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PRELIMINARY STUDIES ON HEPATOPROTECTIVE ACTIVITY OF LIMONIA ACIDISSIMA AND CLITORIA TERNATAEA IN CARBON TETRA CHLORIDE INDUCED EXPERIMENTAL RATS

V.Elango and D.Eazhisai vallabi

Department of Siddha Medicine, Faculty of Sciences, Tamil University, Thanjavur -613010

Abstract:

Objective: *To evaluate the hepatoprotective activity of Limonia acidisssima leaves (LAL) and flowers of Clitoria ternatae (CTF) in experimental animals.*

Materials and Methods: *The leaves and flowers of the selected plant were shade dried and the plant extract was prepared by using soxhlet apparatus using the solvent ethanol at constant temperature and it was used for the in-vivo work. Biochemical parameters like bilirubin, albumin, total protein, transaminase, phosphatase and LPO were tested in carbon tetra chloride induced experimental animals were tested.*

Results: *The ethanolic plant extract of both plant parts (leaves of LA and flowers of CT) were found to be effective against the carbon tetra chloride induced hepatotoxicity in experimental male albino rats. The results shows the elevated level of total protein, albumin, liver glycogen and reduced level of phosphatase, transaminase, lipid peroxide enzyme, and bilirubin than the carbon tetra chloride treated experimental animals.*

Conclusion: *The leaves of Limonia acidisssima and flowers Clitoria ternatae has the efficacy to protect against hepato-toxic disease and hepatic necrosis may due to the presence of phyto-constituents present in it.*

Keywords: *Limonia acidisssima, Clitoria ternatae, biochemical estimations, carbon tetra chloride.*

Corresponding author:

Dr.V.Elango M.Sc., Ph.D

Associate professor,

Department of Siddha Medicine,
Faculty of Sciences,
Tamil University, Thanjavur -613010
E mail.id: drelangovantu@gmail.com
Contact number: 9843725592

QR code

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INTRODUCTION:

The Indian Traditional Medicine like Siddha, Ayurveda and Unani are predominantly based on the use of plant materials. Herbal drugs have gained importance and popularity in recent years because of their safety, efficacy and cost effectiveness. Several Indian medicinal plants have been extensively used in the Indian traditional system of medicine for the management of liver disorder [1]. In Siddha traditional system of medicine, plant were claimed to be effective and used successfully to alleviate multiple liver disorders. There are number of phytoconstituents from plants which have exhibited hepatoprotective activity. Recent progress in the study of such plants has resulted in the isolation of about 170 different phytoconstituents from plants belonging to about 55 families, which exhibit hepatoprotective activity [2] .

Liver is one of the vital organ in human body for intense metabolism and excretion. It has a surprising role in the maintenance, performance and regulating homeostasis of the body. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction .The major functions of the liver are carbohydrate, protein and fat metabolism, detoxification, secretion of bile and storage of vitamin. Thus, to maintain a healthy liver is a crucial factor for overall health and well-being. But when it is continuously and variedly exposed to environmental toxins, chemicals like carbon tetra-chloride, drug habits, alcohol, infections and autoimmune disorders, prescribed (antibiotics, chemotherapeutic agents) cum over-the-counter drugs can eventually lead to various liver ailments like hepatitis, cirrhosis and alcoholic liver disease [3].

Several phyto-medicines (medicinal plants or herbal drugs) are now used for the prevention and treatment of various liver disorders. Although experimental studies have been conducted on a number of these plants and their formulations, however, only some plants have clearly shown the hepatogenic / hepatoprotective effects against liver diseases or hepatotoxicity caused by variety of hepatotoxic agents such as chemicals, drugs, pollutants, and infections from parasites, bacteria or viruses (e.g., hepatitis A, B and C), etc. Indeed, to obtain satisfactory herbal drugs for treating severe liver diseases, the medicinal plants must be evaluated systematically for properties like antiviral activity (Hepatitis B, Hepatitis C, etc.), antihepatotoxicity activity (antioxidants and others), stimulation of liver regeneration and choleretic activity. A combination of different herbal extracts / fractions is likely to provide desired activities to cure severe liver diseases. The medicinal plants contain several phytochemicals which possess strong antioxidant property, leading to anti hepatotoxic activity.

