepatocytic binucleation (Fig. 2b) at 4-6 weeks. After 6 weeks onwards, hepatocytes showed rarefaction of cytoplasm and disappearance of nuclei at 8 weeks (Fig. 2c). At 12 weeks, severely swollen and degenerated hepatocytes with indistinct cell boundaries and frequent karyomegaly with distortion of hepatic cord pattern was observed in three animals (Fig. 2d). Group II animals revealed moderate changes in addition to above with an increase in number of bile duct and MNC's infiltration in portal areas at 2 weeks of T-2 toxin feeding (Fig. 3a). More or less similar type of changes was observed at 4-6 weeks. Severe hepatocytic degeneration with rarefied cytoplasm, indistinct cell boundaries and binucleation (Fig. 3b) was seen at 8 weeks of T-2 feeding. At 10 weeks, the changes were similar to those observed at 8 weeks but with increase in severity (Fig. 3c). Severely degenerated hepatocytes with rarefaction and condensation of cytoplasm, presence of necrosed hepatocytes and frequent karyomegaly (Fig. 3d) was seen at the end of trial. Group III animals revealed variable degree of severe degenerative/necrotic changes appeared earlier than group I and II liver at 2 weeks. At 4 weeks, changes with increased severity such as foamy cytoplasm of degenerated hepatocytes, indistinct cell boundaries, binucleation and karyomegaly and engorged sinusoids (Fig. 4a) were observed. Mild fibroblastic proliferation, MNC's infiltration and cell debris in lumen of bile duct (Fig. 4b) was observed at 6 weeks of toxin feeding. Clumping of degenerated and necrotic hepatocytes with MNC's infiltration in portal region (Fig. 4c) was observed at 8 weeks. At 10 weeks, majority of cases showed loss of hepatocytes causing dilatation and engorgement of sinusoids, variable sized

Table 1: Effect of T-2 on plasma liver biomarkers in rats of different treatment groups

