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Nootropic and behavioural actions of saponins isolated from bark of *Albizzia lebbeck*

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Abstract

Objectives: In the previous study saponins isolated from leaves of *Albizzia lebbeck* exhibited nootropic activity in mice. The objectives of the present study were therefore to assess the nootropic activity of saponin containing fraction isolated from the bark of *Albizzia lebbeck* using different paradigms and to study its effect on the behaviour mediated by various neurotransmitters like acetylcholine, noradrenaline, serotonin, dopamine and gamma-aminobutyric acid. Materials and methods: The nootropic effect of saponin containing n-butanolic fraction (ALBF) obtained from dried bark of *A. lebbeck* was studied in male albino mice using passive shock avoidance, elevated plus maze, and object recognition. The effect of ALBF was studied on sodium nitrite-induced respiratory arrest (acetylcholine mediated behaviour), clonidine-induced hypothermia and passivity (noradrenaline mediated behaviour), baclofen-induced hypothermia and passivity (gamma-aminobutyric acid mediated behaviour), haloperidol-induced catalepsy (dopamine mediated behaviour), and lithium-induced head twitches (serotonin mediated behaviour). The effects were compared with those of piracetam. Results: ALBF reduced latency to reach shock free zone and number of mistakes in the passive shock avoidance paradigm. ALBF antagonized amnesic effect of scopolamine in this test. ALBF *per se* reduced transfer latency on the elevated plus maze. The inflexion ratio reduced by scopolamine was not increased significantly by ALBF. In the object recognition test, ALBF explored the familiar object significantly earlier compared to that by piracetam. Both piracetam and ALBF antagonized the amnesic effect of scopolamine in this experiment. ALBF potentiated hypothermic effect of clonidine but could not antagonize the baclofen-induced hypothermia significantly. ALBF increased clonidine-induced passivity and inhibited baclofen-induced passivity. ALBF was without any effect on sodium nitrite-induced respiratory arrest and haloperidol-induced catalepsy but reduced lithium-induced head twitches. Conclusion: The ALBF, saponins containing fraction of *A. lebbeck per se* exhibited nootropic activity and reversed amnesic effect of scopolamine. The ALBF exhibited decreased noradrenergic and serotonergic transmission and was without any effect on cholinergic and dopaminergic transmission. The study indicates that the saponins isolated from *A. lebbeck* possess potential nootropic activity. The absence of cholinergic and dopaminergic involvement in nootropic activity of ALBF may be utilized to study the involvement of other neurotransmitters in the cognitive function.

Keywords: *Albizzia lebbeck*, Nootropic activity, Behaviour, Saponins.

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1. Introduction

In recent years, there has been a phenomenal rise in the interest of scientific community to explore the pharmacological actions of herbs and to confirm the claims made about them in the official books of Ayurveda. Several plants are known to possess nootropic activity [1]. Nalini *et al.*, [2] have reported nootropic activity of oil obtained from *Celastrus paniculatus.*

This laboratory has reported nootropic activity of leaves of *Lawsonia inermis* [3]. Drugs having different chemical structures exhibit the nootropic activity. The alkaloids from *Vinca minor* and *Secale cornutum* [4], and saponins, bacoside A and B from *Bacopa monnieri* [5], and ginsenoside Rb1 and Rb 1- influene, the saponins from *Panax ginseng* [6] are the active principles responsible for enhancing cognitive function. The leaves of *Albizzia lebbeck* Benth (Fam. Mimosaceae) are rich in saponin [7]. We have previously studied nootropic activity of saponin containing fraction of *A. lebbeck* leaves (in press).

Since bark of *A. lebbeck* is also a rich source of saponins we studied the nootropic activity of saponins isolated from the bark using passive shock avoidance, elevated plus maze test, and object recognition test in mice.

To correlate the nootropic activity with behaviour mediated by some of the neurotransmitters involved in cognitive function, the effect of ALBF was studied on sodium nitrite induced respiratory arrest (acetylcholine mediated behaviour), clonidine-induced hypothermia and passivity (noradrenaline mediated behaviour), baclofen-induced hypothermia and passivity (gamma-aminobutyric acid mediated behaviour), haloperidol-induced catalepsy (dopamine mediated behaviour), and lithium-induced head twitches (serotonin mediated behaviour).

