INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

www.ijabpt.com Volume-3, Issue-4, Oct-Dec-2012 Coden : IJABPT Copyrights@2012

ISSN : 0976-4550

Received: 26th August-2012 *Revised: 28th August-2012* *Accepted: 30th August -2012*

Research article

EVALUATION OF ANXIOLYTIC EFFECT OF PET–ETHER EXTRACT OF PORTULACA OLERACEA (LINN.) IN MICE

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ABSTRACT: The usage of benzodiazepines, the major class of anxiolytic drugs is invariably accompanied by many side effects like sedation and myorelaxation leading to incoordination of movements. Intense research is going on all over the world to find out most effective and safer anxiolytic compounds. In the present study, portilaca oleracea has been investigated in detail for its anxiolytic in mice. The anxiolytic activity was screened by using two well validated methods namely elevated plus maze, stair case test. Portilaca oleracea displayed a dose-dependent anxiolytic effect similar to diazepam in the animal models of anxiety as revealed by a significant increase in the time spent in open arms of the elevated plus maze and significant reduction in the number of rearing responses in staircase test, the results of the present study have identified the novel anxiolytic compound.

Keywords: Portulaca oleracea (Linn.), petroleum-ether extract, Anxiolytic activity, Diazepam

INTRODUCTION:

Anxiety is an emotional state caused by the perception of real or perceived danger that threatens the security of an individual. Anxiety disorders are considered the most common mental illness present in 15-20% of medical clinic patients (Reus 2008). Drugs like benzodiazepines, buspirone and propranolol are often used as first line approach in the management of anxiety related disorders (O' Donnell and Shelton 2011). These medications have many undesirable side effects and a significant number of patients are resistant to these drugs (Fernandez et al., 2009). Hence there is a need for robust anxiolytic compounds that have lesser side effects.

Portulaca oleracea (P. oleracea) belonging to the family "Portulacaceae" is an herbaceous plant widely distributed throughout the world. It contains many biologically active compounds and is a source of many nutrients like free oxalic acids, alkaloids, omega-3 fatty acids, coumarins, flavonoids, cardiac glycosides, anthraquinone, protein, (Ezekwe MO, et al., 1999) a-linolenic acid and b-carotene (Liu LX, et al., 2000, Barbosa – Filho JM, et al., 2008) mono terpene glycoside (Sakai NK, et al., 1996) N-trans-feruloyltyramine (Mizutani M, et al., 1998). It was also found to contain vitamin C, oleoresins-I and II, saponins, tannins, saccharides, triterpenoids, 0a-tocopherol and glutathione (Chatterjee A, et al., 1956, Simopoulous AP, et al., 1992, Prashanth KL, et al., 2005). The high contents of a variety of phytoconstituents present in this plant were considered to be responsible for the biological

activities like antibacterial, antifungal (Oh KB, et al., 2002), anti-fertility (Verma OP, et al., 1982), muscle relaxant (Parry O, et al., 1993) and wound healing properties, (Rasheed AN, et al., 2003) analgesic and antiinflammatory activity (Jagan Rao N, et al., 2012). This plant which is normally used as a vegetable to prepare curry by the native people of Andhra Pradesh is used in combination with tomato. Earlier studies revealed the above pharmacological properties of Portulaca oleracea. However, no study was done on its anxiolytic activity of petroleum-ether extract of Portulaca oleracea. Therefore, the present study has been designed to investigate the petroleum-ether extract of Portulaca oleracea for its anxiolytic activity.

MATERIALS AND METHODS

The leaves of Portulaca oleracea were collected from a local vegetable market in Kanchipuram in the month of January 2011. The identification and authentication of the plant done at the department of Botany, Government Degree College, Kanchipuram.

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Animals

Adult male Swiss albino mice weighing 20 – 25g were procured from the institutional animal house. The animals had free access to standard pellet feed (Provomi) and water *ad libitum* under strict hygienic conditions, and maintained in room temperature of 25±1°C; relative humidity 45-55% and a 12:12 light/dark cycle. All the experiments were conducted in strict compliance according to ethical principles and guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and the study protocol was approved by the institutional animal ethical committee.

