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ANXIOLYTIC ACTIVITY OF AQUEOUS AND METHANOLIC EXTRACTS OF *IPOMOEA CARNEA* LEAVES

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KEYWORDS:

Ipomoea carnea, acute toxicity study, Anxiolytic activity.

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ABSTRACT

Ipomoea carnea Jacq (Convolvulaceae), locally known as Beshram and is used traditionally and having sedative hypnotic property. *Ipomoea carnea* appears to fall under the sedative-hypnotic category of central depressants activity, so the present work was undertaken to investigate the anxiolytic activity by using scientific models. The anxiolytic effects of the aqueous and methanolic extract of *Ipomoea carnea* leaves (32.50 and 16.25mg/kg i.p.) was evaluated in mice using elevated plus maze, open field test and hole board test models, diazepam was used as positive standard. The intra-peritoneal (i.p.) LD_{50} of the *Ipomoea carnea* leaf aqueous extract (ICLAE) and *Ipomoea carnea* methanolic extract (ICLME) in mice was found to be 325 mg/kg i.p. body weight. Administration of ICLAE and ICLME at doses 32.5mg/kg, 16.2mg/kg significantly increased time spent in open arms, number of entries in open arms and percentage entries in open arms of the elevated plus maze. Treatment of ICLAE and ICLME at doses 32.5mg/kg, 16.2mg/kg) significantly increased the number of crossed squares, decreased the immobility, and decreased the fecal pellets in open field test. ICLAE and ICLME at doses 32.5mg/kg, 16.2mg/kg) significantly increased the hole-poking response in hole board test. All results are compared to vehicle and diazepam as a standard drug for anxiolytic activity. ICLME showed greater anxiolytic effect as compared to ICLAE (doses of 32.5mg/kg and 16.2mg/kg) and diazepam.

1. INTRODUCTION :

The normal fear response to threatening stimuli comprises several components including defensive behaviors, autonomic reflexes, arousal, alertness, corticosteroid secretion and negative emotions. In anxiety states, these reactions occur in an anticipatory manner, independently of external events. The distinction between a 'pathological' and a 'normal' state of anxiety is not clear-cut but represents the point at which the symptoms interfere with normal productive activities. Despite (or perhaps because of) this loose distinction, anxiolytic drugs are among the most frequently prescribed substances, used regularly by upwards of 10% of the population in most developed countries [1]. Anxiety disorders in a modern society have relatively high prevalence affecting between 10 and 30% of the general population with considerable financial resources [2]. Anxiolytics are agents used to relive stress, tension and anxiety generated by the complex and hectic modern daily life. The ideal anxiolytic drug should calm the patient without causing too much daytime sedation and drowsiness and should not produce psychological or physical dependence [3]. Almost all pharmacological treatment used to diminish anxiety may produce side effects [4]. Benzodiazepines have well known benefits for anxiety but their side effects are prominent, including sedation, muscle relaxation, physical dependence and risk of cognitive dysfunction [5-7]. To overcome these side effects the move has been made towards herbal medicines which are well acquainted with safety, efficacy and reasonable.

Ipomoea carnea Jacq family Convolvulaceae is a common weed which is locally known as Beshram. Due to its high adaptability and resistance towards adverse climatic condition it may grow in all types of climate and soils, marsly as well dry [8]. The plant has allelopathic effect; boiled roots are used as laxative and it provokes menstruation. Other parts of the plant are used by traditional healers for treatment of leucoderma and other skin diseases. While screening its different parts for enzymatic activity, the latex exhibited a considerable amount of chitinase lysozyme activity [9]. Two kinds of toxic principle well isolated from the plant the nortropane alkaloids calystegines B_1, B_2, C_3 and mainly the indolizidine alkaloid Swainsonine [10-11]. Immunomodulatory activity [12], Antioxidant activity [13]. Drug appears to fall under the sedative-hypnotic category of central depressants with muscle relaxant property [14]. Hence, the rational of present study was to evaluate the anxiolytic activity of *Ipomoea carnea* jacq.

2. MATERIALS AND METHODS

2.1. Plant material

Fresh leaf samples of *Ipomoea carnea* (Convolvulaceae) were collected from North Pune district region of Maharashtra and authenticated by Mr. P.G.Diwakar, Deputy Director, Botanical survey of India (BSI), Pune. The voucher specimen of the plant was deposited at BSI Pune. The voucher no. Bhujbal-1**, (**vide letter No. BSI/WRC/Tech/2010/410 dated 30th Aug.2010).

