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Hepatoprotective effect of *Vernonia cinerea* and Cumin seeds on Carbon Tetrachloride Induced Hepatic Oxidative Stress

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Abstract: In this study, we have examined the protective effect of *Vernonia cinerea* against carbon tetrachloride (1.0ml / kg b.wt / day) administered intraperitoneally for 2 days in male albino Wistar rats. The levels of aspartate transaminase, alanine transaminase, lactate dehydrogenase, alkaline phosphatase, bilirubin, creatinine, and urea were determined. The activities of glutathione, Vitamin C and the levels of lipid peroxides in 10% w/v liver homogenate were also determined. The CCl_4 induction resulted a significant elevation in the levels of serum marker enzymes, bilirubin and creatinine with decreased urea. The activities of hepatic glutathione and vitamin C were also significantly depleted with increased lipid peroxides in CCl_4 intoxicated rats. The oral administration of herbal drug alone did not show any toxicity in the liver tissue. These results suggest that the herbal drug may probably act as a natural antioxidant against CCl_4 induced hepatic oxidative stress.

Keywords: *Vernonia cinerea*, carbon tetrachloride, oxidative stress, lipid peroxides, antioxidant.

Introduction

Liver, the largest organ in Vertebrate body, is the major site of intense metabolic activities. The liver plays an astonishing array of vital functions in the maintenance and performance of the body. Some of these major functions include carbohydrate, protein and fat metabolism, detoxification, and secretion of bile. Unfortunately, the liver is often abused by environmental toxins, poor eating habits, alcohol, and over the counter drug abuse, which can damage and weaken the liver and eventually lead to hepatitis, cirrhosis and alcoholic liver disease [1].

Liver diseases, especially viral hepatitis occurs predominantly in the developing world [2] with an enormous impact on public health and economy. Plant drugs in Indian Ayurvedic system of medicine and Chinese herbal medicine have long been used for liver and biliary diseases. Some plants have also been found to possess hepatoprotective activity and the underlying mechanism of action involves their antioxidant property [3]. The oxidative stress is not only the causative for liver damage but also implicated in the pathogenesis of cancer, diabetes, cardiovascular disorders as well as in the process of aging [4].

In recent years many researchers have examined the effects of plants used traditionally by indigenous healers and herbalists to support liver function and treat disease of the liver. In most cases, research has confirmed traditional experience and wisdom by discovering the mechanisms and modes of action of these plants as well as reaffirming the therapeutic effectiveness of certain plants or plant extracts in

clinical studies [5]. Realizing the fact, this research was carried out to evaluate the hepatoprotective activity of herbal powder of *Vernonia cinerea* and cumin seeds against CCl_4-induced liver damage in rats.

Materials and Methods

Preparation of herbal powder

The whole plant of *Vernonia cinerea* was collected from Valparai, Western Ghats of Anamalai Hills, Coimbatore district of Tamilnadu, India. The plant was identified and authenticated by Dr.V.S. Ramachandran, Taxonomist, Postgraduate and Research Department of Botany, Kongunadu Arts and Science College, Coimbatore district of Tamilnadu, India. The cumin seeds were purchased from the local market and it was mixed with plant powder of *V.cinerea* in the ratio of 1:1. The collected materials were thoroughly washed in water, chopped, air dried at 35-40°C for a week and pulverized in electric grinder and tend in a closed container for future use.

Experimental Animals

Adult male albino wistar rats, each weighing 150-200g were used in the present investigation. They were purchased from the P.S.G. Institute of Medical Sciences, Coimbatore District of Tamilnadu (India) and housed in clean polypropylene cages (38 x 23 x 10 cm) with not more than six animals per cage. They were allowed free access to standard pellet diet (AVM feeds and foods, Hindustan lever limited (Mumbai, India) and water *ad libitum*. The pellet composition was found to be similar to RDA (Recommended Dietary Allowances) for laboratory animas, studies were carried out according to the recommendations of animals ethical committee.

Carbon tetrachloride – Induced hepatotoxicity

Hepatic injury was induced in rats through intraperitoneal administration of CCl_4 diluted with liquid paraffin oil (1:1) in a dose of 1ml/kg body wt. for 2 days to all the animals except for control (group I) as standardised by Rao and Mishra [6] .