2. Materials and methods

2.1 Preparation of extract

Saponins were extracted using method described by Pal *et al.*, [7].

In brief, shade dried powdered bark (0.6 kg.) of *Albizzia lebbeck* was defatted with petroleum ether (60°-80°C) in Soxhlet's apparatus. The marc was dried and extracted with methanol. The methanolic extract was evaporated to dryness in vacuum. The residue (15g) was suspended in water, extracted successively with ethyl acetate and n-butanol. (3x100 ml each) and solutions were evaporated to dryness in vacuum to provide ethyl acetate soluble (2.0g), n-butanol soluble (9.6g) and water soluble portions (2.7g), n-butanol soluble fraction (ALBF) was tested for the presence of saponins and used for pharmacological studies.

2.2 Animals

Male albino mice (20-25g) and albino rats (125-150g) were used. The animals were housed in cages at ambient temperature of 25 ± 2°c with a 12 h light: dark cycle. Experiments were carried out between 0900 to 1400 h. Food but not water was withheld 12 h before experimentation. Animals were tail-marked and handled daily for 5 min during the last three days before experiment.

2.3 Drugs

Piracetam (Uni-UCB Ltd., India), clonidine, (Unichem Laboratories Ltd., India), baclofen (Sun Pharma Ltd., India), scopolamine (Inj. Buscopan, German Remedies, India) were dissolved in de-ionized water. All drugs except ALBF were administered intraperitoneally. ALBF was admnistered orally. The injections were given in a volume of 2.0 ml/kg body weight. The n-butanolic fraction of methanolic extract of *A. lebbeck* (ALBF) was given orally.

2.4 Passive shock avoidance [8]

The apparatus consisted of an electric grid (24x30 cm) with a shock free zone (SFZ, 2x3x1 cm) in the center and the entire grid having perplex enclosure. Mice were placed individually on electric grid and allowed to explore the maze for one minute.

The stimulus (20V) with AC current of 5mA was then applied and latency to reach the shock free zone (SFZ) was recorded three consecutive times as basal reading. Animals that reached the SFZ in 2 min in the first trial were selected for the study.

After 1 h of the first trial, each animal was put on the grid again and the latency to reach SFZ and number of mistakes (descents) the animal made in 15 min were recorded as parameters for acquisition and retention respectively. Piracetam (100mg/kg) and scopolamine (0.3mg/kg) were administered 30 min before test while ALBF (0.1mg/kg) was administered 60 min before test. One group received vehicle only. All groups consisted of five animals.

2.5 Elevated plus maze

An elevated plus-maze consisting of two open arms (35x6 cm) and two enclosed arms (35x6x15 cm) was used. The maze was elevated at a height of 25 cm. Mice were placed individually at the end of open arm facing away from central platform and the time it took to move from open arm to either of the closed arm (transfer latency, TL) was recorded. On the first day, the mouse was allowed to explore the plus maze for 5min and sent back to their home cages after the first trial [9].

After 24 h mice were placed again on the elevated plus maze individually as before and TL was noted again. Transfer latency measured on first day served as parameter for acquisition. TL was expressed as retention scores after 24 h or one week for each mouse by calculating the "inflexion ratio" [10],

Inflexion ratio = $(L_1-L_0) / L_0$

Where,

L_0 = Transfer Latency after 24 h as week in seconds.

L_1 = initial transfer latency in seconds.

The animals (n = 6) received vehicle, ALBF (0.05 or 0.1mg/kg), piracetam (100mg/kg), scopolamine (0.3mg/kg) with or without piracetam / ALBF. Vehicle and ALBF were administered orally 60 min prior to the first trial whereas in another set of experiments ALBF/ piracetam was administered 30 min before scopolamine and the trial was performed 30 min later. No treatment was given on the 2^{nd} and 9^{th} day.

