Evaluation of *In-Vivo* Antioxidant Potential of *Ecbolium viride* (Forssk.) Alston Roots on Carbon Tetra Chloride Induced Oxidative Stress in Wister Rats

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Abstract
Aim: To evaluate the antioxidant activity of the methanolic extract of *Ecbolium viride (Forssk.) Alston* roots *in vivo*. **Methods:** The levels of catalase, superoxide dismutase, thiobarbituric acid reactive substance and glutathione were estimated in Carbon tetra chloride treated rats. **Results:** The extract at 400 mg/kg significantly increased the levels of catalase, superoxide dismutase and glutathione and significantly decreased the thiobarbituric acid reactive substance level when compared with the toxicant group. **Conclusion:** These findings propose that the methanolic extract of *Ecbolium viride* (Forssk). Alston has persuasive antioxidant activity and can be used as antioxidant supplement which would be beneficial in the treatment of various diseases.

Key words: *Ecbolium Viride (Forssk.) Alston, Catalase, Superoxide Dismutase, Glutathione*

1. INTRODUCTION

Free radicals are known to cause various deleterious effects in a biological system and antioxidants protect these effects through various defence mechanisms.[1] In the cells various factors like stress, radiation, smoking, alcohol, toxins and psychological stress produce Reactive oxygen species (ROS) and overproduction of this leads to complications of some disease conditions like, diabetes, cardiac related diseases, cancer, arthritis, neurodegenerative disease and aging process.[2,3] Antioxidants act as oxygen scavengers thereby prevent oxidative damage caused by ROS [4, 5] and also play a major role in the prevention of various intimidating diseases[6]. Plants with affluent phenolic compounds like tannins, flavonoids etc have been known to posses, anti-inflammatory, anti diabetic, anti aging and anti carcinogenic activities can be accredited to their free radical scavenging potential. [6, 7]

Ecbolium viride (Forssk).Alston is a low shrub with erect branches and large leaves mainly found in Western Ghats, Chittoor district and North East India. It is among one of the plant belonging to Acanthaceae family. Different parts of *Ecbolium viride* (Forssk). Alston are reported to possess various pharmacological activities and importantly roots of the plant used in the treatment of jaundice and other liver related diseases.[8,9]The occurrence of glycoflavones such as orientin, vitexin, isoorienten, and isovitexin and lignan ecbolin.[10] A prompt us to take up this study to prove its antioxidant potential.

2. MATERIALS AND METHODS

Collection of Plant Material

The plant material was collected from Tirumala hills of Chittoor district, AP which was identified and authenticated by Dr. Madhavachetty (Voucher specimen No: EV-1768).

Preparation of Plant Extracts

The roots of the plant were thoroughly washed with water and dried in oven at low temperature. It was then powdered in a multi mill grinder and sieved to obtain coarse powder. Different extracts were prepared by successive solvent extraction

using solvents n-hexane, chloroform, ethyl acetate and methanol in the increasing order of polarity and aqueous extract was prepared with chloroform water.

Phytochemical Screening

Phytochemical screening was performed on different extracts for the detection of various phytoconstituents.[11, 12]

Animal Studies

Experimental Animals

Albino Wister rats of either sex weighing 150-225 g were housed in the animal house of MSRCP and maintained under controlled temperature (27±2 ^{0}C) and light. Animals were fed with rat feed and water ad *libitum*. The approval was obtained from the IAEC of MSRCP (MSRCP/P-11 2010, dated 3/12/2010).

Acute Toxicity Studies

Acute toxicity study was performed following OECD-423 guidelines [13]. After fasting overnight, rats were administered orally with a single dose of 300 mg and 2000 mg extract separately. The animals were observed continuously for 2 hours for behaviour and neurological profile. The animals were further observed for a period of 24 h and till end of the study to observe the toxic symptoms or lethality.

Experimental Design

Animals were divided into 5 groups containing six animals each.

Group I was served as normal control and received acacia solution (2% w/v) orally in distilled water. Group II was maintained as positive control. Group II animals were administered with CCl_4 1 ml/ kg, p.o. once on 7th day. Group III was orally administered with 25 mg/kg b.w silymarin once daily for 7 days. Group IV and V were orally administered with MEEV at the dose of 200 & 400 mg/kg body weight respectively once daily for 7 days. All the groups were administered with CCl_4 1 ml/ kg, p.o. once on 7th day except the normal control group.[14]

Preparation of Liver Homogenate

Liver was isolated on 8th day under excess of anesthetic agent, it was then dissected and washed thoroughly with ice cold saline solution. The weight was taken after pressing between filter paper pads. The isolated liver was divided in to 2 parts for the preparation of liver homogenates.

