INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

HEPATOPROTECTIVE EFFECT OF *INDIGOFERA LINNAEI* ALI. ON CARBON TETRACHLORIDE INDUCED WISTAR ALBINO RATS

M. Akila and G. Prasanna*
P. G and Research Department of Biochemistry, Sengamala Thayaar Educational Trust Women's College, Mannargudi, Tamil Nadu, India
*Corresponding Author Email: prasannakeertana@yahoo.in

Article Received on: 18/03/14 Revised on: 11/04/14 Approved for publication: 02/05/14

DOI: 10.7897/2230-8407.050581

ABSTRACT
The present investigation has been carried out the hepatoprotective activity of *Indigofera linnaei* Ali on carbon tetrachloride induced wistar albino rats. Carbon tetrachloride induced liver damage was well manifested by significant increase in the activities of Aspartate transaminae (AST), Alanine transaminase (ALT), Alkaline phosphatase (ALP), Gamma glutamyl transferase (GGT), Total Bilirubin, Cholesterol, Triglycerides, Lipid peroxide (LPO) also decreased the levels of Total protein, Glycogen, Superoxide dismutase (SOD) and Reduced glutathione (GSH). The oral administration of *Indigofera linnaei* Ali (100 mg/kg bw) along with carbon tetrachloride for 21 days reversed these altered parameters to normal level which indicating the hepatoprotective efficacy of *Indigofera linnaei* Ali against carbon tetrachloride induced liver injury.
Keywords: Hepatoprotective, *Indigofera linnaei* Ali, Carbon tetrachloride.

INTRODUCTION

Liver plays a pivotal role in the regulating various physiological process in the body. It is involved with almost all biochemical pathways to growth, fight diseases, nutrients supply, provision and reproduction[1]. Liver diseases are some of the fatal diseases in world today; they pose 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[2]. Liver diseases are the most serious ailment and are mainly caused by toxic chemicals [excess consumption of alcohol, high doses of paracetomol, carbon tetrachloride, chemotherapeutic agents, peroxidised oil etc. Most of the chemicals damage liver cells mainly by including lipid per oxidation and other oxidative damage[3]. Carbon tetrachloride is one of the most commonly used hepatotoxin in the experimental study of liver diseases since the changes associated with CCl_4 induced liver damage is similar to that of viral acute hepatitis[4]. Carbon tetrachloride is metabolically activated by the cytochrome p-450 dependent mixed oxidase in endoplasmic reticulam to form trichloromethyl free radical which combined with cellular lipids and proteins in the presence of oxygen to induce lipid peroxidation[5]. In the absence of reliable liver protective drug in modern medicine there are a number of medicinal preparations in Ayurveda recommended for the treatment of liver disorders[6]. Liver protective plants contain a variety of chemical constituents like phenols, coumarins, lignans, essential oil, monoterpenes, carotinoids, glycosides, flavanoids, lipids, alkaloids and xanthenes[7]. *Indigofera linnaei* Ali (Fabaceae) is a small trailing, much branched annual or biennial herb, distributed throughout India. The root, stem and leaves are biter, thermogenic, laxative, trichogenous, expectorant, anthelmintic, tonic and diuretic are useful for promoting the growth of hair and in gastropathy, splenomegaly, cephalagia, cardiopathy, ulcer and skin diseases. The leaf juice is given in the dose 10-20 ml along with honey twice daily for jaundice, inflammation of liver etc. The leaves are useful to treatment of hydrophobia. An extract of plant is good for epilepsy, neuropathy and antioxidant activity[8]. The present study is aimed to evaluate the hepatoprotective activity of aquous extract of the leaves of *Indigofera linnaei* Ali against CCl_4 induced hepatotoxicity in rats.

MATERIALS AND METHODS

Collection of Plant

Plant source selected for the present study is *Indigofera linnaei* Ali. Whole plants of the *Indigofera linnaei* Ali was collected from in and around Trichy, identified and authenticated with RAPINAT Herbarium, St. Joseph's College, Trichy, Tamil Nadu, India.