2.6 Object recognition test [11]

The apparatus was formed by polyvinyl chloride box (70x60x30 cm) with a grid floor that could be easily cleaned. The apparatus was illuminated by 75W lamp suspended 50 cm above the box. The objects to be discriminated were also made of polyvinyl chloride, gray colored and were in three different shapes: cubes of 8 cm side, pyramids and cylinders of 8 cm height. The day before testing mice were allowed to explore the box for 2 min.

On the day of the test in the first trial (T_1) two identical objects were presented in two opposite corners of the box, and the amount of the time taken by each mice to complete 20s of object exploration was recorded. Exploration was considered directing the nose at a distance < 2 cm to the object and / or touching it with the nose.

During the second trial (T_2, 90 min after T_1) one of the object presented in T_1 was replaced by new object and mice were left in the box for 5 min. The times spent for the exploration of

the familiar (F) and the new object (N) were recorded separately and discrimination index (D) was calculated (N-F/N+F). Care was taken to avoid place preference and olfactory stimuli by randomly changing the role (familiar or new object) and the position of the two objects during T_2, and cleaning them carefully.

ALBF was administered orally 60 min prior to trial T_1 while scopolamine (0.3mg/kg) was administered intraperitoneally 30 min before the trial T_1. Each group consisted of 6 mice.

2.7 Sodium nitrite intoxication [12]

Sodium nitrite ($NaNO_2$, 250 mg/kg s.c.) was used to induce chemical hypoxia. $NaNO_2$ reduces the oxygen carrying capacity of the blood by converting hemoglobin to methemoglobin. This dose produces 100% death in all vehicle treated animals by respiratory arrest. The test drug or vehicle was injected 60min before $NaNO_2$. The time between injection of $NaNO_2$ and cessation of respiration was recorded. Pilocarpine (10mg/kg i.p.) was used as reference standard. Each group consisted of 5 mice.

2.8 Clonidine-induced passivity and hypothermia [13]

Albino rats (150-200g) were taken into groups of 6 each. Passivity and rectal temperature were measured every 30 min after clonidine (0.1mg/kg i.p.) till 180 min ALBF (0.1 mg/kg p.o.) was administered 30 min before clonidine (0.1mg/kg). Passivity was scored as described by Turner [14].

The rat was grasped with the thumb and index finger, which held the dorsal skin of the neck, while the rat was in walking position. An unaffected rat moved its head and limbs in trying to escape (0). The rat still grasped in the same manner was held in a vertical position, it struggled (2). When the unaffected rat was placed on the observer's fist held in the supine position with head resting on the thumb, it tried to escape (4).

The unaffected rat tried to escape when held vertically by one fore paw (6) or by one hind paw (8). Intermediate scores were used when struggle was diminished but not abolished.

Table 1.
Effect of n-butanolic fraction (ALBF) of methanolic extract of *A. lebbeck* on passive shock avoidance in mice.

Treatment (mg/kg)	**Latency to reach SFZ in sec (mean ± SEM)**	**Mistakes in 15 min (mean ± SEM)**
Vehicle	12.8 ± 1.2	13.0 ± 0.9
Piracetam (100)	9.4 ± 0.9*	6.6 ± 0.9**
ALBF (0.1)	3.6 ± 0.51**	6.8 ± 1.5**
Scopolamine (0.3)	19.2 ± 1.4**	19.0 ± 1.4**
Piracetam (100) + Scopolamine (0.3)	6.2 ± 0.97#	7.2 ± 2.08#
ALBF (0.1) + Scopolamine (0.3)	9.4 ± 3.14##	7.0 ± 2.26#

n = 5, SFZ - shock free zone; *P<0.05, **P<0.01compared to vehicle treated group (Student's *t* - test); #P<0.05, ##P<0.01 compared to scopolamine treated group (Student's *t* - test).

Table 2.
Effect of n-butanolic fraction (ALBF) of methanolic extract of *A. lebbeck* on transfer latency (TL) and inflexion ratio in mice.

