Hepatoprotective activity of Stem of *Abutilon indicum* (L.) Sweet an indigenous medicinal plant against Paracetamol induced Liver toxicity in Albino rats

Sumeet Dwivedi[1*], Prakash Chandra Patel[2], Arvind Kumar Tripathi[3], Rahul Shriramsa Bijwar[4], Pankaj Kumar Sahu[5] and Surbhi Gujrathi[6]

1, Acropolis Institute of Pharmaceutical Education and Research, Indore (M.P.) – India
2, Department of Botany, Govt. S.K. College, Mauganj, Rewa, (M.P.) – India
3, Department of Biotechnology, APS University, Rewa, (M.P.) – India
4, Jagadambha Institute of Pharmacy & Research, Kalamb, Yavatmal, (M.H.) – India
5, Department of Botany, Govt. S.S.P. College, Waraseoni, Balaghat, (M.P.) – India
6, Faculty of Pharmacy, Oriental University, Indore (M.P.) – India

Abstract

Liver disease is a collective term for a group of disorders that damage the tissues, structures, and cells of the human liver. Many important functions are performed by the liver, so it is open to many mistakes. One of the most common causes of liver disease is inflammation, often caused by alcohol, poor diet, or malnutrition. drug-induced liver injury or liver failure is a major health crisis that challenges not only medical professionals, but also the pharmaceutical industry and the Drug Control Board. According to the American Acute Liver Failure Study Group, more than 50% of cases of acute liver failure involve idiosyncratic liver injury caused by other drugs, including hepatotoxicity caused by acetaminophen overdose (39%).The present paper deals with the hepatoprotective activity of hydro-alcoholic extract of *Abutilon indicum* (Stem) against paracetamol induced liver toxicity in albino rats. The results indicate that hydro-alcoholic extract showed significant hepatoprotective activity as compared to the hepatotoxic control at the dose of 200 mg/kg.

Keywords: Liver disorders, *Abutilon indicum*, Paracetamol induced

***Corresponding Author**

E.mail: herbal0914@rediffmail.com, drsumeetdwivedi@acropolis.edu.in

Introduction

In the last few years there has been an exponential growth in the field of herbal medicine and these drugs are gaining popularity both in developing and developed countries because of their natural origin and less side effects. Many traditional medicines in use are derived from medicinal plants, minerals and organic matter. A number of medicinal plants, traditionally used for over 1000 years named rasayana are present in herbal preparations of Indian traditional health care systems. In Indian systems of medicine most practitioners formulate and dispense their own recipes. The World Health Organization (WHO) has listed 21,000 plants, which are used for

medicinal purposes around the world. Among these 2500 species are in India, out of which 150 species are used commercially on a fairly large scale. India is the largest producer of medicinal herbs and is called as botanical garden of the world. [1-3]

Indian Indigenous medicinal plants are most widely used for the treatment of several diseases either in alone or in combination in raw as well as their extract. Synthetic hepatoprotective agents can produce several serious effects and also they are not suitable to use during pregnancy. In this light herbals are preferred in the treatment of liver disorders. Ancient ayurvedic literature reveals that the selected plant i.e., Abutilon indicum (Stem) have been widely used in the treatment of liver disorders. The plants have been extensively used in ayurveda and traditional system of medicine for the treatment of liver disorders and found to be efficient and inexpensive as compared to synthetic drugs and not evaluated scientifically. Therefore, it was worthwhile to investigate the hepatoprotective activity of *Abutilon indicum* (stem).

