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In-vitro and *in-vivo* antioxidant activity of *cordia subcordata* whole plant

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A B S T R A C T

Phytochemical is non nutritive chemical constituent of plants which occur naturally in it termed as phytochemical, or the chemical which is derived from plants are called phytochemical. In Indian system of medicine the whole plant of *cordia subcordata Lam*, belonging to family Boragenacaeae used for the treatment of various diseases. The objective of the present study was to investigate the antioxidant activity of whole plant of *Cordia subcordata*. The CCl_4 induced erythrocyte damage in rates models were used to study the in vivo anti oxident activity and H_2O_2 free radical scavenging activity and DPPH free radical scavenging activity model was used to study *in-vitro* antioxidant activity of methanol extract of whole plant. Wister strain albino rats with 200mg/kg dose were used for all studies. Ascorbic acid was used as the standard drug.

Keywords: anti oxident, ethanol, erythrocyte damage, free radical scavenging activity, DPPH.

A R T I C L E I N F O

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1. Introduction

Antioxidants are radical scavengers, which are protecting the human body against free radicals. Free radicals may cause pathological conditions like anemia, asthma, inflammation, neurodegeneration, Parkinson's disease,

dementia, ageing, mongolism, etc. Oxidation reactions can produce free radicals, which starts chain reactions that damage cells. Plants and animals maintain complex systems of multiple types of antioxidants such as, Vitamin C, E, and glutathione as well as enzymes catalase, superoxide dismutase. Low levels or inhibition of antioxidants cause oxidative stress and may damage or kill cells. Oxidative stress might be an important role in human diseases. The use of antioxidants can treat the stroke, dementia and neurodiseases. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants terminate these chain reactions being oxidized themselves. As a result, antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols. Plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases. Low levels of antioxidants, or inhibition of the antioxidant enzymes, cause oxidative stress and may damage or kill cells.

2. Materials and Methods

***In-vitro* antioxidant activity:**

DPPH Radical scavenging test:

The free radical scavenging activity of the methanol extracts of *Cordia subcordata.L*. (MEOC) was determined by using 2, 2 Diphenyl-1-picryl hydrazyl radical (DPPH) using UV-Spectrometry at 517nm. The DPPH solution was prepared in 95% methanol. The MECS was mixed with 95% methanol to prepare the stock solution (10mg/100ml or 100µg/ml). From the stock solution 2ml, 4ml, 6ml, 8ml and 10ml of this solution were taken in five test tubes and by serial dilution with same solvent were made the final volume of each test tube up to 10ml whose concentration was then 20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml and 100µg/ml respectively. Freshly prepared DPPH solution (0.004% w/v) was added in each of these test tubes. Containing MECS (20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml and 100µg/ml) and after 10 min, the *absorbance* was taken at 517nm, using a spectrophotometer (SHIMADZU UV-1700, UV-visible spectrophotometer). Ascorbic acid was used as a reference standard. It is dissolved in distilled water to make stock solution with the same concentration of MECS control sample was prepared without extract and reference ascorbic acid. 95% methanol was used as blank % scavenging of the DPPH free radical was measured using following equation.

% DPPH radical-scavenging=

$$\frac{(Absorbance\ of\ control - Absorbance\ of\ test\ sample)}{(Absorbance\ of\ control)} \times 100$$

H_2O_2 Radical Scavenging Activity

The ability of extracts to scavenge H_2O_2 was determined using H_2O_2-phosphate buffer 15. A solution of H_2O_2 will be prepared in phosphate buffer (pH 7.4). H_2O_2 concentration will be determined spectrophotometrically measuring absorption with extinction coefficient for H_2O_2 of 81 M^{-1} cm^{-1}. Extracts (10-320µg/ml) in distilled water will be added to a H_2O_2 solution (0.6 ml, 40 mM). Absorbance of H_2O_2 at 230 nm will be determined 10min later against a blank solution containing the phosphate buffer without H_2O_2. The % of H_2O_2 scavenging of both the extracts and standard compounds will be calculated.