Part A: 0.15 M potassium chloride buffer was used to prepare liver homogenate and centrifuged at 8000 rpm for 10 min at 4 ^{o}C. Catalase and lipid peroxidation were estimated by using the supernatant.

Part B: 0.25 % sucrose in 5M phosphate buffer was used to prepare liver homogenate and centrifugation was carried out at 10,000 rpm for 10 min. Super oxide dismutase and glutathione were estimated using the supernatant.

Evaluation Studies

Lipid Peroxidation (Malondialdehyde)

Liver homogenate (500 µl) was mixed with 1 mL of 14% trichloroacetic acid and 1 mL of 0.6% thiobarbituric acid. The mixture was heated on 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 colored product (TBARS) was measured at 535 nm with a UVspectrophotometer[15]. The concentration of TBARS was calculated using the molar extinction coefficient of malondialdehyde (1.56×10^5 mol/L/cm) using the formula, $C = A / \in t$, where A = absorbance, Σ = molar coefficient, C = concentration, and L = path length. The results were expressed in nmol/mg of protein.

Catalase

100 µl of 10% liver homogenate was mixed with 1.9 ml of phosphate buffer (pH 7) and the absorbance was measured at 240 nm. Reading was again taken 1 min after adding 1 ml of 10 mM hydrogen peroxide solution [16]. Catalase activity was calculated by using

the formula. Catalase unit per ml of sample = $(\Delta A_s - \Delta A_o)\times 3\times DF/\in\times 2$, where ΔA_s = Absorbance difference of sample, ΔA_o= Absorbance of Control, DF = Dilution factor, $\in$ = Molar coefficient of H_2O_2

Superoxide Dismutase

To 100 µl of 10 % w/v liver homogenate, 1 ml of sodium carbonate, 400 µl nitro blue tetrazolium and 200 µl EDTA were added. The absorbance was measured at 560 nm. 400µlof hydroxylamine hydrochloride was added to initiate the reaction and incubated for 5 min at 25 ^{o} C. The reduction of NBT was measured after 5 min at 560 nm [17]. Percentage inhibition was calculated using the formula ΔA_C/min - ΔA_T/min× 100 /ΔA_C/min and SOD activity was calculated using the formula % inhibition × 1/50× DF × 1/0.1× 1/ 0.1 mg of tissue /ml, where ΔA_C = Absorbance difference of control, ΔA_T= Absorbance difference of sample, DF = Dilution factor

Glutathione

To 1 ml of 10 % w/v liver homogenate, 1.8 ml of distilled water and 2 ml of phosphate buffer (pH 7) were added and the absorbance was read at 412 nm. Five min after adding 0.2 ml of DTNB reagent, the intensity of yellow colour was measured at 412 nm [18]. The amount of glutathione was determined using its molar extinction coefficient of 13,060 /m/cm and expressed in terms of µ mol/ mg of protein. It can be estimated by using the following formulaC = A / $\in$t, where, A = Absorbance of sample, t = Path length, C = Concentration of sample, $\in$ = Molar coefficient of GSH (13.6×10^3 /Moles/cm)

Statistical Analysis: The data were expressed as Mean values ± S.E.M and were tested with one way ANOVA followed by Tukey-Kramer multiple comparison test.

3. RESULTS

Among different extracts, methanolic extract was found to be rich in flavonoids, tannins, alkaloids and other phenolic compounds. Phenolic compounds, flavonoids and saponins are reported for their potent antioxidant activity [19] and hence, the methanolic extract was selected for further studies. Acute toxicity studies of MEEV at the dose of 300mg/kg and 2000mg/kg showed no toxic symptoms or death in any of the animals, hence 1/10 and 1/5th of highest dose were selected for the studies. The results of the *in vivo* antioxidant effect of MEEV on rats are presented in Table 1 and Fig 1.Carbon tetra chloride significantly increased the level of lipid peroxidation and reduced catalase, SOD and glutathione levels in toxicant animals by 51.22%, 43.15% and 46.21% respectively. Lipid peroxidation was significantly decreased ($p<0.001$) with MEEV at 200 mg and 400 mg/kg. Catalase levels were significantly increased by MEEV 400 mg/kg and the results were not significant at 200 mg/kg. Levels of SOD and glutathione were increased significantly with MEEV ($p<0.01$ for 200 mg/kg and $p<0.001$ for 400 mg/kg) and silymarin showed significant effect on all the parameters ($p<0.001$).

