

**HEPATOPROTECTIVE ACTIVITY OF *ADHATODA VASICA* LEAVES
AGAINST CARBONTETRACHLORIDE INDUCED TOXICITY**

G.Vinothapooshan* and K.Sundar¹

**Arulmigu Kalasalingam College of Pharmacy, Krishnankoil – 626 190*

¹*Department of Biotechnology, Kalasalingam University, Krishnankoil – 626 190
TamilNadu, India.*

Summary

The methanolic, chloroform, and diethyl ether extracts of leaves of *Adhatoda vasica* at the dose of 200mg/kg body weight per oral was studied for the hepatoprotective effect using Carbontetrachloride induced liver damage in wistar albino rats. Methanolic extract showed significant ($p < 0.05$) hepatoprotective effect when compare to other two extracts by lowering the serum levels of various biochemical parameters such as serum glutamic oxaloacetate transaminase (SGOT), serum glutamic pyruvates transaminase (SGPT), alkaline phosphatase (ALP), total bilirubin (TBL), in the selected model. These biochemical observations were in turn confirmed by histopathological examinations of liver sections and are comparable with the standard hepatoprotective drug Silymarin (100mg/kg body weight i.p.) which served as a positive control. The overall experimental results suggests that the biologically active phytoconstituents such as Alkaloids-Quinazoline, Flavonoids, Tannins, Vasicinone, Essential oil present in the various extracts of *Adhatoda vasica* plant may be responsible for the significant hepatoprotective activity and the results justify the use of *Adhatoda vasica* as a hepatoprotective agent.

Keywords: *Adhatoda vasica*; Hepatoprotective activity; Methanolic, Chloroform, Diethyl Ether extracts; Carbontetrachloride.

Corresponding author: ***G.Vinothapooshan**, *Arulmigu Kalasalingam College of Pharmacy, Krishnankoil – 626 190, India Mobile No.: 09003925162*

E Mail: g_pooshan@yahoo.com

Introduction

Liver is the largest and most complex internal organ of the body. It plays a vital role in the metabolic activities and important bio-chemical conversion. But at the same time many factors have been reported which cause the hepatitis, such as prolonged drug therapy, alcoholism, certain disease state and toxic industrial chemical. Liver being a vital organ, its protection has a special status in therapeutics. Liver disease is a serious health problem. In the traditional system of medicine liver diseases had been successfully treated by using medicinal plants and their formulations. However, there is no satisfactory therapy for serious liver diseases; mostly the herbal drugs increase the rate of natural healing process of liver. Hence the searches for effective liver protective drug persist.

Adhatoda vasica(acanthaceae) known as chue Mue, is a stout stragling prostrate shrubby plant with the compound leaves which gets sensitive on touching, spinous stipules and globose pinkish flower heads, grows as weed in almost all parts of the country (Ghani, 2003). Leaves and stems of the plant have been reported to contain an alkaloid mimosine, leaves also contain mucilage and root contains tannins. *Adhatoda vasica* is used for its anti – hyperglycemic (Uma maheswari, 2007), anti – diarrhoeal (Balakrishnan, et al., 2006), anti – convulsant (Bum, et al., 2004) and cytotoxic properties (Sadia Afreen Chowdhury, et al., 2008). The plant also contains turgorins, leaves and roots are used in treatment of piles and fistula. Paste of leaves is applied to hydrocele. Cotton impregnated with juice of leaves is used for dressing sinus. Plant is also used in treatment of sore gum and is used as a blood purifier. In ayurvedic and Unani system of medicine, this plant has been used in diseases arising from corrupted blood and bile, bilious fever, piles, jaundice, leprosy, ulcers, small pox. The objective of present investigation was to study Hepatoprotective activity of *Adhatoda vasica* has not been experimentally evaluated so far, hence the present studies were performed to assess the hepatoprotective activity in rats against Carbontetrachloride as hepatotoxin to prove its claim in folklore practice against liver disorders.

PLANT NAME	SYNONYM	FAMILY	CHEMICAL CONSTITUENTS
<i>Adhatoda vasica</i>	<i>Adhatoda zeylancia</i>	Acanthaceae	Alkaloids- Quinazoline, Flavonoids, Tannins, Vasicinone, Essential oil.

Materials & methods

Plant material: The plant material was collected in Arulmigu kalasalingam college of pharmacy Medicinal Garden, Krishnankoil, Tamilnadu. It was authenticated by Dr. Stephan, Dept of Botany, The American college, Madurai.

