ORIGINAL ARTICLE

Comparative evaluation of manuka honey with honey in antitubercular drug-induced hepatotoxicity in rats

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Abstract

Objective: The objective of this study is to compare the effect of manuka honey with honey in hepatotoxicity induced by antitubercular drugs in rats. **Materials and Methods:** Hepatotoxicity was induced in rats by a combination of isoniazid, rifampicin, and pyrazinamide given orally as suspension for 30 days. Treatment groups received honey and manuka honey along with anti-tubercular drugs. Liver damage was assessed by biochemical and histological parameters. **Results:** Concurrent administration of manuka honey along with anti-tubercular drugs significantly prevented the rise in levels of serum alanine aminotransferase, serum aspartate aminotransferase, and tissue malondialdehyde. It reduced inflammation, degeneration, and necrotic changes in hepatocytes. Similarly, manuka honey significantly prevented fall in serum total protein and superoxide dismutase as compared to the group receiving anti-tubercular drugs alone. However, the effects produced by manuka honey were not statistically different from those of honey and silymarin. **Conclusion:** Manuka honey is effective as a hepatoprotective agent as it significantly prevented the hepatotoxic damage induced by antitubercular drugs in rats. However, the comparison between the effects produced by manuka honey and honey or silymarin showed that the difference was not statistically significant. Hence, contrary to the popular belief, it is only as good and effective as honey.

Key words: Antitubercular drug-induced hepatotoxicity, hepatoprotection, honey, manuka honey

INTRODUCTION

Drug-induced hepatotoxicity is an injury to the liver caused by drugs or herbal medicines ultimately leading to liver dysfunction. As liver plays an important role in the metabolism and elimination of drugs, it is very susceptible to the toxicity induced by them.[1] Drug-induced liver injury is a leading health problem globally. Approximately, more than 1000 drugs have been reported to induce liver disease.[2] Currently used first-line antitubercular drugs such as isoniazid, rifampicin, and pyrazinamide induce hepatotoxicity as a serious adverse effect. They individually have been associated with hepatotoxicity and the risk is enhanced when these drugs are used in combination.[1] Antitubercular drug-induced hepatotoxicity is a common serious adverse drug reaction. It is one of the most challenging clinical problems. It is a main cause of treatment interruption during tuberculosis treatment course that may even cause hospitalization and life-threatening events.[3,4]

Manuka honey is a monofloral honey produced in New Zealand and Australia. It is produced from nectar collected from *Leptospermum scoparium*, which grows wild on undeveloped and unspoilt land. Manuka honey is rich in carbohydrates, fatty acids, proteins, and a high amount of phenolic compounds such as flavonoids.[5] It consists of a high amount of phenolic compounds such as flavonoids, methyl syringate, and a methoxylated benzoic acid, a structural isomer of syringic acid. The phenolic compounds are more potent antioxidants than the non-phenolic antioxidants in honey. In addition, it also contains a bioactive fraction called methylglyoxal that exhibits non-peroxide antibacterial activity.[6] Furthermore, manuka

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honey has demonstrated broad-spectrum antibacterial activity with an inhibitory effect on around 60 species of bacteria.[7] *In vitro* studies have indicated methylglyoxal is an effective antimicrobial agent against forms of methicillin-resistant *Staphylococcus aureus*.[8] Hence, manuka honey is considered as a superior form of honey. As manuka honey is rich in phenolic antioxidants, it is suggested that it may possess hepatoprotective activity. Furthermore, due to its broad-spectrum antibacterial activity, it may be beneficial in resolving the tubercular infection also. Various studies have shown hepatoprotective property of honey. An earlier study has reported the beneficial effects of honey in antitubercular drug-induced hepatotoxicity also.[9] However, the hepatoprotective activity of manuka honey in antitubercular drug-induced hepatotoxicity has not been investigated yet. Hence, this study was carried out to compare the effect of manuka honey with honey in hepatotoxicity induced by antitubercular drugs in rats.

MATERIALS AND METHODS

Healthy adult Sprague-Dawley rats of either sex weighing between 200 and 300 g were used after approval of Institutional Ethics Committee. They were housed in standard laboratory conditions at 25 ± 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 anti-tubercular 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] Manuka honey was purchased from L.I.I. Exports Pvt. Ltd. Delhi, India and honey (manufactured by a reputed Indian manufacturer) were purchased from the local market. 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, i.e., 2% gum acacia orally daily for 30 days.
Group II: (H + R + Z) suspension orally daily for 30 days.
Group III: (H + R + Z) suspension + manuka honey (5 g/kg) orally daily for 30 days.
Group IV: (H + R + Z) suspension + honey (5 g/kg) orally daily for 30 days.
Group V: (H + R + Z) suspension + silymarin (50 mg/kg) orally 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. They were weighed after blotting with filter paper. The liver indices were calculated as the percentage of the body weight.[11] Then gross morphological assessment was done for hepatic lesions based on the qualitative procedure developed by Mitchell *et al*.[12] 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 (MDA) and superoxide dismutase (SOD) assays.

