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## Original Article

# Effect of goat milk on hepatotoxicity induced by antitubercular drugs in rats



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## ABSTRACT

Aim of the present study was to assess the hepatoprotective activity of goat milk on antitubercular drug-induced hepatotoxicity in rats. Hepatotoxicity was induced in rats using a combination of isoniazid, rifampicin, and pyrazinamide given orally as a suspension for 30 days. Treatment groups received goat milk along with antitubercular drugs. Liver damage was assessed using biochemical and histological parameters. Administration of goat milk (20 mL/kg) along with antitubercular drugs (Group III) reversed the levels of serum alanine aminotransferase ( $82 \pm 25.1$  vs.  $128.8 \pm 8.9$  units/L) and aspartate aminotransferase ( $174.7 \pm 31.5$  vs.  $296.4 \pm 56.4$  units/L,  $p < 0.01$ ) compared with antitubercular drug treatment Group II. There was a significant decrease in serum alanine aminotransferase ( $41.8 \pm 4.1$  vs.  $128.8 \pm 8.9$  units/L,  $p < 0.01$ ) and aspartate aminotransferase ( $128.8 \pm 8.54$  vs.  $296.4 \pm 56.4$  units/L,  $p < 0.001$ ) levels in Group IV (goat milk 40 mL/kg) compared with antitubercular drug treatment Group II. Goat milk (20 mL/kg and 40 mL/kg) was effective in reversing the rise in malondialdehyde level compared with the antitubercular drug suspension groups ( $58.5 \pm 2$  vs.  $89.88 \pm 2.42$   $\mu\text{mol/mL}$  of tissue homogenate,  $p < 0.001$  and  $69.7 \pm 0.78$  vs.  $89.88 \pm 2.42$   $\mu\text{mol/mL}$  of tissue homogenate,  $p < 0.001$ , respectively). Similarly, both doses of milk significantly prevented a fall in superoxide dismutase level ( $6.23 \pm 0.29$  vs.  $3.1 \pm 0.288$  units/mL,  $p < 0.001$  and  $7.8 \pm 0.392$  vs.  $3.1 \pm 0.288$  units/mL,  $p < 0.001$ ) compared with the group receiving antitubercular drugs alone. Histological examination indicated that goat milk reduced inflammation and necrotic changes in hepatocytes in the treatment groups. The results indicated that goat milk prevented the antitubercular drug-induced hepatotoxicity and is an effective hepatoprotective agent.

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## 1. Introduction

Antitubercular drug-induced hepatotoxicity is one of the most challenging clinical problems. It is the main cause of interruption during a tuberculosis treatment course and may lead to hospitalization or life threatening events [1,2]. Antitubercular drug-induced hepatotoxicity has a wide spectrum of presentations, ranging from an asymptomatic mild rise in liver biochemical tests to acute hepatitis and acute liver failure. It is reported to be mediated through oxidative stress, which leads to lipid peroxidation and an alteration in antioxidant levels in the body [3]. Lipid peroxidation results in cell damage due to oxidative degradation of lipids present in cell membranes. In response to deleterious effects of free radical-induced lipid peroxidation, cells activate antioxidant defense mechanisms in which superoxide dismutase and reduced glutathione act synergistically to detoxify the effects of lipid peroxidation [3]. Therefore, it is suggested that the agents which reduce the lipid peroxide content in tissue and increase the intracellular antioxidant defenses may have protective effects on the liver in people taking antitubercular treatment. Currently very few reliable liver-protective drugs are available in the allopathic armamentarium. Their effects are unsatisfactory and they add to the pill burden. Management of drug-induced hepatotoxicity is still a challenge to modern medicine. Therefore, there is a strict need to screen herbal products and nutraceuticals which can be taken by the patients as food during the treatment of tuberculosis.

Goat milk is a food of high nutritional value as it is rich in various physiologically functional components, including proteins, vitamins (such as vitamins E and C), flavonoids, and carotenoids with antioxidant properties [4–7]. Goat milk is considered to possess high antioxidant activity that resists oxidative stability and highly protects consumers from exposure to oxidative stress [8]. It has been reported that goat milk consumption potentiates liver divalent metal transporter 1 expression thereby enhancing Fe metabolism and storage indicating its potential in anemia [9]. Furthermore, few studies have also demonstrated its anti-inflammatory and antioxidant properties which indicate that goat milk may possess hepatoprotective activity [10–13]. Goat milk is easily available, easy to digest, and can be taken as food during drug treatment of tuberculosis. The hepatoprotective activity of goat milk in antitubercular drug-induced hepatotoxicity has not yet been investigated. The rationale of the present study was to explore the effect of goat milk on antitubercular drug-induced hepatotoxicity in rats.

