ORIGINAL ARTICLE

Protective potential of royal jelly against hepatotoxicity

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Abstract

Objective: The objective is to study the hepatoprotective effect of royal jelly (RJ) against antitubercular drug-induced hepatotoxicity in rats. **Materials and Methods:** A total of 30 Wistar albino rats of either sex were divided into five groups (n = 6): Group I - Vehicle control (gum acacia, 1%), Group II - combination of isoniazid (H), rifampicin (R), and pyrazinamide (Z) (H + R + Z suspension – 27 + 54 + 135 mg/kg), Group III - H + R + Z suspension (27 + 54 + 135 mg/kg) + RJ (50 mg/kg), Group IV - H + R + Z suspension (27 + 54 + 135 mg/kg) + RJ (100 mg/kg), and Group V - H + R + Z suspension (27 + 54 + 135 mg/kg) + silymarin (50 mg/kg). The animals were treated for 30 days with H + R + Z suspension and the test group was concomitantly administered RJ. After 30 days, the animals were sacrificed for the investigation of morphological, histopathological, and biochemical parameters. **Results:** Antitubercular drug-induced hepatotoxicity was successfully reproduced. Concurrent administration of RJ along with antitubercular drugs significantly prevented the rise in levels of serum alanine aminotransferase, serum aspartate aminotransferase, and tissue malondialdehyde. Administration of RJ reduced inflammation, degeneration, necrotic changes in hepatocytes and significantly prevented fall in superoxide dismutase as compared to the group receiving antitubercular drugs alone. **Conclusion:** RJ is an effective hepatoprotective agent and prevented the antitubercular drug-induced hepatotoxicity.

Key words: Hepatoprotection, hepatotoxicity, isoniazid, pyrazinamide, rifampicin, royal jelly

INTRODUCTION

Tuberculosis remains a huge public concern worldwide.[1] About one-third of the world's population is infected with this and almost three million people per year are killed by this disease.[2] *Mycobacterium tuberculosis* is the most common cause of tuberculosis as well as tuberculosis associated morbidity and mortality. Tuberculosis is a curable disease and can be properly treated with antitubercular drugs.[3] However, these antitubercular drugs are responsible for some frequent adverse effects such as skin reactions, hepatotoxic, gastrointestinal, and neurological disorders.[4] The first-line treatment currently recommended for tuberculosis is a regimen of isoniazid (H), rifampicin (R), pyrazinamide (Z), and ethambutol (E) for 2 months, followed by H + R + E for 4 months. One of the most frequent and serious adverse effects of antitubercular drugs is hepatotoxicity and any compromise in treatment regimens may reduce treatment effectiveness. Among the first-line quadruple therapy drugs: H, R, and Z are mainly metabolized by the liver, and thus are potentially hepatotoxic.[1] Individually, these drugs are reported to cause hepatotoxicity, but the risk is heightened when these drugs are used in combination.[5] Antitubercular drugs mainly induce hepatic cytochrome P450 (CYP450) enzymes, for example, H induces CYP2E1 while R is a potent inducer of CYP2E1 and CYP3A4. CYP2E1 is actively involved in hepatotoxicity. Thus, multi-drug resistance and increased drug disposition are most likely to becaused by the induction of CYP450 enzymes.[6] Antitubercular drug therapy leads to serious adverse complications, that is, hepatotoxicity which ranges from asymptomatic elevation of serum transaminase to acute hepatic failure.[7] Currently, antitubercular drug-induced

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hepatotoxicity is one of the most challenging clinical problem. Moreover, it is the principal cause of treatment interruption that may cause hospitalization and life-threatening events.[8] Drug-induced hepatotoxicity is mediated through oxidative stress, lipid peroxidation, and antioxidant alteration.[9] Therefore, it is proposed that the agents which reduce the lipid peroxidation level in tissue and elevate in tracellular antioxidant defenses may have hepatoprotective effect in people taking antitubercular drugs. To solve this problem, use of silymarin is suggested in clinical practice. A few pre-clinical studies have reported some herbal hepatoprotectives also, but as their effects are far from satisfactory, a continuous search is going on for newer hepatoprotective agents.

