RESEARCH PAPER

Protective effect on *Hygrophila auriculata* leaf extraction cadmium chloride induced hepatotoxicity in albino wistar rats

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Protective effect of *Hygrophila auriculata* leaf extract on cadmium chloride induced hepatotoxicity in albino wistar rats were investigated by analyzing various biochemical parameters. cadmium chloride induced hepatic damage was well manifested by significant increase in the activities of SGOT, SGPT, ALP, ACP, total bilirubin, MDA and LDH and also decreased in total protein and GSH. The oral administration of aqueous extract of *Hygrophila auriculata* (100mg/kg body wight) along with cadmium chloride for 7 days reversed these altered parameters to normal level which indicating the hepatoprotective efficacy of *Hygrophila auriculata* against cadmium chloride induced liver injury. Phytochemical constituents such as flavonoids are responsible for the hepato protective activity of *Hygrophila auriculata*. Further extensive studies are required for its potential uses in clinical practice.

Key words **:** linseed , *Hygrophila auriculata*, Cadmium chloride, Hepatotoxicity, Hepatoprotectivity

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INTRODUCTION

The main industrial centre of the body is the liver and weighs about 1.5kg making up about 23 per cent of the total body weight. Liver plays a pivotal role in the regulating various physiological process in the body. It is involved with almost all the biochemical pathways to growth, fight against diseases, nutrient supply, energy provision and reproduction (Ward and Daly, 1999). Liver diseases are some of the fatal diseases in world today they pose a serious challenge to international public health. An injury to it or impairment of its function may lead to many complications. About 20,000 deaths occur every year due to liver diseases (Laurence and Beunett, 1992). Hepatitis (Inflammation of the liver), Jaundice (Yellow discoloration of skin, mucous membranes), chirrhosis (Formation of Fibrous tissue is the liver), Hepatomegaly (liver enlargement) etc.

Liver diseases are mainly caused by toxic chemicals, excess consumption of alcohol, infectious and auto immune disorders. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damage (Reelmagel, 1983; Wendal *et al*., 1987; Diazani *et al*., 1991).

Heavy metal:

Heavy metals cause liver damage. The most harmful heavy metals enter the liver and affect the liver cells, can produce the liver diseases, such as hepatitis, cirrhosis, liver enlargement, cholestasis. Ex: lead acetate, carbon tetra chloride, cadmium chloride, magnesium chloride.

Cadmium chloride:

Human uptake of cadmium takes place mainly through food. Food stuffs that are rich in cadmium can greatly increase the cadmium concentration in human bodies. Example is liver, mushroom, shellfish, mussels, cocoa powder and dried seaweed. Food and cigarette smoke are the largest potential sources of cadmium exposure for members of the general population (Goyer *et al*.,1997). The average person ingests about 30 micrograms of cadmium from food each day. Smokers absorb an additional 1 to 3 µg/day from cigarettes. Cadmium can enter the blood by absorption from the stomach or intestines after ingestion of water or food, by absorption from

the lungs after inhalation. Cadmium is present in the plasma of blood within minutes after its exposure, from which cadmium is readily taken up by the liver. Cadmium enters the liver where it become bound to metallothionein and is released to the blood stream, 24 hours after exposure most of the cadmium is distributed in blood cells probably bound to metallothionein, a cystine rich metal binding protein (Norberg *et al*., 1971). Further, Cadmium transported to the Kidneys and eliminated.

Health effects of cadmium:

Cadmium administration has been shown to deplete glutathione (GSH) and protein binding sulfhydryl groups, which resulted in an increase in reactive oxygen species leading to such events as an increase in lipid peroxidation, a change in intracellular stability and apoptosis. Cadmium initially accumulates in the liver and, therefore, acute exposure to toxic of cadmium produces apoptosis and necrosis in the liver.

Hemodialysis may be used to remove circulating cadmium from the blood stream, although the literature on the subject in scarce. Other than dialysis dimercaptosuccinic acid (DMSA) on oral chelating agent, has been recommended for removal of cadmium from the blood. These treatments are only effective for oral poisoning and have no demonstrated benefit in cadmium fume inhalation(Blaha *et al*.,1995). Now-a-days the use of herbal medicine has gained more momentum owing to the general awareness of its safety towards the human systems in comparison to the synthetic drugs for cadmium induced toxicity.

Hygrophila auriculata (syn: *Astercantha longifolia*) belongs to Acantheceae family is widely distributed throughout tropical and sub-tropical regions of India and it has the several mediunal properties including hepato protection.

The roots are useful in dropsy of chronic bight's disease, inflammation, ascites, hyperdipsia, vesical calculi, strangury, jaundice, flatulence, dysentery. The leaves are useful in jaundice, dropsy, rheumatism, arthralgia, arthritis, cough, gastropathy, anaemia and ophtalmopathy. The seeds are arresting abortion, burning sensation, anaemia, diarrhoea, dysentery.

