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Research Article

ESTIMATION AND EVALUATION OF BRAIN BIOGENIC AMINES FOR ANTICONVULSANT ACTIVITY OF *ANNONA SQUAMOSA* LINN. LEAVES IN MICE

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ABSTRACT

The ethanol extract of the leaves of Annona squamosa Linn. showed marked protection against convulsions induced by chemo convulsive agents in mice. The catecholamines contained were significantly increased in the processed extract treated mice. The amount of GABA, which is most likely to be involved in seizure activity, was increased significantly in mice brain after six week treatment. Results of the present study revealed that the processed extract showed a significant anticonvulsive property by altering the level of catecholamine and brain amino acids in mice.

Keywords: *Annona squamosa, Catecholamines, GABA, Pentylenetetrazol.*

INTRODUCTION

More than a century and a half ago, Hughlings Jackson defined an epileptic seizure as the clinical phenomenon resulting from 'an occasional, sudden and excessive discharge of gray matter' [1], a definition that has stood the test of time. Over the years, the motor, sensory and autonomic phenomena that are produced by epileptic brain discharges have been identified and classified. Today, as in Jackson's time, seizures remain important signals to the possibility of underlying brain disorders that need to be identified and treated. Seizures are symptoms of abnormal brain function. Epilepsy is the condition of spontaneously recurrent seizures and is one of the major neurological disorders of the brain, affecting approximately 0.5-1.0% of the world population.

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Seizure is the characteristic feature in epilepsy and is associated with disordered and rhythmic high frequency discharge of impulses by a group of neurons in the brain. Abnormal cellular discharge may be associated with a variety of causative factors such as- trauma, oxygen deprivation, tumors, infection and metabolic derangements. However, no specific factors are found in about half of patients suffering from epilepsy [2]

Annona squamosa Linn., Annonaceae, commonly known as sitaphal and custard-apple or sugar-apple, is a native of West Indies and is now cultivated throughout India, mainly for its edible fruit. The leaves contains several alkaloids [3] (annonaine, roemerine), flavanoids and acetogenins. *Annona squamosa* is reported to have numerous therapeutic uses viz: leaves extract has been used as – antidiabetics, hypolipidemic, anticancer, expectorant and insecticidal agents. Ethanol extract of *A.squamosa* showed anticonvulsant effect [4] However, the mechanism

of action responsible for CNS activity has not been present study was undertaken. In this communication, an effort was made to find the biochemical parameters including catecholamine, 5-HT and brain amino acids in mice brain and to correlate them with the anticonvulsive property of extract.

MATERIALS AND METHODS

Ethanol extracts of leaves of A.squamosa(AS) dissolved in tween 80 (Ranbaxy India Ltd.) were used for *p.o.* administration. Pentylene tetrazole (PTZ) (Himedia Laboratories Pvt Ltd, Mumbai) was used as chemoconvulsive agent. Epinephrine, norepinephrine, dopamine, 5-HT, GABA, glutamic acid (Central DrugLaboratory, Kolkata) were used as standard catecholamine and diazepam as reference drug.

Preparation of extract

The leaves of *Annona squamosa* were collected during the month of april-may. and authenticated by Regional Research Institute, Bangalore (voucher no. RRI/BNG/SMP/Drug authentication/2008-09/266). The leaves were shade dried and grounded. The powdered material was then extracted twice using hydroalcoholic (30:70) solvent system in a soxhlet apparatus for 12 hrs. The extract was concentrated under reduced pressure using rotary evaporator and stored at 10^0C (yield: 10%, w/w). The extract was reconstituted by dissolving it in 0.9% NaCl solution and then suspending the resultant solution in 0.5% tween 80 suspensions freshly before use.

