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Study of Passiflora incarnata Alone and with Asparagus Racemosus for Anxiolytic Activity in Mice

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Abstract
In this study, we have evaluated anxiolytic activity of treatment of Passiflora incarnata alone and with Asparagus racemosus in mice, to find whether the treatment produce synergistic anxiolytic activity or not. Anxiolytic activity was evaluated using marble bury test, elevated plus maze and effect of drugs on GABA levels in mice brain using UV-Vis spectrophotometer. Drugs were administered orally as per mg/kg body weight, one time a day for 10 days. Methanolic extract of Passiflora incarnata leaf-stem (MEPILS) and Methanolic extract of Asparagus racemosus roots (MEARR) was used as test drugs. Lorazepam 0.05 mg/kg was used as standard drug. In marble bury test and elevated plus maze, treatment of MEPILS with MEARR showed significant synergistic anxiolytic activity in mice. Increase in GABA levels in mice brain after the treatment of MEPILS with MEARR was observed. This study suggests that treatment of MEPILS with MEARR produces significant synergistic anxiolytic activity in mice.

Keywords: Anxiolytic, Neurotransmitters, sedative-hypnotic, Benzodiazepines, Passiflora incarnata, Asparagus racemosus

Introduction
Anxiety disorders are recognized as one of the most common type of psychiatric disorders in the world. The worldwide prevalence of anxiety disorders is about 7.3% (4.8%-10.9%). Prevalence of anxiety disorders in females is about 1.5 to 2 times more than the males [1].
Specific phobia, generalized anxiety disorder (GAD), social anxiety disorder (SAD), post-traumatic stress disorder (PTSD), panic disorder (PD) and obsessive-compulsive disorder (OCD) are the most common types of anxiety disorders [1-5].
According to recent study, an estimated 284 million people have experienced anxiety disorders in the year 2017 [6]. Anxiety disorders can affect person's daily works. Furthermore, anxiety disorders may also responsible for cardiovascular and immune problems [7].
Causes for anxiety disorders includes stress factors, biological and genetics factors. Current data suggests that disturbance in the GABAergic, glutamatergic, serotonergic and noradrenergic transmission could be responsible for anxiety disorders [7, 8].
Increased oxidative stress can cause cellular damage, also cause damage to the membrane proteins which results into inactivation of receptors, ion channels and enzymes. This causes disturbance in the neurotransmission, neuronal work and brain functions [9]. Evidence suggests that inflammation and inflammatory cytokines may affect brain functions related to threat sensitivity and reward functions. This could result into some behavioural changes. The parts of brain which have been observed to be get affected by inflammation includes basal ganglia, cortical reward, fear and anxiety related parts including amygdala, insula and anterior cingulate cortex [10].
Passiflora incarnata (Family: Passifloraceae) has been used in the Unani, Ayurveda and Siddha medicinal system as a medicine [11]. It has been known to have anxiolytic, anticonvulsant, sedative-hypnotic, antitussive, aphrodisiac, antiasthmatic activity [12]. Passiflora incarnata contains alkaloids (harmol, harman, harmine, harmaline and harmalol), flavonoids (Chrysin, benzoflavone, apigenin, orientin, isoorientin, vitexin, and isovitexin) and other constituents like carbohydrates and essential oils. GABA is also found in the extract of Passiflora incarnata [11, 13-15]. Methanolic leaf extract of Passiflora incarnata has shown sedative, anticonvulsant and CNS depressant activities in mice at a dose of 200 mg/kg (intraperitoneally). The extract also showed analgesic activity in mice and anti-inflammatory activity in rats [16]. Ethanolic

Ethanolic extract of Passion flower (*Passiflora incarnata* L.) have shown anxiolytic activity at a dose of 375 mg/kg (orally) in mice using elevated plus maze model [17].

Asparagus racemosus (Family: Asparagaceae) also called as 'shatavari' has been used in the Ayurveda and different medicinal systems from years. In Ayurveda it has been used as an Ayurvedic rejuvenative tonic for females. In Ayurveda it has been medicinally used in treatment of madhur rasam, madhur vipakam, seet-veeryam, som rogam, chronic fever and internal heat [18-20].

