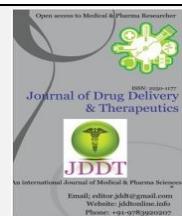


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Research Article

In vivo antioxidant activity of Ethanolic extract from root of *Smilax zeylanica* on Aluminium Chloride Induced oxidative stress in Wistar rats

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ABSTRACT

Objective: The objective of the present study was to investigate the Evaluation of In vivo antioxidant activity of Ethanolic extract of root of *Smilax zeylanica* (EESZ) on Aluminium Chloride Induced apoptosis suppressing oxidative stress in Wistar rats. **Materials and Methods:** The ethanolic extract from the roots of *S. china* by hot continuous percolation method. The rats were divided into 5 groups and each group consists of 6 animals. Rats were treated with EESC for 150 and 300 mg/ kg of body weight and piracetam, 0.5 mg/ kg of body weight for 14 successive days after inducing oxidative stress with aluminium chloride (100 mg/ kg of body weight) for 60 days. The lipid peroxidation level (TBARS) and antioxidant activities like Superoxide dismutase (SOD), Catalase (CAT) and reduced Glutathione (GSH) were estimated in rats. **Results:** AlCl₃ induced rats showed increased the TBARS and decreased the antioxidant enzymes like Superoxide dismutase (SOD), Catalase (CAT) and reduced Glutathione (GSH) when compared with the control group. The EESZ at higher dose 300 mg/ kg of body weight animals were significantly (P < 0.001) reduced the TBARS and increased the anti oxidant enzymes Superoxide dismutase (SOD), Catalase (CAT) and reduced Glutathione (GSH) when compared with the AlCl₃ treated group **Conclusion:** Findings of the present study revealed that Ethanolic extract from roots of *Smilax zeylanica* may be used as a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses.

Keywords: *S. zeylanica*, antioxidant, ethanolic extract, TBARS, rats.

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INTRODUCTION

Reactive oxygen and nitrogen species play key roles in normal physiological process, including cellular life/death process, protection from pathogens, various cellular signaling pathways, and regulation of vascular tone¹. Oxidative stress is caused by an insufficient capacity of biological systems to neutralize excessive free radical production, which can contribute to cardiovascular disease², neurodegenerative disease and age-related cognitive decline³, as well as immune system dysfunction⁴. Considerable evidence have accumulated to implicate cellular damage arising from reactive oxygen species (ROS), at least in part, in the etiology and pathophysiology of human diseases such as neurodegenerative disorders (e.g. Alzheimer disease, Parkinson disease, multiple sclerosis, Down's syndrome), inflammation, viral infections, autoimmune pathologies, and digestive system disorders such as gastrointestinal inflammation and ulcer⁵.

The roots of *Smilax zeylanica* is belong to the Smilacaceae family. It is commonly distributed in the forest and hills of south India and found in tropical and subtropical hills from

Himalayan region in the north to Kerala in south. It is widely in hilly region of Karnataka, Kerala and Tamil Nadu between altitudes of 500-1800 meter. *S. zeylanica* is used ethnomedicinally for the treatment of different conditions such as abscesses, boils, dysentery, psoriasis, rheumatism, skin diseases, swellings, toothache and venereal diseases⁶⁻¹⁰. This plant is shown to exhibit several bioactivities such as antimicrobial and analgesic^{11,12}, antioxidant^{12,13}, cytotoxic¹³, immunomodulatory and antiarthritic¹⁴, anthelmintic¹⁵, antipyretic¹⁶, anticonvulsant¹⁶, antidiabetic¹⁷, cytoprotective¹⁸, hepatoprotective¹⁹, anti-inflammatory²⁰, antiepileptic²¹, pesticidal²², thrombolytic²³, and antidepressant²⁴ activities. Therefore, the present investigation focused to evaluate the invivo antioxidant potential of ethanolic extract of roots of *Smilax zeylanica* in different screening methods.

