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Investigation of neuropharmacological activities of ethanolic extract of *Dodonaea viscosa* seeds

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Abstract

Objective: To study the neuropharmacological activities of the ethanolic extract of *Dodonaea viscosa* seeds. Materials and methods: Neuropharmacological activities includes behavioural assessment, pentobarbitone-induced sleeping time, motor coordination activity and locomotor activity of the *Dodonaea viscosa* seeds ethanolic extract was evaluated at the dose of 30 mg/kg and 20 mg/kg (p.o.) respectively. Results: Ethanolic extract of *Dodonaea viscosa* seeds potentiated phenobarbitone - induced sleeping time, reduced locomotion and the same extract did not induce motor in coordination at dose levels of 30 mg/kg and 20 mg/kg (p.o.). Conclusion: The results of present study have revealed that the ethanolic extract of *Dodonaea vsiscosa* showed excellent neuropharmacological activities and there is a scope for further envisage.

Key words: *Dodonaea viscosa* seeds, Ethanolic extract, Hypnosis, Rotarod, Actophotometer.

1. Introduction

Dodonaea viscosa (family- Sapindaceae) is an erect and broad evergreen shrub, widely distributed in India [1]. The various parts of this plant enjoys wide reputation in the traditional system of medicines to cure different human ailments including rheumatism, febrifuse, antimicrobial, anodyne, antipruritic, discutient, hypotensive and antiviral [2, 3].

Phytochemical investigations have revealed the presence of traces of alkaloids, saponin glycosides etc. The plant is also reported to contain flavonoids (isorhamnetin, penduletin, quercetin, doviscogenin, sakuranetin, quercetol, hyperin, kaempferol, rutin and cyaniding), saponins (dodonoside A, B), triterpenes, phenols, coumarins, essential oil,

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fixed oil and beta-sitosterol [4]. These active constituents and above-mentioned activities in turn appear to correlate with some other biological activities. Hence the present study is focused on the evaluation of neuropharmacological activity of ethanolic extract of *Dodonaea viscosa* seeds (DV) in animal models.

2. Materials and methods

2.1 Plant material

Fresh dried seeds were procured during early winter season from young matured plants in Alagilawada, Davangere District, Karnataka, India. The plant was authenticated (voucher specimens: 4/2004) by taxonomist of Botany department of DRM Science College Kuvempu University and Department of Pharmacognosy, Bapuji Pharmacy College Davangere, India. Garbled seeds were powdered, passed through sieve no. 40 to get coarse powder and was used for studies.

2.2 Preparation of extract

The coarse powdered material was subjected to Soxhlet extraction successively with petroleum ether (60-80°C), chloroform, ethanol (95%) and distilled water [5, 6]. The ethanolic extract was evaporated to dryness under reduced pressure in a rotary flash evaporator to get the extract in powdered form and preserved in a desiccator for further screening. The test samples were suspended in 1% Tween-80 solution.

2.3 Animals used

Adult mice (25-30g) of either sex were used in the studies. The selected animals were kept in standard polypropylene cages at room temperature of 27 ± 2°C with water *ad libitum*.

3. Experimental

LD_{50} was carried out on mice [7] of either sex and doses were fixed as 30 mg/kg (p.o.) and 20 mg/kg (p.o.) for neuropharmacological studies on animal models.

3.1 Behavioural assessment

Behavioural effect of ethanolic extract of *Dodonaea viscosa* seeds at 20 mg/kg and 30 mg/kg (p.o.) was assessed as per the method described by Irwin *et al* (1968) [8]. The albino mice were divided into 3 groups (n=6). After treatment with ethanolic extract of DV, the animals were observed for gross behavioural assessment after administration from 30 min for 3 h. The observed parameters are includes depressant effect associated with auditory depression, loss of muscle tone, traction, loss of pinna and righting reflex.

3.2 Pentobarbitone-induced sleeping time [9]

Mice (18) were divided into 3 groups (n=6). Group I received pentobarbitone 45 mg/kg (i.p.). Group II received pentobarbitone 45 mg/kg (i.p.) along with DV 30 mg/kg (p.o.). Group III received pentobarbitone 45 mg/kg (i.p.) along with DV 20 mg/kg (p.o.). Pentobarbitone 45 mg/kg (i.p.) was administered 30 min before oral administration of ethanolic extract of *Dodonaea viscosa* seeds. The time elapsed between loss and recovery of righting reflex was noted and taken as sleeping time. The results are indicated in Table 1 and Fig. 1.

3.3 Motor coordination

Dunham and Miya [10] suggested that skeletal muscle relaxation induced by a test compound could be evaluated by testing the ability of mice or rats to remain on the rotarod. For this purpose, groups of animals were trained to remains on the rotarod for 1 min.

Table 1. Effect of ethanolic extract of *Dodonaea viscosa* on pentobarbitone induced sleeping time in albino mice.