Histopathological examination

All the groups were subjected to histological examination. Microscopic examination was done by a qualified pathologist using hemotoxylin 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.[13] Tissue MDA and SOD activity were estimated by Biuret method, Kakkar *et al.,* method and Ohkawa *et al.,* method, respectively.[14,15]

Statistical Analysis

The values were expressed as mean ± standard error of the 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 and liver weight noted in all groups is shown in Table 1. One rat each from the group I and IV died at the beginning of the experiment for unknown reasons. There was a significant reduction in the body weight and increase in the liver weight in H + R + Z suspension

treated rats when compared to corresponding control rats. Treatment with manuka honey, honey, and silymarin caused a marked increase in the body weight and decreased the liver weight.

Gross Morphological Assessment

The severity of liver necrosis was assessed qualitatively following an inspection of the liver gross morphology [Table 2]. Gross morphological scores indicated that manuka honey significantly decreased the degeneration and tissue necrosis in liver as compared to Group II. However, the hepatoprotective effect was not statistically significant as compared to the Group IV [Table 2].

Histopathological Analysis

In the histopathological studies, the liver sections of rat treated with vehicle showed normal hepatic architecture [Figure 1a]. Administration of anti-tubercular drugs for 30 days to Group II produced changes of inflammation, degeneration, and necrosis on histological examination of rat livers [Figure 1b]. Co-administration of manuka honey along with anti-tubercular drugs reversed histological changes such as inflammation, degeneration, and necrosis [Figure 1c]. Its effect in reversing the cell damage and cell infiltration was comparable to silymarin [Figure 1d and e].

Biochemical Estimations

Serum ALT and AST

Anti-tubercular drugs cause a substantial degree of hepatotoxicity and tissue injury in the rat liver. Hence, there is an increase in serum ALT and AST. In this study, group II which received anti-tubercular drugs for 30 days showed a significant rise in serum ALT and AST as compared to control group [Table 2]. Treatment with manuka honey along with anti-tubercular drugs (Group III) for 30 days significantly reversed level of serum ALT ($P < 0.01$) and AST ($P < 0.01$) as compared to anti-tubercular drug treatment Group II.

MDA and SOD activity

Table 2 shows the effect of different pharmacological interventions on (MDA) levels in rats after 30 days of treatment. The level of MDA in Group II (47.59 ± 8.27) was significantly higher ($P < 0.05$) than the healthy control rats (41.27 ± 8.20). Administration of manuka honey at a dose of 5 g/kg was moderately effective in reversing the rise in MDA level as compared to healthy and H + R+ Z suspension group. Similarly, honey and silymarin were found to be effective in treatment as compared to group II. However, the effect of manuka honey was not statistically significant as compared to honey and silymarin. The level of the antioxidant enzyme SOD showed a significant decrease in Group II as compared to control group. Co-administration

Table 1: Body weight and liver weights of the rats in different groups

Experimental groups	Initial body weight (g)	Final body weight (g)	Percent change	Liver weight (g)	Liver index (%)
Group I	326±3.33	352±9.69	7.97	11.86±0.51	3.36
Group II	293±14.7	260±17.7	−11.26*	11.65±0.52	4.48*
Group III	200±3.33	225±8.46	12.5$^{\#,\dagger}$	6.95±0.32	3.08
Group IV	241±8.33	266±8.71	10.37$^{\#,\dagger}$	8.75±0.35	3.28
Group V	310±8.47	330±7.71	6.45$^{\#}$	9.44±0.69	2.86$^{\#}$

Liver index was calculated as (liver weight/body weight×100%). The values were expressed as mean±SEM ($^{*,\#,\dagger}P<0.05$) *when compared with vehicle control group, $^{\#}$when compared with anti-TB drug group, †when compared with silymarin group. The data was analyzed using one-way ANOVA followed by *Tukey HSD test*. HSD: Honestly significant difference, SEM: Standard error of the mean

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)	28.38±1.18	181.83±18.72**	36.70±4.71$^{\#\#}$	45.46±5.33$^{\#\#}$	41.19±3.00$^{\#\#}$
AST (units/L)	25.01±3.18	55.61±36.55**	37.19±3.56$^{\#\#}$	56.58±3.18$^{\#\#}$	42.06±3.03$^{\#\#}$
MDA (µmol/ml of tissue homogenate)	41.27±8.20	47.59±8.27*	37.59±2.71$^{\#}$	51.03±4.85$^{\#}$	26.73±4.33$^{\#\#}$
SOD (Units/ml)	33.31±0.60	13.61±0.39*	26.78±0.51$^{\#}$	28.60±0.89$^{\#}$	30.11±0.76$^{\#,++}$
MI (0-3)	0	2.83±0.16**	1±0.36$^{\#\#,\dagger\dagger}$	1±0.24$^{\#\#,\dagger\dagger}$	0.33±0.21$^{\#\#}$

