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Hepatoprotective and Antioxidant activity of *Alpinia purpurata (Vieill)* against Carbon tetrachloride induced in Albino rats

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ABSTRACT

Hepatoprotective activity and antioxidant status of ethanolic extracts of 200,400,600 mg/kg of *Alpinia purpurata rhizome* was evaluated against Carbon tetrachloride (CCl_4) in rats. Acute and short-term toxicity studies were performed initially in order to ascertain the safety of ethanolic extracts of *Alpinia purpurata rhizome*. After 48 hrs of CCl_4 induced, the extract was administered daily for 15 days. After administration of the last dose followed by 18 h fasting, rats were then sacrificed for observation of hepatoprotective activity. The effect of ethanolic extracts of *Alpinia purpurata* on the CCl_4 treated rats. Ethanolic extract showed significant ($p<0.05$) hepatoprotective effect by lowering the serum levels of various biochemical parameters such as serum glutamic oxaloacetate transaminase (SGOT), serum glutamic pyruvate transaminase (SGPT), alkaline phospatase (ALP), total bilirubin (TBL), total cholesterol (CHL) in the selected model and liver biochemical parameters (lipid peroxidation, antioxidant enzymes) were estimated. These biochemical observations were in turn confirmed by histopathological examinations of liver sections and are comparable with the standard hepatoprotective drug Silymarin (25.0 mg/kg body weight, p.o) which served as a positive control. Treatment with ethanolic extracts of *Alpinia purpurata* decreased the levels of lipid peroxidation and increased the levels of glutathione, superoxide dismutase and catalase. The ethanolic extracts of *Alpinia purpurata rhizome* exhibited hepatoprotective effect by modulating lipid peroxidation and augmenting antioxidant defense system in CCl_4 treated rats.

Introduction

Liver is the most important organ, which plays a pivotal role in regulating various physiological processes in the body. It is involved in several vital functions, such as metabolism, secretion and storage. It has great capacity to detoxicate toxic substances and synthesize useful principles [1]. Therefore, damage to the liver inflicted by hepatotoxic agents is of grave consequences. Liver diseases are mainly caused by toxic chemicals, excessive consumption of alcohol, infections and autoimmune disorders. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damages [2]. Liver damage is always associated with cellular necrosis, increase in tissue lipid peroxidation and depletion in the tissue GSH levels. In addition serum levels of many biochemical markers like SGOT, SGPT, triglycerides, cholesterol, Bilirubin, alkaline phosphatase, are elevated in liver disease [3, 4]. There are numerous plants and traditional formulations available for the treatment of liver diseases [5, 6]. About 600 commercial herbal formulations with claimed hepatoprotective activity are being sold all over the world. Around 170 phytoconstituents isolated from 110 plants belonging to 55 families have been reported to possess hepatoprotective activity. In India, more than 93 medicinal plants are used in different combinations in the preparations of 40 patented herbal formulations [7]. However, only a small proportion of hepatoprotective plants as well as formulations used in traditional medicine are pharmacologically evaluated for their safety and efficacy [8]. Some herbal preparations exist as standardized extracts with major known ingredients or even pure compounds which are being evaluated [6]. Alpinia purpurata is a popular tropical landscape ornamental that has been grown in gardens which is very recent and incipient and results show the presence of flavonoids rutin and kaempferol-3-O-glucuronide [9]. The rhizomes of Zingiberaceae plants are widely used as spices or traditional medicine in Asian countries, eaten raw, or cooked as vegetables and as flavouring. Leaves of several Zingiberaceae have also been used for food flavouring and in traditional medicine [10]. In the phytochemical study was focused to reveal the quantitative and qualitative analysis, Tannins, Resins, Proteins, Alkaloids, Flavonoids, Glycosides, Phenols and Saponins presented in ethanolic extract of parts of *Alpinia purpurata* rhizome[11]. Antioxidant and Freeradical scavenging activity also were done which the results showed tremendous activity. The objective of the present work is to explore the antioxidant and hepatoprotective activity of *Alpinia purpurata* rhizome against CCl_4 induced rats.

Materials and Methods

Plant Material:

Collection:

Fresh plant material was collected from Kovaipudhur, Coimbatore District, Tamil Nadu State, India. Efforts were made to collect the plant in rhizomes and flowering conditions for the correct botanical identification. The plant material was brought to the laboratory and identified with the help of Agriculture university of Coimbatore, Tamil Nadu State.

