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Hepatoprotective activity of Asteracantha longifolia (Nees.) extract against anti-tuberculosis drugs induced hepatic damage in Sprague-Dawley rats

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Summary

Asteracantha longifolia Nees. (Family-Acanthaceae) is a wild herb commonly used as aphrodisiac, tonic, sedative and blood diseases etc. The first line anti-tuberculosis drugs isoniazid (INH), rifampicin (RIF) and pyrazinamide (PZA) continues to be the effective drugs in the treatment of tuberculosis, however, the use of these drugs is associated with toxic reactions in tissues, particularly in the liver, leading to hepatitis. *Asterantha longifolia* (AL) plant extract is known to be an effective agent for liver protection and liver regeneration. The aim of this study was to investigate the protective actions of hydroalcoholic extract of AL against hepatotoxicity caused by different combinations of anti-tuberculosis drugs. Male Sprague-Dawley rats weighing 170–230 g were used to form three study groups, each group consisting of 6 rats.

Animals were treated with oral administartion of isoniazid (50 mg/kg) and rifampicin (50 mg/kg). Hepatotoxicity was induced by INH + RIF's combination. Hepatoprotective effect of AL was investigated by co-administration of AL together with the drugs. Serum biochemical tests for liver functions and histopathological examination of livers were carried out to demonstrate the protection of liver against antituberculosis drugs by silymarin. Treatment of rats with INH+RIF induced hepatotoxicity as evidenced by biochemical measurements: serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activities and the levels of total bilirubin were elevated, and the levels of albumin and total protein were decreased in drugs-treated animals. Histopathological changes were also observed in livers of animals that received drugs. Simultaneous administration of AL plant extract significantly decreased the biochemical and histological changes induced by the drugs. The active components of AL plant extract had protective effects against hepatotoxic actions of drugs used in the chemotherapy of tuberculosis in animal models.

Keywords: Hepatotoxicity, Histopathology, Tuberculosis, Aminotransferase

Introduction

Management of liver disease is still a challenge to the modern medicine. In the absence of reliable liver-protective drugs in allopathic medical practices, herbs play a vital role in the management of liver disorders. Many indigenous plants are used for the treatment of liver disorders [1].

Asteracantha longifolia (AL) Nees. (Syn. Hygrophila spinosa T. Anders.; Hygropihila auriculata [K. Schum.] Heine) (known as Kokilaksa in Sanskrit and Talmakhana in Hindi, Family-Acanthaceae) is a common weed growing in marshy and water logged areas. The plant is an important medicinal herb, widely distributed in India and used by local population for different medicinal purposes. It is also used commercially as ingredient of some over the counter (OTC) formulations used in liver disorder and those prescribed as general tonic. The herb has been reported to contain chemical constituents such as β-sitosterol, lupeol [2-4], both have been reported to have antipyretic [5, 6] and hepatoprotective activity [7, 8]. The plant contains flavonoids, terpenoids, steroids and betulin. The roots, seeds and ashes of the plant are extensively used in traditional system of medicine for various ailments like jaundice, hepatic obstruction, rheumatism, inflammation, pain, urinary infections, edema and gout. The plant is known to possess antitumor [9-10], hypoglycaemic [11], antibacterial [12-13], free radical scavenging and lipid peroxidation activities [14]. The hepatoprotective activity of seeds of *Asteracantha longifolia* in thioacetamide and paracetamol-induced liver damage in rats [15]. It has been investigated the haematopoietic activity of the chloroform and petroleum ether extract of *Asteracantha longifolia* Nees [8]. *Asteracantha longifolia* also played a role as a mitigator/attenuating agent in perchloroethylene-induced hepatotoxicity [16].