## 2. Materials and methods

Healthy adult albino rats of either sex of Wistar strain weighing 200–300 g were used after approval of the Institutional Ethics Committee. They were housed in standard laboratory conditions at  $25 \pm 2^\circ\text{C}$  with a 12 hour light/dark cycle. Animals were given free access to a rat chow diet and water *ad libitum*. Before conducting experiments, animals were acclimatized to laboratory conditions for 7 days.

### 2.1. Induction of hepatotoxicity

Experimental antitubercular drug-induced hepatotoxicity was produced by administration of isoniazid, rifampicin, and pyrazinamide (H+R+Z) suspension daily orally for 30 days. The doses of antitubercular drugs (H: 27 mg/kg, R: 54 mg/kg, Z: 135 mg/kg/d; Kwality Pharmaceuticals Pvt. Ltd., Amritsar, Punjab, India) were extrapolated from daily human doses using a conversion table [14]. Gum acacia was used as a suspending agent. In the vehicle control group, 2% gum acacia (10 mL/kg) was administered to the rats. For induction of hepatotoxicity, a combination of calculated doses of H, R, and Z suspended in 2% gum acacia were administered to rats orally.

The animals were divided into five groups ( $n=6$ ). The groups were treated as follows:

Group I: vehicle control, i.e., 2% gum acacia orally for 30 days.

Group II: (H+R+Z) suspension orally for 30 days.

Group III: (H+R+Z) suspension + goat milk 20 mL/kg orally for 30 days.

Group IV: (H+R+Z) suspension + goat milk 40 mL/kg orally for 30 days.

Group V: (H+R+Z) + silymarin (standard) 50 mg/kg orally for 30 days.

Blood samples of animals from all the groups were taken on 30<sup>th</sup> day by cardiac puncture under ether anesthesia. After sacrificing the animals, livers were removed for histopathological examination and biochemical parameters were investigated.

### 2.2. Assessment of liver damage

#### 2.2.1. Gross morphological assessment

Livers were excised from the rats and were rinsed with normal saline. They were weighed after blotting with filter paper. The liver indices were calculated as a percentage of the body weight [15]. A gross morphological assessment was then performed for hepatic lesions based on the qualitative procedure developed by Mitchell et al [16]. They were graded as follows: 0, no lesions; 1+, minimal damage; 2+, mild to moderate damage; and 3+, severe damage.

Each liver was excised into two pieces. The right lobe was immersed in isotonic 10% buffered formalin fixative for histological assessment while the left lobe was rinsed using cold physiological saline and then homogenized with cool phosphate buffer saline for malondialdehyde and superoxide dismutase (SOD) assays.

#### 2.2.2. Histopathological examination

All the groups were subjected to histological examination. Microscopic examination was done by a qualified pathologist using hematoxylin and eosin staining in a blinded fashion.

#### 2.2.3. Biochemical estimations

Serum alanine aminotransferase (ALT) and serum aspartate aminotransferase (AST) were estimated using the Reitman and Frankel method [17]. Tissue malondialdehyde and SOD

activity were estimated by the biuret method, Kakkar et al [18] method, and Ohkawa et al [19] method.

### 2.3. Statistical analysis

The values were expressed as mean  $\pm$  standard error of the mean. One-way analysis of variance, followed by an appropriate post hoc test (Tukey's test) were used for analysis.

## 3. Results

The mean body and liver weights noted in all groups are shown in Table 1. One rat from Group I and Group II died at the beginning of the experiment for unknown reasons. There was a statistically significant reduction in the percentage change of the mean body weight in the H+R+Z suspension group compared with the vehicle control group ( $-11.26$  vs.  $7.97$ ,  $p < 0.05$ ). Treatment with goat milk and silymarin (Table 1) caused a marked increase in the body weight compared with the H+R+Z suspension group ( $12.5$  vs.  $-11.26$ ,  $p < 0.05$ ;  $10.37$  vs.  $-11.26$ ,  $p < 0.05$ ; and  $6.45$  vs.  $-11.26$ ,  $p < 0.05$ , respectively).

### 3.1. Gross morphological assessment

The morphological index in Group II was significantly high compared with Group I ( $2.83$  vs.  $0$ ,  $p < 0.001$ ). Group III revealed a significant reduction in morphological score compared with Group II ( $0.83$  vs.  $2.83$ ,  $p < 0.001$ ). Group IV showed a significant decrease in morphological score compared with Group II ( $0.5$  vs.  $2.83$ ,  $p < 0.001$ ). Group V also showed a decrease in morphological score but it was still higher than Group III and Group IV (Table 2).

