ANTIDOPAMINERGIC ACTIVITY OF ISOFLAVONE ISOLATED FROM *BUTEA MONOSPERMA* FLOWERS

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Summary

Bioassay guided fractionation of methanolic extract of *Butea monosperma* flowers was carried out using inhibition of dopamine-induced contraction of rat vas deferens as a biological end point. The antidopaminergic activity was present in the isoflavone isolated from ethyl acetate soluble fraction of methanolic extract. The methanolic extract, its ethyl acetate soluble fraction and the isoflavone potentiated haloperidol-induced catalepsy and inhibited foot shock-induced aggression in rats in a dose dependent manner confirming the antidopaminergic activity.

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Introduction

Butea monosperma Kuntz (family: Fabaceae) is common throughout the Asia, upto 3,000 ft. Flowers are astringent, sweet, and acrid. They are traditionally used in treatment of leprosy, strangury, gout, and as astringent, depurative, diuretic, aphrodisiac and antidiarrhoeal. In Unani system of medicine, flowers are used as aphrodisiac, expectorant, tonic, and diuretic (1). Gawale et al., (2) reported nootropic activity of *Butea monosperma* flowers. *Butea monosperma* flowers also exhibit anticonvulsant activity in laboratory animals against seizures induced by maximum electroshock and also inhibited seizures induced by pentylenetetrazole, electrical kindling, and combination of lithium sulphate and pilocarpine (3). The ethanolic extract also possessed antistress activity (4). Soman et al., (5) reported antistress, anxiolytic, and memory enhancing activity of ethanolic extract of *Butea monosperma*. Manish et al., (6) have reported free radical scavenging activity of ethyl acetate and butanol fractions derived from total methanolic extract of *Butea monosperma* flowers using *in-vitro* models like reducing power assay, scavenging of 2,2- diphenly-picrylhydrazyl (DPPH) radical, nitric oxide radical, hydroxyl radical and inhibition of erythrocyte hemolysis using 2,2'-azo-bis-amidinopropane dihyrochloride (APPH).

In our previous studies (7) we observed that ethanolic extract reduced locomotion, which is indicative of diminished dopaminergic transmission. Therefore in the present study, we investigated antidopaminergic activity of methanolic extract of *B. monosperma* with the objective of isolating a pure compound having antidopaminergic activity. The antidopaminergic activity was assessed by observing the effect on rat vas deferens, haloperidol-induced catalepsy and foot shock-induced aggressive behaviour.

Materials and Methods

Drugs

Haloperidol (RPG Life Sciences, India) and dopamine hydrochloride (SG Pharma, India) were used in this study. The drugs were freshly prepared in water for injection.

Animals

Male Wistar rats and Swiss mice were used in this study. Animals were acclimatized for 8 days after their arrival, under standard laboratory conditions of light and temperature. The Institutional animal Ethics Committee constituted according to the provisions of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) the approved the protocol of this study. The animals were handled as per the Indian Council of Medical Research guidelines.

Extraction of Butea monosperma flowers

Petals of *B. monosperma* flowers were separated; shade dried and defatted with petroleum ether (60-80°c) by maceration. The marc obtained was dried and extracted with methanol. The methanolic extract (ME, 50 g) was evaporated to dryness in vacuum. The residue was suspended in ethyl acetate, filtered and the filtrate was vacuum dried to provide ethyl acetate soluble (EAS, 10 g). The EAS was fractionated further by preparatory thin layer chromatography using silica gel as stationary phase and benzene: ethyl acetate in the ratio of 4:1 as mobile phase and major bands of compounds were scrapped and dissolved in distilled water to study their effect on rat vas deferens. The fraction which inhibited dopamine-induced contraction of the rat vas deferens was found to be isoflavone (FEAS, weight 700 mg) having Rf of 0.8 and was used in further studies.