Treatment	Transfer latency in sec (mean (SEM) on			Inflexion Ratio	
(Dose:mg/kg)	Day 1	Day 2	Day 9	Day 2	Day 9
Vehicle	26.0 ± 2.7	16.4 ± 1.84*	24.0 ± 4.41	0.7 ± 0.3	0.08 ± 0.1
Piracetam (100)	30.4 ± 7.31	11.5 ± 1.5	11.5 ± 1.6	1.9 ± 0.6*	1.85 ± 0.5*
ALBF (0.05)	28.0 ± 2.9	14.6 ± 3.0*	15.2 ± 2.5*	1.2 ± 0.4	0.9 ± 0.3*
ALBF (0.1)	28.2 ± 5.7	9.8 ± 1.0**	13.8 ± 1.5	2.0 ± 0.5*	1.0 ± 0.3*
Scopolamine (0.3)	40.8 ± 8.6	30.4 ± 4.8	22.0 ± 0.7	0.7 ± 0.5	0.8 ± 0.4
Piracetam (100)+ Scopolamine (0.3)	42.8 ± 13.8	26.6 ± 9.7	16.7 ± 2.7	0.65 ± 0.4	2.3 ± 0.1#
ALBF (0.1) + Scopolamine (0.3)	38.5 ± 17.1	18.4 ± 3.2	13.2 ± 1.0	1.2 ± 0.6	3.1 ± 1.6

n = 6; * P <0.05 and ** P < 0.01, compared to respective control (Student's *t* - test).
#P<0.01 compared to scopolamine group (Student's *t* - test).

Rectal temperature was measured by inserting the probe four inches inside rectum of rat till constant reading appears in telethermometer.

2.9 Baclofen - induced passivity and hypothermia

Albino rats (150-200g) were taken into groups of five each. Passivity and rectal temperature were measured every 30 min after baclofen (10mg/kg i.p.) till 180 min as described earlier by Turner [14]. ALBF (0.1mg/kg p.o) was given 30 min before baclofen.

2.10 Haloperidol-induced catalepsy [15]

Rats were divided into groups of 5 each. The control group received vehicle (1ml/kg orally) and haloperidol (3mg/kg i.p.). The other group received ALBF (0.1mg/kg p.o) 30 minutes before haloperidol. Catalepsy was scored at 0, 30, 60, 90, 120, 150 and 180 min using following scoring system: score 0 - rat moved normally when placed on the table, score 0.5- rat moved only when touched or pushed, score 0.5- rat placed on the table with front paws set alternately on a 3 cm high wooden block fails to correct the posture in 10 sec for each paw (total score of 1 per animal).

Score1 - rat fails to correct posture in 10 sec when front paws are placed on a 9 cm high wooden block , score 1 for each paw (total score of 2 per animal). Thus, for single rat maximum possible cumulative score of catalepsy was 3.5. A lower score would mean an apparently lesser degree of catalepsy.

2.11 Lithium- induced head twitches [16]

Rats were divided into groups of six each. Lithium sulphate (200mg/kg i.p.) was given 60 min after ALBF (0.1 mg/kg. p.o.) or vehicle and the number of head twitches were observed for 60 min.

2.12 Acute toxicity study

The ALBF was administered intraperitoneally in descending order of doses, starting from a dose of 100mg/kg i.p. followed by 30, 5, 1 and 0.1 mg/kg body weight to different groups of mice (n = 5 in

each group). The effect of ALBF was also studied on gross behaviour as described by Turner [17].

2.13 Statistical Analysis

The observations are mean ± S.E.M. Significant differences were evaluated using Mann-Whitney U-test, one way ANOVA and Student's *t* - test. P<0.05 was considered significant.

3. Results

3.1 Acute toxicity

The ALBF administered intraperitoneally in doses of 100, 30, 5mg/kg body weight was found to be lethal and all animals died within 6 h. Autopsy study indicated visceral hemorrhage (may be due to the haemolysis by saponins present in the extract). In a dose of 1mg/kg i.p. ALBF induced fighting behaviour in animals and the animals exhibited hyperactivity, increased grooming, convulsions, and hypothermia and no mortality was observed with the dose of 0.1mg/kg and was therefore used for further study.

3.2 Passive shock avoidance test

The vehicle treated mice required 12.8 ± 1.2 sec to reach the shock free zone (SFZ) and the animals committed 13.0 ± 0.9 mistakes (number of step-downs) in 15 min. Scopolamine increased the latency to reach the SFZ and also increased errors.