Preparation of Extract:

About 300g of the coarse dried powder of the rhizome of *Alpinia purpurata* was taken in soxhlet apparatus and extracted

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using 95% ethanol. The extraction was carried out for about 72 hours. The extract was collected by the filtrate was pooled and the solvents were evaporated in a rotatory evaporator at temperature below 50°C and the extracts were freeze-dried. The residue was used to analyse the various *in vivo* hepatoprotective activity.

Chemicals:

The drugs and fine chemicals were purchased from Sigma Chemical Company, St. Louis, USA. All other chemicals and solvents were obtained from Himedia and SD Fine Chemicals, Mumbai, India and were of the highest purity and analytical grade.

Experimental Animals:

The male albino rats of Wistar strain weighing 180g-230g were obtained from Kovai medical centre of research and hospital (KMCH) Pharmacy College, Coimbatore. The animals were housed in polypropylene cages at controlled temperature (27 ± 2° C), relative humidity (60 ± 5%) and light conditions(12 -12 hours day night cycle). The rats were fed with standard laboratory diet and drinking water was given through a drinking bottle, throughout the experiment. They were given a week's time to get acclimatized to the laboratory conditions. All animal experiments were conducted with the permission from Institutional Ethical Committee (KMCRET/Ph.D/07/2011).

Preliminary Phytochemical Analysis:

A preliminary phytochemical screening of the ethanolic extract of *Alpinia purpurata* rhizome was carried out. The phytochemical profile was performed as described by Mukherjee *et al*., 2002 [12]. It showed the presence of active constituents such as Alkaloids, Flavanoids, Steroids, Carbohydrates, Proteins, Tannins, Glycosides, Saponins and Phenols [13].

Acute oral toxicity study

Acute oral toxicity of *Alpinia purpurata* extract was performed on Swiss albino rat, according to OECD guideline 423. Two groups of six rats each were used for the study. Group I served as control and received distilled water. Group II received single oral dose of ethanolic extract of *Alpinia purpurata* rhizome (2000 mg/kg). The animals were observed for gross behavioural, neurological, autonomic and toxic effects at short intervals of time for 24 h and then daily for 15 days. Food consumption was monitored daily and body weights were recorded weekly. On 15th day, animals were sacrificed and all the organs were removed for gross pathological examination.

Experimental Design:

The rat dose was calculated on the basis of the surface area ratio. Animals were divided into five groups, consisting of six animals each. The groupings of the experimental rats were as follows, CCl_4 (1mg/kg b.w) was administered to all groups except group I. Group I served as control group, receiving carboxy methyl cellulose, Group II served as $CC1_4$ control, receiving alone at a concentration of (1ml/ body weight (b.w)), Group III CCl_4 treated rats receiving the extract of *Alpinia purpurata* (200mg/kg Probationary Oral (p.o)) Group IV CCl_4 treated rats receiving the extract of *Alpinia purpurata* (400mg/kg/d (p.o)), Group V $CC1_4$ treated rats receiving the reference drug silymarin (25.0 mg/kg intra peritoneal (i.p)) for a period of 15 consecutive days. All the animals were sacrificed at the end of 15th day after $CC1_4$ administration. Blood samples were drawn by puncturing the retro-orbital plexus, serum separated and estimated for biochemical parameters. Liver tissues were removed for the determination of antioxidant enzyme levels and histopathological examinations.

Measurement of Biochemical Parameters:

Biochemical analysis were carried out to asses liver function viz., Serum transaminases Aspartate transaminase (AST) IFCC method (1986) [8], Alanine aminotransferase (ALT) IFCC method (1986) [8], Alkaline phosphatase (ALP) King, Wootton,(1964) [13], Acid phosphatise (ACP) King,1965 [14], Total Bilirubin Daumas *et al*.,(1971) [15], Total Cholesterol, Allain *et al*.,1974 [16], Urea, Wybenga et al.,1971 [17] by enzymatic method (Tietz, 1987) [18], Creatinine (Bowers, L.D. 1980) [19] and protein Lowry *et al*.,1951[20] the enzyme levels were assayed. The liver homogenate was prepared and the clear supernatant was used for the estimation of lipid peroxidation (MDA) Hogberg *et al*.,(2004) [21], Reduced Glutathione (GSH), Moron et al.,1979 [22], Glutathione peroxidase, Rotruck et al., 1973 [23] and antioxidant enzymes viz. Catalase (CAT), Sinha,1972 [24] and Superoxide dismutase (SOD) (Das *et al*.,2000) [25] levels.

