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Evaluation of Anti-anxiety activity of *Achillea millefolium* Linn.

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Abstract

Anxiety is one of the most common mental disorders, characterized by changes in mood, behavior, somatic function, and cognition. Benzodiazepines and SSRIs are most commonly employed drugs for the treatment of anxiety. Synthetic drugs available for treatment of anxiety have various adverse effects. Drugs obtained from natural sources are known to cause fewer side effects compared to synthetic drugs despite of same ability to cure disease. *Achillea millefolium* commonly known as 'yarrow",bhutkesi, puthkanda, gandrain in India is a plant belonging to family Astaraceae. Traditionally it has been used to treat skin diseases, gastrointestinal diseases, insomnia, and inflammation. The aim of present study was to investigate the anxiolytic activity of ethanolic extracts of *Achillea millefolium* in mice. Ethanolic extracts were administered orally at a dose of 30 and 60mg/kg bw. The results were analyzed by One Way Analysis ofVariance (ANOVA).The results showed the extracts of leaves showed significant anxiety relatively ethanolic extract. The findings concluded that *Achillea millefolium* exhibit anxiety and further studies are suggested to isolate the active principles responsible for the activity.

Keywords: Ethanolic extract, Anxiety, *Achillea millefolium*

Introduction

Achillea milefolium is native to temperate regions of the Northern Hemisphere in Asia, Europe, and North America It has been introduced as a feed for livestock in places like New Zealand and Australia. However, it is a weed in those places and sometimes also in its native regions. In New Mexico and southern Colorado, it is called plumajillo from its leaf shape and texture. In antiquity yarrow was known as herbal militaris, for its use in stanching the flow of blood from wounds. Other common names for this species include gordaldo, nosebleed plant, old man's pepper, devil's nettle, sanguinary, milfoil, soldier's woundwort, thousand-leaf, and thousand-seal.

Traditionally it has been used to treat skin diseases, gastrointestinal diseases, insomnia, and inflammation. The aim of present study was to investigate the anxiolytic activity of ethanolic extracts of *Achillea millefolium* in mice. Ethanolic extracts were administered orally at a dose of 30 and 60mg/kg bodyweight. The results were analyzed by One Way Analysis ofVariance (ANOVA).The results showed the extracts of leaves showed significant anxiety relatively ethanolicextract. The findings concluded that *Achillea millefolium* exhibit anxiety and further studies are suggested to isolate the active principles responsible for the activity.

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Material and Methods

Selection of plant

The plant was selected based on the traditional claims

Collection, identification and authentication of Plant material

Plant material (fruits) were collected from local market in Indore and authenticated by Dr. S. N. Dwivedi, Professor of Botany, Janata PG College, APS University, Rewa, (M.P.).

Preparation of extracts

Coarsely powdered plant was macerated in 90% ethanol. After it was filtrated. The ethanolic solution(filtrate) was evaporated to dryness in rotatory evaporator to get ethanolic extract. The ethanolic extract obtained was screened for phytochemical analysis.

Animal

Albino mice, weighing 20-30gm, were obtained from the animal house of the Department of Pharmacology of the Swami Vivekanand College of Pharmacy, Indore, India. Animals was housed at four per cage, allow them, free access to water and food, and was maintained under constant temperature (23±1^{0}C) and humidity (60±10%) under 12-h light/dark cycle. Animal treatment and maintenance was conducted accordance to the Principles of Laboratory Animal Care.

Anti-anxiety activity Experimental design

Elevated plus maze (EPM) test

This model is based on natural behavior of mice for open spaces and fear of height. Mice always tend to avoid the open areas and stay in darker areas, more enclosed spaces. When animal is placed on EPM anxious animals spend more time in enclosed arms and non-anxious animals explore and spend more time open arm.

The plus-maze consists of two open arms, 43 × 15 cm (L ×W), and two enclosed arms, 43 × 15×23cm(L×W×H), opened to the top, arranged in such way that the two open arms are faced opposite to each other. The maze is elevated to a height of 70 cm. The mice weighing 25-30gms body weight are randomly selected irrespective of sex and grouped into 4 groups so that each group consisting of 5mice. After one hour of oral administration of the test drug or the standard, the rat is placed at the centre of the maze, facing towards one of the enclosed arms. After 5min of observation the following parameters are noted: The number of entries into open arm and closed arms and time spent in the open and enclosed arms.