### 3.2. Histological examination

The histological examination of the liver tissue showed normal hepatic architecture in Group I (Figure 1). Administration of antitubercular drugs for 30 days to Group II produced hepatocellular damage indicated by necrosis with hepatocyte infiltration. Coadministration of goat milk (20 mL/kg) with antitubercular drugs reduced histological changes like inflammation and necrosis. In Group IV, after coadministration of goat milk (40 mL/kg) with antitubercular drugs

very scanty inflammation was observed. It maintained the normal histology of liver tissue and showed almost a reversal from the degenerative and necrotic changes caused by antitubercular drugs. The effects of goat milk were comparable to silymarin.

### 3.3. Biochemical estimations

#### 3.3.1. Serum ALT and AST

In the present study, Group II, which received antitubercular drugs for 30 days, showed a rise in serum ALT ( $128.8 \pm 8.9$  vs.  $50.75 \pm 3.40$  units/L,  $p < 0.01$ ) and AST ( $296.4 \pm 56.4$  vs.  $181.75 \pm 7.88$  units/L,  $p < 0.05$ ) compared with the control group. Administration of goat milk (20 mL/kg) along with antitubercular drugs reversed the levels of serum ALT ( $82 \pm 25.1$  vs.  $128.8 \pm 8.9$  units/L) and AST ( $174.7 \pm 31.5$  vs.  $296.4 \pm 56.4$  units/L,  $p < 0.01$ ) compared with the antitubercular drug treatment group. There was also significant decrease in serum ALT ( $41.8 \pm 4.1$  vs.  $128.8 \pm 8.9$  units/L,  $p < 0.01$ ) and AST levels ( $128.8 \pm 8.54$  vs.  $296.4 \pm 56.4$  units/L,  $p < 0.001$ ) in Group IV (goat milk 40 mL/kg) compared with the antitubercular drug treatment group, i.e., Group II. Moreover, the effects were comparable to Group V, i.e., the silymarin treated group (Table 2).

#### 3.3.2. Malondialdehyde and SOD activity

The lipid peroxidation activity of liver homogenate in Group II ( $89.88 \pm 2.42$   $\mu\text{mol/mL}$  of tissue homogenate) was significantly raised compared with Group I ( $60.1 \pm 3.24$   $\mu\text{mol/mL}$  of tissue homogenate;  $p < 0.001$ ). Lipid peroxidation reduced significantly in both the goat milk treated groups compared with the antitubercular drug treatment group ( $58.5 \pm 2$  vs.  $89.88 \pm 2.42$   $\mu\text{mol/mL}$  of tissue homogenate,  $p < 0.001$  and  $69.7 \pm 0.78$  vs.  $89.88 \pm 2.42$   $\mu\text{mol/mL}$  of tissue homogenate,  $p < 0.001$ ; Table 2). The reduction was higher in Group III ( $58.5 \pm 2.0$   $\mu\text{mol/mL}$  of tissue homogenate) than in Group IV ( $69.7 \pm 0.78$   $\mu\text{mol/mL}$  of tissue homogenate) and was similar to Group V ( $54.55 \pm 1.96$   $\mu\text{mol/mL}$  of tissue homogenate). Table 2 shows the effect of different pharmacological interventions on SOD activity in liver homogenate. SOD activity showed a significant decrease in Group II compared with the vehicle control group ( $3.1 \pm 0.28$  vs.  $8.15 \pm 0.99$  units/mL,  $p < 0.001$ ). Coadministration of goat milk with the antitubercular drugs significantly increased SOD activity in Group III and Group IV ( $6.23 \pm 0.29$  and  $7.8 \pm 0.39$  units/mL) compared with antitubercular drug treatment

**Table 1 – Body weight and liver weights of the rats in different groups.**

Serial no.	Experimental groups	Initial body weight (g)	Final body weight (g)	Percent change (%)	Liver weight (g)	Liver index (%)
1	Group I	326 $\pm$ 3.33	352 $\pm$ 9.69	7.97	9.76 $\pm$ 0.51	2.77
2	Group II	293 $\pm$ 14.7	260 $\pm$ 17.7	$-11.26^{a,*}$	11.65 $\pm$ 0.52	4.48 <sup>a,*</sup>
3	Group III	200 $\pm$ 3.33	225 $\pm$ 8.46	12.5 <sup>b,c,*</sup>	10.95 $\pm$ 0.32	4.86 <sup>a,*</sup>
4	Group IV	241 $\pm$ 8.33	266 $\pm$ 8.71	10.37 <sup>b,c,*</sup>	9.95 $\pm$ 0.35	3.74
5	Group V	310 $\pm$ 8.47	330 $\pm$ 7.71	6.45 <sup>b,*</sup>	9.84 $\pm$ 0.69	2.98 <sup>b,*</sup>

Liver index was calculated as (liver weight/body weight  $\times$  100%). The values were expressed as mean  $\pm$  standard error of the mean. The data was analyzed using one-way analysis of variance followed by Tukey honest significant difference test.