Its roots have been used in the Ayurveda for treatment of various disorders and diseases. Asparagus racemosus is used to treat nervous disorders, mental problems, dyspepsia, inflammation, tumors, neuropathy and hepatopathy. The plant has antioxidant, antidiabetic, antidiarrhoeal, immunomodulatory, antispasmodic, demulcent, digestive, aphrodisiac, diuretic, galactogogue properties. It has been used to treat infirtility in females [21, 19, 22]. Its major chemical constituents includes steroidal saponins (Shatavarin I to VI), polycyclic alkaloids (Asparagamine A), Isoflavones-8-methoxy-5, 6, 4-trihydroxy isoflavone-7-0-beta-D-glucopyranoside [18-20], flavonoids (Kaempferol, quercetin, rutin), shatavaroside, secoisolariciresinol, immunosides, beta sitosterol, sitigmaterol, daidzein, genistein, racemoside A-C and other compounds like essential oils, resins, tannins, tyrosine, arginine, asparagine are found [19, 23, 24]. In one invitro study, methanolic extract of Asparagus racemosus roots have shown antioxidant activity in DPPH free radical scavenging activity, hydroxyl radical scavenging activity, superoxide radical scavenging activity, hydrogen peroxide radical scavenging activity and also found to have a high antioxidant capacity by phosphomolybdenum method [25]. In another invitro antioxidant study, ethanolic extract of Asparagus racemosus (EEAR) roots have shown antioxidant activity in DPPH radical scavenging assay, hydroxyl radical scavenging assay, nitric oxide scavenging assay and total reducing ability [26]. Ethanolic extract of leaf of Asparagus racemosus wild has shown acute anti-inflammatory activity against carrageenan-induced paw oedema in rats [27].

Current treatment for anxiety disorders includes use of benzodiazepines, selective serotonin reuptake inhibitors (SSRI's), serotonin-norepinephrine reuptake inhibitors (SNRI's), azaspirones, monoamine oxidase inhibitors and tricyclic antidepressants [5, 28]. Herbal drugs can be used effectively for the treatment of anxiety disorders as they are easily available and generally have less or no side effects if used properly.

Oxidation and inflammation has found to affect anxiety levels, use of an antioxidant and anti-inflammatory agent could be effective in the treatment of anxiety disorders when used with anxiolytic agent. As Asparagus racemosus have antioxidant and anti-inflammatory activities, it could increase anxiolytic activity when used with anxiolytic agent Passiflora incarnata. In this study, we have find whether the oral treatment of methanolic extract of Passiflora incarnata leaf and stem (MEPILS) with methanolic extract of Asparagus racemosus roots (MEARR) produces synergistic anxiolytic activity in mice or not. For evaluation of anxiolytic activity of drugs, marble bury test, elevated plus maze and effect of drugs on GABA levels in mice brain using UV-Vis Spectroscopy was used. Lorazepam 0.05 mg/kg was used as standard drug.

Materials and methods

Collection of plants and Authentication

Plant of Passiflora incarnata and asparagus racemosus was collected from local areas in Purandar, dist. Pune, Maharashtra. Identification of Passiflora incarnata L. and Asparagus racemosus wild was done by the Botanical Survey of India, Pune.

Experimental Animals

Swiss Albino Mice (Male) weighing 20-30 gm, age 4-5 weeks was obtained from animal house of PDEA's Seth Govind Raghunath Sable College of Pharmacy, Saswad, Pune, Maharashtra. Mice were housed in the animal house at PDEA's Seth Govind Raghunath Sable College of Pharmacy, Saswad, Pune, Maharashtra. Standard pellet feed and water ad libitum with 12 hour light-dark cycle, temperature 24 ℃±2 and proper ventilation was provided to mice. Experimental protocol no. SGRS/IAEC/01/2020-21 was approved by the Institutional Animal Ethics Committee (IAEC).

Extraction of Plant materials

A) Extraction of Passiflora incarnata

Leaves and stem of Passiflora incarnata was isolated, washed with water and dried in shade. After drying, equal quantity of leaves and stem was powdered with the help of morter pastel. Weight of power obtained was 135 gm. The powder was moistened with methanol for 24 hours, then placed into conical percolator for percolation. Methanol was used as menstruum for extraction by percolation method. After four days, drain valve was opened. methanolic extract was filtered by passing through whatman filter paper. Filtered extract was collected in a glass beacker then placed in petri plates. The methanol was allowed to evaporate in the air flow at room temperature to obtain dry extract. The weight of the dried extract was found to be 6.1 gm. % yield was found to be 4.51% w/w.

