Research Article **Biochemistry**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

IN VITRO HEPATOPROTECTIVE ACTIVITY OF *APLOTAXIS AURICULATA* IN HEPATOCYTES

NANDHINI R, JEYADOSS T# AND VELAVAN S*

Department of Chemistry & Biosciences, Srinivasa Ramanujan Centre, SASTRA University, Kumbakonam, Tamil Nadu-612 001, India.
**Department of Biochemistry, Marudupandiyar College, Thanjavur, Tamil Nadu, India*

ABSTRACT

Liver plays a major role in detoxification. Any impairment to it or dysfunction may lead to many complication on one's health. Treatment of liver diseases is still a challenge to modern medicine. The commerecially available medicine has little to offer for the alleviation of hepatic ailments but severe side effects. The study was aimed to evaluate *in vitro* hepatoprotective activity of *Aplotaxis auriculata* rhizome (200 and 400µg/ml) through CCl_4 induced toxicity in hepatocytes. The phytochemical screening revealed the presence of flavonoids, terpenoids, triterpenoids, polyphenol and tannins. All the variables tested such as LPO, GSH, Protein, Bilirubin, ALP, SGOT and SGPT recorded a significant alteration observed in CCl_4 exposed hepatocytes. However treatment with *Aplotaxis auriculata* extract restored the level and to near normal values was observed. The potential hepatoprotective activity of *Aplotaxis auriculata* is due to the presence of phytochemical constituents present in plant. Some of these phytochemicals such as flavonoids and polyphenolic compounds might have possessed hepatoprotective activity.

KEY WORDS: ***Aplotaxis auriculata,*** **Phytochemicals, Hepatocytes, Carbon tetrachloride,**

JEYADOSS T
Department of Chemistry & Biosciences, Srinivasa Ramanujan Centre, SASTRA University, Kumbakonam, Tamil Nadu-612 001, India.
Email: jeyadoss@yahoo.co.in

#Corresponding author

INTRODUCTION

Liver is considered to be one of the most vital organs that functions as a centre of metabolism of nutrients such as carbohydrates, proteins and lipids and excretion of waste metabolites. Liver cell injury caused by various toxicants such as certain chemotherapeutic agents, carbon tetrachloride, thioacetamide, chronic alcohol consumption and microbes is well-studied. Enhanced lipid peroxidation during metabolism of ethanol may result in development of hepatitis leading to cirrhosis [1]. A number of natural products are used in the traditional medicine system for various ailments. Alternative medicine for treatment of various diseases is getting more popular with no side effects. Therefore, agents of natural origin with no side effects are required as substitute chemical therapeutics. Search for herbal remedies with potent modulatory activity received momentum recently. Plants have basic nutritional importance by their content of protein, carbohydrate, fats and oils, minerals, vitamins and water responsible for growth and development in man and animals. Phytochemical simply means plant chemicals. “Phyto” is the Greek word for plant. Phytochemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. Primary metabolism is important for growth and development of plants include the common sugars, amino acids, proteins, purines and pyrimidines of nucleic acids, chlorophylls etc. Secondary metabolism in a plant plays a major role in the survival of the plant in its environment [2]. Attractions of pollinators, natural defense system against predators and diseases, etc., are examples of the roles of secondary metabolites. The secondary metabolites formed also are an important trait for our food plants (taste, colour, scent etc.) and ornamental plants. Moreover, numerous plant secondary metabolites such as flavonoids, alkaloids, tannins, saponins, steroids, anthocyanins, terpenoids, rotenoids etc. have found commercial application as drug, dye, flavour, fragrance, insecticide, etc. Such fine chemicals are extracted and purified from plant materials. Plant produces these chemicals to protect itself but recent research demonstrates that many phytochemicals can protect human beings against diseases including cancer, cardiovascular, arthritis, diabetic, aging etc. In the present study the chosen medicinal plant *Aplotaxis auriculata* is belonging to the family of Zingiberaceae. In the present investigation deals with *in vitro* hepatoprotective activity and comprehensive phytochemical studies on rhizome of this plant, including fluorescence analysis, as well as behaviour of rhizome powder with different chemical reagents and histochemical analysis.

