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Evaluation of the locomotor and skeletal muscle relaxant activities of ethanolic extracts of *Acacia Nilotica* Linn. BARK. (Mimosaceae)

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Abstract

Acacia nilotica Linn. Subsp. indica (Family- Mimosaceae) bark has many activities like anti-plasmodial, molluscidal, anti-fungal, anti-microbial activity. The plant has no CNS depressant action found yet, but having such good number of beneficial action to human being has drawn our interest to study the same. The aim of the present study is to investigate the CNS depressant activity of ethanolic extracts *A.nilotica* bark on mice. The CNS depressant activity was determined by muscle relaxant property, reduction in spontaneous motility. The investigations had brought out that ethanolic extract of *Acacia nilotica* bark has significant CNS depressant property.

Keywords: *Acacia nilotica*, CNS depressant activity, Muscle-relaxant, Locomotor, Ethanolic extract.

Introduction

Acacia nilotica (Linn.) commonly known as gum arabic tree belonging to the genus of Acacia, family Mimosaceae, found throughout the India especially in West Bengal.(1) In Bengali it is known as Babla/Babul. Various parts of this plant is used as anticancer, anti tumours, antiscorbutic, astringent, anti-oxidant, natriuretic, antiplasmodial, diuretic, intestinal pains and diarrhea, nerve stimulant, cold, congestion, coughs, dysentery, fever, hemorrhages, leucorrhea, ophthalmia and sclerosis.(2) The leaves and pods are an excellent fodder with anti inflammatory properties, rich in protein. The pods have molluscicidal and algicidal properties. The leaves are used as gargle for sore throat, tonic to liver, enriches blood.(3)

There are some reports that the bark extracts possesses the potential CNS depressant activity, but enough research data is unavailable. This present study is carried out to study the neurological activity of the crude ethanolic extract of *Acacia nilotica* Linn. Bark.

Materials and Methods

Plant Material: The Barks of *Acacia nilotica* (local name Babool) collected from the local market of Barasat, North 24 Parganas in the month of December 2015.

Plant Extract Preparation: For the preparation of extract about 200 g of air dried, powdered bark were charged in to Soxhlet's apparatus and successively extracted with 95% ethanol at room temperature for 7 days. The extract was evaporated to dryness in rotary evaporator. The yield of ethanolic extract was obtained as 4.5% w/w. Moreover, the extract was subjected to preliminary phytochemical screening for the detection of various plant constituents.

Preliminary Phytochemical Studies: The EEAN (ethanolic extract of *Acacia nilotica*) was tested for the presence of alkaloids with Dragendorff's reagent, Mayer's reagent and Hager's reagent; flavonoids with the use of lead acetate, Magnesium and HCl; tannins with ferric chloride and potassium dichromate solutions; glycosides with Baljet's test and Keller-Killiani test and saponins by using standard phytochemical screening procedure.(4)

Experimental Animals: Swiss Albino Mice (20-25gm) of either sex were used. The animals were obtained from M/s Chakraborty Enterprises, A CPCSEA registered company (Regn No 1443/PO/b/11/CPCSEA). They were kept under standard environmental conditions (24.0±0°C and 55-65% relative humidity with a 12 h light/dark cycle) for two weeks for acclimatization. They were feed rodent food and had access to tap water ad libitum. All the animals were fasted over night prior to each test but had free access to water.

Acute Toxicity Study: The EEAN was employed for the determination of acute oral toxicity and LD_{50} (lethal dose) by using female, non pregnant mice weighing 18-20 g as per revised OECD guidelines No.425. The animals were fasted overnight and then were administered with the ethanolic extract at the following doses; 175, 550, 1750 and 2000 mg/kg by oral route. Animals were observed for their mortality during 48h study period (short term) toxicity and the final LD_{50} values were calculated as per the OECD guidelines 425.(5)

Assessment of Locomotor Activity: This test involves placing a number of mice-activity cages, which enables movement of the animal across a light beam to be recorded as a locomotion count. This test can demonstrate a CNS depressant or stimulan t activity profile. The animals were allowed to adapt to the new environment for at least 10 min and then the locomotor activity was counted. The plant extract (100,200 and 400 mg/kg,

i.p.) or the standard drug Diazepam 4 mg/kg (i.p.) was administered 30 min before the assessment of locomotor activity. Counts were then taken after 30 and 60min.[6]

Assessment of Skeletal Muscle Relaxant Activity: Animals remaining on Rota-Rod (16 rpm) 2 min or more in low successive trials were selected for testing; 30 min after t he injection of test material or control vehicle the same test was repeated at intervals of 15 min for 1 h.

