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Effect of *Cissampelos Pareira* Leaves on Anxiety-like Behavior in Experimental Animals

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ABSTRACT

The present study was undertaken to evaluate anxiolytic effect of 70% hydroethanolic extract of leaves of *Cissampelos pareira* in murine models. *C. pareira* (Menispermaceae) is rich in alkaloids, and phytochemical results showed that it contains alkaloids, flavanoids, terpenoids, steroids, etc., Anxiolytic activity was evaluated by using elevated plus maze test (EPM), light dark (LandD) model, and forced swim test (FS) models in rats. The efficacy of extract (100, 200, 400 mg/kg) was compared with control as well as standard diazepam (DZ; 2 mg/kg, p.o.) in EPM, LandD model, and imipramine (IM; 2.5 mg/kg, p.o.) in FS model. The results showed that DZ and extract significantly increased the number of entries, time spent in open arm, head dip counts, and rearing time, while they decreased fecal count in EPM. DZ and extract also significantly increased the number of crossings and time spent in light compartment, while they decreased duration of immobility in LandD model. In case of FS model, IM and extract significantly increased mobility and swimming time. Thus, the results confirm that hydroethanolic extract of *C. pareira* has the potential to be used in the management of anxiety-like behavior in a dose of 200 and 400 mg/kg. Further study is required to explore the plant and its parts for anxiolytic potential.

Key words: Anxiolytic, *Cissampelos pareira*, Elevated plus maze, Forced swim test, Light dark model

INTRODUCTION

Anxiety is a complex physiological and behavioral alteration of the organism, which ultimately leads to wide variety of central nervous system (CNS) disorders, if untreated. In addition to individual genetic factors, external influences such as nutrition, smoking, alcohol, socioeconomic status, environmental conditions, etc., can strongly contribute to its anticipated appearance.[1] Globally, anxiety affects one-eighth of the population and has become an important research area for brain disorders and psychopharmacology.[2] Excessive anxiety can debilitate and damage the quality of healthy and wealthy life. Anxiolytic drugs are one of the most frequently prescribed drugs as the disease is highly prevalent in the society.[3]

Benzodiazepines (BZDs), gamma-aminobutyric acid $(GABA)_A$ receptor agonist, and buspirone, a 5-hydroxytryptamine $(5\text{-HT})_{1A}$ receptor agonist, are mainly used in the clinical treatment of anxiety and their regular use results in physical and pharmacological dependence. They have other serious concerns and problems such as rebound insomnia, sedation, muscle relaxation, withdrawal, tolerance (BZDs, barbiturates, and alcohol), and sexual dysfunction. Anticholinergic and antihistaminic effects (tricyclic antidepressants, TCAs) have limited their use in patients.[3,4] Psychopharmacological research in the treatment of anxiety and stress is very much influenced by the availability of anxiolytic drugs.[5] Drawbacks of the medicines have forced many pharmaceutical companies to conduct research for alternative medicines or plant-derived medicines to reduce the CNS disorders like anxiolytic effects.[6]

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India has a rich repository of biodiversity and knowledge in the use of "folk" herbal medicines to cure many ailments in various cultures and tribes.[5] *Cissampelos pareira*, belonging to family Menispermaceae, is one of the important plants in Indian history, which is traditionally used in the treatment of anxiety, psychological and brain disorders. Historically, it has been used as an anti-inflammatory, smooth muscle relaxant, antispasmodic, as a uterine muscle relaxant, for menstrual disorders (including cramping and pain), and is reported to have antiulcerous, anti-hemorrhagic, anti-fertility, anti-nociceptive, antimalarial, antibacterial activities, etc.[7-9] *C. pareira* has also been reported to possess immunomodulatory activity.[10] The roots and leaves contain several alkaloids and volatile oil. *C. pareira* is reported to contain stetradrine, berberine, cissampeline, bulbocapnine, cissamine, cissampareine, corytuberine, curine, 4-methylcurine, cyclanoline, cycleanine, dicentrine, dehydrodicentrine, dimethyltetrandrinium, grandirubrine, hayatine, hayatinine, insularine, isochondodendrine, isomerubrine, laudanosine, linoleic acid, magnoflorine, menismine, norimeluteine, nor-ruffscine, nuciferine, pareirine, pareirubrine, pareitropone, quercitol, and stearic acid.[8] Literature survey of *C. pareira* has revealed that its CNS activity or on traditional use validation has not been studied much, so the present study was conducted to determine the anxiolytic effect of hydroethanolic extract of leaves of *C. pareira* by using different animal models of anxiety.