Whereas, ALBF *per se* reduced the time required to reach SFZ significantly (P<0.01). Both piracetam and ALBF reversed the amnesic effect of scopolamine as indicated by significant decrease in the latency to reach SFZ and the number of mistakes significantly. The observations are summarized in Table 1.

3.3 Elevated plus maze

The transfer latency (T_1) on the first day was not significantly affected by any treatment, though scopolamine treated group showed a tendency to increase it. The transfer latency on the second day (T_2) was significantly reduced by vehicle, piracetam, and both the doses of ALBF (0.05 and 0.1mg/kg; P<0.05).

Table 3.
Effect of n-butanolic fraction (ALBF) of methanolic extract of *A. lebbeck* on object recognition in mice.

Treatment (Dose: mg/kg)	T_1 session time in sec (mean ± SEM)	T_2 exploration time in sec (mean ± SEM)		D
		F	N	
Vehicle	279.6 ± 22.7	24.4 ± 3.5	28.9 ± 6.5	0.17 ± 0.0
Piracetam (100)	286.5 ± 18.6	10.8 ± 1.6@	30.0 ± 4.1**	0.47 ± 0#
ALBF (0.1)	293.3 ± 6.0	6.6 ± 0.6@	27.4 ± 3.4**	0.6 ± 0.0#
Scopolamine (0.3)	325.2 ± 36.0	22.0 ± 7.3	15.8 ± 3.8	-0.14 ± 0.1#
ALBF (0.1)+ Scopolamine (0.3)	442.0 ± 34.4*	11.0 ± 2.1	13.7 ± 1.49	0.13 ± 0.09[a]
Piracetam (100) + Scopolamine (0.3)	369.8 ± 30.3	28.0 ± 7.9	21.8 ± 2.5	-0.07 ± 0.1[a]

n = 6; T_1 = First exploration session; F = Familiar object; N = Novel object; D = Discrimination index; ALBF = n-butanolic fraction of *A. lebbeck*. Scopolamine was administered 60 min before first exploration session.
*P< 0.05, N *vs.* F (Student's *t* - test); **P< 0.01, N *vs.* F (Student's *t* - test); # P<0.01, *vs.* vehicle (ANOVA, followed by Dunnett's test); @ P<0.05 *vs.* vehicle (Student's *t* - test); [a] P<0.001 *vs.* scopolamine (ANOVA, followed by Dunnett's test).

However, piracetam and ALBF could not reverse the effect of scopolamine on day 2 and only piracetam reversed amnesic effect of scopolamine on the 9th day. The observations are given in Table 2.

3.4 Object recognition test

Except the group that received ALBF and scopolamine, none of the treatments exhibited any significant increase in the time required to explore the objects in the first trial (T_1). In the second trial, the animals treated with piracetam and ALBF required significantly less time to explore the familiar object and both the drugs significantly increased the discrimination index ($P < 0.05$).

Scopolamine decreased the discrimination index indicating amnesia and both the piracetam and ALBF could antagonize the amnesic effect of scopolamine significantly ($P<0.05$) as indicated by increased discrimination index. ALBF was more effective than piracetam. The observations are given in Table 3.

3.5 Sodium nitrite intoxication

The mice, receiving vehicle died due to respiratory arrest 23.4 ± 1.2 min after sodium nitrite whereas mice pretreated with ALBF ceased respiration after 20.6 ± 0.81min. Pilocarpine significantly delayed the effect of sodium nitrite till 40.0 ± 5.0 min ($P<0.05$).

3.6 Clonidine-induced passivity and hypothermia

ALBF significantly increased the passivity induced by clonidine. The effect was maximum 30 min after clonidine and then started fading. Significant ($P<0.05$) effect was noted till 90min after clonidine (Fig.1).

The peak hypothermic effect was noted 60 min after clonidine in both vehicle and ALBF treated mice. ALBF per se reduced rectal temperature significantly and potentiated hypothermia caused by clonidine at 60 and 90 min after clonidine ($P<0.05$). The observations are given in Table 4.

