Senthil Nagaraj D. / *International Journal of Preclinical & Pharmaceutical Research.* 2013; 4(2): 81-84.

e-ISSN 2249-7552
Print ISSN 2229-7502

International Journal of
Preclinical & Pharmaceutical Research

Journal homepage: www.preclinicaljournal.com

EFFECT OF *FAGONIA CRETICA (L.)* EXTRACTS ON BIOGENIC AMINES CONCENTRATIONS IN RAT BRAIN AFTER INDUCTION OF SEIZURE

D. Senthil Nagaraj

Faculty of Bio-chemistry, Madawalabu University, Bale Robe, Ethiopia.

ABSTRACT

The present study is to investigate the effect of aqueous extract of *Fagonia cretica (L.)* (AEFC) on biogenic amines concentrations in rat brain after induction of seizures by MES. Our aim of study was relationship between seizure activities and altered the monoamines such as noradrenaline (NA), dopamine (DA), and serotonin (5-HT) in forebrain of rats in MES seizure model. In MES model, AEFC (250 & 500 mg/kg) showed significantly restored the decreased levels of brain monoamines such as NA, DA, & 5-HT. Hence, this study confirmed that aqueous extract of *Fagonia cretica (L.)* increased the monoamines on rat brain, which may be decreased the susceptibility to MES induced seizure in rats.

Key Words: Antiepileptic activity, *Fagonia cretica (L.)*, Biogenic amines.

INTRODUCTION

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. Therefore, the present study was performed to examine the effect of *Fagonia cretica (L.)* on biogenic amines concentrations in rat brain after induction of seizure by MES model.

Fagonia cretica L., a member of the family Zygophyllaceae, is a small spiny undershrub. It is reputed to be a medicinal plant in scientific and folkloric literature, and its medicinal values are well documented. *Fagonia cretica* is astringent, febrifuge and prophylactic against small-pox. The plant is bitter and used for the treatment of fever, thirst, vomiting, dysentery, asthma, urinary discharges, liver trouble, typhoid, toothache, stomach troubles, convulsion and skin diseases [1-5]. Boiled residue of the plant in water is used to induce abortion. It is externally applied as a paste on tumors and other swellings of the neck. An aqueous decoction of the considering the medicinal activity of *Fagonia cretica* based on traditional information. Hence in this study *Fagonia cretica* L. whole plant was evaluated for its effects on epilepsy.

Corresponding Author

D. Senthil Nagaraj
Email: dsenthilnagaraj@gmail.com

MATERIALS AND METHODS

Plant collection

The whole plant of *Fagonia cretica* L. has been collected from Sri Venketeswara University near Tirupati, Andhra Pradesh during the month of August 2012 and dried under shade. The plant was authentified by Mr. K. Madhava chetty, Assistant Professor, Department of Botany of S. V. University, Tirupati. The voucher specimen of the plant was deposited at the college for further reference.

Preparation of plant extract

The whole plant of *Fagonia cretica* L. are properly washed in tap water and then rinsed in distilled water. The rinsed plants are dried in an oven at 35^{o}C for 4 days. The dried plant of *Fagonia cretica* L. was crushed to obtain powder. These powdered samples are then stored in airtight polythene bags protected from sunlight until use. The aqueous extract of each sample was prepared by soaking 10g of powdered sample in 200ml distilled water for 12h. The extracts are then filtered using Whatmann

filter paper. Percentage yield of aqueous extract of whole plant of *Fagonia cretica* L. was found to be 12% w/w. The aqueous extract was administered to the animals by suspending each time in 1%w/v carbaoxy methyl cellulose (CMC).

Animals used

Adult albino rats (Wister strain) of either sex with weighing 150 - 180gm were used. The animals were maintained on the suitable nutritional and environmental condition throughout the experiment. The animals were housed in polypropylene cages with paddy house bedding under standard laboratory condition for an acclimatization periods of 7 days prior to performing the experiment. The animals had access to laboratory chow and water *ad libitum*. The experimental protocols were approved by institutional Animal Ethical Committee & a written permission from Institutional ethical committee has been taken to carry out and complete this study.

Acute Toxicity Study

The acute toxicity of aqueous extract of whole plant of *Fagonia cretica* L. was determined as per the OECD guideline no. 423 (Acute Toxic Class Method). It was observed that the test extract was not lethal to the rats even at the 2000 mg/kg doses. Hence, $1/8^{th}$ (250mg/kg) and $1/4^{th}$ (500mg/kg) of this dose were selected for further study [6].

