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RESEARCH ARTICLE

ANTIOXIDANT ACTIVITIES OF ETHANOLIC EXTRACT OF *VERNONIA CINEREA* IN CARBON TETRACHLORIDE INDUCED HEPATIC DAMAGE IN RATS

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ABSTRACT

In this study, the ethanolic extract of V*ernonia cinerea* plant leaves were investigated for the antioxidant and hepatoprotective effects in male Wistar strain albino rats. The extract at a dose of 100mg/Kg body wt was administered orally to the CCl_4 treated rats. The effect of extract on the activities of superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), glutathione S-transferase (GST) and reduced glutathione (GSH) content were estimated in the liver of rats intoxicated with CCl_4. When compared to CCl_4 treated group of rats, the extract significantly ($p<0.05$) increased the levels of SOD, CAT, GR, GSH, and GST. The extract also showed antioxidant effects by significantly decreasing ($p<0.05$) on $FeCl_2$-Ascorbate induced lipid peroxidation in rat liver homogenate. these suggest that extract at a dose could protect the liver cells from CCl_4 induced liver damages perhaps, by its antioxidant effect on hepatocytes and hence eliminating the deleterious effects of toxic metabolites from CCl_4.the protective effect of extract in respect to biochemical changes were also confirmed by histopathological study in the liver sections.

INTRODUCTION

Vernonia cinerea LESS. of compositae family is a perennial herbaceous plant. It is widely distributed in India. The whole plant possesses medicinal properties such as analgesic, antipyretic and anti-inflammatory effects (Iwalewa *et al*., 2003; Mazumdar *et al*., 2003; Gupta *et al*., 2003). Liver diseases, especially viral hepatitis occurs predominantly in the developing world with an enormous impact on public health and economy (Simonsen *et al*., 1999). Plant drugs in Indian ayurvedic system of medicine and Chinese herbal medicine have long been used for liver biliary diseases. Some plants have also been found to possess hepatoprotective activity and underlying mechanism of action involves their antioxidant property (Gupta *et al*., 2004). The experimental intoxication induced by carbon tetrachloride (CCl_4) is widely used for modeling liver injury in rats. Hepatoxicity is connected with severe impairment of cell protection mechanisms. The location of liver injury is defined mainly by the biotransformation of CCl_4, which is cytochrome p-450 dependant. Free radicals initiate the process of lipid peroxidation, which generally cause inhibition of enzyme activity (Mac Cay *et al*., 1984; Poli *et al*., 1985).

It is now generally accepted that the hepatotoxicity of CCl_4 is the result of reductive dehalogenation, which is catalyzed by p-450 and formed highly reactive trichloromethyl free radical. This readily interacts with molecular oxygen to form the trichloromethyl peroxy radical. Both trichloromethyl and its peroxy radicals are capable of binding to proteins and lipids, or of abstracting a hydrogen atom from an unsaturated lipid, initiating lipid peroxidation and liver damage and by doing so playing a significant role in pathogenesis of diseases (Recknagel *et al*., 1989). Antioxidants are compounds that help prevent free radical damage.

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These protective compounds are common in foods and they include vitamins A, C, E and Co enzyme Q_{10}. It is well known that the hepatotoxic effect of carbon tetrachloride is due to the oxidative damage by free radical generation and antioxidant property is claimed to be one of the mechanisms of hepatoprotective drugs (Muriel *et al*., 2001**)**. Therefore, the aim of the present study was to evaluate the antioxidant effect of herbal powder of *Vernonia* and *cumin* seeds in carbon tetrachloride (CCl_4) induced hepatotoxicity in rats.

MATERIALS AND METHODS

Preparation of herbal powder

V.cinerea was leaves were collected in the month of March 2012 from the Anamalai hills, Coimbatore district of Tamilnadu, India. The plant was identified and authenticated by experts in the Taxonomy division, Department of Botany, Kongunadu arts and science college, Coimbatore. The plant leaves were picked up and dried under shade, powdered and passed through 40-mesh sieve and kept in a closed container for future use. The dried powder material of the leaves of *Vernonia cinerea* was extracted with ethanol (yield 26.3%) in a soxhlet apparatus. The ethanolic extract was then distilled, evaporated and dried in vacuum.

Experimental animals

Studies were carried out using male Wistar strains adult albino rats weighing 150-180g were obtained from PSG Institute of Medical Sciences & Reasearch, Coimbatore, Tamil Nadu, India. The animals were grouped and housed in polyacrylic cages(38 x 23 x 10 cm) with not more than six animals per cage and maintained under standard laboratory conditions (temperature 25 ± 2°C, 60-70% RH) with dark and light cycle (12:12 hr). These rats were fed with standard pellet diet from Hindustan lever limited (Mumbai, India) and water *ad libitum.* Rats 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 (Thomas *et al*., 1983). The study was carried out based on the guidelines for the use and care for laboratory animals. All the chemicals used in the present study were of analytical grade and was obtained from various sources. Solutions, buffers and reagents were prepared with glass-distilled water.

Experimental design

The selected rats were divided into five groups of six animals each as given below.