Isolated rat vas deferens

Male Albino Wistar rats weighing 200-300 g were sacrificed. The abdominal wall was quickly opened and the vas-deferens was isolated from the animal. They were mounted in the isolated organ bath containing 30ml of Kerbs- Henselit solution of the following composition (mM): NaCl, 115; KCl, 4.7; $CaCl_2$, 2; $NaHCO_3$, 25; KH_2PO_4, 12; $MgCl_2$, 1.2; glucose 11.5 maintained at 37 ± 1^0c and the solution was continuously bubbled with carbogen. The tissue was stabilized for 30 minute during the period solution was changed at every 10 minutes as described by Goyal (8). The effect of dopamine (10, 20, 40, and 80 µg/ml) in presence or absence of ME, EAS and the individual bands obtained from the preparatory thin layer chromatography of ethyl acetate fraction (0.5 ml of 25 mg/ml of each) was observed on the vas deferens. The contact time was maintained for 60s and the log dose response curve was plotted. The fraction FEAS (obtained from ethyl acetate fraction and identified spectroscopically as isoflavone) inhibiting dopamine-induced contraction was tested in further animal models indicative of antidopaminergic activity i.e. haloperidol-induced catalepsy and footshock-induced aggression in mice.

Haloperidol- induced catalepsy in mice

Albino Swiss mice (20-30 g) were divided into several groups. The forepaw of each mouse was placed on a bar elevated at 3.5 cm above the ground. The duration for which the animal maintained the imposed posture was noted as the time required for removing the forepaw from the bar. The vehicle or ME, EAS or the isoflavone FEAS (50 and 100 mg/kg) were administered 30 min prior to administration of haloperidol. Both the forepaws were placed twice on the bar and the higher value was considered for calculation. The duration of catalepsy was measured at 0, 30, 60, 90, 120, 150, and 180 min using the Bar test (9).

Footshock - induced aggressive behaviour in mice

Male albino Swiss mice weighing 20-30 g were divided into 8 groups, each containing 6 pairs. Mice were placed in pair in a box with a grid floor attached to a constant current shocker. A 60 Hz current was delivered for 5 seconds followed by 5 seconds intermission for 3 minutes as described by Datla et al., (10). Each pair of mice was dosed and tested without previous exposure. The total number of fights was recorded for each pair during the 3 min period. The vehicle or ME, EAS or FEAS (50 and 100 mg/kg) were administered 30 min prior to administration of haloperidol.

Phytochemical tests

The ME and EAS were subjected to chemical identification as described by Harborne, (1973).

Ultra- Violet Spectroscopy

The fraction FEAS showing inhibition of dopamine-induced contraction of vas deferens was dissolved in methanol and the solution was scanned between 200-500 nm to obtain absorption maxima of fraction (UV-Shimadzu 2501 a model). Since the compound showed presence of flavonoids, its type was determined using chemical shifts as described by Harborne (11).

Statistical analysis

Numerical results were expressed as mean ± SEM. The data was analysed by one-way analysis of variance (ANOVA) followed by Dennett's test or student's t-test (unpaired), $P < 0.05$ being the criterion for statistical significance.

Results

Isolated rat vas deference

Dopamine produced dose dependent contraction of isolated rat vas deferens preparation. ME significantly ($P< 0.05$) inhibited dopamine-induced contraction of vas deferens. In presence of the extract, the agonist could not produce maximal effect. ME, *per se* did not produce any contraction of vas deferens. EAS significantly ($P< 0.05$) inhibited dopamine-induced contraction of vas deferens. In presence of the extract, the agonist could not produce maximal effect. EAS *per se* did not produce any contraction of vas deferens. The isoflavone significantly inhibited dopamine-induced contraction of vas deferens. In presence of the extract, the agonist could not produce the same response as in its absences. The isoflavone *per se* did not produce any contraction of vas deferens (Fig 1).