2.2. Protocol for Successive Extraction

Fresh leaves of *Ipomoea carnea* were shade dried and powdered (100 gm) were defatted in soxhlet apparatus by using Petroleum ether (60-80ºC). The mark is dried and subjected to soxhlet extraction using methanol as solvent for 72 hrs. After completion of extraction the extract is filtered, marc is air dried and subjected for maceration with distilled water for 72 hrs. After completion of extraction, solvent was distilled off under reduced pressure and concentrated extract was air-dried [15]. The extract yields were as follows: Methanol (7.11g), Distilled water (7.91g).

2.3. Animals

Male Swiss mice (20-25 g) (n=6 per group) purchased from Lacsmi Biofarms animal centre, Pune. Animals were kept in colony cages at 25±2ºc, relative humidity 50-55% maintained under 12 h light and dark cycles. The animals were fed with standard animal feed and water ad libitium. Each animal was used only once. The experimental protocol was approved and conducted as per the guidelines of Institutional animal ethical committee CPCSEA Req. No: - (1197/c/08/CPCSEA).

2.4. Sample preparation

Fresh leaves of *Ipomoea carnea* were shade dried and powdered (100 gm) were defatted in soxhlet apparatus by using Petroleum ether (60-80ºC). The mark is dried and subjected to soxhlet extraction using methanol as solvent for 72 hrs. After completion of extraction the extract is filtered, marc is air dried and subjected for maceration with distilled water for 72 hrs. After completion of extraction, solvent was distilled off under reduced pressure and concentrated extract was air-dried [15].The extract yields were as follows: Methanol (7.11g), Distilled water (7.91g).

2.5. Drugs

Diazepam injection (1 mg/kg, 2 mg/kg) diluted upto the desired concentrations with the help of normal saline was administered intraperitoneally (i.p.) and it was purchased from local market. Different dose of *Ipomoea carnea* leaves of methanolic and aqueous extracts were made by serial dilution from a stock solution of 5 mg/ml of extract in saline. All solutions of extracts were prepared freshly and administered by i.p. route.

2.6. Acute toxicity study

The acute toxicity study was carried out as per the Lorke (1983) method. Twenty one mice were used in 7 divided groups of 3 each. In each step three animals were used, fasted overnight and maintained free access with water. Starting dose of test drug (alcoholic extracts, aqueous extracts) was selected to be 50, 100, 150, 200, 250, 300, 325, 350, mg/kg b.w. by i.p. respectively. After administration of the test compounds, animals were observed individually and continuously for 30 min, 2 h and 24 h to detect changes in the autonomic or behavioral responses and also for tremors, convulsion, salivation, diarrhoea, lethargy, sleep and coma and then monitored for any mortality for the following 14 days [16]. According to the results of acute toxicity test, doses were chosen for pharmacological evaluations.

2.7. Evaluation of anxiolytic activity

2.7.1. Elevated plus-maze test

The anxiolytic activity of *Ipomoea carnea* leaves methanolic extract (ICLME) and *Ipomoea carnea* leaves aqueous extract (ICLAE) was evaluated in elevated plus-maze method described earlier [17]. The maze consisted of two open (30cmx5cmx0.2cm) and two closed (30cmx5cmx15cm) arms, extending from central platform (5cmx5cm) and elevated to a height of 45cm above the floor. The entire maze was made of clear plexiglass. The animals were divided in to control, test groups and positive control containing six mice each group. The test groups received two different extracts i.e. ICLME and ICLAE having doses 32.50mg/kg, 16.25mg/kg respectively by i.p whereas the control group received vehicle (Normal saline) and standard group received diazepam in two different dose 1mg/kg b.w, 2mg/kg i.p. Half an hour after treatments, the animal was placed at the center of the plus maze. During the 5 minute test period, the number of open and closed arms entries, time spent in open and closed arms was recorded. Entry into an arm was defined as the point when the animal

places all four paws on to the arms. The procedure was conducted in a sound attenuated room; observations made from an adjacent corner. The area was carefully cleaned with 10% ethanol solution after every test.

2.7.2. Open field test

Apparatus consisted of a square area 76×76 cm with walls 42 cm high. The floor was divided by lines into 25 equal squares [18]. Half an hour after i.p. treatments, each mouse was placed in the center of the open field, which was novel to the animals and following behavioral aspects were noted, activity in center i.e. number of central crossed by the animal, fecal dropping and immobility in 5 minute test period [19]. The apparatus was carefully cleaned with 10% Ethanol solution after every test.