Abutilon indicum (Linn.) Sweet commonly known as Kanghi (H) belongs to family Malvaceae**.** The plant is Found in wild state in Central India. The plant contains saponins, flavonoids, alkaloids. The important constituents reported in the plant are β-sitosterol, vanillic acid, p-coumaric acid, caffeic acid, fumaric acid. The leaves of the plant contain steroids, sapogenins, carbohydrates and flavonoids. Almost all the parts are of medicinal importance and used traditionally for the treatment of various ailments. The roots of the plant are considered as demulcent, diuretic, in chest infection and urethritis. The infusion of the root is prescribed in fevers as a cooling medicine and is considered useful in strangury, haematuria and in leprosy. The leaves are found to be good for ulcer and to treat liver disorders. The bark is used as febrifuge, anthelmintic, alexeteric, astringent and diuretic. The seeds are used in piles, laxative, expectorant, in chronic cystitis, gleet and gonorrhea. [4]

Material and Methods

Collection of herbs and their authentication

The stem of *Abutilon indicum* was collected from local sites of Malwa region of Madhya Pradesh, India during January 2023 and identified morphologically, microscopically and compared with standard pharmacopoeial monograph and authenticated by Dr. S. N. Dwivedi, Retd. Prof. and Head, Department of Botany, Janata PG College, A.P.S. University, Rewa, (M.P.) and was deposited in our Laboratory. Voucher specimen No. J/Bot./AIS-033 was allotted to the selected plant parts.

Extraction of selected herbs

The shade dried coarsely powdered plant material (250 gms) of stem of *Abutilon indicum* was loaded in Soxhlet apparatus and was extracted with ethanol:water (90:10) for 48 hour. After completion of extraction, the solvent was removed by evaporation. The extracts were dried using rotator evaporator. The residue was then stored in dessicator. [5-6]

Pharmacological screening

Acute Toxicity Studies of Extracts

The mice were used for acute toxicity study as per OECD guidelines 423. The animals were fed with standard pellet diet (Hindustan lever Ltd. Bangalore) and water *ad libitum*. All the animals were housed in polypropylene cages. The animals were kept under alternate cycle of 12 hours of darkness and light. The animals were acclimatized to the laboratory condition for 1 week before starting the experiment. The experimental protocols were approved by Institutional Animal Ethics Committee after scrutinization. IEAC approval. [7]

Hepatoprotective activity of extracts

Test Compounds

The hydroalcoholic extracts of *Abutilon indicum* and standard drug silymarin (50 mg/kg body weight) were used.

Chemicals and Reagents

Paracetamol, Silymarin.

Experimental Animal

Albino rats (200-250 g) used in the present studies was procured. The animals were fed with standard pellet diet (Hindustan lever Ltd. Bangalore) and water *ad libitum*. All the animals were acclimatized for a week before use.

Paracetamol Induced Model

The rats were divided into 11 groups of 6 animals in each. [8-10]

S/No.	Group	Treatments
1.	Group I (Normal)	Received vehicle gum acacia (5mg/kg p.o) for 7days
2.	Group I (Control)	Received vehicle gum acacia (5 mg/kg p.o) for 7 days once daily and paracetamol 500mg/kg once daily
3.	Group III (Standard)	Received silymarin as standard (50 mg/kg) for 7 days once daily and paracetamol 500mg/kg once daily
4.	Group IV	Received HAEAIS (100 mg/kg) once daily and paracetamol 500mg/kg once daily
5.	Group V	Received HAEAIS (200 mg/kg) once daily and paracetamol 500mg/kg once daily

On the seventh day, the blood samples were collected via orbital sinus puncture for the estimation of biochemical marker enzymes and allowed to clot and serum was separated by centrifuge at 2500 rpm for 15 min and analyzed for various biochemical parameters. Then the liver was carefully isolated and cleaned off extraneous tissue and preserved in 10% neutral formalin and then subjected to histopathological studies. [8-10]

Statistical Analysis

All the values ware statistically analyzed by one-way analysis of variance (ANOVA) followed by Dunnette multiple Comparisons test. Statistically significance of * $P<0.01$, ** $P<0.001$, when compared with respective control. All values are expressed as mean ± SEM.

