



DETERMINATION OF BRAIN BIOGENIC AMINES IN *CYNODON DACTYLON* PERS. AND *CYPERUS ROTUNDUS* L. TREATED MICE

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ABSTRACT

The ethanol extract of aerial parts of *Cynodon dactylon* (EECD) and roots & rhizomes of *Cyperus rotundus* (EECR) 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 both the processed extract showed a significant anticonvulsive property by altering the level of catecholamine and brain amino acids in mice.

Keywords : *Cynodon dactylon*, *Cyperus rotundus*, Biogenic amines, Catecholamines, GABA.

INTRODUCTION

Cynodon dactylon Pers. (Family: Graminae, Purba in Bengali, Dhub in Hindi, Bermuda grass in English) is a creeping grass found in warm climates all over the world between 45° south and north attitude. It grows in open areas where there are frequent disturbances such as grazing, flooding and fire. The juice of the plant is astringent and is applied externally to fresh cuts and wounds. It is also used in treatment of catarrhal ophthalmia, dropsy, hysteria, epilepsy, insanity, chronic diarrhea and dysentery. The plant is folk remedy for anasarea, calculus, cancer, carbuncles, cough, hypertension, snakebites, stones,

gout and rheumatic affections^{1, 2, 3, 4}.

Ethanol extract of aerial parts of *C. dactylon* has also marked CNS depressant⁵ and antioxidant activities⁶.

Cyperus rotundus L.(Family: Cyperaceae, Muthagas in Bengali, Motha in Hindi, Dutgrass in English) is a perennial sedge distributed throughout India. Roots and rhizomes of this plant are used in different diseases like chronic diarrhea, inflammation, skin rashes and excess bleeding. It has also antiestrogenic, antimicrobial, anathematic, antihistaminic, antiemetic, antipyretic, antidiabetic and antioxidant activities^{7, 8}.⁹. Ethanol extract of *C. rotundus* showed marked CNS depressant action (Pal et al.

unpublished observations). However, the mechanism of action responsible for CNS activity of *C. dactylon* and *C. rotundus* has not been investigated till date. Keeping this in view, the 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 each extract.

MATERIALS AND METHOD

Materials

Ethanol extracts of aerial parts of *C. dactylon* (EECD) and roots & rhizomes of *C. rotundus* (EECR) dissolved in propylene glycol (Ranbaxy India Ltd.) were used for *i.p.* administration. Pentylene tetrazole (PTZ) (Himedia Laboratories Pvt Ltd, Mumbai) was used as chemoconvulsive agent. Epinephrine, nor epinephrine, dopamine, 5-HT, GABA, glutamic acid (Central Drug Laboratory, Kolkata) were used as standard catecholamine and pentobarbitone sodium as reference drug.

Preparation of extract

The aerial parts of *C. dactylon* and roots & rhizomes of *C. rotundus* were collected from Panua, Bankura district region of West Bengal, India in the month of June and the taxonomic identification was made by Dr H. J.

Chowdhury, Joint Director, Central National Herbarium, Botanical Survey of India, Howrah, West Bengal, India. The voucher specimen of each sample has been preserved in our laboratory for future reference (Ref no: DM1, DPS1).

Sun-dried, powdered plant materials were soxhlet extracted with ethanol to afford a gummy residue. The ethanol extracts were evaporated to complete dryness. The trace amount of ethanol which might be present within the solid mass of extracts was removed under vacuum. The yield of EECD and EECR were 2.6% and 12.3% respectively. Phytochemical screening¹⁰ gave positive tests for saponins, flavonoids and polyphenolic compounds in EECR⁷ and flavonoids in EECD⁶, respectively.

For pharmacological testing, EECD and EECR were dissolved in propylene glycol (PG).

Animal experiments

Adult male albino mice of Swiss strain (body wt 20-25 gm) were acclimatized in standard environmental conditions and kept on a standard commercial diet (Hindustan Lever Ltd.) and water *ad libitum*. The experimental protocol was subjected to the scrutiny of the Institutional Ethical Committee and was cleared before initiating the experiments. The animals were handled as per guidelines of committee for the

purpose of control and supervision on animals (CPCSEA), New Delhi.

Pentylentetrazole (PTZ) at a dose of 80 mg/kg body wt (b.w.), *i.p.* was used as chemo convulsive agent. Pentobarbitone sodium (30 mg/kg) was used as standard. EECD at 0.050, 0.075, 0.10 g/kg b.w. and EECD at 0.040, 0.060, 0.080 g/kg b.w. were given to mice intraperitoneally 30 min prior to the administration of pentylentetrazole.