B) Extraction of Asparagus racemosus

Roots of asparagus racemosus was isolated, washed with water and dried in shade. After drying, roots was grounded into fine powder with the help of morter pastle. The weight of powder was found to be 141 gm. The powder was moistened with methanol for 24 hours, Then placed into conical percolator for percolation. Methanol was used as menstruum for extraction by percolation method. After four days, drain valve was opened. methanolic extract was filtered by passing through whatman filter paper. Filtered extract was collected in a glass beacker then placed in petri plates. The methanol was allowed to evaporate in the air flow at room temperature to obtain dry extract. The weight of the dried extract was found to be 6.7 gm. The % yield was found to be 4.75 % w/w.

Phytochemical tests of plant extract

The Methanolic Extract of Passiflora incarnata leaf and stem (MEPILS) and Methanolic extract of Asparagus racemosus was tested for followin phytochemical tests.

1) Test for alkaloids

Hager's test

Plant extract treated with Hager's reagent (concentrated picric acid). Formation of yellow colour precipitation indicates presence of alkaloids [29].

2) Test for flavonoids

Alkaline reagent test

Plant extract + 2 ml of 2% sodium hydroxide, formation of intense yellow colour, which becomes colourless on addition of few drops (2-3) drops of dilute acid. This indicates

presence of flavonoids [30].

3) Test for steroids
Salkowski test
Plant extract is treated with chloroform and few drops of conc. Sulfuric acid added. Formation of red colour layer shows presence of steroids [29].

4) Test for glycosides
a) Liebermann's test
Plant extract treated with 2 ml chloroform and 2 ml acetic acid. Cool the mixture, add few drops of sulphuric acid. Formation greenish colour indicates presence of glycosides [30].

b) Bromine water test
Plant extract treated with bromine water, formation of yellow colour indicates presence of glycosides [31].

5) Test for saponins
0.5 g of plant extract is shaken with 2 ml distilled water. If the foam remains for 10 minutes, it shows presence of saponins [32].

6) Test for mucilage
50 mg dried mucilage powder is added in 2 ml distilled water, add few drops of ruthenium red solution. Formation of pink colour indicates presence of gums and mucilage [33].

Drugs and chemicals
Lorazepam 0.05 mg/kg was used as a standard drug. Methanol, distilled water, dextrose, ethanol, copper sulfate, P-Amino Butyric Acid (GABA), Ninhydrin reagent, n-butyl alchol, glacial acetic acid, chloroform was obtained from the laberatories of PDEA's Seth Govind Raghunath Sable College of Pharmacy, Saswad, Pune, Maharashtra.

Equipements and apparatus
Equipments used: Elevated Plus Maze, marble bury test assembly (Glass marbles, cages and bedding material), cooling centrifuge, homogenizer, UV-Vis spectrophotometer, conical percolator, hot air oven, analytical weighing balance, sonicator.
Apparatus: Test tubes, micropipette, beacker, petri plates, funnel, Flasks.
Other requirements: Whatman filter paper, mice oral garvage (cannula)

Administration of drugs
5% dextrose in distilled water was used as veichle for dose administration. Drugs were administred orally as per mg/kg body weight, one time a day for 10 days. At day 10 th, after 1 hour of dose administration, mice were evaulated for anxiolytic activity.

Experimental Protocol
Male swiss albino mice weighing 20-30 gm was divided in 5 groups as follows. Each group contain six mice (n=6)

- **Group 1:** Control: 5% Dextrose in distilled water P.O.
- **Group 2:** Standard: Lorazepam 0.05 mg/kg P.O.
- **Group 3:** Methanolic extract of Passiflora incarnata leaves and stem (MEPILS) 300 mg/kg P.O.
- **Group 4:** Methanolic extract of Passiflora incarnata leaves and stem (MEPILS) 300 mg/kg + Methanolic extract of Asparagus racemosus roots (MEARR) 100 mg/kg P.O.
- **Group 5:** Methanolic extract of Passiflora incarnata leaves and stem (MEPILS) 450 mg/kg + Methanolic extract of Asparagus racemosus roots (MEARR) 150 mg/kg P.O.

