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## Assessment of neuropharmacological activities of *Borassus flabellifer* L. roots

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### Abstract

In the present study the methanolic extract of *Borassus flabellifer* L. (MBF) was evaluated for the safety along with neuropharmacological activity by using OECD guidelines, y-maze, elevated plus-maze and open field test respectively. Mortality, sign of any toxicity or behavioral changes were not noticed up to the dose as high as 4000mg/kg. Moreover, in y-maze test, MBF 200 mg/kg and MBF 400 mg/kg exhibited anti-depressive and depressive activities. Again, every extract including both lower and higher doses elicited depressive, anti- depressive and anxiolytic activities in elevated plus maze test. Later, in open field test, significant ( $p < 0.05$ , vs. control) CNS depressive activities were found by both MBF 200 mg/kg and MBF 400 mg/kg. The results obtained in the present study demonstrated that MBF can be the possible sources of CNS depressant, anti- depressant and anxiolytic agents. But further investigation is needed for the identification of the active compounds as well as confirmation of their activities.

**Keywords:** Acute toxicity, *Borassus flabellifer* L., Neuropharmacological study

### 1. Introduction

*Borassus flabellifer* L. belongs to the family Arecaceae is a tall tree, which is 30 m in height possessing black stem. Besides, the plant contains its crown of leaves at the top position. The leaves have a diameter of about 0.9-1.5 m which is palmately fan shaped and petiole edges along with hard horny spinescent serratures. The flowers are unisexual with male spadix branching and female spadix simple. Fruits are large and subglobose drupes, on the greatly enlarged perianth. The plant is grown in various parts of India<sup>[1]</sup>. The plant generally contains gums, steroidal glycosides, albuminoids, carbohydrate like sucrose and fats<sup>[2]</sup>. Traditionally the plant is used as a stimulant, antiphlogistic, diuretic and anti-laprotic. The fruits are naturally sedative, aphrodisiac, stomachic and laxative which are useful in the treatment of dyspepsia, skin diseases, fever, hyperdipsia, hemorrhages, flatulence and general debility. In inflammatory reactions, the roots and juice of the plant are beneficial<sup>[1]</sup>. However, review of the literature of this plant reported that various parts of this plant possessed some pharmacological activities such as analgesic, anthelmintic, antibacterial, antidiabetic, antifungal, antihyperlipidemic, anti-inflammatory, antioxidant, antipyretic, antitumor, antiulcer, cytotoxicity and diuretic<sup>[1, 2, 3, 4, 5, 6, 7, 8, 9, 10]</sup>.

Therefore, the aim of the study was to evaluate acute toxicity as well as neuropharmacological activities of the roots of *Borassus flabellifer* L.

### 2. Materials and Methods

#### 2.1. Collection and Identification of the Plant

*Borassus flabellifer* L. roots were collected from the Jahangirnagar University campus, Savar, Dhaka, Bangladesh in November, 2011. Sarder Nasir Uddin, the Principal Scientific Officer at the Bangladesh National Herbarium assured the verification of the identification of the species. Dried specimens were preserved in the herbarium for future references.

#### 2.2. Extraction

100 g of powdered roots were taken for methanol extraction. Plant parts were rinsed 3–4 times successively with running water and once with sterile distilled water. The plant materials were then dried in the shade for a period of 7 d. A laboratory grinding mill (Model 2000 LAB Eriez®) was utilized for the grinding purpose of dried roots and passed through a 40-mesh sieve to obtain fine powders. A hot extraction procedure along with Soxhlet apparatus was applied for the extraction of powdered roots of *B. flabellifer* L. where 1 L methanol was used. The liquid extract was filtered by using Whatman No.1 filter papers.

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The filtrate was then dried in a hot air oven at 40 °C. The percentage yield of the extract was 13.73 % (w/w). The extract was stored at 4°C for additional studies.

