

NEUROTOXIC EFFECT OF *ALBIZIA MYRIOPHYLLA* BENTH., A MEDICINAL PLANT IN MALE MICE**MAIBAM RASILA DEVI¹, MEENAKSHI BAWARI^{*1}, SATYA BUSHAN PAUL²**¹**Department of Life Science and Bioinformatics, Assam University, Silchar 788011, Assam, India**
²**Department of Chemistry, Assam University, Silchar 788011, Assam, India. Email: meenakshibawari@yahoo.co.in***Received: 28 Feb 2013, Revised and Accepted: 16 Apr 2013***ABSTRACT**

Albizia myriophylla Benth. is used traditionally in the making of Hamei, a fermented product which is added to produce better quality alcohol in Manipur, India. The plant is also known to possess medicinal value in other parts of the world. This study aims to assess the effect of *A.myriophylla* bark aqueous extract on the central nervous system in mice. The extract was administered at a dose of 30 mg/kg and 60 mg/kg BW for 7 days and were subjected to various behavioral study such as locomotor activity, elevated plus maze test and forced swimming test. The activities of the catalase and lactate dehydrogenase were estimated in the cerebral cortex and midbrain of the mice. The ultrastructural changes induced by the plant extract was also studied using transmission electron microscopy. The results showed a decrease in the locomotor activity, an increase in the immobility time and did not cause anxiolytic effect. The plant extract significantly decreased the activity of catalase and increased the activity of lactate dehydrogenase in cerebral cortex and midbrain. The ultrastructural examination of cerebral cortex also showed deleterious effect in the treated mice. Based on the present findings we can conclude that administration of aqueous extract of *A.myriophylla* bark have potential neurotoxic effect on mice.

Keywords: CNS depressant, Enzyme, Ultrastructure, Neurotoxicity**INTRODUCTION**

Albizia myriophylla Benth. is a small tree belonging to the Fabaceae family. The genus comprises approximately 145 species distributed in tropical and subtropical regions in the world which includes India, Thailand, Malaysia, Vietnam and other countries. It is used in traditional medicine as mouth wash in Thailand [1]. The extract of *Amryophylla* has been found to have anticandidal activity against six candida species [2]. This plant is used as an anti diabetic agent and is also used for rice beer production by Thai people [3,4]. In the Thai and Vietnamese systems of traditional medicine, the stems of *Amryophylla* is also used as a substitute for licorice due to its sweet taste [5]. In Manipur, a north-eastern state of India, a fermented product called Hamei is generally added in order to get good quality alcohol in traditional way of making distilled and fermented beverages. In the preparation of Hamei, finely chopped or powdered dried bark of *Amryophylla* plant is mixed with water and the filtrate is used [6]. To the best of our knowledge there is no report on the effect of this plant on the central nervous system. The present study was undertaken to provide scientific basis for a possible behavioral, biochemical and ultrastructural effects of aqueous extract of *A. myriophylla* bark on the mice brain.

MATERIALS AND METHODS**Plant material and preparation of the aqueous extract**

The bark of *A.myriophylla* was collected, washed and dried under room temperature for about 60 days. The air-dried bark was minced into small pieces and macerated in distilled water and the extract was decanted after 24 hours. The filtrate was evaporated to dryness in oven at 40°C. The dried extract was weighed and dissolved in distilled water to give the required concentration before its administration to the experimental animals [7].

Preliminary phytochemical screening

The extract was screened for the presence of various phytochemical constituents viz., alkaloids, tannins, flavonoids, cardiac glycosides, phlobatannins, saponins and terpenoids [8,9,10,11,12].

Animals

Adult male albino mice weighing between 26g and 32g, used for the study were obtained from the Pasture Institute, Shillong. The animals were housed in cages under standard environmental conditions and had free access to food and water ad libitum. The experiments were performed in accordance with the guidelines in the care and use of laboratory animals and were approved by the Ethical Committee of the Assam University, Silchar.