MATERIALS AND METHODS

Chemicals

Thiobarbituric acid, Carbon tetrachloride, glutathione and 2,4-Dinitro phenyl hydrazine were purchased from sigma chemical, Mumbai. All other reagents and chemicals used in this study were of analytical grade with high purity.

Collection and Identification of Plant Materials

The rhizome of *Aplotaxis auriculata* was purchased from a traditional medical shop at Thanjavur. The plant material was identified and authenticated (TUH-87) by Dr. M. Jegadeesan Msc., Ph.D., Professor & Head of environmental & Herbal sciences, Faculty of sciences, Tamil University, Thanjavur.

Preparation of extract

The plant material was washed with water and shade dried at room temperature. The dried plant materials were ground into fine powder in an electric blender and subsequently sieved for obtaining fine powder. The powder material of *Aplotaxis auriculata* was macerated with methanol at room temperature for 3 days. After 3 days, the supernatant was transferred into china dish. The supernatant was completely removed by keeping the china dish over a boiling water bath at 45°c. A semi solid extract was obtained after complete elimination of alcohol. The obtained residue was kept in the refrigerator for further use. The extract was made up to a known volume of distilled water just before the experimental process [3].

In vitro hepatoprotective activity [4]

The liver was excised and weighed in a beaker of cold calcium-free Locke's solution. Sufficient solution was removed to give a ratio of 1 g of liver to 10 ml of final suspension. The liver and solution were then transferred to a homogenizer tube, and the liver broken up by pressing down with a loose-fitting lucite pestle. This was followed by twenty even up and down strokes by hand. Shreds of connective tissue containing many cells remained after this treatment, but they were readily removed by straining through bolting silk. Experience has shown that further homogenization to release more whole cells. The isolated hepatocytes were cultured in Ham's F12 medium, supplemented with 10% newborn calf serum, antibiotics, dexamethasone and bovine insulin. The cell suspension was incubated at 37^{o}C for 30 min in a humidified incubator under 5% CO_2. After incubation of 24 hrs, the hepatocytes were exposed to the fresh medium containing CCl_4 (1%) along with different concentration of *Aplotaxis auriculata* (200 and 400µg/ml). After 60 min of CCl_4 intoxication, the oxidative stress and hepatic markers were determined.

Biochemical estimations

MDA released from endogenous lipoperoxides, reflecting the lipid peroxidation process, were assayed in liver as described by Beuge and Aust, [5]. Serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) activities were measured according to the method described by Reitman and Frankel[6]. The levels of non-enzymatic antioxidants such as GSH, was estimated by the method of Moron *et al*.[7]. The protein and bilirubin content was estimated by the method of Lowry's *et al*.[8]. Determination of Mitochondrial Synthesis by Micro culture Tetrazolium (MTT) Assay[9].

Phytochemical screening

Phytochemical tests were carried out on the methanol extract and on the powdered specimens using standard procedures to identify the constituents as described by Sofowara [10], Trease and Evans [11] and Harborne [11].

RESULTS AND DISCUSSION

Preliminary phytochemical screening

The preliminary phytochemical test was performed on the methanolic extract of *Aplotaxis auriculata* rhizome. Results of the test show the presence of the glycosides, flavonoids, tannin, terpenoids, triterpenoids, carbohydrate, steroids, alkaloids, polyphenol and saponin present in the extract (Table-1). Among the various phytochemicals, glycosides, Terpenoids, Triterpenoids and flavonoids are present in high concentrations as compared to other phytochemicals. Protein, Anthroquinone and phlobatannins are absent. The detailed phytochemical investigation strengthens the resourcefulness of the extract for the further pharmacological evaluations.

