



## ANTIOXIDANT AND HEPATOPROTECTIVE EFFECTS OF TRANILAST AGAINST THIOACETAMIDE-INDUCED LIVER FIBROSIS IN RATS

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### ABSTRACT

The aim of the present study was to investigate the potential antioxidant and Hepatoprotective activities of tranilast against TAA-induced liver fibrosis in rats. Methods: Liver fibrosis was induced in rats by intra peritoneal injection of TAA (200 mg/kg) for 6 weeks. Tranilast was administered orally at a dose of 150 and 300mg/kg for 6 weeks before induction of liver fibrosis. Results: Injection of TAA induced hepatic fibrosis that was manifested by a significant increase in the activities of aminotransferases and total bilirubin with a significant decrease in total protein and albumin in serum. Liver homogenate of TAA rats had the lower content of reduced glutathione (GSH) and superoxide dismutase (SOD) with increased levels of the hepatic malondialdehyde. Histological data presented marked damage in liver sections of TAA rats. Oral dosing of tranilast for 6 weeks before induction of liver fibrosis reversed these altered parameters near to normal values. These results suggest that tranilast could afford significant protection and antioxidant effect in prevention of liver fibrosis.

**Keywords:** Hepatic fibrosis, Thioacetamide, Tranilast, Liver, Rat.

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### INTRODUCTION

Liver fibrosis is defined as the hepatic dynamic response to repeated and chronic liver injury, accompanying the activation of HSCs, the over expression of extracellular matrix (ECM) proteins, ultimately lead to cirrhosis, which is characterized by scar tissue, loss of parenchymal architecture and organ failure (Wang *et al.* 2016). It is known that various agents, including chronic infection by hepatotropic viruses (hepatitis B and hepatitis C viruses), chronic exposure to toxins or drugs (e.g., alcohol abuse), chronic cholestatic diseases and autoimmune chronic hepatitis result in liver damage. When the damage is prolonged, liver fibrosis occurs (Hernandez-Aquino *et al.* 2017). The liver fibrosis affects more than 100 million people worldwide and represents one of the most common causes of death in adults (Rehman *et al.* 2016). In Egypt, up to 85% of HCV infections persist for life, leading to chronic hepatitis

(Elgharably *et al.* 2017).

Thioacetamide (TAA) is a thiono-sulphur containing compound that has been used as a fungicide, a curing ingredient, a chemical reagent, a raw medicine, a textile dye, and a finishing auxiliary (Al-Attar *et al.* 2017). TAA is a model hepatotoxicant and responsible of severe damage of the cells with significant toxic effects on the biosynthesis of macromolecules including proteins and nucleic acid. Its biotransformation to thioacetamide sulfoxide (TAAS) occurs along the cytochrome P-450 (CYP)-dependent pathways (Koblihova *et al.* 2014). Furthermore, different studies showed that the exposure to TAA caused liver injury, fibrosis and cirrhosis in experimental animals. The hepatic toxic chemical TAA has been widely used in the study of the underlying mechanisms of hepatic fibrogenesis and the therapeutic effects of potential antifibrotic drugs (Al-Attar *et al.* 2016).

The prevention of liver fibrosis remains a major problem that troubles the medical profession. Therefore, searching for the safe compound with high preventive and/or therapeutic efficacies is urgently needed (Zhao *et al.* 2017). Tranilast (N-[3, 4-dimethoxycinnamoyl]-anthranilic acid; Rizaben®) is an analog of the amino acid *tryptophan*, that was originally approved as an anti-allergic drug. Tranilast was originally developed as a mast cell stabilizer, since it exerts inhibitory effects on the release of chemokines (Baba *et al.* 2016). Tranilast is currently used in the treatment of inflammatory diseases, such as bronchial asthma, atypical dermatitis, allergic conjunctivitis, keloids and hypertrophic scars (Li *et al.* 2014). It was demonstrated that tranilast suppressed release of profibrotic cytokines from monocyte-macrophages *in vitro* and reduce tubulointerstitial and heart fibrosis in diabetic rat models, highlighting tranilast antifibrotic properties (Swiderski *et al.* 2014).