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 negative control (1% w/v SCMC, 1ml/100 g) Group-III received standard drug (Phenytoin, 25mg/kg) *i.p*, Group-III and IV, received aqueous extract of the whole plants of *Fagonia cretica (L.)* (250 and 500 mg/kg b.w) *p.o* respectively for 14 days. On the 14^{th} day, Seizures are induced to all the groups except Group I by using an Electro convulsiometer.

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 [7].

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 aqueous) 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 [7].

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 [7].

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 Dunnett's test p values less than 0.05 were considered as significance.

RESULTS

Effect of AEFC on monoamines levels in seizure induced rats by MES

Noradrenaline

In MES model, Noradrenaline levels significantly ($p<0.01$) decreased in forebrain of epileptic control animals. AEFC at the doses of 250&500mg/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 model, Dopamine levels significantly ($p<0.01$) decreased in forebrain of epileptic control animals. AEFC at the doses of 250&500mg/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).

Serotonin

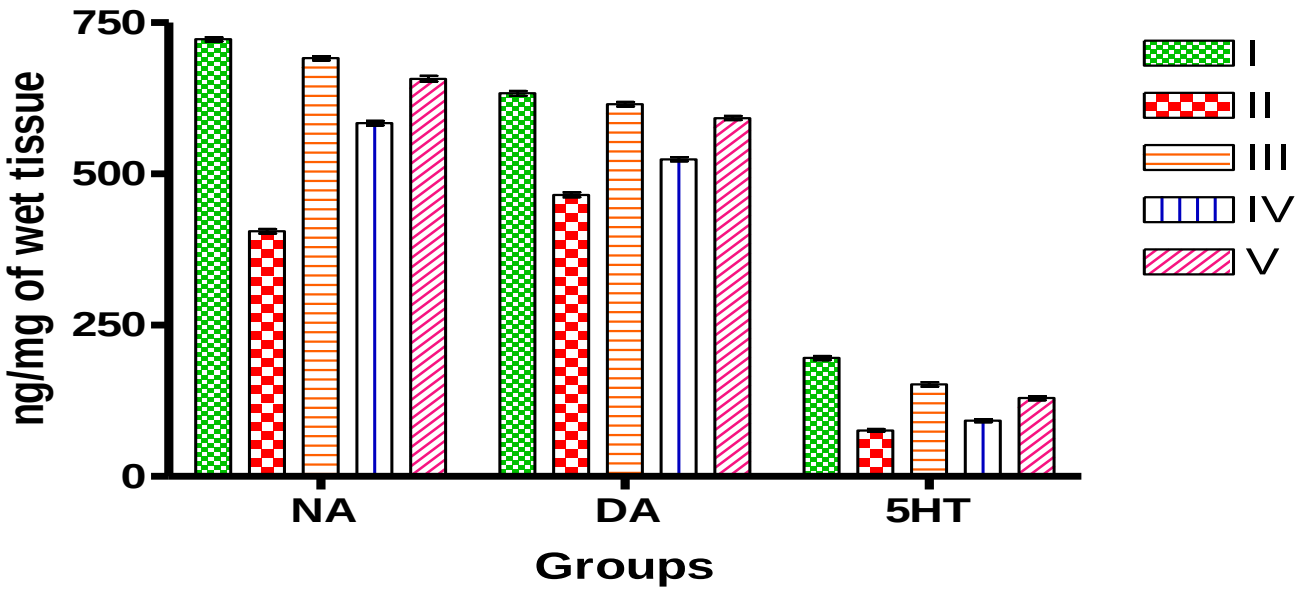
In MES model, Serotonin levels significantly ($p<0.01$) decreased in forebrain of epileptic control animals were observed. AEFC at the doses of 250&500mg/kg, standard drugs phenytoin and diazepam treated animals showed a significantly ($p<0.05$ & $p<0.01$) increased in Serotonin levels in forebrain of rats (Table 1).

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

Group	Design of Treatment	Noradrenaline	Dopamine	Serotonin
I	Vehicle Control(SCMC 1ml/100gm)	722.52±3.19	633.22±4.14	195.64±3.22
II	MES (SCMC 1ml/100gm)	405.24±3.64[a **]	465.58±4.22[a **]	76.22±2.17[a **]
III	Phenytoin 25mg/kg,*i.p*	691.49±3.47[b **]	615.19±3.64[b **]	152.19±3.48[b **]
IV	AEFC 250 mg/kg,*p.o*	584.22±3.67[b**]	524.16±3.46[b**]	92.29±2.33[b**]
V	AEFC 500 mg/kg,*p.o*	657.33±4.57[b*]	592.33±3.36[b*]	129.47±3.46[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 Dunnett's test *p<0.05;** p<0.01;
Units = ng/mg of wet tissue.