Table 1
Preliminary phytochemical screening of rhizome of Aplotaxis auriculata

Phytochemicals	Methanol
Tannin	++
Phlobatannins	-
Saponin	++
Flavonoids	++
Steroids	++
Terpenoids	+++
Triterpenoids	+++
Alkaloids	++
Carbohydrate	+++
Protein	-
Anthroquinone	-
Polyphenol	++
Glycoside	++

Key: [+++] Highly present, [++] Moderately present, [+] Faintly present, [-ve] Absent

In vitro hepatoprotective activity

Liver regulates various important metabolic functions. Hepatic damage is associated with distortion of these metabolic functions. Liver disease is still a worldwide health problem. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects. This is one of the reasons for many people in the world over including those in developed countries turning complementary and alternative medicine (CAM). Many traditional remedies employ herbal drugs for the treatment of liver ailments[13]. To the best of our knowledge, there is no scientific report available in support of the hepatoprotective activity of *Aplotaxis auriculata.* Hence, to justify the herbal claims we have evaluated the hepatoprotective effects of *Aplotaxis auriculata* on CCl_4 exposed hepatotoxicity in hepatocyte. The hepatoprotective activity of the plant reported in this study would provide scientific evidence of its claimed medicinal properties. The present study was carried out to evaluate the *In vitro* hepatoprotective activity of *Aplotaxis auriculata* on CCl_4 induced hepatotoxicity. Table 2 represents the % of viability of control and experimental cell line. Group II CCl_4 induced oxidative stress showed a significant decreased in the % of cell viability when compared to Group I. Group III and IV CCl_4 induced oxidative stress group treated with (200 and 400µg/ml) *Aplotaxis auriculata* significantly increased in % of cell viability when compared to group II.

Table 2
shows the % of viability

Treatment group	Concentration	% of viability
Control (Untreated)	---	100
CCl_4	1%	43.21
CCl_4 + *Aplotaxis auriculata*	200µg/ml	78.45*
CCl_4 + *Aplotaxis auriculata*	400µg/ml	87.43*

Values were expressed as mean ± SD for triplicate in each group.
****Significantly different from Group II (P < 0.05)***

Table 3
Effect of Aplotaxis ariculata on MDA and GSH in experimental hepatocytes

Parameters	Group I	Group II	Group III	Group IV
GSH (mg/dl)	5.86±0.38	3.15±0.38*	6.41±0.70**	5.81±0.67**
MDA (nmol/l)	2.42±0.69	9.29±1.99*	6.66±0.44**	3.51±1.95**

Values were expressed as mean ± SD
**** Significantly different from Group I(P<0.05)***
***** Significantly different from Group II(P<0.05)***

Table 4
Effect of Aplotaxis ariculata on protein and bilirubin in experimental hepatocytes

Parameters	Group I	Group II	Group III	Group IV
Protein (gm/dl)	6±0.74	12.5±1.5*	8±1.14**	7.75±1.14**
Bilirubin(mg/dl)	0.80±0.24	2.30±0.4*	1.15±0.31**	1.08±0.34**

Values were expressed as mean ± SD
**** Significantly different from Group I(P<0.05)***
***** Significantly different from Group II(P<0.05)***

Table 5
Effect of Aplotaxis ariculata on SGOT, SGPT and ALP activities in experimental hepatocytes

Parameters	Group I	Group II	Group III	Group IV
SGOT (IU/dl)	31.22±5.47	45.01±9.06*	31.22±4.53**	26.14±3.77**
SGPT (IU/dl)	16.06±5.50	32.66±5.60*	20.22±3.10**	17.11±3.10**
ALP (IU/dl)	14.33±1.20	23.66±2.07*	15.66±3.78**	14.33±4.93**

Values were expressed as mean ± SD
**** Significantly different from Group I(P<0.05)***
*****Significantly different from Group II(P<0.05)***

It is well established that CCl_4 induces hepatotoxicity by metabolic activation; therefore, it selectively causes toxicity in liver cells maintaining semi normal metabolic function. CCl_4 is biotransformed by the cytochrome *P*450 system in the endoplasmic reticulum to produce trichloromethyl free radical ($^{\bullet}CCl_3$). Trichloromethyl free radical then combined with cellular lipids and proteins in the presence of oxygen to form a trichloromethyl peroxyl radical, which may attack lipids on the membrane of endoplasmic reticulum faster than trichloromethyl free radical. Thus, trichloro methylperoxyl free radical leads to initiate the process of lipid peroxidation., the destruction of Ca^{2+} homeostasis and finally, results in cell death[14, 15]. These result in changes of structures of the endoplasmic reticulum and other membrane, loss of enzyme metabolic enzyme activation, reduction of protein synthesis and loss of glucose-6-phosphatase activation, leading to liver damage [16, 1]. MDA is a secondary product of lipid peroxidation is used as an indicator of tissue damage by series of chain reactions [17]. Hepatotoxic compounds like CCl_4 are known to cause marked elevation in enzyme activities. In the present study, treatment with *Aplotaxis auriculata* attenuated the increased content of MDA in hepatocyte (Table- 3).

