Hepatosuppression by *Adhatoda vasica* against CCl_4 Induced Liver Toxicity in Rat

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Summary

Liver disorder is one of the common thrust area declared by the Indian Council of Medical Research, New Delhi in the reviewed research on traditional medicine. *Adhatoda vasica* have been reported to exhibit varying degrees of hepatoprotection against the CCl_4 induced liver dysfunction in rats. The present work was carried out to investigate the potential hepatoprotective action of *Adhatoda vasica* whole plant powder against CCl_4 induced liver damaged Wister rat model. Blood and tissue biochemical parameters of liver have been examined for evaluating the hepatoprotection action. These biochemical markers are GOT, GPT, Alkaline phosphate, glucose, bilirubin, Triglycerides, γGT, cholesterol, DNA, RNA, total protein etc, The effect of *Adhatoda vasica* whole plant powder is compared with Silymarin by standard protocol and is found to have better hepatoprotective action, thus *Adhatoda vasica* indicating protection in liver may prove promising effect against liver disorders. Thus it may act even in humans as a potent liver tonic**.**

Keywords: *Adhatoda vasica,* CCl_4, Hepatosuppression, biochemical markers, liver tonic.

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Introduction

Liver disorders constitute a major health problem in India. There is scarcity of effective modern drugs for the treatment of liver disorder like Jaundice. Many herbal preparations have been marketed for the same. *Adhatoda vasica* is widely used as one of the component by Folk healers for treatment of jaundice. The current investigation on *Adhatoda vasica* as hepatoprotective action was undertaken as an extension of my earlier work on *Argemone mexicana* and *Centella-asiatica* [1,2].

Adhatoda vasica is a small evergreen perennial shrub used in Indian medicine for over 2000 years. It is commonly used for treatment of respiratory complaints. The leaves are boiled and taken orally for fevers [4]; warmed leaves are applied externally for rheumatic pains and dislocation of joints. The powder boiled in seasam oil is used for ear infection and to stop bleeding, dried leaves powder is used for stomach acidity, decoction of leaves is used to facilitate childbirth or induce abortion [5]. A paste of leaves is applied to the abdomen to treat urinary disorders [8]. The leaves and flowers used as vegetables, as expectorant, antiplasmodic, and febrifuge [6]. The leaves are used in cough and colds, in treating abscesses, antharax, throat diseases, asthma, tuberculosis, jaundice, scabies, rheumatism, pneumonia, haematuria and contagious abortion [7, 8].

Materials and Methods

Plant Material

Adhatoda vasica plants were collected from Avsari forest park Ambegaon, Pune, India. Herbaria of the plant was prepared and authenticated by BSI (Botanical Survey of India), Pune, India. After collection of the required quantity, it was carefully segregated, washed and dried in shade to constant weight. The plant material was kept in preset oven for eight days at 45°C. The dried plant free of moisture was powdered in high speed electronic mixer and sieved through a BSS Mesh No. 80 sieve and stored in an airtight container.

Acute toxicity Study

The acute toxicity study of *Adhatoda vasica* was carried out on Swiss mice with a dose of 3, 5 and 7g/Kg body weight orally. The single administration exposure of the whole plant powder in the form of aqueous slurry was carried out and the exposure route was oral with water as a vehicle. The observations of changes in body weight, food and water intake as well as cage side observations were reported. There was no mortality reported even at the highest dose level i.e. 7g/Kg body weight and the whole plant powder was found to be nontoxic.

Sixty Wistar rats of either sexes (30 male and 30 female) were procured from Raj Biotech (India) Ltd., Pune, India. The animals were housed in standard rat cages and were fed with commercially available rat feed pellets supplied by AMRUT feeds. The rat feed pallets contains 20-22% crude protein, 4% crude fiber, 4-5% ether extractive, 8% ash, 1.2% calcium, 0.6% phosphorus, 54% NFE etc. The animals were given measured volume (250 ml) of drinking water and weighed amount (200 g) of food during the experiment. All animals used for the study were in the weight range of 130-150g. The animals were randomly divided into five groups of twelve (6 male and 6 female) animals each. The male and female rats were housed in separate cages.

Animals were grouped into five groups and administered following dose mentioned in Table 1.