Animals Experiment

Male swiss albino mice (*Mus musculus*), weighing 25-30 g procured from animal house, B.N.College of Pharmacy, Udaipur (Raj.). The animals were acclimatized for ten days under laboratory conditions. They were housed in polypropylene cages and maintained at 27°C ± 2°C, relative humidity 65 ± 10% under 12 hours light/dark cycle. The animals were fed with commercial diet and water ad libitum. Ethical clearance for performing the experiments on animals was obtained from Institutional Animal Ethics Committee (IAEC) with CPCSEA no. 870/ac/05/CPCSEA.

investigated till date. Keeping this in view, the Pentylenetetrazol at a dose of 80 mg/kg, i.p. was given to eleven groups containing five animals in each pretreated 30 mins prior with varying i.p. doses and 60 mins prior with p.o. doses.

Group I received 0.9% NaCl solution as control

Group II received Tween 80

Group III received diazepam (Dzm-1.0 mg/kg, i.p.)

Group IV received AS 1 (A.squamosa)-125 mg/kg, p.o.

Group V received AS 2 (A.squamosa)-250 mg/kg, p.o.

Group VI received AS 3(A.squamosa)-500 mg/kg, p.o.

The injections were given once a week and the experiments were carried out for a period of 6 weeks. Animals from each group were killed by cervical dislocation 30 min after the last dose. The brains were dissected out, weighed and kept on ice for further processing.

Biochemical estimation

Brains were homogenized with dry n-butanol and then centrifuged. About 4ml aliquots of the clear supernatant were extracted with 3 ml of 0.1 M phosphate buffer. Then, after adding 4% EDTA, 0.2 ml iodine solution, 0.5 ml alkaline sulphite and 0.6 ml 5 N acetic acid, the solutions were heated and cooled. Standard solutions of 0.1 mg/ml of epinephrine, nor epinephrine and dopamine were prepared. The intensities of fluorescence in resulting solutions were determined using a spectrophoto-fluorometer (Perkin Elmer MPF-44B, USA) at wavelengths of 400/500 & 310/365 for epinephrine, nor epinephrine and dopamine respectively [4, 5, 6]
The concentration of 5 HT in the solution was calculated from the standard curves. Paper chromatographic method using an undimentional descending technique was adopted for GABA, glutamate and glutamine analysis. The positions of each amino acid in the chromatogram were developed with ninhydrin. The eluted portions were analysed using a spectrocolorimeter (Systonic M- no103 at 570 mg). [7]

STATISTICAL ANALYSIS

The results were expressed as mean ± SEM. followed by the post-hoc Tukey test and the difference was considered statistically significant (P<0.05).

RESULTS

Results are summarized in **Table 1.** Extracts Statistical analysis done by ANOVA

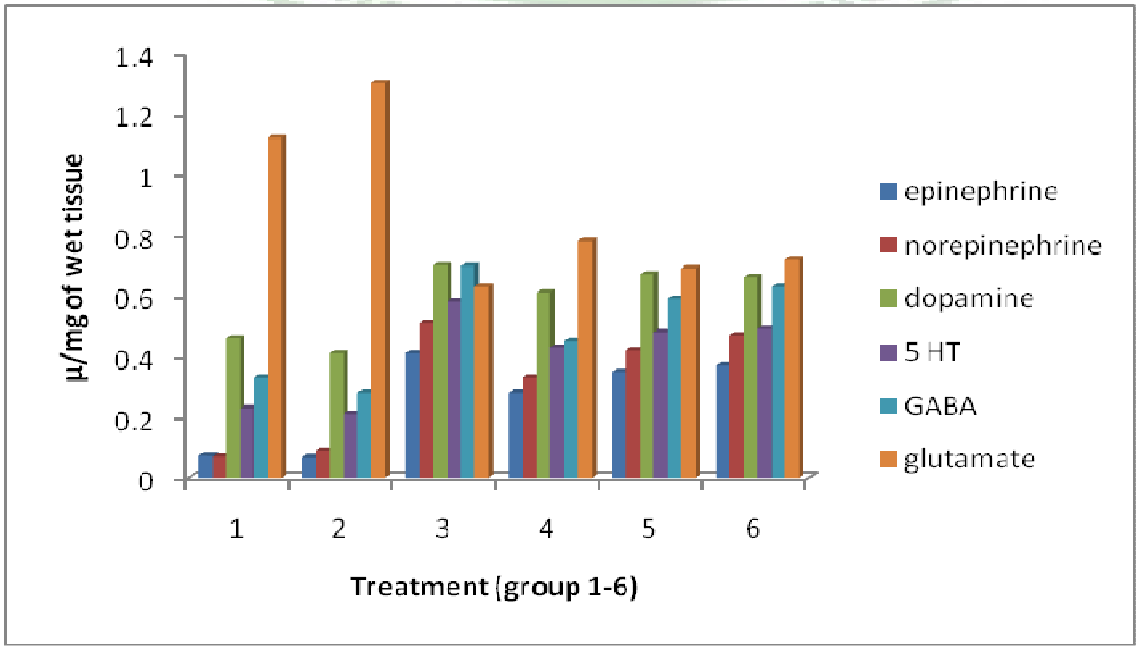
significantly increased (compared to vehicle control mice) the levels of catecholamine in mice brain after a six week treatment in a dose dependent manner. The extract also significantly elevated the levels of GABA ,glutamine and glutamate as compared to their respective control group.

