

STUDIES ON BRAIN BIOGENIC AMINES IN METHANOLIC EXTRACT OF *CUSCUTA REFLEXA* ROXB. AND *CORCHORUS OLITORIUS* LINN. SEED TREATED MICE

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Abstract: The methanolic extract of both *Cuscuta reflexa* stem and *Corchorus olitorius* seed showed marked protection against convulsion induced by chemoconvulsive 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 a six week treatment. Results of the present study revealed that both the processed extracts showed a significant anticonvulsive property by altering the level of catecholamines and brain amino acids in mice.

Keywords: *Cuscuta reflexa*, *Corchorus olitorius*, anticonvulsive, catecholamines.

Cuscuta reflexa Roxb. (Swarnalata in Bengali, Amarvel in Hindi) from the family *Convolvulaceae*, is a golden yellow dodder like a parasite. The plant is common throughout India, found widely in the plains of West Bengal, growing on thorny or other shrubs as parasite annuals. Various parts of this plant were used in tribal medicine for the diseases like fits, melancholy, and insanity (1). It is also useful externally against itch and internally in protracted fevers, retention of wind and induration of the live (2, 3, 4). *Cuscuta reflexa* on preliminary analysis is found to contain 2% cuscutin (a colouring matter), 1% cuscotalin (lactone) and a large quantity of flavonoids (5, 6, 7).

Corchorous olitorius L. (family *Tiliaceae*) is an annual herb with slender stems. It is cultivated in many parts of India. The seeds are used as purgative and leaves as demulscent, diuretic, febrifuge (infusion) and in chronic cystitis and dysuria (8). On preliminary analysis, seeds have been found to contain cardenolide glycosides (9, 10). Both the methanolic extract of *Cuscuta reflexa* stem and *Corchorus olitorius* seed exhibited Central Nervous System (CNS) activities such as behaviour, sedative, hypnosis, analgesic and anticonvulsant effects in mice (unpublished data). However, the mechanism of action responsible for this activity has not been investigated. Keeping this in view, the present study was undertaken. In this communication, an effort

was made to find the biochemical parameters including catecholamines, 5-HT and brain amino acids in mice brain and to correlate them with the anticonvulsive property of each extract.

EXPERIMENTAL

Materials

Methanolic extract of the stem of *C. reflexa* and *C. olitorius*, dissolved in propylene glycol (Ranbaxy India Ltd.) for *i.p.* administration, strychnine (Sigma) was used as chemoconvulsive agent. Epinephrine, norepinephrine, dopamine, 5-HT, GABA, glutamic acid (Central Drug Laboratory, Kolkata) were used as standard catecholamines and Pentobarbitone sodium as reference drug.

Preparation of extract

Sun-dried, powdered plant material was Soxhlet extracted with MeOH to afford a gummy residue. Besides the presence of lactones, phytochemical screening (11) gave positive tests for flavonoids in ME of *Cuscuta reflexa*. For pharmacological testing, methanolic extract of *Cuscuta reflexa* stem (MECR) and *Corchorus olitorius* seed (MECO) were dissolved in propylene glycol (PG).

Animal experiments

Adult male albino mice of Swiss strain (Body

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wt 20–25 gm) were acclimatized in standard environmental conditions and kept on a standard commercial diet and water *ad libitum*.

Strychnine at a dose of 1 mg/kg body weight (b.w.) was used as chemoconvulsive agent. Pentobarbitone sodium (20 mg/kg) was used as standard. MECR at 0.025, 0.050, 0.070 g/kg b.w. and MECO at 0.015, 0.020, 0.025 g/kg b.w. were given to mice intraperitoneally 15 min prior to the administration of strychnine.

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

Subgroups

- 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 strychnine (1 mg/kg *i.p.*)
- IV: Pentobarbitone sodium (30 mg/kg, *i.p.*) administered 30 min before strychnine (1 mg/kg, *i.p.*)
- V: MECR (0.025 g/kg, *i.p.*) + Strychnine (1 mg/kg, *i.p.*)
- VI: MECR (0.050 g/kg, *i.p.*) + Strychnine (1 mg/kg, *i.p.*)
- VII: MECR (0.075 g/kg, *i.p.*) + Strychnine (1 mg/kg, *i.p.*)
- VIII: MECO (0.015 g/kg, *i.p.*) + Strychnine (1 mg/kg, *i.p.*)
- IX: MECO (0.020 g/kg, *i.p.*) + Strychnine (1 mg/kg, *i.p.*)
- X: MECO (0.025 g/kg, *i.p.*) + Strychnine (1 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 (12, 13, 14).

The concentration of 5-HT in the solution was calculated from the standard curves (15).

Paper chromatographic method using an unidimensional descending technique was adopted for

GABA, glutamate and glutamine analysis. The positions of each amino acids in the chromatogram were developed with ninhydrin 90.1%. The eluted portions were analysed using a spectrophotometer (Systonic M – no 103 at 570 mµ) (16, 17).

Statistical analysis

The results were expressed as mean ±SEM and analysed by Student's t test (18) and the difference was considered statistically significant (P<0.05).

RESULTS

Results are summarised in Tables 1 and 2. Methanolic extract of both *C. reflexa* stem (MECR) and *C. olitorius* seed (MECO) significantly increased (compared to vehicle control mice) the levels of catecholamines in mice brain after a 6 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 groups.