After an acclimatization period of fourteen days the rat cages were randomly assigned the following treatments; Group I: Normal control, Group II: toxicant control, Group III: toxicant recovery, Group IV: CCl_4 + plant (*Adhatoda vasica*) control, Group V: CCl_4 + Silymarin treated. The animals from Group I received an intra peritoneal (i.p.) injection of 0.5 mL of liquid paraffin and those from Group II, III, IV and V received an i.p. injection of 0.7 ml/kg of CCl_4 in 0.5 ml liquid paraffin per animal on the first day of the study. The animals from Group I, II and III received an oral dose of 2 ml of Distilled Water (D/W) once daily. The animals from Group IV received an oral dose of 0.50 g/kg of sieved whole plant powder of *Adhatoda vasica* suspended in 2 ml of distilled water per animal. The animals from Group V received an oral dose of 0.007g/kg of Silymarin suspended in 2 ml of distilled water per animal. The animals from Group I, II, IV and V were sacrificed on the fourth day (72 h after dosing) and those from Group III were sacrificed on seventh day of the study [3].

Before sacrificing the animals were weighed. The food supply was stopped twelve hours before sacrifice. All animals were weighed before sacrifice. Blood was collected by cardiac puncture under light ether anesthesia during sacrifice. Blood biochemical assays of some selected parameters like Alkaline Phosphatase (AlkP), Glucose, Cholesterol, Triglycerides (TG), Aspartate Transferase (AST) and Alanine Transferase (ALT), Bilirubin, gama GT was carried out as per the standard kits (Raichem, division of Hemagen diagnostic, inc. San Diego, CA 92111-1203).

Table 1: DAILY DOSE REGIME

DAYS	Group I Normal Control	Group II CCl_4 control	Group III Natural Recovery	Group lV Plant slurry treated	GroupV Silymarin Treated
1	0.5cc liq. Paraffin & 2 cc d/w oral	0.7cc/kg CCl_4 in 0.5cc liq. Paraffin i.p. And 2cc d/w oral	0.7cc/kg CCl_4 in 0.5cc liq. Paraffin i.p. And 2cc d/w oral	0.7cc/kg CCl_4 in 0.5cc liq. Paraffin i.p. and 0.5gm/kg plant material in 2cc d/w oral	0.7cc/kg CCl_4 in 0.5cc liq. Paraffin i.p., 0.007gm/kg Silymarin in 2cc d/w oral
2	2cc d/w oral	2cc d/w oral	2cc d/w oral	0.5gm/kg plant material in 2cc d/w oral	0.007gm/kg Silymarin in 2cc d/w oral
3	2cc d/w oral	2cc d/w oral	2cc d/w oral	0.5gm/kg plant material in 2cc d/w oral	0.007gm/kg Silymarin in 2cc d/w oral
4	Sacrifice	Sacrifice	2cc d/w oral	Sacrifice	Sacrifice
5	-	-	2cc d/w oral	-	-
6	-	-	2cc d/w oral	-	-
7	-	-	Sacrifice	-	-

Note: 1. The above dosage is for an individual animal of the group.
2.The number of animals in each group = 6.
3. i.p. = intra peritoneal.
4. d/w = distilled water
5. liqd. paraffin = liquid paraffin.

After sacrifice, liver was excised, rinsed in saline, blotted and weighed. Liver to body weight ratio was calculated of all animals. A small 5 mm piece of liver from the largest lobe was cut and fixed in Bouin's fixative for 24 h. The tissues after fixations were processed, cut into sections of 1 and 7 micro meters and stained. The stained slides were observed, photographed and preserved. The remaining liver was used for the estimation of tissue biochemical assays, which included DNA, RNA, Alkaline phosphatase, Glycogen, Total Protein and Cholesterol. All the assays were done using standard Diagnostic kits, DNA and RNA was estimated by the method of Munro.

Results

Food consumption and weight gain

It is observed that there was significant decrease in body weight of CCl_4 treated group as compared to normal control group given in Table 2. Treatment of Silymarin and plant powder showed on an increase in body weight as compared to CCl_4 treated group.

Table 2 Effect o*f* *Adhatoda vasica* Whole **Plant powder slurry on body weight**

Groups	Body weights of rats in grams						
	1st Day	2nd Day	3rd Day	4th Day	5th Day	6th day	7th Day
Normal Control	141.4 ± 2.2	142.33 ± 3.2	144.2 ± 2.4	SACRIFICE	-	-	-
CCl_4 Control	145.3 ± 3.2	144.2 ± 3.4	142.2 ± 4.0	SACRIFICE	-	-	-
CCl_4 Recovery	134.4 ± 4.2	135.2 ± 3.5	137.3 ± 3.7	137.8 ± 4.0	139.1 ± 3.2	140.4 ± 2.04	SACRIFICE
Plant control	130.3 ±3.5	131.00 ± 2.4	131.9 ± 5.4	SACRIFICE	-	-	-
Silymarin Control	143.1 ± 3.2	144.7 ± 6.20	146.7 ± 6.2	SACRIFICE	-	-	-