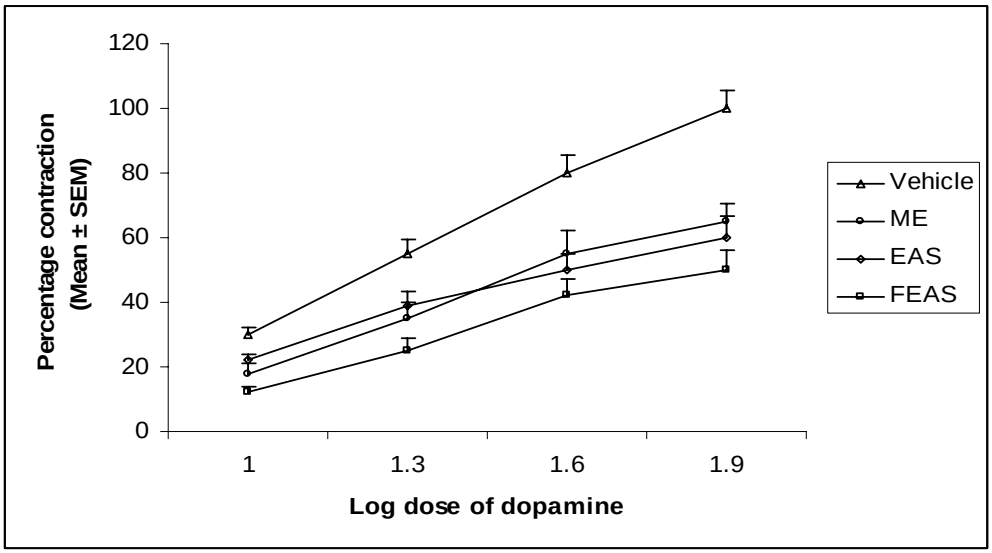


Fig. 1: Effect of ME, EAS and FEAS on dopamine-induced contraction of rat vas deferens. all the treatments inhibited dopamine-induced contraction ($p < 0.05$).

Haloperidol-induced catalepsy in mice

In vehicle treated mice, haloperidol (1mg/kg, i.p.) induced catalepsy and the maximum cataleptic response of 261.25 ± 15.47 s was produce at 60 min, which gradually decreased to 58.0 sec at 180 min. In presence of ME (50 and 100 mg/kg, i.p.), haloperidol induced catalepsy was potentiated significantly ($P<0.001$ and $P<0.05$) and maintained up to 180 min. In presence of EAS and the isoflavone FEAS (50, 100 mg/kg, i.p.), haloperidol induced catalepsy was potentiated significantly ($P<0.001$ and $P<0.05$) and maintained up to 180 min. The ME, EAS, and FEAS (100 mg/kg) per se induced cataleptic response (Fig. 2-4).

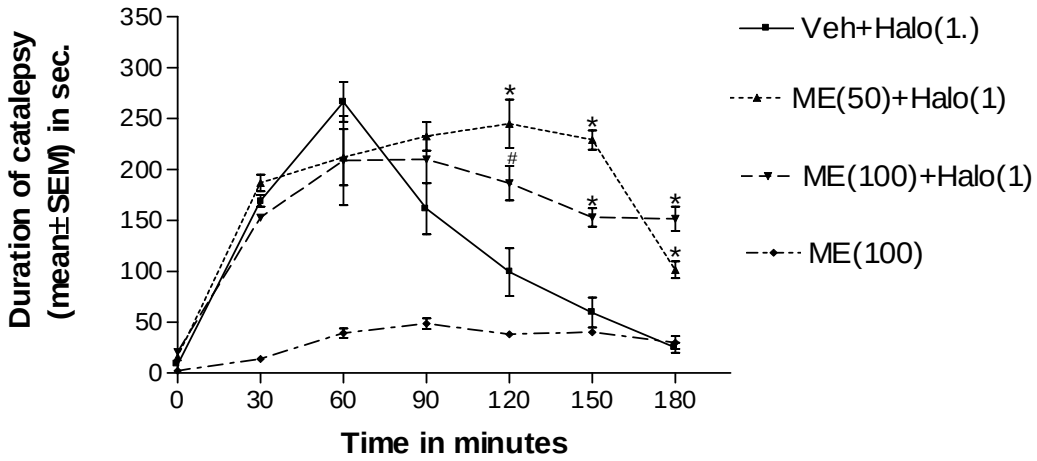


Fig. 2: Effect of ME on haloperidol (Halo) induced catalepsy in mice n = 5, $^{*}P<0.001$, and $^{\#}P<0.05$, compared with vehicle group using one way ANOVA followed by Dunnett's test.

