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IN VITRO MEMBRANE STABILIZING AND *IN VIVO* ANALGESIC ACTIVITIES OF DIPTEROCARPUS TURBINATES GAERTN

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Abstract: *The methanol extract of leaves of Dipterocarpus turbinatus Gaertn. and its Kupchan fractions were screened for in vitro membrane stabilization while the methanolic crude extract was evaluated for in vivo analgesic activity in mice at 200- and 400- mg/kg b.w. In heat- and hypotonic solution- induced conditions, the crude methanol extract inhibited haemolysis of human erythrocyte by 62.40±1.43% and 69.14±2.05%, respectively as compared to 70.37±0.95% and 40.60±1.65% demonstrated by the standard acetyl salicylic acid. Moreover, the analgesic activity was determined for its central and peripheral pharmacological actions using tail immersion and formalin-induced writhing test at 200- and 400- mg/kg b.w. The extract significantly (P<0.05) influenced the formalin-induced writhing in a dose dependent manner with the highest activity observed at 400 mg/kg b.w. (34.33%) when compared with the standard drug, acetyl salicylic acid (42.64%). A significant dose-dependent increase (P<0.05) of latency period was also observed in tail immersion method.*

Keywords: *Dipterocarpus turbinatus, membrane stabilizing, writhing and analgesic.*

Introduction

Dipterocarpus turbinatus Gaertn. (Common Name- Garjan or Gurjan; Family- Dipterocarpacea) is a tree native to western India and mainland Southeast Asia and cultivated in the surrounding areas. The oleo-resin of the trunk of *D. turbinatus* is a stimulant to the mucous surfaces and diuretic and is used externally for ulcers, ringworms and other cutaneous diseases such as eczema, cuts and wounds[1, 2]. It has been used in gonorrhoea, gleet and rheumatism[3]. Previous phytochemical investigations of *D. turbinatus* revealed the trunk to contain oleoresin, known as garjan balsam, which yields an essential oil containing a hydrocarbon, β-caryophyllene, humulene, sesquiterpene derivatives. The bark of *D. turbinatus* contains bergenin and the seeds are a rich source of urease[4]. Since this plant has important medicinal properties, the present study has been undertaken as part of our regular research program[5, 6, 7], and we, herein, report the membrane stabilizing and analgesic activities of the leaf of *D. turbinatus* for the first time.

Materials and Methods

Plant materials: The leaves of *D. turbinatus* were collected from botanical garden in Dhaka and a voucher specimen of the plant sample has been deposited in the Department of Botany, University of Dhaka for future reference.

Extraction and fractionation: The collected plant parts were cleaned under tape water, sun dried for several days and then oven dried for 24 hours at 40° C to facilitate grinding. The powdered whole plant (500 gm) of *D. turbinatus* was soaked in 2.0 L methanol for 7 days and then filtered through a cotton plug followed by Whatman filter paper number 1. The extract was then concentrated by using a rotary evaporator at reduced temperature (40-45° C) and pressure. An aliquot (5.73 gm) of the concentrated methanol extract (ME) was partitionated by the modified Kupchan method[8] and the resultant partitionates i.e., petroleum ether (PE), carbon tetrachloride (CT) and aqueous (AQ) soluble materials were used for biological screenings.

Animals: Swiss albino mice (male), were bred in the animal house of Department of Pharmacy, Jahangirnagar University, Savar, Dhaka, Bangladesh. Mice of weight range of 23-25 gm were used for the experiments. All the animals were acclimatized one week prior to the experiments. The animals were housed under standard laboratory conditions (relative humidity 55-65%, room temperature 25.0±2 ℃, and 12 h light dark cycle). The mice were fed with ICDDRB formulated standard diet and had free access to drinking water but were fasted 12 h prior to each experiment. Standard protocols for animal experiment were followed to minimize discomfort.

Drugs: Acetylsalicylic acid , Tramadol, 1% Formalin.

Measurement of membrane stabilizing activity: The inhibition of hypotonic solution- and heat- induced haemolysis of human erythrocytes was used to determine the membrane stabilizing activity of the extractives[9].

Measurement of analgesic activity

Formalin induced writhing method: The analgesic activity of crude extract was evaluated by using formalin-induced writhing method in mice[10]. Experimental animals (Swiss albino mice) were randomly selected and divided into three groups denoted as group-I, group-II, and group-III consisting of 4 mice in each group. Each group received a particular treatment *i.e.* control, standard and the two doses of the extract, respectively. Each mouse was weighed properly and the doses of the test samples and control materials were adjusted accordingly. Test samples at 200- and 400- mg/kg b.w., control and standard acetyl salicylic acid were given orally by means of a feeding needle. An interval of thirty minutes was given to ensure proper absorption of the administered substances. Then the writhing inducing chemical, formalin solution (1%), was then administered subcutaneously to each of the animals of a group. After an interval of 10 min, which was given for absorption of formalin, the number of squirms (writhing) were counted for 5 minutes.