Mice were subdivided into 10 groups consisting of 10 mice in each group as follows:

I: Normal saline (0.9% NaCl, w/v, 5 ml/kg, *i.p.*).

II: Propylene glycol (5 ml/kg as vehicle, *i.p.*).

III: Only pentylentetrazole(PTZ) (80 mg/kg, *i.p.*).

IV: Pentobarbitone sodium (30 mg/kg, *i.p.*) administered 30 min before pentylentetrazole (80 mg/kg, *i.p.*).

V: EECD (0.050 g/kg, *i.p.*) + pentylentetrazole (80 mg/kg, *i.p.*).

VI: EECD (0.075 g/kg, *i.p.*) + pentylentetrazole (80 mg/kg, *i.p.*).

VII: EECD (0.10 g/kg, *i.p.*) + pentylentetrazole (80 mg/kg, *i.p.*).

VIII: EECD (0.040 g/kg, *i.p.*) + pentylentetrazole (80 mg/kg, *i.p.*).

IX: EECD (0.060 g/kg, *i.p.*) + pentylentetrazole (80 mg/kg, *i.p.*).

X: EECD (0.080 g/kg, *i.p.*) + pentylentetrazole (80 mg/kg, *i.p.*).

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 4 ml 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 µg/ml of epinephrine, norepinephrine and dopamine were prepared. The intensities of fluorescence in resulting solutions were determined using a spectrophotofluorometer (Perkin Elmer MPF-44B, USA) at wavelengths of 400/500 & 310/365 for epinephrine, norepinephrine and dopamine respectively^{11, 12, 13}.

The concentration of 5 HT in the solution was calculated from the standard curves¹⁴.

Paper chromatographic method using an undimensional 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 spectrophotometer (Sytonic M- no 103 at 570 m μ)^{15, 16}.

Statistical analysis

The results were expressed as mean \pm SEM. Statistical analysis done by ANOVA followed by the post-hoc Tukey test¹⁷ and the difference was considered statistically significant (P<0.05).

RESULTS

Results are summarized in Table 1 and 2. EECD and EECR 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 (γ -amino butyric acid), glutamine and glutamate as compared to their respective control group.

Table 1 : Effect of ethanol extract of aerial parts of *C. dactylon* (EECD) and roots & rhizomes of *C. rotundus* (EECR) on brain catecholamine levels in mice after chemoconvulsion

Drug with dose	Epinephrine	Nor-epinephrine	Dopamine	5-HT
Normal saline (5 ml/kg, <i>i.p.</i>)	0.08 \pm 0.03	0.09 \pm 0.12	0.51 \pm 0.12	0.29 \pm 0.22
Propylene glycol (PG) (5 ml/kg, <i>i.p.</i>)	0.07 \pm 0.04	0.10 \pm 0.08	0.46 \pm 0.08	0.27 \pm 0.08
PTZ(80 mg/kg, <i>i.p.</i>)	0.11 \pm 0.02	0.11 \pm 0.04	0.52 \pm 0.05	0.56 \pm 0.06*
Pentobarb (30 mg/kg, <i>i.p.</i>) + PTZ (80 mg/kg, <i>i.p.</i>)	0.42 \pm 0.18*	0.51 \pm 0.12*	0.76 \pm 0.10*	0.69 \pm 0.14*
EECD (0.050 g/kg, <i>i.p.</i>) + PTZ (80 mg/kg, <i>i.p.</i>)	0.23 \pm 0.06*	0.32 \pm 0.04*	0.67 \pm 0.05*	0.51 \pm 0.06*
EECD (0.075 g/kg, <i>i.p.</i>) + PTZ (80 mg/kg, <i>i.p.</i>)	0.31 \pm 0.08*	0.41 \pm 0.09*	0.72 \pm 0.05*	0.58 \pm 0.03*
EECD (0.10 g/kg, <i>i.p.</i>) +PTZ (80 mg/kg, <i>i.p.</i>)	0.40 \pm 0.06*	0.48 \pm 0.08*	0.76 \pm 0.09*	0.65 \pm 0.10*
EECR (0.040 g/kg, <i>i.p.</i>) +PTZ (80 mg/kg, <i>i.p.</i>)	0.25 \pm 0.03*	0.37 \pm 0.07*	0.64 \pm 0.05*	0.52 \pm 0.07*
EECR (0.060 g/kg, <i>i.p.</i>) +PTZ (80 mg/kg, <i>i.p.</i>)	0.36 \pm 0.07*	0.43 \pm 0.10*	0.70 \pm 0.06*	0.60 \pm 0.07*
EECR (0.080 g/kg, <i>i.p.</i>) +PTZ (80 mg/kg, <i>i.p.</i>)	0.41 \pm 0.09*	0.50 \pm 0.12*	0.76 \pm 0.09*	0.68 \pm 0.12*