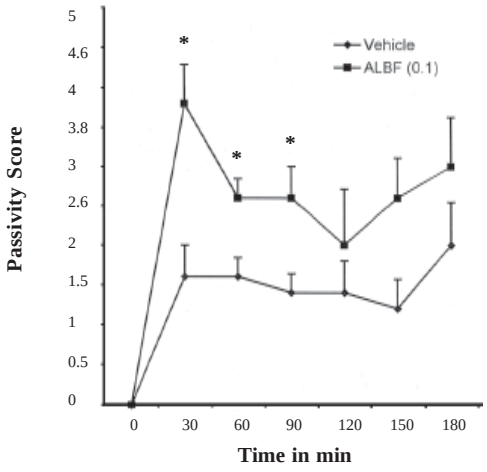
3.7 Baclofen - induced passivity and hypothermia

Baclofen-induced passivity was more severe than the clonidine-induced passivity. Peak effect was observed 150 min after baclofen. ALBF reduced the severity of passivity significantly during the test interval of 180 min (Fig 2). Baclofen induced fall in rectal

Table 4.
Effect of n-butanolic fraction (ALBF) of methanolic extract of *A. lebbeck* on clonidine-induced hypothermia in rats.

	Rectal temperature in °F (mean ± SEM)			
Treatment Time (min)	**Vehicle**	**Clonidine**	**ALBF**	**Clonidine + ALBF**
0	100.1 ± 0.4	98.92 ± 0.4	100.3 ± 0.5	100.0 ± 0.2
30	100.0 ± 0.4	98.10 ± 0.8*	99.5 ± 0.4	98.84 ± 0.2*,#
60	100.5 ± 0.2	97.68 ± 0.9*	98.8 ± 0.2*	96.24 ± 0.4**,#@
120	99.8 ± 0.17	98.62 ± 0.7*	98.9 ± 0.3*	96.72 ± 0.7**,#@
180	100.3 ± 0.3	98.64 ± 0.5*	98.4 ± 0.4*	97.5 ± 0.7*,#

n = 6. * P < 0.05, compared to vehicle treated group (Student's *t* - test).
** P<0.01, compared to vehicle treated group (Student's *t* - test). ; #P<0.05, compared to clonidine treated group (Student's *t* - test).; @P<0.05, compared to ALBF treated group (Student's *t* - test).



n=6, * p<0.05 *vs* Vehicle (Mann-Whitney U test)

Fig. 1: Effect of n-butanol soluble fraction (ALBF) of methanolic extract of *A.lebbeck* on clonidine induced passivity in rats

temperature within 30 min and the peak effect was observed 120 min after baclofen. ALBF *per se* also reduced rectal temperature significantly (P<0.05) but failed to potentiate baclofen-induced hypothermia significantly (P>0.05). The observations are given in Table 5.

3.8 Haloperidol-induced catalepsy

ALBF was without any significant effect on haloperidol-induced catalepsy (P>0.05). ALBF *per se* was without cataleptic activity (Fig 3).

3.9 Lithium-induced head twitches

In vehicle treated rats lithium sulphate induced 70.2 ± 9.07 head twitches whereas in ALBF treated rats only 39.4 ± 7.3 twitches were noted during the test interval of 60 min (P<0.05). Piracetam pretreated animals showed 70.8 ± 7.39 head twitches. The animals receiving ALBF alone did not exhibited head twitches.

4. Discussion

Nootropic drugs belong to the category of psychotropic agents with selective facilitatory effect on intellectual performance, learning and memory [18]. A number of drugs such as piracetam, aniracetam, nefiracetam have now been introduced in therapy to ameliorate cognitive deficits. In our studies on the saponin-containing fraction of *A. lebbeck* leaves, we noted potent nootropic activity (in press).

The study indicated that the saponins isolated from leaves of *A. lebbeck* improved the retention of acquired learning as indicated by decrease in the transfer latency on the elevated plus maze and decreased time required to explore the familiar object.

These observations were in accordance with the hypothesis of Itoh *et al.*, [19]. Since isolation of saponins from bark is more economical, it was our objective to study nootropic activity of saponins (ALBF) isolated from bark.

