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EFFECT OF *OXALIS CORNICULATA L.* EXTRACTS ON BIOGENIC AMINES CONCENTRATIONS IN RAT BRAIN AFTER INDUCTION OF SEIZURE

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ABSTRACT

The leaves of *Oxalis corniculata L.* is used traditional Indian medicine to treat epilepsy. Previous studies have demonstrated that extracts of these plants was subjected to acute toxicity and then screened for antiepileptic activity on Maximal Electroshock (MES) and Pentylenetetrazole (PTZ) induced seizures models in albino wistar rats. The purpose of the present study is to investigate the effect of methanolic (95%) extract of *Oxalis corniculata L.* (MEOC) on biogenic amines concentrations in rat brain after induction of seizures by MES and PTZ. Our aim of study was relationship between seizure activities and altered the monoamines such as noradrenaline (NA), dopamine (DA), serotonin (5-HT) and Gamma amino butyric acid (GABA) in forebrain of rats in MES and PTZ seizure models. In MES model, MEOC (200 & 400 mg/kg) showed significantly restored the decreased levels of brain monoamines such as NA, DA, 5-HT and GABA. Similarly in PTZ model, MEOC showed significantly increased the monoamines in forebrain of rats. Thus, this study suggests that methanol extract of *Oxalis corniculata L.* increased the monoamines on rat brain, which may be decreased the susceptibility to MES and PTZ induced seizure in rats.

Keywords: Antiepileptic activity, Traditional Medicine, *Oxalis corniculata L.*, biogenic amines, NA, DA, 5-HT and GABA

INTRODUCTION

Epilepsy is among the most prevalent of the serious neurological disorders, affecting from0.5 to 1.0% of the world's population (Rapp PR and Bachevalier J, 2003). Interestingly, the prevalence of epilepsy in developing countries is generally higher than in developed countries (Sander JW and Shorvon SD, 1996). Epileptic seizures are paroxysmal clinic events arising from neuronal hyper excitability and hyper synchrony of the cerebral cortex, either locally (partial seizures) or diffusely in both hemispheres (generalised seizures). The agitated neuronal activity that occurs during a seizure is caused by a sudden imbalance between the inhibitory and excitatory signals in the brain with δ-aminobutyric acid (GABA), noradrenaline, serotonin, and dopamine respectively, being the most important neurotransmitters involved (Rang HP, 2007).

The role of dopamine and serotonin in epilepsy remains controversial, but both have convincingly been implicated in the pathophysiology of seizures (Bagdy, 2007; Starr MS, 1996). All the currently available antiepileptic drugs are synthetic molecules. Medicinal plants used for the therapy of epilepsy in traditional medicine have been shown to possess promising anticonvulsant activities in animal models of anticonvulsant screening can be an invaluable source for search of new antiepileptic compounds. In previous study, the methanolic (95%) extract of leaves of *Oxalis corniculata L.* (MEOC) was subjected to acute toxicity and then screened for antiepileptic activity on Maximal Electroshock (MES) and Pentylenetetrazole (PTZ) induced seizures models in albino wistar rats was reported (Senthil Kumar KK and Rajkapoor B, 2010). Therefore,

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the present study was performed to examine the effect of *Oxalis corniculata L.* on biogenic amines concentrations in rat brain after induction of seizure by MES & PTZ model.

***Oxalis corniculata* L. (**Family: Oxalidaceae) is a small creeping perennial herb which forms roots at nodes. Leaves alternate, long-petiolate, trifoliate (clover-like in appearance), the leaflets obcordate with a conspicuous notched apex, each leaflet up to 2 cm long. Flowers yellow, 5-merous, borne in axillary few-flowered inflorescences. Fruit a sub cylindrical capsule up to 20 cm long containing numerous tiny black seeds. Flowers and fruit available throughout the year (Tewari, 1976). It *is* Common in damp shady places, roadsides, pastures, plantations, lawns, etc. This plant reported for Hypoglycemic, antihypertensive, chronotropic effect, uterine relaxant, antipsychotic, CNS-stimulant, antiyeast, inotropic effect, smooth muscle relaxant. In Fiji, the plant is traditionally used as a remedy for convulsions in children and for healing fractured bones (Cambie and Ash, 1994; Weiner, 1984; Weiner, 1971). However there are no reports on the antiepileptic activity of the plant. Hence, the present study was designed to verify the claims of the native practitioners. Therefore, the present study was performed to verify the effect of effect of *Oxalis corniculata L.* on biogenic amines levels in rat brain after induction of seizure by MES and PTZ model.