Treatment	Onset	Av.sleeping time ± SE
I. Pentobarbitone (45mg/kg., i.p.)	5.8 ± 0.4	60.8 ± 1.3
II. Pentobarbitone (45mg/kg., i.p.) + DV (30mg/lkg., p.o.)	5.3 ± 0.8[a]	123.3 ± 5.1[b]
III. Pentobarbitone (45mg/kg., i.p.) + DV (20mg/lkg., p.o.)	5.8 ± 0.4[a]	77.0 ± 1.8[b]
One-way ANOVA	F 101.2 P < 0.001, HS	Studentized Range Test Min. Sig. Range : 15.6 (P < 0.01)

Values are mean ± SE, n=6 in each group, [a]P<0.05, [b]P<0.01 as compared to control

Table 2. Effect of ethanolic extract of *Dodonaea viscosa* on motor coordination

Treatment	Av Fall of Time (Sec) ± SE			
	Before	After	Diff	% decrease
I. Tween 80 (1%) Control	115.8 ± 5.2	-	-	-
II. Diazepam (4mg/kg., i.p.)	116.0 ± 6.0	9.0 ± 0.4	107.0 ± 6.0	92%
III. DV (30mg/kg.,p.o.)	125.0 ± 2.0	116.0 ± 3.7	9.0 ± 4.2	7%
IV. DV (20mg/kg.,p.o.)	122.0 ± 5.3	120.0 ± 2.5	20. ± 3.1	1.%
V. DV (30mg/kg.,p.o.) + Diazepam (4mg/kg., i.p.)	127.0 ± 2.7	1250.0 ± 2.0	2.0 ± 1.1	1.5%
One- way ANOVA, Between groups fall of time	F 131.2 P < 0.001, P < 0.001			Min.Sig. diff 16.5 (P< 0.05)

Table 3. Effect of ethanolic extract of *Dodonaea viscosa* on locomotor activity.

Treatment	Loco motor scores/60min
I. Tween 80 (1%) Control	456.7 ± 4.9
II. Amphetamine (2mg/kg.,i.p.)	1098.3 ± 20.1
III. DV (30mg/kg.,p.o.) + Amphetamine (2mg/kg.,i.p.)	698.0 ± 11.7
IV. DV (20mg/kg.,p.o.) + Amphetamine (2mg/kg.,i.p.)	950.0 ± 18.3
One- way ANOVA	F 356.5
Studentized Range Test MSR: 253.0 P<0.05 320.0 P<0.01	II > IV> IIII > I - Sig

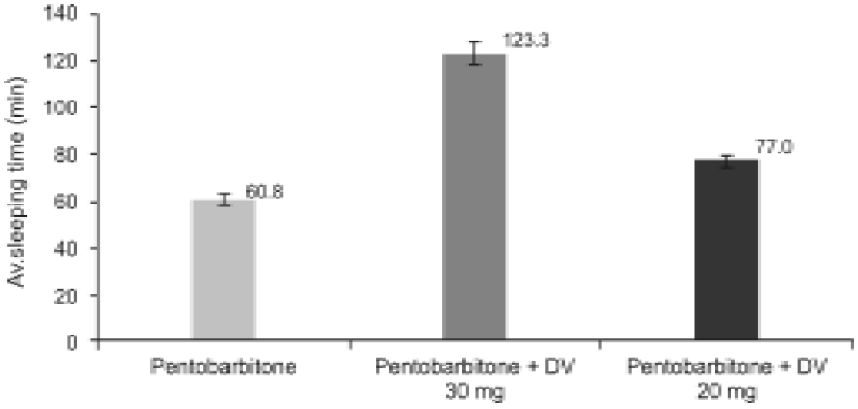

Fig 1: Effect of ethanolic extract of *Dodonaea viscosa* of pentobarbitone-induced sleeping time in mice.

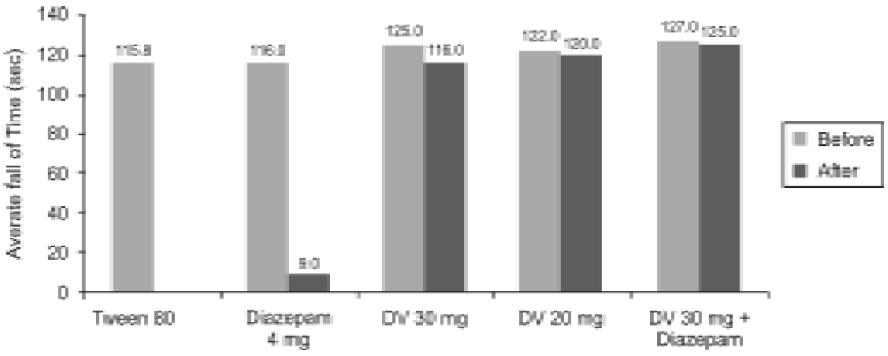

Fig 2: Effect of ethanolic extract of *Dodonaea viscosa* on motor co-ordination.