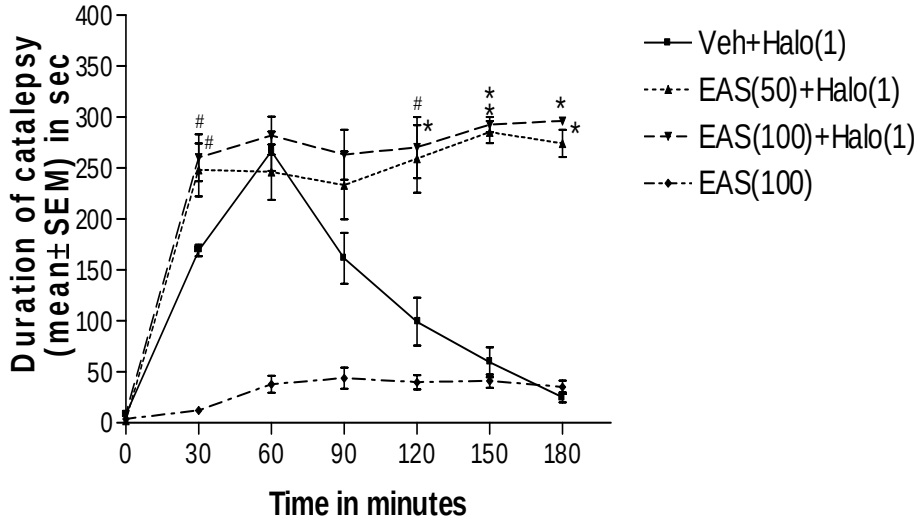


Fig. 3: Effect of EAS on haloperidol (Halo) induced catalepsy in mice n = 5, *P<0.001, and $^{\#}$P<0.05, compared with vehicle group using one way ANOVA followed by Dunnett's test

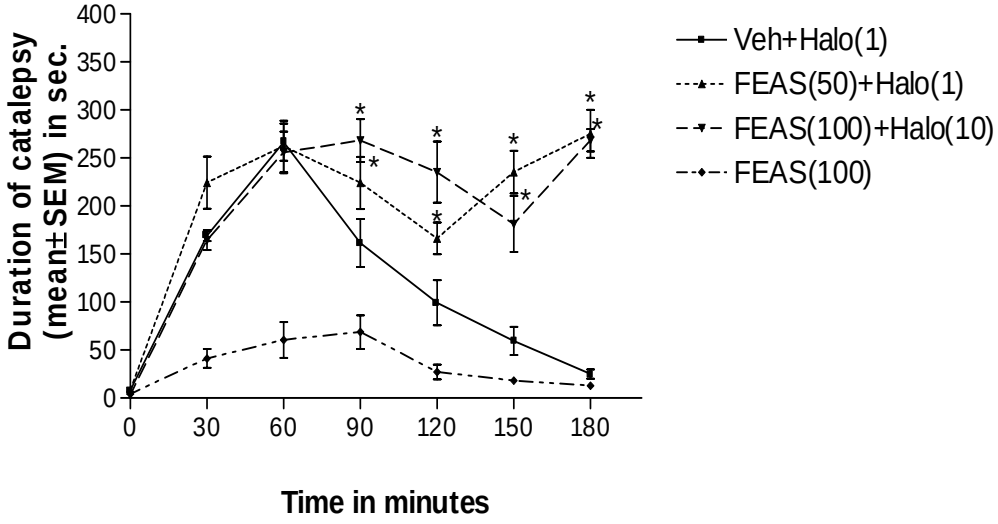


Fig. 4: Effect of FEAS on haloperidol (Halo) induced catalepsy in mice n = 5, *P<0.001 compared with vehicle group using one way ANOVA followed by Dunnett's test.

Foot shock-induced aggression in mice

Number of fights in mice were significantly (P<0.001) reduced by ME, EAS and FEAS (50 and 100mg/kg, i.p.) in comparison with vehicle treated group. Haloperidol, which was used as standard, reduced the number of fights from 35.0 ± 3.5 in vehicle treated group to 13.5 ± 3.2 showing its potent inhibitory effect on aggressive behaviour (Table 1).