Models used to study anxiolytic activity in mice are as follows
Marble Bury Test (MBT)
Marble bury test is an important model for evaulation of anxiolytic drugs. Fear of strange or novel objects (Neophobia) is observed in rodents to any noxious and harmless objects. The marbles works like strange objects to rodents. Rodents may feel noxious or feels fear when they see marbles around them. This triggers digging and burying activities in rodents. If test drug generates anxiolytic activity, decrease in the number of marbles buried by the rodent is observed.

Procedure
Standard cages was filled with husk about 5 cm deep. Bedding material was lightly tamped town to creat even and flat surface. In each cage, 10 glass marbles was placed on the bedding material by grid pattern, maintaining approxmately 4 cm distance between the marble. Mice was treated orally with test and standard drugs. After 1 hour of oral drug administration, mice was places in respected cages. Mice was allowed to explore the cages for 30 minutes. During the experimental period, experimental room was kept silent and undisturbed environment was maintained. After 30 minutes, mice was removed carefully from the cages and placed in their original cages.

Evaluation
Numbers of marbles buried by the mice was measured. Marble was considered buried if 2/3 area of the marble is covered with the bedding material [34-37].

Fig 1: Marble bury test

Elevated Plus Maze (EPM)
Elevated Plus Maze (EPM) is important model to study anxiety related behaviour in rodents. EPM apparatus consist two oppositely faced open arm and two close arms forming + shape. The Maze is elevated at height of 50 cm above the floor. The dimension of open and close arms is 50x10x40 cm with open roof arrangement.

Procedure
Mice were placed in the testing room for about 1 hour before testing to reduce effects of stress on their behaviour during the period of testing. Test drugs were given to mice orally as per mg/kg body weight. 1 hour after drug treatment, mice was placed on the central square part of maze facing towards open arm. Mice were allowed to move freely on maze. Behaviour of mice was recorded for 5 minutes by plus maze controller and video camera mounted over the maze. After completion of 5 minutes mice was removed carefully and placed to their original cages.

Evaluation
Number of entries in open arm and % time spent in open arm was evaluated [38-41].
Percentage of time spent on the open arm was calculated as % of the total time the mice spent on the maze. Open arm time % = 100xOpen arm time/total time [42].

Fig 2: Elevated plus maze

Effect of test drugs on GABA levels in mice using UV-Vis Spectroscopy
Sample preparation
One hour after oral treatment of test drugs, mice was sacrificed and the brain was isolated immediately and transferred to homogenization tube containing 80% double distilled ethanol (for every 100 mg of the brain tissue, 2 ml of 80% alcohol was used). After homogenization, homogenates are transferred to polypropylene tubes and centrifuged at 1200 rpm for 10 minutes. 1 ml of the supernatant was transferred in small test tubes and evaporated to dryness at 70°C temperature in the oven and the residue was reconstituted in 100 ml distilled water. 10 µl was used for spotting on Whatman No. 1 Chromatographic paper. Standard solutions of GABA at a concentration of 2mM along with sample are spotted on Whatman No.1 chromatography paper using Eppendorf micropipette. After that the spots was dried.

Chromatographic condition
The mobile phase was prepared containing Butanol: Acetic acid: Water (12:3:5 v/v) as a solvent. The chamber was saturated with mobile phase and paper chromatography was developed with ascending method. When the solvent front reaches the top of the paper, it was removed and dried. A second run was performed similarly. After which the paper was dried and sprayed with 0.25 % ninhydrin solution (in 95% ethanol). The paper was dried in oven at 100°C for 4 minutes. The area which carries GABA corresponding with the standard are cut and eluted with 0.005% CuSO4 in 75% ethanol. The absorbance was read against blank at 515 nm by UV-Vis spectrophotometer.

