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In vivo sedative and muscle relaxants activity of *Diospyros lotus* L

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PEER REVIEW

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Comments

This is a valuable research work for investigation of safe, effective and potent sedative, and muscle relaxant phytomedicines. In the present research work, the authors reported the sedative, and muscle relaxant effect of the said plant. The sedative effect has been tested using phenobarbitone-induced sleeping time while Rota rod model is used for relaxant activity.

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ABSTRACT

Objective: To evaluate the sedative effect of *Diospyros lotus* L (*D. lotus*) extract in mice using the open field and Rota rod tests.

Methods: For the sedative and muscle relaxants activities of extract/fractions of the plant, *in-vivo* open field and phenobarbitone-induced sleeping time were used, while the Roda rod test was employed in animals for the assessment of muscle relaxant activity.

Results: Results from this investigation revealed that the extracts of *D. lotus* have exhibited significant sedative effect in mice (45.98%) at 100 mg/kg *i.p*. When the extract was partitioned with different solvents, the *n*-hexane fraction was inactive whereas the chloroform fraction was the most active with 82.67% sedative effect at 50 and 100 mg/kg *i.p.* On the other hand, the ethyl acetate and *n*-butanol fractions displayed significant sedative effects (55.65% and 40.87%, respectively) at 100 mg/kg *i.p.* Among the tested extract/fractions, only chloroform and ethyl acetate fractions showed significant ($P < 0.05$) muscle relaxant activity in the Rota rod test.

Conclusions: In short, our study provided scientific background to the traditional uses of *D. lotus* as sedative.

KEYWORDS
Diospyros lotus, Ebenaceae, Sedative, Muscle relaxants activity

1. Introduction

Medicinal plants are a rich source of bioactive molecules which are used approximately 80% of the world population for their basic health needs[1]. The genus *Diospyros* (Ebenaceae) consists of woody shrubs and trees distributed in the tropical and subtropical regions of the world. Around 500 species are known worldwide, 24 species of which are native to India[2]. Among *Diospyros* species, *Diospyros dendo*, *Diospyros mespiliformis*, *Diospyros crassiflora*, *Diospyros ebenum*, *Diospyros melanoxylon*, *Diospyros perrieri* and *Diospyros haplostylis* are used to provide good ebonies. Moreover, the heartwoods of certain *Diospyros* species provide interesting colors, for example, *Diospyros chloroxylon*, *Diospyros rubra* and *Diospyros chrysophyllus* produce green, red, and white colors, respectively[3,4]. Additional constituents found in *Diospyros* are anthraquinones and lignans; these metabolites do not accumulate to a significant extent[4].

Diospyros lotus L. (*D. lotus*) is a deciduous tree that grows in China and Asia and is cultivated for its edible fruits. The fruits of *D. lotus* are used as sedative, astringent, nutritive, antiseptic,

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antidiabetic, antitumor, astringent, laxative, nutritive and as a febrifuge and for the treatment of constipation[5]. In addition, fruits of *D. lotus* have been used to for the treatment of diarrhea, dry coughs, and hypertension, whereas *D. lotus* fruits aqueous extracts have been used to treat streptozotocin-induced diabetes[6,7]. Moreover, the fruit extract of *D. lotus* has also been reported to protect glucose-6-phosphate dehydrogenase-deficient erythrocytes of hemolytic injury in both *in vitro* and *in vivo*[8].

Phytochemical constituents isolated from the *D. lotus* have been reported in the literature[9]. The fixed oil compositional changes and variations in phenolic substances in fruit growth of *D. lotus* have been studied previously. *D. lotus* has also reported for antiradical activity[10]. Phytochemical studies on many *Diospyros* species have revealed the presence of naphthoquinones and naphthalene derivatives, dimeric naphthoquinones, and lupane triterpenes[11]. Similarly, chemical investigation of the fruits of *D. lotus* led to the identification of some fatty acids, sugars, phenolic compounds, and non-volatile acids[12,13]. In view of the activity profile of *D. lotus*, the current study was undertaken to evaluate the sedative and muscle relaxant effects of crude extract and its fractions in *in-vivo* models with the intention of providing a pharmacological rationale for its use.

2. Materials and methods

2.1. Plant material

Roots of *D. lotus* were collected from Toormang Razagram, Dir, KPK, Pakistan, in May 2009. The sample was authenticated by Dr. Abdur Rashid, a taxonomist and botanist at the Botany Department, University of Peshawar, Pakistan. A voucher specimen (Bot/649) has been deposited at the herbarium located at the Department of Botany, University of Peshawar, Pakistan.

2.2. Extraction and isolation

Shade-dried roots of *D. lotus* (14 kg) were powdered and soaked in MeOH for a period of six days with continuous stirring. Then the solution was filtered and the extract was concentrated and dried by means of rotary evaporation at 55 ℃. This process was repeated four times and afforded 202 g of a dark red residue. The MeOH root extract was then suspended in water and successively partitioned with *n*-hexane, $CHCl_3$, EtOAc and *n*-BuOH according to published procedures[14].