Glutathione is a ubiquitous thiol-containing tripeptide, which plays a central role in cell biology. It is implicated in the cellular defence against xenobiotics and naturally occurring deleterious compounds, such as free radicals and hydroperoxides. Glutathione status is a highly sensitive indicator of cell functionality and viability. Glutathione is responsible for the regulation [18]. The toxic activation of CCl_4 via the CYP2E1 (Cytochrome *P450* 2E1) pathway, the detoxification pathway involves GSH conjugation of the trichloromethyl radical, a CYP2E1-mediated CCl_4 metabolite. Previous studies on the mechanism of CCl_4 exposed hepatotoxicity have shown that GSH plays a key role in detoxifying the reactive toxic metabolites of CCl_4 and that liver necrosis begins when the GSH stores are markedly depleted [18]. GSH is largely mediated through the activity of GST and forms adducts with the toxic metabolites of CCl_4. Moreover, GSH contribute to the detoxification of CCl_4, and it has been suggested that one of the principal causes of CCl_4 exposed liver injury lipid peroxidation caused by its free radical derivatives[19,20]. Our results show that a treatment with *Aplotaxis auriculata* significantly inhibited lipid peroxidation (Table 3) and significantly reduces CCl_4 exposed hepatic GSH depletion (Table 4). This was attributed to the decreased bioactivation of CCl_4 caused by the *Aplotaxis auriculata* treatment.

In the assessment of liver damage by carbon tetrachloride, the determination of enzyme activities such as aspartate aminotransferase (AST/SGOT) and alanine aminotransferase (ALT/SGPT) is largely used. Activities of AST, ALT and alkaline phosphatase (ALP) are the most frequently utilized indicators of hepatocellular injury. Necrosis or membrane damage releases the enzymes into circulation and therefore, they can be measured in hepatocyte. ALT is more specific to the liver, and is thus a better parameter for detecting liver injury. Elevated levels of hepatocyte enzymes are indicative of cellular leakage and loss of functional integrity of the cell membrane in the liver [13]. The mechanism by which alkaline phosphatase reaches the circulation is uncertain leakage

from the bile canaliculi into hepatic sinusoids may result from leaky tight junctions and the other hypothesis is that the damaged liver fails to excrete alkaline phosphatase made in the bone, intestine and the liver [21]. Total protein and bilirubin levels, on other hand, are related to the function of hepatic cells i.e they reveal the functional status of the hepatic cells. Decreased levels of total protein and bilirubin are indicative of the failure of the biosynthetic function of the hepatocyte, while increased levels of bilirubin indicate defective hepatocellular uptake, conjugation and excretion of bilirubin due to the failure of hepatic cell function [22].

In the present study, the CCl_4 treated hepatocyte showed a significant elevation (Table- 5) in the activities of ALT, AST, alkaline phosphatase and total bilirubin content, while significantly decreasing the levels of total protein (Table – 4) as compared to the normal control hepatocyte, thereby indicating oxidative damage. Cotreatment with *Aplotaxis auriculata* at doses of 200 and 400µg/ml, significantly prevented the rise in the levels of the marker enzymes and total bilirubin, as well as it significantly prevented the decrease in the total protein. The diminished rise of hepatocyte enzymes, together with the diminished fall in the levels of total protein in the *Aplotaxis auriculata* (Table-4&5) treated groups, is a clear manifestation of the hepatoprotective effect of the *Aplotaxis auriculata.* The phytochemical screening revealed the presence of flavonoids, terpenoids, triterpenoids, polyphenol and tannins. All the variables tested as LPO, GSH, Protein, Bilirubin, ALP, SGOT and SGPT recorded a significant alteration observed in CCl_4 exposed hepatocytes. However treatment with *Aplotaxis auriculata* extract restored the level to near normal was observed. The potential hepatoprotective activity of *Aplotaxis auriculata* is due to the presence of phytochemical constitution present in plant. Some of these phytochemical such as flavonoids and polyphenolic compounds have possessed hepatoprotective activity.