Royal jelly (RJ) is secreted by the mandibular and hypopharyngeal glands of young workers of honey bees.[10] It is a milky white to yellowish creamy acidic material of slightly pungent odor, and taste. Fresh RJ chemically comprises of water (50-70%), carbohydrates (7-18%), fatty acids and lipids (3-8%), proteins (9-18%), mineral salts (1.5%), and small amount of vitamins and polyphenols.[11] Because of its natural source, that is, honeybees, it is supposed to have allergenic potential. To reduce this allergic potential, it is treated enzymatically.[12] RJ is a functional food item that possesses various health promoting, medicinal and cosmetic properties. It has been demonstrated to possess numerous pharmacological activities in experimental animals which include vasodilatory, hypotensive, disinfectant, antitumor, and antihypercholesterolemic activities. RJ has been reported to modulate tissue injury and oxidative stress in rats.[13,14] A study has reported the antioxidant property of RJ by preparing enzyme hydrolysates which are having much higher antioxidant activity.[15] Further anti-inflammatory dose-dependent effect of RJ against formalin in rat hind paw was observed.[16] RJ has been known to exhibit DNA protection effect in experimental animals.[17] It has been reported to contain a glycoprotein (57 kDa), which is considered to initiate hepatic tissue regeneration and hepatocyte development.[18] However, the hepatoprotective activity of RJ in antitubercular drug-induced hepatotoxicity has not been investigated yet. Hence, the present study was carried out to explore the effect of RJ on antitubercular drug-induced hepatotoxicity in rats.

MATERIALS AND METHODS

Experimental animals

Healthy Wistar albino rats of either sex weighing between 180-270 g were used after the approval of the institutional animal ethics committee. They were housed in standard laboratory conditions at 25°C ± 2°C and 12 h light and dark cycle. Animals were given free access to rat chow diet and water *ad libitum*. Before conducting experiments, animals were acclimatized to laboratory conditions for 7 days.

Induction of hepatotoxicity

Experimental antitubercular drug-induced hepatotoxicity was produced by administration of isoniazid (H), rifampicin (R), and pyrazinamide (Z) suspension daily orally for 30 days. The animals were sacrificed after 30 days. The doses of antitubercular drugs (H-27 mg/kg, R-54 mg/kg, Z-135 mg/kg/day; Kwality Pharmaceuticals Pvt., Ltd., Amritsar) were extrapolated from daily human dose using the conversion table based on body surface area.[10] RJ was obtained from Yamada Bee Company, Inc. Institute for Bee Products and Health Science, Okayama, Japan. In total, 30 animals were included in the study. Animals were divided into total five groups (n = 6). The groups were treated as follows:

Group I: Vehicle control, that is, 1% gum acacia orally daily for 30 days.

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

Group III: (H + R + Z) suspension + RJ (50 mg/kg) orally daily for 30 days.

Group IV: (H + R + Z) suspension + RJ (100 mg/kg) orally daily for 30 days.

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

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

Assessment of liver damage

Gross morphological assessment

Livers were excised from the rats and were rinsed with normal saline. Then, gross morphological assessment was done for hepatic lesions based on the qualitative procedure developed by Mitchell *et al*.[19] They were graded as follows:

- 0 - No lesions
- 1 + - Minimal damage
- 2 + - Mild-to-moderate damage
- 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.

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.

Biochemical estimations

Serum alanine aminotransferase (ALT) and serum aspartate aminotransferase (AST) were estimated by Reitman and Frankel Method.[20] Tissue malondialdehyde and SOD activity were estimated by Kakkar *et al*. method and Ohkawa *et al*. method, respectively.[21,22]

Statistical analysis

The values were expressed as mean ± standard error mean. One-way analysis of variance (ANOVA) followed by appropriate *post hoc* test (Tukey's test) were used for analysis. $P < 0.05$ was considered as statistically significant.

RESULTS

The mean body weight noted in all groups is shown in Table 1. There was a significant reduction in the body weight in H+R+Z suspension-treated rats when compared to corresponding control rats. Treatment with RJ and silymarin caused a significant increase in body weight.

Gross morphological assessment

The severity of liver necrosis was assessed qualitatively following inspection of the liver gross morphology (Table 2) gross morphological scores indicated that RJ significantly decreased the degeneration and tissue necrosis in liver as compared to Group II.