Preparation of Plant Extract

Fresh plant material was shade dried and powdered coarsely using electric blender. 300 g of coarse powder of *Indigofera linnaei* Ali was taken and extracted with water. The plant material was mixed with and its six parts of water was added, boiled and reduced to one third and filtrate was evaporated to dryness. Paste form of the extract obtained was subjected to pre-clinical screening. Percentage yield of the plant extract was 25.76 %.

Chemicals

Carbon tetrachloride and all other chemical used in the experiment were of analytical grade, the biochemical reagent used for the assay was purchased from Sri Anchana diagnostic, Trichy, India.

Preliminary Phytochemical Screening

Aqueous extract obtained was subjected to preliminary screening for the presence or absence of active phytochemicals by using the qualitative tests[9,10].

Experimental Animals

Healthy adult Wistar strain of albino rats of both sexes, two to three months old and weighing 150-200 g were obtained

from Tamil Nadu, India Veterinary and Animal Sciences University, Chennai, India. The animals were allowed to acclimatize to laboratory conditions for a period of 5 days prior to the experiment. Animals were housed in standard polypropylene cages. Six animals were housed per cage, so as to provide them with sufficient space, and to avoid unnecessary morbidity and mortality. Animals were maintained under standard condition of 12- hour's light/dark cycle and at an ambient temperature at 23 ± 2°C, with 65 ± 5 % humidity. Animals were fed with standard rat chow pellet obtained from Sai Durga Feeds and Foods, Bangalore, India and water *ad libitum*. All the studies were conducted according to the ethical guidelines of CPCSEA after obtaining necessary clearance from the committee (Approval No: 790/03/ac/CPCSEA).

Assessment of Hepatoprotective Activity

Wistar strains of albino rats were divided into five groups, each comprising of six rats. Group I served as normal control. Group II received CCl_4 at a dose of 0.5 ml/150 g b. w. in olive oil (1:1 v/v) for 3 days. Group III and IV CCl_4 induced hepatotoxic rats treated with aqueous extract of *Indigofera linnaei* Ali (AEIL) at the doses of 150 and 300 mg/kg b.w. Group V CCl_4 induced hepatotoxic rats received standard drug silymarin (25 mg/kg b.w.). After the experimental period (21 days), animals were sacrificed by cervical decapitation. Blood was collected and serum was separated by centrifuging at 3000 rpm for 10 minutes and subjected for the determination of liver markers AST, ALT, ALP, GGT[11], biochemical parameters like Bilirubin[12], Cholesterol[13], Triglycerides[14], Glycogen and Protein[15]. Liver were dissected out and washed in ice-cold saline. Liver tissues were homogenized in 0.1 M phosphate buffer, pH 7.4 and used for studying LPO[16], and antioxidant such as SOD [17] and GSH[18].

Statistical Analysis

All the results were expressed as mean ± S.E. The data were statistically analyzed by one – way analysis of variance (ANOVA) followed by Duncan multiple range test. Statistical presentations were organized using the Statistical Package for Social Sciences (SPSS), Windows version 17.0, 2008, SPSS Inc., New York. Inter group comparison were carried out and P values ≤ 0.05 were considered significant.

RESULT

In this study, we screened the phytochemical compounds and analyze the hepatoprotective activity of *Indigofera linnaei* Ali against carbon tetrachloride induced hepatotoxicity.

Screening of Phytochemical Compounds

The phytochemical compounds of *Indigofera linnaei* were analysed and their results were given in the Table 1. The phytochemical compound such as Tannins, Saponins, steroids, terpenoids, coumarin and alkaloids were found.

