Antianxiety Investigations of Centaurea behen Linn. and Elaeocarpus ganitrus Roxb.

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ABSTRACT

The petroleum ether (PE), chloroform (CE), ethanol (EE) and water extractives (WE) of Centaurea behen and Elaeocarpus ganitrus were prepared and evaluated for antianxiety activity in mice using elevated plus maze model. The results were compared with standard drug, diazepam. The ethanol extractive of C. behen (200 mg/kg) and chloroform and ethanol extractives of E. ganitrus (200 and 400 mg/kg) significantly increased the time spent and percentage of the open arm entries in the elevated plus maze model and hence exhibited antianxiety activity, which was comparable to diazepam. Chemically the extracts of both the plants showed the presence of phytosterols, fats, alkaloids, flavonoids, carbohydrates, proteins and tannins. The anxiolytic effects of the ethanol extractive of C. behen and chloroform and ethanol extractives of E. ganitrus may be related to their algaloidal and flavonoid content. The results indicate that both the plants can be considered as potential candidate for bioactivity guided isolation of natural antianxiety agents.

Key words: Antianxiety, Centaurea behen, Safed behman, Elaeocarpus ganitrus, Rudraksh,

INTRODUCTION

Anxiety is a feeling of apprehension, worry, or uneasiness that may or may not be based on reality. Anxiety may be seen in many types of situations, ranging from the anxiety that may accompany one’s employment to the acute anxiety that may be seen during withdrawal from alcohol. Although a certain amount of anxiety is normal, excess anxiety interferes with day-to-day functioning and can cause undue stress1. Anxiety affects one-eighth of the total population worldwide and has become an important area of re-search in psychopharmacology during this decade2. Benzodiazepines, barbiturates, alcohol and tricyclic antidepressants (TCA’s) have been used for long time to treat anxiety disorders3-4. However, the clinical uses of these drugs are limited because of the serious side effects associated with these drugs, namely rebound insomnia, sedation, muscle relaxation, withdrawal and tolerance, sexual dysfunction, anticholinergic and anti-histamnic effects5.

The use of herbal medications by physicians in Europe and Asia is becoming very common and researchers are exploring the traditional remedies to find a suitable cure for these mind affecting diseases6.

Centaurea behen Linn. (Family Asteraceae, Compositae) is commonly known as safed behmen in Hindi7. It is native to Iran and also occurs in India. Pakistan, Europe, North Africa and Israel8. It is an annual or perennial herb. It is used as Aphrodisiac, antiflatulent, cardiotonic, sedative, emmenagogue, in jaundice, kidney stone and anti-inflammatory9.

Elaeocarpus ganitrus ROXB. (Syn. E. sphaericus (Gaertn): family Elaeocarpacaeae), is popular for its attractive fruit stones. Its beads are covered by an outer shell of blue color on fully ripening; hence also called blueberry beads8. It finds a prominent place in Hindu religion and Ayurveda, the ancient Indian system of medicine. In Hindi it is known as Rudraksha10. The flesh or pulp of drupe is given in epilepsy, and in mental illness11. Besides it is reported to exhibit multifarious pharmacological activities i.e., anti-inflammatory4, analgesic, sedative2, antiulcerogenic, antidepressant3, antiasthmatic15, hypoglycemic11, anti hypertensive16-18, smooth muscle relaxant, hydrocholeteric11, anti convulsant12, etc. Despite the widespread traditional uses of C. behen and E. ganitrus there are no reports of scientific evaluation of their antianxiety activity, therefore the present study has been undertaken to explore anxiolytic potential of the above plants.

MATERIAL AND METHODS

Plant material:

Dried roots of C. behen and dried fruits (beads) of E. ganitrus were procured from a cultivated source, Hind Herb Shop, Saharanpur, Uttar Pradesh, India, in the month of September. Identity of both the drugs was confirmed through Plant Anatomy Research Centre, Medicinal Plant Research Unit, Chennai. Voucher specimen no. PARC/2008/166 and PARC/2008/164 has been deposited in the herbarium of the same institute.

Animals:

The experimental animals [Swiss albino mice (laca strain, 20-25 g) of either sex] were procured from the Central Research Institute, Kasauli, Himachal Pradesh. The animals received a standard pelleted diet and water ad libitum, were maintained under standard environmental conditions (25±2°C with 12hr of light/dark cycle). The experimental protocol was approved by Institutional animal ethical committee and experiments were conducted according to CPCSEA, India guidelines on the use and care of experimental animals.

