

CNS activity of ethyl acetate extract of stem bark of *Dodonaea viscosa* Linn.

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The major extensively recommended treatments for anxiety and insomnia disorders are the benzodiazepines; yet, they have protuberant side effects. Consequently, the progress of new pharmacological agents is well acknowledged and so it is now contemporary to search some safe and effective alternative medicine. The current study is aimed to investigate the CNS effect of the stem bark of *Dodonaea viscosa* in experimental animal models. Preliminary phyto-chemical screening and Thin Layer Chromatography (TLC) of the ethyl acetate extract of stem bark of *Dodonaea viscosa* (EAEDV) were performed. Acute oral toxicity study was performed as per OECD 423 guidelines. The CNS effects were evaluated using Elevated Plus Maze (EPM) and phenobarbitone induced sleeping time using Diazepam (2 mg/kg) as the standard. Phyto-chemical analysis reflects the presence of flavanoids, alkaloids, terpenoids and tannins. The TLC studies confirmed that the isolated compound was found to be quercetin. Mortality and sign of any toxicity were not observed up to the dose orally with 2000 mg/kg. For all the statistical tests performed, $p < 0.05$ is considered to be significant. In EPM, 200 mg/kg and 400mg/kg of EAEDV produced significant $p < 0.0005$, $p < 0.0001$ anti-anxiety effect respectively compared to control group and the activity was similar to that of diazepam. In addition, the extract significantly potentiated the phenobarbitone induced sleeping time.

Keywords: Anxiety, Insomnia, Elevated plus maze, Ethyl acetate extract of *Dodonaea viscosa*, Phenobarbitone induced sleeping time

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Anxiety affects one-eighth of the total inhabitants around the world and has developed a major space for research in psychopharmacology during this decade.¹ Presently, the most generally recommended medications for anxiety disorders are benzodiazepines. Nevertheless, the clinical uses of benzodiazepines present a tapered safety margin by their side effects such as psychomotor impairment, potentiating the other central depressant drugs and dependence liability. Since many distressing side effects of the currently available treatments exist, the improvement of novel treatments without these adverse effects would be of great importance in the management of anxiety related disorders.^{2,3}

Herbal medicine is the ancient form of health care system acknowledged to mankind. The pharmacological management of illness instigated long back with the use of plants. Approaches of traditional healing all over the world usually used herbs as part of their practice. Several pharmacological and pharmacognostical studies of the

therapeutic plants exposed their vigorous application in the arena of treatment as they own definite nutrient and non-nutrient constituents that defends and avoids the body from various diseases.⁴ To encourage the appropriate utilization of herbal medications and to conclude their potential as sources for new drugs, it is crucial to study medicinal plants, which have folklore status in a more intensified way.⁵ On basis of this consideration, it is the purpose of this study to illustrate the anxiolytic activity of the stem bark of *Dodonaea viscosa*.

Dodonaea viscosa, a member of soapberry family, sapindaceae is an evergreen woody shrub with an innate of Australia, indigenous and was later abundantly spread throughout the region of tropics.⁴ It is enormously found in Hijaz region, eastern region and Southern province in Saudi Arabia. Several plant parts such as stem, leaves, seeds, roots, bark, aerial parts are used in traditional system of medicine.⁶ The plant is unique amongst the therapeutic plant with various pharmacological actions that makes it as an effective types midst all. So far, the antimicrobial, anti-inflammatory, anti-ulcer, wound healing, local

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anesthetic, smooth muscle relaxant, gastro-protective outcome, management of rheumatism and antioxidant activities were exposed by the investigational studies. It is also used as an antipruritic in skin rashes and for the treatment of a sore throat, dermatitis and hemorrhoids. In order to discover its pharmacological perspective, still, many study works have to be passed out in the above plant.^{4&7} However, there are only a few reports on the central nervous system activity of this plant. Therefore, in the light of their reported use in traditional medicine, the present study is undertaken to investigate central nervous system activity of the ethylacetate extract of stem bark of *Dodonaea viscosa* in experimental animal models.

Methodology

Collection and identification of plant material

The stem bark of the plant *D. viscosa* was collected from the streets of King Khalid University, Greigor Campus during, September 2017. The plant material was taxonomically identified by Dr N. Mahadevan, Department of Pharmacognosy, King Khalid University, Abha. The collected stem bark material was dried under shade and pulverized in a mechanical grinder.