MATERIALS AND METHODS

1. Collection and Identification of Plant materials

The roots of *Smilax zeylanica* were collected form Kulithalai, Kanyakumari District, Tamil Nadu, India.

Taxonomic identification was made from Botanical Survey of Medical Plants Unit Siddha, Government of India. Palayamkottai. The roots of *Smilax zeylanica* were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve.

2. Preparation of Extracts

The above powdered plant materials were consecutively extracted with pet. ether (40-60°C) by hot continuous percolation method in Soxhlet apparatus²⁵ for one day. The marc was dried out and extracted with chloroform and then marc was extracted with ethyl acetate (76-78°C) for one day, then this marc was dried out after that it was extracted with ethanol for one day and then marc was extracted with water. All the three extracts were concentrated by utilizing a rotary evaporator and undergone to freeze drying using a lyophilizer until dry powder was acquired. The ethanolic extract gave more yield and more phytoconstituents were present. So the ethanolic extract of *Smilax zeylanica* was selected for the further investigation.

Evaluation of *In-vivo* antioxidant activity:

Experimental

Both the genders of Wister rats with 8 weeks weighed between 150 and 200 g of body weight was used for the present study. Rats were acclimatized for experimental conditions for about two weeks. The rats housed in plastic cages at 25°C with relative humidity of 70% under 12/12 hours day/ night cycle. Rats were fed with food and water *ad libitum*. The experiments were approved as per the strategy of CPCSEA, New Delhi, India and approved by the Annamalai University IAEC (Approved number: AU/IAEC/1199/1/18). Animals were grouped randomly into five different groups with six rats in each group:

Group 1: Control – received saline (5 ml/ kg p.o.).

Group 2: Negative control group i.e received aluminium chloride at dose of 100mg/ kg of body weight p.o.

Group 3: Aluminium chloride + Ethanolic extract of *Smilax zeylanica* (150mg/ kg of body weight p.o.)

Group 4: Aluminium chloride + Ethanolic extract of *Smilax zeylanica* (300mg/ kg of body weight p.o.)

Groups 5: Aluminium chloride + piracetam (0.5mg/ kg body weight p.o.)

Oxidative stress was induced to the all the rats, except in the group I, by Aluminium Chloride at a dose of 100mg/ kg/ body weight for 60 days through oral gavage. From 61st day onwards the group 3 and 4 animals were treated with the ethanolic extract of *Smilax zeylanica* for 30 days. At the end of the experimental period, the rats were sacrificed by cervical decapitation after overnight fasting. Dissected hippocampus, cortex and cerebellum were grinded in 10 mM Tris/HCl (pH 7.0) containing 10 µl/ml protease inhibitor and were centrifuged to separate the nuclear debris. Supernatant 1 (S1) was collected and used for quantification of the levels of thiobarbituric acid reactive substances (TBARS)²⁶ and the remaining pellet was further centrifuged to get the post-mitochondrial fraction, which was used for the assay of antioxidants like used for the estimation of Superoxide dismutase (SOD)²⁷, Catalase (CAT)²⁸ and reduced Glutathione (GSH)²⁹.

Statistical analysis

Data were presented as mean ± SEM. One-way ANOVA using Tukey's employed for post hoc test for multiple comparisons. The value of P < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Effect of ethanolic extracts of roots of *Smilax zeylanica* on Brain tissue TBARS levels in AlCl₃ induced rats

Effect of ethanolic extracts of roots of *Smilax zeylanica* on brain tissues like, hippocampus cortex and cerebellum TBARS level results is shown in Tables 1 and Fig.1.. The activities of TBARS in the tissue like hippocampus cortex and cerebellum, significantly (P<0.001) increased in rats fed with AlCl₃-treated animals (group II) than control group animals. AlCl₃ treatment significantly increased TBARS levels, a sensitive marker of the lipid peroxidation process. Previous reports showed the Al exposure could elevate the free radical generation and oxidative damage in specific areas of brain including cerebral cortex^{30,31}, hippocampus³⁰, cerebellum^{31,32}. Administration of ethanolic extract extracts of roots of *Smilax zeylanica* on brain tissues like, hippocampus cortex and cerebellum TBARS levels were significantly reduced when compared to AlCl₃-treated animals.

