



Effect of Methanolic Extract of *Sarcostemma acidum* (Roxb) Voigt on Brain Neurotransmitters for Its Anti-Psychotic Potential

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Abstract

Psychiatric disorders occur due to the disturbances of neuronal network and neurotransmitters. The neurotransmitters, mainly responsible for those actions were Dopamine, Serotonin, γ – amino butyric acid (GABA), acetyl choline and adrenaline. The drugs and medications currently available for the treatment of psychiatric disorders include benzodiazepines and barbiturates, which produce beneficial effects along with severe extrapyramidal side effects. Medicinal plants have reported having CNS activity useful for treating human psychological problems and research as products for neurological disorders has progressed rapidly. *Sarcostemma acidum* Roxb. Voigt a traditional medicinal plant has reported to produce several psychopharmacological effects, which includes CNS inhibitory activity, anti-psychotics and anxiolytics. Even though no proper scientific data exists. In this study, we studied the effect of methanolic extract of *S. acidum* (MESA) on brain neurotransmitter levels using rats. We evaluated the levels of GABA, Dopamine and Serotonin employing standard procedures. From the results, it was found that MESA at the dose of 200 mg/kg produced a significant increase in all the estimated neurotransmitters when compared to that of control. The effect produced is similar to that of haloperidol. On the other side MESA at the dose of 100 mg/kg produced no significant change with that of control, which shows the dose-dependent activity. The concentration of Serotonin and GABA was increased. The anti-psychotic activity may be due to the action on serotonin, and the CNS depressant activity may be due to GABAergic action. Dopaminergic blocking activity may also be a probable mechanism of anti-psychotic activity.

Keywords*Sarcostemma acidum*, Neurotransmitter, Anti-psychotics, Serotonin, Dopamine, GABA.

INTRODUCTION

According to world health report (WHO 2001) about 450 million people are having mental disorder and only a few about 13% receive proper medical treatment [1]. The CNS drugs were the first class of drugs discovered by primitive humans for it produced physiological and psychological effects. From the vast array of medicinal plants of *Materia Medica*, many plants have reported having CNS activity useful for treating human psychological problems. Medicinal plant research as products for neurological disorders has progressed rapidly. Variety of animal models has been used for testing these products. The tests are based as psychomotor performance, motor behaviour and neurotoxicity. Depressive activity of CNS is an indication of level of excitability of CNS [2].

Psychiatric disorders occur due to the disturbances of neuronal network and neurotransmitters. The central nervous system processes information with the help of chemical messengers namely neurotransmitters, neuromodulators, neuro mediators and neurotropic factors like Nor-adrenaline, Adrenaline, Dopamine, Gamma Amino Butyric acid (GABA), Glutamate, Acetyl choline, 5-Hydroxytryptamine (5-HT), Peptides and Prostaglandins [3]. Anti-depressants reduce the symptoms of mood disorders by nor-epinephrine and 5-HT receptor mediation [4]. Anxiolytics are useful in the treatment of anxiety disorders. Antipsychotics are dopamine antagonists; they act on the nigrostriatal system.

The dopamine antagonism must be 60-80-% for D2 receptors. The first-generation anti-psychotics antagonize dopamine transmission, but they can produce Parkinsonism like side effects. The second-generation atypical antipsychotics have a greater serotonin / dopamine ratio. They have fewer extrapyramidal side effects [5]. The effect is due to 5-HT-2 receptor blockade along with D2 blockade [6]. In this study we evaluated, the methanolic extract of *Sarcostemma acidum* (Roxb) Voigt for its anti-psychotic potential via different brain neurotransmitters.

MATERIALS AND METHODS**Materials**

Dihydromyricetin was purchased from Amazon.in; o-Phthaldialdehyde [OPT] (P1378), Dopamine

(D2960000), Serotonin (14927) and GABA (A2129) standards were obtained from Sigma Aldrich, USA.

Experimental design

Wistar albino rats of both sex weighing 150-200 gm were maintained at standard conditions (25°C, RH 55% and 12 hr light dark cycle) and water was given *ad libitum* along with standard animal feed. The studies on animals were conducted only after getting prior approval from the institutional animal ethics committee (Approval No: IAEC/PhD/DPS/2018-02). Toxicological study was conducted as per OECD 423 guidelines [7]. The doses were fixed after the acute toxicity studies. The animals were grouped into five groups each containing six animals (Table 1). After 28 days of drug administration, the animals were sacrificed by cervical dislocation under anaesthetic conditions followed by decapitation, and the brains were isolated for biochemical assays.