Histopathological analysis

In the histopathological studies, the liver sections of rat treated with vehicle showed normal hepatic architecture [Figure 1a]. Administration of antitubercular drugs for 30 days to group II produced changes such as inflammation, degeneration, and necrosis which were visible on histological examination of rat livers [Figure 1b]. Coadministration of RJ along with antitubercular drugs reversed histological changes such as inflammation, degeneration, and necrosis [Figure 1c and d]. Its effect in reversing the cell damage and cell infiltration was comparable to silymarin (Figure 1e and Table 2).

Biochemical estimations

Serum ALT and aspartate aminotransferase

There was a significant increase in serum ALT, AST, and bilirubin in group II as compared to Group I. Treatment with

Table 1: Mean body weight of the rats in different groups

Experimental groups	Initial body weight (g)	Final body weight (g)	Percent change (%)
Group I	208±6.89	234.1±5.32	12.5
Group II	235±14.5	200.3±8.73	−14.8#
Group III	234±7.83	247.6±9.14	5.5*
Group IV	247±6.36	260±6.34	9.7*
Group V	229±4.9	254.5±4.77	10.9*

The values were expressed as mean±SEM. [*,#$P<0.05$)], #when compared with healthy control, *when compared with antitubercular drug group. The data were analyzed using one-way ANOVA followed by Tukey's HSD test. SEM: Standard error mean, HSD: Honest significant difference

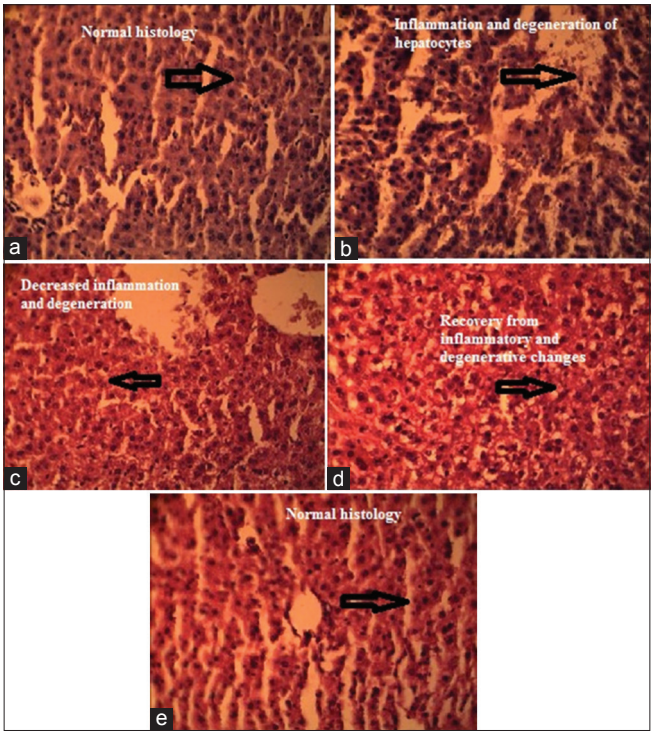

Figure 1: Photomicrograph of the liver tissue of: (a) Vehicle control: Showing the normal histology of liver tissue, (b) H + R + Z group: Showing, inflammation in hepatocytes in the form of degeneration and inflammation, (c) H + R + Z + RJ (50 mg/kg) group: Showing recovery from, degenerative, and inflammatory changes, (d) H + R + Z + RJ (100 mg/kg) group: Shows decreased inflammation, degeneration, and necrosis, (e) H + R + Z + Silymarin group: Shows nearly normal architecture of the liver

RJ (50 mg/kg) along with antitubercular drugs in Group III significantly ($P < 0.05$) reversed the levels of serum ALT, AST, total protein, and serum bilirubin as compared to treatment Group II (Table 2). Treatment with RJ (100 mg/kg) in Group IV significantly reversed the levels of serum ALT ($P < 0.05$), AST ($P < 0.05$), and serum bilirubin ($P < 0.05$) as compared to Group II (Table 2). Treatment with silymarin in Group V also significantly reversed the levels of serum

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)	26.4±0.36	49.4±0.49$^{\#}$	36.6±0.96*	40.2±0.76*	34.6±1.73*$^{\varphi}$
AST (units/L)	119±1.86	287±3.73$^{\#}$	138±2.11*	167±2.69*$^{\delta}$	153±1.81*$^{\delta\varphi}$
MDA (µmol/ml of tissue homogenate)	58.7±2.08	86.2±1.94$^{\#}$	57.4±0.95*	61.0±1.84*	64.5±1.29*
SOD (units/ml)	7.3±0.32	2.8±0.21$^{\#}$	6.43±0.35*	7.0±0.21*	7.16±0.25*
MI (0-3)	0	3$^{\#}$	1	1	0*