Table 1: Effect of ethanolic extracts of *Smilax zeylanica* on tissues TBARS in AlCl₃ induced rats

Groups	TBARS (n mol of MDA formed/g tissue)		
	Hippocampus	Cortex	Cerebellum
Group I	2.36±0.13 a, ¹	6.75±0.22 a, ¹	2.23±0.14 a, ¹
Group II	9.56±0.15 a, ^{1,2}	14.66±0.27 b, ^{1,2}	6.23±0.18 b, ^{1,2}
Group III	7.45±0.16 a, ^{1,2}	12.55±0.16 b, ^{1,2}	4.97±0.16 b, ^{1,2}
Group IV	3.65±0.08 b, ^{1,2}	7.12±0.11 a, ^{1,2}	2.56±0.37 b, ^{1,2}
Group V	3.18±0.19 c, ²	6.89±0.19 c, ²	2.42 ±0.13 c, ²

Values are expressed as mean± SEM of 6 animals. Statistical significance tested by one way ANOVA, followed by Tukey's "t" test. a – P < 0.05; b – P < 0.01; c – P < 0.005; 1 – compared with group I; 2 – compared with group V. Group I: Control – received saline (5 ml/ kg p.o.). Group II: Negative control group i.e received aluminium chloride at dose of 100mg/ kg of body weight p.o., Group III: Aluminium chloride + Ethanolic extract of *Smilax zeylanica* (150mg/ kg of body weight p.o.), Group IV: Aluminium chloride + Ethanolic extract of *Smilax zeylanica* (300mg/ kg of body weight p.o.), Groups V: Aluminium chloride + piracetam (0.5mg/ kg body weight p.o.)

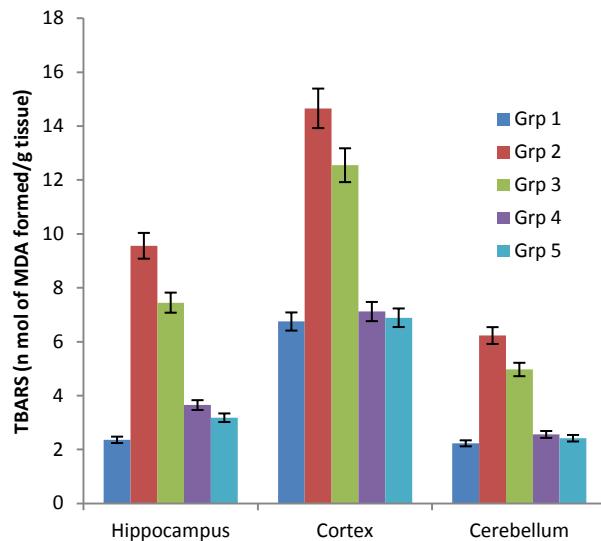


Figure 1: Effect of ethanolic extracts of *Smilax china* on tissues TBARS in AlCl_3 induced rats

Effect of ethanolic extracts of roots of *Smilax zeylanica* on Brain tissue enzymatic antioxidants in AlCl_3 induced rats

Effect of ethanolic extracts of roots of *Smilax zeylanica* on brain tissues like, hippocampus cortex and cerebellum SOD and CAT level results is shown in Tables 2 & 3 and Fig.2 & 3 respectively. The activities of SOD and CAT in the tissue like hippocampus cortex and cerebellum, significantly ($P < 0.001$) lowered in rats fed with AlCl_3 -treated animals (group II) than control group animals. Metals such as copper, iron, cadmium, arsenic, mercury, nickel and Al induced their toxic effects due to their ability to transfer electrons and free radicals production. Chronic exposure of Al could leads to the disruptions of mineral balance by replacing iron and magnesium ions ³³. Previous reports showed the Al exposure could elevate the free radical generation and oxidative damage in specific areas of brain including cerebral cortex, hippocampus, cerebellum. Administration of ethanolic extract extracts of roots of *Smilax zeylanica* on brain tissues like, hippocampus cortex and cerebellum SOD and CAT levels were significantly increased when compared to AlCl_3 -treated animals.