Acute toxicity test

The LD₅₀ of the plant extract was evaluated by using Karber's method [13].

Experimental design

Animals were treated with the extract at sublethal doses of 30mg/kg and 60mg/kg body weight (BW) intraperitoneally. Controls received vehicle (distilled water) through the same route as in the treated groups. Diazepam (DZP) 1 and 2mg/kg BW and Fluoxetine 10mg/kg BW were intraperitoneally injected and the mice were used as standards for behavioural studies. All the treatments were done to the respective groups upto a volume of 5ml/kg BW for a period of seven days. The proposed parameters were performed one hour after the administration of the last dose.

Locomotor activity

All mice were tested in acrylic cages (45×25 cm) divided into 16 equal squares. The number of crossed squares was recorded for each mouse for 5min [14]. Diazepam (2mg/kg i.p.) was used as the positive control drug.

Elevated plus maze test (EPM)

The EPM apparatus consists of two open arms (16×5cm) and two enclosed arms (16×5×12cm) having an open roof. The whole apparatus was elevated 25 cm from the floor in a dimly lit room. The mice were placed individually at the centre of the maze with their heads facing a closed arm and allowed to explore the maze for five min. The number of entries in open arms and time spent in open arms were recorded [15]. Diazepam (1mg/kg i.p.) was used as the positive control drug.

Forced swimming test (FST)

The animals were individually forced to swim in a transparent glass vessel (25 cm high, 15 cm in diameter) filled with (12.5 cm high) water at 21–24 °C. The duration of immobility (in seconds) was measured for 5 min. 'Immobility' was defined as floating and treading water just enough to keep the nose above water. The water was changed for each mice [16]. Fluoxetine (10mg/kg i.p.) was used as positive control drug.

Biochemical estimation

The mice in the experimental group and in control after the behavioral test were sacrificed quickly. The brains were rapidly removed, cerebral cortex and midbrain were then separated. They

were washed quickly with saline, blotted between two damp filter papers, then weighted using electronic balance. Weighed tissue was homogenized in a cool environment and used for catalase (CAT) assay [17] and another known weight of tissue was homogenized for assay of lactate dehydrogenase (LDH) [18]. Protein content was assayed by Lowry's method [19].

Transmission electron microscopy

A specific portion of the cerebral cortex was carefully transferred to glutaraldehyde for transmission electron microscopy by following the standard protocol.

Statistical analysis

The data were expressed as mean \pm SEM. The results were analysed statistically by ANOVA which was followed by Tukey's multiple comparisons test. P values < 0.05 were considered as significant.

RESULTS

Preliminary phytochemical screening

Results obtained in this experiment showed the presence of flavonoids, saponins, tannins, phlobatannins and cardiac glycosides.

Acute toxicity test

The LD₅₀ of the extract was estimated to be 152.31mg/kg BW.

Effect of *Amyriophylla* extract on locomotor activity

The locomotor activity during the 5min duration is shown in Fig.1. In animals treated with *Amyriophylla* bark aqueous extract (30mg/kg and 60mg/kg), the locomotor activity was decreased significantly as compared with the vehicle treated controls ($P<0.001$). Administration of DZP at 2mg/kg BW suppressed the locomotor activity to a greater extent. ($P<0.001$).

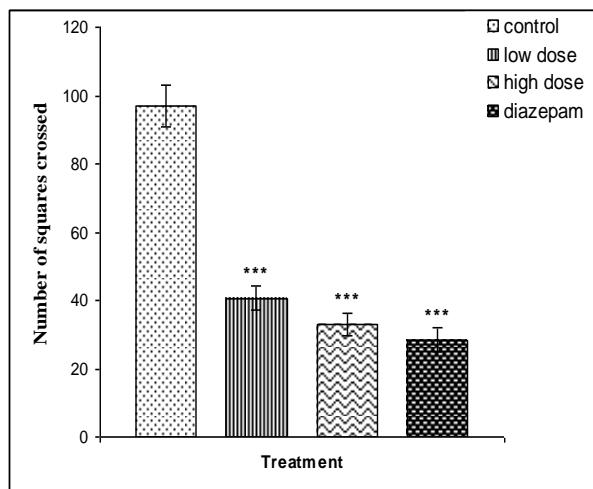


Fig. 1: Each column represent mean \pm SEM (n=6). Comparisons were made by using one way ANOVA followed by Tukey's Multiple Comparison test (***(P<0.001 vs control)).