Therefore, the current study aimed to investigate the potential hepatoprotective and antioxidant effects of tranilast against liver fibrosis induced by TAA in male rats.

## MATERIALS AND METHODS

### Animals

Twenty eight adult male Wistar rats weighing 150-180 g were utilized in the present study. Standard food pellets and tap water were supplied *ad libitum*. Animals and food pellets were obtained from the animal house colony of the National Research Centre (NRC, Egypt). The animals were cared for in accordance with the Guide for the Care and Use of Laboratory Animals (1996, published by National Academy Press, 2101 Constitution Ave. NW, Washington, DC 20055, USA) and that their use was reviewed and approved in accordance with ethical rules for standard experimental animal studies and the Medical Research Ethics Committee (MREC) of the NRC.

### Drugs and chemicals

Thioacetamide (TAA) (C<sub>2</sub>H<sub>5</sub>NS) was obtained from El-Gomhouria Company for drug and chemicals, Egypt. Carboxymethyl cellulose sodium (C<sub>8</sub>H<sub>15</sub>NaO<sub>8</sub>) was purchased from Sigma-Aldrich (Merck Millipore, Darmstadt, Germany). Tranilast (C<sub>18</sub>H<sub>17</sub>NO<sub>5</sub>) was obtained from Xiamen Kerda trade CO., LTD., Xiamen City, China.

### Experimental design

Rats were randomly allocated into four groups (7 rats each). Group I rats (normal control) received vehicle (0.5% CMC). Liver fibrosis was induced in the remaining 3 groups by intraperitoneal injection of TAA at a dose of 200 mg/kg b.wt for six weeks

(Ftahy *et al.* 2013). Group II served as (TAA group). Group III and IV rats were administered with tranilast at a dose of 150 and 300 mg/kg/day, respectively, for 6 weeks then given concomitantly with TAA injection (Abdelaziz *et al.* 2015).

### Serum biochemical analysis

At the end of the experiment (12 weeks), rats were anaesthetized with diethyl ether and blood samples were withdrawn from the retro-orbital venous plexus. Collected blood samples were allowed to stand for 10 min at room temperature then centrifuged at 4°C using cooling centrifuge (Laborzentrifugen, 2k15, Sigma, Germany) at 3000 r.p.m for 10 min. Sera were separated for assessment of levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), total bilirubin, total protein and albumin using commercially available kits according to the manufacturer's instructions as follows: Alanine Aminotransferase EIAab® kit, Aspartate Aminotransferase EIAab® kit, total bilirubin BioAssay Systems® kit, total protein Sigma-Aldrich® kit, and albumin Biovision® kit.

### Hepatic tissue biochemical analysis

Directly after blood sampling, rats were sacrificed by cervical dislocation under ether anesthesia. Liver tissues were collected, washed in normal saline and then divided into 2 parts. The first part was homogenized using MPW-120 homogenizer (Med instruments, Poland); the homogenate was centrifuged using a cooling centrifuge (Laborzentrifugen, 2k15, Sigma, Germany) at 3000 r.p.m for 10 min. and the supernatant was assessed for hepatic levels of reduced glutathione (GSH) (Ellman 1959), lipid peroxides as malondialdehyde (MDA) (Ruiz-Larrea *et al.* 1994), and superoxide dismutase (SOD) (Abdel-Rafei *et al.* 2016). The second part was used for histopathological examination

### Histopathological examination

Tissue specimens were collected from livers of the different experimental groups and were preserved in 10% buffered neutral formalin. Formalin fixed liver specimens were routinely dehydrated by graded series of alcohol, cleared in xylol and finally embedded in paraffin. Paraffin blocks were serially sectioned at 4-5 µm thickness and stained with H&E (Bancroft and Gamble, 2008).

### Statistical analysis

The results were expressed as mean ± SE of the mean. Data analysis was achieved by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test using software program GraphPad Prism (version 5.00). A value of P < 0.05 was considered statistically significant.

