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COMPARATIVE EVALUATION OF ANXIOLYTIC EFFECTS OF VARIOUS EXTRACTS OF OATS (*AVENA SATIVA*), RICE BRAN (*ORYZA SATIVA*) AND SPINACH (*SPINACIA OLERACEA*) IN EXPERIMENTAL ANIMALS

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ABSTRACT: Aim of the present study was to compare antianxiety activity of various extracts of Oats (OA), Rice Bran (RB) and Spinach (SP) which are rich in phenolics mainly flavonoids and possesses excellent antioxidant property. Flavonoids are reported to have effect on central nervous system, mainly in anxiety disorders. The activity of various extracts (petroleum ether and hydroalcoholic) of all the three plants was evaluated at different doses (50, 100, 200 mg/kg of body weight) using EPM model of anxiety. The studies were conducted on swiss albino mice, and the test materials were administered per oral route. Results indicated that out of all the prepared extracts of the three selected plants, hydroalcoholic extract of Spinach (*Spinacia oleracea*) exhibited maximum and significant effect at 200 mg/kg of body weight, the results were comparable to the standard antianxiety drug diazepam (2 mg/kg p.o.).

INTRODUCTION: Anxiety is a feeling of apprehension, uncertainty or tension streaming from the anticipation of an imagined or unreal threat. Anxiety disorders cause immense suffering worldwide and hence search for antianxiety drugs has become important area of research. Benzodiazepenes, barbiturates and tricyclic antidepressents have been used for a long time to treat anxiety disorders. The benzodiazepines act via the benzodiazepine receptors which are present on the GABAA pentameric complex.

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The most used drug is diazepam[1]. However, the clinical uses of benzodiazepines are limited by their side effects such as psychomotor impairment, sedation, myorelaxation, ataxia, amnesia and dependence liability [2, 3].

There is a need of drugs which possesses greater efficacy, lesser undesirable effects with minimum or no tolerance and dependence. Herbs are widely accepted sources of medicine, which play an important role in health care programme worldwide [4].

The brain is highly vulnerable to oxidative stress. Oxidative stress can alter neurotransmission, neuronal function and overall brain activity. Oxidative stress has been implicated in neurodegenerative diseases and neuropsychiatric diseases including stress and anxiety [5]. Antioxidant

therapy has been reported to have a protective effect in anxiety disorders [6]. Polyphenols, flavonoids, specific foods and diets rich in antioxidants have been shown to improve antioxidant status and have anxiolytic effects [7, 8]. For the present investigation, three common dietary plants that contain phenolic compounds and have marked antioxidant activity have been selected.

In the present study, extracts of some common dietary plants, Oats (*Avena sativa*), Rice bran (*Oryza sativa*) and Spinach (*Spinacia oleracea*) have been evaluated for their anxiolytic potential. Oats (OA) belongs to family graminae and is a cereal grain grown for its seed. Chemically it contains proteins, fat, carbohydrates, β-glucan, niacin, iron, potassium [9]. It also contains various other phytoconstituents like avenanthramides, an indole alkaloid-gramine, flavonoids (flavone-C-glycosides such as O-methyl-apigenin-C-hexoside-O-deoxyhexoside, apigenin – C - hexoside-O-pentoside and luteolin-C-hexoside-O-pentoside), flavonolignans, ferulic acid, phenolics, triterpenoid saponins, sterols, and tocols [10, 11]. Traditionally it was used to treat exhaustion, insomnia and weakness of nerves [12]. Therapeutic potentials of oat gain include prevention of cardiovascular diseases [13], breast cancer [14], prevents asthma [15] and chronic constipation [16].

