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Protective effects of *Morus alba* L. (white mulberry) against toxicity induced by Isoniazid in experimental rabbits

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Abstract

The hepatoprotective efficacy of the hydroalcoholic extract of *Morus alba* L. (*M. alba*) was evaluated in rabbits previously induced hepatotoxicity by isoniazid. For this study rabbits were randomly divided into five groups (n=8) in which Group 1 served as control, Group 2 as untreated control on isoniazid, Group 3 as treated control on standard hepatoprotective drug silymarin and Group 4 and 5 as treated control on different doses (400 mg/kg body weight, 800 mg/kg body weight) of the hydroalcoholic extracts of *M. alba* from 0-28 days. Blood samples were taken at 7-day intervals from jugular vein (0-28 days) and serums were separated after centrifugation. Liver enzyme profile including SGPT, SGOT were determined by using reagent kits available in the market. While, hematological parameters including Hb, WBCs, RBCs, ESR, PCV and platelet count were determined. It was observed that hydroalcoholic extract (800 mg/kg body weight) significantly reduced the elevated levels of liver enzymes (SGPT and SGOT) in hepatotoxic rabbits at post treatment day 28. Variation in hematological parameters was observed in various treated groups as compared to each other and control in experimental trial.

Keywords

Hematological parameters
Hepatoprotective drugs
Morus alba L. (White Mulberry)
Tuberculosis

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Introduction

Tuberculosis (TB) is a major global problem which is caused by *Mycobacterium tuberculosis* (Murray, 2004). It is a contagious and airborne disease transmittable by airborne droplet of 1–5 μm diameter (Anonymous, 2010). Essential elements of the treatment of tuberculosis are at least two different effective drugs for appropriate duration to prevent relapse. The treatment therapy for tuberculosis includes isoniazid (INH), pyrazinamide, rifampicin and ethambutol and tremendous results for the patients with non-drug resistant tuberculosis can be attained with a six months course of treatment (Brunton *et al.*, 2006). The side effects may be occurred due to long duration treatment of tuberculosis.

Isoniazid (INH) is the first line antituberculosis drug, mostly used in combination with rifampicin (RIF), ethambutol and pyrazinamide (PZA) or streptomycin (Anonymous, 2007). The anti TB drugs are associated with the adverse reactions in different body tissues, especially in liver (Sude *et al.*, 2008). Serious adverse effect of INH is hepatotoxicity. Approximately 90% of patients treated with first line antituberculosis drugs. INH is converted into its toxic metabolite Hydrazine (HYD) (Sarma *et al.*, 1986). The other adverse reactions of INH are skin rashes, fever, hepatitis, jaundice, skins eruption, vasculitis, arthritis and hematological complications like hemolytic, agranulocytosis, thrombocytopenia, sideroblastic or aplastic anemia and hypersensitivity reactions (Brunton *et al.*, 2006). RIF may causes hepatitis and other hypersensitivity reactions including hemolytic anemia, thrombocytopenia and

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renal failure. There are increased levels of serum enzyme like alkaline phosphatase, Transminase and bilirubin after the use of rifampicin shows its toxicity in liver (Anonymous, 2010). Now a day only a small amount of hepatoprotective drugs and those from natural source are accessible for the treatment of liver disorders (Ross *et al.,* 1996).

Various plant extracts have been used reliably to cure many disorders. *Morus alba* L. also called white Mulberry, silkworm mulberry and Shahtoot in Urdu. It belongs to *Plantae* Kingdom, *Tracheobionta* as subkingdom, division *Magnoliophyta*, class *Magnoliopsida*, Order *Urticales*, Family *Moraceae*, Genus *Morus* and *Morus alba* as species. It contains more than 150 species among these *M. alba* is one of the most prevailing specie (Srivastava *et al*., 2006). It is used in Chinese oriental medicine. Commonly it is used to feed ruminants and silkworms besides its use in nutritional products in various countries. (Arabshahi-Delouee and Urooj, 2007; Ercisli and Orhan, 2007). It has been reported that almost all parts of this plant is very useful in liver, cardiovascular, and spleen disorders (Fukai *et al.,* 2003). The free radical of this herbal plant has shown the properties of, hypolipidemic, antioxidant, antibacterial, antiviral, astringent, emollient, hepatoprotective and anti-inflammatory (Hogade *et al.*, 2003). Presence of polyphenolic constituents in *M. alba* especially quercetin 3-(6-malonylglucoside) and different flavonoids are the important antioxidant components of mulberry plant.

