

Original Research Article

Phytochemical Screening and Hepatoprotective Effect of *Alhagi maurorum* Boiss (Leguminosae) Against Paracetamol-Induced Hepatotoxicity in Rabbits

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Abstract

Purpose: To evaluate the hepatoprotective activity of aqueous-ethanol (30:70 %) extract of *Alhagi maurorum* Boiss. (Leguminosae) whole plant against paracetamol-induced liver injury in experimental rabbits.

Methods: Aqueous-ethanol extract of *Alhagi maurorum* at doses of 250 mg/kg and 500 mg/kg body weight, p.o., was administered for 7 days in paracetamol (2 gm/kg, s.c.) intoxicated rabbits and compared with silymarine (50 mg/kg, p.o.)-treated rabbits. Biochemical parameters, alkaline phosphatase (ALP), serum glutamic oxalacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) and total bilirubin (TB) levels were recorded to investigate the degree of improvement in the conditions of the rabbits. The liver was removed, washed with normal saline and preserved in 10 % formalin and used in histopathological studies of hepatic architecture by microscopy. Phytochemical screening of the extract was also carried out.

Results: The levels of biochemical parameters were increased in paracetamol intoxicated rabbits when compared with the normal group. The extract, at doses of 250 and 500 mg/kg, exhibited significant ($p < 0.001$) reduction in biochemical parameters (ALP, SGOT, SGPT and TB). Hepatoprotective activity was also confirmed by histopathological findings. Furthermore, the phytochemical profile of the extract revealed the presence of tannins, alkaloids, saponins and flavonoids.

Conclusion: These results suggest that *Alhagi maurorum* extract possesses significant hepatoprotective effect against paracetamol-induced hepatotoxicity and this may be due to the presence of flavonoids and tannins.

Keywords: *Alhagi maurorum*, Hepatoprotective, Paracetamol, Silymarin, Histopathology

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INTRODUCTION

Liver disease is a serious problem in developing countries and a cause of morbidity and mortality throughout the world. Liver ailments are frequently caused by hepatitis A, B and C

viruses, carbon tetrachloride (CCl₄), high doses of paracetamol, thioacetamide (TAA) and certain chemotherapeutic agents etc. [1]. It has been estimated in recent reports that 10 % of world population is affected with liver diseases including hepatitis, alcoholic steatosis, fibrosis,

liver cirrhosis and hepatocellular carcinoma. Morbidity and mortality resulting from liver diseases is a major public health problem worldwide [2]. The management of liver diseases is still a challenge to modern medicine. The drugs available for the treatment of liver diseases are only corticosteroids and immunosuppressive agents which exert several serious adverse effects. This has led to increased dependency on complementary and alternative medicine, especially herbal/plant-based medicines. Medicinal plants play a vital role in the management of liver disorder in the developing world for primary health care because they are inexpensive, possessing minimal or no side effect and easy availability in nature [3]. The hepatoprotective effects of several medicinal plants such as *Leptadenia reticulata* stem [4], *Suaeda fructocosa* roots [5], *Cleome viscosa* leaves [6], *Zingiber officinale* and *Piper nigrum* have been evaluated in some previous reports [7].

Alhagi marjoram Boiss., belong to the family Leguminosae or Fabaceae, commonly known as camel thorn or Persian manna plan. This is native to the geographical areas extending from the temperate and tropical Eurasia and the Middle East Pakistan, India, Bangladesh, Iran, Iraq, Jordan, Israel, Kuwait, Lebanon, Cyprus, Turkey, Turkmenistan, Uzbekistan and Russia [8]. It is widely distributed in Cholistan Desert of Bahawalpur (Pakistan) and traditionally used by local inhabitants in various disorders like hepatitis, jaundice, stomatitis, kidney stones, urinary retention, flatulence and piles [9].

Literature search reveals that *Alhagi marjoram* is commonly prescribed in folk medicine as an anti-asthmatic, aphrodisiac, antipyretic, appetizer, anti-rheumatic, diuretic, expectorant and laxative [10]. Alkaloids, flavonoids and fatty acids are the major active constituents of this genus [11,12]. Six main flavonoid glycosides such as kaempferol, chrysoeriol, isorhamnetin, chrysoeriol-7-O-xyloside, kaempferol-3-galactorhamnoside and isorhamnetin 3-O- β -D-apio-furanosyl (1-2) β -D-galactopyranoside have been isolated from the ethanol extract of *A. maurorum* [13].

