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Exploring the Antianxiety Role of *Prunus Domestica* Leaves in Rats

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ABSTRACT

A review of the literature found that *Prunus domestica* has not had any systematic pharmacological research done to support the traditional claims made for the majority of its neuropharmacological activities, particularly for its antianxiety profile. Therefore, it was thought to be beneficial to test *Prunus domestica* aerial parts antianxiety profile. In a Soxhlet device, the plant leaves were extracted one at a time using n-hexane as a defatting solvent. Further, the hydroalcoholic extract and its ethyl acetate fraction were prepared using standard procedures. n-hexnae extract was used to defat the plant matter. After preliminary phytochemical screening revealed the presence of flavonoids, tannins, steroids, and triterpenoids as major bioactive classes of compounds, the hydroalcoholic extract and its ethyl acetate fraction were chosen for further investigation to evaluate its antianxiety activity. Ethyl acetate was used to fractionate the crude hydroalcoholic extract in order to obtain a fraction high in flavonoids. The well-known raised plus maze model was used to test the antianxiety effects of the hydroalcoholic extract and its ethyl acetate fraction. At tested concentrations, the hydroalcoholic extract and its ethyl acetate fraction demonstrated considerable antianxiety effects in comparison to the control. At lower and medium dosages of the hydroalcoholic extract and its ethyl acetate fraction, the activity demonstrated by the test medications was not comparable to that of the standard drug. However, the highest antianxiety activity of the hydroalcoholic extract and its ethyl acetate fraction, which is comparable to the standard medication, is shown at greater doses. It has been proposed that the anxiolytic effect of Prunus domestica leaves is attributed to flavonoids and/or triterpenoids.

Key words: *Prunus domestica*, antianxiety, elevated plus maze, flavonoids, triterpenoids.

Introduction

Traditionally, the plant *Prunus* domestica has been used in the treatment of various ailments such as asthma, joint pain, hypercholesterolemia, alzheimer, iron deficiency, and cardiovascular diseases and mental disorders (Parihaar et al., 2014; Shahidi et al., 2013). The plant is mainly distributed in the areas of Punjab, Himachal Pradesh and Garhwal district of Uttarakhand (Chopra, 2009) and some other wild regions such as Kashmir and Afghanistan (Kritkar and Basu, 1975). The plant has been reported to contain various natural products such as flavonoids (Nagarajan and Parmar, 1977; Parmar et al., 1992), proanthocyanadin (Hillis and Swain, 1959), steroids, terpenes (Stosic et al., 1985), phenylpropanoid esters, phenolic acids (Kikuzaki et al., 2004), coumarins (Nagrajan and Parmar, 1977), carotenoids (Gross, 1984) and carbohydrates (Rosik et al., 1965). The plant has been reported to exhibit various pharmacological activities such as antioxidant (Cardador-Martinez et al., 2002) antimicrobial (Mahmood et al., 2009), antihaemolytic (El-Beltagi et al., 2019), cholinesterase inhibitory (Bonesi et al., 2018), anticancer (Nath et al., 2013), hepatoprotective (Soni et al., 2011), anti-hyperlipidaemic (Tinker et al., 1994; Tinker et al., 1991), anti-inflammatory (Andrade and De-Sousa, 2013), antidiabetic (Bnouham et al., 2006) and larvicidal (Mahn and Tuyet, 2020). A review of the literature found that *Prunus domestica* has not had any systematic pharmacological research done to support the traditional claims made for the majority of its neuropharmacological activities, particularly for its antianxiety profile. Therefore, it was thought to be beneficial to test *Prunus domestica* leaves for its antianxiety profile.

MATERIALS AND METHODS

Collection and identification of plant material

The *Prunus domestica* leaves were collected from local areas of Uttarakhand in January, 2024. The identity of plant was confirmed from online literature related to their microscopic characters.

Preparation of crude extract / fraction and their phytochemical screening

The hydroalcoholic extract from dried plant material was prepared after defating with n-hexnae solvent using Soxhlet apparatus technology. Further the ethyl acetate fraction from hydroalcoholic extract was prepared using reflux technology (Kumar and Kumar, 2015). The hydroalcoholic extract and its ethyl acetate fraction were subjected to chemical screening to check presence and absence of phytomolecules (Farnsworth, 1966).

Antianxiety activity

Animals

Male SD rats of body weight 250-300 g, procured from Akal College of Pharmacy and Technical Education, Mastuana Sahib, Sangrur were used for antianxiety activity using elevated plus maze model (Kulkarni, 2003; Prakash et al., 2015; Kumar et al., 2016). The animals were fed with normal laboratory pellet diet and water *ad libitum*. The approval was taken from Institutional Animal Ethics Committee of Akal College of Pharmacy and Technical Education, Mastuana Sahib, Sangrur before carrying out animal studies (ATRC/29/23, dated 26/12/2023).