Histopathological Examination:

A portion of liver tissue from each group was preserved in a 10% formalin solution for histopathological studies. Haematoxylin and eosin were used for staining and later the Microscopic slides of the liver cells were photographed at a magnification of x100.

Statistical Analysis:

Values were represented as mean±SEM. Data were analysed by one-way analysis of variance (ANOVA) followed by Dennett's test using statistical package for social sciences (SPSS) version 10.0. $P<0.05$ was considered significant. The toxic control group was compared with the normal control group and all other treatment groups were compared with the toxic control group.

Results And Discussion:

Acute Lethal Dosage Study:

When the rats were observed for the behavioural changes after orally administration of a single dose of the extract, none of the rats exhibited any abnormal behaviour responses at doses of 2000 mg/kg. Administration of repeated daily doses of 2000 for 15 days did not influence the body weight of the rats. The weights of liver, kidney, and spleen were also not altered by the treatment. Hematological parameters like haemoglobin and RBC count remained unaltered at the dose of 2000 mg/kg. Thus, it was concluded that ethanolic extract of Alpinia purpurata rhizome extract was safe at 2000 mg/kg.

Biochemical Assessment:

Carbon tetrachloride is a widely used experimental hepatotoxicant. It is bio transformed by the cytochrome P-450 system to produce the trichloromethyl free radical, which in turn covalently binds to cell membranes and organelles to elicit lipid peroxidation, disturb Ca 2+ haemostasis and finally result in cell death [25]. Determination of the activity of hepatic enzymes released into the blood by the damaged liver is one of the most important tools in the study of hepatotoxicity [26].The biochemical assays of hepatoprotective study were performed on 15th day. The results were presented in Table 1. (Assay liver marker enzymes and Bilirubin), Table 2. (Changes in serum cholesterol, urea and creatinine) and Table 3 (Changes in antioxidant enzymes and TBA). Significant acute hepatotoxicity cellular damage, and biliary obstruction was indicated by the elevated level of serum liver marker enzymes, Bilirubin, Cholesterol, Urea and Creatinine in rats induced with CCl_4 (Group II) as compared to control group (Group I).

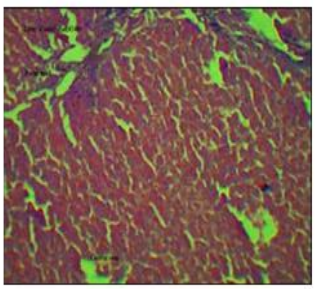
Accumulation of higher concentrations of Cholesterol and Creatinine confirms the depth and intensity of liver damage reduction in the level of liver marker enzymes and Bilirubin, Cholesterol, Urea and Creatinine in the rats (Group III) treated with the CCl_4 + 200 mg of *Alpinia purpurata*, showed the hepatoprotective action of *Alpinia purpurata*. The treatment with standard drug silymarin (Group V) has also significantly reduced these liver enzyme, Bilirubin, cholesterol, urea and Creatinine levels. In the rats treated with the CCl_4 + 400 mg of ethanolic extract of *Alpinia purpurata* (Group IV) the liver marker enzymes, Bilirubin, cholesterol, urea and Creatinine levels are almost near to that of the control group (Group I). It is found that the ethanolic extracts of *Alpinia purpurata* decreased the CCl_4 produced elevation in the enzyme levels, indicating the production of structural integrity of hepatocytic cell membrane or regeneration of damaged liver cells by the *Alpinia purpurata* extracts. From this study, it could be concluded that the ethanolic extracts of *Alpinia purpurata* protects the liver from damage very similar to the protective effect brought about by the standard drug silymarin.