MATERIALS AND METHODS

Plant collection

The leaves of *Oxalis corniculata L.* was collected from Tirupathi, Andhra Pradesh, India. It was identified and authenticated by Prof. Jayaraman, Plant Anatomy Research Centre (PARC), Chennai, Tamil Nadu. The voucher specimen **PARC / 2009 / 352** was preserved in our laboratory for future reference.

Preparation of extracts

The leaves of plants were dried in shade, separated and made to dry powder. It was then passed through the 40 mesh sieve. A weighed quantity (200gm) of the powder was subjected to continuous hot extraction in Soxhlet Apparatus. Percentage yield of MEOC was found to be 19 % w/w.

Animals used

Albino wistar rats (150-200g) of either sex were obtained from the animal house in St. Peter's College of Pharmacy, Warangal. The animals were maintained in a well-ventilated room with 12:12 hour light/dark cycle in polypropylene cages. The animals were fed with standard pellet feed (Hindustan Lever Limited., Bangalore) and water was given *ad libitum*. Ethical committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) of CPCSEA (Ref No. IAEC / XIII / 06/ SPCP / 2009 - 2010).

Experimental design

Albino wistar rats were divided into four groups of six animals each. Group I received vehicle control (1% w/v SCMC, 1ml/100 g) whereas Group-II received standard drug (Phenytoin, 25mg/kg) *i.p*, Group-III and IV, received 95% methanolic extract of the leaves of *Oxalis corniculata L.* (L.) (200 and 400 mg/kg b.w) *p.o* respectively for 14 days. On the 14^{th} day, Seizures are induced to all the groups by using an Electro convulsiometer. The duration of various phases of epilepsy were observed.

Pentylenetetrazole (90mg/kg b.w, *s.c*) was administered to other groups to induce clonic convulsions after above respective treatment. Animals were observed for a period of 30mins post– PTZ administration.

A fluorimetric micromethod for the simultaneous determination of serotonin, noradrenaline and dopamine

On the 14^{th} day after observed the convulsion all groups rats were sacrificed, whole brain was dissected out and separated the forebrain. Weighed quantity of tissue and was homogenized in 0.1 mL hydrochloric acid - butanol, (0.85 ml of 37% hydrochloric acid in one liter *n*- butanol for spectroscopy) for 1 min in a cool environment. The sample was then centrifuged for 10 min at 2,000 rpm. 0.08 mL of supernatant phase was removed and added to an Eppendorf reagent tube containing 0.2 mL of heptane (for spectroscopy) and 0.025 mL 0.1 M hydrochloric acid. After 10 min of vigorous shaking, the tube was centrifuged under same conditions to separate two phases. Upper organic phase was discarded and the aqueous phase (0.02 mL) was used for estimation of Serotonin, Nor Adrenaline and Dopamine assay.

Nor-Adrenaline and Dopamine Assay

The assay represents a miniaturization of the trihydroxide method. To 0.02ml of HCl phase, 0.05ml 0.4M and 0.01ml EDTA/Sodium acetate buffer (pH 6.9) were added, followed by 0.01ml iodine solution (0.1M in ethanol) for oxidation. The reaction was stored after two minutes by addition of 0.01ml Na_2SO_3 in 5m NaOH. Acetic acid was added 1.5 minutes later. The solution was then heated to 100 for 6 minutes. When the sample again reached room temperature, excitation and emission spectra were read in the microcuvette as with 5-HT: in some cases, the readings were limited to the excitation maxima. 395-485nm for NA and 330-375nm for DA uncorrected instrument values.

Serotonin Assay

As mentioned earlier some modifications in reagent concentration became necessary together with changes in the proportions of the solvent, in order to obtain in a good fluorescence yield with reduced volume for 5-HT determination, the O-pthaldialdehyde (OPT) method was employed. From the OPT reagent 0.025ml were added to 0.02ml of the HCl extract. The fluorophore was developed by heating t 100°C for 10 min. After the samples reached equilibrium with the ambient temperature, excitation / estimation spectra or intensity reading at 360-470 nm were taken in the micro cuvette.

