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IN-VIVO ANTIOXIDANT ACTIVITY OF ETHANOLIC EXTRACT FROM ROOTS OF *SMILAX CHINA* ON ALUMINIUM CHLORIDE INDUCED OXIDATIVE STRESS IN WISTAR RATS

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Keywords:

S. china, Antioxidants, TBARS, Oxidative stress, $AlCl_3$ & Wistar rats

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ABSTRACT: To examine the antioxidant property of ethanolic extract of roots of *Smilax china* (EESC) on Aluminium Chloride Induced oxidative stress in Wistar rats. The phytoconstituents are extracted from the roots of *S. china* using ethanol, by hot continuous percolation method. The rats were divided into 5 groups (6 animals/ group) and treated with EESC (150 & 300 mg/kg of body weight) and piracetam (0.5 mg/kg of body weight) for 14 days after inducing oxidative stress with $AlCl_3$ (100 mg/ kg of body weight) for 60 days. The lipid peroxidation level (TBARS) and antioxidants activities like Superoxide dismutase (SOD), Catalase (CAT), and Glutathione (GSH) were estimated. When compared with the control group, $AlCl_3$ induced rats showed increased TBARS and decreased antioxidant enzymes like Catalase (CAT), Superoxide dismutase (SOD) and reduced glutathione (GSH). The EESC at higher dose 300 mg/kg of body weight animals were significantly ($P<0.001$) reduced the TBARS and increased the antioxidant enzymes SOD, CAT, and increased GSH when compared with the control group. The present study results revealed that the ethanolic extract from *Smilax china* roots might be used as a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses and oxidative stress-related diseases.

INTRODUCTION: Free radical is a molecule with an unpaired electron which involved in bacterial & parasitic infections, atherosclerosis, aging, cardiovascular disorders, lung damage, neoplastic diseases, and autoimmune disorders (rheumatoid arthritis). Reactive Oxygen Species (ROS) plays an important key role in normal physiological processes comprising cellular life/death process, defense from pathogens, several cellular signaling pathways like ARE Pathways, and maintenance of vascular tone [1].

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Oxidative stress is a deficient capability of biological systems to counterbalance the disproportionate free radicals and its production, which can lead to cardiovascular diseases, neurodegenerative disease, age-related cognitive decline, and immune system dysfunction [2]. ROS is already proved for its part in the pathophysiology of neurodegenerative disorders (*e.g.* Alzheimer disease, Parkinson disease, Down's syndrome and multiple sclerosis), pathologies of autoimmune and GI inflammations [4]. Antioxidant compounds act as free radical scavengers and initiator of multiplexes of pro-oxidant metals. And also reducing agents and quenchers of singlet oxygen formation [5]. The roots of *Smilax china* belongs to the Liliaceae family which is generally found in South India, namely Andhra Pradesh, Tamil Nadu, and Karnataka.

It is used ethnomedicinally for the treatment of various illnesses such as colic, constipation helminthiasis, dyspepsia, flatulence, skin diseases, leprosy, psoriasis, fever, epilepsy, insanity neuralgia, syphilis, strangury, seminal weakness, and general debility. Detoxifies organs [6], purifies blood, assists absorption and destroys bacteria, excites digestion, increases urination, defends liver, and speeds up perspiration. And this plant is shown to exhibit several bioactivities, for instance, anti-inflammatory, anti-diabetic, anti-psoriatic, diuretic, digestive properties. It is also hepatoprotective, nephroprotective, and used in conditions of infertility [7].

Hence, the present work was focused on the role of antioxidants to heal oxidative stress. Mainly observed for neuro-degeneration disorders like Parkinson's and Alzheimer's diseases. Ethanolic extracts of roots of *Smilax china* (EESC) is used as the antioxidant. Therefore, the present investigation focused on evaluating the *in-vivo* antioxidant potential of ethanolic extract of *Smilax china* roots by different screening methods.

MATERIALS AND METHODS:

Collection and Identification of Plant Materials: The roots of *Smilax china* were collected from Marthandam, Kanyakumari District, Tamil Nadu, India, and authenticated by Botanical Survey of Medical Plants Unit Siddha, Government of India, Palayankottai (Authentication Ref no. 01/08/2016). The roots of *Smilax china* were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40-mesh sieves [8].