In this study elevated levels of LPO observed in CCl_4 administered rats in group II indicates excessive formation of free radicals and activation of LPO system resulting in hepatic damage. Thiobarpituric acid reactive substances (TBARS) are produced as by products of LPO that occur in hydrophobic core of bio-membranes [27]. The significant decline in the concentration of these constituents in the liver tissue of group IV indicates Anti-peroxidative effect of the ethanolic extracts of *Alpinia purpurata*. In this study a significant decrease in the liver GSH was observed in the experimental rats (Group II) administrated with CCl_4, GSH constitutes the first line of defense against the free radical reduction in liver GSH and decreased activity of glutathione peroxidise (GPx) in CCl_4 treated experimental rats (Group II) indicates the damage to the liver cells. But the reconstitution of the levels of the GSH, GPx activity in the rats with ethanolic extract of *Alpinia purpurata* accounts for the protective and antioxidants efficiency of the drug. This increase in hepatic GSH levels in group IV could either be due to an effect on the Lenovo synthesis of GSH, its regeneration or both. As a consequence, hepatic GSH level could be sufficiently maintained to counteract the increased formation of free radicals as in the case of CCl_4 toxicity [28].The result from this study clearly reveals that the *Alpinia purpurata* extracts when administered to rats at a concentration of 200,400 mg/kg (Group III, IV) showed protective effect and the results from the above parameters were compared to that of the normal control vehicle (Group I) and with the group treated with the silymarin (V).

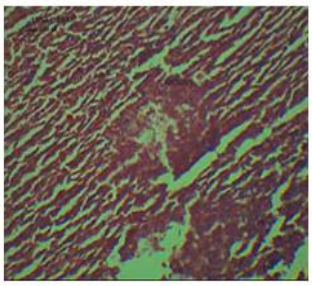
Histopathological assessment

During the histological examination of liver sections of the control group [Figure A], it was observed that the central vein was prominent, with normal hepatocytes. In the carbon tetrachloride intoxicated group [Figure B], centriolobular necrosis was observed. In the histological profile of the Siliymarin treated group [Figure E] and the different groups treated with *Alpinia purpurata* [Figures C & D], there was less centriolobular necrosis and hepatocytes showing regeneration activity figure 1.

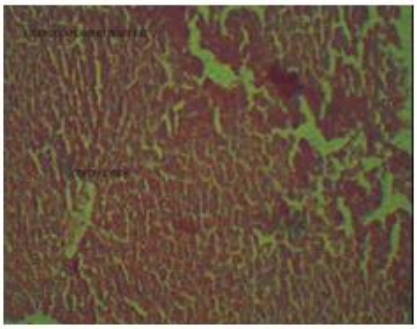
Figure 1. Histopathological observation of liver (H & E × 100)



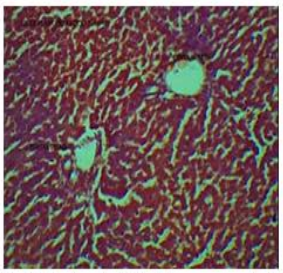
A – Normal liver (Group-I)
Normal cohesive cords of liver cells, central vein and portal triad.



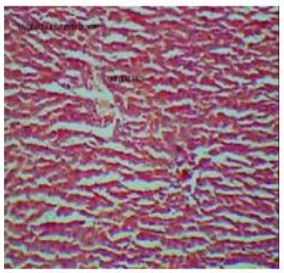
B – CCL_4 Treated (Group-II)
Loosely cohesive cords of liver Cells and necrosis around central vein.



C - CCL_4 treated + *Alpinia purpurata (200 mg)* (Group-III)
Almost Normal except mild congestion



D - CCL_4 treated *Alpinia purpurata (400 mg)* (Group-IV) Normal cohesive cords loosely of liver cells



E – CCL_4 treated + Silymarin (25 mg) (Group-V) Mild central vein congestion and cohesive cords of liver cells

Conclusion

In the present pharmacological evaluation the extracts (ethanolic) of *Alpinia purpurata rhizome* plant was extensively investigated for its hepatoprotective potential against substance (CCl_4) induced hepatotoxicity and also evaluate the lipid peroxidation and anti-oxidant status. At the end of our study, a strong conclusion can be drawn that, the ethanolic extracts of *Alpinia purpurata* possess hepatoprotective activities more or less depending on the dose levels. The ethanolic extracts of the plant at dose levels of 200, 400mg/kg exhibited competent, potent and comparable, promoting *Alpinia purpurata* as a promising hepatoprotective plant species. The hepatoprotective effect of *Alpinia purpurata* might be due to the presence of phycocyanin pigment present in the extract and antioxidant effect. Further study is needed to identify and isolate the active principle of *Alpinia purpurata*, which can have offer antioxidant and hepatoprotective properties. Studies in this direction are progressing in our lab, as this is the first report in the antioxidant and hepatoprotective properties about ethanolic extract of *Alpinia purpurata* rhizome. Therefore, further studies should be conducted to determine the active compounds that are responsible for the hepatoprotective effects and the mechanisms of action involved in the antihepatotoxic effect.