Parameters	Groups	2 wk	4 wk	6 wk	8 wk	10 wk	12 wk
TP (g/dl)	GI	$7.90^{abD}\pm0.21$	$7.30^{Acd}\pm0.21$	$6.77^{aC}\pm0.23$	$5.84^{bB}\pm0.35$	$4.36^{bA}\pm0.28$	$4.01^{cA}\pm0.22$
	GII	$7.67^{abD}\pm0.23$	$6.77^{aCD}\pm0.43$	$6.16^{aC}\pm0.46$	$4.87^{aB}\pm0.62$	$3.83^{aA}\pm0.28$	$3.00^{bA}\pm0.43$
	GIII	$7.38^{aE}\pm0.19$	$6.39^{aE}\pm0.25$	$5.66^{aD}\pm0.59$	$4.26^{aC}\pm0.42$	$2.94^{aB}\pm0.33$	$1.90^{aA}\pm0.06$
	GIV	$8.24^{b}\pm0.17$	$8.26^{b}\pm0.25$	$8.21^{b}\pm0.53$	$8.03^{c}\pm0.54$	$7.58^{c}\pm0.28$	$7.74^{d}\pm0.21$
Alb. (g/dl)	GI	$4.82^{abD}\pm0.20$	$4.17^{abCD}\pm0.32$	$3.73^{abBC}\pm0.34$	$3.17^{bAB}\pm0.44$	$2.93^{bAB}\pm0.31$	$2.59^{bA}\pm0.21$
	GII	$4.63^{abD}\pm0.15$	$3.86^{abCD}\pm0.35$	$3.19^{aC}\pm0.50$	$2.82^{abBC}\pm0.25$	$2.13^{abA}\pm0.34$	$1.99^{abA}\pm0.26$
	GIII	$4.28^{aD}\pm0.12$	$3.33^{aCD}\pm0.43$	$2.88^{aBC}\pm0.65$	$2.09^{aAB}\pm0.22$	$1.54^{aAA}\pm0.18$	$1.22^{aA}\pm0.32$
	GIV	$5.04^{b}\pm0.23$	$4.93^{b}\pm0.44$	$5.09^{b}\pm0.35$	$4.94^{c}\pm0.42$	$5.05^{c}\pm0.56$	$5.12^{c}\pm0.45$
Glob. (g/dl)	GI	$3.58^{bC}\pm0.25$	$3.28^{cBC}\pm0.13$	$2.87^{bAB}\pm0.15$	$2.66^{bAB}\pm0.18$	$2.40^{bA}\pm0.30$	$2.18^{bA}\pm0.31$
	GII	$3.24^{abC}\pm0.17$	$2.43^{abBC}\pm0.37$	$2.14^{aAB}\pm0.28$	$1.69^{aAB}\pm0.21$	$1.37^{aA}\pm0.46$	$1.19^{aA}\pm0.31$
	GIII	$2.96^{aE}\pm0.08$	$2.30^{aD}\pm0.16$	$1.83^{aC}\pm0.19$	$1.34^{aB}\pm0.15$	$1.00^{aAB}\pm0.17$	$0.80^{aA}\pm0.08$
	GIV	$3.64^{b}\pm0.19$	$3.68^{c}\pm0.47$	$3.62^{c}\pm0.32$	$3.71^{c}\pm0.29$	$3.63^{c}\pm0.23$	$3.64^{c}\pm0.30$
A:G	GI	$2.63^{B}\pm0.72$	$2.50^{abAB}\pm0.30$	$2.08^{abAB}\pm0.24$	$1.79^{aAB}\pm0.21$	$1.63^{aAB}\pm0.24$	$1.33^{Aa}\pm0.15$
	GII	$2.38^{B}\pm0.61$	$2.01^{abAB}\pm0.19$	$1.86^{aAB}\pm0.18$	$1.60^{aAB}\pm0.31$	$1.30^{aAB}\pm0.27$	$1.11^{aA}\pm0.16$
	GIII	$2.07^{C}\pm0.43$	$1.76^{aBC}\pm0.44$	$1.49^{aAB}\pm0.07$	$1.23^{aAB}\pm0.19$	$1.24^{aA}\pm0.29$	$1.02^{aA}\pm0.20$
	GIV	$2.81^{a}\pm0.83$	$2.96^{b}\pm0.68$	$2.86^{b}\pm0.45$	$2.96^{b}\pm0.54$	$2.90^{b}\pm0.51$	$2.94^{b}\pm0.54$
ALT (KA units/ml)	GI	$44.20^{abA}\pm3.62$	$52.00^{abA}\pm2.55$	$62.20^{bB}\pm3.65$	$71.60^{bC}\pm1.03$	$81.80^{bD}\pm3.64$	$89.00^{Bd}\pm3.32$
	GII	$52.00^{bcA}\pm2.00$	$61.00^{bcAB}\pm3.67$	$69.20^{bBC}\pm5.67$	$79.60^{bC}\pm3.19$	$92.00^{bD}\pm3.74$	$108.00^{bE}\pm4.90$
	GIII	$59.00^{cA}\pm4.00$	$75.66^{cAB}\pm10.25$	$88.52^{cBC}\pm9.99$	$97.44^{cC}\pm5.14$	$105.20^{cD}\pm7.30$	$120.00^{cE}\pm6.32$
	GIV	$39.60^{a}\pm4.27$	$40.58^{a}\pm5.09$	$39.42^{a}\pm3.90$	$40.36^{a}\pm4.93$	$41.82^{a}\pm4.34$	$42.48^{a}\pm4.20$
AST (KA units/ml)	GI	$49.20^{abA}\pm1.93$	$57.00^{abAB}\pm2.55$	$64.00^{abBC}\pm4.30$	$70.20^{abCD}\pm2.84$	$74.00^{bD}\pm3.13$	$84.80^{bE}\pm0.80$
	GII	$54.00^{abA}\pm4.85$	$59.40^{abAB}\pm2.20$	$71.40^{bcBC}\pm4.50$	$81.86^{bcC}\pm6.90$	$89.10^{bD}\pm7.11$	$95.20^{bE}\pm5.29$
	GIII	$58.20^{bA}\pm2.50$	$65.00^{bAB}\pm5.00$	$83.54^{cBC}\pm9.00$	$91.46^{cCD}\pm9.43$	$99.00^{cCD}\pm9.27$	$109.20^{cE}\pm7.37$
	GIV	$45.22^{a}\pm3.74$	$49.38^{a}\pm3.12$	$48.00^{a}\pm3.57$	$53.48^{b}\pm3.71$	$49.84^{a}\pm3.90$	$50.20^{a}\pm3.44$

Data are presented as Mean + S.E. (*n*=8 rats/group). Means bearing at least one common superscript (a, b, c, d and A, B, C, D, E) do not differ significantly between groups and weeks, respectively (P<0.05). Group I (0.5 ppm), Group II (0.75 ppm), Group III (1.0 ppm), Group IV (Control) **TP** = Total protein**; Alb**. = Albumin; **Glob**. = Globulin; **A:G** = Albumin : Globulin ratio; **ALT** = Alanine aminotransferase; **AST** = Aspartate aminotransferase