Assessment of Liver Function

The liver was removed and weighed**.** Biochemical parameters i.e., Serum glutamic Pyruvate transaminase (SGPT), serum Glutamic Oxaloacetic Transaminase (SGOT), Alkaline phosphatase (ALP) were analyzed according to the reported methods. [8-10]

Histopathological Studies

Liver slices fixed for 12 hrs in Bouin's solution were processed for paraffin embedding following standard micro technique. 5µ section of the livers stained with alum haematoxylin and eosin, were observed microscopically for histopathological changes i.e., normal liver, damaged and recovered liver were studied and compared. [8-10]

Results and Discussion

The hydro-alcoholic extracts of stem of *Abutilon indicum* was screened for acute toxicity study by OECD guideline no. 423 for determination of $LD_{50.}$ The results showed that the hydro-alcoholic extracts were belonging to category-4. Hence, LD_{50} was 2000 mg/kg, therefore, ED_{50} was 200 mg/kg. Therefore, two doses of 100 and 200 mg were selected for present investigation. The results were presented in table 1.

The liver plays an important role in regulating physiological processes. Several functions are involved, including metabolism, secretion, and storage. In addition, detoxification of various drugs and xenobiotics takes place in the liver. Bile, secreted by the liver, plays an important role in digestion, among others. Liver disease is the worst disease. The results of the biochemical parameters revealed that the elevated enzyme levels in the paracetamol-treated group were almost restored to normal levels in the extract-treated group. The hydro-alcoholic extracts of stem of *Abutilon indicum* showed significant hepatoprotective activity as they reduced SGPT as compared to the hepatotoxic control at the dose of 200 mg/kg. The results of treatment and extract are shown in the table. SGPT is a cytosol enzyme present mainly in the liver. Serum SGPT levels are elevated due to plasma leakage of this cellular enzyme in paracetamol-induced liver injury. SGPT serum levels may increase due to tissue damage resulting in hepatic necrosis. Since the extract significantly reduced SGPT levels, this suggests that this extract has significant hepatoprotective activity. The hydro-alcoholic extract of Abutilon leaves showed significant hepatoprotective activity, as hepatotoxicity was reduced compared to the control at a dose of 200 mg/kg. The results of treatment and transplantation are shown in the table. SGOT is a mitochondrial enzyme secreted from the heart, liver, skeletal muscle, and kidney. Hepatotoxicity, such as multiple viral hepatitis and acute cholestasis, has elevated serum SGOT levels due to tissue damage resulting in acute necrosis. Since the extract significantly reduced SGOT levels, this suggests that this extract has significant hepatoprotective activity. The hydro-alcoholic extract of Abutilon leaves showed significant hepatoprotective activity, as hepatotoxicity was reduced compared to the control at a dose of 200 mg/kg. The results of treatment and transplantation are shown in the table. In the case of toxic liver, the level of gastric phosphate is too high and may be due to increased hepatic secretion or increased production of ALP by liver parenchyma or duct cells. Since the extract significantly reduced ALP levels, it suggests that this extract has significant hepatoprotective activity. Liver weight of animals treated with hydro-alcoholic extract of Abutilon stem index compared with treatment of conventional drug silymarin (50mg/kg). The hydro-alcoholic extract showed a significant decrease in liver weight similar to the conventional drug silymarin, thus indicating a significant hepatoprotective activity of the extract. The results was presented in table 2.

Table 1: Determination of LD_{50} and ED_{50} of hydro-alcoholic extract of *Abutilon indicum*

