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ANTIOXIDANT ACTIVITY OF ETHANOLIC EXTRACT OF *CASSIA OCCIDENTALIS* AGAINST CARBON TETRACHLORIDE-INDUCED OXIDATIVE STRESS IN WISTAR RATS

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ABSTRACT

The efficacy of ethanolic extract from *Cassia occidentalis* against CCl_4 induced oxidative stress was tested using wistar albino rats. The antioxidant activity was assessed by monitoring the levels of lipid peroxides, antioxidant enzymes like glutathione peroxidase, glutathione reductase, glutathione-S-transferase, superoxide dismutase and catalase, and non-enzymic antioxidants like reduced glutathione, vitamin-C, vitamin-E, cereloplasmin and uric acid in the liver tissues. Administration of CCl_4 increased the level of lipid peroxides decreasing the activities of enzymic and non-enzymic antioxidants. Pre-treatment with ethanolic extract significantly prevented the alterations induced by CCl_4 and maintained a near normal antioxidant status. Decreased activities of enzymes in CCl_4 intoxicated rats and their reversal in the ethanolic extract treated rats shows the potency of ethanolic extract in combating CCl_4 induced oxidative stress.

Key words: Antioxidant enzymes, Carbon tetrachloride, *Cassia occidentalis*, Ethanolic extract, Lipid peroxidation.

INTRODUCTION

Hepatic injury caused by chemicals, drugs[1], and virus is a well-known toxicological problem[2]. One of the major causes of CCl_4 induced[3] hepatoprotective is lipid peroxidation by its free radical derivative $^{\bullet}CCl_3$. Thus, the free radical scavenging activity plays a crucial role in providing protection against such hepatic damage. Chemical antioxidant such as butylated hydroxyl anisole is found to be toxic[4], when given at higher doses. Therefore, there is a need for the identification of naturally occurring antioxidants[5], as they are non-toxic, and cheap with less side effects.

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Liver diseases[6] remain one of the serious health problem. In the absence of reliable liver protective drugs in allopathic medical practices[7], herbals play an important role in the management of various liver[8] disorders. In the present study, an effort has been made to evaluate the combined antioxidant[9] potential of ethanolic extract of *Cassia occidentalis* against CCl_4 induced oxidative stress. *Cassia occidentalis* is an important medicinal plant, which finds its use in Ayurveda and Unani system of medicine. Literature survey revealed that *Cassia occidentalis* possesses significant hepatoprotective activity

EXPERIMENTAL

Collection of plant material

The plant material used in this study is *Cassia occidentalis* seeds belonging to family Caesalpiniacea collected from Coastal Andhra Pradesh. The chosen plant parts were Sun dried for nine days and made into fine powder using Willey mill.

Preparation of plant extract

Cassia occidentalis plant material about (1Kg) were shade-dried and pulverized to a coarse powder. Equal quantities of the powder was passed through 40-mesh sieve and exhaustively extracted with 90% (v/v) ethanol in Soxhlet apparatus at 60^{o}C. The extract was evaporated under pressure till all the solvent has been removed and further removal of water was carried out by freeze drying to give an extract sample with the yield of 15.6% (w/w). The extract was stored in refrigerator for further use.

Animals

Wister albino rats weighing 200-250 g of either sex, were used and housed in polypropylene cages, maintained with temperature controlled in room at (22 ± 2^{o}C), fed with commercial rat feed and clean drinking water *ad libitum*. They were given a week's time to get acclimatized with the laboratory conditions.

Preliminary phytochemical screening

The crude powder and ethanolic extract of *Cassia occidentalis* was subjected to preliminary phytochemical screening to identify the presence of various phytoconstituents present in the extract and crude powder.