Vehicle and standard drugs

Distilled water + Tween 80 (2%) was used as vehicle for preparing various test doses of crude extract and fraction in such a concentration as to administer a volume ranging 1-2 ml to the rats. Diazepam was used as a standard antianxiety drug at the dose of 2 mg/kg, *p.o.*

Experimental protocol

Experimental protocol comprising groups 1-7 was designed to assess antianxiety activity of various crude extract / fractions of plant leaves.

Group 1 - Control group received vehicle (1-2 mg/kg, *p.o.*).

Group 2 - Standard group received diazepam (2 mg/kg, *p.o.*).

Groups 3 - Test groups received 100 mg/kg doses of hydroalcoholic extract.

Groups 4 - Test groups received 200 mg/kg doses of hydroalcoholic extract.

Groups 5 - Test groups received 400 mg/kg doses of hydroalcoholic extract.

Groups 6 - Test groups received 25 mg/kg doses of ethyl acetate fraction.

Groups 7 - Test groups received 50 mg/kg doses of ethyl acetate fraction.

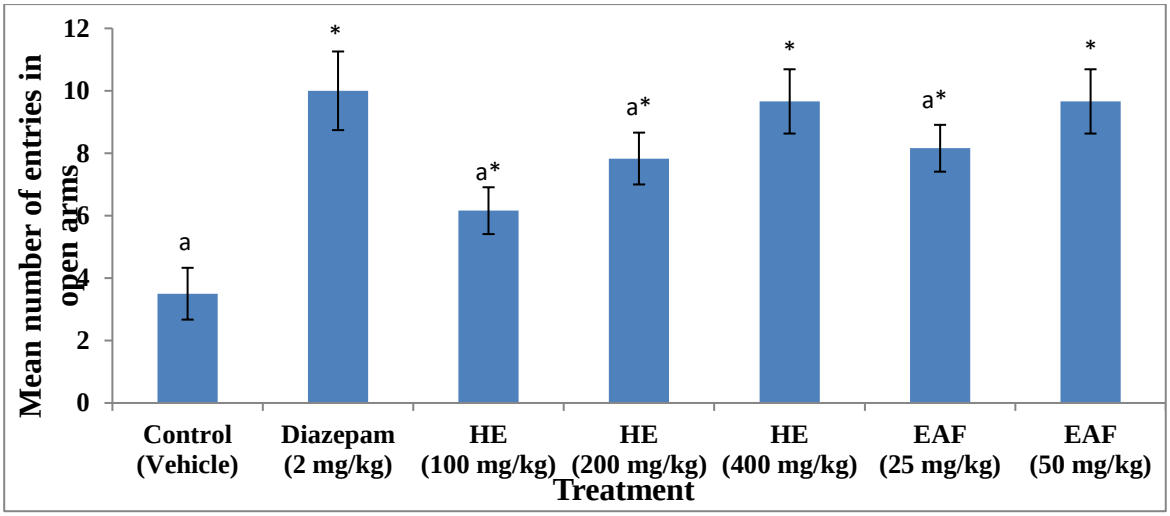
Statistics

The results were expressed as mean ± standard deviation (S.D.). The antianxiety activity of test drugs was compared with that of standard drug and control by one way analysis of variance (ANOVA) followed by Student-Newman-Keul's test (Scheffer, 1980).