### 2.3. Experimental Animals

One hundred and fifteen *Swiss albino* mice of both sexes, 6–7 weeks old, weighting 25–30 g were collected from the Department of Pharmacy, Jahangirnagar University, Savar, Dhaka, Bangladesh. These animals were reserved under standard environmental conditions, having relative humidity 55%–65%, 12 h light/12 h dark cycle and (27.00±1.00) °C temperature. Sufficient supply of foods along with water *ad libitum* was ensured. Prior to the experiment, animals were adapted to the laboratory conditions for 1 week. All the protocols applied in the experiments conducted with these animals were approved by the Institutional Animal Ethical Committee of Jahangirnagar University, Savar, Dhaka, Bangladesh.

### 2.4. Acute Oral Toxicity Study

Acute toxicity refers to the adverse effects that result either from a single exposure or from multiple exposures over a short time (normally less than 24 h). According to Organization of Economic Cooperation and Development (OECD) guidelines, the acute toxicity study was designed to estimate the half lethal dose (LD<sub>50</sub>) of the experimental samples [11]. Fifteen mice were divided into two groups including control group and test group (MBF), with five animals per group. Different concentrations of experimental sample (MBF) such as 100, 250, 500, 1000, 2000, 3000 and 4000 mg/kg body weight were prepared and administered orally. Therefore, the animals were observed every 1 h for next 5–6 h for mortality, behavioral pattern changes such as weakness, aggressiveness, food or water refusal, diarrhea, salivation, discharge from eyes and ears, noisy breathing, changes in locomotor activity, convulsion, coma, injury, pain or any sign of toxicity in each group of animals. At the end of a 2-week observation period, a final evaluation was also conducted [11].

### 2.5. Neuropharmacological Study

#### 2.5.1. Y-maze Test

Y-maze test was conducted according to Mandal *et al.*, 2001; Rushton *et al.*, 1961 and Ma *et al.*, 2007 [12,13,14]. Twenty mice were divided into control group (distilled water, 10 mL/kg, p.o.), positive control or standard group (Diazepam, 1mg/kg, p.o.) and test groups (MBF at 200 and 400 mg/kg body weight, p.o.), containing five mice in each group. The Y-maze apparatus was made of three wooden arms with a dimension of 30 cm x 8 cm x 15 cm (length x width x height) and there was an angle of 120° between each of the two arms. Each mouse was placed in the centre of a Y – shaped runway. The number of entry of the test animals into the closed and open arms was counted at 0, 30, 60, 120 and 180 min after respective treatment and the every counting was continued for 3 min. Arm entry refers to the entry of all four paws into one arm.

#### 2.5.2. Elevated Plus-maze Test (EPM)

According to the method of Lister Elevated plus-maze test was conducted [15]. Grouping of the mice and sample administration were carried out as like as Y-maze test. The apparatus was made of two opposing closed arms with a dimension of 50 cm x 10 cm x 30 cm (length x width x

height) along with two opposing open arms 50 cm (length) x 10 cm (width) and it was placed at 70 cm high from the floor level. Each mouse was placed in the centre of elevated plus-maze apparatus. The number of entry of the test animals into the closed and open arms was counted at 0, 30, 60, 120 and 180 min after respective treatment and the every counting was continued for 3 min. Arm entry refers to the entry of all four paws into one arm.

#### 2.5.3. Open Field Test

The test was conducted following the method of Hawiset *et al* [16]. Twenty mice were divided into control group (distilled water, 10 mL/kg, p.o.), positive control or standard group (Diazepam, 1mg/kg, p.o.) and test groups (MBF at 200 and 400 mg/kg body weight, p.o.), containing five mice in each group. The assessment of the CNS depression activity can be completed by this test. The open field was made of a series of alternating white and black squares with a height of 40 cm. The number of movement of the test animals i.e., total number of squares that every group of animals visited was counted at 0, 30, 60, 120 and 180 min after respective treatment and the every counting was continued for 3 min.

### 2.6. Statistical Analysis

All the results were expressed as mean ± S.E. (Standard Error). One-way ANOVA following Dunnet's test ( $P < 0.05$ , vs. control) and Post-hoc Bonferroni test ( $P < 0.05$ , vs. standard/extract) through the SPSS software (version 20; IBM Corporation, New York, USA). was utilized for statistical analyses of the neuropharmacological studies which was considered statistically significant.