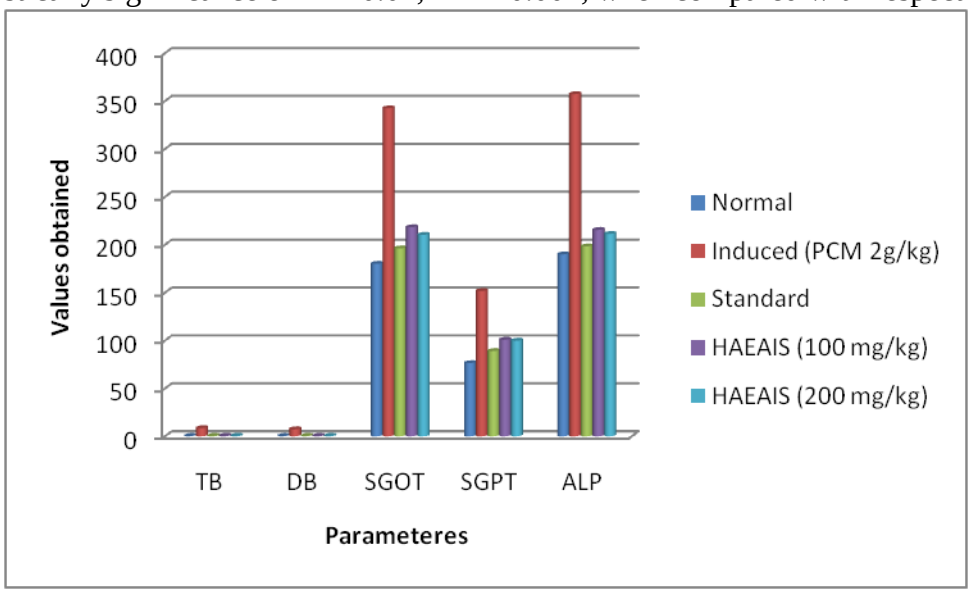
No. of Animals	Extract Dose (mg/kg)	No. of death of animals			
		AEAIL	AEPNF	AEEAL	AEASB
3	5	0	0	0	0
3	50	0	0	0	0
3	300	0	0	0	0
3	2000	0	0	0	0

Table 2: Effect of hydro-alcoholic extract of stem of *Abutilon indicum* on paracetamol induced hepatotoxicity in rats

Treatment	Total Bilirubin (mg %)	Direct Bilirubin (mg %)	SGOT (µ/min/l)	SGPT (µ/min/l)	ALP (µ/min/l)

Normal	0.42 ± 0.20	0.42 ± 0.60	180.12 ± 2.11	76.40 ± 2.22	190.0 ± 6.22
Induced (PCM 2g/kg)	8.60 ± 2.02	7.41 ± 8.61	342.40± 10.02	151.7± 8.04	357.22±8.80
Standard (Silymarin 50mg/kg)	0.51 ±4.09**	0.48 ±0.18**	196.21±9.03**	89.07±8.72**	198.21 ±10.11**
HAEAIS (100 mg/kg)	0.60 ± 4.21*	0.52 ± 0.09*	218.31± 8.18*	100.82± 4.51*	215.49± 9.01*
HAEAIS (200 mg/kg)	0.59 ± 4.11*	0.51 ± 0.12*	210.32± 8.22*	99.82± 4.521*	211.31± 9.02*

Values are mean ±SEM, n= 6. (One way ANOVA Followed by Dunnette multiple Comparisons test). Statistically significance of * P<0.01, ** P<0.001, when compared with respective control

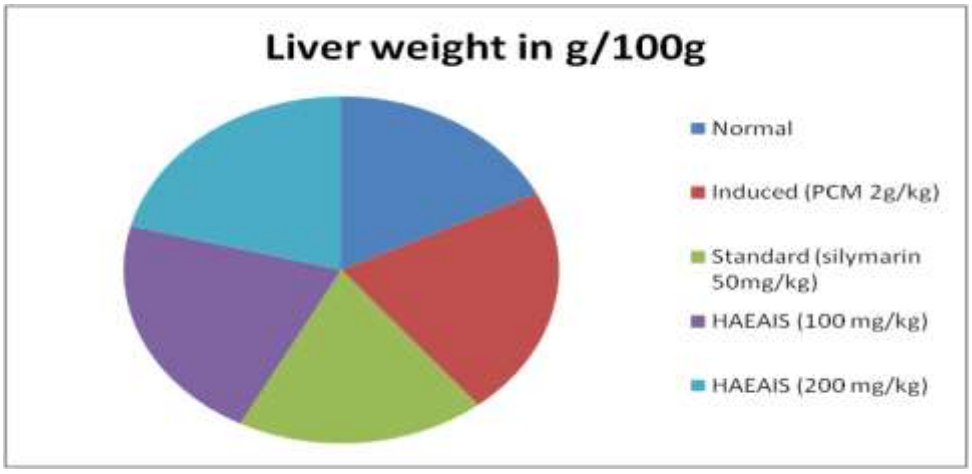


Graph 1: Estimation of parameters of extracts on paracetamol induced hepatotoxicity in rats