M. alba leaves are widely used as animal and insect feed, and also have been extensively consumed in Japan, Chile and Korea as antihyperglycemic and as nutraceutical foods in diabetes mellitus patients. All parts of *M. alba* have immense therapeutic importance due to their antioxidant potential. Old civilization got fresh look and recent findings supported its part against atherosclerosis and diabetes mellitus (Shibata *et al.*, 2006; Enkhmaa *et al.*, 2005). Red and black mulberry are imperative resource of phytochemicals therefore, good for the human body (Cieslik *et al*., 2006).

Studies in animal and human showed that isoniazid-induced hepatotoxicity causes necrosis and hepatocellular steatosis, it had been observed that isoniazid metabolites bind covalently to cell macromolecules which cause toxicity (Sarich *et al.,* 1996; Mitchell *et al.,* 1976). The toxic metabolite of isoniazid is hydrazine and study showed that hydrazine causes steatosis, mitochondrial swelling, depletion of glutathione and hepatocyte vacuolation in midzonal and periportal hepatocytes (Scale and Timbrell, 1982; Sarich *et al.,* 1995).

Materials and Methods

Preparation of dry powder: *M. alba* L. leaves were collected and dried under shade for 2 weeks then made its powdered mechanically by grinder. After passing through 40 no. mesh sieve they were stored in airtight container at 4ºC.

Preparation of Plant extract: For extraction of dry powder ethanol and water was used as ratio 7:3 in Soxhlet apparatus. The dried leaf powder (900 g) was extracted by maceration for three days. 25 g dry powder of *M. alba* was taken in a thimble of Soxhelt apparatus, 400 ml of solvent (Ethyl alcohol + aqueous) was added in the flask. The extraction process was started later on. The extracted fluid was separated after three days and collected in a Petri-dish. The collected material was kept overnight at room temperature and then placed in freezer to solidify. After that lyophilization of solid material was done by drying apparatus (Christ, Germany model # Alpha 1-4 LSC), so that moisture contents were evaporated. 7.75 gm of lyophilized powder was obtained. This process was repeated again and again and until the required extracted was obtained. The resulting solid extract obtained was 279 gm and was stored in an air tight container at 15°C.

***In-vivo* experimental studies:** Isoniazid was administered at dose rate of 100 mg/kg b.w P.O (Yue *et al.,* 2004) to each rabbit through stomach tube for 28 days. The rabbits were divided into 5 groups (n=8) in which group 1 served as normal control, group 2 treated with isoniazid alone, group 3 as treated control on standard hepatoprotective drug silymarin and group 4 and 5 are treated with 400 mg/kg body weight and 800 mg/kg body weight doses of the hydroalcoholic extracts of *M. alba* respectively. Blood samples were collected in EDTA tubes at 0 day and after 1, 2, 3 and 4 weeks of drug administration. The serums were separated by centrifugation at 3500-4000 rpm for 15 minutes and stored at -20°C till further used.

Liver enzymes analysis: The aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzymes were measured in the serum by commercially available kit of (Reference # BT294Q) Randox Laboratories, UK and (Reference # BT294QY) Randox Laboratories, UK respectively.

Hematological analysis: The hematological parameters including Red Blood Cells (RBCs), White Blood Cells (WBCs), Hemoglobin (Hb), Packed Cell Volume (PCV), platelet count and Erythrocyte Sedimentation Rate (ESR) were performed.

Histopathological analysis: The liver biopsies in formalin container were processed in graded ethanolic concentrations and fixed in paraffin blocks. Liver fragments were prepared perpendicular to the plane of the section in the block and 6 micrometer thick oblique fragments were cut and straddling on glass slides and stained with hematoxylin and eosin (H and E stain). Morphometric measurements were accomplished on Olympus PM – 10ADS automatic light microscope (Olympus optical Co., Tokyo, Japan) with a 40X objective and calibrated ocular micrometer.

Statistical Analysis: Statistical analysis was done by one way analysis of variance (ANOVA) and statistical differences between various treatment groups was calculated by Duncan's Multiple Range test at 5% level of significance (Steel *et al.*, 1997).