***In-vivo* antioxidant activity:**

Whole plant of *Cordia subcordata Lam* extract will be demonstrate the antioxidant activity against CCl_4 induced erythrocyte damage in rats. The following anti-oxidant enzymes will be evaluated by standard procedure.

a. Lipid peroxidation
b. Superoxide dismutase
c. Catalase
d. Cholesterol and Phospholipids
e. Cholesterol/Phospholipid ratio

Determination of the Lipid Peroxidation (LPO) in Serum:

The level of thiobarbituric acid reactive substance (TBARS) and malondialdehyde (MDA) production was measured in serum by the modified method as described by Draper and Hadley [18]. The serum (50 µL) was deproteinized by adding 1 mL of 14% trichloroacetic acid and 1 mL of 0.6% thiobarbituric acid. The mixture was heated in a water bath for 30 min to complete the reaction and then cooled on ice for 5 min. After centrifugation at 2000 g for 10 min, the absorbance of the colour product (TBARS) was measured at 535 nm with a UV spectrophotometer. The concentration of TBARS was calculated using the molar extinction coefficient of malondialdehyde (1.56 × 105 mol/L/cm) using the formula,

$A = \Sigma CL$

Where,

A = absorbance,

Σ = molar coefficient,

C = concentration, and L = path length.

The results were expressed in nmol/mg of protein.

Estimation of Superoxide Dismutase (SOD):

Superoxide dismutase activity was assayed according to the method of Sun et al. In this method, xanthine-xanthine oxidase system was used to generate a superoxide flux, and nitro blue tetrazolium (NBT) was used as an indicator of superoxide production. SOD activity was then measured by the degree of inhibition of the reaction unit of enzyme providing 50% inhibition of NBT reduction. Results are expressed as U/mL.

3. Results and Discussion

Estimation of Catalase Activity:

The catalase activity in serum was determined using the modified method as described by the method is as follows: serum (10 µL) was added to test tube containing 2.80 mL of 50 mM potassium phosphate buffer (pH 7.0). The reaction was initiated by adding 0.1 mL of fresh 30 mM hydrogen peroxide and the decomposition rate of hydrogen peroxide was measured at 240 nm for 5 min on a spectrophotometer. A molar extinction coefficient of 0.041 mM−1 cm−1 was used to calculate catalase activity.

***In-vivo* anti oxidant activity:**

The results of preliminary phytochemical screening of the methanolic extract of cardiasubcordata. Revealed that presence of alkaloids, flavonoids, glycosides, steroid, tannins, saponins, terpeniods and absence of steroids.

Table:22 shows the effect of MECS on carbon tetrachloride induced oxidative stress. Treatments with the extracts significantly (P <0.01) prevented the accumulation of lipid peroxidation products in the plasma. Intoxication of the rats with carbon tetrachloride also led to significant increases in superoxide dismutase and catalase activities, while simultaneous administration of carbon tetrachloride with the MECS significantly (P <0.01) decreased these activities. Intoxication with carbon tetrachloride causes an increase in membrane cholesterol, a decrease in membrane phospholipid and a subsequent increase in the cholesterol to phospholipids ratio. At the doses of ethanolic extract 200 & 400 significantly (P<0.01) decreases the cholesterol, phospholipids and cholesterol/phospholipids ratio also (Table:2)

***In-vitro* antioxidant activity:**

DPPH radical scavenging test:

The DPPH radical scavenging activity of MECS root was evaluated and compared with Ascorbic acid and the results are given in Table 3. The % inhibition at various concentration (200-1200 µg/ml) of EEAL root as well as standard Ascorbic acid (12.5 -100 µg/ml) were calculated and plotted in Figure 2 using Microsoft Office Excel 2007. The IC50 values are calculated from graph and were found to be 38.36 µg/ml (Ascorbic acid) and 945.76 (MECS).