Effect of *Amyriophylla* extract on elevated plus maze test

The results of the EPM is reported in Fig.2. The extract treated animals did not show significant increase in the time spent in the

open arms and the number of entries in the open arms as compared to the control group. However the animals which were given DZP at the dose of 1mg/kg BW, an anxiolytic dose significantly increased the time spent and number of entries in open arms ($P<0.001$).

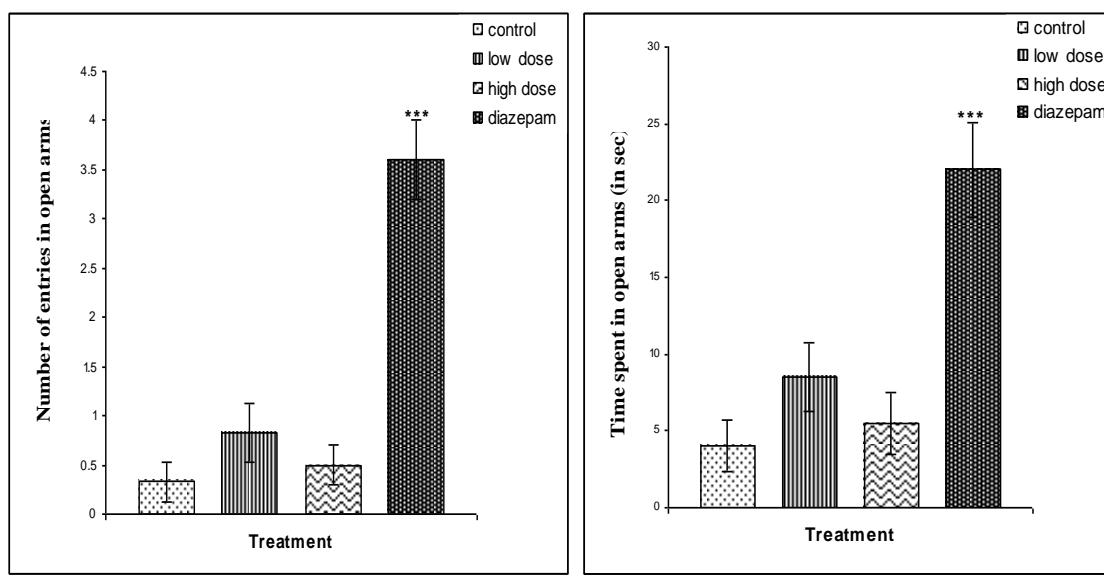


Fig. 2: Number of entries in open arms (A), time spent in open arms (B). Results are presented as mean \pm SEM (n=6). Comparisons were made by using one way ANOVA followed by Tukey's Multiple Comparison test (***(P<0.001 vs control)).

Effect of *Amyriophylla* extract on forced swimming test

The effects of *A. myriophylla* extract and fluoxetine in the FST are shown in Fig.3. The plant extract significantly increased the immobility time in comparison to control ($P<0.05$ for 30mg/kg, $P<0.01$ for 60mg/kg). On the other hand, fluoxetine, a standard antidepressant drug significantly decreased the immobility time during the 5min test session ($P<0.001$).