Results and Discussion

Hematological parameters

Hemoglobin: The Mean ± SEM values of Hb has considerably (P<0.05) reduced in the isoniazid alone treated group (11.09±0.16 g/dl) has compared to the control group (12.04±0.13 g/dl). While the Mean ± SEM values of the group treated with silymarin + isoniazid (12.76±0.47 g/dl) is significantly different from the group treated with isoniazid alone and is comparable with the control group. Mean ± SEM values of the group treated with *M. alba* 400 mg/kg Hydroalcoholic extract (11.67±0.36 g/dl) and the group treated with *M. alba* 800mg/kg Hydroalcoholic extract (11.30±0.22 g/dl) are comparable with each other and group treated with isoniazid alone and control group. But it is significantly different from the group treated with silymarin + isoniazid. Whereas, non-significant change was determined among all the groups in different weeks (Figure 1a).

Erythrocyte Sedimentation Rate (E.S.R): The Mean ± SEM values of E.S.R has considerably (P<0.05) reduced in the isoniazid alone treated (3.33±0.33 mm/hr) has compared to control group (4.16±0.18 mm/hr). While the Mean ± SEM values of the group treated with silymarin + isoniazid (3.24±0.49 mm/hr) is comparable with the control group and isoniazid alone group. Mean ± SEM values of the group treated with *M. alba* 400 mg/kg hydroalcoholic extract (3.53±0.44 mm/hr) is comparable with groups treated with isoniazid alone, silymarin + isoniazid and control group. But the Mean ± SEM values of the group treated with *M. alba* 800 mg/kg hydroalcoholic extract (4.29±0.44 mm/hr) is significantly different from the group treated with silymarin + isoniazid and isoniazid alone and comparable with control group and *M. alba* 400 mg/kg hydroalcoholic extract. Whereas, there was highly significant difference was seen among all the groups of experimental trial at various week intervals (Figure 1b).

Total white blood cell (W.B.C): The Mean ± SEM values of W.B.C are comparable among all treated groups with control group. Non-significantly change was observed in all groups in different weeks (Figure 1c).

Red blood cells (RBC): The Mean ± SEM values of RBC has significantly elevated in the isoniazid alone treated group (5.86±0.24 10^6/µl) as compared to the control group (5.27±0.19 10^6/µl). While the Mean ± SEM values of the group treated with silymarin + isoniazid (5.82±0.16 10^6/µl) is significantly different from the group treated with the control group and is comparable with isoniazid alone. Mean ± SEM values of the group treated with *M. alba* 400 mg/kg hydroalcoholic extract (6.10±0.13 10^6/µl) and the group treated with *M. alba* 800 mg/kg hydroalcoholic extract (5.66±0.06 10^6/µl) are comparable with each other and group treated with isoniazid alone and silymarin + isoniazid group. But Mean ± SEM values of the group treated with *M. alba* 400 mg/kg hydroalcoholic extract is significantly different from control group. Whereas, non-significant difference was seen among all the groups at different weeks interval (Figure 1d).

Table 1: Mean ± SEM values of SGPT and SGOT level (U/L) in different groups of experiment.

Groups	TEST	
	SGPT	SGOT
Control	17.20±1.53	62.20±6.97
Isoniazid	59.13±6.67	79.23±3.73
Silymarin	17.10±1.08	59.40±6.46
M. alba (400 mg/kg)	21.85±5.73	48.30±6.49
M. alba (800 mg/Kg)	22.13±5.25	53.98±6.94

Platelet count: The Mean ± SEM values of platelet count has significantly reduced in the isoniazid alone treated group (157600±46469.91 10^3/µl) as compared to the group treated with *M. alba* 400 mg/kg hydroalcoholic extract (286200±61099.82 10^3/µl) and comparable with group treated with silymarin + isoniazid (141007±26604.97 10^3/µl), the group treated with *M. alba* 800 mg/kg hydroalcoholic extract (258493±34143.52 10^3/µl) and control group (249162±1902.49 10^3/µl). Whereas, the Mean ± SEM values of group treated with silymarin + isoniazid is significantly (P<0.05) different from the group treated with *M. alba* 400 mg/kg Hydroalcoholic extract and *M. alba* 800 mg/kg hydroalcoholic extract But is comparable with isoniazid alone group and control group. Whereas, there was non-significant difference was seen among all the groups of experimental trial at various week intervals (Figure 1e).