Table 3: Blood and tissue biochemical parameters

Parameter	Group I Normal control	Group II CCl_4 control	Group III CCl_4 recovery	Group IV CCl_4 + plant slurry treated	Group V CCl_4 + Silymarin treated
Blood biochemical parameters					
Glucose (B)	74.56	140.48	62.45	81.52	70.74
Alk PO_4 (B)	96.50	110.00	108.40	111.40	96.40
Cholesterol (B)	48.52	68.50	61.40	67.00	58.60
Triglycerides (B)	56.50	89.40	63.50	87.80	61.00
AST (B)	36.00	39.00	36.80	37.50	33.50
ALT (B)	29.00	36.58	38.10	32.40	35.60
Bilirubin(B)	0.69	0.75	0.76	0.64	0.74
γGT	21.80	44.60	40.50	45.40	39.30
Tissue biochemical parameters					
DNA (T)	10.22	10.30	16.20	12.26	8.30
Alk PO_4 (T)	59.50	57.20	37.00	59.80	40.00
RNA (T)	150.50	120.66	78.85	88.51	76.63
Glycogen (T)	6.16	5.65	4.85	5.77	4.88
Cholesterol (T)	35.63	46.27	53.38	37.27	48.45
Total Protein(T)	09.52	18.14	11.21	07.33	08.21
Liver to body weight ratio					
Liver to b/w ratio	0.040	0.037	0.042	0.041	0.041

Biochemical parameters

The use of CCl_4 as a hepatotoxicant in animal models is well documented. Hepatic damage caused by CCl_4 is very specific except at higher doses and is reversible to low doses. The biochemical and histopathological changes in liver exposed to CCl_4 are well documented [3, 4, 5]. It has a destructive effect on the membranes of the hepatocytes and

interferes with cellular metabolism and transport. A single dose leads to centrilobular necrosis and fatty liver. Within few minutes, there is injury to endoplasmic reticulum leading to functional defects of the hepatocyte. Multiple biochemical manifestations of hepatic injury can be recorded. Irrespective of the route of administration, CCl_4 leads to centrilobular necrosis and steatosis. Biochemical changes in the blood adequately reflect status of the injury. Serum enzymes levels increase with cytoplasmic enzymes, reaching their peak level within 12 h of exposure. Mitochondrial enzymes reach their peak level within 36 h. Enzymes common to both mitochondria and cytoplasm reach their peak level around 24 h. The process of recovery begins within 24-48 h after exposure of CCl_4.

CCl_4 causes hepatic damage through the production of trichloromethyl (CCl_3) free radical. The metabolism of CCl_4 releases CCl_3 free radical, which initiates peroxidation and cleavage of fatty acids in the membranes. Observations published earlier indicate that CCl_4 causes accumulation of fat in the liver especially by interfering with the transfer of triglycerides from the liver into the plasma. Many clinical conditions that cause an increase in cholesterol levels also cause increase in Triglycerides (TG). Blockage of the secretion of hepatic TG into the plasma is the basic mechanism underlying the fatty liver induced in the rat by CCl_4. This causes elevated amounts of fats predominantly TG in the parenchymal cells.

Aspartate and Alanine aminotransferase are present in high concentration in liver. Due to necrosis of hepatocyte or abnormal membrane permeability, these enzymes are released from the hepatocytes into the blood and their levels in the blood increase. Alanine Aminotransferase (ALT) is a sensitive indicator of acute liver damage and elevation of this enzyme in non-hepatic disease is unusual. ALT is more selectively a liver parenchymal cell enzyme than is Aspartate Aminotransferase (AST). The transaminases being more stable than other enzymes under the laboratory conditions are better indices for evaluating the extent of hepatic damage. Alkaline phosphatase is a membrane bound enzyme and its elevation in plasma indicates membrane disruption in the organ. Alkaline phosphatase, although is not a liver specific enzyme, but liver is the major source of alkaline phosphatase. The level of this enzyme increases in cholestasis. Levels of Lactic dehydrogenase reach a peak at 12h after CCl_4 treatment.

Discussion

CCl_4 treatment causes significant increase in blood and tissue biochemical parameters which were studied mentioned in Table 3. Reduction in RNA values indicates the toxicant induced changes in protein synthesis. The data in Table 3 indicates that the change in DNA levels of liver observed in the present study indicates CCl_4 induced cellular hepatic damage.