## 3. Results

### 3.1. Acute Oral Toxicity Study

No mortality was noticed up to the dose as high as 4000 mg/kg for MBF or control group after acute oral toxicity study. Sign of any toxicity or behavioral changes were not noticed up to the dose as high as 4000 mg/kg for MBF (test group) or control group, before or after their administration in any animal, which lived up to 14 days. This apparently demonstrated that the test group does not expose acute oral toxicity.

### 3.2. Neuropharmacological Study

#### 3.2.1. Y-maze Test

From table-1 it was noticeable that, diazepam i.e., standard drug elicited time dependent depressive activity. In case of MBF 200 mg/kg, fluctuating effects including both depressive and anti-depressive activities were found during different observations. Besides, third and fourth observations were significant ( $p < 0.05$ , vs. control). Therefore, MBF 400 mg/kg exposed anti-depressive activity with respect time, in which significant activities were noticed at 60 min, 120 min and 180 min respectively ( $p < 0.05$ , vs. control).

The result of table-2 exhibited that, standard drug diazepam revealed depressive activity with time. Moreover, MBF 200 mg/kg disclosed depressive activities during all observations except fourth observation, whereas, significant activities were noticed at 60 min and 120 min respectively ( $p < 0.05$ , vs. control). Furthermore, similar result was obtained by MBF 400 mg/kg as like as MBF 200 mg/kg. Again, second, third and fourth observations were significant ( $p < 0.05$ , vs. control).

### 3.2.2. Elevated Plus-maze Test (EPM)

From table-3 it was clear that, standard drug diazepam exhibited depressive activity with time. Therefore, depressive activities were shown by MBF 200 mg/kg during second, third and fourth observations but it elicited antidepressant activity at 180 min. In case of MBF 400 mg/kg, the number of entries into the closed arm was reduced during second observation. Later, depressive effect was noticed at 60 min, 120 min and 180 min respectively, in which fourth observation was significant ( $p < 0.05$ , vs. control).

The result of the table-4 showed that, standard drug diazepam revealed time dependent depressive activity. Moreover, MBF 200 mg/kg and MBF 400 mg/kg both elicited depressive activities at 30 min, 60 min and 120 min respectively but during fourth observation anxiolytic activity was noticed.

### 3.2.3. Open Field Test

From table-5 it was pronounced that, both the lower and higher doses of MBF elicited depressive activity from 1<sup>st</sup> observation period in the test animals. In this case, significant activities were noticed during all of the observations at MBF 200 mg/kg ( $p < 0.05$ , vs. control). Furthermore, similar result was obtained by MBF 400 mg/kg as like as MBF 200 mg/kg.

## 4. Discussion

Despite of the usage of numerous plant-derived products in traditional medicine system, scientifically intense toxicity studies have been carried out on very few. Moreover, to determine the appropriate range of doses for subsequent usage and to recognize the potential adverse effects of the materials under the test it is crucial to study acute oral toxicity. The acute oral toxicity study has been a vital factor for the investigation of therapeutic index of drugs and xenobiotics [3]. As no mortality was noticed up to the dose as high as 4000 mg/kg, LD<sub>50</sub> of the plant extract could not be obtained. Therefore, the extract was found to be safe with a broad therapeutic range. So, two comparatively high doses (200 and 400 mg/kg) for MBF were used for *in-vivo* doses.

In the current study, the effect of methanolic extracts of roots of *Borassus flabellifer* was evaluated for CNS activities. The assessment of the CNS activity of any drug depends on the locomotor activities of animals. The measurement of the level of excitability of the CNS is termed as the locomotor activity of animal. There is an imminent relationship between sedation generated from CNS depression and reduction of locomotor activity [11]. By observing the number of arm entries of Y-maze and Elevated plus-maze test apparatus locomotor activity was determined [17]. The GABA<sub>A</sub> receptor initiates CNS depressant activity of drugs [11]. CNS depressant activity is exposed due to the elevated concentration of GABA<sub>A</sub> receptor in brain [18].