Table 2: Effect of T-2 on various antioxidant enzymes in liver in rats of different treatment groups

Parameters	Groups	2 wk	4 wk	6 wk	8 wk	10 wk	12 wk
LPO (nM MDA/g)	GI	$28.72^{A}\pm4.88$	$50.95^{abAB}\pm11.54$	$55.52^{abB}\pm6.30$	$60.12^{bB}\pm6.29$	$64.44^{bB}\pm8.43$	$72.23^{Bb}\pm11.58$
	GII	$33.23^{A}\pm5.57$	$52.03^{abAB}\pm7.82$	$59.65^{bB}\pm5.58$	$65.92^{bBC}\pm0.99$	$71.82^{bBC}\pm0.58$	$81.54^{bC}\pm8.79$
	GIII	$37.18^{A}\pm5.24$	$70.51^{bB}\pm6.95$	$80.70^{cBC}\pm3.61$	$91.24^{cC}\pm1.22$	$104.63^{cD}\pm3.15$	$119.25^{cE}\pm2.41$
	GIV	$27.21^{a}\pm4.10$	$39.34^{a}\pm3.74$	$38.71^{a}\pm6.75$	$44.36^{a}\pm5.92$	$45.22^{a}\pm5.54$	$46.46^{a}\pm3.47$
SOD (U/mg)	GI	$30.57^{C}\pm1.09$	$29.57^{abC}\pm0.69$	$28.57^{bB}\pm0.31$	$26.66^{bA}\pm1.27$	$23.89^{bA}\pm1.04$	$1.28^{bA}\pm0.24$
	GII	$29.83^{D}\pm0.74$	$27.95^{abCD}\pm0.30$	$27.04^{bC}\pm0.79$	$24.41^{bB}\pm0.63$	$22.72^{bB}\pm0.38$	$19.36^{bA}\pm1.23$
	GIII	$28.53^{D}\pm0.62$	$26.76^{aD}\pm1.61$	$21.79^{aCD}\pm1.02$	$17.84^{aC}\pm1.16$	$16.99^{aB}\pm0.64$	$15.01^{aA}\pm0.68$
	GIV	31.34 ± 1.09	$31.25^{c}\pm1.40$	$30.78^{c}\pm1.14$	$30.78^{c}\pm1.23$	$30.40^{c}\pm1.72$	$30.13^{c}\pm1.77$
CAT (KA/g)	GI	$244.97^{C}\pm6.63$	$242.51^{BC}\pm6.47$	$240.29^{abBC}\pm5.07$	$235.24^{bBC}\pm3.33$	$227.12^{bAB}\pm3.25$	$212.84^{bA}\pm4.72$
	GII	$244.75^{C}\pm9.13$	$241.14^{C}\pm5.75$	$237.84^{abC}\pm3.71$	$229.70^{bBC}\pm3.20$	$217.17^{aB}\pm4.04$	$199.96^{aA}\pm3.48$
	GIII	$244.19^{D}\pm5.37$	$239.64^{D}\pm4.05$	$224.92^{aCD}\pm6.47$	$207.83^{aBC}\pm7.59$	$192.23^{aC}\pm7.29$	$150.49^{aA}\pm7.72$
	GIV	246.56 ± 6.02	244.25 ± 6.77	$244.14^{c}\pm7.35$	$245.79^{c}\pm5.92$	$244.33^{c}\pm7.26$	$244.43^{c}\pm6.74$
GSH (mM/g)	GI	$0.221^{D}\pm0.018$	$0.213^{aD}\pm0.011$	$0.193^{abCD}\pm0.010$	$0.162^{bBC}\pm0.015$	$0.141^{cAB}\pm0.005$	$0.122^{cA}\pm0.006$
	GII	$0.216^{D}\pm0.011$	$0.184^{abCD}\pm0.030$	$0.176^{abCD}\pm0.012$	$0.136^{abBC}\pm0.015$	$0.110^{bA}\pm0.011$	$0.093^{bA}\pm0.010$
	GIII	$0.203^{D}\pm0.017$	$0.143^{aCD}\pm0.015$	$0.152^{aBCD}\pm0.046$	$0.109^{aABC}\pm0.011$	$0.080a^{AB}\pm0.005$	$0.057^{aA}\pm0.010$
	GIV	0.243 ± 0.010	$0.242^{b}\pm0.008$	$0.246^{c}\pm0.006$	$0.247^{c}\pm0.004$	$0.258^{d}\pm0.005$	$0.257^{d}\pm0.003$