Significant variations in tissue glycogen and plasma glucose levels after treatment with CCl_4 indicate impairment of liver metabolism. CCl_4 treatment causes a drop in tissue glycogen levels. The plant treated group shows a further decrease in the tissue glycogen as compared to CCl_4 control group. From the results given in Table 3 for the blood parameters, it is evident that CCl_4 causes hyperglycemia in animals of CCl_4 control group. Treatment of *Adhatoda vasica* whole plant powder lowers the blood glucose level bringing it close to normal values. Values of blood glucose in recovery group animals are close to control group values.

Treatment of animals having CCl_4 induced hepatic injury with *Adhatoda vasica* causes decrease in tissue alkaline phosphatase and cholesterol levels and blood alkaline phosphatase, TG, AST and ALT levels as compared to those of CCl_4 control group animals. Blood cholesterol levels of *Adhatoda vasica* plant treated group animals showed a significant reduction as compared to CCl_4 control. This reduction in cholesterol levels was lower than those in animals of natural recovery group given in Table 3.

The Group IV animals treated with only the *Adhatoda vasica* plant slurry show significantly comparable levels of blood and tissue biochemical parameters as compared to normal control group animals.

Animals from all groups showed no abnormal behavior in food and water consumption. The food consumptions of animals from CCl_4 control, decreased significantly. The CCl_4 recovery group animals showed significant decrease up to the fourth day of the treatment and then they showed an increase. This indicates that the animals are recovering from the toxicity induced by the CCl_4 similar observations were noted with the trends in water consumption by treated animals.

Liver to body weight ratio of the animals from all the groups showed no significant changes in their values. This observation can be attributed to the lower dose of CCl_4 used for the study given in Table 3.

Conclusion

From the results obtained for Group IV animals, it is evident that treatment with the slurry of *Adhatoda vasica* brings about significant recovery of the liver. This is supported by the observations in Group III animals where the levels of various liver function parameters have recovered almost to control levels after natural recovery. Cellular recovery after CCl_4 administration takes about 14 days, whereas Group III animals underwent a recovery period of 6 days.

The present investigation therefore adequately proves that *Adhatoda vasica* is an effective hepatoprotective agent at the dose (0.50 g kg^{-1}) used in the present investigation. The plant slurry impairs normal liver function inducing distinct toxic changes in hepatocytes. This is the dose which show maximum hepatoprotective action against CCl_4 induced liver toxicity. The study reiterates the importance of standardization while formulating herbal based formulation.

References

1. Pingale Shirish, S., Evaluation of effect of *Centella asiatica* on CCl4 induced rat liver damage. Pharmacologyonline, 3: 2008, 537-543.
2. Pingale Shirish, S., Acute toxicity study for *Centella asiatica* whole plant powder. Pharmacologyonline, 3: 2008, 80-84.
3. Indira Balachandran and V.V. Sivarajan, 1994. *In: Ayurvedic Drugs and their Plant Sources*. 1st Edn., Oxford and IBH Publishing Company Pvt. Ltd., New Delhi.

4. Sane, R.T., V.V. Kuber, M.S. Challisary and S. Menon, 1995. Hepatoprotection by *Phyllanthus amarus* and *Phyllanthus debilis* in CCl_4 induced liver dysfunction. Curr. Sci., 68: 1243-1246.
5. Meghana C. Shah, Prateek H. Patel, Madura M. Phadke, Sasikumar N. Menon and Ramesh T. Sane, 1999. Hepatoprotective action of extracts of phyllanthus debilis in various solvents. Biores. J., 2: 11-26.
6. Pandey, V.N. and G.N. Chaturvedi, 1969. Effect of different extracts of kutaki (picrorhiza kurroa) on experimentally induced abnormalities in the liv er. Ind. J. Med. Res., 57: 503-512.
7. Kirtikar KR, Basu BD, An LCS 1975, Indian Medicinal Plants, Vol 3.
8. Claeson UP, Wikman G, Bruhn JG 2000, Adhatoda Vasica: a critical review of ethanopharmacological and toxicological data, Journal of Ethanopharmacology 72:1.
9. Mailhuri RK, Gangwar AK 1965, Ethnobiological notes on Kashi and Garo tribes of Meghalaya, North East India, Economic Botany, 47; 345.
10. Jha MK, 1972, The Folk Veternary System of Bihar-a research Survey, NDDB, Anand, Gujarat.
11. Chaturvedi GN, Rai NP, Dhani R, Tiwari SK, 1983, Clinical Trial of Adhatoda Vasica syrup in the patient of non ulcer dyspepsia, Ancient Science of Life 3(1);19.