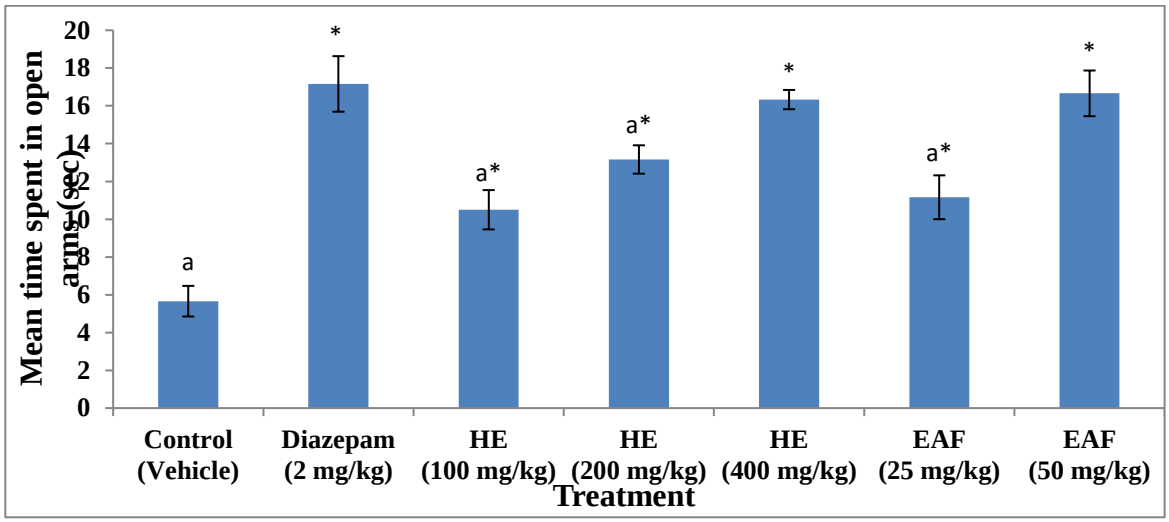
RESULTS AND DISCUSSION

The percentage yield (w/w) of the hydroalcoholic extract and its ethyl acetate fraction (in relation to hydroalcoholic extract) was found to be 12.85 and 40.25 % w/w respectively. n-hexnae extract was used to defat the plant matter. After preliminary phytochemical screening revealed the presence of flavonoids, tannins, steroids, and triterpenoids as major bioactive classes of compounds, the hydroalcoholic extract and its ethyl acetate fraction were chosen for further investigation to evaluate its antianxiety activity. Ethyl acetate was used to fractionate the crude hydroalcoholic extract in order to obtain a fraction high in flavonoids. The well-known raised plus maze model was used to test the antianxiety effects of the hydroalcoholic extract and its ethyl acetate fraction.

Using an elevated plus maze model, rats were given the hydroalcoholic extract and its ethyl acetate fraction of plant leaves to test for antianxiety properties. Figure 1 display the average number of entries and average amount of time the rats spent in the open arms of the elevated plus maze model apparatus following the administration of hydroalcoholic extract (100, 200, or 400 mg/kg, p.o.), ethyl acetate fraction (25 or 50 mg/kg, p.o.), diazepam (2 mg/kg, p.o.), and the control (vehicle, p.o.). At tested concentrations, the hydroalcoholic extract and its ethyl acetate fraction demonstrated considerable antianxiety effects in comparison to the control. At lower and medium dosages of the hydroalcoholic extract and its ethyl acetate fraction, the activity demonstrated by the test medications was not comparable to that of the standard drug. However, the highest antianxiety activity of the hydroalcoholic extract and its ethyl acetate fraction, which is comparable to the standard medication, is shown at greater doses.



(A)



(B)

Figure 1: Antianxiety activity of *Prunus domestica* leaves using elevated plus maze model. HE, Hydroalcoholic extract; EAF, Ethyl acetate fraction.

n=6; The data is expressed as Mean ± S.D.; **P<0.05* vs. Control; [a]*P< 0.05* vs. Standard; one way ANOVA followed by Student Newman Keul's test.

Conclusion

Using a well-known model, namely EPM, the antianxiety activity of test samples was examined by reducing motor activity in animals that increased as a result of acrophobia, or a fear of heights (Belzung and Griebel, 2001).

The benzodiazepine receptors (Avallone et al., 2002; Bergendorff et al., 1997), the serotonergic system (Kim et al., 2004), the regulation of 5-hydroxytryptamaine1A (5HT1A), the gamma-amino butyric acid type A (GABAA) receptor system (Yu et al., 2007), the inhibition of γ-amino butyric acid transmission (Une et al., 2001), their capacity to raise the level of ascorbic acid in the brain (Yanpallewar et al., 2005), or monoamine oxidase inhibition (Saaby et al., 2009) are some of the proposed modes of action for antianxiety activity in plant test samples.

Plant leaves treated with a hydroalcoholic extract and its ethyl acetate fraction included flavonoids and triterpenoids, according to preliminary phytochemical investigations. Our findings are consistent with the literature that has been reported to show anxiolytic activity for flavonoids such as quercetin (Saaby et al., 2009), kaempferol (Grundmann et al., 2009), chrysin (Wolfman et al., 1994), luteolin (Coleta et al., 2006), wogonin (Hui et al., 2002), bacicalin (Liao et al., 2003), apigenin (Kumar & Sharma, 2006) and triterpenoids galphimine A, galphimine B (Herrera-Ruiz et al., 2006), α, β-amyrin (Aragao et al., 2006). It has been proposed that the anxiolytic effect of *Prunus domestica* leaves is attributed to flavonoids and/or triterpenoids.

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