Preparation of extract

The classical chemical procedure for obtaining organic constituents from dried plant tissue (heartwood, dried seeds, root, and stem bark) is to continuously extract powdered material in a Soxhlet apparatus with the range of solvents ethyl acetate. The 100 g of dried powder material of the stem bark of *Dodonaea viscosa* was extracted with 1000 mL of ethyl acetate in a soxhlet apparatus. The powdered bark material was packed in a Soxhlet apparatus and the extraction was carried out using ethyl acetate (1 L). The extract was allowed to cool to room temperature and filtered. The solvent was evaporated (45°C) under vacuum in a rotary evaporator (Buchi Rotavap).

Selection and maintenance of animals:

Adult wistar albino rats weighing between 150-200 g of either sex were used for the study. The animals were housed in standard polypropylene cages at room temperature and provided with standard diet and water *ad libitum*. All experimental process of animals was permitted by the Ethics Committee for Animal Experimentation of the University, and carried out in accordance with the Regulations of Experimental Animal Administration.

Phytochemical Screening²⁴: The purpose of this screening to distinguish the material contained in the plant which is mostly tannins, alkaloids, flavonoids, anthraquinones, sterols, and saponins, they are detected by tests using qualitative methods.

Thin-layer chromatography (TLC)²⁵:

The ethyl acetate extract of the stem bark of *Dodonaea viscosa* is subjected to thin layer chromatography. About 0.1-0.2 mL of ethyl acetate extract of stem bark of *Dodonaea viscosa*, the standards quercetin and rutin were loaded on the plate by using the capillary tube. The spotted plates would be carefully dried and used for elution purpose. Solvent systems as chosen were kept in developing tank for the saturation. The spotting plate was carefully dried and used for elution purpose. The TLC plates were observed under UV light and the separated spots were marked. For the detection, the spraying reagents (10% sulphuric acid in methanol) were applied using the brush on the TLC plates.

Pharmacological Study

Acute Toxicity Study²⁶:

A single dose of ethyl acetate extract of stem bark of *Dodonaea viscosa* was given in enormous amount (2000 mg/kg) using oral gavage to determine the immediate toxic effect. Healthy young and nulliparous, non-pregnant mice weighing from 30-40 g were chosen. Animals should be fasted prior to dosing (mouse, food but not water should be withheld for 3-4 hours). Observed individually once during the first 30 min., periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for the duration of 48 hrs. Any abnormality or mortality must be observed. The following changes must be observed:

- ❖ Pre-terminal deaths: observed daily.
- ❖ Cage-side observation: Changes in skin, fur, eyes
- ❖ General Observation: salivation, lacrimation, piloerection, urinary incontinence, and defecation.

Elevated Plus Maze Assay in rats²⁷:

Elevated plus Maze apparatus (UGO Basil) comprising of two open arms and two enclosed arms. In general, 24 rats were divided into four groups (n=6), and were treated with vehicle (2% Gum Acacia), reference standard diazepam (2 mg/kg, p.o.), 200 mg/kg of EAEDV and 400 mg/kg of EAEDV respectively, 30 min before the test. The rats were

placed separately in the center of the maze, head facing towards the open arm. The number of entries in the open and closed arm and total time spent in open and closed arms respectively were recorded for a period of 5 min (entry into an arm is defined as to point when the animal places all four paws onto the arm). The observations were noted down for each animal and the results were examined.

Potentiation of phenobarbitone induced sleep²⁸:

The ethyl acetate extract of the stem bark of *D.viscosa* were subjected to the phenobarbitone induced sleep test. Selected 24rats were separated into four groups of six (n=6) each. The first group received the vehicle (2% Gum Acacia), the second group received the Diazepam (2 mg/kg) whereas the third and fourth group received 200 mg/kg and 400 mg/kg of EAEDV respectively. Thirty minutes after receiving test samples, each animal was injected intraperitoneally, Sodium phenobarbitone (30 mg/kg). The sleeping time was noted by recording the interval between the losses and regaining of righting reflex.

Statistical Analysis

All the results were expressed as mean $\pm$ SEM. The data were analyzed by one-way analysis of variance (ANOVA) followed by a post-hoc tukey's test.

Results

Phytochemical Analysis

The preliminary phytochemical screening was carried out to find the presence of the active chemical components found in the extract. Phytochemical analysis of the ethyl acetate extract of stem bark of *Dodonaea viscosa* revealed the presence of Flavanoids, Terpenoids, Tannins and Alkaloids and shown in Table 1.