The hydrogen peroxide scavenging activity of MECS whole plant was evaluated and compared with Ascorbic acid and the results are given in Table 5. The percentage inhibition (% inhibition) at various concentration (200-500 µg/ml) of MECS as well as standard Ascorbic acid (12.5-100 µg/ml) were calculated and plotted in Figure 4 using Microsoft Office Excel 2007. The IC50 values are calculated from graph and were found to be 42.92 µg/ml (Ascorbic acid) and 56.49(plant extract).

The hydrogen peroxide scavenging activity of MECS whole plant was evaluated and compared with Ascorbic acid and the results are given in Table 5. The percentage inhibition (% inhibition) at various concentration (200-500 µg/ml) of MECS as well as standard Ascorbic acid (12.5-100 µg/ml) were calculated and plotted in Figure 4 using Microsoft Office Excel 2007. The IC50 values are calculated from graph and were found to be 42.92 µg/ml (Ascorbic acid) and 56.49(plant extract).

Table 1: The effects of MECS on lipidperoxidation products and antioxidant enzymes of the primary erythrocytes of carbon tetrachloride–intoxicated rats.

Group	Design of treatment	Lipid peroxidation x 10 -6 (unit	Enzyme activities (Units/mg protein)	
			Superoxide dismutase	Catalase
I	Control, PG	0.25± 0.01	196.74 ± 4.7	1.8 ± 0.2
II	MECS200mg/kg+PG	0.24 ±0.07	194.7±4 4.7	1.5± 0.2
III	MECS400mg/kg+PG	0.22 ±0.02	188.4±3 3.3	1.5 ±0.4
IV	CCl4+PG	0.45 ±0.04	258.4±7 2.4	4.5± 0.3
V	CCl4+MECS200mg/kg	0.33 ±0.07	230.3±3 2.4*	3.20 ±0.4*
VI		0.31 ±0.06**	208.2±5 1.2**	2.11± 0.2**

PG=Propylene glycol; Carbon tetrachloride= CCl4, MECS=Ethanolic extract of C.subcordata. Values are expressed as mean ± SEM of six observations. Statistical significant test for comparison was done by ANOVA, followed by Dun net's 't' test. a means Comparison between Group IV Vs Group I, II & III. Means Comparison between Group V and VI, Vs Group I, II, III & IV. *p<0.05;** p<0.01; ns-non significant

Table 2: Effect of MECS on erythrocyte membrane lipids and cholesterol/phospholipids ratio of Carbon tetrachloride -intoxicated rats.

Group	Design of treatment	Cholesterol (mg/100µl)	Phospholipid (mg/100µl)	Cholesterol /Phospholipid
I	Control, PG	0.70± 0.02	1.06 ±0.04	0.72 ±0.04
II	MECS200mg/kg+PG	0.69± 0.04	1.13± 0.02	0.74 ±0.02
III	MECS400mg/kg+PG	0.70 ±0.02	1.02± 0.02	0.62± 0.01
IV	CCl4+PG	0.80± 0.04	0.84 ±0.04	0.91 ±0.04
V	CCl4+MECS200mg/kg	0.75± 0.01*	0.92 ±0.05*	0.70 ±0.04*
VI		0.65 ±0.02**	0.93 ±0.01**	0.78 ±0.02**

PG=Propylene glycol; Carbon tetrachloride= CCl4; MECS=Ethanolic extract of C.subcordata. Values are expressed as mean ± SEM of six observations. Statistical significant test for comparison was done by ANOVA, followed by Dunnet's test. a means Comparison between Group IV Vs Group I, II & III. **b**means Comparison between Group V and VI Vs Group I, II, III& IV. ; *p<0.05;** p<0.01; ns-non significant

Table 3: Results of DPPH scavenging activity.