Preparation of extract

Portulaca leaves were shade dried and one kg of coarse powder was soaked in 4 litres of petroleum-ether for 3 days at room temperature. The extract was evaporated to dryness by using a rotary vacuum flash evaporator and the yield was10% w/w.

Phytochemical screening:

The petroleum ether extract of Portulaca oleracea leaves were subjected to qualitative chemical investigation for the identification of phyto constituents (Khandelwal KR, et al., 2000) like triterpenoids, saponins, alkaloids, carbohydrates, tannins, flavonoids and glycosides using appropriate reagents. The extracts were treated with dilute hydrochloric acid and filtered. The filtrate is used in the following tests.

Test for alkaloids (Mayer's test):

The extract was treated with Mayer's reagent and the appearance of cream color indicates the presence of alkaloid.

Test for tannins:

The extract was treated with 10% lead acetate solution; appearance of white precipitate indicates the presence of tannins.

Test for flavonoids (Shinoda test):

To the extract, add 5 ml 95% ethanol, few drops of conc. HCl and 0.5g magnesium turnings. Pink coloration indicates the presence of flavonoids.

Test for saponins (froth test):

1ml of the extract was diluted to 20 ml with distilled water and shaken well in a test tube. The formation of foam in the upper part of the test tube indicates the presence of saponins.

Test for terpenoids (Salkowski test):

Five ml of extract was mixed in 2 ml of chloroform, and concentrated H2S04 (3 ml) was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids.

Test for carbohydrates (Molisch's test):

The extract was treated with 3ml of alpha–napthol in alcohol and concentrated sulphuric acid was added along the sides of the test tube carefully. Formation of violet colour ring at the junction of two liquids indicates the presence of carbohydrates

Test for glycosides (modified Borntrager's test):

To 5 ml of extract add 5ml of 5% FeCl3 and 5ml dil. HCl. Heat for 5 min. in boiling water bath. Cool and add benzene or any organic solvent. Shake well. Separate the organic layer and add equal volume of dil.Ammonia. Ammonical layer shows pinkish red color.

Acute toxicity studies:

Acute oral toxicity studies were performed according to Organization for Economic Cooperation and Development (OECD 423) guidelines (Ecobichon DJ, et al., 1997). Male Swiss albino mice were used to determine the LD50 of petroleum-ether extract of Portulaca oleracea. Tween-80 1% v/v was used as vehicle to suspend the petroleum-ether extract.The petroleum-ether extract was administered in a dose of 2g/kg orally to a group of three rats. The animals were continuously observed for changes in autonomic or behavioral responses for 6hrs.The animals were kept under observation for 14 days to detect any mortality. The petroleum-ether extract were found to be non-toxic up to dose of 2g/kg body weight.

Anxiolytic Activity

Elevated plus maze

The elevated plus maze (EPM) consists of two open arms, 25 x 25 cm crossed with two closed arms of the same dimension having 25cm high walls. The arms are connected with a central square 5 x 5 cm giving the apparatus the shape of a plus sign.

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The maze is kept in a dimly lit room and elevated 25cm above the floor (Pellow & File 1986). Each mouse was placed on the central square facing an open arm and allowed to freely explore the apparatus for five minutes (Fernadez et al. 2009). The duration of time spent by the mouse in the open arm was carefully recorded using a digital stop watch. The percentage of time spent in open arms was calculated from the total duration of exposure (5 min). Different groups of mice (n=6) were treated with vehicle (0.2ml of 1% carboxy methyl cellulose), diazepam (2mg/kg. s.c) or Petroleum ether extract 100mg/kg and 200 mg/kg p.o 30 minutes prior to the experiment. The doses of Portulaca oleracea were selected based on a previous study on its antiinflammatory effect (B. Mallikarjuna Rao et al., 2012). The apparatus was carefully cleaned after every use to remove any residue or odor.