Acute toxicity study

Acute toxicity studies were conducted for *Cassia occidentalis* ethonolic extract in wistar albino mice by staircase method. First group served as normal control. Ethanolic

extract of *Cassia occidentalis* was administered orally to different groups at the dose level of 250, 500, 1000 and 2000 mg/(kg. p.o.) of body weight. All animals were observed for toxic symptoms and mortality for 72 hr. The LD_{50} of ethanolic extract of *Cassia occidenalis* was found to be 500 mg/kg b.w. One tenth of the LD_{50} doses were selected for the evaluation of antioxidant activity.

CCl_4 - induced oxidative stress

The rats were divided into five groups of (six per group). Group I served as normal control and received a single administration of 0.3 mL vehicle (2% v/v aqueous Tween -80, p.o.) on all 14 days; Group II served as CCl_4 control and received a single dose of CCl_4 (2 mL/kg, p.o.) in 0.3 mL of Tween- 80 for 7 days: Group III were pretreated with ethanolic extract of *Cassia occidentalis* (50 mg/kg, p.o.) in 0.3 mL of Tween-80 for 14 days and intoxicated with CCl_4 (2 mL/kg, p.o.) on days 7 to 14. Group IV animals received ethanolic extract of *Cassia occidentalis* (50 mg/kg, p.o.) in 0.3 mL of Tween-80 daily for 14 days. Group V animals were treated with the reference drug silymarin (50 mg/kg, p.o.) for 14 days administered with CCl_4 (2 mL/ kg, p.o.) on days 7 to 14.

After the experimental period, the animals were sacrificed after 12 hrs fasting under mild ether anaesthesia. Liver was excised from the animal, washed in ice-cold saline. A 10% liver homogenate was prepared in HCl buffer (0.1M pH 7.4). The homogenate was centrifuged and the supernatant was used for the assay of following enzymic and nonenzymic antioxidant parameters.

Assessment of antioxidant activity assessment of enzymic antioxidants

Glutathione reductase (GR) activity was expressed in terms of utilisation of GSSG/min/mg of protein in the liver homogenate. Total protein in the liver tissue homogenate was also estimated

Assessment of non-enzymic antioxidants

Ascorbic acid, tocopherol, cereloplasmin and uric acid were assayed by the reported methods. Liver glutathione was estimated by 5,5-dithiobis-2-nitrobenzoic acid (DTNB) according to the method.

Statistical analysis

The data were expressed as mean ± S.E.M. (n = 6). Statistical evaluation was carried out with ANOVA using SPSS software. The difference was considered significant $p < 0.01$.

RESULTS AND DISCUSSION

The ethanolic extract and crude powder of *Cassia occidentalis* revealed the presence of various phytoconstituents, like reducing and non-reducing sugars, polyphenolics (flavonoids and tannins), steroids, saponins and triterpenes, alkaloids, proteins and amino acids.

The levels of various enzymic and non-enzymic antioxidants in normal, CCl_4 controlled, ethanolic extract of *Cassia occidetnalis* treated groups are represented in Tables 1 and 2. The levels of lipid peroxides (LPO) were significantly increased ($p < 0.01$) in the liver tissues of CCl_4 treated Group II rats. Pretreatment with ethanolic extract of *Cassia occidentalis* significantly reversed the increased levels. Increased production of Reactive Oxygen Species (ROS) due to oxidative stress plays an important role in liver diseases. CCl_4 has been reported to induce lipid peroxidation and alter the antioxidant[10] defence system through formation of free radicals, which in turn causes damage and degeneration of hepatic tissues. The significant increased levels ($p < 0.01$) of LPO in Group II animals could be due to the damage caused by CCl_4.

SOD, CAT and GR activities were significantly decreased ($p < 0.01$) in CCl_4 treated rats compared to normal control Group I rats. But the oral administration of the ethanolic extract of *Cassia occidentalis* to such CCl_4 treated rats reversed the enhanced levels of LPO and increased the activities of SOD, CAT, GPX, GST and GR. This indicates that the antilipid peroxidative nature of the system against CCl_4 treatment is enhanced by ethanolic extract of *Cassia occidentalis*. Glutathione (GSH) constitutes the first line of defence against the free radical. Reduction in liver GSH and reduced activity of GPX and GST in CCl_4 treated rats indicates damage to the liver cells. But the reconstitution of the levels of GSH, GPX and GST activity in the rats treated with ethanolic extract of *Cassia occidentalis* proves the protective and antioxidant efficiency of the drug.