\*  $p < 0.05$ .

<sup>a</sup> When compared with vehicle control group.

<sup>b</sup> When compared with the antituberculosis drug group.

<sup>c</sup> When compared with the silymarin group.

**Table 2 – Comparison of different parameters measured in experimental groups of rats.**

Biochemical parameters	Group I	Group II	Group III	Group IV	Group V
ALT (units/L)	50.75 ± 3.4	128.8 ± 8.9 <sup>a,**</sup>	82 ± 25.1	41.8 ± 4.1 <sup>b,**</sup>	38 ± 1.9 <sup>c,***</sup>
AST (units/L)	181.75 ± 7.88	296.4 ± 56.4 <sup>a,*</sup>	174.7 ± 31.5 <sup>b,**</sup>	128.8 ± 8.54 <sup>c,***</sup>	121.2 ± 8.61 <sup>c,***</sup>
MDA (μmol/mL of tissue homogenate)	60.1 ± 3.24	89.88 ± 2.42 <sup>a,***</sup>	58.5 ± 2 <sup>b,c,**,***</sup>	69.7 ± 0.78 <sup>c,***</sup>	54.55 ± 1.96 <sup>c,***</sup>
SOD (units/mL)	8.15 ± 0.99	3.1 ± 0.28 <sup>a,***</sup>	6.23 ± 0.29 <sup>c,***</sup>	7.8 ± 0.39 <sup>c,***</sup>	6.62 ± 0.55 <sup>c,***</sup>
MI (0–3)	0 (0–0)	2.83 (2–3) <sup>a,***</sup>	0.83 (0–2) <sup>c,***</sup>	0.5 (0–2) <sup>c,***</sup>	0.16 (0–1) <sup>c,***</sup>

The values were expressed as mean ± standard error of the mean. The data was analyzed using one-way analysis of variance followed by Tukey honest significant difference test.

\*  $p < 0.05$ .

\*\*  $p < 0.01$ .

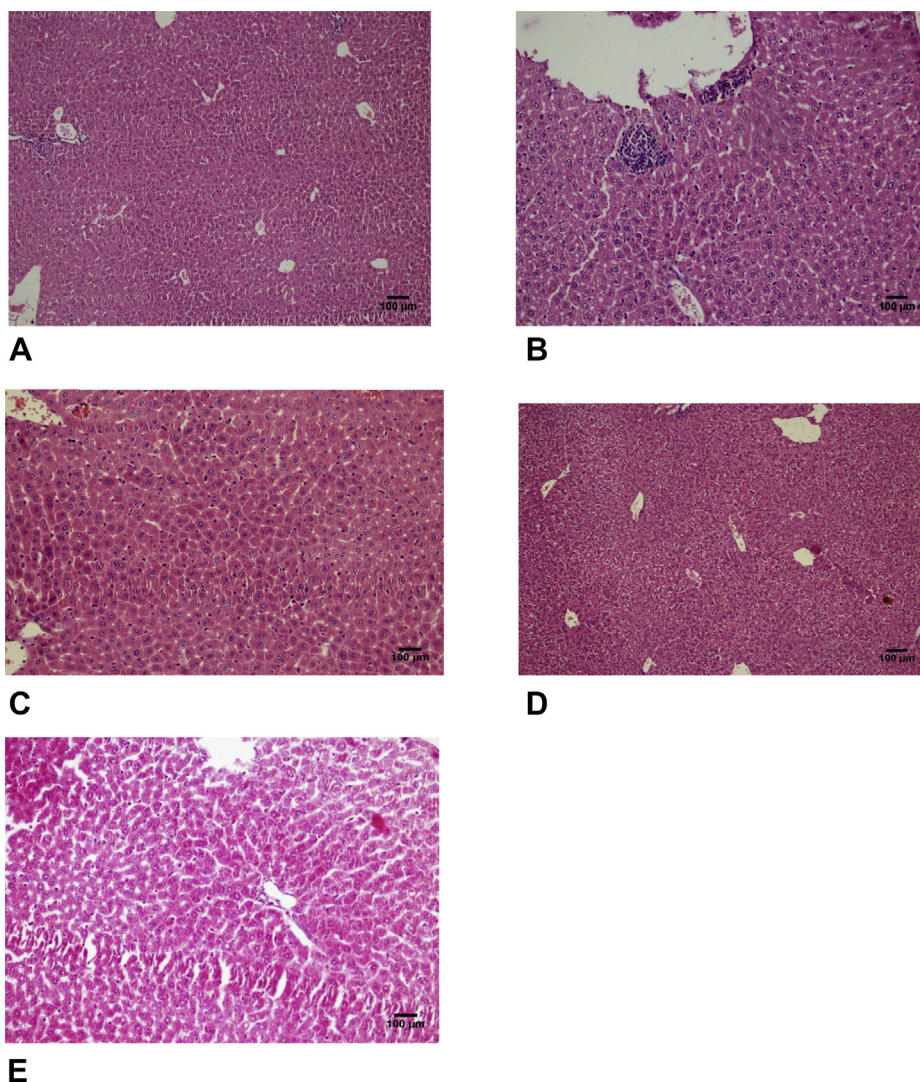
\*\*\*  $p < 0.001$ .