Chemicals:

Solvents viz., petroleum ether (60-80° Merck), chloroform (Merck) and ethanol, All of LR grade were employed for the extraction of plant material. Diazepam was procured from Ranbaxy Laboratories Limited Mumbai.

Preparation of extracts and doses:

Coarsely powdered dried roots of C. behen and dried fruits of E. ganitrus were successively extracted with petroleum ether, chloroform and ethanol for 48 hours each using soxhlet apparatus and finally boiled with distilled water for 6 hours. The extracts were filtered, concentrated in vacuum and dried in an oven at 40-50°C. After removal of solvents under vacuum from various extracts, the dried extractives were preserved in vacuum dessicator. Phytochemical screening of various extracts of C. behen roots and E. ganitrus beads viz petroleum ether, chloroform, ethanol and water was carried out using standard procedures (Evans 1996a; Farnsworth 1966; Evans 1996b; 1996c; Khandelwal 2004)19-25. All the extractives of both the plants were suspended in carboxy methylcellulose for preparation of suspensions of various test doses. Animals were divided into 12 groups of 5 animals each and given following treatments- Group I: vehicle treated animals served as control; Group II: animals received diazepam (2 mg/kg) Group III-XII: were treated with different extractives of both the plants at doses of 50,
Table 1. Phytochemical screening of various extractives of *Centaurea behen* (CB) and *Elaeocarpus ganitrus* (EG).

<table>
<thead>
<tr>
<th>Plant constituent</th>
<th>Test/Reagent used</th>
<th>Pet. ether extract</th>
<th>Chloroform extract</th>
<th>Ethanolic extract</th>
<th>Aqueous extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Alkaloids</td>
<td>CB</td>
<td>E</td>
<td>CB</td>
<td>E</td>
<td>CB</td>
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<tr>
<td></td>
<td>Hager’s regent</td>
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<td>+</td>
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<td></td>
<td>Wagner’s regent</td>
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<td></td>
<td>Mayer’s regent</td>
<td>-</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>2. Carbohydrates</td>
<td>CB</td>
<td>E</td>
<td>CB</td>
<td>E</td>
<td>CB</td>
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<tr>
<td></td>
<td>Molish’s regent</td>
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<td>-</td>
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<td></td>
<td>Fehling regent</td>
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<td>+</td>
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<td>3. Proteins &amp; amino</td>
<td>CB</td>
<td>E</td>
<td>CB</td>
<td>E</td>
<td>CB</td>
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<tr>
<td></td>
<td>Ninhydrin reagent</td>
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<td></td>
<td>Biuret test</td>
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<td>Xanthoprotic test</td>
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<td>4. Phytoestols</td>
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<td>E</td>
<td>CB</td>
<td>E</td>
<td>CB</td>
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<td>Salkowskí test</td>
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<td>Libermann Buchard’s test</td>
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<td></td>
<td>Libermann test</td>
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<td>+</td>
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<tr>
<td>5. Phenolic compds. &amp; tannins</td>
<td>CB</td>
<td>E</td>
<td>CB</td>
<td>E</td>
<td>CB</td>
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<td></td>
<td>Lead acetate test</td>
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<td></td>
<td>Acetic acid test</td>
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<td>Potassium dichromate test</td>
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<td>Nitric acid test</td>
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<td>Ferric Chloride test</td>
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<td>Potassium permanaginate test</td>
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<td>6. Saponins</td>
<td>CB</td>
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<td>CB</td>
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<td>Foam test</td>
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<td>7. Flavonoids</td>
<td>CB</td>
<td>E</td>
<td>CB</td>
<td>E</td>
<td>CB</td>
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<td>Shinoda test</td>
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<td>Sodium hydroxide test</td>
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<td>8. Fixed oils &amp; fats</td>
<td>CB</td>
<td>E</td>
<td>CB</td>
<td>E</td>
<td>CB</td>
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<tr>
<td></td>
<td>Staining test</td>
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<td></td>
<td>Saponification test</td>
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</tbody>
</table>

CB *Centaurea behen*, EG *Elaeocarpus ganitrus*, + Present, - absent

Fig. 1. Effect of Petroleum ether, chloroform, ethanol and water extractive of *Centaurea behen* on elevated plus maze model. The data was analyzed by one way ANOVA followed by Dunnet’s test. Values are expressed mean±SE (n=5) *p< 0.05 as compared to control and *p< 0.05 Diazepam (Standard Drug).
**Elevated plus maze (EPM):**

Anxiolytic activity was measured using elevated plus maze. The EPM test is the most widely used model for the anxiolytic activity assessment of novel substances including herbal remedies in rodents. The EPM apparatus was consisted of two open arms (16 × 5 cm), two enclosed arms (16 × 5 × 12 cm), and the maze was elevated 25 cm from the room’s floor. Each animal was placed at the center of the maze facing one of the enclosed arms. Number of entries and time spent on closed and open arms were recorded for 5 min. Entry into an arm was defined as the animal placing all four paws on the arm. After each test, the maze was carefully cleaned with tissue paper wet with a 10% ethanol solution. The average of number of entries to open arms and the average of time spent in open arms were registered.

