

Effect of Grape Seed Extract on the T-2 Toxicity in mice

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Abstract:

T-2 toxin is a cytotoxic secondary fungal metabolite that belongs to the trichothecenes mycotoxin family. Hepatotoxicity and nephrotoxicity were induced in mice by feeding T-2 toxin contaminated wheat. The serum biochemical analysis was observed after 8 weeks by an increase in the plasma activity of alanine aminotransferase (ALT), where that elevation reached (76.00 IU/L) in G2, in comparison with G3 that the ALT level return back to normal level. While the levels of total protein and albumin appeared no significant change in all groups of study. Serum urea showed a significant increase in the mean serum level of urea (52.70 mg/dl) in G2 whereas the treated with grape seed extract could return back the mean serum level of urea to normal level (38.95 mg/dl). Serum level of total bilirubin was observed increase significantly to reach (0.42 mg/dl) in G2 when compared to third group that the grape seed extract reduce the serum level of total bilirubin to normal level (0.34 mg/dl). There was a significant increase in the mean serum level of creatinine was observed in the T-2 toxin group (0.69 mg/dl) when compared to creatinine level in grape seed extract with T-2 toxin recorded a significant reduction (0.54 mg/dl). Histopathological examination of liver and kidney sections confirmed the serum analysis. These results clearly indicated that T-2 toxin has stressful effects on the hepatic and renal tissues.

Key words: *Trichothecenes, Grape, Mycotoxin toxicity.*

Introduction:

T-2 toxin is a secondary metabolite that belongs to the trichothecene family of mycotoxin. They are produced by various species of *Fusarium* (*F. sporotrichioides*, *F. poae*, *F. equiseti*, and *F. acuminatum*), which can infect corn, wheat, barley and rice crops in the field or during storage (1, 2). T-2 toxin has been implicated mycotoxicoses such as red mold disease in humans and animals (3), bean hull poisoning in horses (4) and inhibit protein synthesis through its high binding affinity to peptidyl transferase which is an integral part of the 60s ribosomal subunit (5,6). Moreover, T-2 toxin interferes with the metabolism of membrane phospholipids and increases liver lipid peroxides (7, 8). Oral, parenteral and cutaneous exposures to T-2 toxin induce lesions in hematopoietic, lymphoid and gastrointestinal tissues and suppress reproductive functions in domestic and laboratory animals (9, 10) and can induce apoptosis in many types of cells bearing rapid rates of proliferation (11, 12) also induces apoptosis and fatty change

in hepatocytes of mice following the increased expression of both oxidative stress and apoptosis-related genes (13). Prenatal exposure of rats to T-2 toxin induces apoptosis in maternal liver, placenta and fetal liver following the increased expression of oxidative stress and apoptosis-related genes (14). Acute toxicity of dermal and subcutaneous exposure of T-2 toxin on brain oxidative stress in adult mice was evaluated by (15). Mice were exposed to T-2 toxin either by the dermal (5.94 mg/kg b.w.) or subcutaneous (1.54 mg/kg b.w.) route and sacrificed at 1, 3 and 7 days post-exposure, they reported that T-2 toxin-treated animals showed a time-dependent increase in ROS generation, glutathione (GSH) depletion, lipid peroxidation and protein carbonyl content in the brain, liver and kidney in both routes of exposure. This indicates that T-2 toxin induces oxidative damage in adult mouse and fetal rat; lipid peroxidation may bring about protein damage and inactivation of membrane-bound enzyme either through direct attack by free radicals or through chemical modification by its end products (16).

Grape (*Vitis vinifera*) is one of the most widely consumed fruits in the world. Grape is known as the “queen of fruits” because of cleansing properties. A “grape cure” or grape fast involves eating 3–6 pounds of grapes to detoxify and prove

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liver function (17). Additionally, it has been reported that grape has important role in controlling of some liver diseases, high blood pressure and anemia. Also fibers and fruit acids in grape have vital role in cleaning blood functions of digestive system and kidney (18). Medically used grape seed extract for its high proanthocyanidin content and it is a naturally occurring plant that contains source of antioxidant like oligomeric proanthocyanidins, which are more powerful antioxidants than vitamins C, E and beta-carotene.