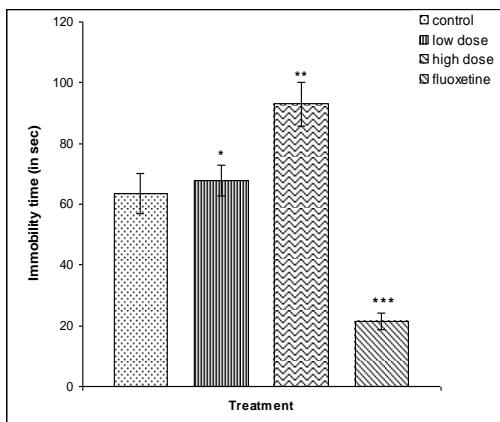


Fig. 3: Each column represent mean \pm SEM (n=6). Comparisons were made by using one way ANOVA followed by Tukey's Multiple Comparison test (*P<0.05, **P<0.01, ***P<0.001 vs control).

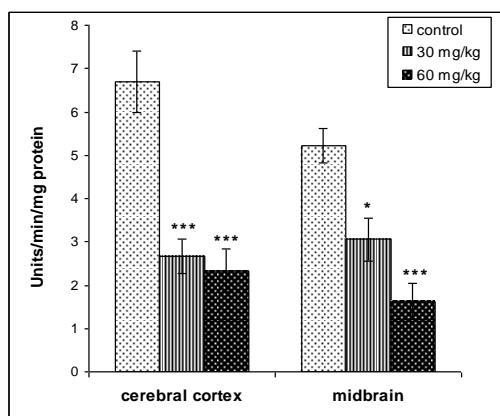


Fig. 4: Graph representing catalase activity in cerebral cortex and midbrain regions. Results are presented as mean \pm SEM (n=6). Comparisons were made by using one way ANOVA followed by Tukey's Multiple Comparison test (*P<0.05, ***P<0.001 vs control).

The result in the Fig.5 shows LDH activities on mice cerebral cortex and midbrain. The activity was significantly increased ($P<0.01$ in cerebral cortex and midbrain) as compared to the control animals.

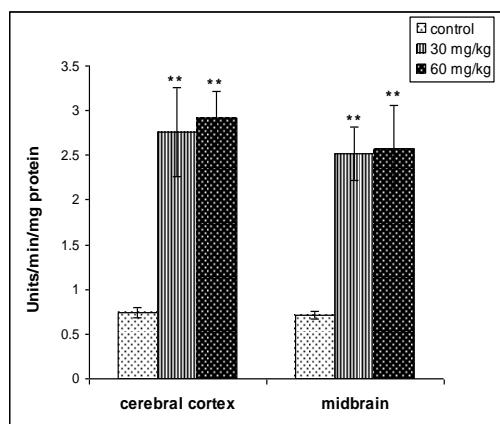


Fig. 5: Graph representing lactate dehydrogenase activity in cerebral cortex and midbrain regions. Results are presented as mean \pm SEM (n=6). Results are presented as mean \pm SEM. Comparisons were made by using one way ANOVA followed by Tukey's Multiple Comparison test (**P<0.01 vs control).

Electron microscopic analysis

Transmission electron microscopic examination of the cerebral cortex of control mice demonstrated the normal architecture of the nerve cells [Fig.6]. The administration of plant extract at the two

doses for 7 days induced ultrastructural alterations in the cerebral cortex. It was observed that the nuclear membrane no longer possessed regular outline and characteristic shape and showed distorted structure of the endoplasmic reticulum and destructed mitochondria in the cerebral cortex [Fig.7 & Fig.8].