2.3. Sedative profile

The apparatus used in this study consisted of an area of a white wood (150 cm diameter) enclosed by stainless steel walls and divided into 19 squares by black lines. The open field was placed inside a light and sound-attenuated room. BALB/c mice of either sex [(22 ± 2) g] were used in this investigation and were divided into groups of 6 mice each. Animals were adapted to being under red light (40 Watt red bulb) for 60 min prior to the start of experiment and had free access to food and water *ad libitum*. Animals were administered with 50 and 100 mg/kg *i.p.* of methanolic extract and its various solvent fractions. After 30 min, each animal was placed in the center of the box and the number of lines crossed was counted for each mouse, according to literature procedures[15,16].

2.4. Muscle relaxant

The Rota rod used in this test was a metallic rod (3 cm diameter) coated with rubber and connected to a motor. The rod was rotated at a constant speed *i.e.* 9 r/min and was about 60 cm above the tabletop in order to prevent the mice from jumping off the roller. Mice were exposed to Rota rod as a pretest before the experiment and only those mice that remained on the rod for 5 min at a speed of 9 r/min were included in the study. All the groups (n = 6) were treated (*i.p.*) with diazepam (0.20 or 0.25 mg/kg), distilled water (10 mL/kg), and various solvent fractions at the dose of 50 and 100 mg/kg, *i.p.* 30, 60, and 90 min before the experiment. Each mouse was allowed for 5 min on the revolving rod and the time spent on the rod was recorded[17,18].

2. 5. Statistical analysis

Results were expressed as mean ± SEM. One-way ANOVA was used for analysis of data followed by Dunnet's multiple comparisons. Differences were considered significant at $P \leqslant 0.05$.

3. Results

3.1. Effect of extracts in locomotive test

Locomotive activity in mice at test doses of extract/fractions of the plant is depicted in Figure 1. Our findings revealed that extract and its fraction showed significant sedative effect of 40.43% and 45.98% at 50 and 100 mg/kg *i.p.*, respectively as displayed in Figure 1A. When the extract was fractioned with different solvents, the *n*-hexane fraction was inactive whereas the chloroform fraction was the most active with 80.01% and 82.67% sedative action at 50 and 100 mg/kg *i.p.*, respectively (Figure 1B). On the other hand, the ethyl acetate fraction showed significant effect with 48.09% and 55.65% activity at 50 and 100 mg/kg *i.p.*, respectively (Figure 1C), whereas the *n*-butanol fraction, exhibited 33.98% and 40.87% sedative effect at 50 and 100 mg/kg *i.p.*, respectively (Figure 1D); the standard drug exhibited the most dominant effect (Figure 1E).

3.2. Effect of extracts in muscle relaxant activity

When evaluated for muscle relaxant effect using the Roda rod test, only chloroform and ethyl acetate fractions demonstrated some activity. As shown in Figure 2, the chloroform fraction displayed significant ($P < 0.05$) muscle relaxant effect after 60 and 90 min of drug administration at both doses of 50 and 100 mg/kg *i.p*. The ethyl acetate fraction was more effective in its muscle relaxant effect and exhibited significant activity even after 30 min of drug administration at both test doses of 50 and 100 mg/kg *i.p*. (Figure 2).

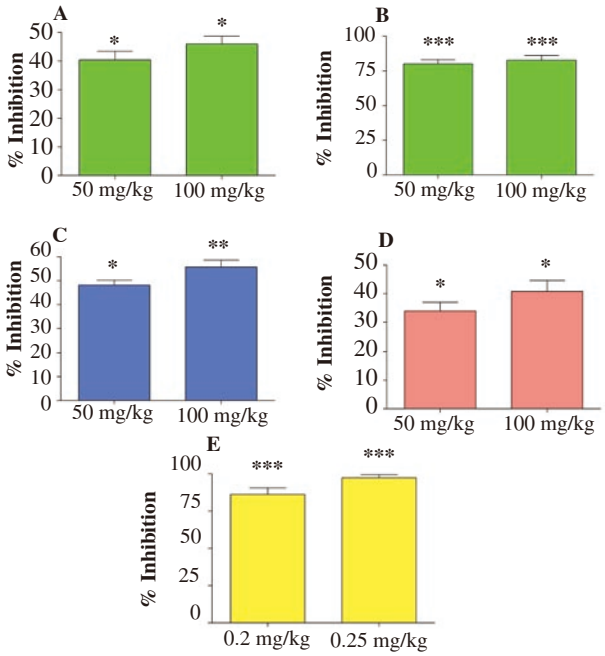

Figure 1. Percent effect of extracts in locomotive test.
A: crude extract; B: chloroform fraction; C: ethyl acetate fraction; D: butanol; E: diazepam. Values represent the percent sedative effect. Data presented as mean ± SEM, ($n = 6$). *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$, all compared with control.