Rice Bran (RB), a by-product of rice (*Oryza sativa*) milling industry belongs to Graminae family. It is rich in micronutrients like tocopherol, oryzanol, phytosterols which comprises of Vitamin E due to which it posseses excellent antioxidant activity. It also contains oil, protein, carbohydrates dietry fibers like pectin, β-glucan and gum [17-20]. The most abundant phenolic acid found in rice bran is ferulic acid. Flavones are most commonly present among the flavonoids. Tricin appears to be the major flavonoid present in the bran [21, 22]. Traditionally it was used for digestion, toning muscles, increasing apetite, treating dysentery and as eye lotion [23]. It has shown to decrease risk of coronary heart disease, reduction of blood cholesterol [24-27], controls Type I and Type II Diabetes Mellitus [28].

Spinach (SP) is a green leafy vegetable that came originally from south-western Asia, belongs to family Chenopodiaceae. A powerful, water-soluble, natural antioxidant mixture (NAO), which specifically inhibits the lipoxygenase enzyme, has been isolated from spinach leaves. NAO is composed of the active compounds mainly flavonoids and coumaric acid derivatives. Various flavonoids reported to be present are quercetin, myricetin, kampferol, apigenin, luteolin, patuletin, spinacetin, jaceidin, glucuronides and acylated diand triglycosides of methylated and methylene dioxide derivatives of 6-oxygenated flavonols [29]. Traditionally it was used as antipyretic, diuretic, laxative, for sore eyes, vomiting, flatulence and urinary calculi 30. It is a good source of the bioflavonoid quercetin with many other flavonoids which exhibits anti-oxidant [31], antiproliferative [32], CNS depressant [33], hepatoprotective [34], anticancer [35] and anthelmintic activity [36].

So, the aim of the present study is to evaluate the antianxiety activity of petroleum ether (PE), and hydroalcoholic (HA) extracts of OA, RB and SP using EPM model of anxiety in experimental animals.

MATERIALS AND METHODS:

Collection and authentication of plant material:

Plants were procured from Punjab Agricultural University, Ludhiana, India in the month of January 2015. Identity of the plants was confirmed by Head, Raw Materials, Herbarium & Museum at National Institute of Science Communication and Information Resources (NISCAIR), New Delhi, India, vide letter numbers:

Avena sativa

(NISCAIR/RHMD/Consult/2014/2444/23-1) dated 13/05/2014,

Oryza sativa

(NISCAIR/RHMD/Consult/2014/2505/84) dated 2/9/2014,

Spinacea oleracea

(NISCAIR/RHMD/Consult/2014/2444/23-2) dated 13.05.2014

Drugs and chemicals:

Diazepam was obtained from GlaxoSmithKline Pvt. Ltd. and petroleum ether, methanol (LR grade) were procured from S.D. Fine-Chem Ltd, Mumbai.

Pharmacognostic evaluation:
Various pharmacognostic parameters of all the three plants were studied following standard procedures [37]. All parameters such as moisture content (LOD method), ash values, extractive values were determined in triplicate.

Preparation of extract:
The plants (Oats, Spinach) were dried in shade and powdered. Rice bran was used as such. The plant materials were separately subjected to successive exhaustive extraction with petroleum ether and methanol:water (70:30) in the Soxhlet apparatus. Petroleum ether (PE) and hydroalcoholic (HA) extracts were dried using rotary evaporator and yield calculated on dry weight basis. Dried extracts were preserved in vacuum desiccator for further use.

Phytochemical screening:
A phytochemical examination was carried out for both the extracts as per standard methods. [38]

Animals:
Swiss albino mice (weighing 25-30g) of either sex were procured from the animal house facility of the Chandigarh College of Pharmacy, Landran (Regd. No. 1201/a/08/CPCSEA). The animals were housed in standard polypropylene cage (two animal/cage) and maintained under controlled room temperature (22 ± 1ºC) with a 12:12h light and dark cycle. Water and food were available *ad libitum*. Groups of six mice were randomly assigned to different treatment groups. Control group received vehicle, one group was given standard drug Diazepam (2mg/kg p.o.) while test groups received PE and HA extract (50, 100 and 200 mg/kg p.o.).