Table 1: Effect of ME, EAS, FEAS, and haloperidol on footshock induced aggression in mice

Treatment (mg/kg i.p.)	No. of Pairs	No. of fights in 3 min	Percentage inhibition of aggression
Vehicle	6	35.0 ± 3.5	--
ME (50)	6	25.0 ± 2.25*	28.57
ME (100)	6	22.0 ± 1.4*	37.14
EAS (50)	6	24.0 ± 1.2*	31.42
EAS (100)	6	23.0 ± 1.1*	34.28
FEAS (50)	6	20.0 ± 1.5*	42.85
FEAS (100)	6	17.33 ± 1.21*	51.42
Haloperidol (1)	6	13.5 ± 3.2*	64.28

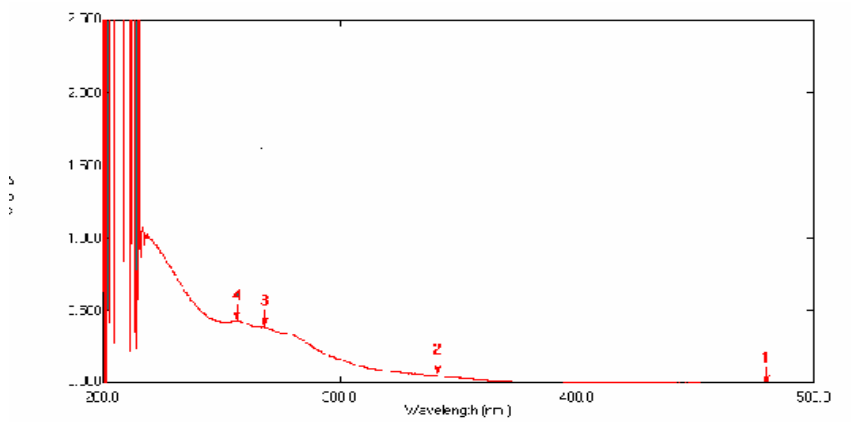
*P<0.001 compared with vehicle group using one way ANOVA followed by Dunnett's test.

Phytochemical tests

The ME and EAS contained glycosides and flavonoids.

Ultra- Violet spectroscopy

The ultra-violet spectrum of the FEAS showed two intense peaks with maximum absorption at 256-268 nm indicating possible presence of isoflavones which showed maximum absorption between 255- 265 nm as described earlier by Harbrone (11) (Fig. 5).



UV Spectrum

Fig. 5: Ultra-violet spectrum of the FEAS isolated from ethyl acetate fraction of methanolic extract of *Butea monosperma* flowers

Discussion

The results obtained in the present study show that the methanolic extract, its ethyl acetate soluble fraction and the isoflavone (FEAS) potentiated haloperidol-induced catalepsy and inhibited foot shock-induced aggression in rats in a dose dependent manner indicating antidopaminergic activity. Hideji, (12) reported existence of postsynaptic DA D_2 receptor as an enhancer of contractile response in Vas deferens. Inhibitory effect of the plant flavonoid galangin on rat vas deferens in vitro has been reported Capasso and Mascolo, (13). Inhibition of methanolic extract, its ethyl acetate fraction and the isoflavone suggests antidopaminergic activity at the D_2 receptors.

Inhibitors of DA D_2 receptors are known to induce catalepsy. Catalepsy is long-term maintenance of an animal in an abnormal posture (14). Costall et al., (15) reported induction of catalepsy and circling behaviour after intrercerabral injection of neuroleptics, cholinergic and anticholinergic agents into the caudate neucleus putamen, globus pallidus and substantia nigra of rat brain. Ossowsk et al., (16) have shown involvement of striatal and nucleus accumbens D_1/D_2 Dopamine receptors in neuroleptics catalepsy. This behaviour is being used since long time as a model for the extrapyramidal side effect associated with antipsychotic usage in humans (17). Thus the methanolic extract, its ethyl acetate fraction, and the isoflavone which inhibit dopamine-induced contraction of vas deferens also act on the central nervous system and induce catalepsy by blockade of dopaminergic receptors.

