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Photochemical and Hepatoprotective Activity of *Artemisia Dracunculus* L Leaves Extract

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ABSTRACT

Several diseases such as liver diseases are caused due to free radicals formation, which leads to decrease in antioxidant enzymes in the body. Carbontetrachloride is metabolized in the liver to generate free radicals, which react with cellular lipids and proteins, eventually leading to cell death. The present work is carried out to investigate the hepatoprotective effect of Artemisia dracunculus L leaves against carbon tetrachloride induce liver toxication in rats. Carbon tetrachloride treatment group showed significantly elevated the alanine aminotransferase, aspartate aminotransferase, alkaline phosphatie.. Ethanolic leaves extract of Artemisia dracunculus L (250 and 500 mg/kg) significantly restored the carbon tetrachloride-induced alterations in the biochemical and cellular constituents of blood. The hepatoprotective effect of Artemisia dracunculus L was also confirmed by the histopathological examination of liver tissue.

KEYWORDS : *Artemisia dracunculus* L, Carbontetrachloride , Oxidative stress, hepatotoxicant.

INTRODUCTION

Free radicals are atoms or groups of atoms with an odd (unpaired) number of electrons and can be formed when oxygen interacts with certain molecules. Once formed these highly reactive radicals can start a chain reaction, like dominoes. Their chief danger comes from the damage they can do when they react with important cellular components such as DNA, or the cell membrane. Cells may function poorly or die if this occurs. To prevent free radical damage the body has a defense system of antioxidants [1]. Oxidative stress in cells and tissues results from the increased generation of reactive oxygen species or free radicals reactive oxygen species and decrease in antioxidant defense potential[2]. The role of free radicals in disease pathology is well established. Liver disease remains a serious health problem [3]. Liver plays an vital role in the metabolism, synthesis, storage and also detoxification of many endogenous and exogenous compounds and converting to less toxic substances for excretion [4]. CCl4(Carbon tetrachloride) is toxic to the liver and its toxicity is dose dependent and is based on the time of exposure[5]. According to the findings by the International Programme on Chemical Safety (IPCS 1999), CCl4 was shown to be an outstanding and potent hepatotoxicant[6]. In the liver, CCl4 is metabolized into highly reactive trichloromethyl radical. This free radical causes autooxidation of the fatty acids present in the cytoplasmic membrane phospholipids, resulting in functional and morphological changes in the cell membrane. Trichloromethyl free radical combines with lipids and proteins in the presence of oxygen to form trichlomethylperoxyl radical .This radical elicits lipid peroxidation, destruction of Ca 2+ homeostasis and finally results in cell death.

Artemisia dracunculus L. or tarragon belongs to the Anthemideae tribe of Asteraceae family. *A. dracunculus* is a woody, perennial subshrub with stem heights ranging from 40 to 150 cm. Aerial stems arise from thick, horizontal rhizomes growing in clusters and singly. Basal leaves are cleft with one to three lobes. The inflorescence is a panicle with numerous flowers [7, 8]. Its main source is alluvial alleys and various parts of Russia and Siberia. But nowadays it has become a native to the western regions of North America. Also, it is grown in the most areas of Iran and has dispersed everywhere. The fresh and dried leaves are commonly used in salads and soups. This plant has been used in traditional folk medicine as appetizer, gastric tonic, diuretic, anti-scurvy and antiworm [9]. The important groups of the *A. dracunculus* bioactive secondary metabolites, are essential oil, coumarins, flavonoids and phenolic acids [10] and also reported that hydroxycinnamates such as 1-caffeyolquinic acid, chlorogenic acid, caffeic acid, caffeoyltaric acid, 5- feruloylquinic acid, 1-4-Dicaffeoylquinic acid are main phenolic components of tarragon leaves [11]. This herb has antifungal and antioxidant [12], anti-bacterial, antiinflammatory, and hepatoprotective [13] as well as Antihyperglycemic activities [14]. Significant differences in phytochemical profile and pharmacological properties between different varieties occur[15,16,17].