No scientific validation has been done on the whole plant materials of *A. maurorum* as a hepatoprotective agent until now. Therefore, the present study was aimed to evaluate its hepatoprotective activity against paracetamol-induced toxicity in experimental rabbits.

EXPERIMENTAL

Plant collection and identification

The plant was collected from Cholistan Desert of Bahawalpur, Pakistan and the plant material was identified and authenticated by Dr. Shazia Anjum, Director, Cholistan Institute of Desert Studies (CIDS), The Islamia University of Bahawalpur. A specimen (3428/CIDS/IUB) deposited in the Herbarium of CIDS, The Islamia University of Bahawalpur, Pakistan.

Extract preparation

The authenticated shade-dried whole plant of *A. maurorum* approximately (1000 g), was powdered to coarse particle size no. 40. The plant material was soaked in 3L of 70 % aqueous-ethanol for 72 h with occasional shaking and stirring in 5 L beaker. The extracts were separated from the residues by filtering through several layers of muslin cloth for coarse filtration and then through Whatman No. 1 filter paper. The residues were extracted twice with the same fresh solvent and all extracts were then combined. These filtered extracts were concentrated and solvents were evaporated under reduced pressure at 40 °C, using a rotary evaporator (EYELA, CA-1111, Rikakikai Company Limited Tokyo, Japan). The dried crude concentrated extracts were weighed to calculate the percentage yield and stored in a refrigerator (- 8 °C), until used for analyses.

Chemicals

Ethanol, distilled water, paracetamol, diagnostic kits (TB, SGPT, SGOT and ALP) by Human (Germany), xylene, paraffin wax, sodium hydroxide, sodium chloride, aluminum chloride, potassium hydroxide, benzene, ether, sulphuric acid, hydrochloric acid, potassium iodide, mercuric chloride, potassium bromide, eosin, hematoxylin and canada balsam. These chemicals were purchased from the Merck, Germany. Silymarin and pentothal sodium was obtained from the Abbott laboratories, Pakistan. All of the above mentioned chemicals were of analytical grade.

Animals

Healthy adult rabbits of (1000 – 1200 gm) were housed in cages with free access to standard rodent chow diet and water *ad libitum* and acclimatized to the surroundings for one week prior to the experiment. Animals were harbored on a light/dark cycle (12/12 h) at a constant

temperature (25 ± 3 °C) and relative humidity (50 ± 20 %).

Hepatoprotective activity

In the dose-response experiment, rabbits were randomly assigned into 5 groups of 6 individuals each. Group I: Animals (+ve control) were administered 1 mL distilled water p. o, for 7 days. Group II: Animals (negative control) were administered 1 mL distilled water p. o. for 7 days. Group III: Animals were administered silymarin 50 mg/kg p.o, for 7 days. Group-IV: Animals were administered 70 % ethanol extract at 250 mg/kg p. o, for 7 days. Group V: Animals were administered with 70 % ethanol extract 500 mg/kg p.o. for 7 days. Paracetamol (PCM 2 g) was administered orally on day 8th to each group except group 1 in divided doses of 400 mg/kg at 0, 6, 12, 18 and 24 h. The animals were observed for 24 h after the PCM induction. The rabbits were sacrificed under mild ether anesthesia. Blood samples were collected by heart puncture for evaluating serum biochemical parameters like SGOT, SGPT, ALP and TB [14]. The liver samples were dissected out, washed with normal saline and preserved it in 10 % formalin for histopathological studies to evaluate microscopically the details of hepatic architecture in each group [15].

Histopathological examination

A section of the liver was collected and immediately fixed in 10 % formalin, and then dehydration in ascending grades of alcohol (ethanol) of 70, 80 and 95 % and absolute alcohol for 2 changes each. The tissues were cleared in xylene and embedded in paraffin wax. Serial section of 5-6 microns in thickness were obtained using rotary microtome and stained with hematoxylin and eosin. The stained sections were examined under microscope for analyzing any changes in the architecture of the liver tissue

due to paracetamol challenge and improved liver architecture due to pre-treatment with test extracts and standard drug [15].

