Role of 5-hydroxytryptamine in *Moringa oleifera* induced potentiation of pentobarbitone hypnosis in albino rats

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The role of 5-hydroxytryptamine (5-HT) in pentobarbitone (PB) sleeping time, gross behaviour, electrical activity of the brain and serum 5-HT level was studied in Holtzman strain adult albino rats following treatment with *M. oleifera* (MO). MO (350mg/kg) caused inhibition of awareness, touch response, motor activity, righting reflex, and grip strength. It significantly increased the PB sleeping time, serum 5-HT level (P<0.001) and a-wave activity. These observations indicate that the aqueous extract of MO potentiated PB induced sleeping time and increased the a-wave activity through 5-HT.

Keywords: *Moringa oleifera*, PB sleeping time, Serotonin, EEG.

5-hydroxytryptamine (5-HT) is a monoamine neurotransmitter of the central nervous system. It is present primarily in the raphae nucleus, hippocampus, amygdala, blood platelets, and mast cells. Besides neurotransmission, 5-HT plays an important role in a variety of physiological responses like mood, appetite, sleep, depression and cognitive dysfunction. That central 5-HT has a role in the process of sleep is strongly supported by evidence that p-chlorophenylalanine, a selective 5-HT synthesis inhibitor regularly precipitates nearly total insomnia in cats. Similarly, reserpine a central monoamine depletor, produces insomnia in cats. 5-hydroxytryptophan restores sleep temporarily in p-chlorophenylalanine pretreated cats and on chronic administration, prevents p-chlorophenylalanine insomnia. Conclusive evidence for serotonergic mediation of sleep in cats, has been provided by destructive lesions of raphae areas, which lead to marked reduction of 5-HT synthesis, release and metabolism, where total insomnia lasting for several days is seen. Several studies reported that 5-HT plays an essential role for the production of sleep.

*Moringa oleifera* (Ver. Sajnae; MO) a perennial plant is found and cultivated in West Bengal. Flowers and leaves of the plant possess depressant property whereas root of the plant is used by tribal groups of India as antiepileptic agent. Majumdar et al reported that crude methanolic extract of MO possess less toxic action (LD₅₀: 2.8g/kg, ip) in mice. Ray et al reported that MO decreased the locomotor activity and penicillin induced convulsion by altering brain 5-HT, dopamine and norepinephrine. Thus it seemed pertinent to explore its effect on pentobarbitone (PB) induced hypnosis and electrical activity. An attempt has been done to relate the effect with 5-HT.

*Preparation of extract*—The root pieces (1 kg) of MO were purchased from the locally. The plant material was identified, authenticated and kept in Department of Physiology, Calcutta University. The root bark was discarded and the root was sundried, ground and spread over tray with shifting of materials every day to avoid growth of fungus. The powder was soaked in water overnight and the solution was filtered with Whatman No. 1 filter paper and subjected to lyophilization. The final yield was 13%.

*Animals and treatment*—Adult Holtzman strain albino rats of either sex (108) weighing 150±10g were used. The rats were housed in groups in cages at an ambient temperature of 25±1°C and 45.5% RH, with a 12:12 hr L:D cycle. The animals had free access to standard laboratory diet and tap water ad libitum. All animal studies were performed in accordance with institutional ethical committee and all procedures were followed as per rules and regulations.

In the first set of experiment, behavioural effect and PB sleeping time were noted. Fifty four (54) rats were divided into 9 groups—control (group I) and 8 experimental groups (group II-IX). Each group consisted of 6 animals. Group I rats were treated with saline (5ml/kg, po). Groups II-IX rats were treated with MO orally using orogastric cannula in the doses of 50, 100, 150, 200, 250, 300, 350 and 400mg/kg respectively between 0900-1100hrs and 30 min later.

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the gross behavioural responses were noted. For PB sleeping time, MO was given orally 4hr prior to PB administration in experimental rats.

In the second set of experiment, electrical activity and serum 5-HT were noted. Another fifty four (54) rats were divided same as the first set of experiment i.e; control and experimental group. The control rats were treated with saline (5ml/kg,po) and experimental rats were treated with MO (50-400mg/kg, po) and 30 min later electrical activity was recorded (8 Channel EEG Medicare & Recorder, Chandigarh) for 5-6 hr without interruption. After EEG studies, rats were sacrificed and blood was collected from jugular vein for serum separation and 5-HT was estimated.

**Behavioural effects**—The effects of MO on awareness, grip strength, touch response, righting reflex and spontaneous motor activity were observed by conventional methods.

**PB induced sleeping time**—The PB (40mg/kg, ip, Abbott India Ltd) induced sleeping time was measured as the time interval between the loss and the regain of the righting reflex. The righting reflex was considered to be lost when the animal placed on its back failed to regain its normal posture within 10 sec. The control rats received only PB. The experimental rats were treated with aqueous extract of MO and after 4hr PB was given intraperitoneally.

**Surgical procedures for electroencephalographic studies**—Prior to surgery all the animals were fasted overnight but had free access to water. Under pentobarbital anaesthesia (40mg/kg, ip; Abbott India Ltd) rats were mounted on the stereotaxic apparatus (INCO, India Ltd). Care was taken 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. The scalp was incised in the midline and the pericranial muscles were retracted laterally. After retracting the nuchal musculature the overlying bone was drilled at the specific loci (surface cortex). Bipolar electrodes were implanted on the surface of the cortex through trephined holes and fixed with dental cement. A reference electrode was implanted over the frontal bone and all electrodes were then soldered to a multiple plug which was fastened to the calvarium with dental cement. Penicillin (10,000 IU) was injected intramuscularly on the day of operation and for the next two consecutive days as antibiotic measure. The electrical activities from cerebral cortex were monitored through an 8 channel EEG machine (Recorder & Medicare, Chandigarh).

