ISSN 0976 – 3333

Available Online at www.ijpba.info.

International Journal of Pharmaceutical & Biological Archives 2011; 2(1):541-545

ORIGINAL RESEARCH ARTICLE

Hepatoprotective and Antioxidant Role of Flower Extract of *Abutilon indicum* .

P.Revansiddaya.[1],Biradar Kalyani.*[2], A.Veerangouda[1].H.Shivkumar[1]., Payghan Santosh .[3]

1) Department of pharmacology SCS College of pharmacy Harapanahalli Karnataka
2) HKES'S Matoshree Taradevi Rampure Institute of Pharmaceutical Sciences Gulbarga, karnataka
3.) Dr. V.K. Patil Pharmacy College, Aurangabad, (M.S).

Received 18 Dec 2010; Revised 16 Jan 2011; Accepted 28 Jan 2011

ABSTRACT

The hepatoprotective activity of the 70% Ethanolic extract of *Abutilon indicum* flowers was assessed using CCl_4 induced hepatotoxicity in albino rats. The degree of protection against liver toxicity was determined by measuring the biochemical markers such as SGPT, SGOT, ACP, ALP and Bilirubin (Direct and Total). In addition morphological changes of liver like wet liver volume and wet liver weight were recorded. Further, histopathological examination of the liver was also studied. Silymarin at the dose of 25 mg/kg, p.o. was used as reference standard drug and it exhibited significant protection. In addition to the screening of hepatoprotective activity, in vitro antioxidant activity was studied by reducing power activity, superoxide anion scavenging activity and hydroxyl radical scavenging activity methods. Sodium meta bisulphate was used as a reference standard drug.

Keywords: *Abutilon indicum*; Hepatoprotective; Antioxidant; CCl_4.

INTRODUCTION:

Liver has a pivotal role in regulation of physiological processes. It is involved in several vital functions such as metabolism, secretion and storage. Furthermore, detoxification of a variety of drugs and xenobiotics occurs in liver. Liver diseases are among the most serious ailments. They may be classified as acute or chronic hepatitis (inflammatory liver diseases), hepatosis (non inflammatory diseases) and cirrhosis (degenerative disorder resulting in fibrosis of the liver). Liver diseases are mainly caused by toxic chemicals (certain antibiotics, chemotherapeutics, peroxidised oil, aflatoxin, carbon-tetrachloride, paracetamol, chlorinated hydrocarbons, etc.), excess consumption of alcohol, infections and autoimmune disorder. So it has become very much necessary to protect the liver from all these agents.

In spite of the tremendous advances made in allopathic medicine, no effective hepatoprotective medicine is available till date. Plant drugs are known to play a vital role in the management of liver diseases. In India, more than 87 medicinal plants are used in different combinations in the preparation of 33 patented herbal formulations[1-4].

In the traditional system of medicine there are numerous plants and polyherbal formulations have been used in liver diseases. But only a small portion of them have been

Pharmacologically evaluated for their efficacy. Still more number of medicinal plants is needed to be investigated for their antihepatotoxic effect.

Abutilon indicum (Malvaceae) is distributed throughout the hotter parts of India. It has been reputed in Siddha system of medicine as remedy for jaundice, piles, ulcer and leprosy[5]. The plant is reported to be having analgesic[6] and antifertility[7] properties. The flowers of the *Abutilon indicum* are known to contain flavonoids[8]. The leaf extract of *Abutilon indicum* has been already reported for the hepatoprotective activity[9,10].However, the literature survey afforded no scientific investigation on hepatoprotective property of flowers of the title plant so far. Hence, the present study was undertaken.

MATERIALS AND METHODS:

The plant material and preparation of extracts:

***Corresponding Author:** BiradarKalyani, **Email:** biradarkb76@gmail.com, **Contact No:** +91-9620631443

The flowers of *Abutilon indicum* were collected during the month of Feb-March, 2007 from the surrounding fields of Harapanahalli. The plant was authenticated by Professor K. Prabhu, Department of Pharmacognosy, S.C.S.College of Pharmacy, and Harapanahalli. A voucher specimen has been deposited at the museum of our college. The dried powder of flowers was extracted by using 70% ethanol in soxhlet apparatus (Yield 12.52%). The preliminary phytochemical screening was carried out on 70% ethanolic extract of *Abutilon indicum* flowers for the detection of photo chemicals present by following the literature reported methods [11,12].