Experimental Study

The selected rats were divided into four 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 wt. for 2 days]. Group III: Orally treated with herbal powder (10mg/100g body wt daily for a period of 15 days) after the CCl_4 intoxication (1ml / kg body wt. intraperitoneally). Group IV: Orally administered with herbal powder alone (10mg / 100g body wt. daily for a period of 15 days). At the end of the experimental period, the animals were fasted overnight and then sacrificed by cervical decapitation.

Collection of Liver and Serum

After 24 hours of the last dose, rats were sacrificed under mild chloroform anesthesia. Blood was collected from retro-orbital plexus. The blood samples were allowed to clot and the serum was separated by centrifugation at 2500 rpm at 37°C and used for the assay of biochemical marker enzymes. The livers were immediately excised, washed with cold saline, blotted and weighed. Then 10% w/v liver homogenates were prepared with 0.15M Tris HCl buffer (pH 7.4) and used for the biochemical estimations.

Measurement of biochemical parameters

The liver homogenate was used for the study of non enzymic antioxidants total reduced

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glutathione [7] and Vitamin C [8]. The collected serum was used for the assay of marker enzymes namely aspartate transaminase (AST) alanine transaminase (ALT) [9], alkaline phosphatase (ALP) [10], lactate dehydrogenase (LDH) [11], Bilirubin [12], Creatinine [13] and Urea [14].

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) followed by students't' test for significant difference between group means. *p* values less than 0.05 were considered significant.

Results and Discussion

As shown in the table 1, Group II rats treated with CCl_4, exhibited a significant increase ($P<0.05$) in serum marker enzymes, bilirubin and creatinine. This suggests that the CCl_4 has developed a severe hepatic damage as compared to the group I.

Table 1: Effect of *Vernonia cinerea* and cumin seeds on marker enzymes in serum of CCl_4 induced liver injury.

Treatment	AST µ moles of pyruvate liberated / L	ALT µ moles of pyruvate liberated / L	ALP µ moles of phenol liberated / L	LDH µ moles of pyruvate liberated / dl.
Control	72.5 ± 4.93	28.3 ± 2.94	100.4 ± 6.06	163.3 ± 14.7
Toxic	146.3 ± 13.1a*	110.6 ± 3.92a*	175.0 ± 8.71a*	294.8 ± 13.6a*
Treatment	78.4 ± 5.16b*	43.5 ± 3.67b*	120.3 ± 7.28b*	194.6 ± 12.1b*
Positive control	74.1 ± 6.28c^{ns}	25.8 ± 2.96c^{ns}	92.5 ± 9.56c^{ns}	143.5 ± 16.3c^{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 I with group IV.

In the assessment of liver damage by CCl_4 hepatotoxin, the hepatospecific serum markers aspartate transaminase (AST**)**, alanine transaminase (ALT), lactate dehydrogenase (LDH), and alkaline phosphatase (ALP) elicit as a result of significant hepatic damage. These changes in the marker levels will reflect in hepatic structural integrity. These markers are cytoplasmic in origin and released into the circulation after cellular damage [15]. High levels of AST indicate liver damage, such as that due to viral hepatitis as well as cardiac infarction and muscle injury. ALT catalyses the conversion of alanine to pyruvate and glutamate, and is released in a similar manner. Therefore, ALT is more specific to the liver, and thus a better parameter for detecting liver damage. The rise in the AST is usually accompanied by an elevation in the levels of ALT, which plays a vital role in the conversion of aminoacids to ketoacids [15]. The treatment with the herbal powder of *Vernonia cinerea* and Cumin seeds attenuated the elevated levels of serum markers. Serum ALP and bilirubin levels on the other hand are related to the function of hepatic cells. Increase in serum levels of ALP is due to increased synthesis, in the presence of increasing biliary [16]. Our results using the modes of CCl_4 induced hepatotoxicity in rats demonstrated that the herbal powder caused a significant reduction in serum ALP and bilirubin levels. Effective control of bilirubin level

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and alkaline phosphatase activity points towards an early improvement in the secretary mechanism of the hepatic cell. The normalization of urea, bilirubin and creatinine in herbal powder treated rats further indicates the protective nature of the herbal powder on hepatic cells when compared with CCl_4 intoxicated rats.