## RESULTS

### Effect on serum biochemical parameters

The data in table (1) revealed that, after 6 weeks of TAA injection, there was a significant elevation of serum levels of hepatic enzymes (ALT and AST) and total bilirubin with a significant reduction of total protein and albumin levels compared to normal control. In the present study, the reduction in levels of transaminases, total bilirubin and increased levels of total protein and albumin by pre-treatment of rats with tranilast were recorded, compared to TAA control group.

### Effect on reduced glutathione (GSH), malondialdehyde (MDA) and superoxide dismutase (SOD) levels in liver homogenate

The results showed that GSH content and SOD activity were significantly reduced and MDA level was significantly increased in TAA induced liver fibrosis rats, compared to normal control. Administration of tranilast for six weeks prior to liver fibrosis induction significantly brought back these oxidative stress markers toward the range of the corresponding normal control values and significantly different from the TAA group as shown in (figures 1 A,B,C).

### Histopathological examination of liver tissues

In regards to the histopathological examination, different liver sections of negative control rats revealed normal histological hepatic structure (Figure 2 A) while, examination of the livers of TAA rats revealed severe tissue alterations as marked hepatocellular swelling with vacuolar to ballooning degeneration and cytoplasmic reticulation. Some hepatocytes showed karyocytomegaly, others appeared with pyknotic nuclei, while some others appeared necrotic. The portal areas in those livers showed congested blood vessels, hyperplastic bile duct with multiple newly formed bile ductules as well as fibrous proliferation with its extension toward the parenchyma.

The later extension resulted in marked portal to portal fibrosis and pseudolobulation of the hepatic parenchyma. The hepatic cells inside the lobules showed vacuolar degeneration with cytoplasmic reticulation and pyknotic nuclei, many necrotic cells

appeared homogenous eosinophilic masses with the appearance of multiple apoptotic bodies (Figure 2, B-F). Some livers of tranilast administrated rats showed very fine strands of fibrous tissue extending from the portal areas toward the parenchyma with good protection of the hepatic parenchymal cells as well as marked apoptosis. The portal areas showed mild portal fibrosis, few inflammatory cells with the surrounding many apoptotic cells (Figure 3, A-D).

Table 1: Effect of pretreatment with tranilast (150 and 300 mg/kg) for 6 weeks on serum biochemical parameters in control healthy and TAA liver intoxicated rats.

	Normal control	TAA group	Tranilast 150 mg/kg	Tranilast 300 mg/kg
<b>AST (U/L)</b>	21.40 ± 1.00 <sup>b</sup>	126.20 ± 4.1 <sup>a</sup>	53.00 ± 2.00 <sup>ab</sup>	36.40 ± 1.00 <sup>ab</sup>
<b>ALT (U/L)</b>	19.40 ± 1.10 <sup>b</sup>	117.10 ± 3.4 <sup>a</sup>	43.10 ± 2.00 <sup>ab</sup>	29.90 ± 0.30 <sup>ab</sup>
<b>Total Bilirubin (mg/dl)</b>	0.29 ± 0.01 <sup>b</sup>	1.85 ± 0.03 <sup>a</sup>	0.87 ± 0.01 <sup>ab</sup>	0.59 ± 0.02 <sup>ab</sup>
<b>Total Protein (g/dl)</b>	7.52 ± 0.16 <sup>b</sup>	5.08 ± 0.07 <sup>a</sup>	6.28 ± 0.08 <sup>ab</sup>	6.80 ± 0.03 <sup>ab</sup>
<b>Albumin (g/dl)</b>	4.79 ± 0.08 <sup>b</sup>	2.19 ± 0.03 <sup>a</sup>	3.34 ± 0.02 <sup>ab</sup>	3.80 ± 0.04 <sup>ab</sup>