Table 1: **Effect of ethanol extract of leaves of *Annona squamosa* on brain biogenic amines levels in mice after chemo convulsion**

S.no	Doses	Epinephrine	Norepinephrine	Dopamine	5 HT	GABA	Glutamate
1	Normal saline	0.073±0.02	0.07±0.11	0.46±0.12	0.23±0.19	0.33±0.05	1.12±0.06
2	Tween 80	0.068±0.03	0.09±0.02	0.41±0.06	0.21±0.06	0.28±0.05	1.3±0.20
3	DZM + PTZ	0.41±0.12*	0.51±0.16*	0.70±0.09*	0.58±0.11*	0.698±0.15*	0.63±0.21*
4	AS1 + PTZ	0.28±0.01*	0.33±0.04*	0.61±0.04*	0.43±0.06*	0.45±0.11*	0.78±0.27*
5	AS2+PTZ	0.35±0.03*	0.42±0.12*	0.67±0.56*	0.48±0.07	0.59±0.01	0.69±0.51
6	AS3+PTZ	0.37±0.02*	0.47±0.18*	0.66±0.09*	0.49±0.11*	0.63±0.09*	0.72±0.52

ANOVA followed by the post-hoc Tukey test. n=10 for each group; *P<0.05 as compared with PG

Fig: 1 Showing the levels of various brain biogenic amines after treating with the chemoconvulsive agent



DISCUSSION

It is understood from the literature that GABAergic neurotransmission is closely associated with the induction of epilepsy in the animals. [8] GABA is the major inhibitory neurotransmitter in the central nervous system and even slight deficiencies in GABAergic transmission may lead to hyper excitability and pathological neuronal discharges leading to epilepsy. GABA is an endogenous agonist at GABAA receptor (ionotropic receptor) thereby opening the channels to Cl^- ions in the neuronal membrane. [9]

In the present study, the biogenic amines were estimated in the whole brain. Epinephrine and nor epinephrine are essentially excitatory substances, but both catecholamine often have depressant action. [10] Both catecholamine and 5-HT appear to play roles in determining the seizure thresholds for electroshock. [11] Dopamine also functions independently as a neuroregulator. It not only increases the level of 5-HTpromote sleep, but the melatonin, which is synthesized from 5-HT in the pineal gland and may also occur in other parts of the brain, also have a role in sleep and as a potent inducer of sleep. In humans, decreased activity of nor adrenaline and dopamine has been found in some epileptic patients. [12] So, the protection offered by A.squamosa against chemo convulsions in mice probably is due to the increased levels of catecholamine's and 5-HT in brain. On the other hand, it is well established that GABA protects the mice against the convulsion induced by leptazole, etc. As far as GABA is concerned, the following facts support its involvement:

- Lowering levels of GABA in the brain results in the appearance of convulsion;
- Some convulsive drugs found to be GABA antagonists;
- Certain antiepileptic drugs enhance the synaptic action of GABA.[13,14]

On the basis of experimental evidences, it may be concluded that the catecholamine and GABA systems have significant role with respect to CNS depressant and anticonvulsive properties of the processed extracts.

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