Figure 1. Effect of AEFC on neurotransmitters levels in rat brain after MES induced epilepsy



DISCUSSION AND CONCLUSION

In present study, the established antiepileptic drugs such as phenytoin restored the monoamine levels on brain [8]. Similarly AEFC significantly ($p<0.05$ & $p<0.01$) increased monoamines levels in forebrain of rats [9]. MES is probably the best validated method for assessment of anti-epileptic drugs in generalized tonic-clonic seizures [10]. Spontaneous and experimentally induced deficiencies in 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 [11-16]. The biogenic amines participate in the control of Maximal electroshock induced seizure in rat model. Our findings support the hypothesis that decreased the monoamines levels in rat brain after induction of seizure. In *Fagonia cretica (L.)* extract treated rats, monoamines such as NA, DA, & 5-HT levels significantly restored on forebrain. Thus AEFC increases the seizure threshold and decreased the susceptibility to MES induced seizure in rats.

Hence we concluded that aqueous extract of whole plants of *Fagonia cretica (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.

REFERENCES

1. Chopra RM, Handa KL, Kapur LD et al. Indigenous Drugs of India (2nd ed). Academic Publisher. New Delhi; 1982.
2. Hooker JD, Thomson T. The Flora of British India. William Clowes and Sons Ltd. London; 1881.
3. Saeed MA. Hamdard Pharmacopoeia of eastern medicine. Hamdard Pharmacopoeia. 1,1969.
4. Baquar SR. Medicinal and Poisonous Plants of Pakistan. Printas. Karachi; 1989, pp. 198-199.
5. Meyer BN, Ferrigni NR, Putnam JE et al. Brine shrimp: a convenient general bioassay for active plant constituents. *Planta Med*, 45, 1982, 31-34.
6. OECD, 2002. Acute oral toxicity. Acute oral toxic class method guideline 423 adopted in: Eleventh Addendum to the OECD, guidelines for the testing of chemicals organisation for economical co-operation and development.
7. Schlumpf M, Lichtensteiger W, Langemann H, Waser PG, Hefti F. A fluorimetric micromethod for the simultaneous determination of serotonin, noradrenaline and dopamine in milligram amounts of brain tissue. *Biochem Pharmacol*, 23, 1974, 2337-46.
8. Applegate CD, Burchfiel JL, Konkol RJ. Kindling antagonism: effects of norepinephrine depletion on kindled seizure suppression after concurrent, alternate stimulation in rats, *Exp. Neurol*, 94, 1986, 379–390.
9. Corcoran ME. Characteristics of accelerated kindling after depletion of noradrenaline in adult rats, *Neuropharmacology*, 27, 1988, 1081–1084.
10. McIntyre DC, Edson N. Kindling-based status epilepticus: effects of norepinephrine depletion with 6-hydroxydopamine, *Exp. Neurol*, 104, 1989, 10–14.
11. Pelletier MR Corcoran ME. Infusions of a2 noradrenergicagonists and antagonists into the amygdala: effects on kindling. *Brain Res*, 632, 1993, 29–35.
12. Yan QS Jobe PC. Dailey JW. Further evidence of anticonvulsant role for 5- hydroxy tryptamine in genetically epilepsy-prone rats. *Br. J. Pharm*, 115, 1995, 1314–1318.
13. Zis AP, Nomkos GG, Brown EE, Damsa G, Fiberger HC. Neurochemical effects of electrically and chemically induced seizures: and in vivo microdialysis study in hippocampus. *Neuropsychopharmacology* 7, 1992, 189–195.
14. Clinckers R, Smolders I, Meurs A, Ebinger G, Michotte Y. Hippocampal dopamine and serotonin elevations as pharmacodynamic markers for the anticonvulsant efficacy of oxcarbazepine and 10, 11- dihydro-10-hydroxycarbamazepine. *Neuroscience Letters*, 390, 2005, 4853.
15. Fisher RS. Animal models of the epilepsies. *Brain Res Rev*, 14, 1989, 245-78.
16. Loscher W, Fassbender CP, Nolting B. The role of technical, biological and pharmacological factors in the laboratory evaluation of anticonvulsant drugs II. Maximal electroshock seizure models. *Epilepsy Res*, 8, 1991, 79-94.