Preparation of Extracts: The above-powdered plant materials were sequentially extracted with petroleum ether (40-60 °C) by hot continuous percolation method in the Soxhlet apparatus (1 day). The marc was dried out and extracted with $ChCl_3$, extracted with ethyl acetate (76-78 °C, 1 day), then this marc was dried out and extracted with ethanol (1 day) and extracted with water. Until dry powder was acquired, all these extracts were concentrated by means of a rotary evaporator and undergone to freeze-drying by lyophilizer. The ethanolic extract gave more phytoconstituents yield comparing to other extracts. So, for further investigation, the ethanolic extract of roots of *Smilax china* was selected.

Evaluation of *in-vivo* Antioxidant Activity: Both the genders of Wister rats with 8 weeks (between 150 & 200 g of body weight) were used for the present study [9]. For about two weeks, these rats were acclimatized for experimental conditions. The rats housed in plastic cages at 25 °C with a relative humidity of 70% under 12/12 hours a day/night cycle. Rats were fed with food and water *ad libitum*. As per the policy of CPCSEA, New Delhi, India, the experiments were agreed. And permitted by the Annamalai University IAEC (Approved number: AU/IAEC/1199/1/18).

Animals have grouped arbitrarily into five diverse groups with six rats in apiece:

Group I: Control Animal [treated with saline (5 ml/ kg p.o.)].

Group II: Negative control animal [received $AlCl_3$ (100mg/kg of body weight p.o.)].

Group III: $AlCl_3$ + Ethanolic extract of *Smilax china* (150mg/kg of body weight p.o.).

Group IV: $AlCl_3$ + Ethanolic extract of *Smilax china* (300mg/ kg of body weight p.o.)

Group V: $AlCl_3$ + Piracetam (0.5mg/kg body weight p.o.).

Except for group I rats, all the other four group rats were oxidative stress induced by Aluminium Chloride (100 mg/kg/body weight) through oral gavage for 60 days [10]. Group III and IV animals were administered for 30 days with the ethanolic extract of *Smilax china* roots from the 61st day onwards. At the experimental period ended, the rats were fasted overnight and sacrificed by way of cervical decapitation. Dissected cortex, hippo-campus, and cerebellum were grinded in 10 mm Tris/HCl (pH 7.0) containing 10 µl/ml protease inhibitor and centrifuged to distinct the nuclear debris. Supernatant 1 (S1) was collected and used for quantification of the levels of thiobarbituric acid reactive substances (TBARS) [11] and the remaining pellet was additionally centrifuged to get the post-mitochondrial fraction, used for the antioxidants assay and estimations of Superoxide dismutase (SOD), Catalase (CAT) and reduced glutathione (GSH) [12].

Statistical Analysis: The statistical analysis was used by Graph Bad prism software. The Data were stated as mean ± SEM. One-way ANOVA (Analysis of Variance) followed by Tukey's post hoc test for multiple comparisons.

Effects of Ethanolic Extract of Roots of *Smilax china* on Brain Tissue TBARS Level in $AlCl_3$ Induced Rats: Effects of ethanolic extract of roots of *Smilax china* on brain tissues like hippocampus, cortex and cerebellum- TBARS level results is shown in **Table 1** and **Fig. 1**.

TABLE 1: EFFECTS OF ETHANOLIC EXTRACT OF ROOTS OF *SMILAX CHINA* ON TISSUES TBARS IN $AlCl_3$ INDUCED RATS

Groups	TBARS (n mol of MDA formed/g tissue)		
	Hippocampus	Cortex	Cerebellum
Group I	2.36 ±0.13 ^{a,2}	6.75 ±0.22 ^{a,2}	2.23 ±0.14 ^{a,2}
Group II	9.56 ±0.15 ^{a,1}	14.66 ±0.27 ^{b,1}	6.23 ±0.18 ^{b,1}
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 stated as mean ± SEM of 6 rats. Statistical significance verified by One-way ANOVA, shadowed by Tukey's test. a – P<0.05; b – P<0.01; c – P<0.001; 1 – equated with group II, III, IV; 2 – compared with group I, III, IV, V.

