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EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF *VERNONIA CINEREA* (L.) LESS AND *CUMINUM CYMINUM* IN CARBON TETRACHLORIDE INDUCED LIVER DAMAGE IN RATS

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ABSTRACT

The present study deals with the effect of herbal powder of *Vernonia cinerea* and *Cuminum cyminum* on tissue defense system against CCl_4 (carbon tetrachloride) induced hepatic injury in rats. CCl_4 diluted with liquid paraffin oil (1:1 ratio) in a dose of 1ml / kg body wt was given intraperitoneally for 2 days. Herbal powder as a suspension of water (10mg / 100g body wt / day) was administered orally for 15 days to rats intoxicated with CCl_4. Induction with CCl_4 resulted in an increase in the levels of aspartate transaminases, alkaline phosphatase, alanine transaminases and total bilirubin in serum. Post treatment with herbal powder reversed the deleterious effects of CCl_4 and the enzymes were found to be near normal. These findings demonstrate the protective nature of herbal powder against CCl_4 induced liver damage.

KEYWORDS : Hepatoprotective, carbon tetrachloride, *Vernonia cinerea* and *Cuminum cyminu*

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INTRODUCTION

Liver plays an astonishing array of vital functions in the maintenance and performance of the body. Some of these major functions include carbohydrates, protein, fat metabolism, detoxification and secretion of bile. Therefore, the maintenance of a healthy liver is vital to overall health and well being [1]. Liver injury caused by toxic chemicals and certain drugs has been recognized as a toxicological problem. Herbal drugs are playing an important role in healthcare programmes in worldwide, and there is a resurgence of interest in herbal medicines for treatment of various ailments including hepatopathy [2]. Traditionally, plants have been used as medicines against various types of diseases [3]. In the present study the efficacy of the selected herbal powder prepared from *V. cinerea* and *C. cyminum* has been studied against experimental liver injury induced in rats. Carbon tetrachloride is a widely used chemical to induce liver damage in experimental studies and its toxicity has been extensively reported. The resulting hepatic injury was characterized by leakage of cellular enzymes into the blood stream and by centrilobular necrosis. [4,5] Carbon tetrachloride toxicity requires cleavage of the carbon-chlorine bond and the cleavage takes place after binding of carbon tetrachloride to cytochrome p-450 apoprotein in the mixed function system located in the hepatocellular endoplasmic reticulum [6]. The present study was undertaken to evaluate the use of *V. cinerea* and *C. cyminum* as a hepatoprotective agent in carbon tetrachloride induced hepatotoxicity in rats.

MATERIALS AND METHODS

Preparation of herbal powder

V.cinerea were collected from Anamalai hills, Coimbatore district of Tamilnadu. The plant was identified and authenticated by experts in the postgraduate and Research, Department of Botany, Kongunadu Arts and Science College, Coimbatore. The plants were picked up and dried under shade, powdered and passed through 40 mesh sieve and kept in a closed container for future use. The cumin seeds were purchased from the local market and it was powdered and mixed with plant powder of *V.cinerea* in the ratio of 1:1 (w/w).

Drugs and chemicals

All the chemicals used in the present study were of analytical grade.

Experimental animals

Wistar strains of adult male albino rats weighing 150-200g were obtained from PSG Institute of Medical Sciences, Coimbatore, Tamil Nadu. These animals were fed with standard pellet diet from Hindustan lever limited (Mumbai, India) and water *ad libitum*. Animals were maintained at laboratory conditions for a period of 30 days. The pellet composition was found to be similar to RDA (Recommended Dietary Allowances) for laboratory animals [7]. The study was carried out based on the guidelines for the use and care for laboratory animals.

Experimental design

The selected rats were divided into six groups of six animals each as given below. Group I: control group fed with normal diet. Group II: toxic group [rats treated Intraperitoneally (ip) with CCl_4 diluted with liquid paraffin oil (1:1 ratio) in a dose of 1ml/kg body weight for 2 days]. Group III: post-treated group [toxic group treated with herbal powder (10mg/100g body weight/day) orally as a suspension of water for 15 days from second day after sensitization]. Group IV: pretreatment group [the herbal powder was administered orally as a suspension of water for 15 days (10mg/100g body weight/day); and then treated Intraperitoneally (ip) with CCl_4 for next two days similar to group II]. Group V: positive control group [rats treated only with herbal powder (10 mg/100g body weight/ day: orally) for 15 days] Collection of serum After 24 hrs of the last dose, blood was collected from retro-orbital plexus under mild chloroform anesthesia. The blood samples were allowed to clot and the serum was separated by centrifugation at 2500 rpm at 37ºC and used for the assay of biochemical marker enzymes

such as AST, ALT [8], ALP [9], total bilirubin [10] and total protein.[11]

Statistical analysis

Results of the biochemical estimations were reported as mean±SD and the data obtained were analyzed by one-way analysis of variance (ANOVA) [12].