Preparation of ethanolic extract

The shade leaves of *Artemisia dracunculus L* were subjected to pulverization to get coarse powder. The coarsely powder leaves of were used for extraction. *Artemisia dracunculus L* leaves powder (250 g) was loosely packed in the thimble of soxhlet apparatus and extracted with ethanol at 55°C for 18 h. The extract was air dried at 25-30°C and weighed. For oral administration, extract was dissolved in 10 mL Phosphate Buffer Saline (PBS) at different concentrations. To make the extract soluble in PBS, 1% tween 80 was used.

Experimental Animals

Wistar albino rats (150-200 g) of both sexes were obtained from the Osmania university animal house,Hyderabad. Before and during the experiment, rats were fed with standard diet. Before initiation of experiment, the rats were acclimatized for a period of 7 days under standard environmental conditions of temperature, relative humidity, and dark/light cycle. Animals described as fasting were deprived of food and water for 16 h ad libitum. All animal experiment was carried out in accordance with the guidelines of CPCSEA.

Experimental design for hepatoprotective activity[18]

Animals are divided into 5 groups, each comprising 6 rats.

Group I : Control group

Group II : CCl4 treated group

Group III : CCl4 + *Artemisia dracunculus L* leaves extract (250mg/kg, p.o)

Group IV : CCl4 + *Artemisia dracunculus L* leaves extract (500mg/kg, p.o)

Group V : CCl4 + Silymarin (100 mg/Kg)

The first group was fed with 1 ml/kg p.o of saline solution (S.S.) for 4 days. The second group was fed with 1 ml/kg p.o. of S.S. for 4 days along with 2 ml/kg of CCl_4 by S.C. on the second and third days. The third group and fourth group was fed with *Artemisia dracunculus L* leaves extracts (250mg/kg,p.o and 500mg/kg, p.o) for 4 days along with 2 ml/kg of CCl_4 by S.C. on the second and third days. Fifth groups were fed with Silymarin (100mg/kg, p.o) for 4 days along with 2 ml/kg

of CCl_4 by S.C. on the second and third days. On the fifth day, all the animals were sacrificed by mild ether anesthesia.

Blood biochemistry

Blood samples were collected from retro-orbital puncture to obtain haemolysis free clear serum for the estimation of SGOT and SGPT[19], ALP[20] and bilirubin[21] by standard method. Serum total protein was measured according to the method of Lowry et al, 1951[22].

Histopathology

Histopathology of liver was carried out by method described by Luna LG., 1999 [23]. The autopsied livers were washed in normal saline and fixed in 10% formalin for 2 days followed with bovine solution for 6 h.Then the livers were paraffin embedded and 5 µ thickness microtone sections were made[24]. The sections were processed in alcohol-xylene series and stained with haematoxylin and eosin. The slides were studied under a light micro-scope for any histological damage/ protection.

Statistical analysis

The data obtained were analyzed by One way analysis of variance (ANOVA) followed by Tukey's multiple comparison test using computerized program. P-value <0.05 or was taken as the criterion of significance.

RESULTS

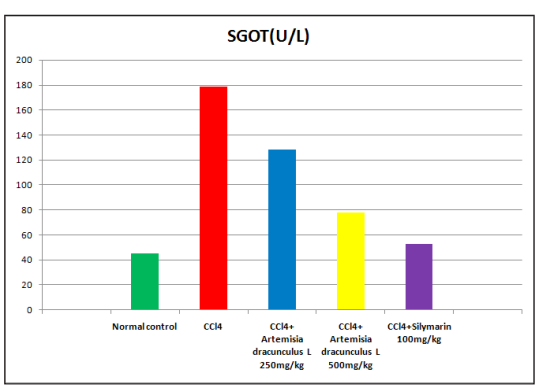
The effect of ethanol extract of *Artemisia dracunculus L*an on alkaline phosphates, serum transaminases, bilirubin and total protein level in CCl4 intoxicated rats are summarized in Table 1. There was a significant increase in bilirubin level, SGOT, SGPT and ALP, in CCl4-intoxicated group, when compared to the normal control group. The total protein levels were significantly decreased in CCl4 intoxicated rats as compared with normal group. On the other hand the group with received leaves extracts and CCl4 (Group III and Group IV) and CCl4+Silymarin(100 mg/Kg) (GROUP V) showed significantly decreased the elevated serum marker enzymes when given orally and reversed the altered total protein to almost normal level (Table 1).