Hepatoprotective Activity

The administration of CCl_4 in-group II rats resulted in significant hepatic damage and it was observed from the elevated levels of AST, ALT, ALP, GGT, and bilirubin,cholesterol and triglycerides in serum and lipid peroxide in liver tissue. The reduced levels of antioxidants (super oxide dismutase and reduced glutathione), total protein were also observed in group II rats when compared to normal rats (Table 2, 3, 4 and 5). Treatment with AEIL (Aqueous Extract of *Indigofera linnaei* Ali) brought the altered levels of the above parameters to near normal level thereby causing significant protection against CCl_4 induced liver damage. However, highly significant effect was observed in group IV rats treated with high dose of AEIL (300 mg/kg b.w.)

Table 1: Phytochemical screening of aqueous extracts of *Indigofera linnaei* Ali

S. No.	Phytochemicals	Results
1	Tannins	+
2	Phylobatannins	-
3	Saponins	+
4	Flavonoids	-
5	Steroids	+
6	Terpenoids	+
7	Coumarins	+
8	Alkaloids	+
9	Glycosides	-

(+)-positive; (-)-negative

Table 2: Changes in the level of liver marker enzymes in control and experimental rats

Groups	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	GGT (IU/L)
I	23.87 ± 1.95*	33.90 ± 1.52*	82.77 ± 1.81*	17.76 ± 1.86*
II	105.49 ± 1.04*,**	94.03 ± 1.53*,**	215.16 ± 1.78*,**	67.57 ±1.85*,**
III	78.11 ± 1.50	77.15 ± 2.28	176.66 ± 1.45	50.94 ± 1.79
IV	41.83 ± 1.97**	55.06 ± 1.13**	90.50 ± 2.20**	32.85 ± 1.74**
V	34.72 ± 1.99	41.87 ± 1.68	88.78 ± 1.61	19.80 ± 1.86

Values are expressed as mean ± SEM n = 6, *Significant when compared between Group I and Group II ($p \leq 0.05$)
**Significant when compared between Group II and Group IV ($p \leq 0.05$)

Table 3: Changes in the levels of bilirubin and liver glycogen in control and experimental rats

Groups	Bilirubin (mg/dl)		Liver Glycogen (mg/g tissue)
	Total Bilirubin	Direct Bilirubin	
I	0.68 ± 0.05*	0.55 ± 0.02*	8.41 ± 0.06*
II	1.88 ± 0.02*, **	1.28 ± 0.02*, **	4.33 ± 0.04*, **
III	1.49 ± 0.02	0.99 ± 0.49	6.53 ± 0.03
IV	0.77 ± 0.02**	0.58 ± 0.02**	7.34 ± 0.02**
V	0.74 ± 0.05	0.57 ± 0.02	7.47 ± 0.02

Values are expressed as mean ± SEM n = 6, *Significant when compared between Group I and Group II ($p \leq 0.05$),
**Significant when compared between Group II and Group IV ($p \leq 0.05$)

Table 4: Changes in the levels of biochemical parameters in control and experimental rats

Groups	Serum Cholesterol (mg/dl)	Serum Triglyceride (mg/dl)	Serum protein (g/dl)
I	163.86 ± 1.50*	54.19 ± 1.74*	7.98 ± 1.91*
II	247.5 ± 1.17*, **	170.08 ± 1.68**	4.5 ± 1.18*, **
III	210.88 ± 1.72	151.81 ± 1.96	6.59 ± 1.85
IV	154.46 ± 1.36**	96.05 ± 2.36**	7.58 ± 1.83**
V	139.15 ± 1.23	49.71 ± 1.70	7.82 ± 1.90

Values are expressed as mean ± SEM n = 6, *Significant when compared between Group I and Group II ($p \leq 0.05$)
**Significant when compared between Group II and Group IV ($p \leq 0.05$)

Table 5: Changes in the levels of LPO, SOD and GSH in control and experimental rats

Groups	Lipid Peroxide (ng of MDA/g tissue)	Superoxide Dismutase (mM of epinephrine oxidised/min/mg)	Reduced Glutathione (µg of GSH/g tissue)
I	1418.64 ± 9.53*	7.24 ± 0.08*	2794.30 ± 12.36*
II	8362.50 ± 14.95*, **	2.06 ± 0.05*, **	570.8 ± 11.4*, **
III	6889.28 ± 10.01	4.08 ± 0.06	680.84 ± 10.44
IV	2662.79 ± 14.31**	6.80 ± 0.07**	2486.95 ± 10.84**
V	1584.81 ± 7.80	6.94 ± 0.26	2575.90 ± 9.04