N = 7; Values are expressed as mean ± SEM

a = significant at P<0.05 when compared with control group

b=significant at P<0.05 when compared with TAA group

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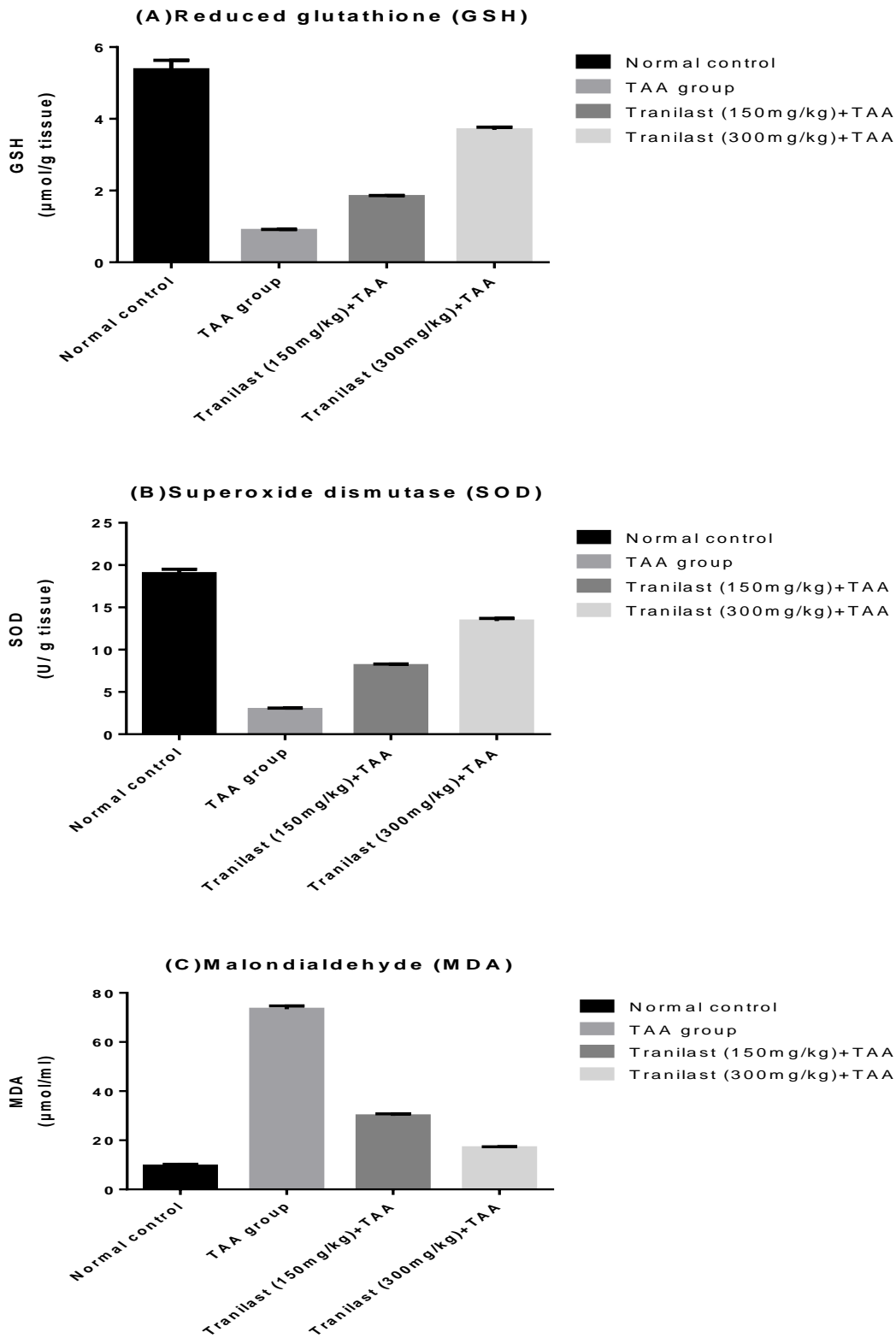


Fig.1. Effect of oral administration of tranilast for 6 weeks before liver fibrosis induction on GSH, SOD and MDA in liver tissue of TAA group.