Table 2: Effect of ethanolic extracts of *Smilax zeylanica* on tissues superoxide dismutase in AlCl_3 induced rats

Groups	SOD (unit min/mg protein)		
	Hippocampus	Cortex	Cerebellum
Group I	4.66±0.22 a,1	3.65±0.18 a,1	2.87±0.12 a,1
Group II	2.11±0.17 b,1,2	1.96±0.23 a,1,2	1.67±0.20 a,1,2
Group III	2.92±0.16 b,1,2	2.34±0.18 b,1,2	1.98±0.16 b,1,2
Group IV	3.92±0.22 a,1,2	3.68±0.19 b,1,2	2.45±0.22 b,1,2
Group V	3.86±0.18 c,2	3.45±0.14 c,2	2.40 ±0.18 b,2

Values are expressed as mean± SEM of 6 animals. Statistical significance tested by one way ANOVA, followed by Tukey's "t" test. a – $P < 0.05$; b – $P < 0.01$; c – $P < 0.005$; 1 – compared with group I; 2 – compared with group V.

Table 3: Effect of ethanolic extracts of *Smilax china* on tissues catalase AlCl_3 induced rats

Groups	CAT (μ moles of H_2O_2 , consumed min/mg protein)		
	Hippocampus	Cortex	Cerebellum
Group I	3.52±0.12 a,1	3.23±0.18 a,1	2.12±0.18 a,1
Group II	1.78±0.12 b,1,2	1.48±0.16 a,1,2	1.08±0.16 b,1,2
Group III	2.02±0.14 a,1,2	2.14±0.12 b,1,2	1.34±0.13 b,1,2
Group IV	2.98±0.14 b,1,2	3.02±0.18 a,1,2	2.03±0.12 b,1,2
Group V	2.68±0.20 c,2	2.98±0.22 c,2	2.08±0.17 c,2

Values are expressed as mean± SEM of 6 animals. Statistical significance tested by one way ANOVA, followed by Tukey's "t" test. a – $P < 0.05$; b – $P < 0.01$; c – $P < 0.005$; 1 – compared with group I; 2 – compared with group V.

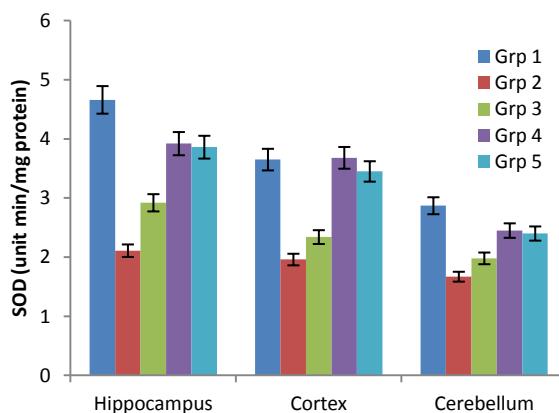


Figure 2: Effect of ethanolic extracts of *Smilax china* on tissues superoxide dismutase in AlCl_3 induced rats

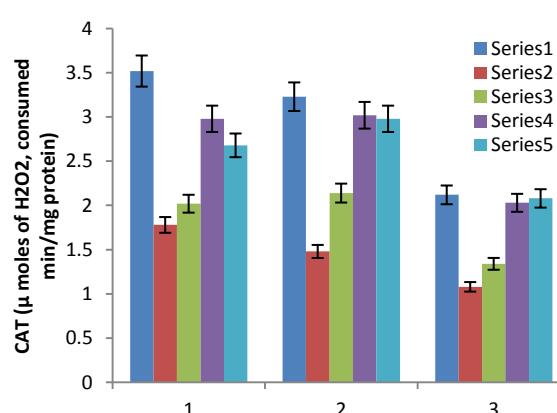


Figure 3: Effect of ethanolic extracts of *Smilax china* on tissues CAT in AlCl_3 induced rats