Values are expressed as mean ± SEM n = 6, *Significant when compared between Group I and Group II ($p \leq 0.05$)
**Significant when compared between Group II and Group IV ($p \leq 0.05$)

DISCUSSION

In our current investigation it was observed that the protective effect of *Indigofera linnaei* Ali evaluated against carbon tetrachloride induced hepatotoxicity in wistar albino rats. Phytochemical were also analysed and the result showed the presence of tannins, saponins, steriods, terphenoids, coumarin and alkaloids.

AST and ALT

Estimating the activity of serum marker enzymes like AST and ALT can make the assessment of the liver function. When the liver cell 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[19]. 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 parenchymal and regeneration of liver cells[20]. In our study, the increased activity of AST and ALT were observed in carbon tetrachloride induced rats compared with control rats. The elevated activities of these enzymes are due to inflammation in the liver. Administration of aqueous extract of *Indigofera linnaei* Ali significantly decreased ($P \leq 0.05$) the level of AST and ALT.

ALP and GGT

ALP is a membrane bound enzyme and released to unequally depending on the pathological phenomenon. GGT present in many tissue. Serum ALP concentration are known to be markedly elevated in cholestasis and to be minimally increased in chronic hepato cellular diseases[21]. In our present study, administration of carbon tetrachloride leads to the assimilation of fat in the liver leads to increase. After treatment with aqueous extract of *Indigofera linnaei* Ali decreased ($P \leq 0.05$) the level of ALP and GGT remarkably.

Total Bilirubin and Glycogen

The estimation of serum total bilirubin confirms the intensity of jaundice. Bilirubin is transported mainly in the portal system to the liver, were it enter the hepatocyte on its membrane surface in contact with the sinusoids. Bilirubin level is very high in the hepatocellular leasion i.e. both conjugated and unconjugated bilirubin[22]. In the present study, the level of total bilirubin increased in carbon tetrachloride induced rats compared to control rats. The increased level of bilirubin indicated in the abnormal liver function and administration of aqueous extract of *Indigofera linnaei* Ali significantly ($P \leq 0.05$) restored in the level of bilirubin in carbon tetrachloride induced rats. CCl_4 induced damage of hepatocytes is also a reason behind decreased glycogen content of liver tissue. Significant increase ($P \leq 0.05$) in hepatic glycogen level was observed after administration of the extract indicating improvement in hepatic states.

Total Cholesterol, Triglycerides and Protein

Inhibition of bile acids synthesis from cholesterol which is syntheses in liver or derived from plasma lipids leading to increase in the cholesterol levels were also resulted due to carbon tetrachloride intoxication. Suppression of cholesterol levels by the extract suggest the bile acids synthesis inhibition was reversed. Triglycerides are mainly stored in the adipose tissue. Triglycerides levels found to be higher during liver injury. The plasma lipoprotein is the major source of fatty acids to synthesis triglycerides. The disorder of lipid metabolism, which is characterized by increased level of triglycerides[23]. In the present study the levels of total cholesterol and triglycerides were increased in carbon tetrachloride induced rats compared to normal rats. Administration of aqueous extract of *Indigofera linnaei* Ali is significantly decreased ($P \leq 0.05$) the level of cholesterol and triglycerides. Diminution of total protein by CCl_4 is a further indication of liver damage[24]. Aqueous extract of *Indigofera linnaei* Ali has increased the level of serum protein towards the respective normal level, which indicates hepatoprotective activity. Stimulation of protein synthesis have been advanced as a contributary hepatoprotective mechanism which accelerates the regeneration process and the production of liver cells[25,26].