Different treatments:
The following treatments were employed in the study:

Group 1- vehicle (1% CMC in distilled water)

Group 2- Standard drug (Diazepam 2mg/kg p.o. suspended in vehicle)

Group 3-21- Test groups (PE and HA extracts of each plant at three doses- 50,100,200 mg/kg p.o. suspended in vehicle)

The effects of the drugs were estimated 45 min after the administration of dose. In each experiment, apparatus was cleaned using 5% ethanol before introducing the next animal to preclude the possible cueing effects of odours left by previous subjects.

Animal Model for Anxiety:
Elevated Plus Maze: [39]
The plus maze apparatus consisting of two open arms (16 x 5 cm) and two closed arms (16 x 5 x 12 cm) having an open roof with a plus maze, elevated 25 cm from the floor was used to observe anxiolytic behaviour of animals. The animals were fasted 18 h prior to experiment. The dose administration schedule was so adjusted that each mice was having its turn on plus maze after 45 min of administration of dose. Each animal was placed in the centre of the elevated plus maze with its head facing the open arms.

During the 5 min experiment, behaviour of the mice was noted as a) preference of the animal for its first entry to open/closed arm b) number of entries into the open arm c) average time spent by the animal in open arm. The antianxiety activity was recorded as average time spent by the animals in the open arms of the EPM. During the entire experiment each animal was allowed to socialize. Every precaution was taken to ensure that no external stimuli disturbed the animal.

Statistical Analysis:
All the values were expressed as mean ± SEM. Statistically significant difference between the groups were calculated by the application of one way analysis of variance (ANOVA) followed by Tukey's, post-hoc test. $^{***}P<0.001$ and $^{**}P<0.01$ were considered statistically significant.

RESULTS:
Pharmacognostic evaluation:
The results of various pharmacognostic parameters assessed are shown in **Table 1**.

TABLE 1: PHARMACOGNOSTIC PARAMETERS

Pharmacognostic Parameters Mean±SEM (n=3)	OA *(Avena sativa)*	RB *(Oryza sativa)*	SP *(Spinacia oleracea)*	Limits (I.P. 2014)
1.Loss on Drying (%w/w)	0.4%	0.5%	0.25%	Not more than 0.5% w/w
2. Ash value (%w/w)				
a) Total ash	3.8	5	4	Not more than 5% w/w
b) Acid insoluble ash	0.2	0.8	0.5	Not more than 1% w/w
c) Water soluble ash	2.8	3.9	3	Not more than 4% w/w
3. Extractive value (% w/w)				
a) Water soluble extract	5	9.8	9.2	
b) Alcohol soluble extract	4	10.5	6.8	

Yield of extracts: The percentage yields of various extracts of *A. sativa*, *O. sativa* and *S. oleracea* are shown in **Table 2**.

TABLE 2: YIELD OF EXTRACTS

Extract	% Yield (w/w) on dry weight basis
Oats PE	2
Oats HA	5
Rice Bran PE	5
Rice Bran HA	10
Spinach PE	4
Spinach HA	9.5

PE: Petroleum ether
HA: Hydroalcoholic

Phytochemical Screening: The results of phytochemical screening of various extracts of *A. sativa*, *O. sativa* and *S. oleracea* are shown in **Table 3**.

TABLE 3: PHYTOCHEMICAL ANALYSIS OF EXTRACTS OF OATS, RICE BRAN AND SPINACH

Tests	Oats (PE)	Oats (HA)	Rice bran (PE)	Rice bran (HA)	Spinach (PE)	Spinach (HA)
Alkaloids						
a) Mayer's Test	+	+	+	+	+	+
b) Wagner's Test	+	+	+	+	+	+
c) Hager's Test	+	+	+	+	+	+
d) Dragendroff Test	+	+	+	+	+	+
Carbohydrates						
a) Molisch's Test	-	-	-	-	-	+
b) Benedict's Test	-	-	-	-	-	+
c) Fehling Test	-	-	-	-	-	+
Glycosides						
(Anthraquinone glycosides)						
a) Borntrager's Test	-	-	+	+	-	+
b) Modified Borntrager's test	-	-	+	+	-	+
(Cardiac glycosides)						
a) Killer Killiani's Test	-	-	+	+	-	+
b) Legal's Test	-	-	+	+	-	+
Saponins						
Froth test	+	+	+	+	-	+
Phytosterols						
a) Salkowski's Test	+	+	+	+	-	+
b) Libermann Burchard's Test	+	+	+	+	-	+
Phenols						