The Experimental animals and acute toxicity studies:

The male albino rats weighing 150 – 200 g and female albino mice 20–30 g was used for the experimentation. The animals were procured from Venkateshwara associates, Bangalore, Karnataka. After randomization into various groups, animals were acclimatized for the period of 10 days under standard husbandry conditions.

- Room temperature 27 ± 3^0c.
- Relative humidity $65 \pm 10\%$.
- 12 hrs. Light / dark cycle.

All the animals were fed with rodent pellet diet (Gold Mohr, Lipton India Ltd.,) and water was allowed *ad-labium* under strict hygienic condition. Ethical clearance was obtained from Institutional Animal Ethics Committee (IAEC) before initiation of the experiments.

The acute toxicity of 70% ethanolic extract of *Abutilon indicum* flowers was determined in female albino mice weighing 25 – 30 g. The animals were fasted over night prior to the dosing. Fixed dose (OECD Guideline No. 420) method of CPCSEA was adapted for toxicity studies.

CCl_4 induced hepatotoxicity[14, 15]

Albino rats weighing 150 – 200 g were divided into six groups of each containing six animals.

Group I – Negative control (received vehicle, distilled water 1 ml/kg, p.o.),
Group II – Positive control (CCl_4 1 ml/kg, i.p.),
Group III – Standard (Silymarin 25 mg/kg, p.o.),
Group IV – EEAIF (100 mg/kg, p.o.),
Group V – EEAIF (250 mg/kg, p.o.) and
Group VI – EEAIF (500 mg/kg, p.o.)

Animals were treated as shown above for a period of 10 days. At the end of every 72 hrs. i.e. 4th day, 7th day and 10th day CCl_4 (30% in liquid paraffin 1 ml/kg, i.p.) was administered to all groups other than group I. Group III received standard drug silymarin25 mg/kg p.o. once a day and CCl_4 as mentioned above. Whereas group IV, V and VI were treated with test extract dose of (100, 250 and 500 mg/kg, p.o.) respectively. During this period of treatment, the rats were maintained under normal diet and water. The biochemical parameters were determined after 24 hrs. After the last dose of CCl_4 i.e. on 11th day. All the animals were sacrificed.

Blood was collected by carotid bleeding under mild ether anesthesia using disposable syringe and needle. Blood was allowed to clot at room temperature for 30 min. then subjected to centrifugation (3000 rpm for 15 min.) and estimation of biochemical parameters namely SGPT, SGOT, ALP, ACP, Bilirubin (Total and Direct).

The liver was dissected out and subjected for morphological study such as wet liver weight and wet liver volume. The volume of wet liver was measured by displacement method and further the livers were placed in 10% formalin solution for histopathological study[16].

In vitro antioxidant activity

Reducing power[17]

Different doses of ethanolic extract of *Abutilon indicum* flowers were mixed in 1 ml of

Distilled water so as to get 10 μg, 25 μg, 50 μg and 100 μg concentration. This was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 ml, 1%). The mixture was incubated at 50^0c for 20 minutes. A portion (2.5 ml) of trichloroacetic

Acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%), and the absorbance (OD) was measured at 700 nm.

% inhibition was calculated by using the following formula

$$\% \text{ Inhibition} = \frac{\text{Control OD} - \text{Test OD}}{\text{Control OD}} \times 100$$

Superoxide anion scavenging activity[18]

About 1 ml of nitro blue tetrazolium (NBT) solution (156 μM NBT in 100 mM phosphate buffer, pH 7.4). 1 ml NADH solution (468 μM in 100 mM phosphate buffer, pH 7.4) and 0.1 ml of sample solution of ethanolic extract of *Abutilon indicum* flowers in water was mixed. The reaction was started by adding 100 μl of Phenazine Methosulphate (PMS) solution (60 μM PMS in 100 mM phosphate buffer, pH 7.4) to the mixture. The reaction mixture was incubated at 25^0c for 5 minutes and the absorbance at 560 nm was measured against blank. Increased absorbance of

the reaction mixture indicates increase in reducing power. The percentage increase was calculated by using the formula mentioned in the estimation of reducing power scavenging activity. Decreased absorbance of the reaction mixture indicates increased superoxide anion scavenging activity.