To the best of our knowledge, in the literature survey, there is no scientific study on evaluation of hepatoprotective activity of *Asteracantha longifolia* incase of Isoniazid and Rifampicin induced hepatotoxicity. Therefore, considering the high incidence of tuberculosis as well as hepatitis in Bangladesh and insufficiency of any cheap and effective modern drug, the present study was focused to evaluate alcoholic extracted from *Asteracantha longifolia* on Isoniazid and Rifampicin induced hepatotoxicity in adult rat (*Rattus novergicus*: Sprague-Dawley strain) by assessing biochemical and histopathological tests.

Materials and Methods

Plant material collection and extraction

Asteracantha longifolia were collected in powdered form from commercial source. The powdered aerial parts (350 gm) of *Asteracantha longifolia* (AL) was successively extracted in a soxhlet apparatus for 24 h with ethanol (95%) which was then concentrated and dried under reduced pressure. The yield was found to be 4.15% (w/w) as semisolid mass. Two grams of semisolid ethanolic extract was dispersed in 1% Tween-80 [17].

Experimental animal

Adult male rats (*Rattus novergicus*: Sprague-Dawley strain) were collected from central animal house of the department of Pharmacy, Jahangirnagar University, Savar, Dhaka-1342, and were used for the present study. The animals were randomized and separated into normal and experimental groups of body weight ranging from 170 to 230 g.

Isoniazid and Rifampicin-induced hepatotoxicity

Experimental animals were also divided into following three groups:

Group I: Normal control (n=6, the animals were given normal saline only for 28 days).

Group II: Hepatotoxic control (n=6, the animals were given INH+RIF for 28 days).

Group III: Treatment group (n=6, the animals were given INH+RIF+AL for 28 days).

Rats were treated as per the treatment protocol. Body weights of these rats were monitored sequen-

tially in control and experimental animals for a period of 28 days.

Dose and Route of Administration

Isoniazid and rifampicin (50 mg/kg body weight) solutions were prepared separately in sterile distilled water. Rats were treated with isoniazid, co-administered with rifampicin for 28 days by oral route [18]. For hepatotoxic model, 50 mg/kg per day of INH and RIF each was used in the study [19]. For the hepatoprotective model, 500 mg/kg per day of freshly prepared *Asteracantha longifolia* homogenate along with INH+RIF solution was administered orally.

Preparation of the samples for biochemical Studies

From the post vena cava of the animal, blood sample were collected and immediately blood was transferred to the tubes having heparin. Blood samples were centrifuged for 10 minutes at 3000 rpm to separate serum for biochemical analysis. The liver was dissected out for histopathological examinations.

Biochemical estimations

Biochemical parameters like serum enzymes, serum Alanine aminotransferase (ALT) serum Aspartate aminotransferase (AST), serum bilirubin (SB) and serum alkaline phosphatase (SAKP) [20-23], total protein (TP) [24-25] and albumin [25] were determined by Humalyzer-3500 auto-analyzer using kits manufactured by HUMAN GmbH, Germany in Pharmacology Lab at Jahangirnagr University, Savar, Dhaka, Bangladesh.

Histopathological studies

The liver specimens obtained from the control and treated groups of animals were fixed in 10% buffered formalin for 24 h. The formalin-fixed liver samples were stained with haematoxylin–eosin for photomicroscopic observations of the liver histological architecture [26].

Estimation of In-vitro antioxidant activity

The method of Ottolenghi [27], was used to determine the TBA values of the samples. Two milliliters of 20% trichloroacetic acid and 2ml of TBA aqueous solution were added to 1ml of sample solution prepared as in FTC procedure, and incubated in a similar manner. The mixture was placed in boiling water bath for 10 min. After cooling, it was centrifuged at 3000 rpm for 20 min and the absorbance of the supernatant was measured at 532 nm. Antioxidant activity was based on the absorbance on the final day. The inhibition of lipid peroxidation in percentage was calculated by the following equation:

$$\text{Percent inhibition} = 1 - \frac{A_1}{A_0}$$

Where, A_0 is the absorbance of control and $A1$ is the absorbance of sample [28].