TLC Analysis of Flavonoids

The results of TLC analysis presented in the table 2. Rf value of Quercetin was found to be 0.93. The green colored compounds in the ethyl acetate extract having the Rf values of 0.93 may be Quercetin.

Pharmacological Study

Acute Toxicity

As per the OECD Guidelines 423, Wellness parameters of animals were observed continuously during the first 30 min after dosing and observed periodically (with special attention given during the

first 4 hours) for the next 24 hours and for 48 hours. All observations were systematically recorded with individual records being maintained for each animal and presented in Table 3. Observations included changes in skin and fur, eyes and mucous membranes and behavioral pattern. Attention was given for observations of tremors, convulsions, salivation, diarrhea, lethargy, sleep, coma and mortality.

Effect of EAEDV on elevated plus maze assay in rats:

In the elevated plus-maze as shown in the Table 4 and 5, the behavior observed confirmed the anxiolytic activity of diazepam as reported previously. The behavioral alterations induced by the extract of stem bark of *Dodonaea viscosa* in the elevated plus maze provided the anxiolytic effect. Administration of *D.viscosa* extract 200 mg/kg and 400 mg/kg significantly ($p<0.0005$, $p<0.0001$ respectively) increases the number of open arm entries and the time

Table 1 — Phytochemical Screening of *D.viscosa* stem bark

Secondary Metabolites	Ethyl acetate Extract
Flavonoids	+
Alkaloids	+
Terpenoids	+
Saponins	-
Tannins	+
Steroids	-

Note: "+" indicates the presence and "-" absence

Table 2 — TLC Analysis

Solvent System	Spots	Rf values	Color
Ethyl acetate-Formic acid-1	1	0.93	Dark green
Glacial acetic acid-Water (100:11:11:26)			

Table 3 — Acute Toxicity Observations

Observations	30 min	4 hours	24 hours	48 hours
Skin Fur	N	N	N	Slightly less
Itching	Present	Nil	Nil	Nil
Alertness	N	N	N	N
Salivation	N	N	N	N
Lacrimation	N	N	N	N
Urination	Slightly higher	N	N	N
Diarrhea	N	N	N	N
Hyperactivity	Nil	Slightly higher	Nil	Nil
Sleep	N	N	N	N
Tremors	Nil	Nil	Nil	Nil
Pinna Reflex	N	N	N	N
Mortality	Nil	Nil	Nil	Nil

Table 4 — Effect of EAEDV on elevated plus maze assay in rats (Number of entries in open arms and closed arms)

Groups	Treatment	No: of entries in the closed arm	No: of entries in the open arm
Control (2% acacia solution)	0.5mL	2.67±0.21	0.66 ± 0.21
Standard Diazepam	2 mg/kg	1.5±0.22	4.33±0.33**
EAEDV	200 mg/kg	1.8 ± 0.40	2.83 ± 0.31*
EAEDV	400 mg/kg	1.5 ±0.32	3.5 ± 0.22**

Each value is the mean ± SEM for 6 rats.

*P < 0.0005 compared with control. Data were analyzed by using one-way ANOVA.

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Table 5 — Effect of EAEDV on elevated plus maze assay in rats (Time Spent in open arms and closed arms)

Groups	Treatment	Time spent in the closed arm (in seconds)	Time spent in the open arm (in seconds)
Control (2% acacia solution)	0.5 mL	219.5 ±4.07	80.5 ± 4.07
Standard Diazepam	2 mg/kg	113.5 ± 3.54**	186.5 ± 3.537**
EAEDV	200 mg/kg	131.83 ± 2.90*	168.17±2.90*
EAEDV	400 mg/kg	118.6 ± 2.86**	182.3 ± 3.45**

Each value is the mean ± SEM for 6 rats.

*p < 0.0005 compared with control. Data were analyzed by using one-way ANOVA.

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spent in open arms as compared with control. They also showed a reduction in the number of entries and correspondingly the time spent in closed arm.

Effect of EAEDV on phenobarbitone-induced sleeping time in rats

Administration of the extract influenced both the onset of phenobarbital-induced sleeping and as well prolonged the representative loss of righting reflex. The administration of the extract (200 mg/kg and 400mg/kg) shortened the sleep start time and also increased the sleep duration significantly ($p < 0.0001$) as compared to the control group of animals. Prolongation of loss of righting reflex in the animals was statistically similar to that of the animals which had received diazepam (2 mg/kg) as the positive control. The result in Fig. 1 indicates that the administration of the extract of *D. viscosa* significantly augmented phenobarbital-induced sleep.