Group I: control group [rats received liquid paraffin (1ml/Kg body wt, ip) daily for 14 days.
Group II: CCl_4 treated [rats received liquid paraffin (1ml/Kg body wt,ip) daily for 7 days and from 8^{th} day, it was followed by treatment with CCl_4 in liquid paraffin (1:1; 2ml/Kg body wt, ip), upto 14^{th} day.
Group III: post-treated group [rats received CCl_4 in liquid paraffin (1:1; 2ml/Kg body wt, ip), upto 7^{th} day and they were treated with 100mg/Kg body wt of ethanolic extract of *V. cinerea* orally (as a suspension in a distilled water) upto 14^{th} day.
Group IV: pretreatment group [animals were pretreated with 100mg/Kg body wt of ethanolic extract of *V. cinerea* orally(as a suspension in a distilled water) from day 1 till day 7 and from 8^{th} day they were treated with CCl_4 in liquid paraffin (1:1; 2ml/Kg body wt, ip), upto 14^{th} day.
Group V: standard group[rats received CCl_4 in liquid paraffin as similar to group III upto 7^{th} day and from 8^{th} day they were treated with silymarin (100mg/Kg body wt, ip) upto 14^{th} day.

Collection of rat liver

Animals were kept starved overnight on the 14^{th} day, on the next day, after recording the weight in each case; they were sacrificed under mild chloroform anesthesia. The livers were immediately excised, washed with cold saline, blotted and weighed. Then 10% w/v liver homogenates were prepared with ice cold 0.1M phosphate buffer (pH 7.4) using potter Elvehjem tefton homogenizer. The homogenate was centrifuged at 5000rpm at 4°C for 30min and supernatant obtained was used for the assay of various enzymes. For Histopathology small pieces of liver tissues were fixed in 10% formalin solution, dehydrated with 90% ethanol, embedded in paraffin, cut into thin sliced section (5µm thickness), and stained with haematoxylin-eosin dye (Bacroft and Stevents, 1996**)**. Lipid peroxidation (Narayanasamy and Selvi, 2005). Superoxide dismutase (Buege and Aust, 1978), Catalase (Sinha, 1972), Glutathione peroxidase (Das *et al*., 2000), Glutathione reductase (Rotruck *et al*., 1973) Glutathione s-transferase (Moron *et al*., 1979), and Reduced glutathione (Torres *et al*., 2004) were studied by using liver sample.

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) (Snedecor and William, 1994).

RESULTS

In the present study, rats induced with CCl_4 (group II) showed a significant ($P<0.05$) increase of MDA release both under basal and induced conditions, when compared to control (Group I) rats (Table 1).

The treatment with herbal powder (Group III) resulted in the significant fall in the level of MDA ($P<0.05$) as compared to Group II. This indicates better antioxidant property of herbal powder in the moderation of hepatic damage due to CCl_4. The animals treated only with herbal powder (Group V) have shown no significant increase in LPO level when compared to control (Group I) rats. It has been shown that protective agents exert their action against CCl_4 induced liver injury by impairment of CCl_4 mediated LPO, either through decreased production of free radical derivatives or due to the antioxidant activity of the protective agent itself.

Table 2. Activities of herbal powder on the levels of antioxidant in the liver of control and experimental rats

Marker enzymes in liver	Group-I Control	Group II Toxic	Group III Post treatment	Group IV Pre treatment	Group V Positive control
CAT	1.46 ±0.16	0.77 ±0.07a*	1.16 ±0.06b*	1.18 ±0.02c*	1.39 ±0.19d^{ns}
SOD	1.61 ±0.64	0.71 ±0.03a*	1.30 ±0.19b*	0.90 ±0.08c*	1.59 ±0.19d^{ns}
GSH	3.96 ±0.33	1.63 ±0.14a*	3.08 ±0.11b*	2.62 ±0.24c*	3.94 ±0.22d^{ns}
GST	6.57 ±0.52	2.42 ±0.15a*	5.47 ±0.24b*	4.70 ±0.75c*	6.20 ±0.55d^{ns}
GR	10.2 ±1.11	4.97 ±0.40a*	9.03 ±0.46b*	7.70 ±0.69c*	10.2 ±1.42d^{ns}

Units
CAT- µ moles of H_2O_2 decomposed/min/mg protein
SOD- 50% inhibition of nitrite/min/ mg protein
GSH- µg/ mg protein
GST- µ moles of CDNB –GSH conjugate formed/ min/ mg protein
GR- n mole NADPH breakdown / min/ mg protein
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, and d- group I with group V.

The changes in the enzymatic antioxidants like catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH)), glutathione s-transferase (GST) and glutathione reductase (GR) in the liver of normal and experimental rats are illustrated in Table 2. The levels of CAT, SOD, GSH, GST and GR recorded a significant decline in CCl_4 administered rats (Group II) when compared with normal controls. In group III rats, the activities of these enzymes attained near normalcy. Both pretreated (Group IV) and post treated (Group III) group of rats showed an elevated levels of these antioxidant enzymes when compared to toxic group (Group II) of rats. No significant difference was noticed between group V and group I which indicates that the selected herbs have no side effects.