Calculation
The levels of GABA was calculated by following formula

$$A = \frac{\text{Unknown OD}}{\text{Standard OD}} \times \frac{\text{Standard in mg}}{\text{Volume Spotted (10µl)}} \times \frac{1000}{W}$$

Where,
A= Amino acid content (GABA) in µmoles/gram wet weight tissue.
1000= Conversion factor for gram wet tissue
W= Weight of the tissue in gram [43, 44]

Statistical analysis
Statistical analysis was done by using one way ANOVA followed by Dunnett's test, compared with control.

Result
Phytochemical tests of plant extract MEPILS are mentioned in table 1

Table 1: Test result for methanolic extract of Passiflora incarnate leaf stem

Sr. No	Phytochemicals	Test results for MEPILS
1	Alkaloids	+
2	Flavonoids	+
3	Steroids	+
4	Glycosides	+
5	Saponins	+
6	Mucilage	-

(Present +; Absent -) Methanolic extract of Passiflora incarnata leaves and stem (MEPILS) shows presence of alkaloids, flavonoids, steroids, glycosides and saponins

Phytochemical tests result for MEARR are mentioned in table 2

Table 2: Test result for methanolic extract of Asparagus racemosus roots

Sr. No.	Phytochemicals	Test result for MEARR
1	Alkaloids	+
2	Flavonoids	+
3	Steroids	+
4	Glycosides	+
5	Saponins	+
6	Mucilage	+

(Present +; Absent -) Methanolic extract of Asparagus racemosus roots (MEARR) shows presence of alkaloids, flavonoids, steroids, glycosides, saponins and mucilage.

Marble Bury Test
Number of marble buried
Results for Marble Bury Test are expressed in table 3

Table 3: Result for number of marble buried by mice.

Group No.	Treatment	Number of Marble Buried (Mean±SEM)
1	Control: 5 % dextrose in distilled water P.O.	7.83±0.47
2	Standard: Lorazepam 0.05 mg/kg P.O.	4.16±0.47 **
3	MEPILS 300 mg/kg P.O.	5.33±0.95 *
4	MEPILS 300 mg/kg + MEARR 100 mg/kg P.O.	5.33±0.71 *
5	MEPILS 450 mg/kg + MEARR 150 mg/kg P.O.	4.83±0.47 *

Results are expressed in Mean±SEM; n=6, in each group; for numbers of marble buried; *$P \leq 0.05$, **$P \leq 0.01$, When compared with control, One way ANOVA followed by Dunnett's test)

In marble bury test, Mice treated with MEPILS 450 mg/kg + MEARR 150 mg/kg shows significant synergistic anxiolytic activity ($P \leq 0.05$), Mice treated with MEPILS 300 mg/kg (group 3) and MEPILS 300 mg/kg + MEARR 100 mg/kg (group 4) shows significant anxiolytic activity. Highest Significant anxiolytic activity was observed with the treatment of Lorazepam 0.05 mg/kg, $P \leq 0.01$ when compared with control.