2.7.3. Hole board test

Anxiety levels were also evaluated in mice using hole board apparatus. The hole board consisted of clear plexiglass and the floor was constructed from black plexiglass and divided in 16 equal squares with 16 hole (diameter 3.5 cm) [20]. The mice were injected with drugs or vehicle and, thirty minutes later, each animal was placed in the center of the hole board, and allowed to freely explore the apparatus for five minutes and number of hole pocked were recorded for 5 min. [21]. An increase of the hole-poking response reveals a positive anxiolytic like [22]. After the test, the hole-board was carefully cleaned with 10 % ethanol solution.

2.8. Statistical analysis

Results are expressed as mean ± standard error of mean (S.E.M.). Statistical significance was determined using one ANOVA followed by Dunnets t test for multiple comparisons. Values with $P<0.05$, $P<0.01$ compared with control were considered as statistically significant in all cases.

3. RESULTS

3.1. *Acute toxicity studies*

The intra-peritoneal LD_{50} of the ICLAE and ICLME in mice was found to be 325mg/kg body weight i.p.

3.2. Evaluation of Anxiolytic Activity

3.2.1. Elevated plus-maze test

The behavioral effects of ICLAE and ICLME on mice behavior in the elevated plus-maze are summarized in Table 1. Administration of ICLAE and ICLME (32.50mg/kg, 16.2mg/kg) significantly($P<0.01$) increased the amount of time spent in open arms of the elevated plus maze and number of entries in open arms, compared to vehicle administration and also compared to diazepam as a standard drug for anxiolytic activity. Additionally compared to vehicle, the administration of ICLAE and ICLME (32.50mg/kg, 16.25mg/kg) promotes significantly ($P<0.01$) greater percentage of entries into the open arms, suggesting an anxiolytic effect. ICLAE and ICLME (32.5mg/kg, 16.25mg/kg), showed greater anxiolytic effect which was dose dependent as compared to diazepam (1mg/kg, 2mg/kg). Moreover, it is known that anxiolytic agent increases the frequency of entries and the time spent in open arms of the elevated plus maze.

3.2.2. Open field test

The results for the open field test are shown in Table 2. Treatment of ICLAE and ICLME (32.50mg/kg, 16.25mg/kg) significantly ($P<0.01$) increased the number of crossed squares compared to vehicle administration and also compared to diazepam as a standard drug for anxiolytic activity. Additionally compared to vehicle and diazepam the administration of ICLAE and ICLME (32.5mg/kg, 16.2mg/kg) promotes significantly ($P<0.01$) decreased the immobility, and decreased the fecal pellets suggesting an anxiolytic effect. ICLME (32.5mg/kg, 16.2mg/kg), showed greater anxiolytic effect which was dose dependent as compared to ICAE (doses of 32.5mg/kg and 16.2mg/kg).

3.2.3. Hole board test

The effects of ICLAE and ICLME on mice behavior in the hole board test are summarized in Table 3. Administration of ICLAE and ICLME (doses of 32.5mg/kg, doses of 16.2mg/kg) significantly increased the hole-poking response ($P<0.01$) compared to vehicle administration and also compared to diazepam as a standard drug for anxiolytic activity, suggesting an anxiolytic effect. ICLME (32.5mg/kg and 16.2mg/kg), showed greater anxiolytic effect which was dose dependent as compared to ICLAE (doses of 32.5mg/kg and 16.2mg/kg). Moreover, it is known that anxiolytic agent increases the hole-poking response in hole board test.

4. DISCUSSION

The elevated plus maze is currently one of the most popular in vivo animal tests currently in use. The test was further validated as an animal model of anxiety on pharmacological, physiological and behavioral grounds, [23] and has been validated for use with both rats and mice [24]. Therefore, we choose this test to investigate the anxiolytic potential of the ICLAE and ICLME. The indices of anxiety in this test, percent of open arm sensitive to agents thought to act via the $GABA_A$ receptor complex, justifying the use of diazepam as a positive control in this study [25].

In agreement with previously published reports, diazepam increased the number of open arm entries and the time spent in the open arms [26] confirming its anxiolytic effects. The ICLAE and ICLME showed a dose dependent significant increase in the time spent in open arms and percent entries in open arms of elevated plus maze at doses 32.5 and 16.2 mg/kg when compared with control and standard (Diazepam 1mg/kg, 2mg/kg).These observations clearly indicate that ICLAE and ICLME exerts an anxiolytic activity. In open field test, when animals are exposed to a novel environment, it was associated with ‘emotionality’. An anxious animal is one which shows reduced ambulation associated with periodic freeze and reduced normal behaviours like rearing associated with increased defecation and urination. Diazepam increased the number of square crossed, latency time and decreased the fecal pellets [27]. The ICLAE and ICLME had promisable effects on these parameters.

