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Research Article

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Evaluation of effect of *Oroxylum indicum* leaves on Central Nervous System with special emphasis on epilepsy

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ABSTRACT

The plant of Oroxylum indicum is reported to possess anti-epileptic activity in ethnomedicine books. So, looking for dire need of a new, safe and economical anti-epileptic agent we focused to investigate anti-epileptic potential of Oroxylum indicum. Methanolic extract of Oroxylum indicum leaves was prepared & subjected for various animal models to evaluate anti-epileptic activity. Anti-epileptic activity was evaluated by PTZ and MES induced seizure model followed by CNS-depressants action by Actophotometer apparatus. Methanolic extract of Oroxylum indicum shown significant decrease in time of Myoclonic jerk and tonic flexation in PTZ model & decrease in time of tonic convulsion and clonic expansion in MES model. This study also shown that number of cut-off is also decrease in actophotometer. (Significance level $P < 0.005 \approx$ highly significant). Animal model show remarkable beneficial effect in PTZ induced seizure as well as MES induced seizure. Along with that study on Actophotometer revealed that MEOI can act as CNS-depressants. Considering all these findings, we can conclude that Methanolic extract of Oroxylum indicum leaves possess CNS-depressant activity which can be therapeutically useful in management of Epilepsy.

Keywords: *Oroxylum indicum*, Anti-epileptic, CNS depressants, Maximum electric shock, Pentylenetetrazole.

INTRODUCTION

Epilepsy is one of the most common neurologic disorders of the brain, affecting about 50 million individuals worldwide and 90% of them are from developing countries. Causative factors for Epilepsy includes Genetic factors as well as infection in brain, stroke, tumor and high fever. [1] Epilepsy is characterized by an enduring predisposition to generate epileptic seizures, and by the neurobiological, cognitive, psychological and social consequences of this condition. An epileptic seizure is the clinical manifestation of an abnormal and excessive discharge of a set of neurons in the brain. [2]There are number of drugs available as for management of Epilepsy like Use dependant Na^+ channel blockers (Phenytoin, Carbamazepine, etc), T-type Ca^{2+} channel blocker (Ethosuximide, Gabapentin), GABAnergic drugs (Diazepam, Viagabatrine, Tiagabine), Miscellaneous – Na.Valproate [3, 4] but these have major drawbacks like Nystagmus, thrombocytopenia, Thinning & curling of hair, vaginal bleeding, hepatotoxicity, teratogenicity like Cleft palate, spina bifida, etc. [3, 4] So, looking for dire need of a new, safe and economical anti-epileptic agent, we resolved to investigate beneficial effects of *Oroxylum indicum.* Traditional knowledge indicating that leaves of *Oroxylum indicum* can be used in management of epilepsy [5, 6] as well as literature review suggests that *Oroxylum indicum* contain Baicalein as a chemical constituent [7] which can act as positive allosteric modulator of $GABA_A$ [8] and can aid better tool for management of Epilepsy. *Oroxylum Indicum* is one of the important Rasayana herb mentioned in Ayurveda [5] belongs to family Bignoniaceae [6]. *Oroxylum Indicum* is a native tree often grown as an ornamental for its strange appearance. Mostly sighted along the river banks or slopes of the hills [7]. A tree of *Oroxylum Indicum* is about 8-15 m tall, brown or grayish brown bark with lenticels and branched at top [8]. The leaves are very large, about 90-180 cm long 2-3 pinnate, cylindrical, swollen at the junction of branches [8]. The large leaf stalks wither and fall off the tree and collect near the base of the trunk, appearing to look like a pile of broken limb bones [8].

EXPERIMENTAL SECTION

Collection & Authentification of Plant (*Oroxylum Indicum* Leaves)

The leaves of *Oroxylum indicum* was collected from medicinal garden of School of Pharmacy, RK University. The plant material was carried by Mrs. Trupti Marakana where the herbarium voucher (No. SOP/COG/471/2015) has been kept. The Oven-dried leaves of *Oroxylum indicum* were pulverized into powder by using a blender and stored in an airtight container.

Figure - 1: Plant - Oroxylum indicum

Plant of *Oroxylum indicum*

Preparation of Methanolic extracts of leaves of *Oroxylum Indicum* using Soxhlet apparatus

The finely ground crude drug was placed in thimble which was made of filter paper and placed in soxhlet apparatus. The extracting solvent Methanol was heated in round bottom flask and its vapors condense in condenser. The condensed extract drops into the thimble containing the crude drug. When the level of liquid in soxhlet apparatus rises to the top of siphon tube, the liquid contents of apparatus siphon in to the round bottom flask. The process is continuous and carried out till green pigments disappear. The methanolic extract was used as test extract. All doses were expressed in terms of crude extract (mg/kg body weight).