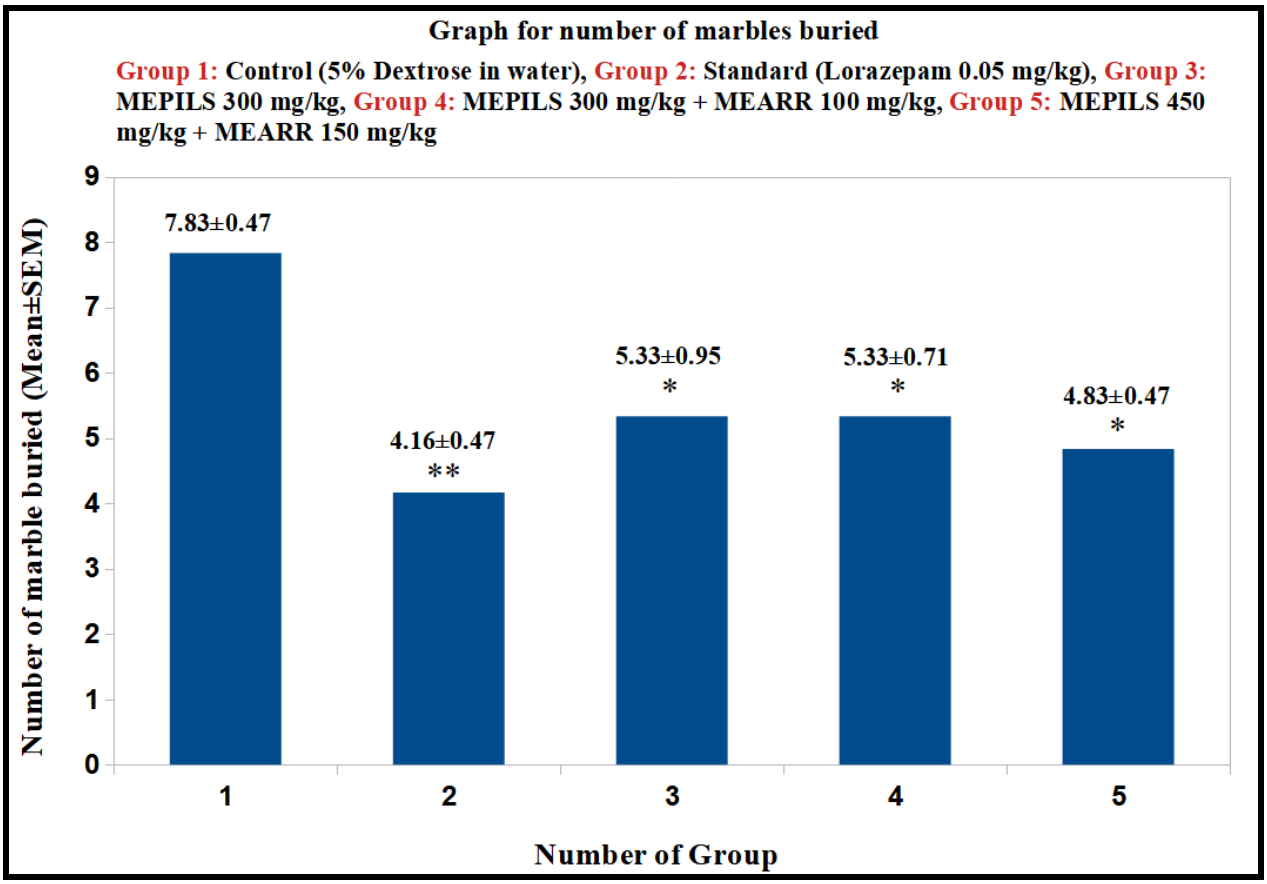

Fig 3: Graph for number of marble buried (Mean±SEM)

Elevated Plus Maze

Number of entries in open arm

Result for Number of entries in open arm are presented in table 4.

Table 4: Result for number of entries in open arm by mice.

Group No.	Treatment	No. of entries in open arm (Mean±SEM)
1	Control: 5 % dextrose in distilled water P.O.	2.33±0.61
2	Standard: Lorazepam 0.05 mg/kg P.O.	5.66±0.61 *
3	MEPILS 300 mg/kg P.O.	6.00±1.41 *
4	MEPILS 300 mg/kg + MEARR 100 mg/kg P.O.	6.66±0.55 **
5	MEPILS 450 mg/kg + MEARR 150 mg/kg P.O.	6.83±0.94 **

Results are expressed in Mean±SEM; n=6, in each group; for number of entries in open arm; *$P \leq 0.05$, **$P \leq 0.01$ when compared with control, One way ANOVA followed by Dunnett's test.

In elevated plus maze, for number of entries in open arm, treatment of lorazepam 0.05 mg/kg (group 2) and MEPILS 300 mg/kg (group 3) shows significant anxiolytic activity ($P \leq 0.05$), as significant increase in number of open arm entries was observed.

Treatment of MEPILS 300 mg/kg + MEARR 100 mg/kg (group 4) and MEPILS 450 mg/kg + MEARR 150 mg/kg (group 5) shows significant synergistic anxiolytic activity ($P \leq 0.01$).