RESEARCH METHODOLOGY

Animals :

Albino rats of male sex, approximately 8-10 weeks of age, weighing 100-150g were obtained from animal house, Tamil University, Thanjavur, Tamilnadu, India. The animals were housed in groups of three in standard cages, at room temperature (25 ± 30^{0}C), with 12hr dark and 12hr light cycles, all the animals were fed with standard pellet diet. They were given 2 weeks time to get acclimated with laboratory conditions.

Experimental design :

The animals were randomized and divided into 4 groups, containing 6 rats in each groups.

Group-I : Animals received standard diet act as a control.

Group-II : Animal received cadmium chloride (10mg /kg body wt) along with normal diet for 7 days.

Group-III : Animals received aqueous extract of *Hygrophila auriculata* (10mg/kg body wt) after induction with cadmium chloride.

Group-IV: Animals received commercial drug for hepatotoxicity (Silymarin 10mg /kg body wt).

Collection of blood:

On the end of experiment, animals were sacrified and the blood was collected by sino-orbital puncture and allowed to clot for few minutes. The clotted blood was transformed to centrifuge tube. The blood was centrifuged at 3000rpm for 5 minutes. The serum was used for estimation of biochemical parameters, such as SGOT, SGPT, ALP, ACP, MDA, total protein, triglycerides, GSH, LDH and total bilirubin.

RESEARCH FINDINGS AND ANALYSIS

In this study, we screened the phytochemical compounds and analyze the hepatoprotective activity of *Hygrophila auriculata* against cadmium chloride induced hepatotoxicity (Table 1).

Screening of phytochemical compounds:

The phytochemical compounds of *Hygrophila auriculata* were analysed and their entire phytochemical compound such as tannins, phylobatannins, saponins, flavonoids, steroids, terpenoids and cardiac glycoside were found.

Plant contains various phytochemical in varying amounts in its different parts. The entire plant has been reported to contain lupeol, stigmasterol, an isoflavon glycoside, an alkaloid and small quantities of uncharacterized bases (Sondhi and Agarwal,1995), isolated a number of minerals from the plant (Mishra *et al*., 1991) and isolated two aliphatic esters from the aerial part of the plant.

MDA and GSH:

Lipid peroxidation is a complex and natural deleterious process. Increasingly the free radicals to formation which increases the level of lipid peroxides in hepatic tissue and causes hepatic cell injury (Liber,1991). MDA is a major oxidation end product of lipid peroxidation and oxidative stress.

In our study, the increased level of MDA in cadmium chloride intoxicated rats observed when compared to control rats. The increase in MDA level in the rats suggested

enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanism to formation of excessive free radicals. Administration of aqueous extract of *Hygrophila auriculata* is significantly decreased the level of MDA in cadmium chloride rats. Level was nearly similar to that of standard drug silymarin.

GSH is a major non-enzymatic antioxidant in living organism, which play a central role in co-ordinating the body's antioxidant defense process and is found in high concentration (Grosshan and Calvin, 1985).

Glutathione is highly sensitive indicator of cells functionality and viability. GSH depletion is linked to number of diseases states including cancer, cardiovascular diseases. It is implicated in the cellular defense against xenobiotics naturally occurring deleterious components, such as free radicals and hydroperoxides. Thus the GSH concentration in liver cells is important.

In the present study, the decreased content of GSH in cadmium chloride intoxicated rat were observed compared to control rats. The decreased plasma GSH content, implying increased consumption for oxygen radical scavenging activity. Administration of aqueous extract of *Hygrophila auriculata* significantly increased in the level of GSH as in standard drug in cadmium chloride intoxicated rats.

AST and ALT:

Estimating the activity of serum markers enzyme like SGOT and SGPT can make the assessment of the liver function. When the liver cell is damaged, a variety of enzymes normally located in cytosol and released in to the blood stream. The estimation in serum is useful quantitative marker of the intent type of hepatocellular damage(Mithra *et al*.,1998).

AST is a cytosolic enzyme, which is more specific for the liver than ALT. Transaminase has been reported to attain normal levels with the healing of liver parenchyma and regeneration of liver cell (Shenoy *et al*., 2001).

Present study showed that increased activity of AST and ALT in cadmium chloride induced rats compared with control rats. The elevated activities of these enzymes due to inflammation in the liver. Administration of aqueous extract of *Hygrophila auriculata* significantly decreased the level of AST and ALT in rats and these levels slightly increased when compared to standard drug.

ALP and ACP:

ALP and ACP is a membrane bound enzyme and is released unequally depending on the pathological phenomenon. ACP present in many tissues, it is not a very specific enzyme (Raymand *et al*., 1996), but a family of enzyme which differing pathological properties. Serum ALP concentration is known to be markedly elevated in chloestasis and to be minimally increased in chronic hepato cellular disease (Wilkinson,1976).