MATERIALS AND METHODS

Plant material

The plant material was collected from Bilaspur district in Himachal Pradesh, India. Plant material was identified and authenticated by Prof. Zulfiquar Ali Bhat, Department of Pharmaceutical Sciences, University of Kashmir, Srinagar, India (voucher specimen number AUKU/012).

Drying and size reduction

Plant leaves were subjected to shade drying for about 1 week. The dried plant material was further crushed to powder and the powder was passed through sieve mesh no. 40 and stored in an air-tight container for further analysis.

Extraction of plant material

The coarsely powdered material (100 g) was extracted using 70% ethanol as a solvent with simple maceration process at room temperature. Extract was concentrated and dried at 40-50°C using vacuum distillation assembly. The hydroethanolic extract of *C. pareira* (HEECP) yielded a thick brown semisolid residue (20%). The extract was subjected to preliminary phytochemical screening.[11,12]

Animals

Adult albino rats (130–160 g) of either sex were obtained from the animal house of Sanjay Biological in Amritsar, Punjab, India. The animals were maintained in a well-ventilated room with 12:12 h light/dark cycle in polypropylene cages with standard pellet chow and water *ad libitum*. Animals were divided into five groups with five rats in each group. Institutional Animal Ethical Committee approved the protocol of the study (approval no. RIP/IAEC/2012-13/11). The experiments were conducted as per the approved protocol. Animals were acclimatized to laboratory conditions 1 week prior to initiation of experiments. This institution is approved by (CPCSEA) Committee for the Purpose of Control and Supervision of Experiments on Animal Government of India, for carrying out animal studies.

Drugs and chemicals

Diazepam (DZ) was purchased from Ranbaxy Laboratories Limited, Himachal Pradesh State Industrial Development Corporation (HPSIDC), Baddi, Solan, India and imipramine (IM) from Pfizer Ltd., Mumbai, India. Ethanol and sodium carboxymethyl cellulose were purchased from Spruce Enterprises Ambala Cant, Haryana, India. DZ, IM, and test drugs (100, 200, and 400 mg/kg) were suspended in a 1% sodium carboxymethyl cellulose solution. All the dosages were prepared immediately before use and administered orally. Control rats received 1% aqueous sodium carboxymethyl cellulose solution only. The effects of the drugs were estimated 60 min after drug administration. Tests were performed only after the rats had been acclimatized to the experimental environment for 7 days. All experiments were carried out between 09:00 and 16:00 h. In each experiment, the apparatus was cleaned using 5% ethanol.

Acute toxicity study

Acute toxicity study was conducted as per the internationally accepted protocol drawn under the OECD (Organisation for Economic Co-operation and Development) guidelines 425 (OECD, 2001). Overnight fasted, healthy rats (n = 3) were administered orally the extract of hydroethanolic plant material in doses of 1600 and 3200 mg/kg body weight and observed continuously for 4 h and after 24 h for any abnormality and mortality. Hydroethanolic extract at a dose level of 3200 mg/kg was found to be safe. Doses of 100, 200, and 400 mg/kg were selected as the experimental dose of extracts for anti-anxiety studies.

Elevated plus maze model

Elevated plus maze

In brief, the apparatus was composed of two open arms (50 × 10 cm) and two enclosed arms of the same size with 40-cm-high wall arranged in such a way that the arms of the same type were opposite to each other with a central square of 10 cm to form a plus sign. The apparatus was wooden and was elevated to a height of 50 cm above the floor level by a single central support. A slightly raised edge on the open arms (0.25 cm) provided additional grip for the animals, whereas open arm activity was further encouraged by testing in a dimly lit room. The experiment was conducted between 9:00 and 16:00 h. To facilitate adaptation to new surroundings, rats were transported to the laboratory at least 1 h prior to testing. The trial was started by placing an animal on the central platform of the maze facing an open arm. Standard 5-min test duration was used for the animals. The maze was thoroughly cleaned. Rats were randomly allocated to the following groups: vehicle control; positive control: DZ (2 mg/kg, p.o.); and test drugs. The experiments were performed with an observer aware of the treatment of the rats inside the room. The following parameters were classically measured in this test: frequency and duration (s) of