The values were expressed as Mean±SEM. ($^{\#,*,\delta,\varphi}P<0.05$), $^{\#}$when compared with healthy control, *when compared with antitubercular drug group, $^{\delta}$when compared with RJ (50 mg/kg), $^{\varphi}$when compared with RJ (100 mg/kg). The data were analyzed using one-way ANOVA followed by Tukey's HSD test. ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, MDA: Malondialdehyde, SOD: Superoxide dismutase, MI: Morphological index, HSD: Honest significant difference

AST ($P < 0.05$), ALT ($P < 0.05$), and bilirubin ($P < 0.05$) as compared to Group II.

Malondialdehyde levels

The level of malondialdehyde in Group II (86.2 ± 1.94) was significantly higher ($P < 0.05$) than the Group I (58.7 ± 2.08). RJ administration at a dose of 50 mg/kg was significantly ($P < 0.05$) effective in reversing the rise in malondialdehyde level as compared to antitubercular drug-treatment group (Table 2). Similarly, RJ (100 mg/kg) and silymarin were found to significantly ($P < 0.05$) reverse malondialdehyde levels as compared to Group II.

SOD activity

Table 2 shows the effect of different pharmacological interventions on SOD levels in rats after 30 days of treatment. The levels of the antioxidant enzyme, SOD showed a significant decrease ($P < 0.05$) in Group II as compared to Group I. Coadministration of RJ (50 mg/kg as well as 100 mg/kg) along with the antitubercular drugs (Group III and Group IV) significantly ($P < 0.05$) increased the levels of SOD as compared to antitubercular drugs group (Group II). Similarly, silymarin along with antitubercular drugs significantly ($P < 0.05$) reversed the level of SOD as compared to antitubercular drugs group.

DISCUSSION

Currently used antitubercular therapeutic regimen includes combination of H, R, and Z which may lead to drug-induced hepatotoxicity, a potentially serious adverse effect. All these drugs are hepatotoxic individually and toxic effects are elevated in synergistic order, when given in combination. The antitubercular drug-induced hepatotoxicity is mediated through oxidative stress and free-radical damage to hepatocytes.[23] This oxidative injury leads to loss of structural integrity of the cells that may cause marked increase in the leakage of hepatocellular enzymes.[24] Further, decrease in antioxidant defences or elevation in freeradical production disturbs the prooxidant-antioxidant balance, leading to oxidative stress-induced cell death. Thus, surge in concentration of thiobarbituric acid reactive substances in Group II represents enhanced lipid peroxidation.[25] In the present study, administration of antitubercular drugs for 30 days significantly increased the levels of serum bilirubin, serum ALT, serum AST, and malondialdehyde while the levels of SOD dismutase were significantly reduced. These findings along with the histopathological changes depict the induction of hepatotoxicity.

Concurrent administration of RJ along antitubercular drugs significantly prevented the rise in levels of serum ALT, AST, bilirubin, and tissue malondialdehyde. Similarly, RJ significantly prevented fall in SOD as compared to group receiving antitubercular drugs alone. Administration of RJ reduced inflammation, degeneration, and necrotic changes. These results showed that RJ is an effective hepatoprotective agent and possesses the potential to prevent the antitubercular drug-induced hepatotoxicity. It is well-established fact now that drug-induced hepatotoxicity is mediated through oxidative stress, lipid peroxidation, and antioxidant alteration. Hence, on the basis of current findings, it may be suggested that RJ provides hepatoprotection by reducing the lipid peroxidation level in tissue and elevating intracellular antioxidant defenses. In other words, the protective potential of RJ is associated with its antioxidant property which is mainly due to the presence of polyphenolic compounds.

Since silymarin is a standard hepatoprotective agent in hepatotoxicity, the effect of RJ was compared with it also. However, the effects produced by RJ were not statistically different from silymarin. The results showed that the effects produced by RJ were at par with silymarin and it was equally effective.

CONCLUSION

The results suggest that RJ possesses hepatoprotective activity as demonstrated by significant attenuated antitubercular drug-induced hepatotoxicity. It should be investigated further as a promising option for the treatment of hepatotoxicity.

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