2.9 Statistical analysis

All the grouped data were statistically evaluated with SPSS (Chicago, IL) version 16.5 software. All the results were expressed as mean ±SEM (Standard error of mean) values for six animals in each group. Means were compared by independent sample t-test [29]. Probability (p) value of 0.05 or less ($p<0.05$) was considered as significant. Here [a] indicates $p<0.001$, [b] indicates $p<0.01$ and [c] indicates $p<0.05$.

Result

Administration of INH and RMP combination only, showed a significant derangement of liver function as assessed by change in serum enzymes (AST, ALT and ALP) as well as bilirubin, total proein, albumin and also liver histopathology.

Table-1 shows the levels of ALT, AST, ALP, bilirubin, total protein, and albumin in serum. There was a significant increase in the levels of ALT, AST, ALP, and bilirubin in serum of rats treated with Isoniazid

and Rifampicin when compared with control rats, whereas the levels of total protein and albumin in serum were decreased. Pretreatment of rats with AL extract caused a significant reduction in the levels of enzymes, and bilirubin leading to a significant reversal of hepatotoxicity.

see Table 1.

The histological architecture of Isoniazid and Rifampicin treated liver sections showed massive hepatic necrosis with dilated blood vessels (Figure. 2) in comparison with normal control (Figure. 1). The AL treated groups showed regeneration of hepatocytes, normalization of fatty changes and necrosis of the liver (Figure. 3).

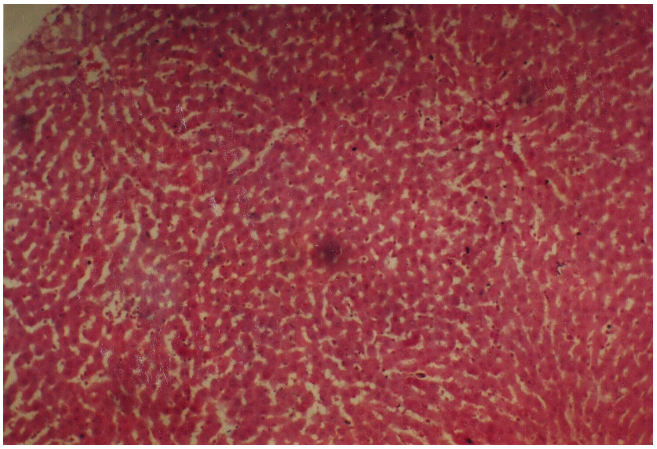

Figure 1: Section of Control rat liver, showing normal architecture of hepatic cell

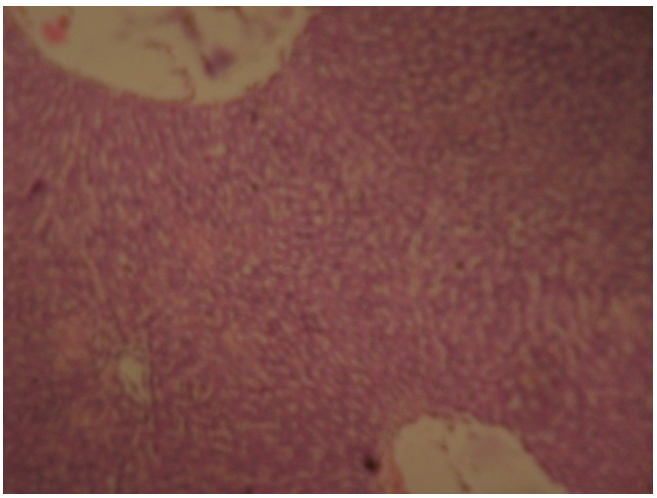

Figure 2: Section of INH-RIF treated rat liver, showing massive hepatic necrosis with dilated blood vessels

