Central inhibitory effect of *Moringa oleifera* root extract: Possible role of neurotransmitters

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Effect of chronic treatment of standardized aqueous extract of *Moringa oleifera* (MO) root (100, 200, 300, 350, 400, 450 mg/kg, p.o) on pentetrazol (PCN) induced convolution, locomotor behaviour, brain serotonin (5-HT), dopamine (DA) and noradrenaline (NE) level was studied in Holtzman strain adult albino rats. The result revealed that pretreatment with MO inhibited PCN-induced seizure and markedly reduced locomotor activity. Chronic treatment with MO significantly increased the 5-HT and decreased the DA level in cerebral cortex (CC), midbrain (MB), caudate nucleus (CN) and cerebellum (CB). NE level was significantly decreased in CC but no appreciable change was observed in MB, CB and CN. Thus the central inhibitory effect of MO is discussed in the light of the disturbed balance between 5-HT, DA and NE.

**Keywords**: Central inhibitory effect, *Moringa oleifera*, Neurotransmitters

*Moringa oleifera* (MO), a plant the family Morinicaeae, is found almost all over the plains of India, particularly in West Bengal. Folkmedicine studies revealed that its roots are useful in hysteria, flatulence and epilepsy. Extensive pharmacological investigation has been reported on flower, pod, seed, leaves and roots of MO. The root extracted in 50% ethanol has no antibacterial property while aqueous extract possesses anti-inflammatory and antifertility action. The aqueous root extract of MO changes the histarchitecture of the genital tract of ovarietomized rats. It also possesses antimplantation action by altering the hormonal properties. The bark of the root has toxic action and also causes hepatorenal and hematological dysfunction. The alcoholic root extract reduced amphetamine-induced pressure response. Sporadic information is reported on CNS calming and adrenenergic neurone blocking effect.

In our preliminary studies, effect of MO on pentobarbital-induced sleeping time has been reported, but little or no systematic study has been done on its action on the CNS. Thus, the present study was undertaken to determine the effect of MO root extract on CNS.

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**Materials and Methods**

**Animals**

Pure (colony) bred Holtzman strain adult albino rats (150-200 g) of either sex were housed individually in a photoperiod cycle of 12 hr:12 hr (light and dark), at room temperature (about 28°C) and maintained on a daily schedule of standard laboratory diet. Drinking water was supplied ad libitum. Body weight of the rats were recorded daily and maintained throughout the experimental period.

**Preparation of extract**

The roots of MO were purchased in bulk from the local market and the identity of the plant was authenticated by the Botanical Survey of India, Howrah and kept in the Department of Physiology, Calcutta University. This bulk amount was used throughout the experimental study. The root bark was discarded because it has toxic action. The woody portion of the roots (1 kg) was crushed, sun-dried, ground and spread over tray with shifting of materials daily to avoid growth of fungus. The powder was soaked in water overnight and the extractive solution was filtered with Whatman No. 1 filter paper and subjected to lyophilization. The final yield was 13%. The aqueous root extract of MO was standardized to contain 0.2% moringine and moringinine.
Animal treatment

Schedule-1: Forty two Holtzman strain adult albino rats (150-200 g) of either sex were divided into control (group I) and experimental (group II, III, IV, V, VI and VII). Group I rats were treated with saline (5 ml/kg, po) for a period of 14 days. Group II, III, IV, V, VI and VII rats received the MO extract 200 μl-300 μl (100, 200, 300, 350, 400 and 450 mg/kg, po) once daily for 14 consecutive days between 9:00 and 11:00 am. The locomotor activity (both control and experimental groups) was recorded on the 14th day. On the 15th day, the animals were sacrificed by cervical dislocation (between 11:00 and 12:00 am) and the biochemical estimation of serotonin (5 HT), dopamine (DA) and noradrenaline (NE) in different brain areas was performed.

Schedule-2: Forty two Holtzman strain adult albino rats (150-200 g) of either sex were divided into saline pretreated (5 ml/kg, po) epileptic control (group I) and MO extract pretreated experimental epileptic rats (group II, III, IV, V, VI and VII). Rats of group II, III, IV, V, VI and VII received MO extract (100, 200, 300, 350, 400 and 450 mg/kg, orally) once daily for a period of 14 days. On the 15th day epileptic animals were prepared by intracerebral injection of penicillin (PCN).