arm visits, separately for open and closed arms. A rat was considered to have entered an arm when all four paws were on the arm. The number of entries into open arms, closed arms), and the time spent in open or closed arms were used as traditional indices of the anxiety. In addition, the latency time (time spent at the center of the maze), head dip count, rearing count and duration, and fecal bolus (stool bal) count were also recorded.[2,13-15]

Light dark (LandD) model

The apparatus consisted of an open top wooden box. Two distinct chambers, a black chamber (20 × 30 × 35 cm) painted black and illuminated with dimmed red light and a bright chamber (30 × 30 × 35 cm) painted white and brightly illuminated with 100 W white light source, were located 17 cm above the box. The two chambers were connected through a small open doorway (7.5 × 5 cm) situated on the floor level at the center of the partition. Each animal was in bright and dark arena paradigm. Sixty minutes after the drug administration [DZ (2 mg/kg, p.o.), test drugs (100, 200, and 400 mg/kg, p.o.)] or vehicle administration, the animal was placed at the center of the brightly lit arena in the light and dark box. Time spent in the light arena, time spent in dark arena, number of crossings, and duration of immobility were noted for 10 min for each trial. Following each trial, the apparatus was cleaned to mask the odor left by the animal in the previous experiment. Hand-operated counters and stop watches were used to score the behavior of animals, and experiments were performed with an observer inside the room.[2,4,14,15]

Forced swim test (FST)

Rats' responses were evaluated in a glass tank (23 cm × 30 cm; height, 40 cm; Techno) filled to a depth of 28 cm with water at 22°C. The rats could not touch the bottom of the glass tank. The glass tank was illuminated indirectly and surrounded by dark brown shading walls (distance from the tank, 20 cm) to screen the view from the experimenter. The experiments were done between 9:00 and 16:00 h in accordance with a described method.[15] On the first experimental day, rats were placed gently in the water for a 15-min period of habituation. Upon removal from the water, they were placed in a standard Plexiglas box with the floor covered with paper towels. They were then placed under an infrared heater for 30 min to dry. The next day, they were placed once more gently into the glass tank and observed for 5 min. The behavior of the rats was recorded. At the end of the 5-min period, the rats were transferred to the infrared heated box and allowed to dry. During the experiment, the data noted or recorded were evaluated manually and the duration of the following behaviors was recorded, i.e. immobility (floating and making only those movements necessary to keep the nose above the water and swimming) and active motions (i.e., moving around the tank, including diving). Rats were randomly allocated to the following groups: vehicle control, IM (2.5 mg/kg, p.o.), and test drug (100, 200, and 400 mg/kg, p.o.). The experiments were done with an observer in the room.[5,16]

Statistical analysis

All observations were presented as Mean ± SEM and were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's "*t*"-test (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). *P* values lower than 0.05 were considered statistically significant.

RESULTS

Phytochemical screening

Preliminary phytochemical screening of HEECP showed that the plant contains alkaloids, terpenoids, carbohydrates, and phenolic/tannins, but proteins, saponins, steroids, and glycoside were absent [Table 1].

Effect of DZ (2 mg/kg, p.o.) and HEECP on the time spent in open/closed arm and latency (time spent in the center of maze) in animals of the EPM model