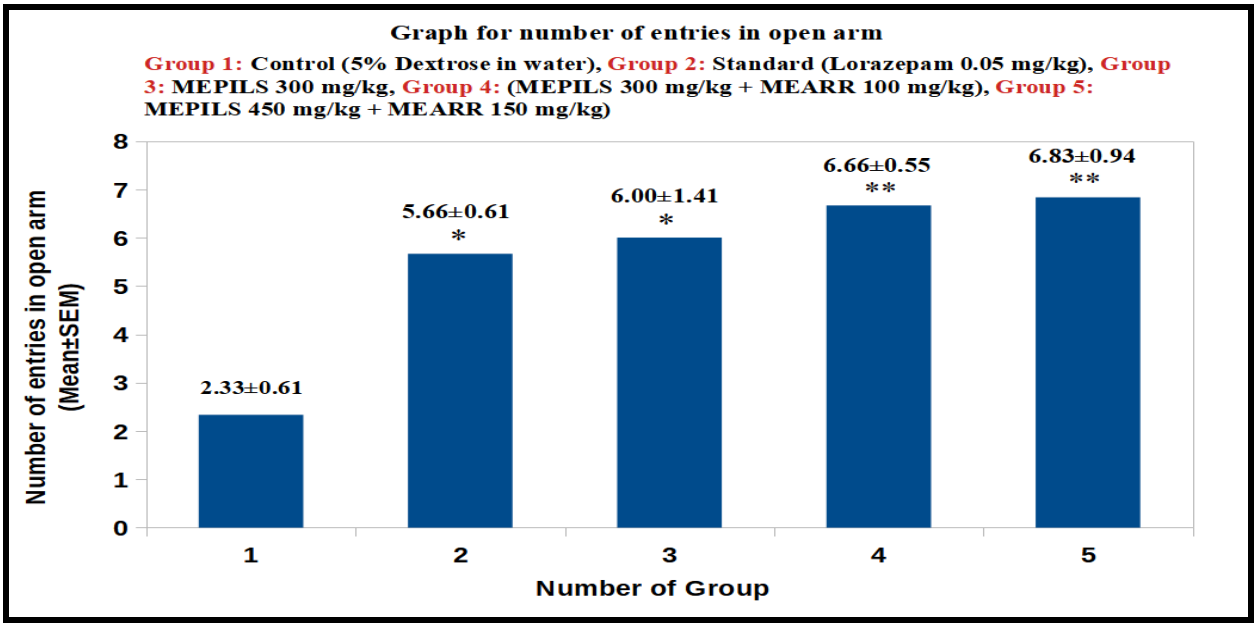

Fig 4: Graph for number of entries in open arm (Mean±SEM)

Percent (%) time spent in open arm

% time spent in the open arm was calculated as % of the total time the mice spent on the maze.

Result for Percent (%) time spent in open arm are expressed in table 5

Table 5: Result for Percent (%) time spent in open arm

Group No.	Treatment	% time spent in open arm (Mean±SEM)
1	Control: 5% dextrose in distilled water P.O.	7.84±1.76
2	Standard: Lorazepam 0.05 mg/kg P.O.	16.15±2.25 *
3	MEPILS 300 mg/kg P.O.	17.96±2.61 **
4	MEPILS 300 mg/kg + MEARR 100 mg/kg P.O.	17.82±2.00 **
5	MEPILS 450 mg/kg + MEARR 150 mg/kg P.O.	20.15±1.34 ***

Results are expressed in Mean±SEM; n=6, in each group; for number of entries in open arm, *$P \leq 0.05$, **$P \leq 0.01$, *** $P \leq 0.001$ when compared with control, One way ANOVA followed by Dunnett's test.

In elevated plus maze, for Percent (%) time spent in open arm, treatment of Lorazepam 0.05 mg/kg (group 2) significantly increases % time spent in open arm ($P \leq 0.05$). Treatment of MEPILS 300 mg/kg (group 3) significantly increases % time spent in open arm ($P \leq 0.01$). Treatment of MEPILS 300 mg/kg + MEARR 100 mg/kg shows significant increase in % time spent in open arm ($P \leq 0.01$) but no synergistic activity observed. Significant synergistic anxiolytic activity was observed with the treatment of MEPILS 450 mg/kg + MEARR 150, ($P \leq 0.001$).