Material and Methods:

Chemicals: All chemicals were purchased from Biosolve BV (France) and the standard mycotoxin purchased from Santa Cruz Company (USA).

Production, Extraction and Purification of T-2 toxin: The production, extraction and purification of T-2 toxin according to the procedure by (19).

Detection of T-2 toxin: Detected by thin layer chromatography (TLC) technique.

Animals and experimental design: Forty albino BALB/C male mice average weight (22-25g) were used. Animals were caged in groups of four and given water. The first group (G1) of 10 mice was used as control (untreated) were fed on uncontaminated wheat; the second group (G2) of 10 mice served as the treated group were fed on contaminated wheat with at least 1mg/ kg body weight T-2 toxin for 8 weeks served as positive control, the third group (G3) of 10 mice were fed on mix of grape seed extract and contaminated wheat and the fourth group (G4) of 10 mice were fed on grape seed extract alone (50mg/kg body weight). At the end of experimental period, the mice were sacrificed. Blood samples were collected in sterile test tubes (20) for analysis.

Extraction of grape seed extract: Red grape were obtained from Baghdad markets and the extraction was performed by (21).

Serum analysis: All biochemical parameters for liver and kidney function:

(Alanine aminotransferase, total proteins, albumin, serum urea, total bilirubin and serum creatinine) were determined with the blood autoanalyzer (Liza 200).

Histopathological examination: Tissue processing and staining of (liver and kidney) depending on (22).

Statistical analysis: Differences in the mean levels of biochemical parameters were analyzed by (ANOVA) one way.

Results:

Determination of T-2 toxin by TLC: TLC technique showed a mild T-2 toxin concentration in wheat in comparison with standard accordingly to their spot size and intensity of color.

Biochemical Results:

1. Serum levels of alanine aminotransferase activity (ALT):

The mean serum activity level of (ALT) in treated group on which the mice fed on T-2 toxin contaminated wheat (76.00 IU/L), where significantly higher ($P \leq 0.05$) than control mice (28.00 IU/L). When grape seed extract used with T-2 toxin, the grape seed extract could significantly lower the serum activity level of ALT to reach (41.50 IU/L), but significantly higher than that of control group, while the serum activity level of ALT in mice treated with grape seed extract alone reach to (29.25 IU/L) (Fig.1).

2. Serum levels of total protein:

There was no significant change in the mean serum level of total protein in the T-2 toxin group (5.98 g/dl), when compared to that of control group (4.58 g/dl). The grape seed extract with T-2 toxin group has been recorded also no significant difference in the mean serum level of total protein (4.66 g/dl) when compared to that of control group and T-2 toxin group, while the grape seed extract recorded about (4.60 g/dl) (Fig.2).

3. Serum levels of Albumin:

There was no significant difference in the mean serum level of albumin in the T-2 toxin group (2.45 g/dl) in comparison with that of control group (2.20 g/dl). Grape seed extract plus T-2 toxin group showed no significant difference in the mean serum level of albumin (2.33 g/dl) in respect to that of control group and T-2 toxin group, but mean serum level of albumin in mice treated with grape seed extract alone reached to (2.23 g/dl) (Fig.3).

4. Serum Urea level:

The T-2 toxin group showed a significant increase in the mean serum level of urea (52.70 mg/dl) when compared to control group (39.54 mg/dl). The treated with grape seed extract could return back the mean serum level of urea to normal level (38.95 mg/dl) when compared to grape seed extract (39.43 mg/dl) and control groups (Fig.4).