Standard group (DZ, 2 mg/kg) showed significant increase in the time spent in open arm (171.67 ± 20.5 sec; ***$P < 0.001$) as compared to the control group (10.6 ± 2.92 sec), which indicates reduction in the anxiety. Significant increase in the time spent in open arm was observed with test doses of HEECP at 100, 200, and 400 mg/kg, i.e. 73.5 ± 5.61 sec (***$P < 0.001$), 84 ± 5.4 sec (***$P < 0.001$), and 87.8 ± 3.24 sec (***$P < 0.001$), respectively, in comparison with the control group. In a similar fashion, to determine the potency of the extract, test groups were compared with standard group. Test group doses (100, 200, and 400 mg/kg) showed significant difference (***$P < 0.001$) compared to the standard drug in reduction of anxiety. Potency of the test drug (400 mg/kg) was found to be lesser than the standard group (DZ). DZ and test doses showed significant decrease in the time spent in closed arm as compared to the control group, which indicates anxiolytic effect. Test group doses (100, 200, and 400 mg/kg) showed significant difference (***$P < 0.001$) compared to the standard drug in reduction of anxiety. DZ and HEECP (100, 200, and 400 mg/kg) showed significant decrease in latency as compared to the control group. Test groups (100 and 400 mg/kg) showed insignificant difference compared to the standard, but the other test group (200 mg/kg) showed significant difference (**$P < 0.01$) in comparison. Potency of the test group (200 mg/kg) was found to be greater than the standard group. Results are presented in Figure 1. Absence of *a* and *b* values in the graph indicate insignificant or insignificant group.

Effect of DZ and HEECP on the number of entries in open/closed arm in animals of the EPM model

Standard group (DZ, 2 mg/kg) showed significant increase in the number of entries in open arm (8.50 ± 1.50; $P < 0.001$) as

Table 1: Phytochemical screening of hydroethanolic extract of *Cissampelos pareira*

Extract constituents	Present (+)/Absent (-)
Alkaloids	+
Terpenoids	+
Carbohydrates	+
Phenolic/tannins	+
Proteins	–
Saponins	–
Steroids	–
Glycosides	–

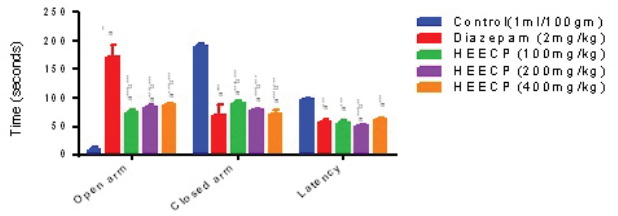
compared to the control group (0.33 ± 0.01), which indicates reduction in anxiety. Insignificant increase in the number of entries in open arm was observed with HEECP 100, 200, and 400 mg/kg, i.e. 3 ± 0.33, 3 ± 0.03, and 4 ± 0.23, respectively, in comparison with the control group. Test groups (100 and 200 mg/kg) showed significant difference (***$P < 0.001$), while 400 mg/kg showed significant difference of **$P < 0.01$ on comparing with the standard group. Potency of the test group (400 mg/kg) was found to be lesser than the standard group [Figure 2]. Standard group showed significant decrease in the number of entries in closed arm (1.83 ± 0.40; $P < 0.01$) as compared to the control group (7.17 ± 0.79). There was insignificant decrease in the number of entries in closed arm with HEECP at doses 100 and 200 mg/kg, i.e. 5 ± 0.89 and 4.17 ± 0.70, respectively, and a significant decrease was observed with 400 mg/kg of HEECP, i.e. 2.6 ± 0.67 ($P < 0.05$), when compared with the control group. HEECP (100, 200, and 400 mg/kg) showed insignificant difference compared to DZ. Potency of the extract (400 mg/kg) was found to be lesser than DZ [Figure 2].

Effect of DZ and HEECP on fecal bolus, rearing count, and head dip count in animals of the EPM model

The standard group (DZ, 2 mg/kg) showed significant decrease in fecal bolus count (0.4 ± 0.20; ***$P < 0.001$) as compared to the control group (6 ± 0.20), which indicates reduction in anxiety. Equal significant decrease in fecal bolus count was observed with two different doses of HEECP (100 and 200 mg/kg, i.e. 1 ± 0.10 (***$P < 0.001$), while no fecal bolus count was observed with HEECP dose 400 mg/kg. Test groups (100, 200, and 400 mg/kg) showed insignificant difference compared to the standard group. Potency of the extract (400 mg/kg) was found to be greater than the standard group [Figure 3]. DZ showed significant increase in rearing count (4 ± 1.32; ***$P < 0.001$) as compared to the control group (1 ± 0.45). Insignificant increase in rearing count was observed with HEECP 100 mg/kg (2 ± 0.32), while significant increase was observed for HEECP 200 and 400 mg/kg, i.e., 3 ± 0.40 (*$P < 0.05$) and 4 ± 1.0 (***$P < 0.001$), respectively, in comparison with the control group. Test group dose of 100 mg/kg showed significant difference (*$P < 0.05$), while the test groups of doses 200 and 400 mg/kg showed insignificant difference in comparison to the standard group. Extract (400 mg/kg) was found to be equipotent to the standard group [Figure 3]. DZ and HEECP at 100, 200, and 400 mg/kg showed significant increase in head dip count (25 ± 1.1; ***$P < 0.001$) as compared to the control group (5 ± 1.0). Test group of 100 mg/kg showed significant difference (*$P < 0.05$), while the test groups of doses 200 and 400 mg/kg showed insignificant difference in comparison with the standard group. Extract (400 mg/kg) was found to be a little bit lesser potent than the standard group [Figure 3].