Administration of cadmium chloride leads to the assimilation of fat in the liver leads of the increased ALP, ACP activity may also be due to the cytosomal imbalance, resulting in the destruction of the intact membranes. After treatment with aqueous extract of *Hygrophila auriculata.* there is decreased in the ALP and ACP level remarkably as in standard drug.

Triglyceride and LDH:

Triglycerides are mainly stored in the adipose tissue

Table 1 : Changes in the levels of biochemical patameters such as GSH, MDA, AST, ALT, ALP, ACP, TG, LDH, BR, total protein in control and experimental rats

Groups	GSH (mg/dl	MDA mg/dl)	AST IU/L	ALT (IU/L)	ALP (IU/L)	ACP (IU/L)	TG (mg/dl)	LDH (mg/dl)	Total bilirubin (mg/dl)	Total protein (g/dl)
Control	1.93 ± 0.30	0.80 ± 0.08	66.74 ± 2.81	23.28 ± 1.39	30.63 ± 0.23	3.98 ± 0.09	70.12 ± 0.03	126.32 ± 0.25	0.11 ± 0.03	6.12 ± 0.02
Cadmium chloride	0.73 ± 0.28*	2.470± 0.15*	96.87 ± 2.87*	30.47 ± 1.21*	103.21 ± 0.56*	8.39 ± 0.12*	180.32 ± 0.12*	301.13 ± 0.69	0.4 ± 0.13*	5.18 ± 0.06*
Cadmium + chloride Hygophila auriculata	1.47 ± 0.28**	1.40 ± 0.07**	76.32 ± 2.91**	23.72 ± 4.11**	36.38 ± 0.75**	4.01 ± 0.23**	74.23 ± 0.35**	131.31 ± 0.76	0.18 ± 0.04**	7.12 ± 0.05**
Cadmium Chloride + Standard drug (silymarin)	1.65 ± 0.35**	1.32 ± 0.15**	73.96 ± 4.59**	24.38 ± 1.83**	32.21 ± 0.65**	4.00 ± 0.26**	67.61 ± 0.45**	121.23 ± 0.09	0.15±0.01**	6.15 ± 0.04**

Values are expressed as mean ± standard deviation for 6 animals in each groups.* as compared to control group: ** as compared to toxicant group: Analysis by one way ANOVA followed by Dunnet's test and significant at P<0.05

(Ashakumary and Vijayammal, 1993). Triglycerides level found to be higher during liver injury. The plasma lipoprotein major sources of fatty acids to synthesis triglycerides. The disorder of lipid metabolism, which is characterized by increased level of triglycerides. Hence level may be high in cadmium chloride induced hepatoprotectivity damaged rats. Administration of aqueous extract of *Hygrophila auriculata* markedly reduces the level of triglycerides and the values were slightly increased than that of standard drug.

Serum LDH isoenzyme appear to be a sensitive indicator of hepatocyte damage and elevation of this isoenzyme is commonly seen after liver hypoxia, a common equal of congestive cardiac failure(Riley *et al*., 1960). In our study, the decreased content of LDH in cadmium chloride intoxicated rats compared to control rats. The evaluated activities of this enzymes due to inflammation in the liver. Administration of aqueous extract of *Hygrophila auriculata* markedly reduces the level of LDH.

Total bilirubin:

The estimation of serum total bilirubin confirms the intensity of jaundice. Bilirubin is transported mainly in the portal system to the liver, where it enters the hepatocyte on its membranes surface in contact with the sinusoids. Bilirubin level is very high in the hepatocellular lesions *i.e.*, both conjugated and unconjugated bilirubin(Raymand *et al*.,1996).

Total serum bilirubin concentration indicate the functional transport capacity of liver, the degree of increased in serum bilirubin values as prognostic significant of liver injuries. In this study, the level of total bilirubin increased in cadmium chloride induced rats compared to control rats which indicated the abnormal liver function. Administration of aqueous extract of *Hygrophila auriculata* significantly restored in the level of bilirubin in cadmium chloride induced rats. This level was nearly similar to standard drug.

Total protein:

Serum protein reflects liver synthetic function rather than cell injury because protein metabolism was occurring in the liver. Accordingly, changes in the serum proteins form the basis for important laboratory aids to the diagnosis of hepatic disease.

The site specific oxidative damage of some of the susceptible amino acid of protein is now regarded as the major cause of metabolic dysfunction during pathogenesis (Srivastava *et al*., 1999). In the context in cadmium chloride induced rats, protein level was decreased than control rats. Administration of aqueous extract of *Hygrophila auriculata* is significantly increased the level of protein as in standard silymyrin drug.

On basis of above results, it can be concluded that aqueous extract of *Hygrophila auriculata* is a valuable protection against hepatotoxicity by cadmium chloride in animal model by normalizing biochemical parameters. The hepatoprotective activity of *Hygrophila auriculata* may be due to the phytochemical constituents such as flavonoids present in it. Further extensive studies are required for its potential uses as a hepatoprotetive drug in clinical practice.

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