Data are presented as Mean + S.E. (n=8 rats/group). Means bearing at least one common superscript (a, b, c, d and A, B, C, D) do not differ significantly between groups and weeks, respectively (P<0.05). Group I (0.5 ppm), Group II (0.75 ppm), Group III (1.0 ppm), Group IV (Control) **LPO** = Lipid peroxidation; **MDA** = Malondialdehyde; **SOD** = Superoxide dismutase; **CAT** = Catalase; **GSH** = Reduced glutathione

hepatocytes with deep eosinophilic cytoplasm and condensation of nuclei (Fig. 4d) were frequently recorded. Almost all animals showed degenerated hepatocytes with marked bile duct hyperplasia, MNC's infiltration in portal areas with presence of necrotic cell mass with deep eosinophilic cytoplasm and remnants of nuclei (Figs. 4e, f, g) at 12th week. Group IV (control) animals showed distinct lobules with normal cord arrangement of hepatocytes, vasculature (sinusoids and vessels) and biliary system had normal histologic details (Fig. 5).

Table 3: Effect of T-2 on hepatosomatic index (% of bw) in rats of various treatment groups

Groups	2 wk	4 wk	6 wk	8 wk	10 wk	12 wk
GI	$3.651^{A}\pm0.176$	$4.048^{abAB}\pm0.199$	$4.541^{abB}\pm0.120$	$4.974^{bB}\pm0.421$	$5.252^{bC}\pm0.154$	$5.747^{bD}\pm0.596$
GII	$3.683^{A}\pm0.219$	$4.428^{bcAB}\pm0.151$	$4.952^{bcB}\pm0.241$	$5.552^{bcB}\pm0.712$	$6.072^{cC}\pm0.321$	$7.246^{cD}\pm0.262$
GIII	$3.661^{A}\pm0.294$	$4.723^{B}\pm0.249$	$5.147^{cB}\pm0.411$	$7.066^{cB}\pm0.541$	$8.047^{cC}\pm0.229$	$8.670^{dD}\pm0.353$
GIV	$3.631^{A}\pm0.326$	$3.716^{aAB}\pm0.305$	$3.958^{aAB}\pm0.285$	$4.160^{aAB}\pm0.171$	$4.260^{aAB}\pm0.136$	$4.500^{aAB}\pm0.316$

Data are presented as Mean + S.E. (n=8 rats/group). Means bearing at least one common superscript (a, b, c, d and A, B, C, D) do not differ significantly between groups and weeks, respectively (P<0.05). Group I (0.5 ppm), Group II (0.75 ppm), Group III (1.0 ppm), Group IV (Control)