Figure - 2: **Soxhlet apparatus – Continuous Extraction Process**

Preparation of the Drug

The fraction of extract was weighed (200mg) and dissolved in 1 ml of distilled water to prepare required concentration in mg/kg and administered by gavage. Fraction was stored and protected from direct sunlight until use.

Experimental Animals

Albino Wistar rats (200-250 gm) were used in this study. The animals were procured from animal house, Department of Pharmacology, School of Pharmacy, RK University, Rajkot, India. Animals were housed at a temperature of 24±20˚C and relative humidity of 30 – 70 %. A light and dark cycle was followed. All animals were fed on standard balance diet and provided with water. The animals were used after getting approval from Institutional Animal Ethics Committee (IAEC) of School of Pharmacy, RK University. (Protocol no. RKCP/COL/RP/16/72)

Pharmacological Evaluation

Model I: Maximal Electro Shock Induced seizures (MES) model [9, 10]
Animal: Either sex of Wistar rat (200-220gm)
Animals were divided into Three groups containing six animals each.
Group I: Disease control group was given vehicle (0.2 ml)
Group II: Standard control group was given Clonazepam (0.1 mg/kg, i.p.)
Group III: Test group was given methanolic extract of leaves of *Oroxylum indicum* (MEOI) (200mg/kg, i.p.)

Procedure: After dividing animals into their respective groups, animals were subjected for maximal electroshock seizures (MES) (150 mA for 0.2sec) using an electroconvulsometer via Crocodile ear clips or eye electrodes, 60 minutes after respective treatment. The number of animals protected from tonic hind limb extension seizure (i.e. abolition of tonic hind limb extension within 10 sec after delivery of the electroshock was considered as protected rat) and duration of observed tonic hind limb extension seizure (HLTE) was recorded.

For recording various parameters, rats were placed in clear rectangular plastic cages with an open top, permitting full view of the animal's motor responses to seizure. Various phases of convulsions, viz., tonic flexion, extension, clonus, stupor and mortality due to convulsions were measured and analyzed. Statistical analysis was done by ANOVA followed by Tuckey's Test.

Model II: Pentylenetetrazole (PTZ) induced Seizure [9, 10]
Animal: Either sex of Wistar rat (200-220gm)
Animals were divided into Three groups containing six animals each.
Group I: Disease control group was given vehicle (0.2 ml)
Group II: Standard control group was given Clonazepam (0.1 mg/kg, i.p.)
Group III: Test group was given methanolic extract of leaves of *Oroxylum indicum* (MEOI) (200mg/kg, i.p.)

Procedure: After dividing animals into their respective groups, animals were subjected for treatment with PTZ (85 mg/kg, i.p.), 30 minutes after respective treatment. The parameters of absence seizure viz., onset of myoclonic jerk, clonus, tonic flection and mortality were observed and compared statistically. Statistical analysis was done by ANOVA followed by Tuckey's test.

Model III: CNS – depressant action in mice [11,12]
Animal: Either sex of Swiss mice (20-25 gm)
Animals were divided into Three groups containing six animals each.
Group I: Normal control group was given vehicle (0.2 ml)
Group II: Standard control group was given Clonazepam (0.1 mg/kg, i.p.)
Group III: Test group was given methanolic extract of leaves of *Oroxylum indicum* (MEOI) (200mg/kg, i.p.)

Procedure: 18 animals (mice) were divided in to 3 groups containing 6 animals each, which was subjected for respective treatment. After 30 minutes, allow the animal to freely move in the model and data of number of cut off (crossing) of lesser for 2 minutes were collected and compared statistically. Statistical analysis was done by ANOVA followed by Tuckey's Test.

Statistical Analysis

Statistical analysis of results was done by ANOVA test for determination of variance. Data were considered significantly different from each other if $p \leq 0.05$ and if $p \leq 0.001$ then the difference between data were consider highly significant.

RESULTS

Table 1: Pharmacognostic evaluation Of *Oroxylum indicum*

Type of Extract	% W/W	Color	Consistency
Methanolic	7.77	Dark green	Semi-Solid

Effect of MEOI on CNS by Actophotometer:

The study revealed that the test group animals had number of cut-off 56.00±1.90 at 90min., Which is significantly lesser compared to that of control viz, control 87.33±3.66 at 90min.During this study, Standard group had 44.33±1.08 at 90min. number of cut-off which were significantly lesser than that of the control. (See figure 3.)