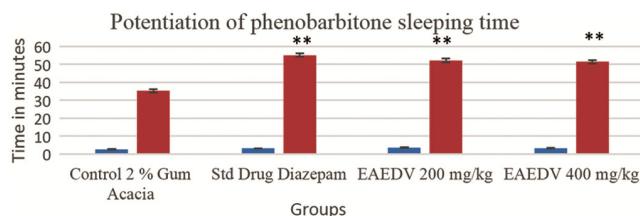


Fig. 1 — Effect of EAEDV extract on the onset and duration of sleep in rats in phenobarbitone induced sleeping time:

**P < 0.0001 compared with control. Data were analyzed by using one-way ANOVA

Discussion

Anxiety, Seizures, Mental illness, and Insomnia complications are in common and senile neurological syndrome in specific, are broadly leading in current fast-paced life with the assembly of stressful conditions. It is now becoming exceptionally apparent that existing psychotherapeutic does not appropriately encounter the burdens of a vast majority of patients with mental health problems, and that herbal preparations continue to be the crucial beneficial treatment for many such patients in the world. Nevertheless, up-to-date, very little attention has been rewarded to progress structurally and or functionally novel CNS active drugs from psychoactive medicinal plants.²⁹

In this study, ethyl acetate extract of *Dodonaea viscosa* was found to contain flavonoids, terpenoids, alkaloids, and tannins. Flavonoids may be accountable for the neuropharmacological activity of the plant.³⁰ Several flavonoids and neuroactive steroids were established to be ligands for the GABA receptors in the CNS; which led to the assumption that they may act as benzodiazepine-like molecules (which act through GABA receptor).³¹

Qualitative chromatographic screening of these extracts involved thin layer chromatography showed 1 spot (Rf values 0.93). Since secondary metabolites are accountable for biological activity, this study would take a prominent trail for giving the data for pharmacological activity and the constituents responsible for the activity.

The elevated plus maze is one of the most widely used models in experimental pharmacological studies on anxiety and it is known that anxiolytic drugs increase entries and time into open arms of this apparatus³². Diazepam increases the percentage of open arms entries and the time spent in the open arms, confirming its anxiolytic effects. The result of this study show that the animals treated with 200 mg/kg

and 400 mg/kg of *Dodonaea viscosa* increased the entries into and time spent in the open arms, indicating an anxiolytic-like effect, in addition to the fact that this reduction in an anxiety behavior was much similar to the one perceived following the predictable treatment with the diazepam^{33,34}.

Barbiturates are recognized sedatives inducing sleep in mammals by depressing the CNS³⁵. Phenobarbitone, even though a long-acting barbiturate, at lesser doses it can act as short to intermediate-acting barbiturate^{36,37}. In the present study, in the negative control group, phenobarbitone (30 mg/kg) produced intermediate onset and duration of sleep as indicated by the loss of righting reflex (inability to maintain posture) and awakening or regaining righting reflex subsequently. Both the standard drug and EAEDV treatment at 200 mg/kg and 400 mg/kg effectively reduced the sleep induction time in rats and as well as prolonged the duration of sleep. Thus, Potentiation of phenobarbitone induced sleeping time indicated the anxiolytic or sedative property of ethyl acetate extract of *Dodonaea viscosa*.

The efficacy of most herbal remedies is attributed to various active principles in combination, in the present study ethyl acetate extract from the stem bark part of *Dodonaea viscosa* containing significant amounts of flavonoid components which are probably more responsible for the anxiolytic activity of *D.viscosa*.

Conclusion

From the above experiments, it could be concluded that ethyl acetate extract of *D. viscosa* contains significant neuro-pharmacological activities. Therefore, we advance the suggestion that this extract may fulfill the therapeutic need for the treatment of anxiety and related neuropsychiatric disorders.

Future Work

However, further studies are required to evaluate the contribution of other substances for the anxiolytic and sedative activity seen in our study as it is not clear which components were exactly responsible for these effects. To elucidate the exact mechanism action and bioactive compounds responsible for the neuro-pharmacological activities of this stem bark extract, further pharmacological studies must be performed.

Ethical Issue

All experimental process of animals was approved by the Ethics Committee for Animal Experimentation

of the King Khalid University, and carried out in accordance with the Regulations of Experimental Animal Administration issued by the University.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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