Estimation of brain GABA content

The brain amino butyric acid (GABA content was estimated according to the method of **Lowe, (1958)** Animals were sacrificed by decapitation and brains were rapidly removed, and separated forebrain region. It was blotted, weighed and placed in 5ml of ice-cold trichloroaceticacid (10% w/v), then homogenized and centrifuged at 10,000rpm for 10min at 0°C. A sample (0.1ml) of tissue extract was placed in 0.2ml of 0.14 M ninhydrin solution in 0.5M corbonate-bicorbonate 1 buffer (pH9.95), kept in a water bath at 60°C for 30min, then cooled and treated with 5ml of copper tartrate reagent (0.16% disodium carbonate, 0.03% copper sulphate and 0.0329% tartaric acid). After 10min fluorescence at 377/455nm in a spectofluorimeter was recorded.

Statistical Analysis

The data were expressed as mean ± standard error mean (S.E.M).The Significance of differences among the group was assessed using one way and multiple way analysis of variance (ANOVA). The test followed by Dunnet's test p values less than 0.05 were considered as significance.

RESULTS

Effect of MEOC on monoamines levels in seizure induced rats by MES and PTZ:

Noradrenaline

In MES and PTZ models, Noradrenaline levels significantly ($p<0.01$) decreased in forebrain of epileptic control animals. MEOC at the doses of 200&400mg/kg, standard drugs phenytoin and diazepam treated animals showed a significantly ($p<0.05$ & $p<0.01$) increased in Noradrenaline levels in forebrain of rats. **Table 1 and 2.**

Dopamine

In MES and PTZ models, Dopamine levels significantly ($p<0.01$) decreased in forebrain of epileptic control animals. MEOC at the doses of 200&400mg/kg, standard drugs phenytoin and diazepam treated animals showed a significantly ($p<0.05$ & $p<0.01$) increased in Dopamine levels in forebrain of rats. **Table 1 and 2.**

Serotonin

In MES and PTZ models, Serotonin levels significantly ($p<0.01$) decreased in forebrain of epileptic control animals were observed. MEOC at the doses of 200&400mg/kg, standard drugs phenytoin and diazepam treated animals showed a significantly ($p<0.05$ & $p<0.01$) increased in Serotonin levels in forebrain of rats. **Table 1 and 2.**

Gamma amino butyric acid

In MES and PTZ models, GABA levels significantly ($p<0.01$) decreased in forebrain of epileptic control animals were observed. MEOC at the doses of 200&400mg/kg, standard drugs phenytoin and diazepam treated animals showed a significantly ($p<0.05$ & $p<0.01$) increased in GABA levels in forebrain of rats. **Table 1 and 2.**

Table: 1. Effect of MEOC on neurotransmitters levels in rat brain after MES induced epilepsy

Group	Design of Treatment	Noradrenaline	Dopamine	Serotonin	GABA
I	Vehicle Control(SCMC 1ml/100gm)	745±5.52	631.50±3.18	171±2.01	265±1.49
II	MES (SCMC 1ml/100gm)	413.66±2.32 ^{a**}	445.66±4.49 ^{a**}	63±1.80 ^{a**}	212.16±2.18 ^{a**}
III	Phenytoin 25mg/kg,*i.p*	578±2.17 ^{b**}	698.50±3.59 ^{b**}	99.16±2.78 ^{b**}	289.83±1.38 ^{b**}
IV	MEOC 400 mg/kg,*p.o*	567.16±4.45 ^{b**}	636±1.77 ^{b**}	90.33±0.88 ^{b**}	258.5±1.17 ^{b**}
V	MEOC 200 mg/kg,*p.o*	756.33±4.46 ^{b*}	562.83±2.3 ^{b*}	83±0.81 ^{b*}	270.66±1.3 ^{b**}

Values are expressed as mean ± SEM of six observations. Comparison between: **a**- Group I Vs Group II, **b**- Group III Vs Group IV and Group V. Statistical significant test for comparison was done by ANOVA, followed by Dunnet's test *$p<0.05$;** $p<0.01$;

Units = pg/mg of wet tissue.

Table: 2. Effect of MEOC on neurotransmitters levels in rat brain after PTZ induced epilepsy

Group	Design of Treatment	Noradrenaline	Dopamine	Serotonin	GABA
I	Vehicle Control(SCMC 1ml/100gm)	758±5.12	847.50±3.08	181±2.01	281.83±1.42
II	MES (SCMC 1ml/100gm)	517.16±2.37 $^{a\,**}$	567.83±4.33 $^{a\,**}$	94.5±3.23 $^{a\,**}$	207.16±1.52^{a**}
III	Diazepam (4mg/kg), *p.o*	612±2.3 $^{b\,**}$	892.16±3.43 $^{b\,**}$	136.83±2.18 $^{b\,**}$	284.33±1.22^{b**}
IV	MEOC 400 mg/kg,*p.o*	752.42±4.14^{b*}	884.66±2.44^{b**}	125.50±1.28^{b**}	232±1.35^{b**}
V	MEOC 200 mg/kg,*p.o*	767.16±4.22^{bns}	771.50±4.35^{b**}	106.33±1.64 ^{b*}	276.34±1.22^{b**}

Values are expressed as mean ± SEM of six observations. Comparison between: **a** - Group I Vs Group II, **b-** Group III Vs Group IV and Group V. Statistical significant test for comparison was done by ANOVA, followed by Dunnet's test *p<0.05;** p<0.01;
Units = pg/mg of wet tissue.