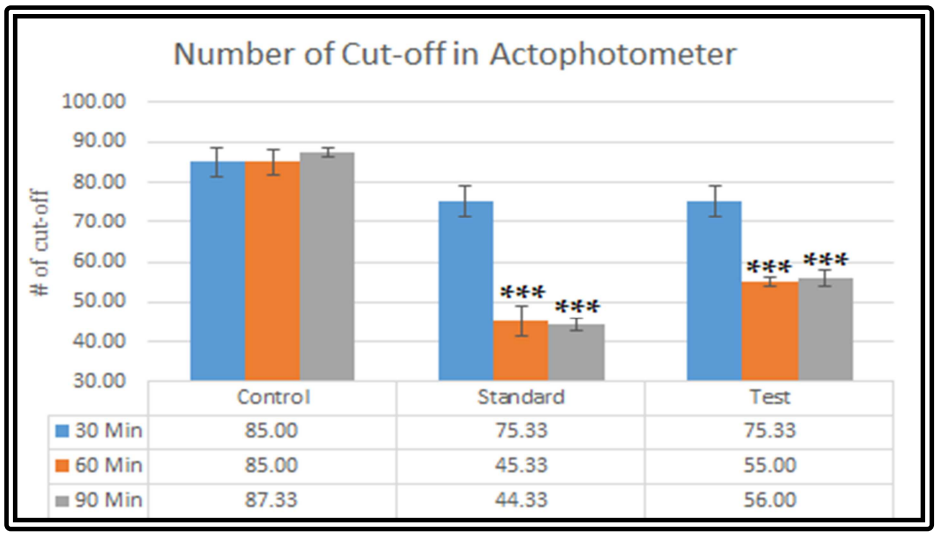

Figure – 3: Effect of MEOI on CNS by Actophotometer

(Indicate significant difference in data compared to disease control group and the level of significance was P<0.001 Highly significant)***

Table 2: Effect of MEOI on CNS by Actophotometer			
Group	30min	60min	90min
Control	85±3.65	85±3.65	87.33±3.66
Standard(Clonazepam 0.1mg/kg)	75.33±3.04***	45.33±3.77***	44.33±1.03***
Test(MEOI 200mg/kg)	75.33±1.04***	55.00±1.00***	56.00±1.90***
F	258.85	977.9424779	1557.763158
df	17 (2,15)	17 (2,15)	17 (2,15)
P-value	0.0000000000024	0.00000000000000013	0.0000000000000000040

Effect of MEOI on PTZ induced seizure

The study shown test group had significant reduction in myoclonic jerk 6.67±0.56 & tonic flexation 4.67±0.56 compared to that of control group had myoclonic jerk 9.33±0.21 & tonic flexation 6.67±0.21.During this study standard group had significant reduction in myoclonic jerk 4.00±0.37 & tonic flexation 1.33±0.21 compared to that of control group. Remarkably recover earlier than control. (See Figure 4a, 4b, 4c)

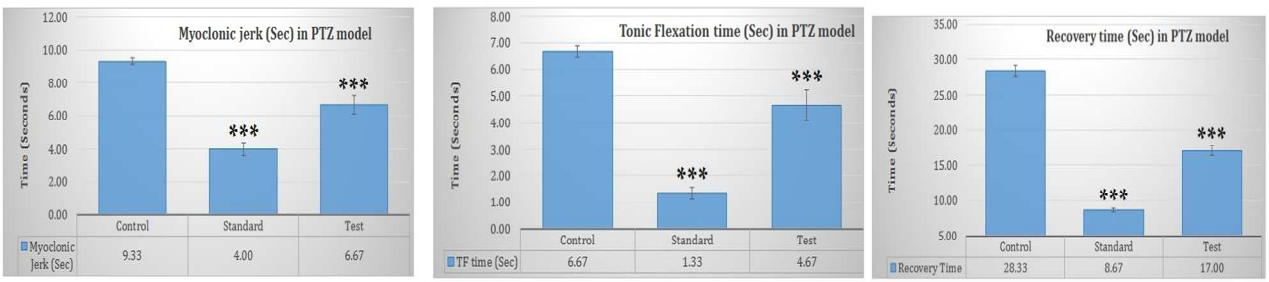

Figure 4: (a – Myoclonic jerk, b – Tonic flexation, c - Recovery)Effect of MEOI on PTZ induced seizure

(Indicate significant difference in data compared to disease control group and the level of significance was P<0.001 Highly significant)***