4. DISCUSSION

The in vivo antioxidant assay showed that the extract increased the activity of superoxide dismutase and catalase and decreased the level of TBARS. Catalase is a ubiquitous enzyme that catalyses the decomposition of hydrogen peroxide, a reactive oxygen species, which is a toxic product of both normal aerobic metabolism and pathogenic ROS production[20, 21]. SOD prevents the formation peroxynitrite by catalyzing the dismutation of superoxide to hydrogen peroxide and oxygen, thereby reducing the possibility of superoxide anion reacting with nitric oxide [21]. The enhanced levels of catalase and SOD as observed in this study suggest that the methanolic extract has potent antioxidant activity and

capable of reducing the effect of ROS in cells. [22]

Lipid peroxidation was induced by ROS by reacting with polyunsaturated fatty acids (PUFA) [23] . An enhanced level of lipid peroxidation decrease membrane fluidity and changing the activity of membrane-bound enzymes and receptor.[24] Thiobarbituric acid reactive substance (TBARS) levels were measured as a marker of LPO and malondialdehyde (MDA) production. In our study, the level of TBARS in the extract treated groups decreased significantly when compared to positive control. This decrease in the

Table 1. *In vivo* antioxidant activity of methanolic extract of *Ecbolium viride* (Forssk.) Alston in CCl_4 treated rats

Groups	Lipid Peroxidation (µ mol MDA/mg of protein)	Catalase (unit/mg of tissue)	SOD (Units/mg of protein)	Glutathione (µ mol/mg of protein)
Normal Control	0.3018±0.0103	9.949±0.4064	2.911±0.077	20.19±0.3645
Positive Control (CCl_4)	1.379±0.1072	4.713±0.2153	1.614±0.1549	11.06±0.4054
Standard (Silymarin)	0.4403±0.0290***	7.415 ±0.2859***	2.705±0.1405***	16.98±0.2747***
MEEV (200mg)	1.085±0.0425***	4.920 ±0.1588 ns	2.276±0.0837**	12.96±0.3076**
MEEV (400mg)	0.6808±0.0517***	6.348 ±0.1321***	2.553±0.047***	14.52±0.3095***

Values are expressed as Mean ± SEM. Data compared against positive control group. One way analysis of variance (ANOVA). * p< 0.05,** p< 0.01, *** p< 0.001 Tukey-Kramer multiple comparison test

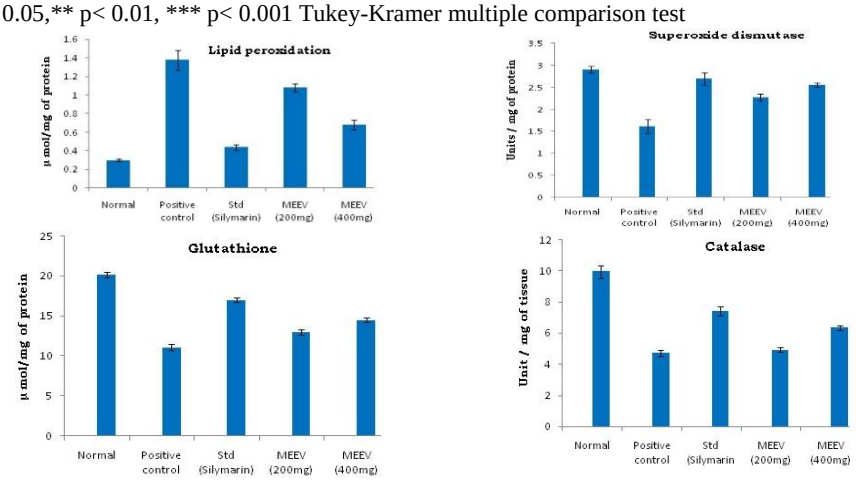

Fig. 1 Effect of methanolic extract of *Ecbolium viride*(Forssk.) Alston on Lipid peroxidation, Catalase, SOD and Glutathione in CCl_4 treated rats

TBARS levels may indicate increase in the activities of glutathione peroxidase and hence inactivation of LPO reactions [25].

5. CONCLUSION

A significant antioxidant activity exhibited by methanolic extract of *Ecboliumviride* (Forssk.) Alston roots may be due to the presence of flavonoids, tannins, alkaloids and other phenolic compounds

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