Lipid peroxide (MDA)

Lipid per oxidation is a complex and natural deleterious process. Increasingly the free radicals to the formation which increases the level of lipid peroxides in the hepatic cell injury[27]. MDA is a major oxidative end product of lipid per oxidation and oxidative stress. In this study, the increased levels of MDA were observed in carbon tetrachloride

intoxicated rats compared to normal rats. The increase in the MDA levels in the rats suggested enhanced lipid per oxidation leading to tissue damage and failure of antioxidant defence mechanism to the formation of excessive free radicals. Administration of aqueous extract *Indigofera linnaei* Ali is significantly decreased ($P \leq 0.05$) the level of MDA in carbon tetrachloride intoxicated rats.

SOD

SOD is major enzymatic antioxidants in living organism. SOD activities and this depletion may result from oxidative modification of this proteins[28]. In this study, we observed decreased content of SOD in carbon tetrachloride intoxicated rats compared to normal rats. Administration of aqueous extract of *Indigofera linnaei* Ali significantly increased ($P \leq 0.05$) in the level of SOD in carbon tetrachloride intoxicated rats.

GSH

GSH is a major non- enzymatic antioxidant in living organism, which a central role in co-ordinating the body's antioxidant defence process and is found in high concentration[29]. Glutathione is highly sensitive indicator of cells functionality and viability. GSH depletion is linked to a number of diseases states including cancer, cardiovascular diseases. It is implicated in the cellular defence against xenobiotics naturally occurring deleterious components, such as free radical and hydroperoxides. Thus the GSH concentration in the liver cells is important. In the present study, decrease in the level of GSH in carbon tetrachloride induced rats when compared to normal rats. After administration of aqueous extract *Indigofera linnaei* Ali significantly increased ($P \leq 0.05$) in the level of GSH in carbon tetrachloride intoxicated rats. On basis of above result, it can be concluded that aqueous extract of *Indigofera linnaei* Ali is a valuable protection against carbon tetrachloride induced hepatotoxic animal model by normalizing various biochemical parameters and tissue injury. The hepatoprotective activity of *Indigofera linnaei* Ali may be due to the phytochemical constituents present in it. Further extensive studies are required for its potential uses as a hepatoprotective drug in clinical practice.