Tail immersion test: According to the method described[11], the tail immersion test was used to measure response latency. Mice were divided into 4 groups of 4 animals each. Group 1, the control group received normal saline, p.o. (20 ml/kg b.w.); group 2, the standard group received Tramadol (10 mg/kg b.w.). Groups 3 and 4 received crude extract at 200- and 400- mg/kg b.w., respectively. The lower 5 cm portion of the tail was marked and his part of the tail was immersed in to the warm water bath (55 ± 2 °C). Within a few seconds the rat were seen to react by withdrawing the tail. The reaction time was recorded in 0.5 seconds units by a stopwatch. After each determination the tail was

carefully dried. The reaction time was determined periodically such as 0, 30, 60 and 90 minutes after oral administration of the test substances. The cut-off time for tail immersion latency was set at 15 seconds. The reaction time was also determined prior to the administration of any substance.

Statistical analysis: The values are presented as mean ± SEM and student's *t*-test was used to determine a significant difference between the control group and experimental groups. $p < 0.05$ was considered to be statistically significant.

Result

The methanol extract of *D. turbinatus* as well as different Kupchan partitionates derived from it were subjected to assay for membrane stabilizing activity while the methanolic crude extract were evaluated for analgesic activity.

Table 1: Percentage (%) inhibition of heat- and hypotonic solution- induced haemolysis of erythrocyte membrane by standard and different partitionates

Sample code	% inhibition of haemolysis	
	Heat induced	Hypotonic solution induced
Hypotonic medium	--	--
ME	62.40±1.43	69.14±2.05
PE	57.46±1.63	14.08±2.06
CT	40.00±1.60	18.36±1.63
AQ	19.00±0.71	1.43±0.80
Acetyl salicylic acid	40.60±1.65	70.37±0.95

ME= Methanol extract, CT= Carbon tetrachloride soluble fraction, PE= Petroleum ether soluble fraction, AQ= Aqueous soluble fraction, ASA= Acetyl salicylic acid.

Table 2: Analgesic activity of *D. turbinatus* in formalin induced writhing test

Groups	Dose (mg/kg)	% Writhing	% Inhibition
Control (vehicle)	-	100	-
Acetyl salicylic acid	50	57.01	42.64 *
Methanol extract of *D. turbinatus*	200	76.06	23.94 *
	400	65.67	34.33 *

n=4, * P<0.05, significant compared to control

Table 3: Analgesic activity of *D. turbinatus* in tail immersion test

Groups	Dose (mg/kg)	Reaction time in seconds at time			
		0 min	30 min	60 min	90 min
Control (vehicle)	-	5.02±0.84	4.71±0.62	2.82±0.62	1.96±0.04
Tramadol	10	7.82±1.77	7.50±0.76 *	5.43±0.23 *	3.90±0.55 *
Methanol extract of *D. turbinatus*	200	5.03±1.34	4.63±0.23	4.56±0.56	4.78±0.40 *
	400	4.16±0.74	5.67±1.20	5.67±0.33 *	6.37±0.32 *

All values are expressed as mean ± SEM; n=4, * P<0.05, significant compared to control.

Discussion

At concentration 1.0 mg/ml, the different fractions of *D. turbinatus* protected the haemolysis of RBC induced by heat and hypotonic solution as compared to the standard acetyl salicylic acid. The crude methanol extract inhibited 62.40±1.43% and 69.14±2.05% of haemolysis of RBC induced by heat and hypotonic solution as compared to 40.60±1.65% and 70.37±0.95% by acetyl salicylic acid, respectively (Table 1).

In formalin-induced analgesic activity test the test samples of *D. turbinatus* exhibited dose dependent inhibition of writhing. The extract inhibited 23.94% and 34.33% of writhing at a dose of 200 and 400 mg/kg b.w., respectively while the standard acetyl salicylic acid demonstrated 42.64% inhibition of writhing (Table 2).

The extract of *D. turbinatus* when administered orally at 200- and 400- mg/kg body weight, respectively showed significant analgesic activity in tail immersion method as supported by the increase in latency time when compared to control. The increase in latency was found to be dose dependant. However, it was the maximum at the dose of 400 mg/kg b.w. and was comparable with the standard drug (Table 3).

Conclusion

The results demonstrated significant membrane stabilizing and analgesic activities of the methanol extract of *D. turbinatus*. These indicated that this plant could be a potential source for the discovery of new analgesic and anti-inflammatory "leads" for drug development. These findings support its traditional claims for using it in rheumatic problems and provide a scientific basis for analgesic and anti-inflammatory effect of *D. turbinatus*. However, further detailed studies are necessary to identify the active principle(s) and elucidate the exact mechanism(s) behind these pharmacological properties.

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