The effect of the ICLAE and ICLME showed a dose dependent significant increased the number of square crossed, latency time and decreased the fecal pellets in open field test at doses 32.5 and 16.2 mg/kg when compared with control and diazepam 1mg/kg, 2mg/kg as a standard. These data indicate that ICLAE and ICLME exerts an anxiolytic activity.

The hole- board test provides a simple method for measuring the response of an animal to an unfamiliar environment and is widely used to assess emotionality, anxiety and / or responses to stress [28-29]. Several behaviors can be readily observes and quantified in the test, which makes a comprehensive description of the animals behavior possible, It has been established that head-dipping behavior in mice and rats reflects exploration distinct from general locomotor activity [30].

Head dipping behavior was sensitive to changes in the emotional state of the animal and suggested that the expression of an anxiolytic state of the animal, and suggested that the expression of an anxiolytic state in animals might be reflected by an increase in head- dipping behavior [29]. Based on previous report diazepam increased the number of head-dipping behavior. [31]. The ICLAE and ICLME results are consistent on those parameters.

The effect of the ICLAE and ICLME showed a dose dependent significant increased the number of head dipping behavior in hole board test at doses 32.5 and 16.2 mg/kg when compared with control and diazepam 1mg/kg, 2mg/kg as a standard. These observations indicate that ICLAE and ICLME showed an anxiolytic activity.

CONCLUSION

Using behavioral pharmacological models ICLME (doses of 32.5mg/kg and 16.2mg/kg), showed greater anxiolytic effect which was dose dependent as compared to ICLAE (doses of 32.5mg/kg and 16.2mg/kg). However, further studies are required to understand the underlying mechanism of anxiolytic activity and to isolate the active phytoconstituents(s) responsible for anxiolytic activity.

REFERENCES

1. Rang H.P.,.Dale M.M., Ritter J.M., Moore P.K.(2003); Pharmacology. 5th edition, pp. 517-524.
2. Kumar R., Murugananthan G., Nandakumar K., Talwar S. (2011); Isolation of anxiolytic principle from ethanolic root extract of *Cardiospermum halicacabum*. Phytomedicine18: 219-223.
3. Barar FSK (1985), Essential of Pharmacotherapeutics. S Chand Publication, 4th edition, pp. 83, 89.
4. Molina-Hernandez M., Tellez-Alcantara N.P., Perez Garcia J., Olivera Lopez J.I., Teresa Jaramillo M., (2004); Anxiolytic actions of leaves of *Casimiroa edulis* (Rutaceae) in male Wistar rats. Journal of Ethnopharmacology93: 93-98.
5. Masoumeh E., Mohammad K., Maryam F.A., (2005); *Coriandrum sativum*: evaluation of its anxiolytic effect in the elevated plus- maze. Journal of Ethnopharmacology 96: 365-370.
6. Chandana C.B., Jayanti D.R., Bhaben B., Achinta G.B., Prabodh B., Mangala L., (2009); Anxiolytic effect of hydroehanolic extract of *Drymaria codata* L Willld. Indian Journal of Experimental Biology 47: 969-973.
7. Girdhari L.G., Avtar chand R., (2007); Protective effect of *Withania Somnifera* Dunal root extract against protracted social isolation induced behavior in rats. Indian J Physiol Pharmacology 51(4): 345-353.
8. Nandakumar P., (2009); Lignin from soda liquor and soda anthraquinone liquor of *Ipomoea carnea* Jacq. Aust. J. Basic App. Sci 3: 474-476.
9. Patel A.K., Sing V.K., Yadav R.P., Moir A.J.G., Jagannadhan M.V., (2009); A glycosylated chitinase from the latex of *Ipomoea carnea*. Phytochemistry 70: 1210-1216.
10. Balogh D.,Dimande K.I.M., van der Lugt A.P., Molyneux J.J., Naude R.J., Welman T.W.,(1999); Lysosomal storage disease induced by *Ipomoea carnea* in goats in Mozambique. Journal of veterinary diagnostic investigation 11: 266-273.
11. Haraguchi M., Gorniak S.L., Ikeda K., Minami Y., Kato A., Watson A.A., Nash R.J., Molyneux R.J., Asano N., (2003); Alkaloidal components in the poisonous plants, *Ipomoea carnea* (Convolvulaceae), J. Agric. Food Chem. 51: 4995-5000.