Phytochemical screening

The extract of *A. maurorum* was screened for the presence of carbohydrate, alkaloids, triterpenoids, saponins, phenols, sterols and flavonoids by following standard procedures [16].

Statistical analysis

Results were expressed as Mean \pm SEM, (n=6). Statistical analyses were performed with one way analysis of variance (ANOVA) followed by tukey's multiple comparison test by using Graph Pad Prism Software. $P < 0.05$ was considered to be statistically significant.

RESULTS

Phytochemical profile

The extract of *A. maurorum* was positive for alkaloids, saponins, tannins, triterpenoids and flavonoids.

Hepatoprotective activity

A significant increase in serum SGPT, SGOT, ALP and total bilirubin level was observed in paracetamol (2 gm/kg s. c.) intoxicated rabbits. Pretreatment with *A. maurorum* (250 mg/kg p.o.) for 7 days decreased the above parameters significantly ($p < 0.01$) while pretreatment with *A. maurorum* (500 mg/kg p.o.) for 7 days produced maximum reduction in liver enzymes and considered statistically more significant ($p < 0.001$). The hepatoprotective effect at dose of 500 mg/kg was almost comparable to standard drug silymarin (Table 1).

Table 1: Effect of *Alhagi maurorum* on liver enzymes in paracetamol induced hepatotoxicity

Group	Treatment	SGPT (IU/L)	SGOT (IU/L)	ALP (IU/L)	Total bilirubin (mg/dL)
I	Normal	70.5 \pm 1.88	71.1 \pm 1.31	190 \pm 2.66	0.71 \pm 0.05
II	Paracetamol	145 \pm 10.47	128.8 \pm 1.98	305 \pm 2.12	1.29 \pm 0.18
III	Sylimarin	94 \pm 5.41**	83.8 \pm 3.37**	270 \pm 3.55**	0.62 \pm 0.03**
IV	<i>Alhagi maurorum</i> 250 mg/kg	108.3 \pm 5.72*	99.17 \pm 1.9**	286 \pm 5.20*	0.98 \pm 0.03*
V	<i>Alhagi maurorum</i> 500 mg/kg	99.5 \pm 5.65**	98.33 \pm 2.24**	269 \pm 1.89**	0.90 \pm 0.03**

* $p < 0.01$, ** $p < 0.001$ compared to paracetamol group (one-way ANOVA followed by Tukey's multiple comparison tests); values are mean \pm SEM (n = 6)