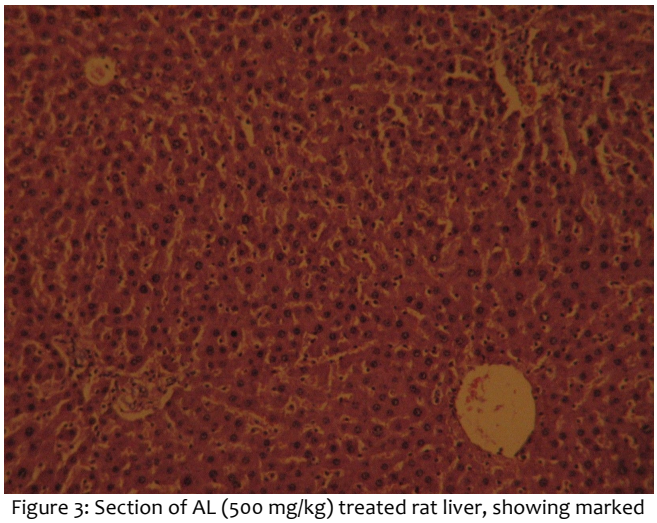

Figure 3: Section of AL (500 mg/kg) treated rat liver, showing marked improvement over INH + RIF group

The antioxidant activity exhibited by AL in TBA methods (in vitro) is represented in Table 2.

Sample/Standard	IC_{50} (µg/ml)
Asteracantha longifolia	342.62
TBA	53.36

Table 2: IC50 values of plant extract and standard

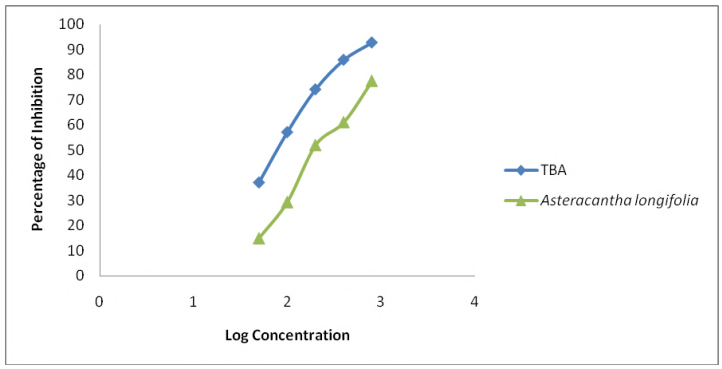

Figure 4: Lipid peroxidation inhibition capacity of *Asteracantha longifolia* and TBA

Discussion

Drug-induced HT is a potentially serious adverse effect of the currently used anti-tubercular chemotherapeutic regimens containing INH, RIF and PZA [30]. All these drugs are potentially HT independently, when given in combination their toxic effects are enhanced in a synergistic manner. It interrupts the treatment regime and compromises its efficacy, leading to grave consequences. The conversion of

monoacetyl hydrazine, a metabolite of INH, to a toxic metabolite via cytochrome P450 leads to hepatotoxicity. RIF-induces cytochrome P450 enzyme causing an increased production of toxic metabolites from acetyl hydrazine (AcHz). RIF can also increase the metabolism of INH to isonicotinic acid and hydrazine, both of which are hepatotoxic. The plasma half life of AcHz (metabolite of INH) is shortened by RIF and AcHz is quickly converted to its active metabolites by increasing the oxidative elimination rate of AcHz, which is related to the higher incidence of liver necrosis caused by INH and RIF in combination . In addition to these mechanisms; oxidative stress induced hepatic injury is one of the important mechanisms in HT produced by antitubercular drugs [31].

Rats have been used successfully to investigate INH and RIF-induced hepatotoxicity models [32-37]. Therefore, we selected rats to study the hepatotoxic effect of antituberculosis drugs and hepatoprotective action of Ayurvedic medicinal plant. Numerous tests have been developed and employed to evaluate liver function or diseases. There are several pathological mechanisms on which these tests are based. Damaged hepatocytes or biliary epithelium may release cell constituents (e.g. enzymes) into blood resulting in increased levels of these analytes. The more commonly measured 'liver' enzymes are alanine aminotransferase (ALT, formerly sGPT), aspartate aminotransferase (AST, formerly known sGOT), Alkaline phosphatase (ALP), sorbitol dehydrogenase (SDH), and gamma-glutamyl transferase (GGT). There are some other tests used less frequently. e.g. ornithine carbamyl transferase, isocitrate dehydrogenase, and arginase.Increased levels of ALT, AST, and SDH are usually associated with damage to hepatocytes [38-41].