12. Hueza I.M., Jose L.G., Mitsue H., Naoki A., Gorniak S.L., (2005); The role of alkaloids in *Ipomoea carnea* toxicosis: A study in rats. Experimental toxicologic pathology57: 53-58.
13. Elija K., Adsul V.B., Rasika R., Deshpande N.R. Kashalkar R.V., (2011); Antioxidant activity of *Ipomoea carnea* stem. Journal of Pharmacy research 4 (1): 161-163.
14. Bhattachary S.K., Ray A., Dasgupta B., (1975); CNS depressant activity of *Ipomoea carnea* jacq. Ind. J Pharmac 7 (4): 31-34.
15. Mukharjee PK, (2002); Quality Control of Herbal Drug, first ed, Business Horizon Publication, New Delhi: pp.132-142, 168-172.
16. Lorke D., (1983); Approach to acute-toxicity testing. Arch. Toxicol. 54 (4): 275-287.
17. Lister R.G., (1987); The use of a plus maze to measure anxiety in the mouse. Psychopharmacology 92:180-185.
18. Farhat B., Asad H.S., Syed D.A., Zafar S.S., Darakhshan J.H., (2009); Possible anxiolytic profile of aqueous fruit extracts of a medicinal plant Sea Buckthorn (*Hippophae rhamnoides* L. Spp. Turkestanica) in experimental models. Pak. J. Bot.41 (6): 2791-2800.
19. Bhattamisra S.K., Sing P.N., Sing S.K., Kumar V., (2007) Anxiolytic activity of *Marsilea minuta* Linn in rodents. Journal of Herbal Medicine and Toxicology 1(1): 15-20.
20. Mora S., Diaz-veliz S., Lungenstrass H., Garcia-Gnzalez M., Coto-Morales T., Poletti C., De Lima TCM., Herrera Ruiz M., Tortoriello J., (2005); Central nervous system activity of the hydroalcoholic extract of *Casimiroa edulis* in rats mice. Journal of Ethnopharmacology 97: 191-197.
21. Xiuyan Wei, Jingyu Y., ChunF.W., (2006); Anxiolytic effect of Baicalin in mice. Asian Journal of Traditional Medicines 1: 3-4.
22. File S.E., Pellow S., (1985); The effects of triazolobenzodiazepines in two animals tests of anxiety & in the hole board. British Journal of Pharmacology 86: 729-735.
23. Fabiana C.V., Roseli S., Alexandre G.P.,(2009); Anxiolytic- like effect of *Sonchus oleraceus* L. in mice.Journal of Ethnopharmacology 124: 325-327.
24. Masoumeh E., Mohammad K., Maryam F.A., *Coriandrum sativum:* evaluation of its anxiolytic effect in the elevated plus-maze. Journal of Ethnopharmacology 96: 365-370.

25. Moser P.C., (1987); An evaluation of elevated plus –maze using the novel anxiolytic buspirone. Psychopharmacology 99: 48-53.
26. Talwar S., Kumar R., Murugananthan G., Nandakumar K., (2011); Isolation of anxiolytic principle from ethanolic root extract of *Cardiospermum halicacabum*. Phytomedicine 18: 219-223.
27. Hooi hoon A., Hung S.C., (1999); Studies on the anxiolytic activity of *Eurycoma longifolia* Jack roots in mice. Jpn. J. Pharmacology 79: 497-500.
28. Rodriguez E.L., Broitman S.T., Foscolo M.R., (1987); Effect of chronic ingestion of chlorimipramine and desipramine on the hole board response to acute stresses in male rats. Pharmacology Biochem Behav, 26: 207-210.
29. Takeda H., Tsuji M., Matsumiya T., (1998); Changes in head dipping behavior in the hole board test reflects the anxiogenic and / or anxiolytic state in mice. Eur J. Pharmacology 350: 21-29.
30. File S.E., (2001); Factors controlling measures of anxiety and responses to novelty in the mouse. Behav Brain Res 125: 151-157.
31. Ji Wook J., Nam Y.A., Hye R.O., Bo KL., Kang J.L., Sun Y.K., Jae H.C., Jong H.R.,(2006); Anxiolytic effects of the aqueous extract of *Uncaria rhynchophylla.* Journal of Ethnopharmacology 108: 193-197.