Herbal-based therapeutics for liver disorders has been in use in India for a long time and has been popularized world over by leading pharmaceuticals. The limiting factors that contribute to this eventuality are (i) lack of standardization of the herbal drugs; (ii) lack of identification of active ingredient(s)/principles(s); (iii) lack of randomized controlled clinical trials (RCTs), and (iv) lack of toxicological evaluation [4] .

The main causes of liver damage are Chemical agents, certain antibiotics, peroxidase oil, aflatoxins, CCl_4, and chlorinated hydrocarbon etc. excess consumption of alcohol, infection and autoimmune disorders. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid per oxidation and other oxidative damages in liver. Enhanced lipid per oxidation produced during the liver microsomal metabolism of ethanol may result in hepatitis and cirrhosis [5].

Carbon tetrachloride CCl_4 is widely used for experimental induction of liver damage [6]. The principle cause of carbon tetrachloride (CCl4) is induced hepatic damage in lipid peroxidation and decreased activities of antioxidant enzymes and generation of free radicals [7]. Various medicinal plants have been used to treat for various diseases in all over the world. Nowadays, Indian medicinal plants are belonging to about 40 families were investigated as liver protective drugs [8].

The preliminary phytochemical analysis of *Limonia acidissima* plant parts showed the presence of alkaloids, flavonoids, phenols, terpenoids, tannins, fats steroids, saponins, glycosides, gum, mucilage and fixed oils [9].

The unripe fruits contain stigma-sterol. Fruit pulp contains large quantity of citric acid and other fruit acids, mucilage and minerals. Alkaloids, coumarins, fatty acids and sterols have been detected in the pericarp. It also contains umbelliferone, dictamnine, xanthotoxol, scoparone, xanthotoxin, isopimpinellin, isoimperatorin and marmin [10].

Leaves contain stigmasterol, psoralen, bergapten, orientin, vitedin, saponarin, tannins and an essential oil [21]. Marmesin, feronolide and feronone have been isolated from the bark [11].

A wide range of secondary metabolites including triterpenoids, flavones glycosides, anthocyanins and steroids has been isolated from *Clitoria ternatea* Linn. Four kaempferol glycosides I, II, III and IV were isolated from the leaves of *Clitoria ternatea* L. Kaempferol-3- glucoside (I), kaempferol- 3-rutinoside (II) and kaempferol-3- neohesperidoside (III) were identified by Ultra Violet, Protein Magnetic Resonance and Mass Spectrometry. (IV),

C33H40O19, mp: 198, was characterized as Kaempferol-3- orhamnosyl glucoside from spectral data and was named clitorin.

MATERIALS AND METHODS:

Plant Materials

Fresh plant sample *Limonia acidissima* (leaves) and *Clitoria ternatea* (flowers) were collected from various parts of Thanjavur district, South India. The leaves and flowers were washed for any contaminants, dried thoroughly under shade and powdered finely. The powdered leaves of LA flowers of CT were used for ethanol extraction. 500 g of powered leaves and flowers material was extracted with 2.5 L of ethanol using Soxhlet apparatus at constant temperature until the powdered plant parts became colorless. The dried extracts were used for the experiment.

Experimental Methods

Male albino rats of 8 -10 weeks of age weighing between 100 and 120g were used for the study. The animals were housed in polypropylene cages. Animals were divided into 4 groups of five animals. The animals were acclimatized for a week under laboratory conditions. All experiments were performed according to the norms of the local ethical committee.