5. Serum Total Bilirubin level:

The mean serum level of total bilirubin in group of mice treated T-2 toxin in wheat increase significantly ($P \leq 0.05$) to reach (0.42 mg/dl) when compared to control group (0.32 mg/dl), but the grape seed extract return the serum level of total bilirubin to normal level (0.34 mg/dl) in third group, while mice treated grape seed extract alone showed value about (0.33 mg/dl) (Fig.5).

6. Serum Creatinine level:

A significant increase ($P \leq 0.05$) in the mean serum level of creatinine was observed in the T-2 toxin group (0.69 mg/dl) when compared to that of control group (0.42 mg/dl). The mean serum level of creatinine in grape seed extract with T-2 toxin recorded a significant reduction (0.54 mg/dl), but the mean serum level of creatinine in fourth group was (0.44 mg/dl) (Fig.6).

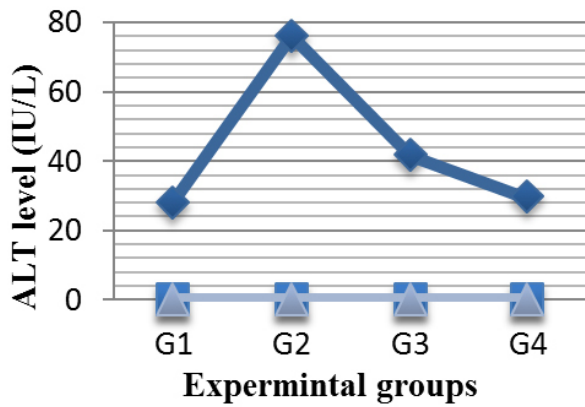


Figure (1): Mean serum level of ALT in experimental mice groups

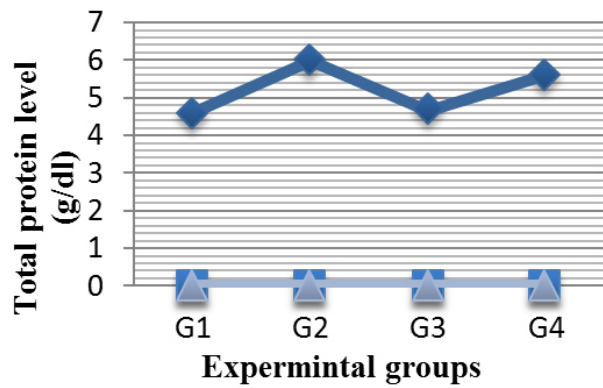


Figure (2): Mean serum level of total protein in experimental mice groups

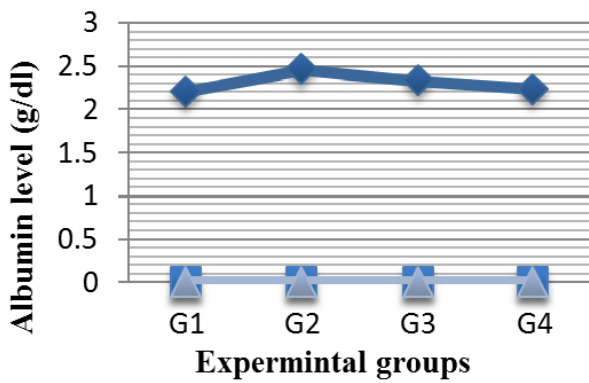


Figure (3): Mean serum level of albumin in experimental mice groups

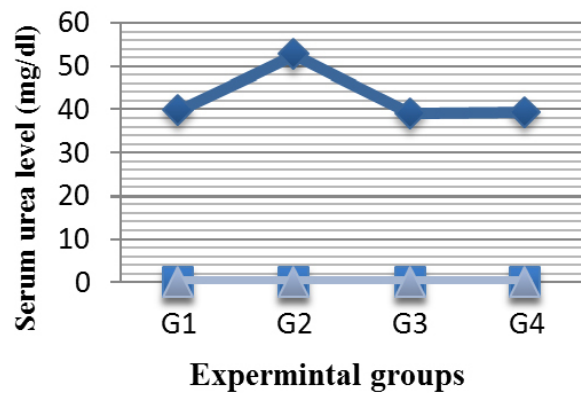


Figure (4): Mean serum level of urea in experimental mice groups

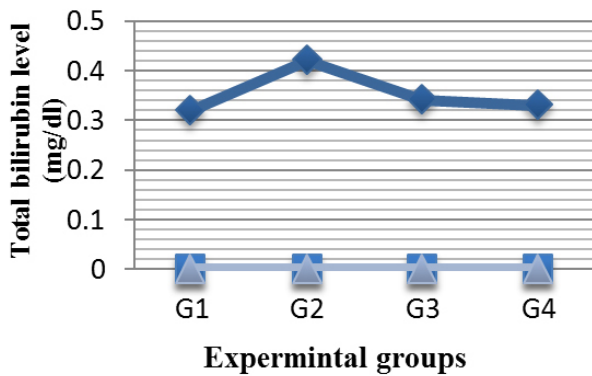


Figure (5): Mean serum level of total bilirubin in experimental mice groups

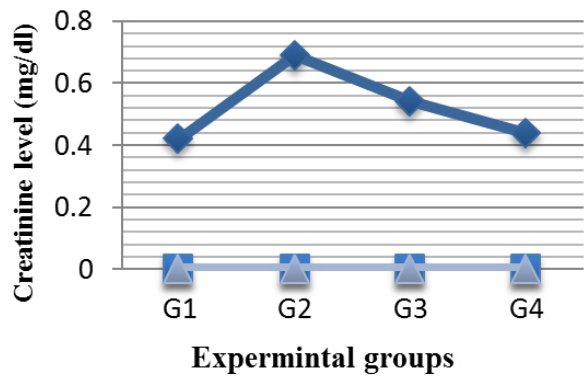
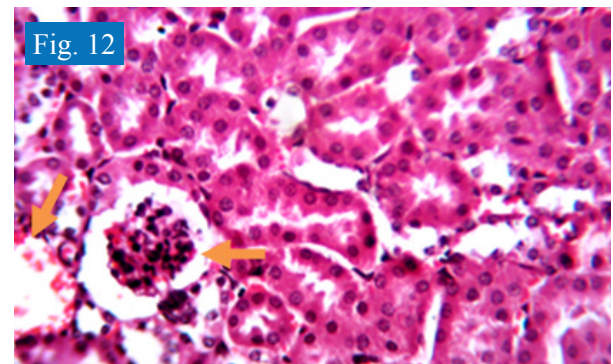
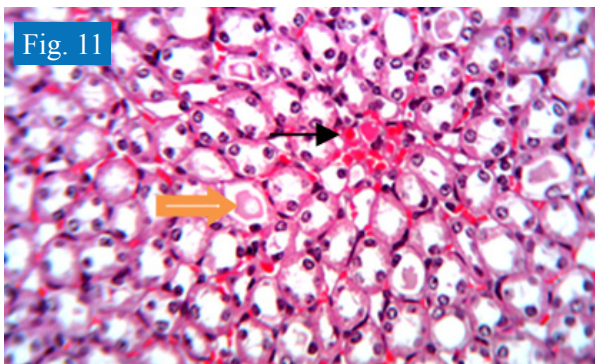
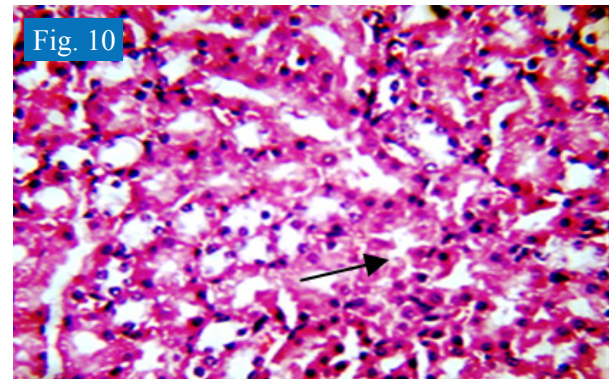
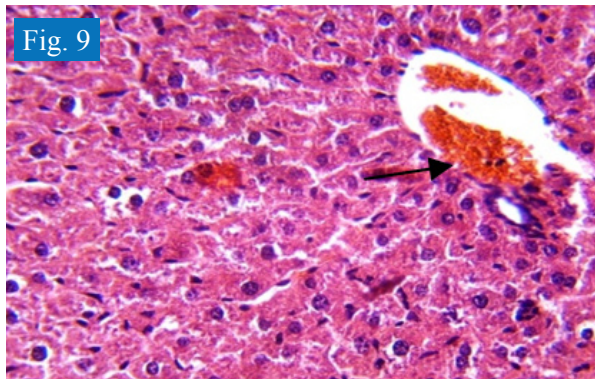
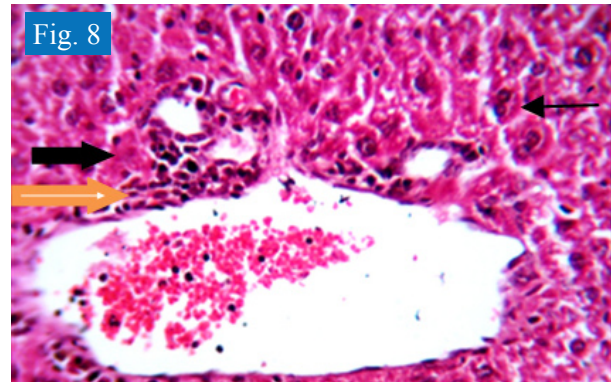
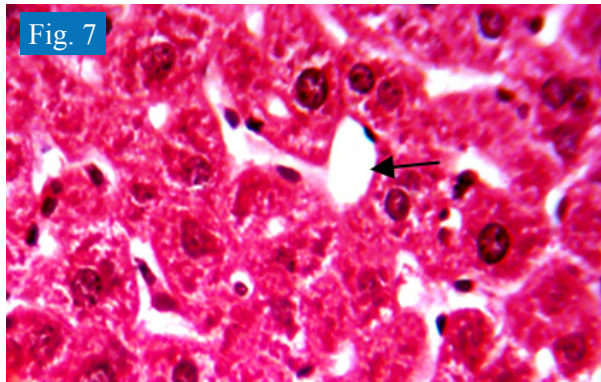