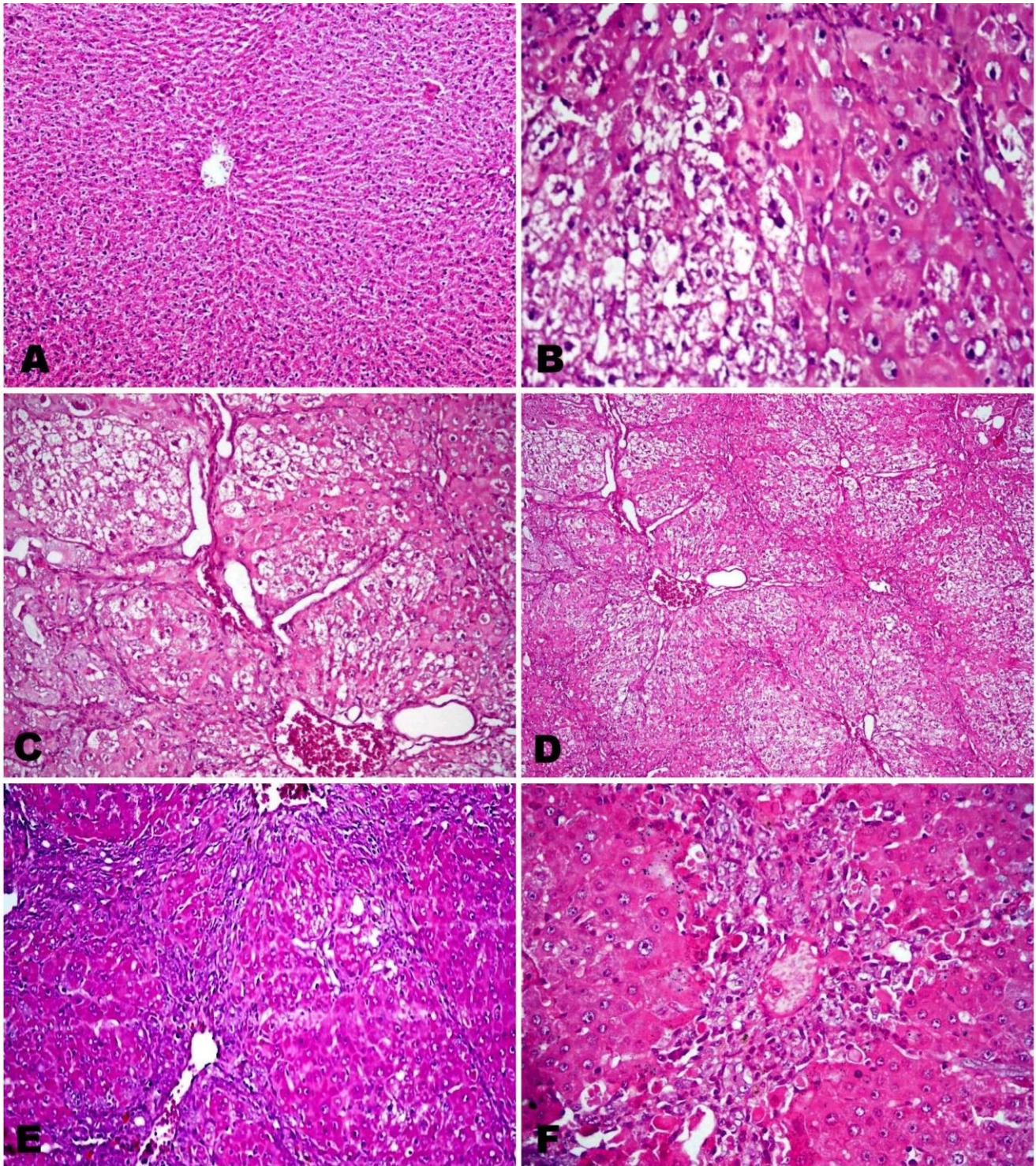


Fig.2. Liver of normal control (A). Liver of thioacetamide administrated rat showing; hepatocellular swelling with vacuolar to ballooning degeneration and cytoplasmic reticulation (B), portal triads showing congested blood vessels, proliferation of bile duct epithelium with multiple newly formed bile ductules as well as fibrous proliferation with its extension peripherally (C), marked portal to portal fibrosis and pseudolobulation (PS) of the hepatic parenchyma (D), many necrotic cells appeared homogenous eosinophilic masses (E), the fibrous septa of the pseudolobulation contained newly formed bile ductules and mononuclear inflammatory cells (F). (H&E, X400, 200, and 100).