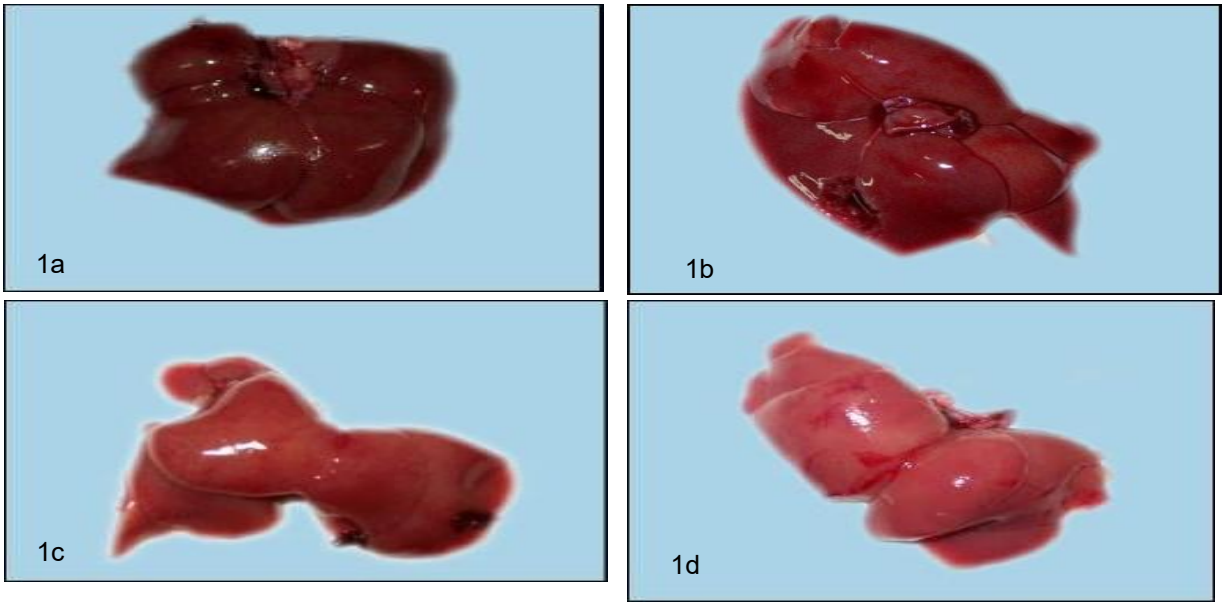

Fig. 1. Gross appearance of liver in different T-2 treatment groups at 12 weeks of feeding. a & b Livers of group IV (control) and I rats, respectively exhibited normal shape, colour, consistency, size and smooth surfaces. c Group II liver were comparatively enlarged with rounded border and mild pale discoloration. d Group III were moderately enlarged with rounded borders and pale discoloration.

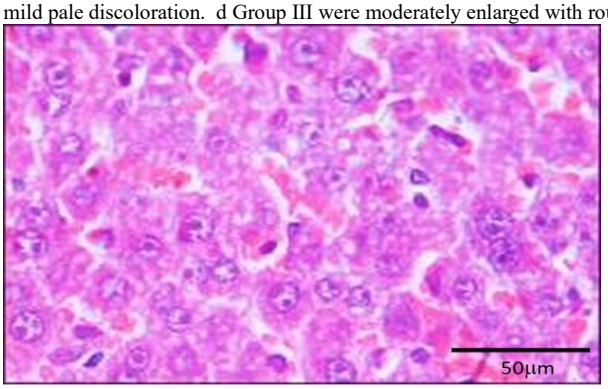

Fig. 2a Liver, group I, 2nd week: Showing engorged sinusoids and swollen hepatocytes with foamy cytoplasm. H&E x 400

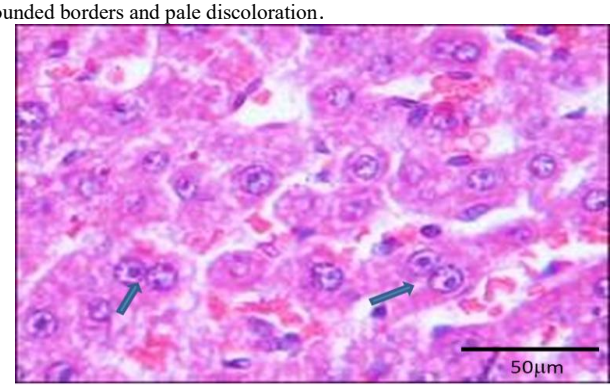

Fig. 2b Liver, group I, 4th week: Showing hepatocytic degeneration, binucleation (arrow) and congestion of sinusoids. H&E x 400.

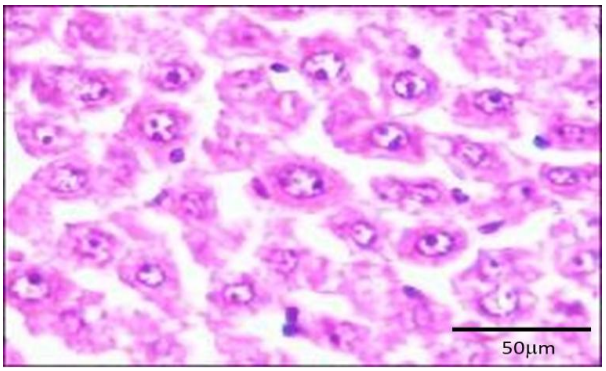

Fig. 2c Liver, group I, 8th week: Showing severe hepatocytic degeneration with rarefaction of cytoplasm, indistinct cell boundaries and disappearance of nuclei. H&E x 400

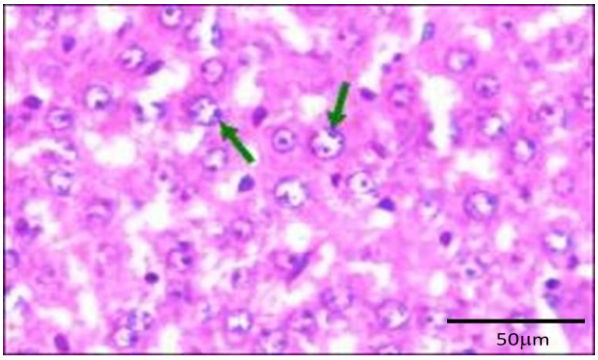

Fig.2d Liver, group I, 12th week: Showing severely swollen, degenerated hepatocytes with indistinct cell boundaries and frequent karyomegaly (arrow). H&E x 400