The decreased levels of vitamin C, vitamin E and cereloplasmin were observed in CCl_4 treated Group II rats. Group III animals showed near normal levels of vitamin C, vitamin E and cereloplasmin activities ($p < 0.01$), when compared to group II animals. Thus, the free radical scavenging property of ethanolic extract of *Cassia occidentalis* could have maintained the near normal levels of non-enzymic antioxidants in group III animals. The decreased levels of GSH in Group II animals may be due to the increased utilization or lower expression of GSSH. The unavailability of GSH reduces the activities of GR, GPX and GST. No significant change was observed in Group III animals, when compared with Group I normal animals.

Table 1: Levels of catalase (CAT), superoxide dismutase (SOD), gluthione-S-tranferase (GST), glutathione peroxidase (GPx), glutathione reductase (GR), and lipid peroxidase (LPO) activitiy of *Cassia occidentalis*

Parameter	Group-I	Group-II	Group-III	Group-IV	Group-V
CAT	66 ± 1.20	47.06 ± 1.87a*	56.13 ± 1.39b*	65.27 ± 1.25c^{NS}	68.39 ± 1.79b*
SOD	12.39 ± 0.28	9.31 ± 0.43 a**	11.47 ± 0.28b*	15.30 ± 0.45c^{NS}	13.47 ± 0.95b*
GST	0.36 ± 0.2	0.24 ± 0.01 a**	0.32 ± 0.01b*	0.40 ± 0.010c^{NS}	0.35 ± 0.19b**
GPX	12.60 ± 0.60	9.06 ± 0.29 a**	12.53 ± 0.43b**	14.09 ± 0.66c^{NS}	12.45 ± 0.40 b**
GR	0.63 ± 0.02	0.12 ± 0.25 a**	0.48 ± 0.10b**	0.54 ± 0.02c^{NS}	0.46 ± 0.88 b**
LPO	140.42 ± 1.16	169.15 ± 1.72 α**	151.50 ± 1.38 β*	139.59 ± 1.02χ^{NS}	149.36 ± 1.22 β*

Values are mean ± SEM of 6 animals each in a group; Statistical significant test for comparison was done by ANOVA, followed by Dunnet's `t' test (n = 6); Comparison between: a–Group I and Group II, b–Group II; vs Group III and Group V; c–Group I and Group IV, *$p < 0.05$, **$p < 0.01$, NS–Not significant; GP_x–n moles of GSH oxidised/min/mg protein; GST – n moles of CDNB conjugate formed/min/mg protein; GR– n moles of GSSG utilized/min/mg protein; SOD-units/mg protein; 1 unit of enzyme activity is the amount of enzyme required to inhibit 50% of epinephrine auto-oxidation, CAT–n moles of H_2O_2 utilized/min/mg protein; LPO-nano moles of MDA/hr/100 mg tissue weight

Preventive action of liver damage by CCl_4 has been widely used as an indicator of liver protective activity of drugs in general It has been established that CCl_4 is accumulated in hepatic parenchyma cells and metabolically activated[6] by cytochrome P_{450}-dependent monooxygenases to form a trichloromethyl radical $^{\bullet}(CCl_3)$. The $^{\bullet}CCl_3$ radical alkylates cellular proteins and other macromolecules with a simultaneous attack on polyunsaturated fatty acids, in the presence of oxygen[11], to produce lipid peroxides, leading to liver damage. Thus, antioxidant or free radical generation inhibition is important in protection against CCl_4-induced liver[12] lesions. Free radicals oxidative stress has been implicated in disorders, resulting usually from deficient natural antioxidant defences. Potential antioxidant therapy should therefore include either natural free radical scavenging antioxidant enzymes or agent

capable of augmenting the activity of this enzymes, which include SOD, CAT and GPX . The end of lipid peroxidants are known to induce cellular damage and have been shown to be responsible for oxidative free radicals induced human disease. The enzyme superoxide dismutase and glutathione constitute the first line of defence against free radical induced damage and a restoration of the superoxide dismutase activity and glutathione level by the ethanolic extract of *Cassia occidentalis* may account for its protective effects. Increase in CAT and GPX activities are essential, if a beneficial effect from increase in SOD activity is to be expected.