Ferric Chloride Test	-	-	+	-	-	+
Tannins	+	-	+	-	-	-
Gelatin Test						
Flavonoids						
a) Alkaline Reagent Test	-	+	-	+	-	+
b) Lead Acetate Test	-	+	-	+	-	+
c) Shinoda Test	-	+	-	+	-	+
Proteins and aminoacids						
a) Xanthoproteic Test	-	-	-	+	-	+
b) Ninhydrin Test						
c) Ninhydrin Test	-	-	-	+	-	+
Diterpenes						
Copper acetate Test	-	+	+	-	+	+

+ : Positive, - : Negative

Elevated Plus maze model: Diazepam (2 mg/kg) showed anxiolytic activity, by showing significant (***P < 0.001) increase in average time spent and frequency in open arms. Out of all, the group of mice treated with SP HA extract (200 mg/kg) have shown maximum anxiolytic activity, significant (***P < 0.001) increase in average time spent and mean number of enteries in the open arms followed by Rice Bran and Oats (**P<0.01) as compared to group administered with the standard drug diazepam as shown in **Fig. 1** and **2**.

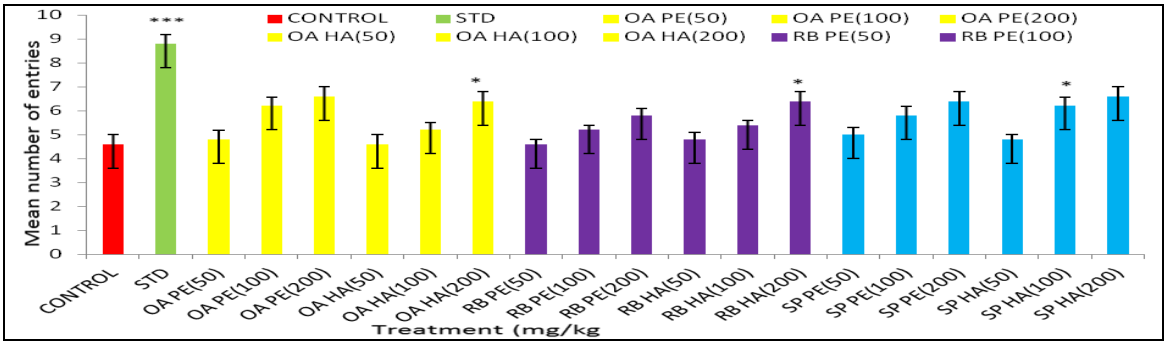

FIG. 1: EFFECTS OF VARIOUS TREATMENTS ON MEAN NUMBER OF ENTRIES BY THE EXPERIMENTAL ANIMALS IN OPEN ARM OF EPM

Control= 1% CMC, STD=Diazepam (2 mg/kg p.o.), OA PE= oat petroleum ether extract (50, 100 and 200 mg/kg p.o.), OA HA= Oats hydroalcoholic extract (50, 100 and 200 mg/kg p.o.), RB PE= Rice Bran petroleum ether extract (50, 100 and 200 mg/kg p.o.), RB HA= Rice Bran hydroalcoholic extract (50, 100 and 200 mg/kg p.o), SP PE=spinach petroleum ether extract (50, 100 and 200 mg/kg p.o.), SP HA= spinach hydroalcoholic extract (50, 100 and 200 mg/kg p.o). Results are expressed as mean±SEM (n=5); *** P<0.001 and ** P<0.01 as compared to control..