Liver cell injury induced by CCl_4 involves initially the metabolism of CCl_4 to trichloromethyl free radical by the mixed function oxidase system of the endoplasmic reticulum. It is postulated that secondary mechanisms CCl_4 metabolism to the widespread disturbances in hepatocyte function. These secondary mechanisms could involve the generation of toxic products arising directly from CCl_4 metabolism or from peroxidative degeneration of membrane lipids (17).

The urea levels were found to be decreased in group II rats as compared with their control. The activities of glutathione and vitamin C were also depleted with increased levels of lipid peroxidation in liver tissue, whereas treatment with herbal powder decreased the activity of serum marker enzymes, bilirubin and creatinine and increased the urea levels in CCl_4-induced liver damage in rats compared to that of toxic group II ($p<0.05$) experimental rats[table 2 and 3]

Table 2: Effect of *Vernonia cinerea* and cumin seeds on non-enzymic antioxidants and lipid peroxide levels in CCl_4 induced liver injury.

Treatment	GSH µg / mg protein	Vitamin C mg / gm tissue	Lipid peroxidation µ moles MDA / hr / gm tissue
Control	3.96 ± 0.33	3.32 ± 0.37	48.72 ± 4.65
Toxic	1.63 ± 0.14a*	1.63 ± 0.18a*	177.2 ± 14.4a*
Treatment	3.08 ± 0.11b*	2.59 ± 0.29b*	65.63 ± 1.36b*
Positive control	3.94 ± 0.22c^{ns}	3.48 ± 0.16c^{ns}	48.07 ± 3.22c^{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 I with group IV.

Table 3. Effect of *Vernonia cinerea* and cumin seeds on Bilirubin, urea and creatinine in CCl_4 induced liver injury.

Treatment	Bilirubin mg / dl	Urea mg / dl	Creatinine mg / dl
Control	0.92 ± 0.25	0.502 ± 0.01	4.23 ± 0.53
Toxic	3.66 ± 0.14a*	0.745 ±0.01a*	8.38 ± 0.18a*
Treatment	1.20 ± 0.24b*	0.574 ±0.01b*	4.55 ± 0.84b*
Positive control	0.87 ± 0.22c^{ns}	0.491±0.02c^{ns}	6.04 ±1.86c^{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 I with group IV.

CCl_4 treated group rats show elevated levels of Malondialdehyde (MDA), a product of lipid peroxidation. Therefore, an increase in the liver MDA level indicates an increase in the degree of lipid peroxidation, a well known mechanism of liver damage [18]. In addition, the extensive lipid peroxidation results in membrane disorganization by peroxidising the highly unsaturated fatty acids, which in turn alters the ratio of polyunsaturated to other fatty acids leading to decrease in the membrane fluidity which may be sufficient to cause cell death [19]. A significant decrease in the levels of lipid peroxides in herbal powder treated rats suggests that the herbal powder may have the ability to protect the liver from free radical injury caused by CCl_4.

As the hepatotoxic doses of CCl_4 will deplete the normal levels of hepatic glutathione, it is widely known that a deficiency of GSH within

living organisms can lead to tissue disorder and injury. For example, liver injury caused by consuming alcohol or by taking drugs like acetaminophen, lung injury by smoking and muscle injury by intense physical activity [20], 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.

The levels of vitamin C were significantly depleted in CCl_4 intoxicated rats. This depletion might be due to the excessive utilization of non-enzymic antioxidants involved in quenching the enormous free radicals produced during CCl_4 intoxication [21]. Vitamin C can protect cell membranes and epiprotein particles from oxidative damage by regenerating the antioxidant form of Vitamin E [22]. The recoupment of vitamin C to near normal level in drug treated rats was found to be due to potent antioxidant activity of the herbal drug, which may induce the regeneration of ascorbic acid.

It has been reported the *Vernonia cinerea* contains triterpenoid [23]. A number of scientific reports indicated that certain flavonoids, triterpenoids and steroids have protective effect on liver due to its antioxidant properties [24, 25]. Presence of those compounds in herbal drug may be responsible for the protective and free radical scavenging property on CCl_4 induced liver damage in rats.

Conclusion

The results of this study demonstrate that the herbal powder of *Vernonia cinerea* and a cumin seeds has a potent hepatoprotective action upon carbon-tetrachloride induced hepatic damage in rats. Further investigation is underway to determine the exact phytoconstituent that is responsible for its hepatoprotective and antioxidant activity.

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