In the present study, the hepatic injury induced by Isoniazid and Rifampicin (I+R) combination is evident by an increase in the levels of serum enzymes. This is in agreement with the results obtained in other previous investigations [42, 43]. The increased levels of AST and ALT are indicative of cellular damage and loss of functional integrity of the cell membrane in the liver [44]. The increase in ALP in liver disease is the result of increased synthesis of the enzyme by cells lining the canaliculi, usually either intra- or extrahepatic, which reflects the pathological alteration in biliary flow [45]. Prior oral administration of AL extract exhibited significant protection against I+R-induced hepatotoxicity. The extract-mediated reduction in the levels of these enzymes towards respective normal values is an indication of stabilization of the plasma membrane as well as repair of hepatic damage caused by I+R. An abnormal increase in the levels of bilirubin in serum indicates hepatobiliary disease and severe disturbance of hepatocellular function [46]. Reversal of the bilirubin level to near normal upon administration of AL extract clearly indicates improvement of the functional status of the liver cells. The histopathological observations suggested the possibility of the plant extract being able to condition the hepatic cells to a state of accelerated regeneration thus decreasing the leakage of ALT, AST and ALP into the circulation.

In TBA method formation of malonaldehyde is the basis for evaluating the extent of lipid peroxidation. At low pH and high temperature (100 °C) malonaldehyde binds TBA to form a red complex that can be measured at 532 nm. The increase of the amount of red pigment formed correlates with the oxidative rancidity of the lipid. Most of the hepatotoxic chemicals including Isoniazid and Rifampicin damaged liver mainly by inducing lipid peroxidation directly or indirectly. In higher animals, lipid peroxidation was known to cause destabilization and disintegration of the cell membrane, leading to liver injury, arteriosclerosis and kidney damage [47]. Peroxy radicals are important agents that mediate lipid peroxidation thereby damaging cell membrane. The IC50 values of the plant extract and standard are presented in the Table 2. % of inhibition of lipid peroxidation was found to rise with increasing concentration of plant extract (Figure 4).

Pre-treatment of AL extract attenuated the changes induced by I+R, and a subsequent recovery towards normalization was achieved. This strongly suggests the possibility of the AL extract being able to condition the hepatocytes so as to cause accele-

rated regeneration of parenchymal cells, thus protecting against cell membrane disturbances induced by I+R. On the basis of the above results it can be concluded that AL exhibit a significant hepatoprotective property.

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Group	ALT (IU/L)	AST (IU/L)	ALP (KA Units/100 ml)	Bilirubin (mg/dl)	Total Protein (mg/dl)	Albumin (mg/dl)
Control	22.666±1.632	44.166±10.127	108.500±1.870	.766±.103	6.704±.219	4.236±.053
I+R	51.000±5.059	106.333±4.179	172.5±4.415	1.800±.089	5.362±.413	3.845±.201
I+R+Al	36.833±6.853[b]	81.833±10.647[a]	152.500±10.251[b]	1.383±.132[a]	6.053±.094[b]	4.201±.218[c]

Table 1: Effect of Al on serum ALT, AST, ALP, bilirubin, TP and Albumin levels in Isoniazid (I) and Rifampicin (R) induced sub acute liver injury in rats

Results are represented as mean ± SEM (n = 6)
[a] $p \leq 3d0.001$, compared to I+R
[b] $p \leq 3d0.01$, compared to I+R
[c] $p \leq 3d0.05$, compared to I+R
ALT- Alanine transaminase, AST- Aspartate aminotransferase, ALP- alkaline phosphatase.