Locomotor activity

The effect of MO on locomotor activity was recorded by using open field test. The open field test apparatus (1 x 1 m) was made up of plywood surrounded by wall 40 cm high with inside surface painted black. It was strongly lit by two 150 W lamps from 150 cm above. The surface of the floor was divided into 25 squares of same size. The open field was placed in a sound proof room. The animals was placed gently in the center of the apparatus, where they were free to walk and to get adjusted to the new environment. After the training, the animals were treated with saline or MO (100, 200, 300, 350, 400 and 450 mg/kg, po) and 30 min later the animals were placed individually in the apparatus and the number of squares traversed in 5 min was noted. The same protocol was followed for a period of 14 days.

Preparation of epileptic animals

Prior to surgery, all the animals were fasted overnight but had free access to water. Rats were anaesthetized with ether (Kabini Drugs Ltd, India). The anaesthetized animals were mounted on stereotaxic apparatus (INCO, India Ltd) with care, to prevent the damage of the tympanic membrane. Head was fixed in such a position that lambda and bregma were in the same horizontal plane. All surgery was performed under strict aseptic conditions. The scalp was incised in the midline and the pericranial muscles were retracted laterally. After retracting the meningeal muscles, the overlying bone was drilled at the specific loci (somatosensory cortex 3.2 mm posterior to bregma, 2.25 mm lateral to the midline and depth 1.5 mm) following the coordinates of the stereotaxic atlas. Bleeding, if any, was controlled by aseptic bone wax. After a trephine hole was bored in the skull, dura mater was split by the tip of a needle and 100 units (100 μl) of freshly prepared Benzyl penicillin G-Sodium salt solution (Alimbic Ltd, India) was injected intracortically in the somatosensory cortex perpendicular to the surface, taking great care so as to keep the deeper brain areas undisturbed. Care was also taken to prevent any reflex of the solution by holding the needle in position for 15-20 sec. After injecting penicillin, the trephine hole was covered with sterile bone wax and skin and muscle were sutured back separately. Neosporin powder was applied on the wound site as antiseptic measure. Penicillin (10,000 IU) was injected intramuscularly on the day of the operation and for the next two consecutive days as antibiotic measure. After surgery, the rats were transferred to the observation cage and changes in behaviour pattern were noted every 15 min for 2 hr, according to a modified version of scale as—no response = 0; gustatory movements and fictive scratching = 1; tremors = 2; head bobbing = 3; forelimb clonus = 4; rearing, falling and clonus = 5. The onset of convulsions and duration of seizures were recorded.

Biochemical estimation of 5-HT, DA and NE

The animals were sacrificed by cervical dislocation (between 11:00 and 12:00 am). Brain tissues were dissected out, washed in ice cold saline and homogenised in 10 ml acidified butanol. Homogenate (4 ml) was mixed with 10 ml 10% heptane and 5 ml 0.003 N HCL and then shaken for 5 min and centrifuged at 2000 rpm for 10 min. Acid layer (4.5 ml) was eluted and mixed with 200 mg alumina and 1 ml of 2 M sodium acetate. The mixture was shaken for 5 min and centrifuged at 2000 rpm for 10 min.

Supernatant was taken for estimation of 5-HT and precipitate was used for estimation of DA and NE.

Supernatant was mixed with 3 volume of 10% isobutanol, shaken twice with equal volume of salt
saturated buffer at pH 10. Then 2 volumes of 10% neat water was added to the butanol phase and 5 ml of 0.1 N HCl was added and shaken well and then the mixture was made 0.3 N with respect to HCl. This was taken for estimation of 5 HT.

Cold distilled water (5 ml) was added to the precipitate, shaken well and then centrifuged at 2000 rpm for 30 sec. 3 ml of 0.33 N acetic acid was added and centrifuged at 2000 rpm for 3 min. Supernatant was transferred to glass stoppered centrifuge tube. 1.2 ml of freshly prepared ethylenediamine and ethylenediamine dihydrochloride mixture (7:5) was added to it and incubated at 50°C for 40 min. Mixture was cooled at room temperature and saturated with sodium chloride and then 4 ml 10% isobutanol was added. It was centrifuged at 2000 rpm for 3 min. The supernatant was taken for estimation of DA and to the precipitate 4 ml of distilled water was added. This was taken for estimation of NE. The fluorescence of 5 HT, DA and NE was measured in the Perkin Elmer MPF 44B Fluorescence spectrophotometer with activation and emission wavelength set at 295 and 550 nm (for 5 HT), 320 and 370 nm (for DA) and 385 and 485 nm (for NE).