There are various subtypes of GABA<sub>A</sub> receptor in which at least 17 subunits including  $\alpha_{1-6}$ ,  $\beta_{1-3}$ ,  $\gamma_{1-3}$  and others (single  $\epsilon$ ,  $\theta$ ,  $\pi$  and  $\delta$ ) are responsible for diversity in the arrangements of its subtypes. Binding of benzodiazepines with  $\alpha_2$  and  $\alpha_1$  containing subunits of GABA<sub>A</sub> receptor generates anxiolytic and sedative, amnesic effects respectively. The anxiolytic effects of benzodiazepines are related with the secondary suppression of nonadrenergic and/or serotonergic and other

excitatory systems [19, 20]. The evaluation of memory function, exploratory behaviors and memory function in rodents are usually performed by Y-maze test [21]. The well-known Elevated plus maze model is utilized for the assessment of anxiety-like behaviour in rodents in which elevated and open place entry is omitted [22, 23]. Numerous antidepressant medications including selective serotonin reuptake inhibitors (SSRIs) or N-methyl-D-aspartate (NMDA) receptor antagonists, tricyclic antidepressants (TCA) occur overturning of immobility position and enhancement of the instance of escape-related behavior. Generation of depression is caused due to fluctuation in functions of some neurotransmitters such as serotonin, noradrenalin and dopamine. Depression is caused because of depletion of serotonin, one of the crucial etiological features. Accessibility of extracellular serotonin is boosted up by SSRIs [21]. Antidepressant action is exerted due to interruption of uptake of 5-HT and/or noradrenaline by SSRIs and TCA.

Antidepressant action is also elicited due to enhancement of some endogenous amines like serotonin, catecholamines etc. which is occurred as a result of downfall in the metabolism of MAO (monoamine oxidase) enzyme system by MAO inhibitors [24].

In case of Y-maze test, MBF 200 mg/kg and MBF 400 mg/kg exposed significant ( $p < 0.05$ , vs. control) anti-depressive activity with time which mechanism of action may be as like as TCA, MAO inhibitors, SSRIs or atypical antidepressants whose mode of action is not understood. Moreover, MBF 200 mg/kg also exhibited depressive activity by binding with GABA<sub>A</sub> receptor (table-1). In table-2, the mechanism of depressive activity of MBF 200 mg/kg and MBF 400 mg/kg may follow MBF 200 mg/kg of table-1. Again, from table-3, it can be understood that all the extracts including both low and high doses exhibited depressive and anti-depressive activities which mechanism of action may be as like as MBF 200 mg/kg of table-1. Therefore, every extract including both 200 and 400 mg/kg doses revealed anxiolytic and depressive activities which mode of action may be due to binding with GABA<sub>A</sub> receptor. Generally  $\alpha_2$  subunit of GABA<sub>A</sub> receptor is responsible for anxiolytic activity (table-4). In case of table-5, both the lower and higher doses of MBF elicited significant ( $p < 0.05$ , vs. control) depressive activity from 1<sup>st</sup> observation period in the test animals which may be due to binding of MBF with GABA<sub>A</sub> receptor.

## 5. Conclusion

The current study demonstrated that the extract of *Borassus flabellifer* might have numerous neuropharmacological activities, such as depressive and/or anti-depressive and anxiolytic activities. But further investigations are essential to authenticate which neuropharmacological property become prominent and to identify the active component of the extract for exploring the mode of actions in the development of neuropharmacological agents. Moreover, genotoxicity study of the extract may be a promising area for the researchers.

## 6. Conflict of Interests

The author declares that there is no conflict of interests regarding the publication of this paper.

**Table 1:** Effect of test groups on Y-maze apparatus after entrance into closed arms.

No. of movement in closed arm						
Group	Dose(mg/kg)	0 min	30 min	60 min	120 min	180 min
Control	10 mL/kg	4.38±0.45	4.34±0.32	4.34±0.40	4.38±0.51	4.30±0.13
Standard	1 mg/kg	1.2±0.37*	1.8±0.22	2.42±0.31*	2.70±0.42*	3.0±0.49
MBF	200 mg/kg	3.4±0.60	2.6±0.33	3.4±0.68	1.4±0.14*	2.4±0.57*
MBF	400 mg/kg	3.4±0.72	3.4±1.32	1.6±0.26*	1.6±0.53*	1.2±0.13* □

Number of movement in closed arm present as mean ± standard error of mean. \* $P < 0.05$ , vs control (Dennett's  $t$  test); □  $P < 0.05$ , vs standard (pair-wise comparison by post-hoc Bonferroni test).