Extraction process

The leaves were shade-dried and made into a coarse powder which was passed through a 40-mesh sieve to get a uniform particle size and then used for extraction. A weighed quantity (500 g) of the powder was then subjected to continuous hot extraction in Soxhlet apparatus with methanol, chloroform and Diethyl ether and the residual marc was collected. The extract was filtered through a cotton plug, followed by Whatman filter paper (no.1). The extract was evaporated under reduced pressure using a rotovac evaporator at a low temperature (40-60oC) until all the solvent had been removed to give an extract sample with a yield of 16% w/w, 14 %w/w and 12% w/w in relation to the dried starting material. Preliminary Phytochemical analysis was carried out to identify presence of Phytoconstituents in the crude extract.

The percentage yields of Adhatoda vasica Extract

WEIGHT OF DRUG	EXTRACTION PATTERN	SOLVENT USED	WEIGHT OBTAINING)	PERCENTAGE YIELD
500gms <i>Adhatoda vasica</i> powder	Soxhlet apparatus	Methanol	16gm	7%
		Chloroform	14gm	6.2%
		Diethyl Ether	12gm	5.2%

Preliminary phytochemical analysis

The various extracts of *Adhatoda vasica* were then subjected to preliminary phytochemical (Harbone, 1984) analysis to assess the presence of various phytoconstituents, it revealed that the presence of Alkaloids-Quinazoline, Flavonoids, Tannins, Vasicinone, Essential oil. Preliminary Thin layer chromatography studies also confirmed these constituents (Wagner and Blatt, 1996).

Animals

Wistar albino rats weighing 150-200g of either sex maintained under standard husbandary conditions (temp 23±2oC, relative humidity 55±10% and 12 hours light dark cycle) were used for the screening. Animals were fed with standard laboratory food and ad libitum during the study period. The experimental protocol has been approved by institutional animal ethics committee, Arulmigu Kalasalingam College of Pharmacy, Krishnankoil. (Regd No.509/02/C/CPCSEA/2002.) India.

Toxicity studies

Acute toxicity study was performed for various extracts of *Adhatoda vasica* according to the acute toxic classic method as per OECD guidelines (Ecobichon, 1997). Female albino rats were used for acute toxicity study. The animals were kept fasting for overnight providing only water, after which the various extracts were administered orally at the dose of 300mg/kg and observed for 14 days. If mortality was observed in two animals out of three animals, then the dose administered was assigned as toxic dose. If the mortality was observed in one animal, then the same dose was repeated to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher doses such 50,200 & 2000mg/kg body weight. The animals were observed for toxic symptoms for 72 h.

Carbontetrachloride induced hepatotoxicity

Rats were divided into six groups of six animals each. Group I served as solvent control, which received normal saline (3ml/kg, p.o). Group II received CCL₄ (0.5ml/kg, i.p) for 7 days. Group III received CCL₄ (0.5ml/kg, i.p) and Silymarin (100mg/kg, p.o), Group IV CCL₄ (0.5ml/kg, i.p) and *Adhatoda vasica* Methanolic Extract (AVME 200mg/kg,p.o.), Group V CCL₄ (0.5ml/kg, i.p) and *Adhatoda vasica* Chloroform Extract (AVCE 200mg/kg,p.o.), Group VI CCL₄ (0.5ml/kg, i.p) and *Adhatoda vasica* Diethyl

Ether Extract (AVDEE 200mg/kg,p.o). Simultaneously for 7 days. After 7 days of treatment, the rats were kept overnight fasting and blood samples were collected from retro-orbital plexus under mild ether anesthesia and the serum was separated and used for determination of biochemical parameters.

Assessment of liver function

Blood was collected from all the groups by puncturing the retro-orbital plexus and was allowed to clot at room temperature and serum was separated by centrifugation at 2500rpm for 10 min. The serum was used for estimation of biochemical parameters to determine the functional state of the liver. Serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) were estimated by a UV kinetic method based on the reference method of International federation of Clinical Chemistry in which both SGOT and SGPT were assayed based on enzyme-coupled system; where keto acid formed by the aminotransferase reacts in a system using NADH. The coenzyme is oxidized to NAD and the decrease in absorbance at 340 nm is measured. For SGOT malated dehydrogenase is used to reduce oxaloacetate to malate where as for SGPT the pyruvated formed in the reaction is converted to lactate by lactate dehydrogenase. Alkaline phosphatase (ALP) was estimated by method described by (Comb and Bowers, 1972) involving hydrolysis of p-nitrophenol which gives strong yellow colour in alkaline solution. The increase in absorbance due to its formation is directly proportional to ALP activity; while total bilirubin (TBL) by (Jendrassik and Grof, 1938) which involves the reaction of bilirubin with diazotized sulphanilic acid to form an azo compound, the colour of which is measured at 546 nm.