Quantification of GABA

The preparation of the stock solution was done by dissolving GABA (10 mg) in 0.1N HCl acid (10 mL). Then the working standard of concentration ranging from 1 ng to 200 ng/5µL was produced from it. The final volume was made up to 10 mL with 0.1N HCl. The rats were fasted for a whole night and then euthanized. Immediately their brains were dissected and washed in ice cold saline to clean and remove blood clots. The cortex, hippocampus and striatum of the brain have been dissected on ice cold platform. Integration of tissues in 0.1N HCl was completed using a manual tissue homogenizer. The homogenates were collected in polypropylene tubes and centrifuged at 4500 rpm for 20 min at room temperature. The clear supernatant was then transferred into micro centrifuge tubes and used immediately for spot application. Ninhydrin solution was prepared by dissolving of Ninhydrin (200 mg), and its dissolution was done in a small volume of acetone. Then the volume was made upto 100 mL with acetone by adding one mL of pyridine. It also made use of pre coated HPTLC silica gel GF 254 plates. Mobile phase was n-butanol: glacial acetic acid: water 65:18:28 in through glass chamber (20×10) in ascending mode, saturation time three hours. The instrument was HPTLC (Camag) Camag TLC scanner III and detection using 0.2% w/v ninhydrin at 486 nm. Relative humidity was 35%–65% GABA standard solution (5 µL) was used. Triplicate spotting was done for standard (1 ng to

200 ng) on pre-coated HPTLC plate. Spots were dried. The mobile phase was n-butanol: glacial acetic acid: water (65: 18: 28). When the elution was complete, the plates were dried in an oven. The process was repeated using sample and the developed plates was

dipped in Ninhydrin solution and dried at 60–65°C. The band scanning was done at 486 nm and the peak areas were recorded. The calibration curve was plotted using applied concentration versus areas [8].

The levels of GABA are calculated by using the following formula;

$$A = \frac{\text{Unknown OD}}{\text{Standard}} \times \frac{\text{Standard (3}\mu\text{g)}}{\text{Volume spotted (10}\mu\text{l)}} \times \frac{1000}{W}$$

A = Amino acid content in $\mu\text{moles/gram}$ wet weight tissue

1000 = Conversion factor for gram wet weight tissue

W = weight of the tissue in grams.

Quantification of Dopamine and Serotonin

On the day of experiment, the rats were sacrificed by cervical dislocation under anaesthesia followed by decapitation. The whole brain was dissected out without any injury, and the sub cortical region (including the striatum) was separated. Butanol–HCl (5mL) was used to homogenize tissue. An aliquot of supernatant liquid (1mL) was removed after centrifuging at 2000 rpm and was mixed with 2.5 mL heptane and 0.31 mL of 0.1 molar HCl in a centrifugal tube. After 10 min of vigorous shaking, the tube was centrifuged under the same conditions as above in order to separate the two phases, and the overlaying organic phase were discarded. The aqueous phase (0.2 mL) was then taken either for 5-HT and DA assay. All steps were carried out at 0°C. Tissue (50-75 mg) was taken for homogenizing with five mL of HCl-Butanol in correlation of same tissue concentration 1.5-5 mg/0.1 mL of HCl-butanol [9].

Estimation of Dopamine

Hydrochloric acid (0.05 mL) and of Sodium acetate buffer pH 6. 9 (0.1 mL) were added to the aqueous phase (0.2 mL). Then, iodine solution (0.1 mL) was added for oxidation. Sodium sulphite solution (0.1 mL) was added after two min to stop the reaction. After a time period of 1.5 min, acetic acid solution (0. 1mL) was added. Next the heating of the solution to 100°C was done for 6 min. Excitation and emission spectra were checked from the spectrofluorometer (Shimadzu RF-6000); the readings were taken at 330-375 nm for dopamine. The reagents of the oxidation were added in the reverse order to prepare the tissue blanks for dopamine (sodium sulphite before iodine).

Estimation of Serotonin

OPT reagent (0.25 mL) was added to the aqueous extract (0.2 mL). Fluorophore was developed when the solution was heated to 100°C for 10 min. The readings were taken at 360-470 nm in the spectrofluorometer (Shimadzu RF-1600). When the

samples reached equilibrium with the ambient temperature, conc. HCl (0.25 mL) without OPT was added for serotonin tissue blank. Preparation of internal Standard: (500 $\mu\text{g/mL}$ each of dopamine and serotonin were dissolved out in distilled water: HCl-butanol at 1:2 ratios).

Statistical analysis

The statistical analysis was carried out by one-way ANOVA followed by Tukey's multiple comparisons *post-hoc* test using GraphPad Prism software (version 7.0). All the Values were expressed as Mean $\pm$ SEM, n=6; with $p < 0.05$ considered as significantly different. * - $p < 0.05$, ** - $p < 0.01$, *** - $p < 0.001$, ns – non-significant.

RESULTS AND DISCUSSION

The important neurotransmitters in CNS include acetyl choline, biogenic amines, neuropeptides and a number of other amino acids. In CNS; acetyl choline acts as an excitatory or inhibitory neuro transmitter of several neuronal tracts, including that present in hippocampus, cerebral cortex and basal ganglia. The amino acids are important neurotransmitters in brain and spinal cord. GABA and Glycine are inhibitory whereas Glutamate and Aspartate are excitatory. GABA is synthesized from glutamic acid. Its receptors are inotropic GABA-A and metabotropic GABA-B. GABA neurotransmission gets altered usually when drugs activate or inhibit GABA-A chloride ion channel complex. The ion channel complex contains a receptor for many drugs and their medication together with general anaesthetics, alcohol, benzodiazepines and barbiturates. GABA regulates neuronal excitability throughout CNS and motor co-ordination [10,11]. In our study Haloperidol and MESA 200 was found to increase the GABA levels in the brain [0.3625 and 0.3578 mg/g wet tissue respectively] ($p < 0.001$) when compared to that of control whereas Dihydromyricetin increased the GABA levels of up to 0.3175 ($p < 0.01$). On the other side, MESA 100 haven't produced significant activity, which shows the dose-dependent activity of MESA (Fig. 1).