Table 1. Effect of *Artemisia dracunculus L*on some serum chemical parameters of CCl4 intoxicated rats.

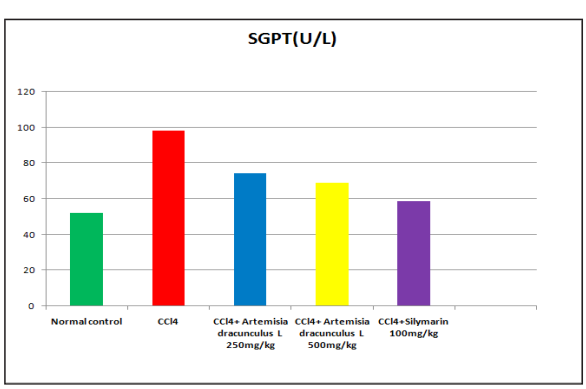
GROUPS	SGOT(U/L)	SGPT(U/L)	ALP(U/L)	Bilurubin (mg/dl)	Total protein(g/dl)
Normal control	45.32± 3.32	52.20±4.2	132.40± 2.35	0.19± 0.20	7.20± 0.01
CCl_4	178.83±5.02	98.40± 2.2	229.22± 2.09	3.38± 0.21	4.15± 0.47
CCl_4+ *Artemisia dracunculus L* 250mg/kg	128.38± 2.01	74.32±3.6	198.43±5.28	2.01±2.52	5.32±0.25
CCl_4+ *Artemisia dracunculus L* 500mg/kg	78.20± 3.43	68.82±2.65	152.46±5.04	0.88±0.28	6.72±0.29
CCl_4+Silymarin (100 mg/Kg)	52.82±2.25	58.74±2.01	141.63±2.08	0.28± 0.17	7.01± 0.28

Values are mean ± S.E.M. number of rats = 6.

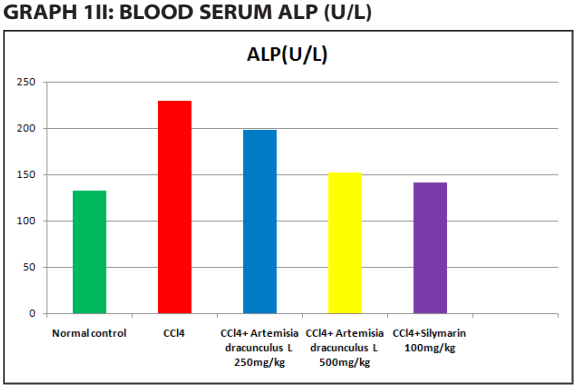
GRAPH I: BLOOD SERUM SGOT (U/L)



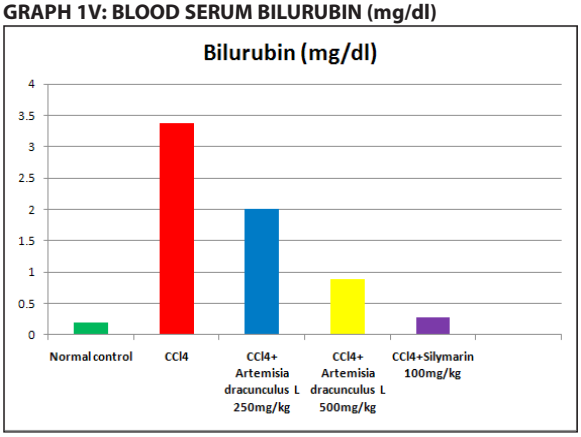
GRAPH II: BLOOD SERUM SGPT (U/L)