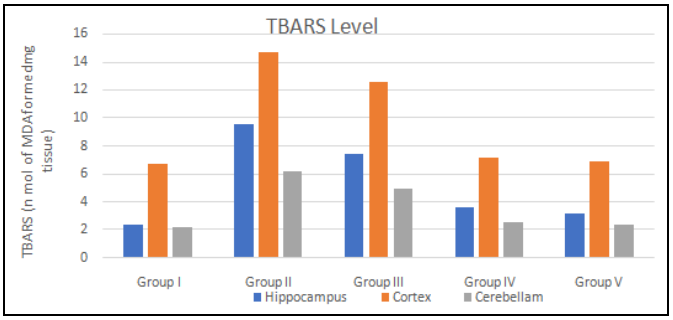

FIG 1: EFFECTS OF ETHANOLIC EXTRACTS OF ROOTS OF *SMILAX CHINA* ON TISSUES TBARS IN $AlCl_3$ INDUCED RATS

The TBARS activities in the tissue significantly (P<0.001) increased in rats fed with $AlCl_3$-treated animals (Group II) than control group rats. $AlCl_3$ treatment increased the level of TBARS, a sensitive marker of the lipid peroxidation process. Previous reports showed that exposure could elevate the free radical generation and oxidative damage in specific areas of the brain, including the cerebral cortex, hippocampus, cerebellum [14]. On brain tissues like hippocampus, cortex, and cerebellum - TBARS levels were significantly reduced on the administration of ethanolic extract of roots of *Smilax china* when compared to $AlCl_3$-treated animals.

Effects of Ethanolic Extract of Roots of *Smilax china* on Brain Tissue Enzymatic Antioxidant SOD & CAT in $AlCl_3$ Induced Rats: Effects of ethanolic extract from roots of *Smilax china* on brain tissues such as cortex, hippocampus, and cerebellum - SOD and CAT level results are shown in **Table 2-3** & **Fig 2-3** respectively. The SOD and CAT activities in the tissues significantly (P<0.001) lowered in the negative control (Group II) than the control group (Group 1) rats. Aluminum could primes to the disturbance of mineral balance by replacing iron and magnesium ions during chronic exposure.

TABLE 2: EFFECTS OF ETHANOLIC EXTRACTS OF ROOTS OF *SMILAX CHINA* ON TISSUES SUPEROXIDE DISMUTASE IN $AlCl_3$ INDUCED RATS

Groups	SOD (n mol of MDA formed/g tissue)		
	Hippocampus	Cortex	Cerebellum
Group I	4.66 ±0.22 ^{a,2}	3.65 ±0.18 ^{a,2}	2.87 ±0.12 ^{a,2}
Group II	2.11 ±0.17 ^{b,1}	1.96 ±0.23 ^{a,1}	1.67 ±0.20 ^{a,1}
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 byone-way ANOVA, followed by Tukey's test. a – P<0.05; b – P<0.01; c – P<0.001; 1 – compared with group II, III, IV; 2 – compared with group I, III, IV, V.

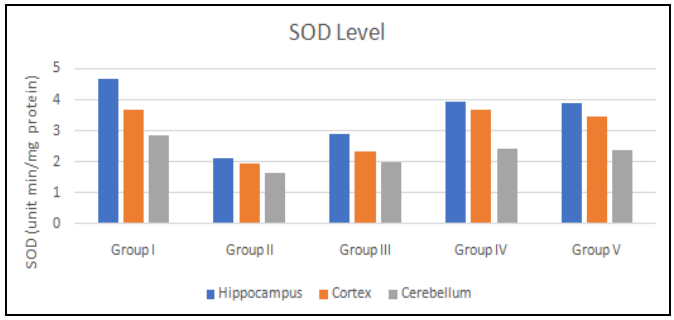

FIG 2: EFFECT OF ETHANOLIC EXTRACTS OF *SMILAX CHINA* ON TISSUES SUPEROXIDE DISMUTASE IN $AlCl_3$ INDUCED RATS

Previous reports proved that exposure of A would increase the generation of free radical and oxidative damage in several areas of the brain, including the cerebral cortex, hippocampus, cerebellum. While

treatment of EESC on brain tissues like hippocampus, cortex, and cerebellum - SOD and CAT levels were significantly increased when compared to $AlCl_3$-treated animals.

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,2}	3.23 ±0.18 ^{a,2}	2.12 ±0.18 ^{a,2}
Group II	1.78 ±0.12 ^{b,1}	1.48 ±0.16 ^{a,1}	1.08 ±0.16 ^{b,1}
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 byone-way ANOVA, followed by Tukey's test. a – P<0.05; b – P<0.01; c – P<0.001; 1 – compared with group II, III, IV; 2 – compared with group I, III, IV, V.