Experimental animals were distributed randomly, in 4 groups, containing 5 animals each. The first group followed by normal animals provided with usual rat feed and water *ad libitum.* Group II animals provided with rat feed and water along with CCl_4, 0.3ml per animal daily for ten days mixing with paraffin in the ratio of 3:1.Third and fourth group animals were treated with normal rat feed, water, CCl_4 and extract of *Limonia acidissima* (LAL) leaves and *Clitoria ternatea* (CTF) flowers at the dose of 25mg/ 100 kg.body.weight given separately according to the body weight and the drug followed by it. At the end of treatment, animals were fasted overnight, anaesthetized with ether the blood serum was collected for biochemical analysis.

Biochemical Parameters

After the collection of the blood serum the antioxidant assay lipid peroxide content was assayed by thio-barbituric acid method colorimetrically. Transaminases (ALT) activities were estimated by Reitman and Frankel method and which was measured spectrometrically. The acid phosphatases (ALP) was estimated and the absorbance was read at 405nm. Albumin level was measured spectrometrically at 600nm and total protein by biuret method a blue purple colored complex with absorbance at 550nm. Bilirubin (Total and Direct bilirubin) level was estimated by Anderson method [20] and the Glycogen was estimated by Anthrone reagent method. Mean values standard were calculated and for all the values carried out [12].

RESULTS AND DISCUSSION:

Table 1: Hepatoprotective activity of LAL and CTF in CCl_4 induced experimental rats.

Group	Normal	Ccl_4 treated	*Limonia acidissima* Treated	*Clitorea ternatea* Treated
Dose	Saline	0.3ml	25mg/kg.b.wt	25mg/kg.b.wt
Total Protein gm/dl	8.30±0.64	4.29±0.261	7.35±0.511	7.93±0.436
Albumin gm/dl	5.39±0.318	1.56±0.19	5.23±0.41	4.05±0.32
Total Bilirubin mg/dl	0.26±0.028	0.590±0.052	0.46±0.039	0.37±0.025
Direct Bilirubin mg/dl	0.70±0.042	0.407±0.0043	0.56±0.033	0.50±0.025
ALT U/l	111.1±7.70	197.71±21.55	88.3±4.40	122±7.32
ALP U/l	47.28±2.75	92.20±4.70	55.28±2.75	64.4±4.48
Glycogen U/l	30.2±3.11	18.9±1.56	27.3±1.38	26.0±1.74
LPO in serum nM/ml MDA	1.60±0.13	2.715±0.179	1.750±0.145	1.425±0.145

Each values is the Mean ± SEM of five animals statistically significant from control

Bilirubin is the most important excretory product of bile, formed as a result of breakdown of hemoglobin. It is formed in reticulo- endothelial system i.e. spleen, bone marrow and kupffer cells in the liver and it circulates attached to the plasma albumin, in low concentration in the blood [13].

Hepatic or hepatocelluar disease is associated with damage to the parenchymal cells of liver by toxic and infective agents, the power to transfer bilirubin from the blood to the biliary canaliculi being diminished. Drugs or poisons causing liver cell necrosis include CCl_4, cytotoxins, sulphonamides, paracetamol, tetracycline, alcohol, chloroform and phosphorous [14]. There are reports to indicate that serum bilirubin level is elevated in CCl_4 poisoning. The cellular degeneration and necrosis permit diffusion of bilirubin into the blood that has reached the canaliculi. Swelling of cells and edema add an intrahepatic obstructive element which causes leakage of bilirubin from the canaliculi into the blood. When there is increase in bilirubin content, there is visible coloration of the skin, sclera and mucous membranes and clinical jaundice is present [15].

In the present study the normal animal shows the 0.26mg/dl (TB), 0.70 mg/ dl (DB) and the CCl_4 induced animals shows 0.590 mg/dl (TB), 0.407 mg/dl (DB). The CTF and LAL treated animal shows the reduced level of total bilirubin and direct bilirubin 0.46 mg/ LAL, 0.37mg/dl CTF and 0.56mg /dl LAL 0.50 mg /dl CTF respectively.