The fall off time from the rotating rod was noted. The difference in the fall off time from the rotating rod between the control and the treated mice (standard-Diazepam/
extract) was taken as an index of muscle relaxation.[7]

Statistical Analysis: All data are presented as the mean ± standard error of mean (SEM). The results were analyzed for statistical significance by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test of significance. P values less than 0.001 were considered as statistically significant.

Results

Preliminary Phytochemical Screening

Table 1: Phytochemical analysis of ethanolic extracts of stem bark of *Acacia nilotica*

Sl. No.	Phytochemicals	Analysis
1.	Alkaloids	Present
2.	Glycosides	Present
3.	Carbohydrates	Present
4.	Sterols	Present
5.	Flavonoids	Present
6.	Tannins	Present
7.	Saponins	Present
8.	Proteins	Absent
9.	Anthraquinones	Present
10.	Fixed Oils and Fats	Absent
11.	Cardiac Glycosides	Present
12	Triterpenoids	Present

Acute Toxicity Study: There was no mortality amongst the graded dose in groups of animals and they did not show any toxicity or behavioral changes at a dose level of 5000 mg/kg. This finding suggests that EEAN safe in or non-toxic to mice up to 2000 mg/kg. Hence, in our study 100mg/kg, 200mg/kg and 400 mg/kg doses of extract were selected.

Locomotor Activity

Table 2: Effect of ethanolic extract of *A.nilotica* bark on locomotor activity in mice

		Number of movements (for 10 minutes)			% Reduction Activity	
Groups	**Dose(mg/kg BW)**	**Before treatment**	**After 30 min treatment**	**After 60 min treatment**	**After 30 min**	**After 60 min**
Control (Normal saline)	-----	210±0.984	206±0.886	208±1.203	----------	----------
Diazepam	4	257±1.234	96±0.768	56±1.875	62.64%	78.21%
EEAN	100	287±0.987	230±1.876	199±1.063	19.86%	30.66%
EEAN	200	255±1.323	180±0.323	130±1.245	29.41%	49.01%
EEAN	400	211±0.876	144±1.234	92±1.854	31.75%	56.39%

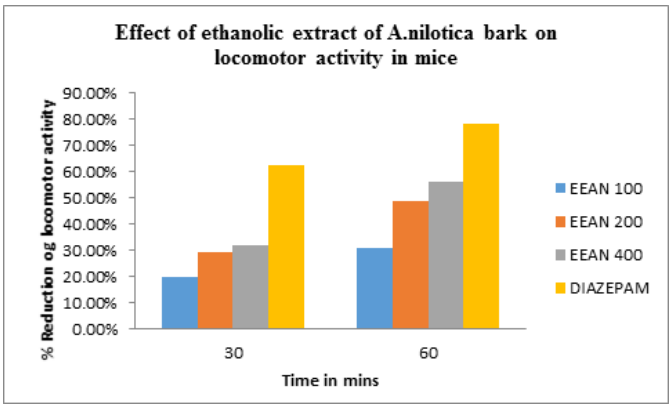

Fig. 1: Effect of ethanolic extract of *A.nilotica* bark on locomotor activity in mice

From Table 2 and Fig. 1, EEAN (100, 200 and 400 mg/Kg BW) and Diazepam (4mg/Kg BW) produced highly significant ($p<0.01$) reduction in locomotor activity as compared to control. EEAN showed the reduction in spontaneous motility in dose dependent manner.