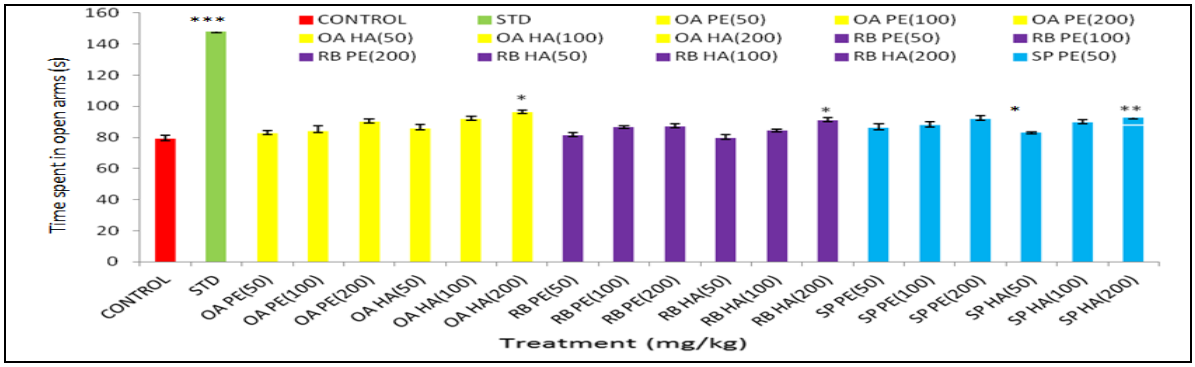

FIG. 2: EFFECTS OF VARIOUS TREATMENTS ON AVERAGE TIME SPENT BY THE EXPERIMENTAL ANIMALS IN OPEN ARM OF EPM.

Control= 1% CMC, STD=Diazepam (2 mg/kg p.o.), OA PE= oat petroleum ether extract (50, 100 and 200 mg/kg p.o.), OA HA= Oats hydroalcoholic extract (50, 100 and 200 mg/kg p.o.), RB PE= Rice Bran petroleum ether extract (50, 100 and 200 mg/kg p.o.), RB HA= Rice Bran hydroalcoholic extract (50, 100 and 200 mg/kg p.o), SP PE=spinach petroleum ether extract (50, 100 and 200 mg/kg p.o.), SP HA= spinach hydroalcoholic extract (50, 100 and 200 mg/kg p.o). Results are expressed as mean±SEM (n=5); *** P<0.001 and ** P<0.01 as compared to control

DISCUSSION: In the present study, EPM model of anxiety was used to evaluate the anxiolytic effects of petroleum ether and hydroalcoholic extracts of Oats (*Avena sativa*), Rice Bran (*Oryza sativa*), Spinach (*Spinacia oleracea*). This is a model which uses the natural fear of rodents to avoid open and elevated places. The ratio of open and closed area entries reflects a specific effect on anxiety, provided there is no concomitant change in the total number of entries (open + closed). As expected, diazepam produced significant increase in time spent and number of entries into the open arms. Among all the extracts, hydroalcoholic extract of Spinach exhibited maximum antianxiety activity at 200 mg/kg dose followed by Rice Bran and Oats, and the activity was statistically comparable to diazepam.

Some of the plants rich in phenolics, specially flavonoids and possessing antianxiety activity are *Pulsatilla nigricans* [40] (Goyal & Kumar 2010), *Tephrosia purpuria* [41] (Sathish et al. 2001), *Coriandrum sativum* [42] (Mahendra & Bisht 2011), *Gastrodia elata* [43] (Jung et al. 2006), *Citrus paradise* [44] (Gupta et al. 2010). Flavonoids have been attributed to have its effect on central nervous system. Flavonoids and Diazepam are structurally similar. The anxiolytic effects of hydroalcoholic extract of *Spinacia oleracea* may be related to their flavonoid content. This effect has been attributed to the affinity of flavonoids for the central benzodiazepine receptors [45, 8]. However further studies are needed to explore exact mechanism of action.

From our research study, it can be concluded that out of all the three plant extracts, hydroalcoholic extract of Spinach possesses significant anxiolytic activity at the dose of 200 mg/kg, highest among all the extracts. So it can be concluded that spinach can be a good alternative and can be included in the diet to help relieve anxiety.

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