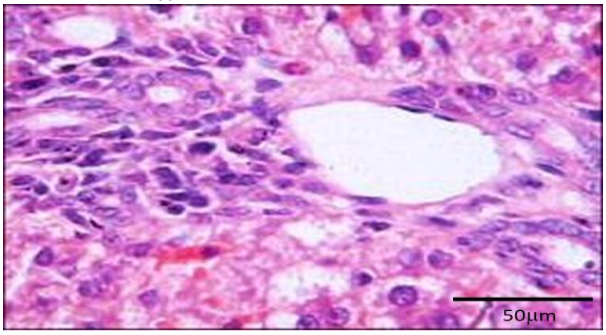

Fig. 3a Liver, group II (0.75 ppm), 2nd week: Showing hepatocytic degeneration with increased number of bile ductules and MNC infiltration in portal area. H&E x 40

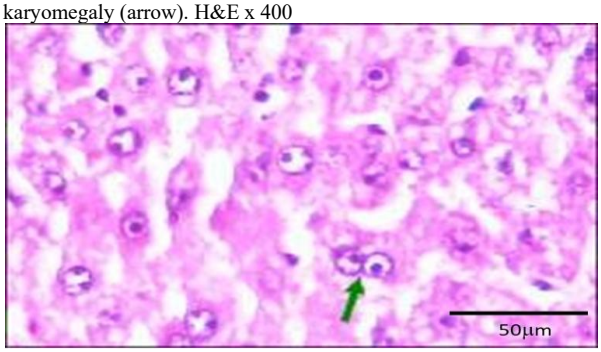

Fig. 3b Liver, group II, 8th week: Showing severe hepatocytic degeneration with rarefied cytoplasm, indistinct cell boundaries and binucleation (arrow). H&Ex 400

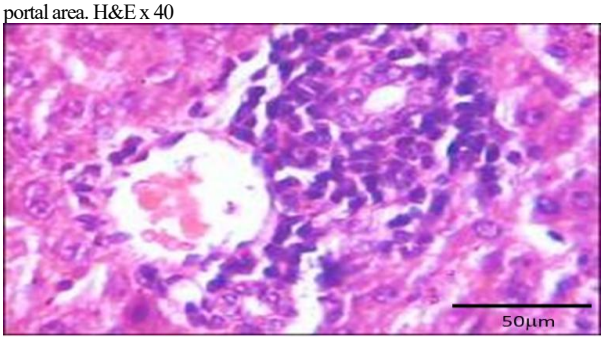

Fig. 3c Liver, group II, 10th week: Showing severe hepatocytic degeneration with finely granular cytoplasm. Note MNC infiltration, mild bile duct proliferation and dilated portal vein in portal area. H&E x 400.

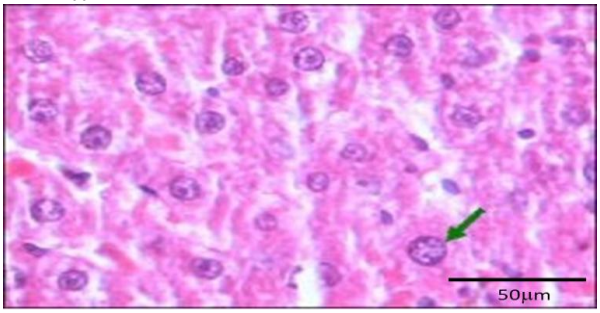

Fig. 3d Liver, group II, 12th week: Showing severely degenerated hepatocytes with rarefaction and condensation of cytoplasm. Note presence of necrosed hepatocytes and karyomegaly (arrow). H&E x 400

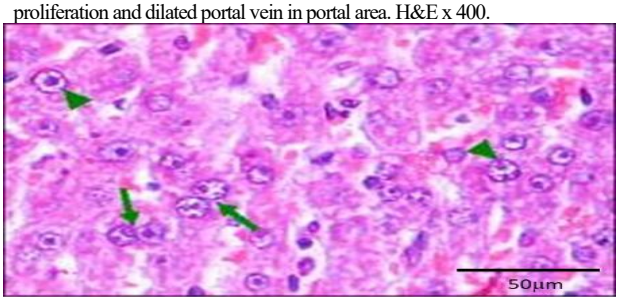

Fig. 4a Liver, group III, 4th week: Showing severe hepatocytic degeneration with foamy cytoplasm, indistinct cell boundaries, binucleation (arrow) karyomegaly (arrow head) and engorged sinusoids. H&E x 400