The latency to reach the shock free zone (SFZ) is considered as a parameter to evaluate effect of drug on acquisition whereas the number of mistakes is a parameter for evaluation of drug effect on retention of the learned task [8].

ALBF was most effective in reducing the latency to reach the SFZ and also reduced the number of mistakes indicating beneficial effect on acquisition as well as retention of learned task. ALBF could antagonize the amnesic effect of scopolamine in all the paradigms used in this study.

The transfer latency (on first day) on elevated plus maze was not affected significantly by ALBF as well as other drugs. The transfer latency on the second day of the experiment was reduced significantly by piracetam as well as ALBF but the improvement in memory as indicated by increased inflexion ratio was not significant when compared with that caused

Table 5.
Effect of n-butanolic fraction (ALBF) of methanolic extract of *A. lebbeck* on baclofen-induced hypothermia in rats.

	Rectal temperature in °F (mean ± SEM)			
Treatment	**Vehicle**	**Baclofen**	**ALBF**	**Baclofen+ALBF**
Time (min)				
0	100.1 ± 0.4	100.52 ± 0.4	100.3 ± 0.5	99.38 ± 0.2
30	100.0 ± 0.4	98.16 ± 0.5*	99.5 ± 0.4	97.78 ± 0.4*
60	100.5 ± 0.2	98.08 ± 0.7*	98.8 ± 0.2*	97.78 ± 0.4*
120	99.8 ± 0.1	97.6 ± 0.4*	98.9 ± 0.3*	98.65 ± 0.7
180	100.3 ± 0.3	97.78 ± 0.3*	98.4 ± 0.4*	97.72 ± 0.3*

n = 5, *P<0.05 compared to vehicle treated group (Student's *t* - test). ALBF was without any effect on baclofen-induced hypothermia.

by vehicle. ALBF and piracetam could not reverse the effect of scopolamine when tested on the second day. However, on the 9th day of the experiment the inflexion ratio was increased by piracetam only and the increase in inflexion ratio by ALBF could not reach statistical significance. This indicates weak nootropic activity of ALBF in this paradigm.

The object recognition test is a simple yet efficient way of observing the effect of drug on episodic memory [11]. Episodic memory means the ability of the mouse to recognize the object previously seen only once. Recognition of the object does not depend upon a reward or punishment but only on the innate exploratory behavior.

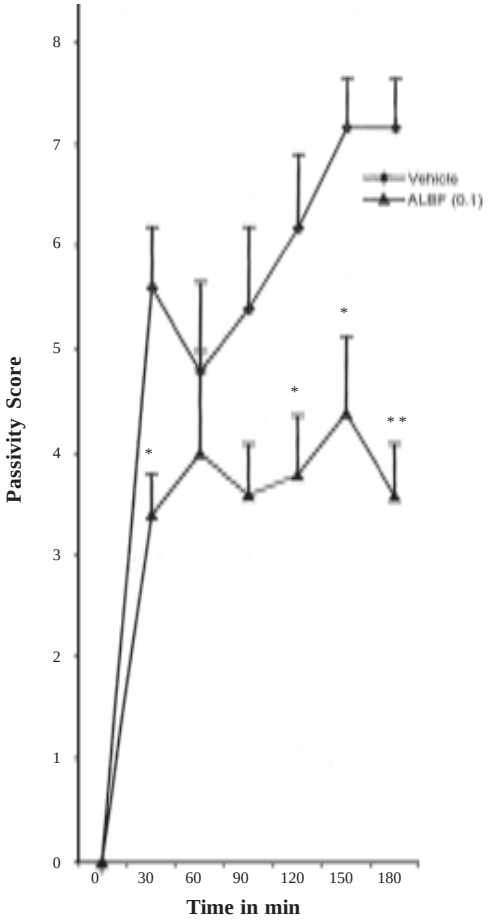
Under the experimental conditions employed in such studies, it has been reported that the episodic memory lasts for 60-90 min [11]. Piracetam as well as the ALBF showed object recognition when given alone and also antagonized impairment of object recognition by scopolamine.

Though several studies have been carried out on the effect of various agents on cognitive behaviour, the mode of action of these agents as well as changes in neurotransmitters in the brain that lead to impairment of memory are still unknown. Agents increasing cholinergic transmission are known to improve memory.