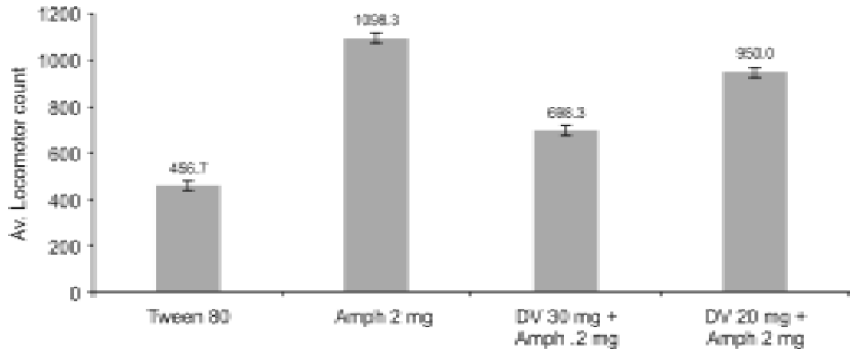

Fig 3: Effect of ethanolic extract of *Dodonaea viscosa* on locomotor activity in mice.

Trained mice (30) of either sex weighing between 25-30g were selected and were divided into 5 groups (n=6). All the animals were fasted for 24h with water *ad libitum*. Group I received Tween-80 (1%, p.o.) as a solvent control. Group II received diazepam (4 mg/kg., i.p.). Group III and IV received ethanolic extract of DV 30 mg/kg (p.o.) and 20 mg/kg (p.o.) respectively. Group V received ethanolic extract of DV 30 mg/kg (p.o.) along with diazepam (4 mg/kg., i.p.). Thirty min after administration of DV and diazepam, animals of all groups including control were placed on rotarod, and the number of animals falling from the rotarod during the scheduled time (1min) were counted and the percentage was transformed into probit and compared with control and standard group. The results are shown in Table 2 and Fig.2.

3.4 Locomotor activity [11,12]

Locomotor activity was assessed by an actophotometer. In which mice or rats are used for screening. Testing animals were placed in an actophotometer for 3h, for the purpose of acclimatization to the testing environment. One day earlier to behavioral testing. Selected animals were randomly divided into 4 groups (n=6). Group I received Tween-80 (1%.,p.o.) served as control group. Group II received amphetamine (2 mg/kg.,i.p.). Group III received DV (30 mg/kg., p.o.) along with amphetamine (2 mg/kg.,i.p.). Group IV received DV (20 mg/kg., p.o.) along with amphetamine (2 mg/kg.,i.p.). The locomotor activity of each was measured for a total duration of 60 min. The results are indicated in Table 3 and Fig.3.

3.5 Statistical analysis

The data obtained were analysed using one-way analysis variance (ANOVA) followed by Student's *t*'test. The minimum level of significance was fixes at $p<0.001$.

4. Results and discussion

LD_{50} studies of ethanolic extract of *Dodonaea viscosa* seeds were carried out according to Reed and Meuch method on albino mice of either sex and doses were fixed as 30 mg/kg (p.o.) and 20 mg/kg (p.o.). The same doses of ethanolic seeds extracts of *Dodonaea viscosa* exhibited CNS depressant action, as they produced reduction in spontaneous behavioural activity. The behavioural assessment was carried out and observed for 2h. The observation parameters includes depressant effect associated with auditory depression, loss of muscle tone, traction, loss of pinna and righting reflex.

Table 1 shows that ethanolic extract of *Dodonaea viscosa* seeds increased the duration of pentobarbitone induced sleeping time significantly ($p<0.01$). While decreaseing the time of onset at both dose levels. Further it supports the assumption of its CNS depressant effect [13, 14]. Locomotor activity test is particularly sensitive to centrally active drugs. Hence this study was also conducted. Dopamergic agonists such as amphetamine or apomorphine exerts extra pyramidal or neurological effects [15]. In this study amphetamine produced hyperactivity and associated with continuous circling activity. When ethanolic extract of DV along with amphetamine was administered, it stopped the agitation and circling of the mice and normalized their response to noxious stimuli. However a higher dose of ethanolic extract of *Dodonaea viscosa* seeds (30 mg/kg) caused a significant ($p<0.05$) decrease in loco motor activity, because it antagonized the amphetamine effects (Table 3). The DV did not induce any motor in co-ordination (Table 2) in animal models.

5. Conclusion

The present findings are indicative of an association of CNS depressant and decreased loco motor activity with ethanolic seeds extract of *Dodonaea viscosa*. Further study regarding the isolation and characterization of the active principle responsible for locomotor activity is currently under progress.

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