REFERENCES

1. Ward FM, Daly MJ. Hepatic Disease, International Clinical Pharmacology and Therapeutics, Churchil livingstone, New York; 1999.
2. Laurence DR, Bennett PN. Churchill living stone. Clinical Pharmacology. 7thed; 1992.
3. Rectnagel RO, Glende EA, Dolak JA and Waller RL. Mechanism of carbon tetra chloride toxicity. Pharmacol Ther 1989; 43: 139. http://dx.doi.org/10.1016/0163-7258(89)90050-8
4. Weremfo A, Asamoah KA, Abassah Oppong. Preliminary study on hepatoprotective activity of Tomato (*Solanum lycopersicum* L.) Pulp against hepatic damage in rats. Advances in Biological 2011; 5(5): 248-250.
5. Rajib Ahsan KM, Monirul Islam Musaddik A and Haque E. Hepatoprotective activity of methanol extract of some medicinal plants against carbon tetrachlorode induced hepatotoxicity in albino rats. Global J. Pharmacol 2009; 3(3): 116-122.
6. Chatterjee TK. Medicinal plants with hepatoprotective properties in herbal opinions. 3rded. Books and Allied (P) Ltd. Calcutta; 2000.
7. Sharma SK, Ali M, Gupta J. Plants having hepatoprotective activity. Phytochemistry and Pharmacology 2002; 2: 253-270.
8. Senthil kumar R, Rajkapoor. Antioxidant potentital of *indicofera linnaei.* Pharmacology online 2011; 1: 710-720.
9. Kokate CK. Practical pharmacognosy. 4thed. (Vallabh Prakashan, Pune); 1995. p. 107.
10. Khandelwal KR. Practical Pharmacognosy. 13thed. (Nirali Prakashan, Pune); 2005. p. 149.
11. King J. Practical clinical enzymology. (D Von Nostrand Co. Ltd, London); 1965. p. 182.
12. Aniya Y, Koyama T, Miyahira M, Inomata C, Kinoshita S Ichiba T. Free radical scavenging actions of the medicinal herb, *Crassocephalum crepidiodes* from the Okinawa Islands. Biol. Pharm. Bull 2005; 28(1): 19-23. http://dx.doi.org/10.1248/bpb.28.19
13. Allain CC, Poon LS, Chan CSG, Richmond W and Fu PC. Enzymatic determination of total serum cholesterol. Clin Chem 1974; 20: 470.
14. Rice EW. Triglyceride in serum. In standard methods of Clinical chemistry. Roderick R Mac Donald. Academic Press 1970; 6: 215-222.
15. Lowry OH, Roesbrough NJ, Farr AL and Randall RJ. Protein measurment with folin phenol reagent. J. Biol Chem 1951; 193: 265.
16. Ohkawa H, Ohishi N and Yagi K. Assay of lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979; 95: 351. http://dx.doi.org/10.1016/0003-2697(79)90738-3
17. Mishra HP and Fridowich I. The role of superoxide dismutase anion in anti oxidation of epinephrine and sample assay in superoxide dismutase. J. Biol Chem 1972; 241: 3170.
18. Moron MA, Depierre JW and Mannervick B. Levels of glutathione S transferase activities in rat liver. Biochem Biophys Acta 1979; 582: 67. http://dx.doi.org/10.1016/0304-4165(79)90289-7
19. Mithra SK, Vengadaragunna MV, Sundaram R and Doupumadhavan S. Protective effect of HD Herbal emulsion against various hepatotoxic agents in rats. J. E. thanopharmacol 1998; 40: 213-218.
20. Shenoy KA, Somaniji SN and Bairy KL. Hepatoprotective effect of ginkgobioba against CCl_4 induced hepatic injury in rats. Indian J Pharm 2001; 33: 260.
21. Wilkinson JH. The principle and practice and diagnosis enzymology. Edward Arnold (publisher) London; 1976. p. 305-348.
22. Raymand RO. A new direction in the study of CCl_4 hepatotoxicity. Life science 1983; 33: 4018.
23. Ashakumary L, Vijayammal PL. Additive effect of alcohol and nicotine on lipid metabolism in rats. Indian J Exp. Biol 1993; 31: 101-114.
24. Navarro VJ, Senior JR. Drug – related hepatotoxicity. N Engl Med 2006; 354: 731-9. http://dx.doi.org/10.1056/NEJMra052270
25. Rip JW, Rupar CA, Ravi K, Carroll KK. Distribution, metabolism and function of dolichol and polyprenols. Prog Lipid Res 1985; 24: 269-309. http://dx.doi.org/10.1016/0163-7827(85)90008-6
26. Tadeusz J, Teresa J, Krzysztof N. The role of polyprenol in modulation of physical properties of model membranes 2001; 25: 33-38.
27. Liber CS. Alchol, Liver JAM cell nutrition 1991; 10: 62.
28. Augustyniak A, Wazkilwicz E, Skrzydlewaka E. Prevention action of Green tea from changes in the liver antioxidant abilities of different aged rats intoxicated with ethanol. Nutrition 2005; 21: 925-932. http://dx.doi.org/10.1016/j.nut.2005.01.006
29. Grosshans K and Calvin HL. Estimation of glutathione in purified population of mouse tesitigerm cells. Biol report 1985; 33: 1997-1205. http://dx.doi.org/10.1095/biolreprod33.5.1197

Cite this article as:
M. Akila and G. Prasanna. Hepatoprotective effect of Indigofera Linnaei Ali. on carbon tetrachloride induced wistar albino rats. Int. Res. J. Pharm. 2014; 5(5):392-395 http://dx.doi.org/10.7897/2230-8407.050581

Source of support: Nil, Conflict of interest: None Declared