Hydroxyl radical scavenging activity[19]

Hydroxyl radical generation has been measured by 2-deoxyribose degradation, assay of Hathwell and Gutterridge in 56 mM phosphate buffer (pH 7.4) containing 1 mM deoxyribose, 0.2 mM phenylhydrazine hydrochloride and other additions as necessary in a total volume of 1.6 ml incubating was terminated after 1 hr or 4 hrs and 1 ml each of 2.8% TCA and 1% (w/v) thiobarbituric acid were added to the reaction mixture and heated for 20 min in a boiling water bath. The tubes were cooled and absorbance taken at 532 nm.Decrease in absorbance indicates the increase in hydroxyl free radical scavenging activity. The percentage reduction in the absorbance is calculated.

Statistical analysis

The results obtained were subjected to statistical analysis using ANOVA followed by Turkey-Kramer Multiple Comparison Test.

Results:

Administration of CCl_4 resulted in a significant rise in the levels of SGPT, SGOT, ALP, ACP and Bilirubin (Total and Direct) when compared to the vehicle treated group (Group-I). Pre-treatment with test extract significantly reduced the elevated levels of biochemical parameters in dose dependent manner. The results indicated that the effect of test extract on biochemical markers was found to be less potent than the reference standard, Silymarin. The results are shown in (**Table-1**)

Intoxication of rats with CCl_4 resulted in enlargement of liver which was pale reddish brown. Rats subjected to the CCl_4 challenge developed significant increase in the morphological parameters like wet liver weight and wet liver volume when compared to negative control group (**Table-2**). Oral administration of the test extract exhibited dose dependent significant reduction in the morphological parameters. Treatment with reference standard, silymarin (25 mg/kg, p.o.) also reversed increased morphological parameters significantly. Organ protective potency of the test extract at the dose of 500 mg/kg was found closer to that of standard.

Histopathological profile of liver in CCl_4 (Group-II) intoxicated rats shown the fatty degeneration of hepatocytes, hepatic cell necrosis, portal tract fibrosis and presence of fatty cyst. The sinusoids of liver were congested and the central vein of globule was constricted. Administration of test extract at the dose of 500 mg/kg shown a significant recovery in the hepatic architecture. The sinusoids are recovered, The globule was normal and hepatocytes are improved. However, there was a improvement in the hepatic architecture observed in rats treated with 100 mg/kg and 250 mg/kg of test extract.

Ethanolic extract of *Abutilon indicum* flowers demonstrated concentration dependent (10, 25, 50 and 100 µg) significant antioxidant activity against reducing power, superoxide anion scavenging and hydroxyl radical scavenging activity. The antioxidant potential of the higher concentration (100 µg) of the test extract was seems to be more potent than that of reference standard drug in case of superoxide anion scavenging activity and hydroxyl radical scavenging activity. But the antioxidant effect of the test extract was found to be less potent than the standard drug in reducing power activity (**Table-3,4 & 5**).

Table 1: Effect of EEAIF on biochemical parameters in CCl_4 induced hepatoxicity

Group	Biochemical parameters Mean ± SEM					
	SGPT IU/L	SGOT IU/L	ALP IU/L	ACP IU/L	TB mg/dl	DB mg/dl
I	105.70 ± 3.251	119.39 ± 6.636	300.37 ± 1.823	30.46 ± 1.821	0.3680± 0.002	0.291 ± 0.003
II	247.77 ± 9.423	367.57 ± 9.112	522.56 ±7.145	56.11 ± 0.749	0.625 ± 0.011	0.582 ± 0.013
III	147.60 ± 3.530***	163.91 ± 3.513***	341.42 ± 3.632***	38.69 ± .210***	0.417 ± 0.004***	0.377 ± 0.016***
IV	228.13±3.536 *	341.29±1.220*	500.56±6.640*	53.78±0.256*	0.591±0.002*	0.542±0.003*
V	221.21 ± 2.950**	332.30 ± 7.281**	474.87 ± 3.141***	51.13 ±0.234**	0.586 ± 0.005**	0.534 ± 0.003**
VI	169.39 ± 0.713***	203.31 ± 4.437***	368.92 ± 4.419***	43.96 ± .445***	0.461 ± 0.009***	0.367 ± 0.004***

Values are mean ± SEM (n = 6)

$P < 0.05$*, 0.01** and 0.001*** as compared to +ve control.