ALT = alanine aminotransferase; AST = aspartate aminotransferase; MDA = malondialdehyde; MI = morphological index; SOD = superoxide dismutase.

<sup>a</sup> When compared with the vehicle control group.

<sup>b</sup> When compared with the antituberculosis drug group.

<sup>c</sup> When compared with the silymarin group.



**Figure 1 – Photomicrograph of the liver tissue. (A) Vehicle control group: showing the normal histology of liver tissue; (b) Isoniazid, rifampicin, and pyrazinamide (H+R+Z) group: showing necrosis with hepatocyte infiltration; c) H+R+Z + goat milk (20 mL/kg): showing normal hepatocytes and decreased necrosis; (D) H+R+Z + goat milk (40 mL/kg): showing almost recovery to normal histology; (e) H+R+Z + silymarin (50 mg/kg): showing normal hepatocytes (hematoxylin and eosin stain, ×50).**

Group II ( $3.1 \pm 0.28$  units/mL,  $p < 0.001$ ). However, there was no statistically significant difference in both groups. Similarly, the effects observed in Group III and Group IV were comparable to Group V ( $6.23 \pm 0.29$  vs.  $6.62 \pm 0.55$  units/mL and  $7.8 \pm 0.39$  vs.  $6.62 \pm 0.55$  units/mL).

#### 4. Discussion

Antitubercular drug-induced hepatotoxicity is mediated through oxidative stress and free radical damage to hepatocytes. Due to the leakage of enzymes from damaged hepatocytes into vascular compartments, serum AST and ALT levels increase in hepatic damage. Treatment with antitubercular drugs increases malondialdehyde concentration which leads to lipid peroxidation. Lipid peroxidation is an important mechanism of hepatotoxicity and plays a key role in the pathogenesis of several human diseases. SOD is an antioxidant enzyme which catalyzes the dismutation of the superoxide ( $O_2^-$ ) radical into either ordinary molecular oxygen or hydrogen peroxide. The decrease in SOD activity could be due to the increased production of reactive oxygen species as evident from the increased lipid peroxidation levels due to antitubercular drug treatment [20,21]. Liver biopsy is the most reliable index of liver damage. Parameters like degeneration, necrosis, and fibrosis indicate liver damage while a reduction in these parameters suggests hepatoprotection. Administration of antitubercular drugs also results in inflammation, degeneration, and necrotic changes in rat liver.

In the present study, concurrent administration of goat milk (20 mL/kg and 40 mL/kg) along with antitubercular drugs was found to be effective in reducing the severity of hepatotoxicity, and the effects were similar to the reference drug silymarin. Administration of goat milk reduced inflammation and necrotic changes. It significantly prevented a rise in levels of serum ALT, AST, and tissue malondialdehyde. Similarly, goat milk significantly prevented a fall in SOD compared with the group receiving antitubercular drugs alone. The beneficial results of goat milk in both groups were equivalent to the reference drug silymarin. However, the difference between the two groups receiving goat milk was not statistically significant. Although the higher dose (40 mL/kg) was safe and tolerable, no additional beneficial effects were observed. These findings suggest that goat milk possesses the potential of an effective and promising option for the treatment of hepatotoxicity.

#### 5. Conclusion

The present study showed that goat milk prevents antitubercular drug-induced hepatotoxicity. Therefore, it may be concluded that goat milk possesses hepatoprotective activity and can be considered as an effective hepatoprotective agent.

#### Conflicts of interest

The authors have no financial or proprietary interest in any of the products mentioned in this manuscript.

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