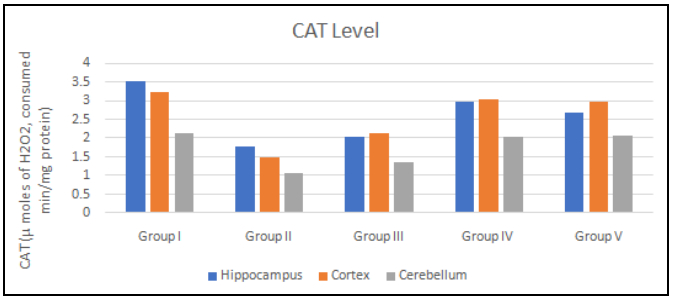

FIG 3: EFFECT OF ETHANOLIC EXTRACTS OF *SMILAX CHINA* ON TISSUES CAT IN $ALCL_3$ INDUCED RATS

Effects of Ethanolic Extract of Roots of *Smilax china* on Tissues Glutathione in $AlCl_3$ Induced Rats: Effects of EESC on brain tissues like hippocampus, cortex and cerebellum-glutathione $AlCl_3$ induced rats results are shown in **Table 4** and **Fig. 4** Glutathione (GSH) a tripeptide, present in cells is an important antioxidant. The activities of glutathione concentration in the tissues signi-ficantly (P<0.001) lowered in the negative control (Group II) than control group animals (Group 1). Due to their capability to electron transferring and production of free radicals, metals such as Cd, Cu, Hg, Ni, and Al-induced their toxic effects. Chronic exposure of Al may tend to the interruptions of mineral balance by replacing iron and magnesium ions. Previous reports showed the Al exposure may raise the generation of free radical and oxidative damage in specific areas of the brain, including the cerebral cortex, hippocampus, cerebellum). $AlCl_3$ exposure was escorted by a decrease in the level of GSH and catalase in the brains of rats [15]. On the treatment of ethanolic extract of *Smilax china* roots (300mg/kg body weight) on brain tissues, GSH levels were significantly increased when compared to $AlCl_3$-treated animals.

TABLE 4: EFFECT ETHANOLIC EXTRACTS OF *SMILAX CHINA* ON TISSUES GLUTATHIONE $AlCl_3$ INDUCED RATS

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

Values are expressed as mean ± SEM of 6 animals. Statistical significance tested by one-way ANOVA, followed by Tukey's test. a – P<0.05; b – P<0.01; c – P<0.001; 1 – compared with group II, III, IV; 2 – compared with group I, III, IV, V.

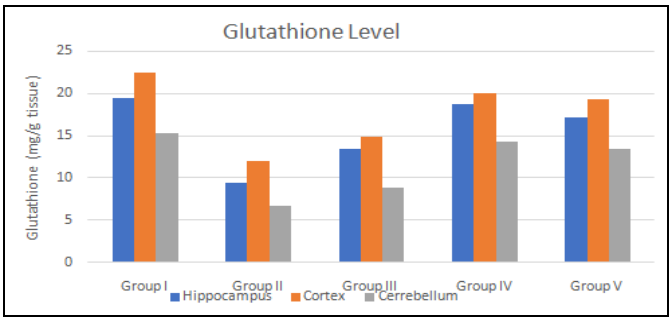

FIG. 4: EFFECTS ETHANOLIC EXTRACTS OF *SMILAX CHINA* ROOTS ON TISSUES GLUTATHIONE $ALCL_3$ INDUCED RATS

CONCLUSION: Oxidative stress was induced in rats by the administration of $AlCl_3$, then it significantly increased and decreased the level of TBARS and antioxidant enzymes, respectively. After the treatment with ethanolic extract of *Smilax china* roots, form the results obtained we can conclude that the ethanolic extract of roots of *Smilax china* had significant *in-vivo* antioxidant and lipids peroxidation activity when compared with AlCl3, treated rats.

These phytoconstituents present in the plant extract may be responsible for the inhibition of lipid peroxidation and enhance the antioxidant activities.

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CONFLICTS OF INTEREST: The authors declare that they have no conflicts of interest.

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