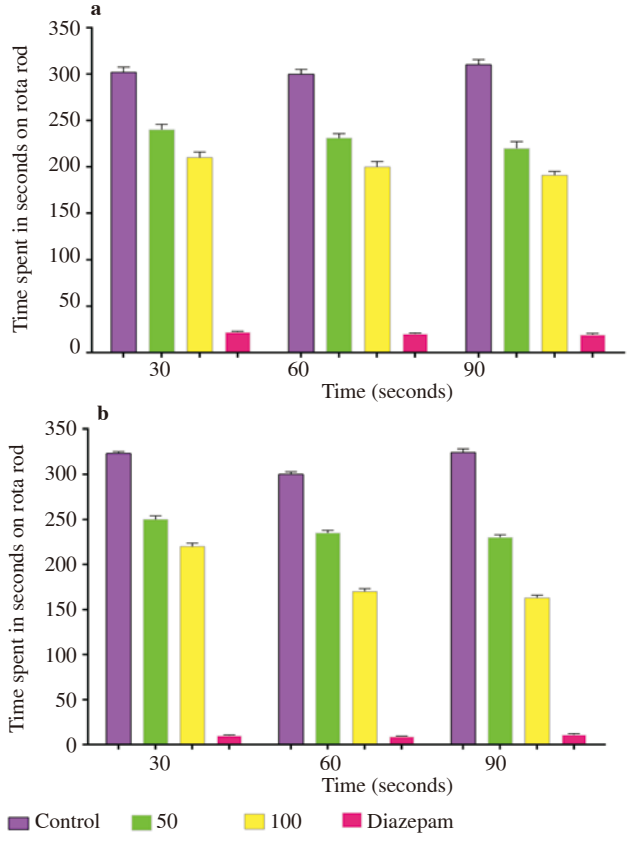

Figure 2. Effect of extracts on muscle coordination in rota rod.
a: chloroform; b: ethyl acetate. Bars represent the time spent in seconds on rota rod, after 30, 60 and 90 min of treatment with distilled water (10 mL/kg), chloroform (50 and 100 mg/kg) or diazepam (0.25 mg/kg). *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$.

4. Discussion

In the light of traditional uses of the *D. lotus* for the treatment of anosmia (as sedative), we employed the open field test to evaluate the sedative potential of the plant, and the Roda rod test to investigate its muscle relaxant effects. Open field test (locomotive activity) assay is frequently employed as a prognostic test for the assessment of sedative properties[16,17]. Pretreatment of mice with extract/fractions showed dose-dependent reduction in locomotive activity in the open field test as compared to control. The reduction in the frequency and amplitude of motion could be attributed to the sedative effect of *D. lotus*. The resulting sedative effect of extract/fractions of the tested plant were similar to the standard drug used (diazepam).

Roda rod test, on the other hand, is primarily employed in animals for the assessment of muscle relaxant properties[18,19]. The animals in this model are allowed to spend time on the revolving rod; less time spent on the rode more indicates a muscle relaxant effect of a tested material. Results obtained from this study reveal that extract/fractions exhibit significant activity only in chloroform and ethyl acetate fractions of the plant. Thus, we can assume that the muscle relaxant constituent(s) of the plant are concentrated in these two fractions. In addition, our results are similar to those of diazepam, the standard drug used in the study.

Researchers believed that the sedative and muscle-relaxant like effects of benzodiazepines such as bromazepam are mostly due to interference with the action of gamma aminobutyric acid (GABAA)[20]. Additionally, studies revealed that benzodiazepines bind to the gamma sub-unit of the GABAA receptor, implicating structural modification of the receptor and thus causing an increase in GABAA receptor activity. Benzodiazepines do not substitute for GABAA, which bind at the alpha sub-unit, but rather increase the frequency of channel opening events, which leads to an increase in chloride ion conductance and inhibition of the action potential. The overall effects of extract/fractions of *D. lotus* were similar to standard drug used (diazepam).

In conclusions, the extract/fractions of *D. lotus* showed significant sedative and muscle relaxant activity in animal models and thus pharmacological rationale for the traditional uses of the plant as sedative. Moreover, the study provided strong evidence for the bioactivity guided isolation of active compounds from the plant to discovery more effective molecules.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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Comments

Background

Medicinal plants have formed the basis of sophisticated traditional medicine systems that have been in subsistence for thousands of years and continue to provide mankind with new remedies. According to the World Health Organization, 80% of the world's population mostly those of developing countries depend on plant-derived medicines for their health care.

Research frontiers

D. lotus is traditionally used as sedative and muscle relaxants; therefore, the authors report the sedative and muscle relaxants effect of crude extract and its fractions in animals model.

Related reports

Open field and phenobarbitone-induced sleeping and Roda rod models were used for sedative and muscle relaxant effects of *D. lotus* extract and its fractions.

Innovations and breakthroughs

The current research work strongly supports the ethno-medicinal use of *D. lotus* valuable plant for its sedative and muscle relaxant properties. The results clearly demonstrate the significant sedation and muscle relaxations property of the *D. lotus*.

Applications

The applications of this manuscript are that *D. lotus* is tested in animal models for their pharmacological activities (sedation and muscle relaxations).

Peer review

This is a valuable research work for investigation of safe, effective and potent sedative, and muscle relaxant phytomedicines. In the present research work, the authors reported the sedative, and muscle relaxant effect of the said plant. The sedative effect has been tested using phenobarbitone-induced sleeping time while Rota rod model is used for relaxant activity.

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