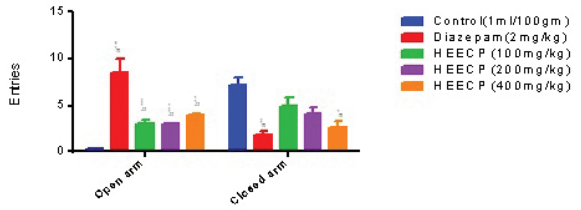
Effect of DZ and HEECP on the time spent in light/dark compartment and duration of immobility in animals of the LandD model

Standard group (DZ, 2 mg/kg) showed significant increase in the time spent in light compartment (450 ± 18.99 sec; ***$P < 0.001$) as compared to the control group (131.8 ± 10.03 sec), which indicates reduction in anxiety. Significant increase in the time spent in light compartment was observed with HEECP at 100, 200, and 400 mg/kg, i.e. 291 ± 14.3 sec ($P < 0.001$), 299 ± 21.5 sec (***$P < 0.001$), and 309 ± 24.3 sec (***$P < 0.001$), respectively, in comparison with the control group. Test groups of HEECP at 100 and 200 mg/kg showed significant difference (***$P < 0.001$), while the test group at 400 mg/kg showed insignificant difference in comparison with the standard group. Test group with 400 mg/kg showed lesser potency than the standard group [Figure 4]. Standard group and test doses showed significant decrease in the time spent in dark compartment (***$P < 0.001$) as compared to the control group. Test groups with 100 and 200 mg/kg showed significant difference ($P < 0.001$), while the test group dose of 400 mg/kg showed insignificant difference in comparison with the standard group. Potency of the test group (400 mg/kg) was found to be lesser than the standard group [Figure 4]. DZ and HEECP at 200 and 400 mg/kg showed significant decrease in the duration of immobility as compared to the control group, while HEECP at 100 mg/kg showed insig-



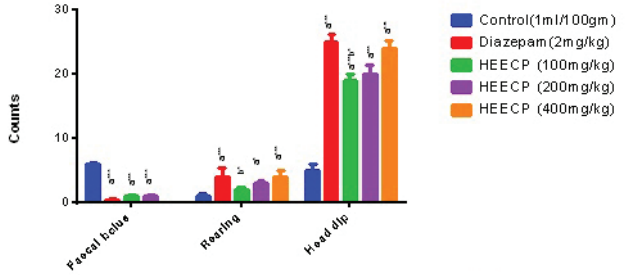
n = 5 (no. of animals), **p<0.05, **p<0.01, ***p<0.001 (one way ANOVA followed by Dunnett's 't' test)*, a = Compared with control; b = Compared with standard. Values are expressed as mean ± SEM.

Figure 1. Effect of Diazepam & HEECP on time spent in open/ closed arm and latency (time spent in the centre of maze) on animals in EPM model



*n = 5, *p<0.05, **p<0.01, ***p<0.001 (one way ANOVA followed by Dunnett's 't' test)*, a = Compared with control; b = Compared with standard. Values are expressed as mean ± SEM.

Figure 2. Effect of Diazepam & HEECP (100,200 & 400 mg/kg) on no. of entries in Open/closed arm on animals in EPM model



*n = 5, *p<0.05, **p<0.01, ***p<0.001 (one way ANOVA followed by Dunnett's 't' test)*

a = Compared with control; b = Compared with standard. All the values are expressed as mean ± SEM.