Concentration (µg/ml)		% Inhibition (mean ±SD)		IC50 (µg/ml)	
Std (Ascorbic Acid)	**Plant extract (c.subcordata)**	**Std (Ascorbic Acid)**	**Plant extract (c.subcordata)**	**Std (Ascorbic Acid)**	**Plant extract (c.subcordata)**
12.5	200	27.95±2.46	16.66 ±1.09		
25	500	45.16±1.61	25.80 ±3.22		
50	800	62.36±1.93	48.92± 2.46	38.36	945.76
100	1200	81.72±2.46	60.75±2.46		

Table 4: Results of Hydrogen Peroxide scavenging activity

Concentration (µg/ml)		% Inhibition (mean ±SD)		IC50 (µg/ml)	
Std (Ascorbic Acid)	**Plant extract (c.subcordata) *C.subcordata***	**Std (Ascorbic Acid)**	**Plant extract (c.subcordata) *C.subcordata***	**Std (Ascorbic Acid)**	**Plant extract (c.subcordata) *C.subcordata***
12.5	200	25.51±0.28	45.90±2.27		
25	300	42.90±1.21	58.59±1.15		
50	400	60.39±1.23	73.49±2.21	42.92	56.49
100	500	75.51±2.08	84.06±2.27		

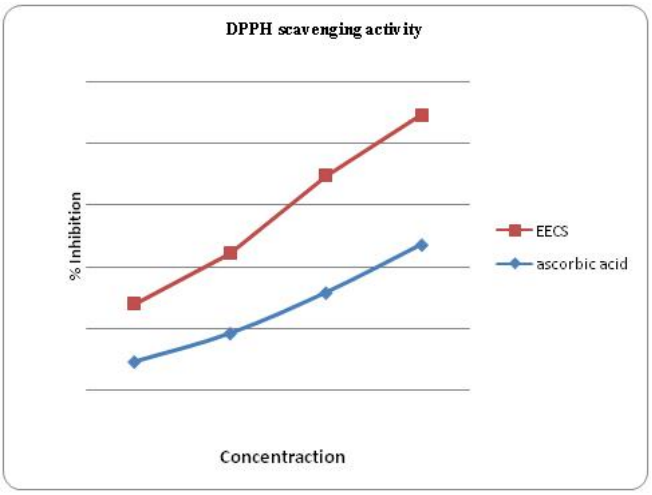

Fig 1: DPPH scavenging activity of ascorbic acid of c.s

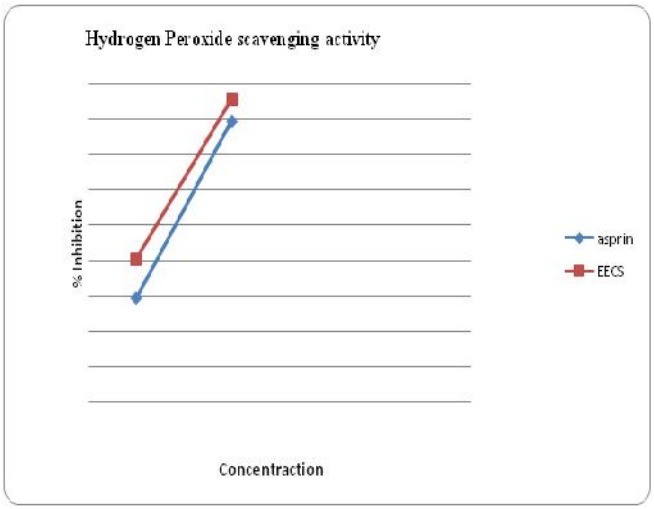

Figure 2: **Hydrogen Peroxide scavenging activity of** Ascorbic acid and C

4. Conclusion

The drug also demonstrated for antioxidant properties in *in-vitro* and *in-vivo* antioxidant models. MECS also showed invivo antioxidant properties by facilitating the increased formation of free radical scavenging enzymes like SOD and catalase and reduced the extant of lipid peroxidation .it can be concluded that all the effects contributes for reducing the free radicals.

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