Skeletal Muscle Relaxant Activity

Table 3: Effect of ethanolic extract of *A.nilotica* bark on time spent on the rotarod in secs

Groups	Dose (mg/kg BW)	0mins	30mins	45mins	60mins
Control	----	345±0.987	375±0.998	328±1.098	330±0.776
Diazepam	10mg/kg BW	328±1.230	233±1.097	190±0.657	87±1.967
EEAN	100mg/kg BW	340±0.780	307±1.232	284±1.005	250±0.775
EEAN	200mg/kg BW	337±0.954	290±0.972	210±1.123	134±1.098
EEAN	400mg/kg BW	360±0.667	270±1.870	231±0.435	110±1.538

Table 4: Muscle relaxant property (% activity) of *A.nilotica* bark ethanolic extracts

Groups	Dose (mg/kg BW)	30mins	45mins	60mins
Diazepam	10mg/kg BW	28.95%	42.07%	73.47%
EEAN	100mg/kg BW	9.70%	16.47%	26.47%
EEAN	200mg/kg BW	13.94%	37.68%	60.23%
EEAN	400mg/kg BW	25.00%	35.83%	69..44%

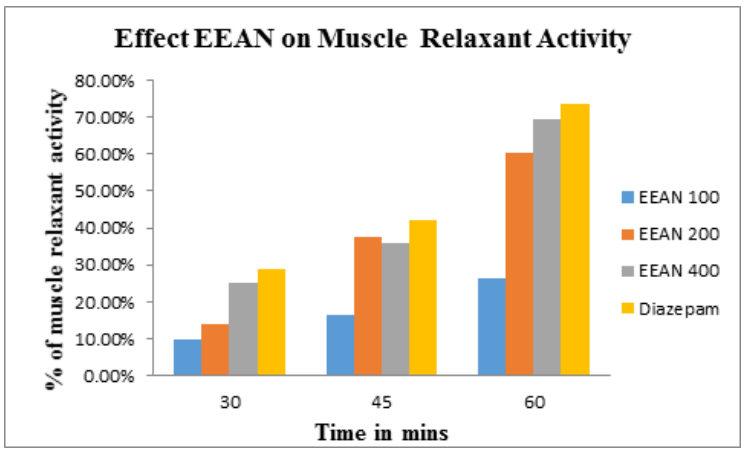

Fig. 2: Muscle relaxant property (% activity) of *A.nilotica* bark ethanolic extracts

From Table 4 and Fig. 2 EEAN at all the doses (100 mg/kg, 200mg/kg and 400mg/kg) showed highly significant reduction in the time spent by the animals on the revolving rod when compared to the control ($P<0.01$). EEAN showed dose-dependent increase in muscle relaxation. The standard drug (Diazepam) also

showed a highly significant effect when compared to the control ($P<0.01$).

Discussion

The locomotor activity was evaluated to assess the CNS-depressant property of EEAN on the motor activity in mice. Most of the centrally active analgesic agents influence the locomotor activities in human beings and rodents mainly by reducing the motor activity because of their CNS depressant property. Locomotor activity is considered as an index of wakefulness or alertness of mental activity and a decrease may lead to calming and sedation as a result of reduced excitability of the CNS. The results of the present study showed significant influence in locomotor activity of mice by EEAN treatment demonstrating decrease in locomotor activity and hence indicating its CNS depressant property in mice.

In muscle relaxant evaluation, the EEAN-induced decrease in fall off time was due to the loss of muscle grip implying skeletal muscle relaxation. Demonstration of marked muscle relaxant effect by the rota-rod study indicated that EEAN induced neurological deficit accompanied with taming or calming effect in mice, thereby further supporting its CNS-depressant effect.

Conclusion

From the present series of experiments, it can be concluded that the barks of *A. nilotica* possessed promising centrally and peripherally mediated locomotor depressant, skeletal muscle relaxant effects in the experimental rodent models demonstrating its prominent depressant action on the central nervous system, as manifested by these important neuro-pharmacological properties in Swiss albino mice. Purification of the plant extract and further definitive studies may reveal the exact mechanisms and constituents behind the observed neuro-pharmacological activities of *A. nilotica* bark.

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