The biogenic amines namely, dopamine and serotonin are formed by the decarboxylation of amino acids and are catabolized by enzyme mono amino oxidase. Dopamine is the major neurotransmitter of CNS, which acts by binding to five types of receptors D₁ to D₅. Clozapine and levodopa have effects on various steps in synthesis and metabolism of Dopamine. Dopamine plays a major role in behavioural and drug reinforcement and regulates emesis, prolactin release, mood states

[12]. Administration of MESA 200 to animals showed significant alteration in the brain dopamine and serotonin levels ($p < 0.01$) (Fig 2 & 3), on the other side MESA 100 showed no significant change ($p > 0.05$) in those neurotransmitter levels when compared to that of control. Dihydromyricetin produced significant increase ($p < 0.01$) in the serotonin levels, whereas on dopamine it showed no significant change ($p > 0.05$).

Table 1. Treatment protocol for the estimation of brain neurotransmitter levels.

S. No	Groups	Dose (mg/kg)
1	Control	-
2	Standard (Haloperidol)	5
3	MESA200	200
4	MESA 100	100
5	Dihydromyricetin	5

Estimation of GABA

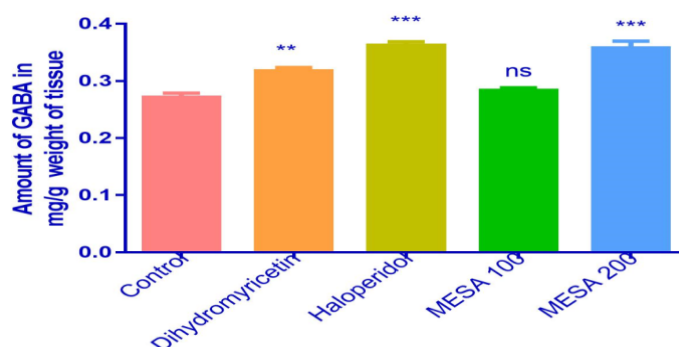


Figure 1. Estimation of γ – amino butyric acid levels in brain

Estimation of dopamine

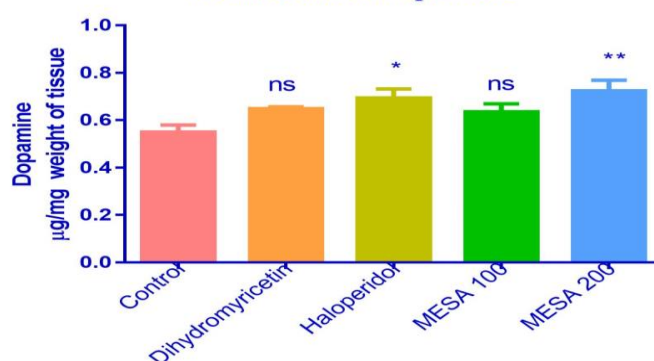


Figure 2. Estimation of dopamine levels in brain

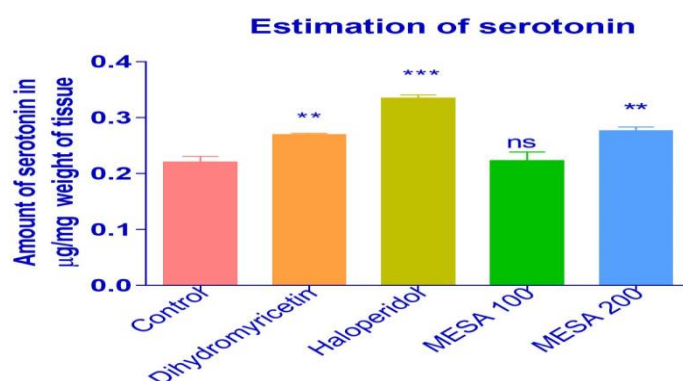


Figure 3. Estimation of serotonin levels in brain

CONCLUSION

In the present study, we estimated the monoamines in the rat brain by using spectrofluorometer. The serotonin and other neurotransmitters do not contribute significantly to fluorescence produced by dopamine. The concentration of neurotransmitter 5-HT and GABA increased followed by the administration of the drug during study. The probable anti-psychotic activity mechanism underlying may be due to the action on serotonin, and the CNS depressant activity may be due to GABAergic action. Dopaminergic blocking activity was also a probable mechanism of anti-psychotic activity. Dihydromyricetin has influenced the neurotransmitter concentration in the rat brain, compared to MESA 200 activities.

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