Table 3: Effect of MEOI on PTZ induced seizure			
Group	Mayoclonic jerk	Tonic flexation	Recovery/Death
Control	9.33±0.21	6.67±0.21	28.33±0.76
Standard(Clonazepam 0.1mg/kg)	4.00±0.37 ***	1.33±0.21***	8.67±0.21 ***
Test(MEOI 200mg/kg)	6.67±0.56 ***	4.67±0.56 ***	17.00±0.37 ***
F	28.82352941	53.61111111	252.9807692
df	17 (2,15)	17 (2,15)	17 (2,15)
P-value	0.0000073	0.00000015	0.0000000000028

Effect of MEOI on MES induced seizure

The study shown test group had significant reduction in Tonic convulsion 2.33±0.21 & Clonic expansion 4.67±0.56 compared to that of control group had Tonic convulsion 4.00±0.37 & Clonic expansion 11.00±0.37. During this study standard group had significant reduction in tonic convulsion 1.33±0.21 & clonic expansion 3.67±0.42 compared to that of control group. Remarkably recover earlier than control. (See the figure no. 5a, 5b, 5c)

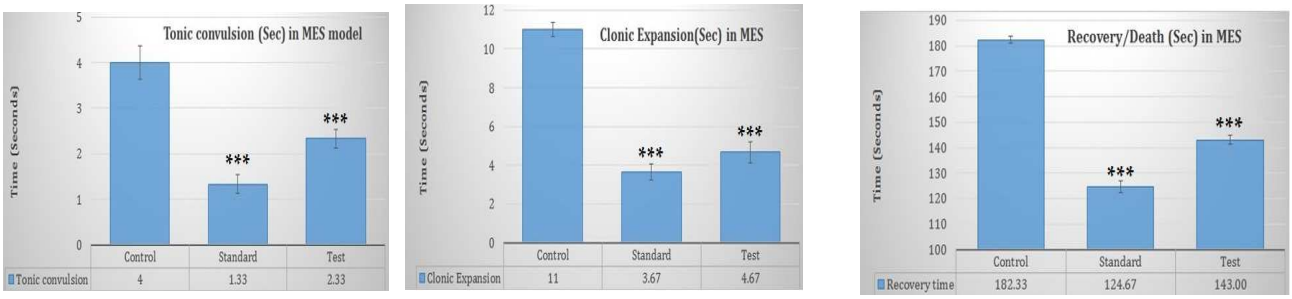

Figure 5: (a – Tonic convulsion, b – Clonic Expansion, c - Recovery) Effect of MEOI on PTZ induced seizure

*(*** Indicate significant difference in data compared to disease control group and the level of significance was P<0.001 Highly significant)*

Table 4:Effect of MEOI on MES induced seizure			
Group	Tonic Convulsion (Seconds)	Clonic Expansion (Seconds)	Recovery/Death (Seconds)
Control	4.00±0.37	11.00±0.37	182.33±1.48
Standard (Clonazepam 0.1mg/kg)	1.33±0.21***	3.67±0.42 ***	124.67±2.25 ***
Test (MEOI 200mg/kg)	2.33±0.21***	4.67±0.56 ***	143.00±1.70 ***
F	24.05	76.25	18.06613226
df	17 (2,15)	17 (2,15)	17 (2,15)
P-value	0.000019	0.000000014	0.00010126

DISCUSSION

The results demonstrate that methanolic extract of *Oroxylum indicum* (MEOI)exhibited anti epileptic activity in experimental animal models. In actophotometer, Study show significant decrease in number of cut-off value compare to control. Which clearly suggest that MEOI possess CNS-depressants activity. In rota-rod test apparatus, Study show similar fall of time compare to control. Which clearly suggest that MEOI does not possess SKM-relaxant activity. In PTZ, Study show significant decrease in myoclonic jerk & tonic flexation compare to control. Which suggest that MEOI can protect the animal against absence seizure (induced by PTZ) & thus it can possess anti-epileptic action. In MES, Study show significant decrease in tonic convulsion & clonic expansion compare to control. Which suggest that MEOI can protect the animal against tonic-clonic convulsion (induced by MES) & thus it can possess anti-epileptic action. Literature review suggest that leaves of OI contain Bacalein[5] , Which is believed to have GABAnergic activity[6] . The present study elearly supported this rationale & leaves of OI exhibit all the pharmacological actions accordingly.

CONCLUSION

Animal model show remarkable beneficial effect in PTZ induced seizure as well as MES induced seizure. Along with that study on Actophotometer reveled that MEOI can act as CNS-depressants. Considering all these findings, we can conclude that Methanolic extract of *Oroxylum indicum* leaves possesses CNS-depressant activity which can be therapeutically useful in management of Epilepsy. The results of various models of epilepsy supported the same and we can conclude that leaves of *Oroxylum indicum* can be used as better, safer & cheaper alternate of currently available treatments of Epilepsy.

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