Young (18) has reported increased extracellular dopamine in nucleus accumbens in response to foot shock. Datla et al., (10) studied dopaminergic modulation of footshock induced aggression in paired rats using several DA receptor agonists and antagonist and concluded that central dopaminergic system induced a facilitatory modulatory effect on foot shock induced aggression and reduction in central dopaminergic activity resulted in attenuation of aggressive behaviour in rats. Thus the inhibition of foot shock induced aggression by the methanolic extract, its ethyl acetate soluble fraction and the isoflavone indicates its antidopaminergic action. Therefore it is concluded that the isoflavone isolated from *Butea monosperma* has antidopaminergic activity.

References

1. Nadkarni KM. Sesbania grandiflora. Indian Materia Medica 3rd edition Mumbai :Popular Prakashan, 1998; 52-54.
2. Gawale NS, Pal SC, Kasture VS, Kasture SB. Effect of *Butea monosperma* on memory and behavior mediated via monoamine transmitters in laboratory animals. J Nat Rem 2001; 1:33-41.
3. Kasture SB, Kasture VS, Chopde CT. Anticonvulsive activity of *Butea monosperma* flowers in laboratory animals. Phamacol, Biochem and Behav 2002; 72;965-972.

4. Bhatwadekar AD, Chintawar sd, Logade, Somani RS, Kasture VS, Kasture SB. Antistress activity of *Butea monosperma* flowers. Indian J Pharmacol. 1999; 31:153-155.

5. Soman I, Mengi SA, Kasture SB. Effect of leaves of *Butea frondosa* on stress, anxiety, and cognition in rats. Pharmacol, Biochem Behav 2004; 79:11-16.

6. Manish SL, Mishra SH. Evaluation of free radical activity of *Butea monosperma* Lam. Indian J of Expt. Biol 2007; 45:376-384.

7. Kasture VS, Chopde CT, Deshmukh VK. Anticonvulsant activity of A*lbizzia lebbeck*, *Hibiscus rosa sinesis* and *Butea monosperma* in experimental animals. J Ethnopharmacol. 2000, 71: 65-75.

8. Goyal RK. Practical in Pharmacology .3rd Ed B.S. Shah Prakashan: Ahmadabad, India, 2002-03; 51-52.

9. Ferre S, Guix T, Jane F, and Casas M. Is experimental catalepsy properly measured? Pharmacol, Biochem Behav 1990: 35:753-757.

10 Datla KP, Sen AP, Bhattacharya SK. Dopaminergic modulation of footshock induced aggression in paired rats. Indian J Exp Biol 1992; 30:587-91.

11. Harbrone JB. Phytochemical Methods.1st Ed Toppan Company, Ltd, Tokyo, Japan 1973; 53-59.

12. Hideji Morishita, Taeshi Katsuragi. Existance of postsynaptic dopamine D2 receptor as an enhancer of contractile respone in Vas deference. Eur. J. Pharmacol. 1998; 344:229-233.

13. Capasso R, Mascolo N. Inhibitory effect of the plant flavonoid galangin on rat vas deferens in vitro. Life sci.2003, 72, 2993-3001

14 Munkvad I, Pakken Berg H, and Randrup A. Aminergic systems in the basal ganglia associated with stereotyped behaviour and catalepsy. Brain Behav. 1968; 1: 89- 100.

15. Costall B, Naylor R J and Olley J E. Catalepsy and circling behaviour after intrercerabral injection of neuroleptics, cholinergic and anticholinergic agents into the caudate neucleus putamen, globus pallidus and substantia nigra of rat brain. Neuropharmacology. 1972; 11:645-63.

16. Ossowsk K, Karcz M, Wardas J, and Wolfarth S. Striatal and nucleus accumbens D_1/D_2 Dopamine receptors in neuroleptics catalepsy. Eur. J. Pharmacol. 1990; 182: 327-334

17. Hornykiewicz O. Dopamine in the basal ganglia. Its role and therapeutic implications (including the clinical use of L- DOPA). Br. Med. Bull. 1973; 29: 172-178.

18. Young AMJ. Increased extracellular dopamine in nucleus accumbens in response to unconditioned and conditioned aversive stimuli: studies using 1 min microdialysis in rats. J Neurosci Meth. 2004; 138: 57-63

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