Table 3: Effect hydro-alcoholic extract of stem of *Abutilon indicum* on liver weight variation of paracetamol induced hepatotoxicity in rats

Treatment	Liver weight in g/100g
Normal	6.80 ± 0.42
Induced (PCM 2g/kg)	8.21 ± 0.22
Standard (silymarin 50mg/kg)	7.11 ± 0.23**
HAEAIS (100 mg/kg)	8.18 ± 0.02*
HAEAIS (200 mg/kg)	8.03± 0.12

Values are mean ±SEM, n= 6. (One way ANOVA Followed by Dunnette multiple Comparisons test). Statistically significance of * P<0.01, ** P<0.001, when compared with respective control.



Graph 3: Liver weight in g/100g of aqueous extracts on paracetamol induced hepatotoxicity in rats

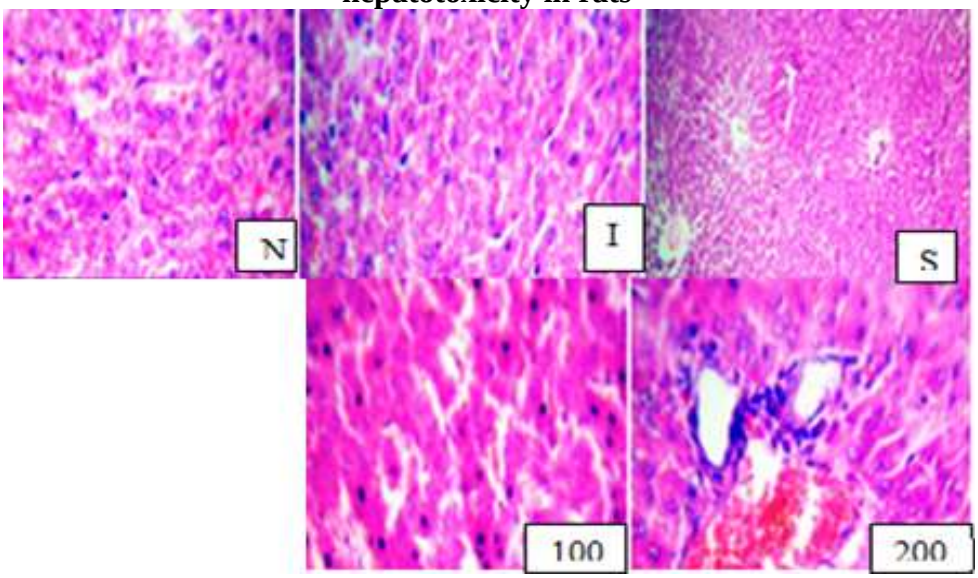


Fig. 1: Histopathologic section of liver of rats in paracetamol induced hepatotoxicity

Normal: The architecture is normal. The central veins, sinusoids and portal triads appear normal. The hepatocytes show moderate cytoplasm and round to oval nuclei. There is no periportal inflammation. **Paracetamol induced (500mg/kg)**: The central veins show dilatation and congestion. The hepatocytes show feathery degeneration. The portal triads show mild peri-portal inflammation composed of lymphocytes. **Silymarin (50mg/kg):** The central veins appear normal. The hepatocytes show feathery degeneration. The portal triads show mild peri-portal inflammation composed of lymphocytes. **HAEAIS (100 mg/kg):** The hepatocytes show moderate cytoplasm and moderately enlarged pleomorphic and hyperchromatic nuclei. The portal triads show mild peri-portal inflammation composed of lymphocytes. The central veins are normal. **HAEAIS (200 mg/kg):** The architecture is normal. The central veins, sinusoids and portal triads appear normal. The hepatocytes show show moderate cytoplasm and round to oval nuclei.

Conclusion

Liver disorders are very common and worrisome. In the present study, the hepatoprotective activity of hydroalcoholic extract of stem of Abutilon indicum an indigenous medicinal plants in the liver treated with paracetamol against. toxicity in albino rats was investigated and the results showed that the extract showed significant hepatoprotective activity compared to the hepatotoxic control at a dose of 200 mg/kg.

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