Table. 1. Level of Liver Marker Enzymes and Bilirubin in Different Experimental Groups of Rats

GROUPS	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	ACP (IU/L)	Bilirubin (mg/100ml)
Group I (Control)	13.18 ± 3.22	11.01 ± 2.93	172.04 ± 4.29	11.45 ± 0.82	0.93 ± 0.98
Group II (CCl_4 treated)	43.47 ± 4.87*	48.70 ± 2.56*	330.52 ± 2.52*	36.86 ± 0.42*	2.56 ± 0.62*
Group III (CCl_4 + 200mg/kg extract)	18.69 ± 3.32*	14.32 ± 2.24*	263.82 ± 3.06*	24.67 ± 2.42*	1.42 ± 0.60*
Group IV (CCl_4+400mg/kg extract)	15.81 ± 2.98*	13.02 ± 4.31*	186.68 ± 2.09*	09.98 ± 1.82*	1.00 ± 0.62*
Group V (CCl_4 + Silymarin)	14.48 ± 2.62^{ns}	11.42 ± 4.09^{ns}	170.42 ± 3.18^{ns}	10.73 ± 2.82^{ns}	0.98 ± 0.68^{ns}
CD ($p<0.05$)	5.39	6.50	4.93	3.57	0.85

Values are mean ± SD of six observations
Groups compared: Group II vs Group I; Group III vs Group II; Group IV vs Group II; Group V vs Group I
Significance: * - Significant at $p<0.05$; ns – Not significant

Table. 2. Level of Cholesterol, Urea and Creatinine in Serum of Different Experimental Groups of Rats

GROUPS	Total Cholesterol (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)
Group I (Control)	102.52 ±7.42	20.81 ± 2.52	0.98 ± 0.06
Group II (CCl_4 treated)	270.98 ± 7.34*	112.13 ± 6.42*	3.27 ± 0.73*
Group III (CCl_4 + 200mg/kg extract)	155.43 ± 4.02*	64.09 ± 1.18*	1.98 ± 0.92*
Group IV (CCl_4+400mg/kg extract)	122.60 ± 4.88*	26.62 ± 2.62*	1.00 ± 0.48*
Group V (CCl_4 + Silymarin)	110.67 ± 2.38^{ns}	22.02 ± 2.42^{ns}	0.96 ± 0.67^{ns}
CD ($p<0.05$)	8.29	7.29	1.05

Values are mean ± SD of six observations
Groups compared: Group II vs Group I; Group III vs Group II; Group IV vs Group II; Group V vs Group I
Significance: * - Significant at $p<0.05$; ns – Not significant

Table. 3. Levels of Antioxidant Enzymes and Thiobarbituric acid Reactive Substances in Different Experimental Groups of Rats

GROUPS	SOD	CAT	GPx	GSH	LPO
Group I (Control)	76.36 ± 4.62	30.02 ± 0.98	9.60 ± 0.91	1.03 ± 0.98	120.0 ± 3.82
Group II (CCl_4 treated)	18.43 ± 3.52*	7.45 ± 0.68*	3.78 ± 0.14*	3.01 ± 0.62*	256.03 ± 3.81*
Group III (CCl_4 + 200mg/kg extract)	42.02 ± 1.08*	18.02 ± 0.02*	5.98 ± 0.96*	2.98 ± 1.09*	98.03 ± 1.09*
Group IV (CCl_4 + 400mg/kg extract)	64.89 ±1.08*	26.98 ± 0.78*	8.38 ± 0.31*	1.98 ± 0.98*	108.60 ± 6.52*
Group V (CCl_4 + Silymarin)	72.98 ± 0.82^{ns}	29.98 ± 5.31^{ns}	9.02 ± 2.31^{ns}	1.18 ± 2.93^{ns}	116.13 ± 2.09^{ns}
CD ($p<0.05$)	5.68	3.46	1.98	2.05	5.63

Catalase (µm of H_2O_2 consumed/ min/ mg protein, SOD- units/ mg protein, GPx (µg of GSH utilized/min/mg protein at 37^0C, LPO (µm of MDA released/mg protein, min at 37^0C
Values are mean ± SD of six observations
Groups compared: Group II vs Group I; Group III vs Group II; Group IV vs Group II; Group V vs Group I
Significance: * - Significant at $p<0.05$; ns – Not significant

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