DISCUSSIONS AND CONCLUSIONS

The role of biogenic amines in epileptogenesis and in recurrent seizure activity is well-documented. Spontaneous and experimentally induced deficiencies in gamma amino butyric acid (GABA), noradrenaline (NA), dopamine (DA) and/or serotonin (5-hydroxy- tryptamine or 5-HT). It has been implicated in the onset and perpetuation of many seizure disorders many experimental procedures designed to increase monoaminergic activity have proven antiepileptic properties (Applegate, 1986; Corcoran ME, 1988; McIntyre DC and Edson N, 1989; Pelletier MR and Corcoran ME, 1993; Yan QS, 1995; Zis AP, 1992).

In present study, the established antiepileptic drugs such as phenytoin and diazepam restored the monoamine levels on brain (Clinckers, 2005). Similarly MEOC significantly (p<0.05 & p<0.01) increased monoamines levels in forebrain of rats. Many drugs that increase the brain contents of GABA have exhibited anticonvulsant activity against seizures induced by MES and PTZ (Fisher RS, 1989). MES is probably the best validated method for assessment of anti-epileptic drugs in generalized tonic-clonic seizures (Loscher, 1991)**.**

GABA is a major inhibitory neurotransmitter of CNS and increase in its level in brain has variety of CNS dependent effects including anticonvulsant effect (Macdonald RL and McLean MJ, 1982). In addition to the GABA binding site, the $GABA_A$ receptor complex appears to have distinct allosteric binding sites for benzodiazepines, barbiturates, methanol etc (Santhakumar, 2007). We therefore studied the effect of *Oxalis corniculata L.* extract on brain GABA content. *Oxalis corniculata L.* extract showed significant (p<0.05 & p<0.01) increased GABA content in brain dose dependently. This suggests that the anticonvulsant activity of *Oxalis corniculata L.* extract is probably through elevation of brain GABA content.

In Norepinephrine-lesioned rats showed a greater susceptibility to seizures induced by the chemoconvulsant PTZ and electroconvulsive shock (Mason ST and Corcoran ME, 1979). The antiepileptic role of endogenous Norepinephrine was inferred from studies that showed harmful effects of a damage of Norepinephrine system on seizures induced by electrical stimulation or systemic administration of chemoconvulsants (Browning, 1989; Dailey JW and Jobe PC, 1986). In present study, MEOC significantly (p<0.05 & p<0.01) increased the NA in forebrain of rats and proves the antiepileptic activity of *Oxalis corniculata L.* extract.

Chen (Chen G, 1954**)** demonstrated that pre-treatment with the monoamine-depleting agent reserpine decreased the epileptic threshold to PTZ and caffeine in mice. Reserpine lacks specificity, since this drug also depletes serotonin (5-HT) and DA, in addition to NE. Therefore, increased seizure susceptibility could be due to a multiple deficit of monoamines (Gross RA and Ferrendelli JA, 1979). Subsequent the present studies confirmed and extended these results. It became clear that MEOC significantly increased the serotonin (5-HT) and DA and NA. It produces significantly decreased the susceptibility to various epileptic stimuli.

In conclusion biogenic amines participate in the control of Maximal electroshock and pentylenetetrazole induced seizure in rat models. Our findings support the hypothesis that decreased the monoamines levels in rat brain after induction of seizure. In *Oxalis corniculata L.* extract treated rats, monoamines such as NA, DA, 5-HT and GABA levels significantly restored on forebrain. Thus MEOC increases the seizure threshold and decreased the susceptibility to MES and PTZ induced seizure in rats. Hence we suggest that methanol extract of leaves of *Oxalis corniculata L.* possess antiepileptic properties that may be due to restored the biogenic amines in rat brain. These results support the ethnomedical uses of the plant in the treatment of epilepsy. However more experimentation, detailed phytochemical and experimental analysis are required for a definitive conclusion.

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