Packed cell volume (P.C.V): The Mean ± SEM values of P.C.V has significantly declined in control group (36.23±0.85 %) as compared to group treated with silymarin + isoniazid (40.06±1.10 %), the group treated with *M. alba* 400 mg/kg hydroalcoholic extract (41.07±0.96 %) and the group treated with *M. alba* 800 mg/kg hydroalcoholic extract (40.12±0.63 %) and is comparable with group treated with isoniazid alone (38.38±1.25 %). Whereas, the Mean ± SEM values of group treated with silymarin + isoniazid is significantly (P<0.05) different from the control group and is comparable with group treated isoniazid alone, group treated with *M. alba* 400 mg/kg hydroalcoholic extract and group treated with *M. alba* 800 mg/kg hydroalcoholic extract. Whereas, non-significant difference was observed among all the groups of experimental trial (Figure 1f).

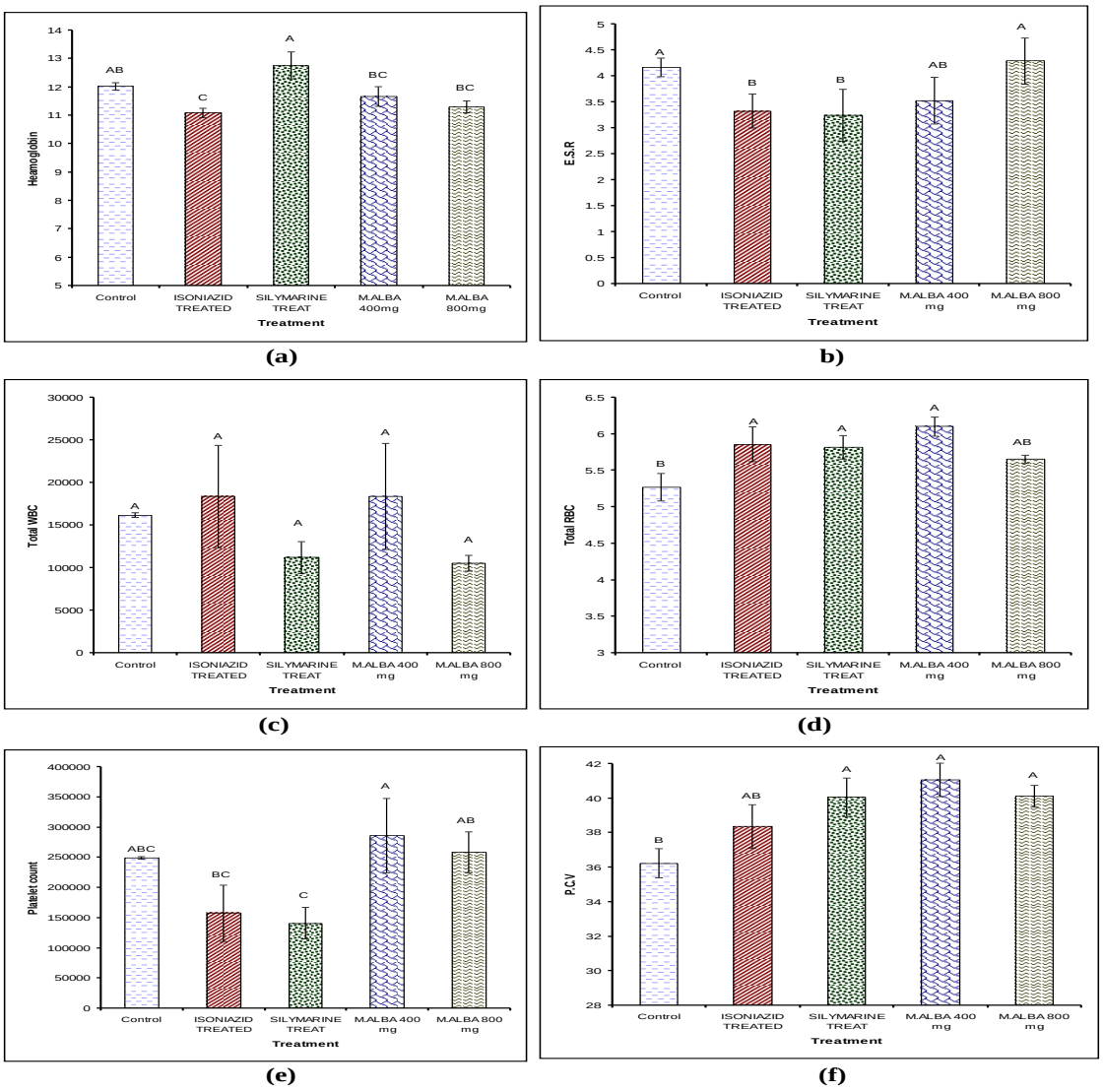

Figure 1: Mean ± SEM values of (a) Hb (b) E.S.R (c) WBCs (d) RBCs (e) Platelet (f) P.C.V with per oral drugs and *M. alba* extract daily administration for 28 days in rabbits (n=8).

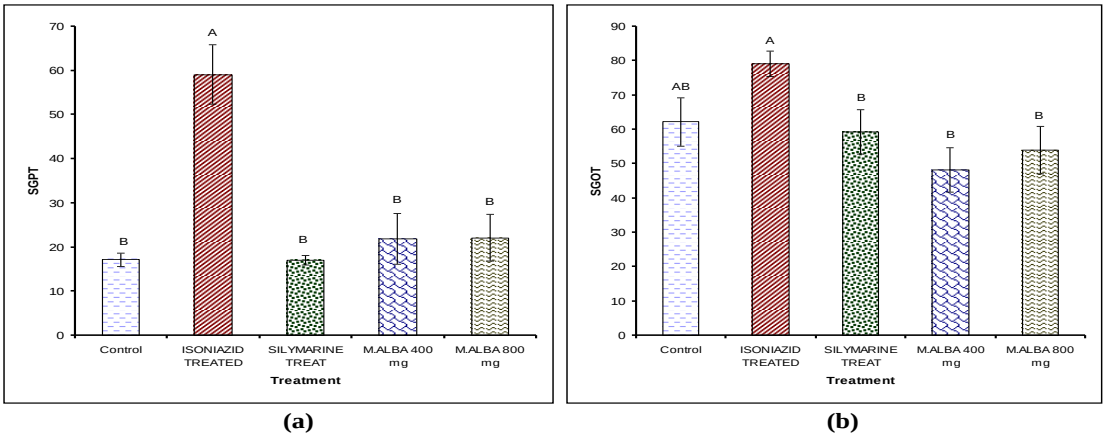

Figure 2: Mean ± SEM values of (a) SGPT level (U/L) and (b) SGOT level (U/L) with per oral drugs and *M. alba* extract daily administration for 28 days in rabbits (n=8).

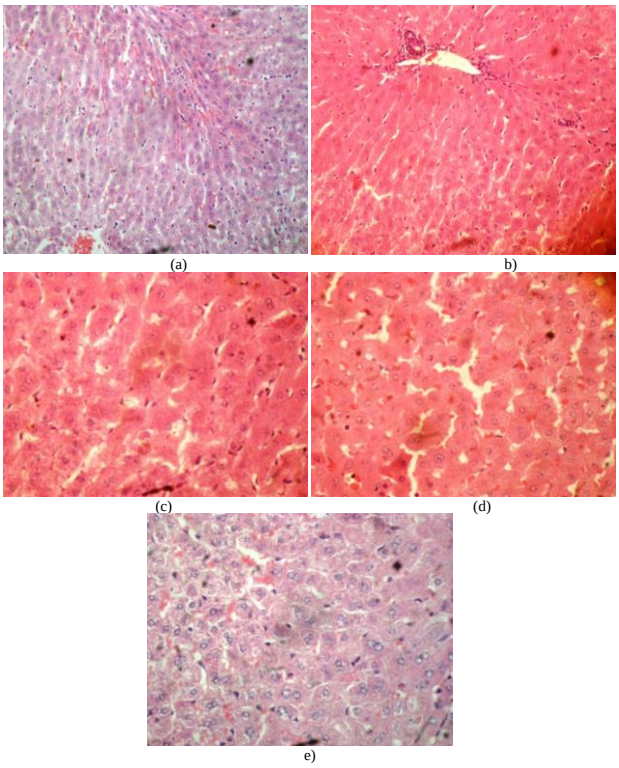

Figure 3 (a, b, c, d, e): Histopathology of liver hepatocytes of in different groups of experiment at (H& E, x 40).