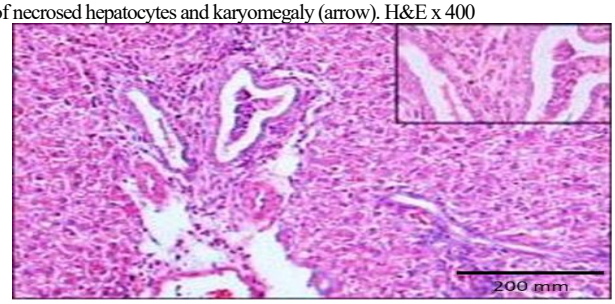

Fig. 4b Liver, group III, 6th week: Portal area showing mild fibroblastic proliferation, MNC infiltration and cell debris in lumen of bile duct. H&E x 100. Inset showing higher magnification of cell debris in the lumen of bile duct. H&E x400

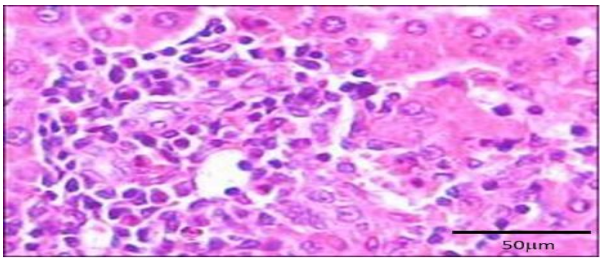

Fig. 4c Liver, group III, 8th week: Showing clumping of degenerated and necrotic hepatocytes with MNC infiltration in portal region. H&E x 400

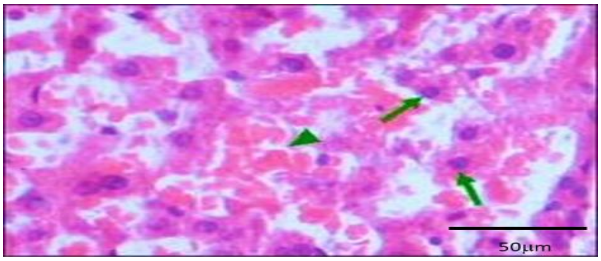

Fig. 4d Liver, group III, 10th week: Showing distorted hepatic cords, loss of hepatocytes causing dilatation and engorgement of sinusoids (arrow head). Note the variable sized hepatocytes with deep eosinophilic cytoplasm and condensation of nuclei (arrow). H&E x 400

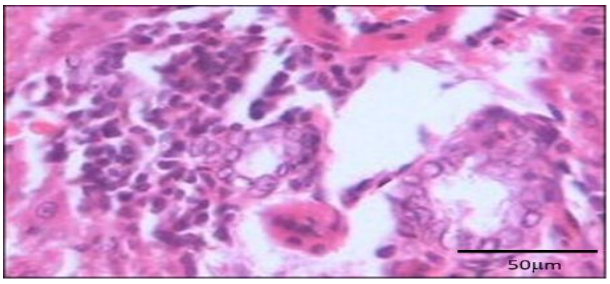

Fig. 4e Liver, group III, 12th week: Showing degenerated hepatocytes with marked bile duct hyperplasia and MNC infiltration in portal area. Note the presence of necrotic cell mass with deep eosinophilic cytoplasm and remnants of nuclei (arrow). H&E x400

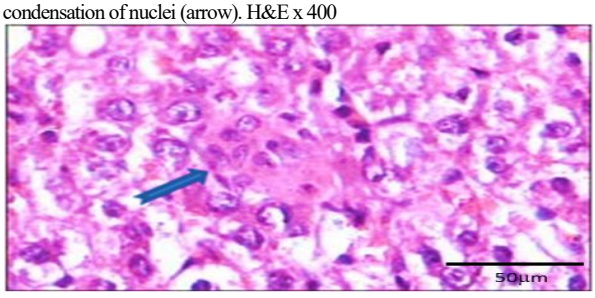

Fig. 4f Liver, group III, 12th week: Showing severe degeneration with rarefied cytoplasm. Note focal clumping of degenerated hepatocytes (arrow). H&E x 400

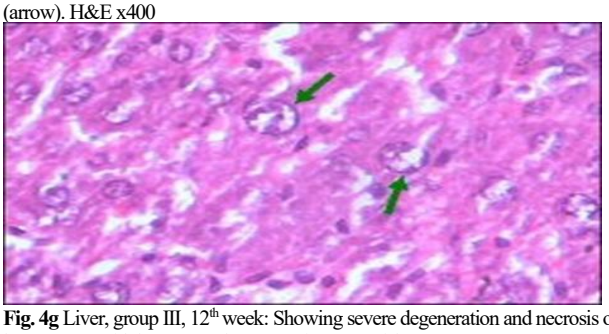

Fig. 4g Liver, group III, 12th week: Showing severe degeneration and necrosis of hepatocytes with karyomegaly (arrow) in some of them. H&E x 400.