Staircase test

The staircase test was carried out by the method described by Simiand et al. (1984). The staircase was made of wood and consisted of five identical steps 2.5cm high, 10cm wide, 7.5cm deep surrounded by walls, the height of which (10cm) was constant along the whole length of the staircase. A wooden box (15 x 10 x 10 cm) with one side open was placed facing the staircase. The mouse was gently placed on the floor of the box with its back to the staircase. During a 3min period, the number of steps climbed and the number of rearings made were recorded. A step was considered climbed when all four paws were placed on the step. Different groups of mice were administered with vehicle, diazepam (2mg /kg, s.c.) or Petroleum ether extract 100mg/kg and 200 mg/kg p.o 30 min prior to the experiment. The number of steps climbed and the rearing responses were recorded for each mouse. The apparatus was cleaned thoroughly between the recordings.

STATISTICS

The results were subjected to analysis of variance (ANOVA) followed by Dunnett's't' test.
A p value of < 0.05 was considered statistically significant.

RESULTS

Phytochemical Screening:

The percentage yield of petroleum-ether extract of leaves Portulaca oleracea was found to be 10.6%w/w.The chemical tests indicate the presence of Phytoconstituents like the flavonoids, tannins, saponins, Terpenoids and Alkaloids in the petroleum-ether extract.

Acute toxicity studies:

There was no significant alteration in autonomic or behavioral responses in the mice treated with pet-ether extract of the leaves of Portulaca oleracea. No mortality was recorded in these animals up to 14 days

Elevated plus maze test

The vehicle treated control animals spent 15.45 ± 1.8% time in the open arm of the elevated plus maze. Treatment with diazepam significantly increased the percentage of time spent in open arm to 48.31± 3.55%. A dose dependent increase in the percent time spent in the open arm was observed in Petroleum ether extract treated mice. The increase was statistically significant in mice after 100 & 200 mg/kg of Portulaca oleracea treatment (36.38± 3.07% and 47.45± 4.3%) when compared with vehicle treated group. The response noted with 200 mg/kg of Portulaca oleracea was comparable to diazepam (2 mg/kg) treatment.

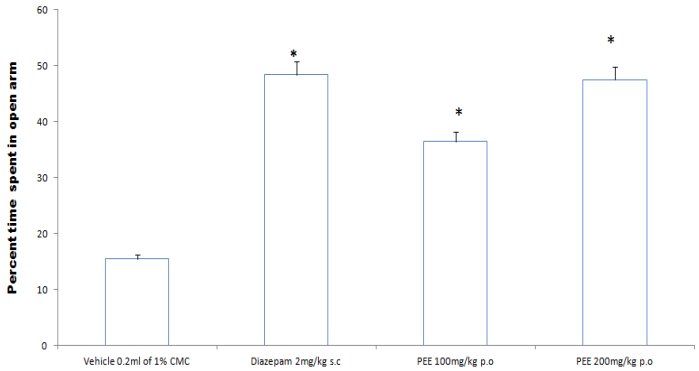

Figure – 1: Effect of petroleum-ether extract of Portulaca oleracea on Elevated plus maze in mice

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Staircase test

The number of steps climbed by vehicle treated mice was 26.4±3.4and the rearing responses were 15.2±1.3. Diazepam treatment significantly reduced the number of steps climbed (15.8±1.6) and rearing responses (6.4±0.9) compared to vehicle group. Portulaca oleracea treatment in different doses did not show any statistically significant change in the number of steps climbed by mice. However, the rearing responses were reduced by Portulaca oleracea in all the doses and statistically significant reduction was observed in doses of 100 & 200 mg/kg (11.8±1.1 and8.2±0.9) compared to vehicle treatment.