The albino mice of either sex divided into 4 groups of 5 animals each. Group I received 0.1ml normal saline (NaCl) orally for seven days as a control group. Group II received 5mg/kg of diazepam orally for seven days as a standard drug. Group III received 200 mg/kg methanol extract orally for seven days. Group IV received 400mg/kg methanol extract orally.

Group I: Control (Normal Saline, 0.1ml/kg)
Group II: Standard (Diazepam5mg/kg)
Group III: Test1 (Ethanolic extract of *Achillea millefolium Linn* 200mg/kg)
Group IV: Test2 (Ethanolic extract of *Achillea millefolium Linn400mg*/kg)

Statistical analysis

All the data represent mean±S.E.M. values. The data were analyzed by means of analysis of variance (ANOVA). Whenever ANOVA was significant, further multiple comparisons were made using Tukey's test as the post hoc test. The levels of statistical significance ranged from $p<0.05$ to $p<0.001$.

Results and Discussion

Ethanolic extract was subjected preliminary phytochemical screening; the results were presented in table 1.

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Table 1: Presence of various phytochemical constituents

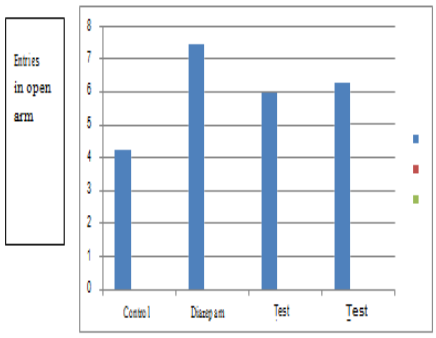
S.No.	Test	Positive/negative
1	Carbohydrate	+
2.	Terpenoids	+
3.	Flavone glycoside	+
4.	Phenolic compound	+
5.	Flavonoids	+
6.	Saponins	-

Present = +, Absent = -

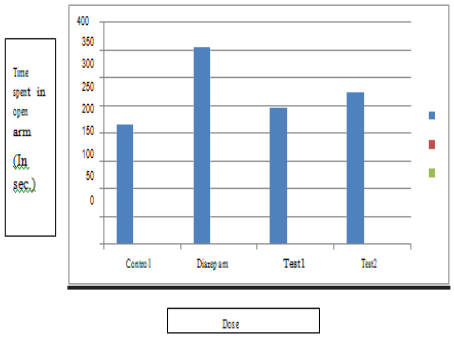
The ethanolic extract of *Achillea millefolium Linn*. were subjected to evaluation of anti-anxiety activity.

Drugs	Dose	No. of entries in open arm	Time spent in in open arms (In sec.)
Control	0.1mg/kg	4.25±0.45	215±8.2
Diazepam	5mg/kg	7.45+0.47	356±7.8
Test 200mg	200mg/kg	5.97±0.40	246±6.9
Test 400mg	400mg/kg	6.27±0.38	275±6.0

Table 2: Result of elevated plus maze



Graph 1: Number of entries of mice in open arm of elevated plus maze



Graph 2: Time spent by mice in open arm of elevated plus maze

Conclusion

The results obtained from these experimental models clearly confirmed that the anti-anxiety activity of ethanolic extracts of *Achillea millefolium*. The acute treatment with (200mg/kg) clearly demonstrates a dose dependant anti-anxiety effect comparable to diazepam (5mg/kg; i.p) in all experimental models of anxiety. The phytoconstituent like flavonoids (cymaroside 1 and cosmosin 2) were reported for their anti-anxiety activity and these constituents were present in *Achillea millefolium*. So this active principle might be responsible for anti- anxiety activity. The mechanism of anti-anxiety activity of *Achillea millefolium* extracts is unclear hence further studies are needed to identify the mechanism and the phyto-constituents responsible for the effects of the ethanolic extract of *Achillea millefolium.*

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