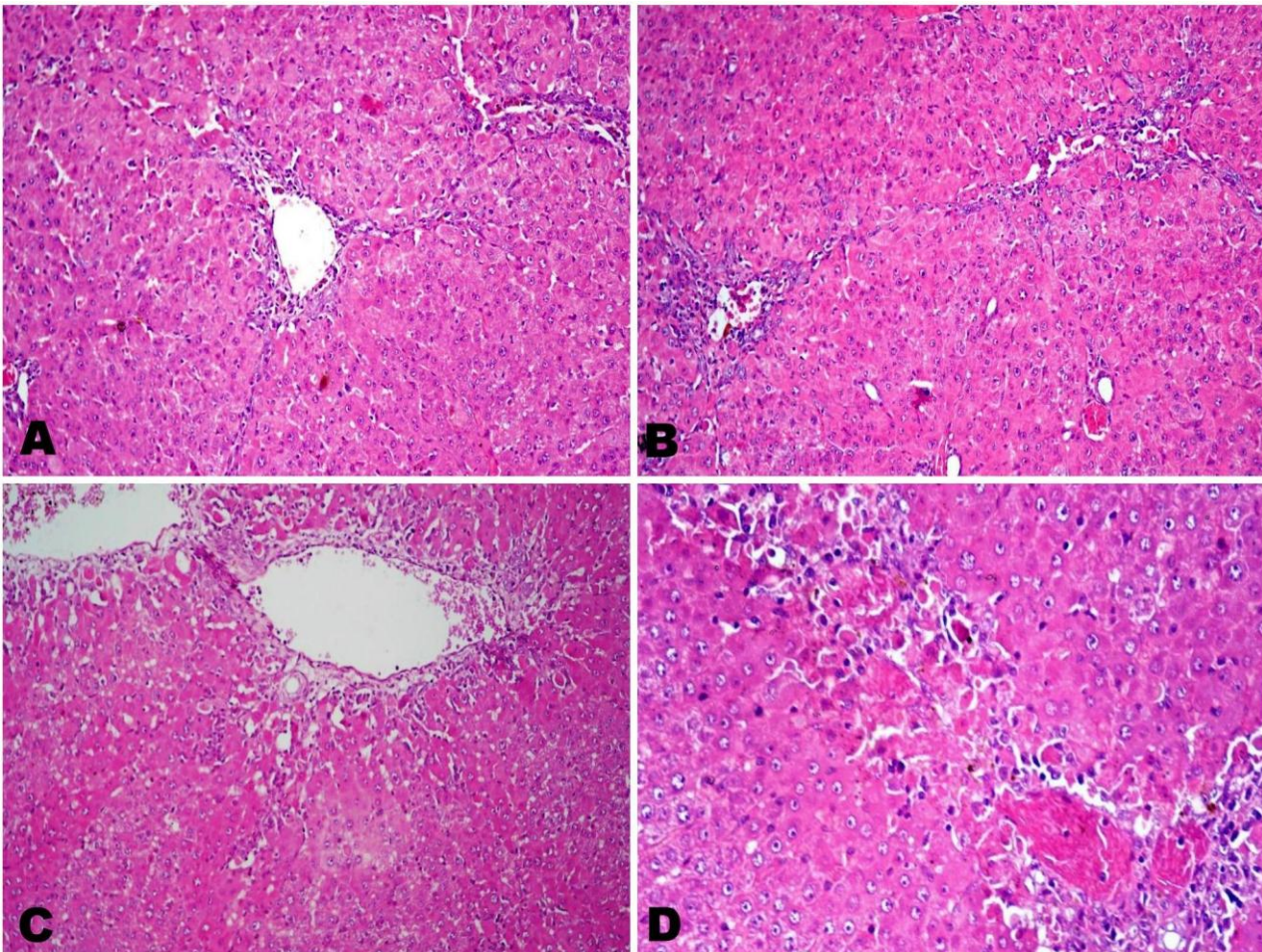


Fig.3. Liver of thioacetamide and tranilast administrated rat showing very fine strands of fibrous tissue (arrow) extending from portal areas toward the parenchyma (A), erasing of fibrotic reaction, near to the normal appearance of the hepatic parenchymal cells, with mild vacuolation (B), portal area of liver of thioacetamide and tranilast administrated rat showing mild portal fibrosis, few inflammatory cells with the surrounding many apoptotic cells (C), good protection of the parenchymal cells with marked apoptosis (D) (H&E, X400, 200, and 100).

## DISCUSSION

In the current study liver fibrosis was induced by i.p. injection of TAA which significantly impaired hepatic integrity, excretory and synthetic functions, with a significant increase in serum liver enzymes, total bilirubin, hepatic oxidative stress markers as well as a significant decrease in serum total protein, albumin, hepatic GSH content and SOD activity. The aim of this study was to investigate the potential hepatoprotective and antioxidant activities of tranilast on liver fibrosis produced after TAA-administration to male albino Wistar rats.

Results showed that administration of TAA significantly elevated the levels of ALT, AST and total bilirubin, suggesting their leakage from injured hepatocytes into the blood, which agrees with the previously reported results (Develi-Is *et al.* 2013). The

current results showed a significant reduction in serum total protein and albumin levels following TAA injections.

These results were in agreement with (Salama *et al.* 2013, Hessin *et al.* 2015). Hypoproteinemia, observed in this study perhaps occur due to inflammatory reaction or might be due to perturbed protein biosynthesis in the cirrhotic liver as demonstrated by (Alshawsh *et al.* 2011). In the present study, the reduction in levels of transaminases, and total bilirubin while, the increase in total protein and albumin levels by treatment with tranilast is an indication of repair of hepatic tissue damage caused by TAA as reported by (Said *et al.* 2012). This was also confirmed by the results of histological examination, as evidenced by a decrease in the incidence and severity of pathological hepatic lesions.