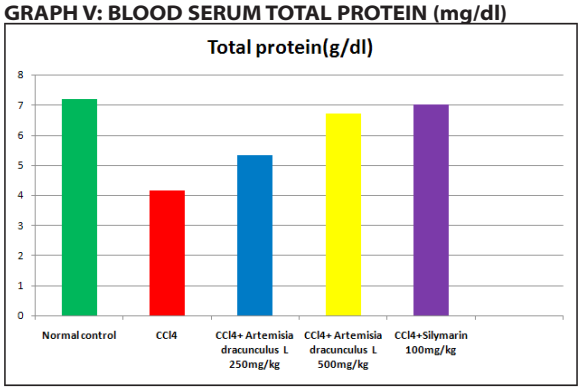
GRAPH 1II: BLOOD SERUM ALP (U/L)



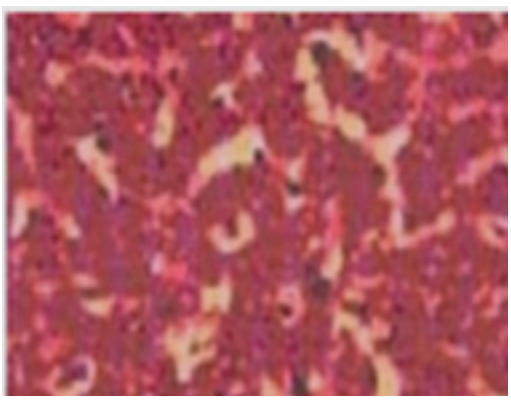
GRAPH 1V: BLOOD SERUM BILURUBIN (mg/dl)



GRAPH V: BLOOD SERUM TOTAL PROTEIN (mg/dl)