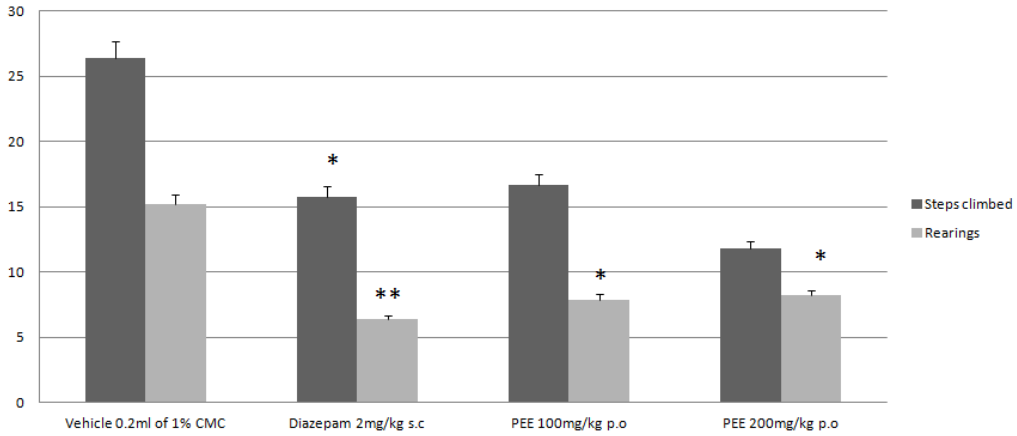

Figure – 2: Effect of petroleum-ether extract of Portulaca oleracea on the responses of mice in a staircase apparatus

DISCUSSION

Anxiety, like all emotions, has cognitive, neurobiological and behavioral components. It is a negative emotion that occurs in response to perceived threats that can come from internal or external sources and can be real or imagined (Moser DK, et al., 2007). The incidence of anxiety in the community is very high and associated with lot of morbidity (Ghoshal S, et al., 1972). Ethnomedical and pharmacological knowledge about the plant under study would allow us to evaluate central nervous system activity, which could be used to treat anxiety type of disorders. The present study has been undertaken to evaluate the anxiolytic effect of petroleum ether extract of Portulaca oleracea in mice.

The effect of Portulaca oleracea was tested on the widely used animal models of anxiety viz; elevated plus maze and staircase test. These tests are based on unconditioned behavior relying on natural behavioral reactions and do not require specific training of the animals (Bhattacharya and Satyan 1997). The elevated plus maze test has been regarded as an 'approach-avoidance' model because it reveals the conflicting tendencies of a rodent to naturally explore novel environment (approach) versus their innate aversion for potentially dangerous open spaces (avoidance) (Crayan and Holmes 2005). Naturally, rodents spend the majority of the test session in the closed arms of the maze. But anxiolytic drugs increase exploration to the open arms. Such an effect has been revealed for Portulaca oleracea in the present study. The percent time spent in open arm by Portulaca oleracea (200 mg/kg) treated mice was comparable to that of a standard anxiolytic drug diazepam. Similarly, the staircase test is considered a simple, rapid and sensitive test and clinically active anxiolytics reduced the rearings at doses which did not reduce the number of steps climbed (Bhattacharya and Satyan 1997). Portulaca oleracea treatment significantly reduced the number of rearings in mice compared to vehicle treatment without much change in the number of steps climbed. Diazepam treatment also resulted in a significant reduction in the number of rearing responses. The reduction in the number of steps climbed in diazepam treated mice could be attributed to its sedative and myorelaxant effects. The results of the above two experiments clearly indicate the anxiolytic property of petroleum ether extract of Portulaca oleracea.

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Phytochemical screening of the pet-ether extract of Portulaca oleracea revealed the presence of flavonoids, tannins, saponins, Terpenoids and Alkaloids. The phytochemical constituents are physiologically active compounds possessing great potential for therapeutic and prophylactic uses. It is possible that the mechanism of anxiolytic action of Portulaca oleracea could be mediated by synergistic action of these phytochemicals especially may be due to flavonoids. Many studies indicate flavones as ligands for the $GABA_A$ benzodiazepine binding site. Both naturally occurring and synthetic flavones have been shown to bind to this site with high affinity and to exert anxiolytic – like effects in rodents (Marder and Paladini 2002; Wang, et al., 2005). In particular, many hydroxy substituted flavones have been shown to exhibit high binding affinity to benzodiazepine receptors (Medina, et al., 1990 and Paladini, et al., 1999).

The results obtained in this study suggest that the petroleum ether extract of Portulaca oleracea possesses anxiolytic and muscle relaxant properties. Thus, Portulaca oleracea has potential clinical applications in the management of anxiety and muscle tension disorders. Further investigations are warranted for elucidating the exact mechanism and bioactive compounds.

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