Histopathological studies

The animals were sacrificed and the abdomen was cut open to remove the liver. The liver was fixed in Boucin's solution (mixture of 75 ml of saturated picric acid, 25 ml of 40% formaldehyde and 5ml of glacial acetic acid) for 12h, then embedded in paraffin using conventional methods (Galighor and Kozloff, 1976) and cut into 5µm thick sections and stained using haematoxylin-eosin dye and finally mounted in di-phenyl xylene. The sections were then observed under microscope for histopathological changes in liver architecture and their photomicrographs were taken.

Statistical analysis

The values Mean±SEM are calculated for each parameter. For determining the significant inter group difference each parameter was analysed separately and one-way analysis of variance (Gennaro, 1995) was carried out and the individual comparisons of the group mean values were done using Dunnet's test (Dunnet, 1964).

Results

Acute toxicity studies

In acute toxicity study, it was found that the animals were safe up to a maximum dose of 2000mg/kg body weight in rats, there were no changes in normal behavioral pattern and no signs and symptoms of toxicity and mortality were observed and hence the extract was considered to be safe and non-toxic for further pharmacological screening.

Hepatoprotective activity: The results of Carbontetrachloride induced hepato-toxicity were shown in table-1. In the Carbontetrachloride control group, the significant acute hepato cellular damage, and biliary obstruction was indicated by the elevated level of SGPT, SGOT, ALP, and TBL. But the group which received the test drug of methanolic extract at the dose of 200mg/kg body weight p.o showed a significant decrease when compare to other two extracts in the elevated levels of SGPT, SGOT, ALP, TBL and these biochemical parameters are comparable with the standard silymarin hepatoprotective drug. Therefore, the silymarin and the methanolic extract of *Adhatoda vasica* restored the altered level of enzymes significantly (P<0.05).

Table-1: Effect of *Adhatoda vasica* on Carbontetrachloride induced hepatotoxicity in rat’s enzymes

Group	Dose	SGPT(U/L)	SGOT(U/L)	ALP(U/L)	TBL(mg/dl)
I-Control	3ml/kg p.o.	127.16±5.12	106.40±2.60	200.80±5.80	1.18±0.6
II-Ccl ₄	0.5ml/kg i.p.	285.10±35.60 ^a	244.30±38.20 ^a	428.60±47.60 ^a	2.98±0.38 ^a
III-Silymarin	100mg/kg p.o	124.10±11.60 ^b	104.60±6.60 ^b	194.60±7.20 ^b	1.38±0.32 ^b
IV-AVME	200mg/kg p.o	130.62±14.12 ^b	114.62±8.14 ^b	203.12±44.62 ^b	1.49±0.62 ^b
V-AVCE	200mg/kg p.o	144.48±10.26 ^b	132.62±14.40 ^b	218.64±36.14 ^b	1.62±0.24 ^b
VI-AVDEE	200mg/kg p.o	165.82±7.16 ^b	158.46±2.62 ^b	240.38±18.14 ^b	1.74±0.26 ^b

Values are expressed as mean SEM; n=6 in each group, ^aP<0.01 Vs control group, ^bP<0.01 Vs CCl₄ – treated group (ANOVA followed by Dunnett’s test).

Histopathological liver sections of control group showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces, and central vein (Fig 1a). Disarrangement of normal hepatic cells with necrosis and vacuolization are observed in Carbontetrachloride intoxicated liver (Fig 1b). The liver sections of the rat treated with 200mg/kg bodyweight p.o of methanolic, chloroform and diethyl ether extracts of *Adhatoda vasica* followed by Carbontetrachloride intoxication (Fig 1c,1d,1e) showed less vacuole formation and absence of necrosis and overall less visible changes observed were comparable with standard Silymarin (Fig 1f), supplementing the protective effect of the test drug of methanolic extract of *Adhatoda vasica* when compare to other two extracts and the standard hepatoprotective drug.

Figure 1
Histopathological sections of Liver



Figure 1a: Control liver tissue showing normal architecture.