RESULTS AND DISCUSSION

The levels of total bilirubin, total protein, AST, ALT, ALP in normal and experimental rats were presented in Table 1. Induction with CCl_4 resulted in an increase in the levels of aspartate transaminases, alkaline phosphatase, alanine transaminases and total bilirubin in serum. Post treatment with herbal powder reversed the deleterious effects of CCl_4 and the enzymes were found to be near normal. The injury and dysfunction of liver caused by the toxic effect of CCl_4 in experimental animals stimulated the human viral hepatitis model [13]. In CCl_4 induced toxic hepatitis, a toxic reactive metabolite, trichloromethyl (CCl_3) radical was produced by the microsomal oxidase system cytochrome p-450. This activated radical binds covalently to the macromolecules of the lipid membranes of endoplasmic reticulum and causes peroxidative degradation of lipids. As a result fats from the adipose tissue were translocated and accumulated in the liver [14]. In most studies this toxic chemical has been used as a tool to induce hepatotoxicity in experimental animals [15]. The estimation of serum total bilirubin confirms the intensity of jaundice in normal population. In viral or toxic hepatitis the degree of excretion of bilirubiun from the intestine is very less and bilirubin present in the liver is excreted into the canaliculi and then regurgitated into the blood stream. Hence hyperbilirubinemia is more common in hepatitis patients [16].

Table 1

Effect of herbal powder on carbon tetrachloride induced hepatotoxicity in rats.

BIOCHEMICAL ASSAYS	GROUP I (CONTROL)	GROUP II (TOXIC)	GROUP III (POST TREATMENT)	GROUP IV (PRE TREATMENT)	GROUP V (POSITIVE CONTROL)
AST	72.57±4.93	146.3±13.1a*	78.41±5.16b*	86.8±3.70c*	74.1±6.28d^{ns}
ALT	28.33±2.94	110.6±3.92a*	43.59±3.61b*	87.0±2.22c*	25.8±2.96d^{ns}
ALP	100.4±6.06	175.0±8.71a*	123.3±7.28b*	132.0±6.70c*	92.5±9.56d^{ns}
TOTAL BILIRUBIN	1.04±0.25	3.78±0.14a*	1.75±0.24b*	1.62±0.25c*	1.02±0.22d^{ns}
TOTAL PROTEIN	7.87±0.65	5.96±0.16a*	7.30±0.38b*	4.97±0.34c*	8.00±0.39d^{ns}

Values are expressed as mean ± SD of six replicates * Significant at 5% level (p<0.05): ns – non significant. Statistical group comparison: a- group I with group II, b-group II with III, c- group II with group IV, d- group I with group V.

Units

AST, ALT= n moles of pyruvate / min/ mg protein. ALP = µ moles of phenol / min /mg protein.Total bilirubin, Total protein= mg / 100 ml. The data in table reveals the decreased level of serum transaminases in animals treated with herbal powder, indicating the stabilization of plasma membrane and hepatoprotection against the effect of CCl_4 and decreased ALP concentration evidences the normal functioning of hepatic cells. The decrease in serum transaminase concentration indicates the stabilization of plasma membrane and protection of hepatocytes against the damage caused by CCl_4. ALP activity on the other hand is related to the functioning of hepatocytes and increase in its activity is due to its increased synthesis in the presence of increased biliary pressure [17]. Blood samples of CCl_4 treated animals showed significant increase in the levels of total bilirubin, alanine transaminase, aspartate transaminase and alkaline phosphatase compared to normal control groups but the total protein level decreased reflecting the liver injury caused by CCl_4. Whereas blood samples from the animals treated with herbal powder of *V.cinerea* and *C.cyminum* showed significant decrease in the levels of serum markers and significant increase in total protein to the near normal, which are comparable to the normal values. Both pretreated and post treated rats (Group IV and Group III respectively) showed a significant reduction in the level of total bilirubin when compared to group II rats.

CONCLUSION

The results of this study demonstrate that herbal powder of *Vernonia cinerea* and *Cuminum cyminum* have potent hepatoprotective action upon carbon tetrachloride induced hepatic damage in rats. The hepatoprotective effects of herbal powder might be due to its antioxidants and free radical scavenging properties. Further investigation is underway to determine the exact phytoconstituents that are responsible for its hepatoprotective effect.

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