DISCUSSION

Many compounds known to be beneficial against carbon tetrachloride mediated liver injury exert their protective action by toxin mediated lipid peroxidation either via a decreased production of carbon tetrachloride derived free radicals or through the antioxidant activity of the protective agents themselves (Maleeka Begum *et al*., 2011). Lipid peroxidation is the oxidative deterioration of lipids containing any number of carbon-carbon double bonds and it is a free radical related process that in biological systems may occur under enzymatic control. In vivo lipid peroxidation destroys biological membrane leading to change in fluidity and permeability of the cell and associated with cellular damage as a result of oxidative stress, which

Table 1. Activities of herbal powder on the levels of lipid peroxidation in the liver of control and experimental rats

Lipid peroxidation	Group I Control	Group II Toxic	Group III post treatment	Group IV pre treatment	GroupV Positive control
Basal	6.60±1.84	21.3±15.5a*	10.60±5.94b*	15.04±3.92c*	6.91±0.97d^{ns}
$Feso_4$	32.70±3.40	134.9±2.32a*	41.19±2.50b*	97.72±9.82c*	40.85±1.36d^{ns}
Ascorbate	48.72±4.65	177.2±14.4a*	65.63±1.36b*	124.95±14.0c*	38.07±3.22d^{ns}

Units: LPO- n moles of MDA formed/ min/ mg protein 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, and d- group I with group V.

also involves cellular antioxidants in this process (Deivanayagam *et al*., 2010). Lipid peroxidation has been linked with changed membrane structure and enzyme inactivation (Thabrew *et al*., 1987). The induction of lipid peroxidation is considered to be important in etiology of many diseases (Memisogullari *et al*., 2003). *In vitro* lipid peroxidation in the liver homogenate can proceed in a non- enzymatic way. The process is induced by ascorbate in the presence of Fe^{2+}/Fe^{3+} and it has been reported that Fe^{2+} and ascorbic acid stimulated lipid peroxidation in rat liver microsomes and mitochondria. In order to clarify the mode of action of herbal powder, in vitro lipid peroxidation experiments were carried out. According to the result obtained, the herbal powder inhibited $FeCl_2$- ascorbic acid-stimulated lipid peroxidation in liver homogenate. Moreover our experimental results demonstrate that the herbal powder exercises free radicals scavenging activity on the superoxide radicals generated using xanthine-xanthine oxidase system and may therefore, act by scavenging free radicals and reactive oxygen species formed during the carbon tetrachloride metabolism. A number of scientific reports indicated certain flavonoids, triterpenoids and steroids have protective effect on liver due to its antioxidant properties (DeFeudis *et al*., 2003). Presence of those compounds in herbal powder may be responsible for the protective effect on CCl_4 induced liver damage in rats. GSH is a naturally occurring substance that is abundant in many living creatures. It is widely known that a deficiency of GSH within living organisms can lead to tissue disorder and injury. For example, liver injury included by consuming alcohol or by taking drugs acetaminophen, lung injury by smoking and muscle injury by intense physical activity (Banskota *et al*., 2000), all are known to be correlated with low tissue levels of GSH. From this point of view, exogenous herbal powder supplementation might provide a mean to recover reduced GSH levels and to prevent tissue disorders and injuries.

In the present study it was demonstrated that the effectiveness of herbal powder by using CCl_4 induced rats, which are known models for both hepatic GSH depletion and injury. Biological systems protect themselves against the damaging effects of activated species by several means. These include free radical scavengers and chain reaction terminators; enzymes such as SOD, CAT and GR system (Leeuwenburgh and Ji, 1995). The SOD dismutases superoxide radicals $O_2^{\cdot-}$ into H_2O_2 plus O_2, thus participating with other antioxidant enzymes, in the enzymatic defense against oxygen toxicity. The observed increase of SOD activity suggests that the herbal powder have an efficient protective mechanism in response to ROS. CAT is a hemeprotein, localized in the peroxisomes or the microperoxisomes. This enzyme catalyses the decomposition of H_2O_2 to water and oxygen and thus protecting the cell from oxidative damage by H_2O_2 and $OH^{\cdot}$. GST plays an essential role in liver by eliminating toxic compounds by conjugating them with glutathione. GR is concerned with the maintenance of cellular level of GSH (especially in the reduced state) by effecting fast reduction of oxidized glutathione to reduced form. The activities of these enzymes were found to be in the reverse order. In liver tissues of CCl_4-- administered rats, level of GST registered a significant increment, whereas that of GR recorded a decline. However, these enzymes restored an almost normal activity in CCl_4 and herbal powder administered rats, thus unearthing the antioxidant effect of herbal powder. And also, these findings indicate that the herbal powder may be associated with decrease in oxidative stress and free radical mediated tissue damage.

Conclusion

The results of this study demonstrate that the herbal powder prepared from *Vernonia cinerea* have a potent hepatoprotective action upon carbon tetrachloride induced hepatic damage in rats. The hepatoprotective effects of the herbal powder might be due to its antioxidant 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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