Table 2: Effect of EEAIF on morphological parameters in CCl_4 induced hepatoxicity

Group	Liver wt. in g /100 g b.w.	Liver volume in ml / 100 g b.w.
I	3.018 ±0.042	3.163 ±0.023
II	4.235 ±0.021	4.685 ±0.037
III	3.182 ±0.313***	3.235 ± 0.026***
IV	4.051 ±0.036*	4.500 ± 0.037*
V	4.015 ±0.048**	4.345 ± 0.040**
VI	3.335 ±0.084***	3.667±0.066***

Values are mean ± SEM (n = 6)
$p < 0.05$*, 0.01** and 0.001*** as compared to +ve control.

Table 3: Effect of EEAIF on reducing power activity

Group	Absorbance Mean ± SEM	% Inhibition
Control	0.146 ± 0.0005	-
Standard 25 µg	0.250 ± 0.004***	75%
Ethanolic extract 10 µg	0.040 ±0.0003*	16%
Ethanolic extract 25 µg	0.093 ± 0.0004**	
Ethanolic extract 50 µg	0.121 ±0.0003***	48%
Ethanolic extract 100 µg	0.166 ± 0.0004***	66%

Values are mean ± SEM
$p < 0.05$*, 0.01** and 0.001*** as compared to control.
Standard – Sodium Meta bi sulphate (SMBS)

Table 4:Effect of EEAIF on superoxide scavenging activity

Group	**Absorbance Mean ± SEM**	**% Inhibition**
Control	0.585 ±0.004	-
Standard 25 µg	0.287 ±0.003***	71%
Ethanolic extract 10 µg	0.390 ±0.004*	62%
Ethanolic extract 25 µg	0.339 ±0.005*	66%
Ethanolic extract 50 µg	0.295 ±0.003***	70%
Ethanolic extract 100 µg	0.274 ±0.004***	73%

Values are mean ± SEM
$p < 0.05$*, 0.01** and 0.001*** as compared to control.
Standard – Sodium Meta bi sulphate (SMBS)

Table 5: **Effect of EEAIF on hydroxyl radical scavenging activity**

Group	Absorbance Mean ± SEM (after 1 hr.)	Percentage inhibition	Absorbance Mean ± SEM (after 4hr.)	Percentage inhibition
Control	0.078 ±0.001	-	0.069 ±0.0005	-
Standard (SMBS)	0.029 ± 0.001***	66.95%	0.027 ± 0.0005***	69.15%
Ethanolic extract 10µg	0.053 ±0.002*	40.6%	0.047 ± 0.004***	41.2%
Ethanolic extract 25µg	0.049 ±0.001**	49.3%	0.041 ± 0.006***	52.1%
Ethanolic Extract 50µg	0.033 ±0.002***	58.79%	0.030 ±0.005***	59.54%
Ethanolic extract 100µg	0.024 ±0.001***	69.9%	0.022 ±0.004***	71%

Values are mean ± SEM
$p < 0.05$*, 0.01** and 0.001*** as compared to control.
Standard – Sodium Meta bi sulphate (SMBS)

DISCUSSION:

Liver injury induced by CCl_4 is a commonly used model for the evaluation of hepatoprotective agents[20,21].

Administration of CCl_4 elevated the serum levels of SGOT, SGPT, ALP, ACP and bilirubin (Total and direct) significantly due to its enzymatic activation of $CCl^{\bullet}_3$ free radical, which in turn alters the structure and function of liver cells[22].

The results of the present study reveal that 70% Ethanolic extract of *Abutilon indicum* flowers (100,250 and 500 mg / kg, p.o.) exhibited protective action against both CCl_4 induced liver damage in a dose related fashion. The amelioration of liver toxicity by the test extract was evident from its significant effect on serum enzyme levels and morphological parameters. These findings were further supported by histopathological observations.

The literature reports indicate that the antioxidant activity is claimed to be one of the mechanisms of the hepatoprotective property of indigenous drugs[23]. Since, in the present investigation the test extract showed significant in vitro free radical scavenging activity in

a concentration dependent manner, this could exert a beneficial effect against liver toxicity in experimental animals.

Further, preliminary photochemical investigation revealed that the extract showed presence of flavonoids, tannins, alkaloids, saponins and glycosides. The literature has already documented the hepatoprotective value of flavonoids[24]. Thus, it appears that the hepatoprotection offered by *Abutilon indicum* flowers extract may be due to its flavonoid content.