Concentrations below the reference range usually reflect low albumin concentration, for instance in liver disease or acute infection. Rarely, low total protein may be a sign of immunodeficiency. Thus the result shows the increased level of albumin and total protein due to the action of the herbal drug CTF and LAL against the CCl_4.

Polyribosomal dissociation and depression of protein synthesis have been reported to occur 5 minutes after CCl4 administration [16]. In patients with acute viral hepatitis serum levels of albumin and pre-albumin were decreased as compared with that of normal subjects. Reversal of serum albumin, globulin ratio (A/G) has been recognized as a significant abnormality associated with liver disease [17]. After the treatment with LAL and CTF extracts the serum total protein level was increase to 7.33gm/dl, 7.93gm/ dl and the albumin level 5.23 gm/dl, 4.05 gm/dl from untreated control animals.

The result shows the significant hepatotoxicity induced by CCl_4 was evidenced by increased in phosphatase and transaminase due to hepatocyte necrosis in lysosomal latency. The drug administration (leaves of LA and flowers of the CT) was able to treat and protect the hepatocyte necrosis and inflammation. The rise in serum levels of ALT and ALP have been attributed to the damaged structural integrity of the liver, because they are located in the cytoplasm and are released into circulation after cellular damages. Elevated serum levels of ALP are found in hepatobilary diseases [18] (Recknagal *et al.*, 1982). In LAL and CTF the serum ALT was decrease to 88 U/l, 122U/l, and the ALP level was 55 U/l, 64U/l from untreated control animals.

In the present experimental study normal animals show 1.60 in serum nM/ml MDA, as the normal level of serum Lipid peroxidation. After the induction of hepatotoxicity with CCl_4 (0.3ml) in animals, it was found that there was an increase by 2.715 nM/ml MDA than the normal level. After the treatment with LAL and CTF extracts, the tissue lipid peroxidation level was reduced to 1.750 nM/ml MDA and 1.425 nM/ml MDA from the untreated control animals.

The result of this study shows that CCl_4 produced hepatotoxicity was evidenced by increasing in lipid peroxidation products suggesting the involvement of oxidative stress and suggestive of tubular damage. The drug treated groups exert a protection against oxidative stress and siddha herbal powder LAL and CTF against CCl_4 induced hepatotoxicity.

It has been known for many years that administration of CCl_4 of rats causes significant depletion of liver glycogen content. The glycogen loss *in vivo* is associated with an increase in the activity of phosphorylase –A, a key enzyme involved in the degradation of glycogen. The glycogen loss *in vivo* is not due to stress induced rise catecholamines. CCl_4 also induces a decrease in glycogen synthetase which is the rate limiting step in glycogen synthesis. Thus CCl_4 induced loss of hepatic glycogen may be due to an increase in glycogenolysis or decreased glycogen synthesis.

CONCLUSION:

Thus liver diseases are one of the fatal diseases in the world today. They pose a serious challenge to international public health. Modern medicines have little to offer for alleviation of hepatic diseases and it is chiefly the plant based preparations which are employed for the treatment of liver disorders. But there are not much drugs available for the treatment of liver disorders.

In the present investigation the carbon tetra chloride injected hepatotoxic animals show involvement of oxidative stress and suggestive liver damage .There was an increase in phosphatase and transaminase enzymes like alkanine phophatase and alanine amino transferase, bilirubin (total and direct bilirubin), tissue glycogen and lipid peroxidase enzyme showing

the impairment of liver function probably as the result of liver damage.The oral administration of crude powder of carbon tetra chloride for 10 days was able to cause liver damage induced of lipid peroxidation and activation of antioxidant enzymes. The siddha herbal drug *Liminia acidissima* (leaves) and *Clitoria ternatea* (flowers) administration was able to protect the liver necrosis and lysosomal latency as evidenced by the inhibitory of the activity of phosphatases and transaminases. Crude powder may be due to the activity of the constituents like flavonoids and phenols present which might have exerted the protection against the liver damage and the subsequent enzyme activities as observed.