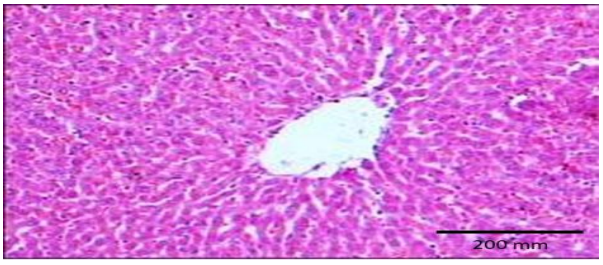

Fig. 5 Liver, group IV (Control): Showing normal hepatic lobules with central vein and hepatocytes with vesicular nuclei and arranged in cord pattern. H&E x 100

Discussion

The current study showed that exposure to T-2 toxin reduced serum levels of total protein, albumin, globulins, and the A:G ratio in a dose- and duration-dependent manner. It also increased serum levels of ALT, AST, and LDH in T-2 treated animals, indicating liver damage, which is further supported by histopathological alterations in liver during the study. These results align with previous findings in rats (Al-Zahrani et al., 2023), and poultry (Singh et al., 2020). Hypoproteinemia, hypoalbuminemia, and hypoglobulinemia may also be caused by decreased feed intake during toxicosis or due to inhibition of biosynthetic enzymes and endoplasmic reticulum degeneration, which impede hepatic protein synthesis (Janik et al., 2021). The hepatic damage seen in all toxin-fed groups histologically in this study and severe tubular degeneration and necrosis in the kidneys of T-2-treated animals reported by Shafiqur Rahman et al. (2016) cause protein loss through urine, particularly albumin, leading to to hypoproteinemia and hypoalbuminemia. T-2 causes lipid peroxidation, inflammation, and apoptosis in liver cells. This weakens the cell membrane, resulting in the release of intracellular ALT and AST into the bloodstream (Janik et al., 2021).

T-2 toxin causes severe oxidative stress in a dose and duration-dependent manner by increasing lipid peroxidation and inhibiting antioxidant defense mechanisms. The findings of the oxidative stress in the present study are in accordance with previous observations reported in rats (Chandratre et al., 2014; Moosavi et al., 2016), and in poultry (Yin et al., 2020). An in vivo investigation using mice showed that T-2 toxicated animals had increased expression of genes linked to apoptosis and oxidative stress. As an amphiphilic molecule, T-2 toxin induces lipid peroxidation by producing free radicals, consequently damaging cellular membranes and also causes depletion of hepatic glutathione (Janik et al.,

2021). Glutathione and lipid peroxidation are sensitive indicators of oxidative stress. The present study observed a duration-dependent increase in LPO in T-2 treated rats, along with a reduction in GSH and antioxidant enzymes (SOD and Catalase), suggesting oxystress-mediated hepatic damage, as also evidenced histopathologically.

Gross and appreciable histological changes were noticed in the liver of the toxin treated animals in a dose and duration dependent manner. These observations found support from earlier reports in poultry (Yin et al., 2020; Verma et al., 2014), and in rats (Chandratre et al., 2014; Moosavi et al., 2016; Al-Zahrani et al., 2023). Hepatocellular damage appears to be mediated by free radical-induced oxidative stress, notably heightened lipid peroxidation observed in the present study.

Decreased serum protein, albumin and globulin levels coupled with elevated ALT, AST levels; oxidative damage (increased LPO, and decreased GSH, SOD and catalase) and patho-morphological changes observed in the liver were suggestive of hepatotoxicity due to T-2 toxicity.

Conclusion

The current study, designed to investigate the impact of graded doses of T-2 toxin on Wistar rats, revealed alterations in serum biochemical parameters, oxidative stress markers, and liver histopathology, indicative of hepatotoxicity. Furthermore, the observed oxidative damage in the liver suggests that oxidative stress plays a crucial role in T-2 toxin-induced hepatotoxicity in Wistar rats.

Acknowledgement

The authors would like to thank all of the workers who participated in this study.

Ethical considerations

All the experimental procedures were approved and conducted as per the guidelines of the Institute Animal Ethics Committee (IAEC) and Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

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