Figure 3. Effect of Diazepam & HEECP (100, 200 & 400 mg/kg) on faecal bolus, rearing count, head dip count on animals in EPM model

nificant activity. HEECP at 100, 200, and 400 mg/kg showed insignificant difference in comparison with DZ. Potency of the test group of 400 mg/kg was found to be lesser than the standard group [Figure 4].

Effect of DZ and HEECP on the number of crossings in animals of the LandD model

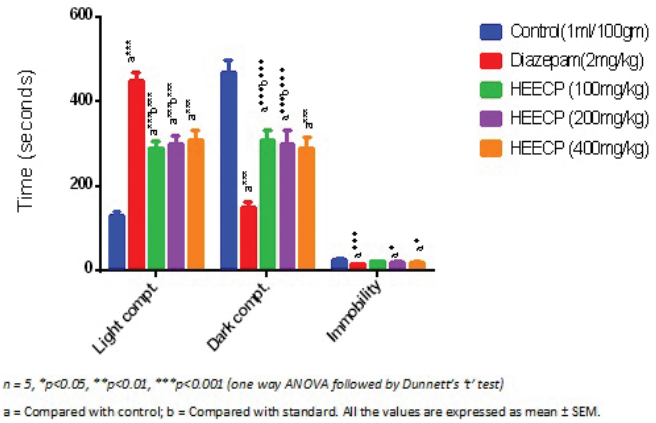
Standard drug (DZ, 2 mg/kg) showed significant increase in the number of crossings (***$P < 0.001$), while HEECP at 100, 200, and 400 mg/kg showed insignificant difference when compared with the control group. HEECP at 100, 200, and 400 mg/kg showed significant difference (**$P < 0.01$, ***$P < 0.001$) when compared to the standard. HEECP at 400 mg/kg showed lesser potency than the standard group [Figure 5].

Effect of IM (2.5 mg/kg, p.o.) and HEECP on immobility/swimming period in animals of FST model

Standard group (IM, 2.5 mg/kg) and HEECP at 200 and 400 mg/kg showed significant decrease in the immobility period (***$P < 0.001$), while HEECP at 100 mg/kg was found to be insignificant when compared to the control group. HEECP (100, 200, and 400 mg/kg) showed significant (***$P < 0.001$, **$P < 0.01$) difference compared to the standard group. Potency of HEECP 400 mg/kg was found to be lesser than the standard group [Figure 6]. DZ and HEECP 200 and 400 mg/kg also showed significant increase in the swimming period (***$P < 0.001$) as compared to the control group, while HEECP 100 mg/kg showed insignificant difference. HEECP at 100, 200, and 400 mg/kg showed significant difference (*$P < 0.05$, ***$P < 0.001$) when compared with the standard group (DZ; 2 mg/kg). Potency of HEECP (400 mg/kg) was found to be lesser than the standard group [Figure 6].

DISCUSSION

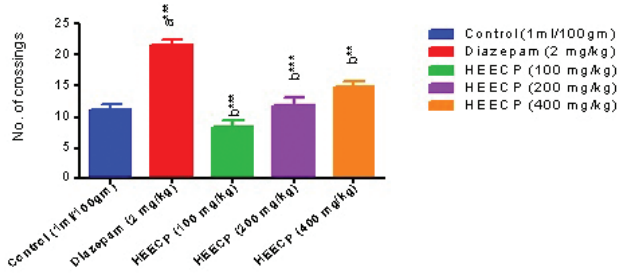
Natural molecules like linalool, hypericin, cardiospermin, chrysin, *p*-coumaric acid, ferulic acid, sanjoinine A, obovatol, kaempferol, apigenin, imperatorin, isoimperatorin, etc., isolated from plants have shown promising anxiolytic activity.[2,12] The etiology of anxiety is not well known, but several studies revealed that dopaminergic and adrenergic systems and GABAergic and serotonergic neurotransmission are involved in the etiology, expression, and treatment of anxiety.[17-19] Despite its widespread traditional use, there are no reports that scientifically provide the proof for the anxiolytic potential of *C. pareira*. The solvent has the capacity to extract the maximum number of constituents. Hydroethanolic extract of leaves of *C. pareira* is found to have anxiolytic-like potential in behavioral models of murines, i.e. EPM, LandD, and FS models. EPM is highly sensitive to the influence of both anxiolytic and anxiogenic drugs acting at the $GABA_A$–BZD complex.[20] EPM is considered one of the most validated models for assaying sedative and anxiolytic activity of drugs such as BZDs.[21] In EPM, the rats normally prefer to spend much of their allotted time in the closed arms. This preference appears to reflect an aversion toward open arms, which is generated by the fears of the open spaces. Drugs that increase open arm exploration are considered as anxiolytics and the reverse holds true for anxiogenics.[22] In this