Results

Locomotor activity

MO (100-450 mg/kg) dose dependently inhibited the locomotor activity of the rats. At lower doses (100, 200mg/kg), the locomotor activity decreased but the decrease was not statistically significant. At higher doses (300, 350, 400 mg/kg), the locomotor activity was significantly decreased as compared to the control rats. The effect of MO was most effective at the dosage of 350mg/kg. At 450 mg/kg dose, the locomotor activity was also decreased but the value was not statistically significant with respect to control (Table 1).

Penicillin-induced epileptic animals

MO at low doses (100, 200mg/kg) did not reveal any change in onset, duration and severity score of the penicillin-induced epileptic animals. At higher doses (300, 350, 400 mg/kg) the root extract showed an increase in onset of convulsion, decrease in duration and severity of score. The dose 350mg/kg prevented the occurrence of seizure. Thus showing protection against penicillin-induced convulsion. Above 400mg/kg, there was no significant change in onset, duration and severity score (Table 2).

Changes in 5-HT, DA and NE level

MO in a dose of 300mg/kg, increased the 5-HT level in CC, MB, CB and CN, but the DA level was decreased in CC, MB, CN and CB. NE level was also decreased in CC while in MB, CN and CB did not alter from control group. The most effective changes in 5-HT, DA and NE level was found at the dose of 350 mg/kg (Table 3).

Discussion

The present study evaluates the effect of aqueous root extract of MO on the locomotor behaviour and PCN-induced seizure activity with the possible involvement of the neurotransmitters. It is evident from the results of the present investigation that pre-treatment with aqueous root extract of MO significantly inhibited both the locomotor behaviour and PCN-induced epileptiform activity in a dose-dependent

Table 1 — Effect of MO on locomotor activity

<table>
<thead>
<tr>
<th>Group</th>
<th>Locomotor activity (No. of squares crossed in 5 min)</th>
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<tbody>
<tr>
<td>Group I (Control)</td>
<td>35.67 ± 2.36</td>
</tr>
<tr>
<td>Group II (100 mg/kg)</td>
<td>31.33 ± 1.76</td>
</tr>
<tr>
<td>Group III (200 mg/kg)</td>
<td>27.17 ± 3.51</td>
</tr>
<tr>
<td>Group IV (300 mg/kg)</td>
<td>23.00 ± 2.86</td>
</tr>
<tr>
<td>Group V (350 mg/kg)</td>
<td>12.17 ± 1.64</td>
</tr>
<tr>
<td>Group VI (400 mg/kg)</td>
<td>20.50 ± 1.73</td>
</tr>
<tr>
<td>Group VII (450 mg/kg)</td>
<td>25.83 ± 4.16</td>
</tr>
</tbody>
</table>

*p < 0.01, *p < 0.001 when compared with control group.

Table 2 — Behavioural changes of penicillin-induced epileptic rats after MO treatment

<table>
<thead>
<tr>
<th>Group</th>
<th>Onset (min)</th>
<th>Duration (min)</th>
<th>Severity score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (epileptic control)</td>
<td>5.13 ± 0.18</td>
<td>123.83 ± 3.11</td>
<td>5.55 ± 0.49</td>
</tr>
<tr>
<td>Group II (100 mg/kg)</td>
<td>5.15 ± 0.31</td>
<td>122.17 ± 3.59</td>
<td>5.13 ± 0.50</td>
</tr>
<tr>
<td>Group III (200 mg/kg)</td>
<td>5.48 ± 0.25</td>
<td>118.67 ± 1.54</td>
<td>4.47 ± 0.22</td>
</tr>
<tr>
<td>Group IV (300 mg/kg)</td>
<td>6.97 ± 0.23a</td>
<td>108.33 ± 6.32a</td>
<td>4.05 ± 0.31a</td>
</tr>
<tr>
<td>Group V (350 mg/kg)</td>
<td>10.77 ± 0.32a</td>
<td>64.00 ± 5.34a</td>
<td>2.93 ± 0.29a</td>
</tr>
<tr>
<td>Group VI (400 mg/kg)</td>
<td>6.45 ± 0.40a</td>
<td>105.5 ± 6.41a</td>
<td>3.45 ± 0.41a</td>
</tr>
<tr>
<td>Group VII (450 mg/kg)</td>
<td>5.77 ± 0.33</td>
<td>120.17 ± 4.76</td>
<td>4.63 ± 0.29</td>
</tr>
</tbody>
</table>

p values: *p < 0.05, *p < 0.02, *p < 0.01, *p < 0.001 when compared with epileptic control group.
manner. These findings can be explaining by alterations of the neurotransmitters 5-HT, DA and NE in different brain regions.