GSH and SOD are considered as the first line of the cellular antioxidant defense system. In the present study, induction of liver fibrosis through TAA caused marked oxidative stress in rat liver which was evidenced by a significant increase in the content of lipid peroxidation end product, malondialdehyde (MDA) combined with a significant decline in GSH content and SOD activity.

Obtained results are in agreement with those of other studies (Kaur *et al.* 2017), who reported that low concentration of GSH and SOD has been implicated in TAA induced liver fibrosis. (Chen *et al.* 2015) reported that increased level of lipid peroxidation in liver after TAA injection is attributed to free radical-mediated lipid peroxidation, leading to tissue injury, mitochondrial dysfunction, and failure of the antioxidant defense mechanism to prevent the formation of excess free radicals.

In the current study, pretreatment with tranilast for 6 weeks followed by liver fibrosis induction exhibited a protective effect against TAA. This protective effect appeared in the form of significant elevation of liver GSH and SOD with significant depletion of liver MDA compared to TAA group. These results are in agreement with (Said *et al.* 2016) who found that tranilast administration significantly decreased MDA content in lung and kidney homogenates, suggesting decreased lipid peroxidation, generation of ROS and a positive influence on the host antioxidant defenses. Moreover, (Miyachi *et al.* 1987) reported that tranilast significantly reduced all ROS levels and this antioxidant effect was related to its ability to scavenge ROS through direct inhibition of nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase) and/or xanthine oxidase activity.

## CONCLUSION

The present findings suggest that pre-treatment with tranilast could be more useful for the prevention of hepatic fibrosis induced by TAA in rats.

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