REFERENCE

1. Wolf CR, Harrelson Jr, Nastainezyk WM, Philpot RM, Kalayanaraman B and Mason RP. Metabolism of carbontetrachloride in hepatic microsome and reconstitution monoxygenase systems and its relationship to lipid peroxidation. Molecular pharmacology, 18: 558, (1980).
2. Kotnis MS, Patel P, Menon SN and Sane RT. Reneprotective effect of *Hemisdesmus indicus*, a herbal drug used in gentamicin-induced renal toxicity. Nephrology (carlton), 3: 142-152, (2004).
3. Selvarani K and Viji Stella Bai G. Toxicity Evaluation Of Ethanolic Extract Of *Cissus Vitiginea* Leaves. International Journal of Toxicology and Applied Pharmacology 4(1): 12-16, (2014).
4. Surendran S, bavani Eswaran M, Vijayakumar and Rao CV. *In vitro* and *in vivo* hepatoprtective activity of *Cissampelos pareir* against carbon tetrachloride induced hepatice damage. Indian Journal of Experimental Biology. 49: p939-945, (2011).
5. Beuge JA and Aust SD. The thiobarbituric acid assay. Method Enzymol, 52: 306-307, (1978).
6. Reitman S and Frankel S. A colorimetric method for the determination of serum glutamate oxaloacetic and glutamate pyruvate transaminases. Am J Clin Pathol, 28: 56-63, (1957).
7. Moron MS, DsePierre JW and Manerwik KB. Levels of glutathione, glutathione reductase and glutathione-s-transferase activities in rat lung and liver. Biochim Biophy Acta, 582: 67-68, (1979).
8. Lowry OH, Rosenbrough NJ, Farr AL and Randall RJ. Protein measurement with the Folin's reagent. J Biol Chem, 193: 265-276, (1951).
9. Masters RW. Animal cell culture, Cytotoxicity and viability assays. 3rd ed.: 202-203, (2000).
10. Sofowara A. Medicinal plants and Traditional medicine in Africa.Spectrum Books Ltd, Ibadan, Nigeria. 191-289, (1993).

11. Trease GE and Evans WC. Phenols and Phenolic glycosides. In:Textbook of Pharmacognosy. (12th ed.). Balliese, Tindall and Co Publishers, London. 343-383, (1989).
12. Harborne JB. Phytochemical methods, London. Chapman and Hall, Ltd. 49-188, (1973).
13. Wolf PL. Biochemical diagnosis of liver diseases. Indian Journal of Clinical Biochemistry, 14:59–90, (1999).
14. De Groot H and Noll T. The crucial role of low steady state oxygen partial pressures in haloalkane free radical mediated lipid peroxidation. Biochemical Pharmacology 35: 15–19, (1986).
15. Clawson GA. Mechanism of carbon tetrachloride hepatotoxicity. Pathology and Immunology Research, 8104–8112, (1989).
16. Recknagel RO, Glende EA, Dolak JA and Waller RL. Mechanism of carbon tetrachloride toxicity. Pharmacological Therapy, 43:139–154, (1989).
17. Recknagel RO and Glende JR. Carbon tetrachloride hepatotoxicity: an example of lethal cleavage. CRC Critical Review Toxicology, 2:263–297, (1973).
18. Ray G and Husain SH. Oxidants antioxidants and carcinogenesis. Indian J Exp Biol. 40:1213-1232, (2002).
19. Pastore A, Federici G, Bertini E and Piemonte F. Analysis of glutathione: Implication in redox and detoxification. Clinical chimica Acta. 333:19-39, (2003).
20. Williams AT and Burk RF. Carbon tetrachloride hepatotoxicity: an example of free radical-mediated injury. Semin. Liver Dis. 10, 279–284, (1990).
21. Thapa BR, Walia A. Liver Function Tests and their Interpretation. Indian Journal of Pediatrics. 74:663-671, (2007).
22. Crawford JM. Liver and biliary tract. In: Kumar V, Abbas AK, Fausto N, editors. Robbins and Cotran pathologic basis of disease. 7th ed. Pennysylvania: Saunders, an imprint of Elsevier, 877–937, (2004).