The results of locomotor activity indicated that MO produced an inhibitory effect on the CNS. The inhibitory effect which occurred did not resemble the sedative effect of benzodiazepines or pentobarbitone as the animals were still responsive and did not show prominent muscle relaxation which is common with the sedatives benzodiazepines and pentobarbitone. Biochemical estimation of neurotransmitters showed an increase in the 5-HT in the cerebral cortex (CC), midbrain (MB), caudate nucleus (CN) and cerebellum (CB). It has been reported by Beninger that the general CNS inhibitors decrease the rearing and locomotion in rats. Takahashi et al. also reported that 5-HT plays an important role in animal behaviour such as locomotor depression. The potentiation of sleeping time are suggestive of CNS depressant effect of the drug. The increase of 5-HT may be suggestive of (a) increased synthesis (b) increased release (c) decreased utilization of the neurotransmitter or (d) may be due to an excess of functional 5-HT. The principal cell bodies of 5-HT are found in the raphae nuclei which are present in the reticular activating system. Afferent 5-HT fibres from the reticular formation (RF) of the brain stem project diffusely to the intralaminar nuclei. These in turn project diffusely to widespread areas of the neocortex and spinal cord. The RF plays a great role in maintaining the awake state. The cerebral cortex also receives a large number of 5-HT fibres from the brain stem (midbrain, pons and medulla). Thus from our biochemical results it may be concluded that MO exerts its action on locomotor behaviour by possibly increasing the level of 5-HT in discrete brain regions through RF. The present study also showed a decrease in the DA level in the CC, MB, CB and CN. Thus a reciprocal relation existed between 5-HT and DA. Locomotor activity is also influenced by DA. DA an immediate metabolic precursor of NE, is an important central neurotransmitter in the regulation of movement. An alteration in locomotor activity is an indicative of decrease in DA transmission in the substantia nigra. The dopaminergic neurons mainly found in the MB and the fibres project to the olfactory tuberculae, cingulate gyrus, entorhinal cortex and perihinal cortex. MO may act through these projection fibres by reducing the DA in the different brain regions such as CC, MB, CN and CB.

The present study also reported that MO dose dependently inhibited the PCN-induced epileptiform activity. The biochemical study showed that NE level was decreased in the CC but there was no appreciable change in the MB, CB and CN. It might be that all the nerve terminals may not be identically susceptible to MO in different regions of brain. The involvement of NE and 5-HT in behavioural disorder has been well studied. Newman reported that the noradrenergic neurons in the brainstem or the axon projecting to the forebrain and the dorsal noradrenergic bundle suppressed the PCN-induced epileptiform activity. It is tempting to suggest that the inhibitory action of MO may be due to modulation of the inhibitory effect of NE. It is well known that PCN acts as a convulsive agent by inhibiting the formation of GABA from
glutamate. Glutamate is an excitatory neurotransmitter and the convulsive effect may be due to excess accumulation of the glutamate. Hence it may be suggested that MO exerts its inhibitory action by activation of GABA receptor which in turn mediates the inhibitory neurotransmission in the CNS. Thus the inhibition of the PCN-induced convulsion may be either due to the modulation of the inhibitory effect by NE or the activation of the GABA receptors or both. In the present study, besides a decrease in the NE, there was increase in the 5-HT which corroborates the findings of Takahashi et al. that the sedative effect may be due to an increase in the 5-HT level. Apart from NE and 5-HT, DA is also involved in regulating behaviour. The role of DA as an inhibitory neurotransmitter is also well known. DA has been traditionally believed to inhibit most hippocampal neurons and has an antiepileptic action. The anticonvulsant action of DA has been attributed to D2 receptor stimulation in the forebrain, while advent of selective D1 agonists with periconvulsant properties revealed for the first time that DA could lower the seizure threshold from the midbrain. Thus from our results and the sporadic work of other investigations it is tempting to suggest that an alteration in these three neurotransmitters (5-HT, DA and NE) may be responsible for the central inhibitory effect of MO. However role of GABA cannot be denied and further studies are needed to elucidate it.

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