Table 2: Levels of ascorbic acid, tochopherol, cereloplasmin, uric acid and glutathione for *Cassia occidentalis*

Parameters	Group-I	Group-II	Group-III	Group-IV	Group-V
Ascorbic acid	3.45 ± 0.29	1.00 ± 0.086a**	1.83 ± 0.17b**	2.72 ± 0.06c^{NS}	1.94 ± 0.16b**
Tochopherol	5.44 ± 0.15	3.42 ± 0.12 a**	4.95 ± 0.20b**	5.40 ± 0.15c^{NS}	4.40 ± 0.11b**
Cereloplasmin	1.67 ± 0.15	0.73 ± 0.09 a**	1.18 ± 0.11b*	1.25 ± 0.06c^{NS}	0.97 ± 0.11b*
Uric acid	3.55 ± 0.15	1.79 ± 0.12 a**	2.39 ± 0.17b*	3.58 ± 0.12c^{NS}	2.69 ± 0.14b**
Glutathione	8.15 ± 0.175	6.50 ± 0.065a*	7.86 ± 0.061b*	8.58 ± 0.30c^{NS}	7.99 ± 0.054b*

Values are Mean ± SEM of 6 animals each in a group; Statistical significant test for comparison was done by ANOVA, followed by Dunnet's `t' test (n = 6); Comparison between: a–Group I and Group II; b–Group II vs. Group III and Group V; c–Group I and Group IV; *$p < 0.05$, * *$p < 0.01$, NS–Not Significant; *Ascorbic acid-expressed as mg/dl; Tochopherol-expressed as mg/dl ; Uric acid-expressed as mg% ; Cereloplasmin-expressed as mg/dl; GSH-micromole GSH/100 mg liver tissue

Since the critical antioxidants superoxide dismutase and glutathione, which are the first line of defence, offer protection against free radicals and thus, maintain low levels of lipid peroxides. Decreased activities of GSH, GR, CAT, SOD, GPX and non-enzymic antioxidants like vitamin E, vitamin C, cereloplasmin and uric acid levels in CCl_4 treated group II rats may increase their susceptibility[13] to oxidative injury. However, over

expression of the antioxidant molecules[14] with ethanlolic extract of *Cassia occidentalis* is indicative of their ability to reactive hepatocellular antioxidant defence in the liver.

Many plant products are known to exert antioxidative effect by quenching various free radicals and singlet form of molecular oxygen[15]. The ethanlolic extract of *Cassia occidentalis* activated antioxidant[16] enzyme that catalyse the reaction of oxidants in diseased liver (CCl_4 treated). The maintenance of GSH levels depends on the activities of various enzymes GR and GST. Since GR effects reduction of GSH[17], the level of this enzyme[18] is also important in detoxification of peroxides.

The present study clearly indicates that the antioxidant effect by ethanolic extract of *Cassia occidentalis* against CCl_4 induced liver damage[19] is possibly due to their ability to activate antioxidant enzymes[20] that catalyse the reaction of oxidants[21] and free radical scavenging activities.

The decreased levels of GSH in Group II animals[22] may be due to the increased utilization or lower expression of GSSH[23]. The unavailability of GSH[24] reduces[25] the activities of GR, GPX andGST. SOD[26], CAT[27] and GR[28] activities were significantly decreased ($p < 0.01$) in CCl_4 treated[29] rats compared to normal control Group1 rats. Glutathione (GSH) constitutes the first line of defence against the free radical.

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