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Anti-anxiety activity of *Eriobotrya japonica* leaf extracts

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ABSTRACT

Eriobotrya japonica leaves has been used traditionally to reduce stress and anxiety; however no pharmacological work has been done to substantiate these claims. The present study was designed to evaluate the anti-anxiety activity of various extracts viz. petroleum ether, toluene, ethyl acetate and methanol of the leaves of *Eriobotrya japonica* using elevated plus maze (EPM) model in Swiss albino mice. Albino mice were treated orally with different doses of the extracts (i.e.100, 200 and 300 mg/kg) and behavior was observed on the EPM. Diazepam (2mg/kg, P.O) was used as a positive control. Results showed that methanol and ethyl acetate extracts at the dose of 300mg/kg of the leaves of *Eriobotrya japonica* markedly increased the average time spent in the open arms of the EPM. This effect was comparable to the effect produced by diazepam. Hence, this plant may be developed as a potentially useful anti anxiety agent.

Keywords: Anxiety*, Eriobotrya japonica,* Diazepam, Elevated plus maze.

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INTRODUCTION

The complexity of daily life in modern society frequently leads to varying degree of anxiety. Mood and anxiety disorders have been found to be associated with chronic pain among medical patients in both developed and developing countries [1-2]. Currently, the most widely prescribed medications for anxiety disorders are the benzodiazepines. However, the clinical uses of benzodiazepines are limited by their side effects such as psychomotor impairment, potentiating of other central depressant drugs and dependence liability [3]. It has lead scientists to investigate plants, which are commonly employed in traditional and alternate system of medicine for sleep disorders and related diseases [4]. Various plants of family Rosaceae are being used in complementary and alternative medicines for management of anxiety [5-6].

So, the present study was designed to evaluate the anti anxiety activity of different extracts of *Eriobotrya japonica* leaves using the EPM, an exteroceptive behavior animal model.

MATERIAL AND METHODS

Plant Material

The leaves of *Eriobotrya japonica* were collected from Hoshiarpur in August 2010 and authentified by Department of Botany, Government College, Hoshiarpur.

Preparation of extracts

Leaves of *Eriobotrya japonica w*ere dried in shade and powdered. The powdered leaves (100g) were subjected to successive Soxhlet extraction by solvents in increasing order of polarity viz. petroleum ether (60-80°C), toluene, ethyl acetate and methanol. Before each extraction the powdered material was dried in hot air-oven below 50° C. Each extract was concentrated by distilling off the solvent and then evaporating to dryness and stored in refrigerator for further use. Extracts were weighed and percentage was calculated in terms of the air-dried weight of the plant material. The yield of the extract petroleum ether (60-80°C), toluene, ethyl acetate and methanol was 3.73%, 3.53%, 5.66%, 3.81% w/w respectively.

All the extracts were subjected to preliminary phytochemical screening [7]

Test Animals

The experimental animals [Swiss albino mice (20-25 gm) of either sex] were procured from the Animal House, SD College, Barnala. The animals were given standard laboratory feed and water *ad libitum*. The experiments were performed between

8.00 am to 1.00 pm. The experiments were conducted in a sound proof laboratory. All the experimental procedures and protocols used in the study were reviewed by the Institutional Animal Ethics Committee.

Treatments

Animals were divided into five (I-V) groups. Group I was a negative control and was given vehicle, consisting of simple syrup IP and carboxy methyl cellulose (2%), in a dose of 0.25ml. Group II was a positive control and was given standard drug, diazepam (2mg/kg, orally), suspended in the vehicle. Group III-V were treated as test groups and were given petroleum ether (60-80°C), toluene, ethyl acetate and methanol extracts of leaves of *Eriobotrya japonica* at different doses of100,200 and 300mg/kg respectively.

All the test solutions, standard drug and control were administered orally 45 minutes prior to elevated plus maze test.