Figure (6): Mean serum level of creatinine in experimental mice groups

Histopathological results:

Liver: Investigation of liver microscopic sections for control group showed normal tissue (Fig.7). While mice treated with T-2 toxin contaminated wheat showed injury in the liver due to toxicity by showing enlargement of hepatocytes and infiltration of mononuclear cells in and around congested blood vessels with apoptotic and mitotic figures of hepatocyte (Fig.8). The mice fed on grape seed extract with toxin showed less to mild signs (Fig.9).

Kidney: Kidney section from control mice (Fig.10) showed normal renal tubules. While kidney section from mice treated with T-2 toxin showed degeneration and necrosis of epithelial lining of proximal and distal convoluted tubules with congestion and wide areas of necrosis in parenchyma in addition for presences of hyaline casts in urinary tubules due to urinary failure (Fig.11). Just congestion was noted in mice fed on grape seed extract with toxin group (Fig.12).



(Fig.7): Histopathological section of liver for control mouse showing central vein (←) (H&E X 400).

(Fig.8): Histopathological section of liver for fed mouse T-2 toxin showing infiltration of mononuclear cells (→) in and around congested blood vessels with apoptotic (→) and mitotic figures (←) of hepatocyte (H&E X 200). (Fig.9): Histopathological section of liver for fed mouse T-2 toxin with extract showing congestion of central vein (→) (H&E X 200). (Fig.10): Histopathological section of kidney for control mouse showing normal tubules (→) (H&E X 200). (Fig.11): Histopathological section of kidney for fed mouse T-2 toxin showing congestion (→) presences of hyaline casts in urinary tubules (→) (H&E X 200). (Fig.9): Histopathological section of kidney for fed mouse T-2 toxin with extract showing congestion of blood vessels and glomerular capillaries (→) (H&E X 400).