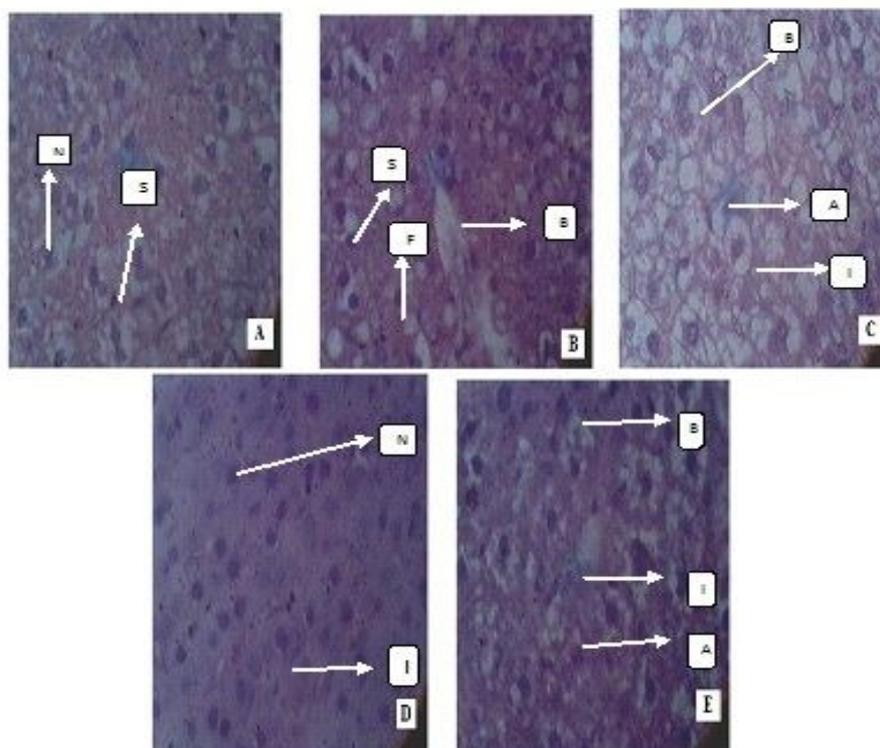


Fig 1: Photomicrography of liver sections in paracetamol induced hepatotoxicity in rabbits. A (Liver architecture of Normal); B: (Liver architecture of paracetamol treatment + 50 mg/kg Silymarin treatment); C: (Liver architecture of paracetamol treatment); D: Liver architecture paracetamol treatment + 500 mg/kg of *Alhagi maurorum*; and E: (Liver architecture paracetamol treatment + 250 mg/kg of *Alhagi maurorum*). **Key:** (N = nucleus, S = sinusoid, B = ballooning-degeneration, F = fibrosis, I = inflammation and A = apoptosis)

Histopathological features

Histopathological examination of liver sections of normal rabbits showed normal hepatic cells with cytoplasm and nucleus whereas paracetamol-treated group showed various degree of fatty degeneration like ballooning of hepatocytes, infiltration of lymphocytes and the loss of cellular boundaries. Administration of *A. maurorum* at dose of 500 mg/kg significantly normalized these defects in the histological architecture of the liver (Figure 1).

DISCUSSION

The current study was designed to evaluate the hepatoprotective potential of the *A. maurorum*. This medicinal plant of Bahawalpur Cholistan desert has been reported to possess hepatoprotective effects in some previous reports. Its hepatoprotective activity of aerial parts in ethanol extract was evaluated against carbon tetrachloride intoxicated Wistar albino rats [17].

Paracetamol is generally used as an analgesic and antipyretic drug [18]. When taken in overdose (200 mg/kg), it produces potent

hepatotoxicity, therefore it is widely used as a hepatotoxicant in experimental animals [19]. SGPT, SGOT, ALP and serum bilirubin level are commonly used biochemical parameters to evaluate liver injury. On induction of paracetamol hepatotoxicity, the SGPT, SGOT, ALP and bilirubin levels increase in the circulation because they are cytoplasmic in location and released into circulation after cellular damage [18]. The elevated level of these entire marker enzymes observed in the group II, paracetamol treated rabbits, corresponded to the extensive liver damage induced by toxin in our study. The levels of these biomarkers in pre-treated animals with *A. maurorum* (500 mg/kg) were found to be lower than the paracetamol intoxicated group indicating that this dose can protect the paracetamol induced hepatic damage. *A. maurorum* possesses flavonoids and tannins which were confirmed by phytochemical analysis of the extract and these both groups are well recognized for their hepatoprotective action. Saponins, alkaloids, flavonoids and triterpenoids are phytochemical constituents of *A. maurorum* possessing antioxidant, free radical scavenging ability and inhibition of lipid peroxidation [20,21]. Silymarin isolated from *Silybum marianum* is a well-known hepatoprotective compound and

shown to have a protective effect on plasma membrane of hepatocytes and possesses multiple inhibitory effects against different hepatotoxic agents. The antioxidant effects and cell regenerating functions of silymarin involved protein synthesis and this was considered as most important actions of silymarin. The hepatoprotective activity observed in the present study may be due to the protective effect of *A. maurorum* on the plasma membrane of the hepatocytes or to cell regenerating function similar to that of silymarin, the reference drug used in the study [22].

The histopathological findings reveal that *A. maurorum* extract showed excellent protection on liver architecture, almost to the level of the silymarin-treated groups, validating its hepatoprotective effects in the experimental animal.

CONCLUSION

The findings of this study indicate that the aqueous-ethanol extract of *A. maurorum* at a dose of 500 mg/kg exhibits significant hepatoprotective activity by reducing elevated levels of biochemical enzymes. Further studies are required to explore the active principle responsible for this hepatoprotective activity.

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