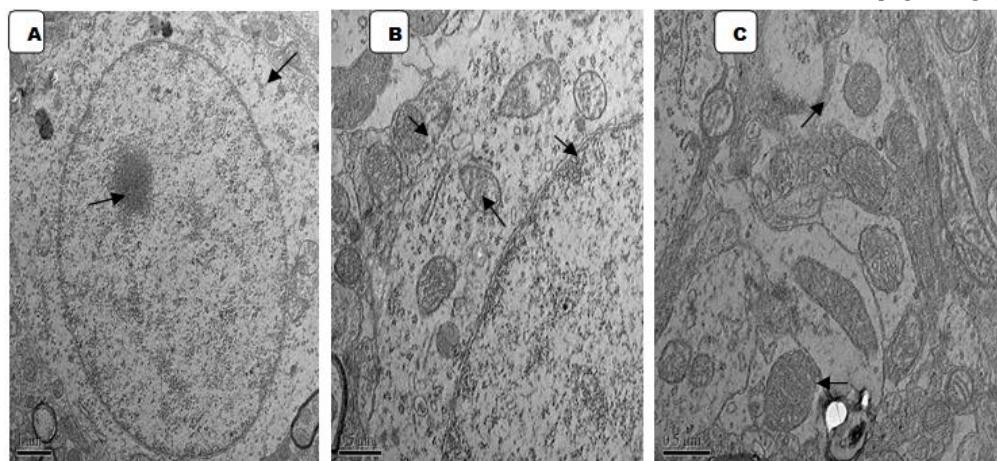


Fig. 6: No abnormal changes were found in the neurons of the cerebral cortex of control animals. It shows an intact nucleus with prominent nucleolus (A), regular nuclear envelop with other cell organelles (B) and portion of the cytoplasm with normal mitochondria (C).

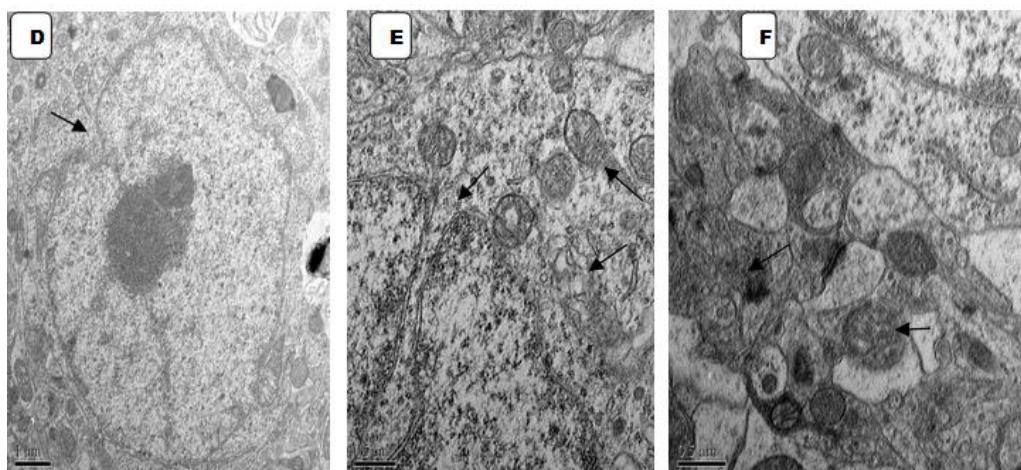


Fig. 7: Electron micrographs of the extract treated animals (30mg/kg). The nucleus is slightly deformed in shape and nuclear membrane is irregular (D), shrinkage of nuclear envelop is seen with many damaged cell organelles (E) and a cytoplasmic part showing some mitochondria with destructed cristae (F).