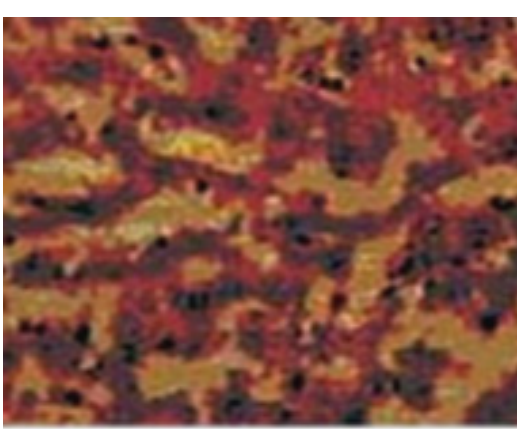


HISTOPATHOLOGY

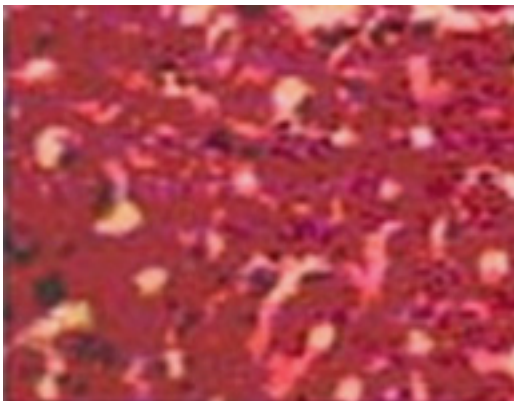


Group I: Control Liver

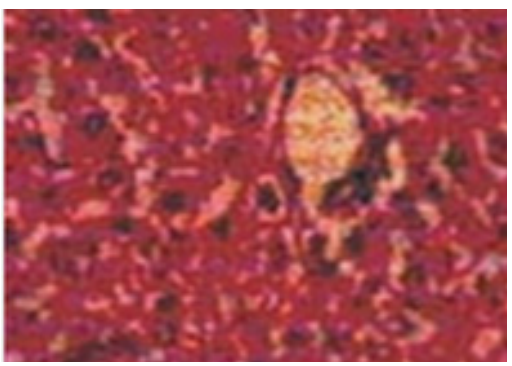
Group II: CCl4 intoxicant Liver



Group 111: CCl4 + *Artemisia dracunculus L* 250mg/kg



Group 1V: CCl4 + *Artemisia dracunculus L* 500mg/kg



Group V: CCl4 + Silymarin (100mg/kg)

Group I: Section shows normal liver tissue; Group II: CCl4 intoxicant liver showing zonal necrosis, vacuolar degeneration engorged and microvesicular fatty changes in hepatocytes; Group III: CCl4 + *Artemisia dracunculus L*(250mg/kg) shows mild fatty hepatocytes; Group IV: CCl4 + *Artemisia dracunculus L*(500mg/kg)shows regeneration of hepatocytes; Group V: CCl4 + Silymarin (100mg/kg)shows regeneration of hepatocytes.

DISCUSSION

Disorders of liver are expressed in several forms;like jaundice, acute and chronic hepatitis, hepatoses and degenerative disorders resulting in fibrosis of the liver,which are still without appropriate therapies. Phenolic compounds are found to be the most important antioxidative components of herbs and other plant materials, and a good correlation between the concentrations of plant phenolic and the total antioxidant capacities has been reported[25,26]. Previous study on some plants, reported that polyphenols can inhibit nitrosation and flavonoides have hepatoprotective activities [27]. Miron et al., (2010) have identified phenolic compounds such as syrinjic acid, vanillic acid, p-hydroxy benzoic acid and p-coumaric acid in Romanian tarragon [28].

The hepatotoxic effects of CCl4 are largely due to biotransformation by the cytochrome P450 system to active metabolite, trichloromethyl radical. Covalent binding of the trichloromethyl radical to cell protein is considered the initial step in a chain of events that eventually leads to lipid peroxidation of the cell membrane and endoplasmic reticulum. Lipid peroxidation in turn gives products like malondialdehyde that cause damage to the membrane. The peroxidative products induce hypoperfusion of the membrane, and finally cytosolic enzymes appear in the blood[29].

Liver injury can be caused by many chemicals and drugs. In the present study, CCl_4 was selected as a hepatotoxicant to induce hepatic damage, since it is clinically relevant, CCl_4 produces a constellation of dose related deleterious effects in the liver[30]. Hepatotoxin CCl4 gets converted into CCl3O– by liver enzymes and attacks the unsaturated fatty acids of cell membrane in the presence of oxygen, which consequently gives rise to lipid peroxides that alter the functional integrity

of liver mitochondria leading to liver damage. During hepatic damage, cellular enzyme like SGPT ,SGOT, ALP and serum bilirubin present in the liver cell, leak into the blood serum resulting to increase in its concentration[31]. Histological changes such as steatosis (fatty changes in hepatocytes) and perivenular fibrosis were observed in CCl_4 treated group. Ethanolic extract of *Artemisia dracunculus L* leaf extract (250mg/kg and 500mg/kg, p.o) prevented these histological changes, further indicating their hepatoprotective activity.

CONCLUSION

In conclusion, the results of the study demonstrate that *Artemisia dracunculus L* leaves extract of dose 500mg/kg, p.o found to be more potent hepatoprotective activity than 250mg/kg, *Artemisia dracunculus L* leaves extract, against CCl_4 induced liver damage in rats. It showed significantly decreased the elevated serum marker enzymes when given orally and reversed the altered total protein to almost normal level. The results also imply that the hepatoprotective effects of *Artemisia dracunculus L* may be due to its antioxidant property. Further investigation is in progress to determine the exact phytoconstituents responsible for hepatoprotective activity.

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