*n = 5, *p<0.05, **p<0.01, ***p<0.001 (one way ANOVA followed by Dunnett's 't' test)*

a = Compared with control; b = Compared with standard. All the values are expressed as mean ± SEM.

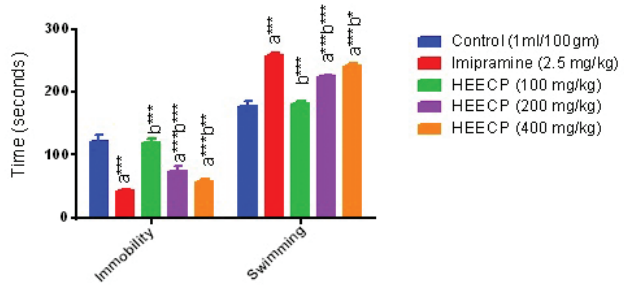
Figure 4. Effect of Diazepam & HEECP (100, 200 & 400 mg/kg) on time spent in light/dark compartment & duration of immobility on animals in light/dark model



*n = 5, *p<0.05, **p<0.01, ***p<0.001 (one way ANOVA followed by Dunnett's 't' test)*

a = Compared with control; b = Compared with standard. Values are expressed as mean ± SEM.

Figure 5. Effect of Diazepam & HEECP (100, 200 & 400 mg/kg) on no. of crossings on animals in light/dark model



*n = 5, *p<0.05, **p<0.01, ***p<0.001 (one way ANOVA followed by Dunnett's 't' test)*

a = Compared with control; b = Compared with standard. All the values are expressed as mean ± SEM.

Figure 6. Effect of Imipramine (2.5 mg/kg) & HEECP (100, 200 & 400 mg/kg) on immobility/swimming period on animals in FST model

study, we observed that HEECP (200 and 400 mg//kg) induced significant activity in the EPM model.

The LandD test is widely used in rodent anxiety models to assess anxiolytic or anxiogenic-like behavior of a drug. In the present study, rats spent more time in the light box and exhibited significant change in the number of transitions between the two boxes, which indicated an anxiolytic activity.[15] Anxiety related to stress is a component of severe depression in human beings. The incidence of anxiety and depression in the community is very high, and

associated with considerable morbidity. Hence, addressing these problems and finding effective remedies is extremely important. Several drugs are available, but all are associated with limitations, and there is an urgent need for alternative medications for these disorders. The FS test has been validated as a suitable tool for predicting the antidepressant properties of drugs. It is a behavioral test in rodents that gives an indication of the clinical effectiveness of various types of antidepressant drugs. This model helps to assess the antidepressant action and identify the anxiolytics for those anxiety which is in combination with depression from nature.[14,15] The active constituents of HEECP have to pass the blood–brain barrier in order to produce any pharmacological effects. Previous reports on the phytochemicals and pharmacology suggest that plants containing alkaloids, flavanoids, and terpenoids possess activity against many CNS disorders.[23] Phytochemical tests of HEECP revealed the presence of alkaloids and flavanoids, terpenoids, phenolics, etc., It may be possible that the mechanism of anxiolytic action of HEECP could be due to the binding of any of these phytochemicals to the $GABA_A$–BZD complex. In support of this, it has been found that berberine alkaloids bind with high-affinity BZD site of the $GABA_A$ receptor[24] and *C. pareira* also contains berberine and flavones which may be responsible for its anxiolytic activity. So, the anxiolytic activity of HEECP might involve an action on GABAergic transmission or effects on serotonergic transmission or may be due to its combinatorial effect. The detailed mechanism of action is needed to be screened and established. Hence, this research paves a way for further evaluation and isolation of active molecules from the plant for anxiolytic potential.

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