REFERENCES:

1.Sampath KP. Swertia chirata, A traditional herb and its medicinal uses, *J Chem Pharm Res*, 2010; 2(1): 262-266.

2.Kiprono, C.P., Midiwo, J.O., Kipkemboi, P.K. and Santino. L. 2004. Larvicidal benzoquinone from *Embelia schimperi*. *Bulletin of Chemical Society of Ethiopia*, *2004;*18(1):45-49.

3.Lamireau, T., Desmouliere, A., Bioulac-Sage, P. and Rosenbaum, J.Mechanisms of hepatic fibrogenesis. *Archives of Pediatrics*, 2002;9(4): 392-405

4.Abajo F.J., Montero D., Madurga M., Garcia Rodriguez L.A. Acute and clinically relevant drug-induced liver injury: a population based case–control study. Br J Clin Pharmacol. 2004; 58: 71–80.

5.Lawrence,O.A.M. and Lawrence, A.D.W.1997. Aflavonol glycoside from *Embelia schimperi* leaves. *Phytochemistry*, *1997;*44(7):1397-1398.

6.Parola, M, Leonarduzz, G, Biasi, F, Albono, M, Biocca, G, Polic, Dianzani, MU. Vitamin E dietary Supplementation. Protects against CCl4 induced chronic liver damage and cirrhosis. Hepatology. 1992; 16: 1014-1021.

7.Castro, JA, De Ferreyra, EC, De castro, CR, Fenoes, OM, Sasame, H, Gillette, JR. Prevention of carbon tetrachloride-induced necrosis by inhibitors of drug metabolism-further studies on their mechanism of action. Biochem pharmacol. 1974; 23:295-302.

8.Handa, SS, Sharma, A, Chakraborti, KK. Natural products and plants as liver protecting drugs.Fitoterapia. 1986; 57:307-45.

9.Official methods of analysis, AOAC, Association of official Analytical Chemists, Edition 16, Arlighton VA, USA, 1995.

10.Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorisation assay. Free Radical Biology and Medicine 1999; 26:1231-1237.

11.Campous D, Betalleluz I, Tauquino R, Chirinos R, and Pedreschi R. Nutritional and functional characterization of Andean chicuru (*Stangear hizanta*). Food Chemistry 2009; 112(1):63−70.

12.Fisher , R.A., In: statistical methods for research workers , Oliver and Boys ; Edinburgh (1950).

13.Varley, H., IN: Practical Clinical Biochemistry , 4th ed , Arnold-Heinemann Publishers, New Delhi,1976,350.

14.Varley, H., IN: Practical Clinical Biochemistry, 5th ed , William-Heinemann Medical Books Ltd, London,1980,1030.

15.Chandra T, Sadique J and Soma Sundram S. Effect of Eclipta alba on inflammation and liver injury. Fitoterapia. 1987;58(1):23-32.

16.Dianzani M.U and Gravella, E., In: Pathogenesis and Mechanisms of Liver Cell Necrosis, D.Keppler (ed.) Univ.Park Press, Baltimore, 1975,225-238.

17.Levina, L.D., Ambalav, Y.U.M and Kartashev, V.V., Klin. Med. (Moscow), 1977: 58(2),126-131.

18.Recknagal, R.O., Glende, Jr.E.A., Waller, R.L and Lowery, K., "Lipid Peroxidation :Biochemistry, Measurement and Significance in Liver cell injury". In Toxicologyof the Liver, Plaa, g. and Hewitt, R., Raven Press, New York, 1982; 213.

19.Dolak , J.A., Glende, Jr.E.A. and Recknagel, R.O., In: Free Radicals in Liver Injury, Poli. G., Cheeseman, K.H., Dianzani, M.U. and Salter T.E. (eds), IRL press, Oxford, 1985, 117

20.Anderson, K.W., *Archiv. Int. Pharmacodyn. Ther.*, 1965;157,191

21.Asp NG. Dietary carbohydrates: Classification by chemistry and physiology. Food Chemistry 1996; 7(1):9-14.