1a – Control

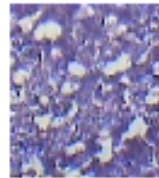


Figure 1b: Liver tissue from the Carbontetrachloride group showing significant damage.

1b-Carbontetrachloride group

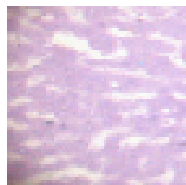


Figure 1c: Liver tissue from the Adhatoda vasica test extract group showing reduced damage.

1c- Test extract of *Adhatoda vasica* group

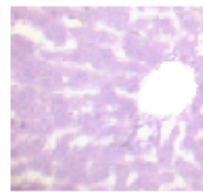


Figure 1d: Liver tissue from the Adhatoda vasica group showing central vein with necrosis.

1d-Test extract of *Adhatoda vasica* group

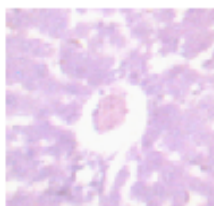


Figure 1e: Liver tissue from the Adhatoda vasica test extract group showing reduced damage.

1e-Test extract of *Adhatoda vasica* group

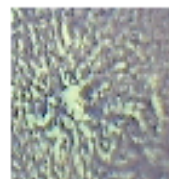


Figure 1f: Liver tissue from the standard silymarin group showing normal architecture.

1f-standard silymarin group

Discussion

The present studies were performed to assess the hepatoprotective activity in rats, against Carbontetrachloride as hepatotoxin to prove its claim in folklore practice against liver disorder. The changes associated with Carbontetrachloride induced liver damage of the present study appeared similar to the acute viral hepatitis (Venukaumar and Latha, 2002). Carbontetrachloride is a widely used experimental hepatotoxicant, is biotransformed by the cytochrome P-450 system to produce the trichloromethyl free radical, which in turn covalently binds to cell membranes and organelles to elicit lipid peroxidation, disturb Ca²⁺ haemostasis and finally result in cell death (Recknag, et al., 1989).

Animals of Group II (received Carbontetrachloride) significantly lost their body weight and showed reduced food consumption as compared to control group. Animals of Group III, IV, V and VI (received Carbontetrachloride plus 200mg/kg body weight of test extracts and standard drug Silymarin 100mg/kg body weight) showed a significant increase in body weight and food consumption when compared to Carbontetrachloride group animals. These findings suggested the extract administered has significantly neutralized the toxic effects of Carbontetrachloride and helped in regeneration of hepatocytes (Farooq, et al., 1997).

Estimating the activities of serum marker enzymes, like SGPT, SGOT, ALP can make the assessment of liver function when liver cell plasma membrane is damaged, a variety of enzyme normally located in the cytosol are released into the blood stream. Their estimation in the serum is a useful quantitative marker of the extent and type of hepato cellular damage (Mitra, et al., 1998). The tendency of these enzymes to return to near normally in extract administered group is a clear manifestation of antihepatotoxic effects of the extract.

Reduction in the levels of SGPT and SGOT towards the normal value is an indication of regeneration process. Reduction in ALP levels with concurrent depletion of raised bilirubin levels suggests the stability of the biliary function during injury with Carbontetrachloride. This hepato protective effect exhibited by the methanolic, chloroform, and diethyl ether extracts of *Adhatoda vasica* at the dose level of 200mg/kg body weight was comparable with the standard drug, Silymarin.

Histopathological liver sections also revealed that the normal liver architecture was disturbed by hepatotoxin in Carbontetrachloride group, whereas in the liver sections of the rat treated with the methanolic, chloroform, and diethyl ether extracts and intoxicated with Carbontetrachloride the normal cellular architecture was retained and it is comparable with the standard Silymarin group, hence confirming the significant hepato protective effect of methanolic extract of *Adhatoda vasica* at the dose of 200mg/kg body weight.

In accordance with these results, it may be confirmed due to the presence of phytoconstituents such as Alkaloids-Quinazoline, Flavonoids, Tannins, Vasicinone, Essential oil which are present in the methanolic extract of *Adhatoda vasica* could be considered as, responsible for the significant hepatoprotective activity. In conclusion, it can be said that the methanolic extract of *Adhatoda vasica* exhibited a hepato protective effect against Carbontetrachloride induced hepatotoxicity when compare to other two extracts. Efforts are in progress to isolate and characterize the active principle, which is responsible for the hepatoprotective efficacy of this valuable medicinal plant.

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