The values were expressed as mean±SEM ($^{*,\#}$ = p<0.05;**, $^{\#\#,\dagger\dagger}$ = p<0.01) *when compared with vehicle control group, $^{\#}$when compared with anti-TB drug group, †when compared with silymarin group, $^{+}$when compared with manuka honey and $^{@}$when compared with honey. The data were analyzed using one-way ANOVA followed by *Tukey* HSD test. ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, MDA: Malondialdehyde, SOD: Superoxide dismutase, MI: Morphological index, HSD: Honestly significant difference, SEM: Standard error of the mean

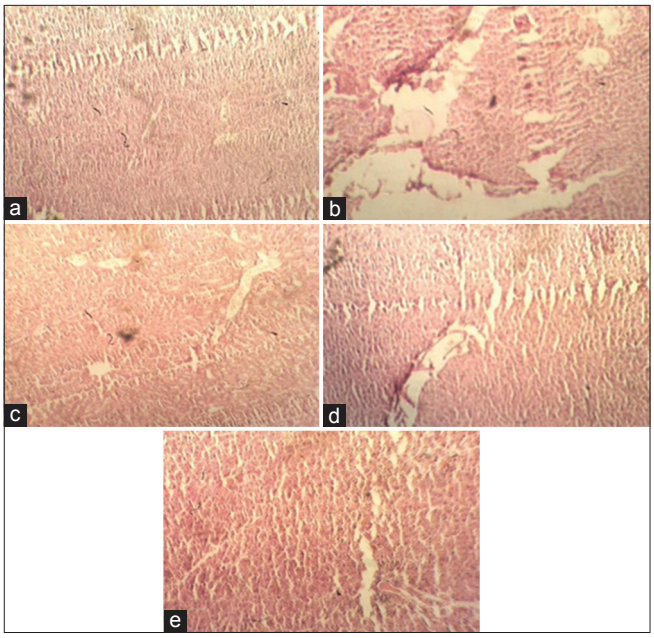

Figure 1: Photomicrograph of the liver tissue of: (a) Vehicle control: Showing the normal histology of liver tissue, (b) H+R+Z group: Showing inflammation in hepatocytes in the form of degeneration, necrosis and inflammation, (c) H+R+Z group + manuka honey (5 g/kg): Showing recovery from degenerative and necrotic changes, (d) H+R+Z group + honey (5 g/kg): Shows decreased inflammation, degeneration and necrosis, (e) H+R+Z group+ silymarin (50 mg/kg): Shows reduced inflammation, degeneration and necrosis (HE, ×40)

of manuka honey along with the anti-tubercular drugs (Group III) significantly ($P < 0.05$) increased the levels of SOD. However, in comparison to silymarin and honey, the effect was not statistically significant.

DISCUSSION

Hepatotoxicity is a serious adverse effect of anti-tubercular drugs such as isoniazid, rifampicin, and pyrazinamide. They alone are hepatotoxic, and when given in combination, their toxic effect is enhanced. The antitubercular drug-induced hepatotoxicity is mediated through oxidative stress and free radical damage to hepatocytes.[16] Hepatic damage is characterized by an increase in serum AST and ALT levels. Free radicals cause lipid peroxidation and subsequently increase serum MDA concentration. Therefore, lipid peroxidation represents tissue injury due to inflammation and serves as an indicator of severe liver damage.[17,18] Liver biopsy is the most reliable index of liver damage. Liver damage is indicated by degeneration, necrosis, and fibrosis while the reduction in these parameters and evidence of regeneration are suggestive of hepatoprotection. Administration of anti-tubercular drugs also resulted in inflammation, degeneration, and necrotic changes in rat liver.

In this study, concurrent administration of manuka honey along with anti-tubercular drugs significantly prevented the rise in the level of serum ALT, AST, and tissue MDA. Similarly, manuka honey significantly prevented fall in serum total protein and SOD as compared to the group receiving anti-tubercular drugs alone. Administration of manuka honey reduced inflammation, degeneration, and necrotic changes. These results showed that manuka honey is effective as hepatoprotective agent and prevented the antitubercular drugs induced hepatotoxicity. Since manuka honey is considered as a superior form of honey and silymarin is a standard hepatoprotective agent in hepatotoxicity, the effect of manuka honey was compared with them also. However, the effects produced by manuka honey were not statistically different from these two interventions. The results showed that the effects produced by manuka honey were on par with these two, and it was equally effective.

CONCLUSION

Manuka honey is effective as a hepatoprotective agent as it significantly prevented the hepatotoxic damage induced by antitubercular drugs in rats. However, the comparison between the effects produced by manuka honey and honey or silymarin showed that the difference was not statistically significant. Hence, contrary to the popular belief, it is only as good and effective as honey.

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