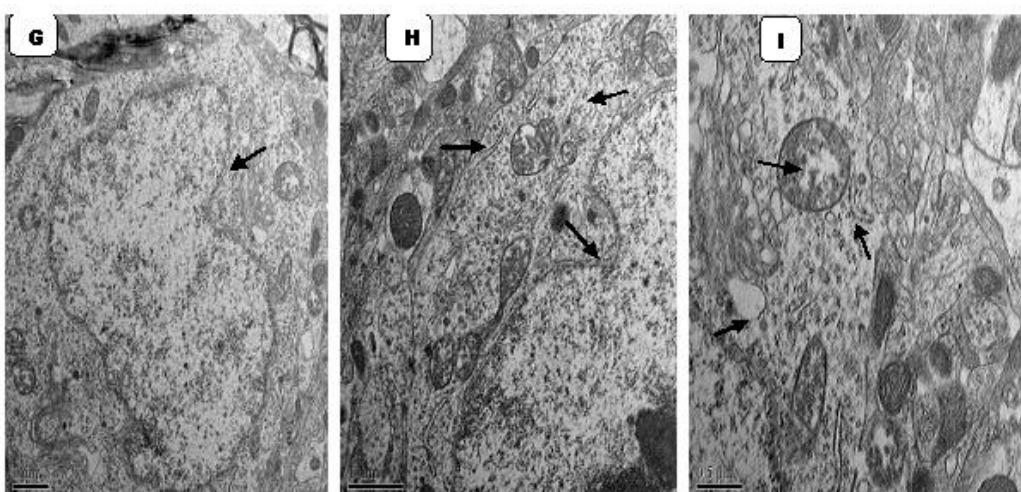


Fig. 8: Electron micrographs of the extract treated animals (60mg/kg). Nucleus with deformed and irregular outline (G), nuclear envelop ill defined, cytoplasm with distorted rough endoplasmic reticulum and dispersed granules (H), mitochondria with disappearance and breakage of cristae, vacuolation and dilated cisternae (I).

DISCUSSION

In the present study, the effect of aqueous extract of *Albizia myriophylla* on certain behavioral paradigms, biochemical and ultrastructural changes has been evaluated. An important step in evaluating the action of a substance on CNS is to observe its effect on behavior of the animal. The result indicated that the extract significantly decreased locomotor activity which showed that it has CNS depressant activity. The decrease in locomotion implies depression effect on CNS [20,21]. It also gives an indication of the level of excitability of the central nervous system and the decrease may be closely related to sedation resulting from depression of the CNS [22]. When mice are forced to swim in an inescapable situation, they tend to become immobile after initial vigorous activity. The immobility reflected a state of lowered mood in which the animals had given up hope of finding an exit and had resigned themselves to the experimental situation. This immobility has been described as a symptom of "behavioral despair", and have been suggested as animal models of human depression [23]. The significant increase in immobility time indicates depressant activity of the plant extract, which is opposite to the effect shown by fluoxetine, a classical antidepressant drug [24,25]. In this study, the extract did not produce any significant change in the elevated plus maze model. The exposure of mice to an elevated and open maze induces an exploratory cum fear drive which results in anxiety [26,27]. Anxiolytic compounds by decreasing anxiety, increase the open arm exploration as well as the number of entries into the open arm. The extract at both doses failed to demonstrate any such effect and thus we can conclude that the plant at the doses studied does not possess anxiolytic activity. The extracellular appearance of LDH is used for detecting cell damage or cell death [28]. Elevation in the specific activity of LDH was observed in the present investigation and this may be due to toxicity and related oxidative stress produced by the plant extract. The LDH activity increases during conditions favouring anaerobic respiration to meet energy demands when aerobic respiration is lowered [29]. Brain cells are damaged by free radicals generated in the body by the oxidation of food substances and other chemical reactions in the cells [30]. Catalase is an antioxidant enzyme that constitute the first line of antioxidant enzymatic defense [31]. A decline in this enzyme activity was observed in this study which is consistent with increased free radical production. The accumulation of free radicals progressively damages brain structure and function [32].

The transmission electron microscopic examination of cerebral cortex in mice treated with the extract revealed features of ultrastructural damage in neurons of the cerebral cortex. All cell organelles, mitochondria and endoplasmic reticulum and nuclear membrane in particular were more vulnerable to extract. Mitochondrial alterations were in the form of destructed cristae and rough endoplasmic reticulum cisternae were dilated. Moreover and the nuclear envelop was also deformed and irregular in shape.

CONCLUSION

Based on the results of the behavioral, biochemical and ultrastructural changes, the present investigation indicated that the brain is particularly susceptible to the plant extract. Therefore, we can infer that the aqueous extract of *Albizia myriophylla* bark have potential to induce neurotoxicity in mice brain. However further study is necessary to understand the mechanism of neurotoxicity and the phytochemical constituents responsible for it.

ACKNOWLEDGEMENTS

The authors are very much grateful to University Grant Commission for financial assistance and SAIF-NEHU, Shillong for electron microscopy.

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