Values are expressed as μ g/mg wet brain tissue and are mean \pm SEM, Statistical analysis done by ANOVA followed by the post-hoc Tukey test. n=10 for each group; *P<0.05 as compared with PG

Table 2 : Effect of ethanol extract of aerial parts of *C. dactylon* (EECD) and roots & rhizomes of *C. rotundus* (EECR) on brain GABA, glutamate and glutamine levels in mice after chemoconvulsion

Drug with dose	GABA	Glutamate	Glutamine
Normal saline (5 ml/kg, <i>i.p.</i>)	0.32 ± 0.03	1.38 ± 0.06	0.68 ± 0.07
Propylene glycol (PG) (5 ml/kg, <i>i.p.</i>)	0.30 ± 0.05	1.40 ± 0.22	0.56 ± 0.10
PTZ (80 mg/kg, <i>i.p.</i>)	0.14 ± 0.03	0.82 ± 0.12	0.39 ± 0.05
Pentobarb (30 mg/kg, <i>i.p.</i>) + PTZ (80 mg/kg, <i>i.p.</i>)	0.74 ± 0.14*	4.00 ± 0.99*	1.33 ± 0.20*
EECD (0.050 g/kg, <i>i.p.</i>) + PTZ (80 mg/kg, <i>i.p.</i>)	0.55 ± 0.08*	2.31 ± 0.18*	0.97 ± 0.15*
EECD (0.075 g/kg, <i>i.p.</i>) + PTZ (80 mg/kg, <i>i.p.</i>)	0.60 ± 0.07*	2.62 ± 0.32*	1.06 ± 0.21*
EECD (0.10 g/kg, <i>i.p.</i>) +PTZ (80 mg/kg, <i>i.p.</i>)	0.73 ± 0.10*	3.43 ± 0.70*	1.31 ± 0.26*
EECR (0.040 g/kg, <i>i.p.</i>) +PTZ (80 mg/kg, <i>i.p.</i>)	0.55 ± 0.07*	2.42 ± 0.39*	0.81 ± 0.06*
EECR (0.060 g/kg, <i>i.p.</i>) +PTZ (80 mg/kg, <i>i.p.</i>)	0.61 ± 0.08*	2.72 ± 0.51*	0.93 ± 0.12*
EECR (0.080 g/kg, <i>i.p.</i>) +PTZ (80 mg/kg, <i>i.p.</i>)	0.66 ± 0.12*	3.31 ± 0.81*	1.21 ± 0.22*

Values are expressed as µg/mg wet brain tissue and are mean ± SEM, Statistical analysis done by ANOVA followed by the post-hoc Tukey test. n=10 for each group; *P<0.05 as compared with PG

DISCUSSION

It is known that anticonvulsants mediate their action through alteration in various neurotransmitter levels in various regions of the brain. 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 action¹⁸. Both catecholamine and 5-HT appear to play roles in

determining the seizure thresholds for electroshock¹⁹. Dopamine also functions independently as a neuroregulator. It not only increases the level of 5-HT promote 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^{20, 21}. So, the protection

offered by EECD and EECR against chemo convulsions in mice probably is due to the increased levels of catecholamines and 5-HT in brain. On the other hand, it is well established that GABA (γ -amino butyric acid) protects the mice against the convulsion induced by leptazole, etc.¹⁶. As far as GABA is concerned, the following facts support its involvement:

- a) Lowering levels of GABA in the brain results in the appearance of convulsion;
- b) Some convulsive drugs found to be GABA antagonists;
- c) Certain antiepileptic drugs enhance the synaptic action of GABA^{20, 22, 23}.

In addition to GABA, the increased level of glutamate and glutamine may also be correlated with the anticonvulsive property of EECD and EECR. Increase in the levels of glutamate and glutamine is possibly a result of accelerated conversion of α -ketoglutarate to glutamine acid transmission of glutamine and reduced oxidation of α -ketoglutarate through the succinate pathway²⁴⁻³³.

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

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