ALBF though effectively antagonized the effect of scopolamine it failed to inhibit the effect of sodium nitrite. Sodium nitrite which, converts hemoglobin to methemoglobin leads to respiratory arrest and agents improving cholinergic transmission delays or prevents the lethal action of sodium nitrite [12].

Piracetam, a well-known nootropic agent, is a GABA analog and antagonizes the effects of GABA [20]. Baclofen induced hypothermia is mediated via $GABA_B$ receptors [21]. In our study we observed additive effect of ALBF on the baclofen-induced hypothermia but the difference in temperature could not reach statistical significance. ALBF also inhibited baclofen-induced passivity. These observations indicate that inhibition of GABAnergic transmission may be (partly) responsible for nootropic activity of ALBF.

Some agents improve memory by increasing release of noradrenaline [2, 22]. We observed that ALBF potentiated hypothermia and passivity induced by clonidine indicating



n=5, *P<0.01 *vs* Vehicle (Mann-Whitney U test)
Fig. 2: Effect of n-butanol soluble fraction (ALBF) of methanolic extra ct of *A.lebbeck* on baclofen-induced passivity in rats.

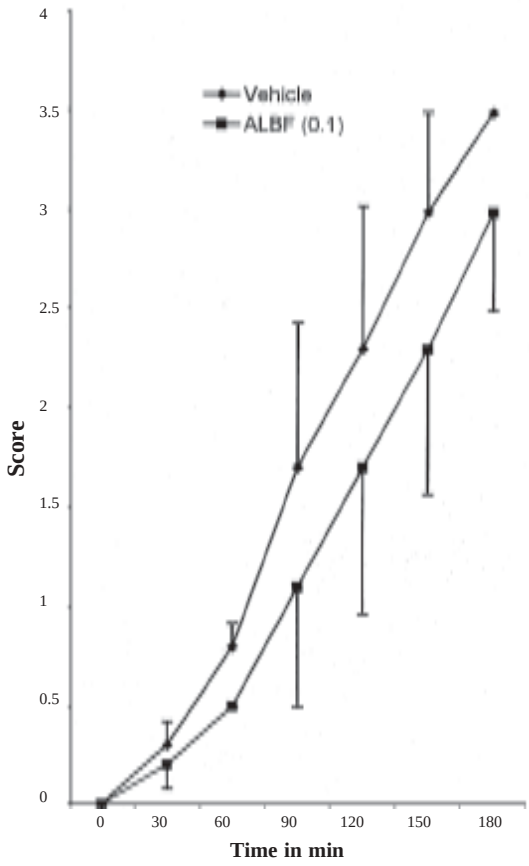


Fig. 3: Effect of n-butanol soluble fraction (ALBF) of methanolic extract of *A.lebbeck* on haloperidol-induced catalepsy in rats

decreased release of noradrenaline. Controversy still exists over involvement of dopaminergic system in cognitive behaviour [23].

In the present study we observed insignificant change in the dopaminergic transmission. Lithium induced head twitches suggests increased serotonin release [16]. Unlike piracetam (which was without any effect on head twitches), ALBF reduced the serotonergic transmission as indicated by decrease in the number of head twitches. Ogren [24] has reported that increase in serotonergic transmission can impair learning and memory.

In our previous study on the nootropic activity of saponin containing fraction of *A. lebbeck* leaves, we observed significant antagonism of scopolamine-induced amnesia in the elevated plus maze, passive shock avoidance and object recognition test. The LD_{50} of the fraction that contained saponins was approximately 50 mg/kg. Whereas in the present study the dose of 5 mg/kg caused death in all the animals within 6 h.

In conclusion, the nootropic activity of ALBF is unrelated to improvement in cholinergic

transmission and the dopaminergic and serotonergic mechanisms may not play any role in its cognitive behaviour.

Inhibition of GABAnergic transmission may be responsible for nootropic activity of saponins of *A. lebbeck*. Lack of cholinergic involvement in nootropic activity of ALBF may be utilized as a tool to study the involvement of other neurotransmitters in cognitive function.

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