**Statistical analysis:**

Each group consisted of five animals. Results were expressed as mean ± S.E.M and all the extractives were compared with standard and control separately using one way analysis of variance (ANOVA) followed by Dunnett’s test. Differences were considered significant at *p* < 0.05 vs. control and *p* < 0.05 towards open arms that is generated by the fears of the open spaces. Drugs that increase the exploratory behavior and preference for open arm are considered as anxiolytics and the reverse holds true for anxiogenics that increase the exploratory behavior and preference for open arm are considered as anxiolytics and the reverse holds true for anxiogenics.

**RESULTS**

Phytochemical screening gave positive tests for phytosterols, fats, alkaloids, flavonoids, carbohydrates, proteins and tannins (Table 1).

**Antianxiety activity:**

Ethanol extractive of *C. behen* showed significant activity at all doses, but maximum activity was noted at a dose of 200 mg/kg which was statistically significant to standard drug diazepam (Fig. 1). Among various extractives of *E. ganitrus* tested maximum anxiolytic activity was observed in the ethanol extractive followed by chloroform extractive (Fig. 2). Ethanol extractive was effective at all doses but a dose of 200 mg/kg was at par with that of diazepam as evident from statistical equivalence between the results of this dose and that manifested by diazepam (Fig. 2). Chloroform extractives of *E. ganitrus* exhibited significant activity at all doses, but a dose of 400 mg/kg exhibited maximum anxiolytic activity.

However, the activity decreased at higher doses in both the plants which might be due to mild sedation. Chloroform extractive of *C. behen* did not show any anxiolytic effect while petroleum ether and water extractives of both the plants were also devoid of activity.

**Discussion**

The fear of 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 increase the motor activity, which manifests as increase in the time spent by the animal in the open arms of EPM. The etiology of most anxiety disorders is not fully understood, but various studies have shown the involvement of GABAergic, serotonergic neurotransmission in etiology, expression and treatment of anxiety. The adrenergic and dopaminergic systems have also been documented to play a role in anxiety. Despite the wide spread use of *C. behen* and *E. ganitrus* in herbal formulations for treating various disorders there are a few scientific evaluation of their antianxiety activity. In the present study the effects of various extractives viz petroleum ether, chloroform, ethanol and water extractives of *C. behen* and *E. ganitrus* were studied in elevated plus maze apparatus. The conventional plus maze is highly sensitive to the influence of both anxiolytic and anxiogenic drugs acting at the GABA-benzodiazepine complex. This animal model is considered one of the most widely validated tests for assaying sedative and anxiolytic substances such as the benzodiazepines.

In EPM, naïve mice will normally prefer to spend much of their allotted time in the closed arms. This preference appears to reflect an aversion towards open arms that is generated by the fears of the open spaces. Drugs that increase the exploratory behavior and preference for open arm are considered as anxiolytics and the reverse holds true for anxiogenics. Phytochemical screening of the plant revealed the presence of phytosterols, fats, alkaloids, flavonoids, carbohydrates, proteins and tannins. The anxiolytic effects of ethanolic extract of *C. behen* and chloroform and ethanolic extract of *E. ganitrus* may be related to their alkaloid and flavonoid content. Studies of *Passiflora* species as an anxiolytic/sedative have been conducted and it was noticed that this species possesses significant activity. Pharmacological effect of passion flower is caused, basically, by flavonoid and alkaloid activity. Flavonoids with anxiolytic activity have...
been described in many plant species used in folk medicine such as Passiflora coerulea\(^{3}\). This effect has been attributed to the affinity of flavonoids for the central benzodiazepine receptors. Furthermore a sedative effect on the central nervous system has been shown for quecrisin and isooquecrasin glycosides in mice. \(^{35-39}\) However, further studies are required to identify the phytoconstituent responsible for the observed anxiolytic effect of ethanol extractive of C. behen and chloroform and ethanol extractives of E. ganitrus to explain exact anxiolytic mechanism.

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