**Table 2:** Effect of test groups on Y-maze apparatus after entrance into open arm.

No. of movement in open arm						
Group	Dose(mg/kg)	0 min	30 min	60 min	120 min	180 min
Control	10 mL/kg	3.96±0.37	3.94±0.55	3.98±0.32	3.98±0.32	3.99±0.51
Standard	1 mg/kg	3.60±0.26	3.24±0.38	2.78±0.12*	2.16±0.09*	1.80±0.20*
MBF	200 mg/kg	3.40±0.49	2.80±0.39	2.20±0.23*	1.20±0.09* □	2.40±0.60
MBF	400 mg/kg	2.80±0.84	2.80±0.92	2.20±0.33*	2.00±0.27*	1.40±0.33*

Number of movement in open arm present as mean ± standard error of mean. \* $P < 0.05$ , vs control (Dennett's  $t$  test); □  $P < 0.05$ , vs standard (pair-wise comparison by post-hoc Bonferroni test).

**Table 3:** Effect of test groups on Elevated plus-maze apparatus after entrance into closed arms.

No. of movement in closed arm						
Group	Dose(mg/kg)	0 min	30 min	60 min	120 min	180 min
Control	10 mL/kg	1.3±0.39	1.36±0.19	1.32±0.25	1.36±0.36	1.3±0.48
Standard	1 mg/kg	0.6±0.19	1.2±0.54	1.8±0.83	2.3±0.97	3.0±0.61
MBF	200 mg/kg	0.4±0.20*	0.4±0.25	0.6±0.30	1.0±0.53	0.4±0.30 □
MBF	400 mg/kg	0.2±0.06*	0.8±0.34	0.5±0.18	0.8±0.23	1.8±0.64*

Number of movement in closed arm present as mean ± standard error of mean. \* $P < 0.05$ , vs control (Dennett's  $t$  test); □  $P < 0.05$ , vs standard (pair-wise comparison by post-hoc Bonferroni test).

**Table 4:** Effect of test groups on Elevated plus-maze apparatus after entrance into open arms.

No. of movement in open arm						
Group	Dose(mg/kg)	0 min	30 min	60 min	120 min	180 min
Control	10 mL/kg	1.6±0.30	1.8±0.34	1.8±0.53	1.7±0.40	1.58±0.44
Standard	1 mg/kg	3±0.40	2.4±0.54	1.0±0.30	0.4±0.14*	0.20±0.05
MBF	200 mg/kg	3.8±0.46*	3.0±0.41	2.0±0.31	1.4±0.17	2.0±0.55 □
MBF	400 mg/kg	3.2±0.55	2.8±0.57	1.6±0.22	1.4±0.16	1.6±0.33

Number of movement in open arm present as mean ± standard error of mean. \* $P < 0.05$ , vs control (Dennett's  $t$  test); □  $P < 0.05$ , vs standard (pair-wise comparison by post-hoc Bonferroni test).

**Table 5:** Effect of MBF on open field test.

No. of movement						
Group	Dose (mg/kg)	0 min	30 min	60 min	120 min	180 min
Control	10 mL/kg	154±2.92	155±1.84	152±1.97	157±1.38	156.2±0.73
Standard	1 mg/kg	119.6±1.63*	114±1.41*	107±0.84*	84.4±1.81*	73.2±1.39*
MBF	200 mg/kg	83.8±1.71* □	61.8±0.73* □	52.6±0.93* □	46±1.30* □	41.2±0.86* □
MBF	400 mg/kg	117±1.14* ■	76.2±0.73* □ ■	72±1.14* □ ■	68±1.76* □ ■	65.2±0.73* □ ■

Number of movement present as mean ± standard error of mean. \* $P < 0.05$ , vs control (Dennett's  $t$  test); □  $P < 0.05$ , vs standard; ■  $P < 0.05$ , vs MBF 200 group (pair-wise comparison by post-hoc Bonferroni test). Here, MBF: Methanolic extract of *Borassus flabellifer*.

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