Recordings were taken approximately every 5 min throughout the session for a period of 5-6 hr.

**Biochemical estimation of 5-HT**—Blood from jugular vein was collected for serum separation. Serum (2 ml) was mixed with 5ml of 10% heptane and 2.5ml of 0.003N HCl. It was then shaken for 5 min and centrifuged at 2000 rpm for 10 min. Acid layer (2.25ml) was eluted and mixed with 100 mg alumina and 0.5ml of 2M sodium acetate. The mixture was shaken for 5 min and centrifuged at 2000 rpm for 10min. The supernatant was used for the estimation of 5-HT. Supernatant was mixed with 1.5 ml of 10% isobutanol, shaken twice with 1 ml of salt saturated buffer. Then 1ml of 10% heptane was added to the butanol phase and 2.5 ml of 0.1 N HCl was added and shaken well and 0.5 ml of 0.3N HCl was added with repeated shaking. This was taken for estimation of 5-HT spectrofluorometrically.

**Statistical analysis**—The results were analysed statistically using Student’s ‘t’ test. Difference below the probability level 0.05 was considered statistically significant.

Studies of gross behavioural changes after treatment with MO in different concentrations (50-400mg/kg) are summarized in Table 1. MO in graded doses produced gradual increase in the depressive effect as indicated by a reduction in behavioural response. With doses 50-150mg/kg there was no appreciable change in behavioural response. But 200-400mg/kg doses produced behavioural changes in awareness, touch response, righting reflex, grip strength and spontaneous motor activity. However MO in the dose of 350mg/kg produced significant depressive effect. There was reduction in the spontaneous motor activity, grip strength and touch response with changes in the awareness and the righting reflex.

MO potentiated PB sleeping time in a dose dependent (50-400mg/kg) manner. The onset of action was after 1-2 min and the duration of action varied from 4-9 hr depending upon the dose of the extract. At 50-150 mg/kg doses there was no appreciable change in sleeping time. However at 200 and 300mg/kg it produced a mild potentiation of sleeping time but at 350 mg/kg dose there was marked potentiation of the sleeping time. However at 400mg/kg dose there was no further increase of sleeping time (Table 2).

The normal EEG pattern showed predominance of low voltage fast waves or β-waves in normal saline treated control rats. MO was administered orally, 30 min before EEG studies. In 50-150mg/kg dose there
was occasional occurrence of α-activity (high voltage slow waves). At 200-300mg/kg doses the α-wave activity increased predominantly, but at 350 mg/kg dose the frequency of α-wave activity increased and persisted for nearly more than 5 hr. At 400mg/kg dose the α-waves decreased with gradual increase in the β-waves or the low voltage fast waves (Fig 1).

Serum 5-HT level was significantly increased at 300-400mg/kg doses as compared to control groups. The most effective changes in 5-HT level were found at the 350 mg/kg dose (Table 2).

The results of the present study indicate that MO decreases touch response, righting reflex of rat in comparison with respective control groups probably due to its depressant action. Reduction of awareness, spontaneous motor activity and depressant action may be due to the action of MO on central nervous system (CNS). Takahashi et al. reported that 5-HT plays an important role in animal behavior such as locomotor depression. In the present study MO prolonged the sleeping time with an increase in serum 5-HT level.

This corroborates earlier studies showing that PB induced sleeping time is a 5-HT mediated response and prolongation of sleeping time may be due to elevation of 5-HT level.

5-HT is mainly found in platelets, enterochromaffin cells (EC cells), throughout the GI tract and in specific region of the CNS. Thus increase in serum 5-HT level may be due to the triggering of the secretion of 5-HT from platelets and EC cells by MO and may be responsible for prolongation of sleeping time. There is considerable evidence which links brain 5-HT with sleep mechanism. 5-HT has direct excitatory and inhibitory action and the 5-HT released from diencephalon and cerebral cortex plays an essential inhibitory role to cause normal sleep. From the present results the exact mechanism by which MO causes depression of locomotor activity and potentiation of the PB induced hypnosis is not clear. However it is well known that Reticular-activating system (RAS) plays an important role in sleep mechanism. It has also been reported earlier that 5-HT is mainly associated with production and prolongation of sleep mechanism.

![EEG study showing (A) low voltage waves in normal control rats and (B) after treatment with MO (350mg/kg), there was high voltage slow waves (occurrence of α-waves).](image-url)

![Table 1—Effect of aqueous extract of MO on behavioural profile in rat.](table-url)

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Control</th>
<th>MO (mg/kg)</th>
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<tbody>
<tr>
<td></td>
<td>50</td>
<td>100</td>
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<tr>
<td>Awareness</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Touch Response</td>
<td>0</td>
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<td>Righting Reflex</td>
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<td>Grip Strenght</td>
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<td>Spontaneous Motor Activity</td>
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0: no effect; +: slight depression; 2+: moderate depression; 3+: strong depression; 4+: very strong depression.

MO (50-150mg/kg) had no effect on the awareness, touch response, righting reflex, grip strength and spontaneous motor activity. 200 and 250 mg/kg indicate slight depression effect. At 300mg/kg showed strong depression effect whereas at 350mg/kg the depressant effect was very strong and effective. At 400mg/kg there was a moderate depression effect.
through activation of RAS. The EEG study showed that there was increase in the occurrence of α-wave activity (high voltage slow waves). The present findings are consistent with reports that α-burst activity recorded from the cortex is produced via the thalamocortical system that is modulated by Reticular formation (RF). Thus it may be suggested that increase serotonin 5-HT may trigger the RAS for potentiation of PB sleeping time.

References