Liver enzyme parameters

Serum Glutamate Pyruvate Transaminase (SGPT): The Mean ± SEM values of ALT has highly significantly elevated in the isoniazid alone treated group (59.13±6.67 U/L) as compared to group treated with silymarin + isoniazid (17.10±1.08 U/L), the group treated with M. alba 400 mg/kg hydroalcoholic extract (21.85±5.73 U/L), the group treated with M. alba 800 mg/kg hydroalcoholic extract (22.13±5.25 U/L) and control group (17.20±1.53 U/L). Whereas, the Mean ± SEM values of group treated with silymarin + isoniazid is highly significantly ($P<0.01$) different from isoniazid alone and is comparable with group treated with *M. alba* 400 mg/kg hydroalcoholic extract, the group treated with *M. alba* 800mg/kg hydroalcoholic extract and control group. Mean ± SEM values of group treated with *M. alba* 400 mg/kg hydroalcoholic extract and the group treated with *M. alba* 800 mg/kg hydroalcoholic extract are is highly significant ($P<0.01$) from isoniazid alone and are comparable with each other and group treated with silymarin + isoniazid and control group. Whereas, non-significant difference was observed among all the groups of experimental trial (Figure 2a and Table 1).

Serum Glutamate Oxaloacetate Transaminase (SGOT): The mean ± SEM values of AST has significantly elevated in the isoniazid alone treated group (79.23±3.73 U/L) as compared with group treated with silymarin+isoniazid (59.40±6.46 U/L), the group treated with *M. alba* 400 mg/kg hydroalcoholic extract (48.30±6.49 U/L), the group treated with *M. alba* 800 mg/kg hydroalcoholic extract (53.98±6.94 U/L) and is comparable with control group (62.20±6.97 U/L). Whereas, Mean ± SEM values of group treated with silymarin + isoniazid is significantly ($P<0.05$) different from isoniazid alone and is comparable with group treated with *M. alba* 400 mg/kg hydroalcoholic extract, the group treated with *M. alba* 800 mg/kg hydroalcoholic extract and control group. Mean ± SEM values of group treated with *M. alba* 400 mg/kg hydroalcoholic extract and the group treated with *M. alba* 800 mg/kg hydroalcoholic extract were significantly different from isoniazid alone and are comparable with each other and group treated with silymarin + isoniazid and control group. Whereas, non-significant difference was observed among all the groups of experiment (Figure 2b and Table 1).

Histopathology: Hepatic parenchyma shows normal appearance of hepatocytes with prominent nucleus having nucleolus blood vessels were mildly hyperemic in control group (Figure 3a). Hepatic cells are swallowing individually; cell necrosis and mild degree of biliary cellular hyperplasia are the prominent features in hepatic toxic group (Figure 3b). In silymarin treated group hepatocytes are normal in appearance. Mild degree of congestion is present in parenchyma. Nucleus is prominent having nucleolus. Coenocitic spaces were normal (Figure 3c). In extract treated group (400 mg/kg) mild degree of vascular degeneration in cytoplasm of hepatocyte, nuclei are normal in appearance having nucleolus. Mild degree of congestion at few places (Figure 3d). In extract treated group (800 mg/kg) blood vessels are congested and hyperemic, hepatocytes are normal in hepatic chords, sinusoidal spaces are normal (Figure 3e).

Conclusion: *M. alba* leaves extract proved protective in reducing hepatotoxicity by the administration of INH as visible through decrease in biochemical parameters. Histological studies have also showed the protective response of hydroalcoholic plant extract against INH induced hepatotoxicity. Hematology was not very much affected by extract treatment significantly. Moreover, the extensive chemical characterization and pharmacological investigations should be accompanied to isolate and evaluate the newer active ingredients that could adequately help in reducing serum lipid levels in humans. Besides this, their medicinal importance as a whole should be assessed and their efficacy should be reputable as hepatoprotective agent.

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