Elevated plus maze model

The elevated plus-maze model is well established animal model for testing anxiolytic drugs [8]. The elevated plus-maze apparatus consist of two open arms (16 x 5 cm for mice and 50 x 10 cm for rats), two closed arms (16 x 5 x 12 cm for mice and 50 x 10 x 40 cm for rats), and an open roof with the entire maze elevated (25 cm for mice and 50 cm for rats) from the floor [9]. The animals were placed individually in the centre of the maze, head facing towards open arms and the stop watch was started and following parameters were noted for 5 min.

a) First preference of mice to open and closed arm.

b) Number of entries in open and closed arms (an arm entry defined as the entry of four paws into the arm)

c) Average time each animal spends in each arm

$$\text{Average time} = \frac{\text{total duration in the arm}}{\text{number of entries}}$$

STATISTICAL ANALYSIS

The anxiolytic activities of the extracts, diazepam and control were analyzed by one-way analysis of variance (ANOVA). The test groups were compared with standard/control by Tukey's Multiple Range Test. Difference were considered significant at $p<0.05$.

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RESULTS

The result of preliminary phytochemical screening is presented in **Table 1**. The results obtained from the EPM model, indicates that methanolic extract showed significant ($p<0.05$) anti anxiety activity as compared to diazepam. The average time spent in open arms increased from 5.73 ± 0.8 (sec) in control to 19.65 ± 0.72 and 23.47 ± 0.12 (sec) in ethyl acetate and methanolic extract (at dose of 300mg/kg) respectively. The petroleum ether and toluene appeared to be devoid of anti anxiety activity. Results obtained are presented in **Table 2**.

Table1: Preliminary phytochemical screening

Constituents/ extracts	EJPE	EJTE	EJEE	EJME
Flavanoids	-	-	+	+
Steroids	-	+	+	+
Triterpenoids	+	+	+	+
Tannins	-	+	+	+
Coumarins	-	-	-	-
Fats	+	+	+	+
Carbohydrates	+	+	+	+
Amino acids	+	+	+	+

+ present, - absent

EJPE-*Eriobotrya japonica* petroleum ether extract, EJTE- *Eriobotrya japonica* toluene extract, EJEE- *Eriobotrya japonica* ethyl acetate extract, EJME- *Eriobotrya japonica* methanolic extract.

Table 2: Anti anxiety activity of various extracts of leaves of *Eriobotrya japonica*

Groups/Extracts	EJPE	EJTE	EJEE	EJME	Negative control	Positive control
Vehicle	-	-	-	-	5.73±0.8	-
Diazepam	-	-	-	-	-	25.11±0.19
100 mg/kg	8.71±0.32	9.63±0.24	7.34±0.45	10.34±0.90	-	-
200 mg/kg	8.34±0.41	12.5±0.12	14.23±0.12	18.36±0.15	-	-
300 mg/kg	9.84±0.76	13.58±0.63	19.65±0.72	23.42±0.12	-	-

Values are Mean±SEM (n=6); one way ANOVA and Tukey's multiple range test. *$p<0.05$ compared to control. EJPE-*Eriobotrya japonica* petroleum ether extract, EJTE- *Eriobotrya japonica* toluene extract, EJEE- *Eriobotrya japonica* ethyl acetate extract, EJME- *Eriobotrya japonica* methanolic extract.

DISCUSSION

The fear due to height induces anxiety in the animals when placed on the EPM. The ultimate manifestation of anxiety and fear in the animals is exhibited by decrease in the motor activity and preference to remain at safer places. Anxiolytic agents are expected to increase the motor activity, which is measured by the time spent by the animal in the open arms [10]. The methanol extract of *Eriobotrya japonica leaves* (300mg/kg) and ethyl acetate extract (300 mg/kg), markedly increased the percentage of average time spent by the animals in the open arms. The anxiolytic effect of the methanolic and ethyl acetate extracts were more prominent

at 300mg/kg and doses higher than this did not show a consistent anxiolytic effects. The lack of significant anxiolytic effects at doses higher than 300mg/kg could be due to strong sedative properties of the plant extracts. Lower doses (less than 100mg/kg) of the plant extract did not show any significant anxiolytic effects. The anxiolytic effects of methanolic and ethyl acetate extracts of *Eriobotrya japonica leaves* may be related to their flavonoid content.

Flavonoids with anxiolytic activity have been described in many plant species used in folk medicine such as *Passiflora coerulea* [11]. This effect has been attributed to the affinity of flavonoids for the central benzodiazepine receptors [12-14]. Furthermore a sedative effect on the central nervous system has been shown for quercetrin and isoquercetin glycosides in mice [15-16]. However, further studies are required to identify the phytoconstituent responsible for the observed anxiolytic effect of the plant and to explain its anxiolytic mechanism.

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