Effect of ethanolic extracts of *Smilax zeylanica* on tissues glutathione AlCl₃ induced rats

Effects of ethanolic extracts of *Smilax zeylanica* on brain tissues like, hippocampus cortex and cerebellum glutathione AlCl₃ induced rats results are shown in Table 4. Glutathione (GSH), a tripeptide which is present in all the cells is an important antioxidant. The activities of activities of glutathione concentration in the tissue like hippocampus cortex and cerebellum, significantly ($P<0.001$) lowered in rats fed with AlCl₃-treated animals (group II) than control group animals. Metals such as

copper, iron, cadmium, arsenic, mercury, nickel and Al induced their toxic effects due to their ability to transfer electrons and free radicals production. Chronic exposure of Al could leads to the disruptions of mineral balance by replacing iron and magnesium ions³³. AlCl₃ exposure was accompanied by decrease in the levels of GSH and the activities of GPx and catalase in brains of rats³⁴ and mice³⁵. Administration of ethanolic extract extracts of roots of *Smilax zeylanica* (300mg/kg body weight) on brain tissues like, hippocampus cortex and cerebellum GSH levels were significantly increased when compared to AlCl₃-treated animals.

Table 4: Effect ethanolic extracts of *Smilax zeylanica* on tissues glutathione AlCl₃ induced rats

Groups	Glutathione (mg/g tissue)		
	Hippocampus	Cortex	Cerebellum
Group I	19.52 \pm 0.33 a, ¹	22.44 \pm 0.42 a, ¹	15.34 \pm 0.32 a, ¹
Group II	9.44 \pm 0.22 b, ^{1,2}	12.12 \pm 0.32 a, ^{1,2}	6.78 \pm 0.24 b, ^{1,2}
Group III	13.53 \pm 0.12 a, ^{1,2}	14.98 \pm 0.45 b, ^{1,2}	8.87 \pm 0.15 a, ^{1,2}
Group IV	18.76 \pm 0.16 b, ^{1,2}	20.08 \pm 0.21 b, ^{1,2}	14.32 \pm 0.18 b, ^{1,2}
Group V	17.22 \pm 0.22 c, ²	19.45 \pm 0.38 c, ²	13.56 \pm 0.18 c, ²

Values are expressed as mean \pm SEM of 6 animals. Statistical significance tested by one way ANOVA, followed by Tukey's "t" test. a – P < 0.05; b – P < 0.01; c – P < 0.005; 1 – compared with group I; 2 – compared with group V.

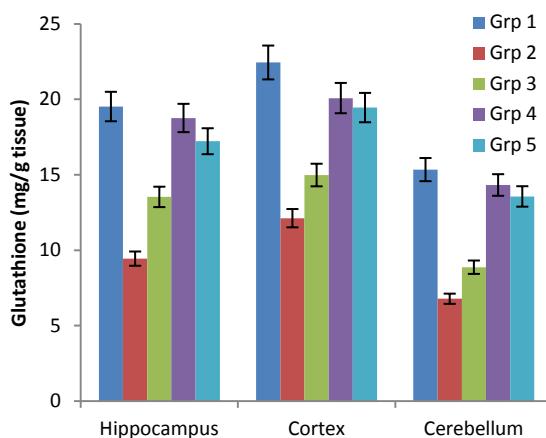


Figure 4: Effect ethanolic extracts of *Smilax china* on tissues glutathione AlCl₃ induced rats

CONCLUSION

AlCl₃ induced the oxidative stress in rats were significantly increased TBARS and reduced antioxidant enzymes. On the basis of the results obtained in the present study, we conclude that the ethanolic extract from roots of *Smilax zeylanica* had significant *in vivo* antioxidant and lipid peroxidation activity when compared with AlCl₃ treated rats. The phytoconstituents may be responsible for the inhibition of lipid peroxidation and enhance the antioxidant activities.

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