Discussion:

The present research demonstrated that the elevation in the serum ALT activity indicated to the damage of hepatocytes as a result of T-2 toxicity. Elevated in the serum ALT activity in mice fed T-2 toxin contaminated diet occurs due to severe damage in the liver (26). On the other hand, there was no significant change in the mean serum levels of total protein and albumin in mice due to the sensitivity differences between mice to their toxicity. In mice treated with T-2 toxin showed nephrotoxic effects as a result to a significant increase in the mean serum levels of urea, total bilirubin and creatinine because that T-2 toxin induces oxidative damage in the brain, liver, kidney and other organs (23, 27). Nephrotoxic occurred due to increase in the creatinine and urea concentrations (28). In the treated group with grape seed extract was significantly reduced the elevation of serum ALT activity suggesting that grape seed extract could return back

the liver to its normal condition. The hepatotoxicity reduced significantly due to the effects of grape seed extract, this effect of extract supports the idea that it is bioavailable and exhibits potent antioxidant and anti-inflammatory effects (29). While the effects of grape seed extract on the serum levels of urea, total bilirubin and creatinine that the extract could return back these levels to normal due to this extract indirectly corrects body homeostasis through its improvement of kidney function (30). The toxic effects of T-2 toxin may depend upon the route of exposure, concentrations in the diet, and the duration of exposure (24). T-2 toxin is associated with a large range of toxic effects, such as weight loss, decreases in blood cell and leukocyte counts, reduction in plasma glucose and pathological changes in the liver, kidney and stomach and neonatal animals are more susceptible than adults to the toxicity of trichothecenes and their toxicity also varies from species to species (25).

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تأثير مستخلص بذور العنب على سمية الدت 2 في الفئران

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الخلاصة:

سم الدت 2 هو أحد نواتج الأيض الثانوي للفطريات والذي يقع ضمن عائلة السموم الفطرية الترايكوثسين. تم أستحداث سمية الكبد والكلية في الفئران بعد تغذيتها بالحنطة الملوثة بالسم ولمدة ثمانية أسابيع. بعدها لوحظت نتائج السمية لكلا العضوين من خلال الفحوصات الكيموحيوية فقد تبين ان هناك زيادة معنوية في فعالية أنزيم (ALT) ليصل (IU/L 76.00) في فئران المجموعة الثانية بالمقارنة مع حيوانات المجموعة الثالثة التي عادت فيها فعالية الأنزيم الى مستواه الطبيعي بفعل المعالجة بمستخلص بذور العنب. فيما لم تظهر مستويات البروتين الكلي و الالبومين أي فروقات معنوية في جميع مجاميع التجربة. بينما أظهر مستوى اليوريا ارتفاع معنوي (mg/dl 52.70) في المجموعة المعاملة بالسم وعودته لمستواه الطبيعي في المجموعة الثالثة بعد المعالجة بالمستخلص (38.95 mg/dl). كما وأرتفعت مستويات البليروبين الكلي بشكل معنوي (mg/dl 0.42) في مجموعة السم بالمقارنة مع المجموعة المعالجة بالمستخلص حيث لوحظ انخفاض في مستواه ليصل الى (mg/dl 0.34). وأرتفعت مستويات الكرياتينين في مصل الفئران المعاملة بالسم الملوثة للعليقة لتصل الى (mg/dl 0.69) عند مقارنتها بالفئران المعالجة بالمستخلص (mg/dl 0.54). وأكدت الفحوصات النسيجية للكبد والكلية ماتوصلت اليه نتائج الفحوصات الكيمياحيوية. نستنتج من هذه تأثير الفعال السمي لسم الدت 2 على كل من كبد وكلية الفئران..