DISCUSSION

It is known that anticonvulsants mediate their action through alternation 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 norepinephrine are essentially excitatory substances, but both catecholamines often have depressant action (19). Both catecholamines and 5-HT appear to play roles in determining the seizure thresholds for electroshock (20). Dopamine also functions independently as a neuroregulator. It not only increases the level of 5-HT promote sleep, but the melatonin, which is synthesised 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 noradrenaline and dopamine has been found in some epileptic patients (21). So, the protection offered by MECR and MECO against chemoconvulsions 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 strychnine, leptazole, etc. (17). 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;

Table 1. Effect of methanolic extract of *C. reflexa* stem (MECR) and *C. oltorius* seed (MECO) on brain catecholamine levels in mice after chemoconvulsion

Drug with Dose	Epinephrine	Norepinephrine	Dopamine	5 HT
Normal saline (5 ml/kg, <i>i.p.</i>)	0.08 ± 0.02	0.09 ± 0.31	0.50 ± 0.13	0.28 ± 0.52
Propylene glycol (PG) (5 ml/kg, <i>i.p.</i>)	0.07 ± 0.03	0.10 ± 0.09	0.46 ± 0.07	0.27 ± 0.09
Strychnine (1 mg/kg, <i>i.p.</i>)	0.10 ± 0.02	0.10 ± 0.04	0.53 ± 0.05	0.55 ± 0.07*
Pentobarb (30 mg/kg, <i>i.p.</i>)	0.42 ± 0.19*	0.50 ± 0.13*	0.75 ± 0.10*	0.68 ± 0.15*
+ Strychnine (1 mg/kg, <i>i.p.</i>) MECR (0.025 g/kg, <i>i.p.</i>)	0.22 ± 0.06*	0.31 ± 0.04*	0.66 ± 0.05*	0.50 ± 0.06*
+ Strychnine (1 mg/kg, <i>i.p.</i>) MECR (0.050 g/kg, <i>i.p.</i>)	0.30 ± 0.09*	0.40 ± 0.10*	0.71 ± 0.06*	0.57 ± 0.03*
+ Strychnine (1 mg/kg, <i>i.p.</i>) MECR (0.075 g/kg, <i>i.p.</i>)	0.39 ± 0.07*	0.47 ± 0.09*	0.74 ± 0.10*	0.64 ± 0.11*
+ Strychnine (1 mg/kg, <i>i.p.</i>) MECO (0.015 g/kg, <i>i.p.</i>)	0.24 ± 0.03*	0.36 ± 0.08*	0.63 ± 0.04*	0.51 ± 0.06*
+ Strychnine (1 mg/kg, <i>i.p.</i>) MECO (0.020 g/kg, <i>i.p.</i>)	0.35 ± 0.08*	0.42 ± 0.11*	0.68 ± 0.07*	0.59 ± 0.08*
+ Strychnine (1 mg/kg, <i>i.p.</i>) MECO (0.025 g/kg, <i>i.p.</i>)	0.40 ± 0.10*	0.49 ± 0.11*	0.73 ± 0.10*	0.66 ± 0.13*

Values are expressed as µg/mg wet brain tissue and are mean ± SEM.
n = 10 for each group, * P < 0.05 as compared with PG.

Table 2. Effect of methanolic extract of *C. reflexa* stem (MECR) and *C. oltorius* seed (MECO) 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>)	0.31 ± 0.04	1.39 ± 0.05	0.67 ± 0.08
Propylene glycol (PG) (5 ml/kg, <i>i.p.</i>)	0.30 ± 0.06	1.40 ± 0.21	0.56 ± 0.09
Strychnine (1 mg/kg, <i>i.p.</i>)	0.13 ± 0.02	0.81 ± 0.13	0.38 ± 0.06
Pentobarb (30 mg/kg, <i>i.p.</i>)	0.73 ± 0.14*	3.99 ± 0.99*	1.32 ± 0.21*
+Strychnine (1 mg/kg, <i>i.p.</i>) MECR (0.025 g/kg, <i>i.p.</i>)	0.54 ± 0.09*	2.30 ± 0.19*	0.96 ± 0.16*
+Strychnine (1 mg/kg, <i>i.p.</i>) MECR (0.050 g/kg, <i>i.p.</i>)	0.59 ± 0.08*	2.61 ± 0.33*	1.05 ± 0.22*
+Strychnine (1 mg/kg, <i>i.p.</i>) MECR (0.075 g/kg, <i>i.p.</i>)	0.71 ± 0.11*	3.41 ± 0.70*	1.29 ± 0.27*
+Strychnine (1 mg/kg, <i>i.p.</i>) MECO (0.015 g/kg, <i>i.p.</i>)	0.55 ± 0.08*	2.41 ± 0.40*	0.80 ± 0.07*
+Strychnine (1 mg/kg, <i>i.p.</i>) MECO (0.020 g/kg, <i>i.p.</i>)	0.60 ± 0.09*	2.71 ± 0.52*	0.92 ± 0.13*
+Strychnine (1 mg/kg, <i>i.p.</i>) MECO (0.025 g/kg, <i>i.p.</i>)	0.64 ± 0.12*	3.29 ± 0.81*	1.19 ± 0.22*

Values are expressed as µg/mg wet brain tissue and are mean ± SEM.
n = 10 for each group, * P < 0.05 as compared with PG.

c) certain antiepileptic drugs enhance the synaptic action of GABA (22).

In addition to GABA, the increased level of glutamate and glutamine may also be correlated with the anticonvulsive property of MECR and MECO. Increase in the levels of glutamate and glutamine is possibly a result of accelerated conversion of α -ketoglutarate to glutamic acid transmission of glutamine and reduced oxidation of α -ketoglutarate through the succinate pathway (23).

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

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