REFRANCESES:

1. Handa SS, Sharma A, Chakraborty KK. Natural products and plants as liver protecting drugs. Fitoterapia 1989; 57: 307-51.
2. Hikino H, Kiso Y. Natural products for liver diseases. Economic and Medicinal Plant Research. Vol.II, London: Academic Press; 1988, 39-72p.
3. Evans WC. An overview of drugs having antihepatotoxic and oral hypoglycaemic activities. Trease and Evans Pharmacognosy. 14th ed. UK: W. D. Saunders company Ltd. 1996.
4. Sharma A, Shing RT, Sehgal V, Handa SS. Antihepatotoxic activity of some

plants used in herbal formulations. Fitoterapia 1991; 62: 131-8.

5. Yoganarasimhan SN. Medicinal Plants of India. Vol.II, Bangalore: Cyber Media; 2000, 10p.
6. Ahmed M, Amin S, Islam M, Takahashi M, Okuyama E, Hossain CF. Analgesic principle from *Abutilon indicum*. Pharmazie 2000; 55(4): 314-6.
7. Johri RK, Pahwa GS, Sharma SC, Zutshi U. Determination of estrogenic/antiestrogenic potential of antifertility substances using rat uterine peroxidase assay. Regional research laboratory (CSIR) Jammu Tawi, India. 1991; 44(5): 549-57.
8. Matlawska I, Sikorska M. Flavonoid compounds in the flowers of *Abutilon indicum.* Acta Pol Pharm 2002; 59(3): 227-9.
9. Porchezhian E, Ansari SH. Effect of liquid extract from fresh *Abutilon indicum* leaves and *Alliums cepa* bulbs on Paracetamol and CCl_4 induced hepatotoxicity. Pharmazie 2000; 55(9): 702-3.
10. Porchezhian E, Ansari SH. Hepatoprotective activity of *Abutilon indicum* on experimental liver damage in rats. Phytomedicine 2005; 12: 62-4.
11. Kokate CK. Pratical Pharmacognosy 4th ed. New Delhi: Vallabha prakashan.1999.169p.
12. Khandelwal KR. Practical Pharmacognosy. 2nd ed. Pune: Nirali Prakashan; 2000, 149p.
13. Mrs. Prema Veeraragavan. Expert consultant, CPCSEA, OECD guideline No. 420, 2000.
14. Gupta M, Mazumder UK, Kumar TS, Periyasamy, Gomathi, Kumar RS. Antioxidant and hepatoprotective effects of *Bauhinia racemosa* against paracetamol and carbon tetrachloride induced liver damage in rats. Iranian J Pharmacol Therapeutics 2004; 3: 12-20.
15. Manoj B, Aqueel K. Protective effect of *Lawsonia Alba* Lam., against CCl4 induced hepatic damage in albino rats. Indian J Expt Biol 2003; 41: 85-7.
16. Luna LG. Manual of histology and staining methods of Armed Forces institute of Pathology, 3rd ed. New York, McGraw Hill Book Co., 1986;1p.
17. Gulcin I, Oktay M, Kufre I, Vioglu O, Ali Aslan. Determination of antioxidant activity of lichen *Cetraria islandica* (L) Ach. J Ethnopharmacol 2002; 79: 325-9.
18. Oyaizu M. Studies on product of browing reaction preparation from glucose amine. Jap J Nutrition 1986; 44: 307-9.
19. Halliwell B, Gutteridge MCJ. Formation of a thiobarbituric acid reactive substance from deoxyribose in the presence of Iron salts. FEBS Letters 1981; 128(2): 347-52.
20. Slater TF. Biochemical mechanism of liver injury. London; Academic press 1965: 1p.
21. Plea GI, Hewitt WR. Toxicology of the liver. Boyer TD; Raven Press Zakim D 1982: 103p.
22. Singh B, Saxena AK, Chandan BK, Suri OP, Suri KA, Sathi NK. Hepatoprotective activity of verbenalin on experimental liver damage in rodents. Fitoterapia 1998; 60: 135.
23. Bhat AD, Bhat S. Indigenous drugs for liver diseases. Indian J Gastroenterol 1996; 15: 63-7.
24. Raj JK, Kapoor S. Flavonoids - Review of biological activities. Indian Drugs 1999; 36(11): 1-8.