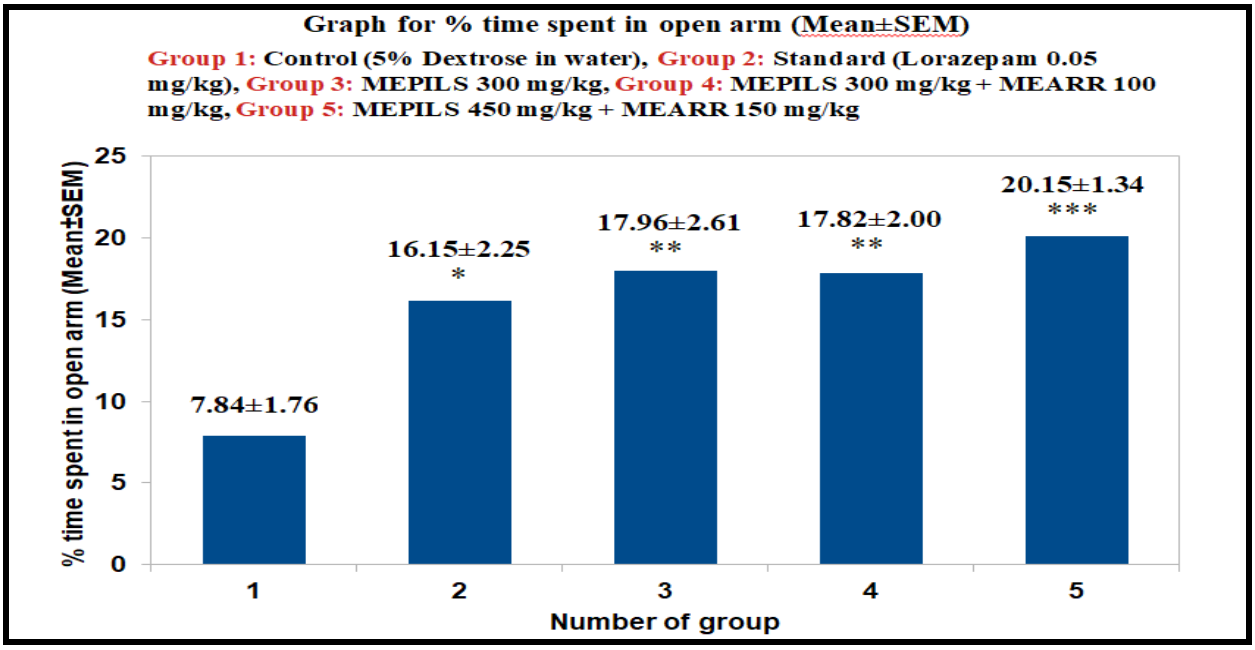

Fig 5: Graph for % time spent in open arm (Mean±SEM)

Effect of test drugs on GABA levels in mice using UV-Vis Spectrophotometer

Table 6: Result for Effect of test drugs on GABA levels in mice brain using UV-Vis Spectrophotometer

Group No.	Treatment	GABA levels in µmoles/gm of wet tissue (Mean±SEM)
1	Control: 5 % dextrose in distilled water P.O.	167.45±3.83
2	Standard: Lorazepam 0.05 mg/kg P.O.	202.09±6.63 *
3	MEPILS 300 mg/kg P.O.	220.46±9.76 ***
4	MEPILS 300 mg/kg + MEARR 100 mg/kg P.O.	223.85±10.08 ***
5	MEPILS 450 mg/kg + MEARR 150 mg/kg P.O.	229.92±4.26 ***

Results are expressed in Mean±SEM; n=6 in each group; for levels of GABA in mice brain (µmoles/gram wet weight tissue), *$P \leq 0.05$, *** $P \leq 0.001$, when compared with control, One way ANOVA followed by Dunnett's test

Significant increase in brain GABA levels was observed with the treatment of lorazepam 0.05mg/kg ($P \leq 0.05$) and MEPILS 300 mg/kg ($P \leq 0.001$).

Mice treated with MEPILS 300 mg/kg + MEARR 100 mg/kg (group 4) and MEPILS 450 mg/kg + MEARR 150 mg/kg (group 5) showed significantly increased brain GABA levels in mice. Significant synergistic activity was observed with the treatment of MEPILS 300 mg/kg + MEARR 100 mg/kg (group 4) and MEPILS 450 mg/kg + MEARR 150 mg/kg (group 5), $P \leq 0.001$ when compared with control.

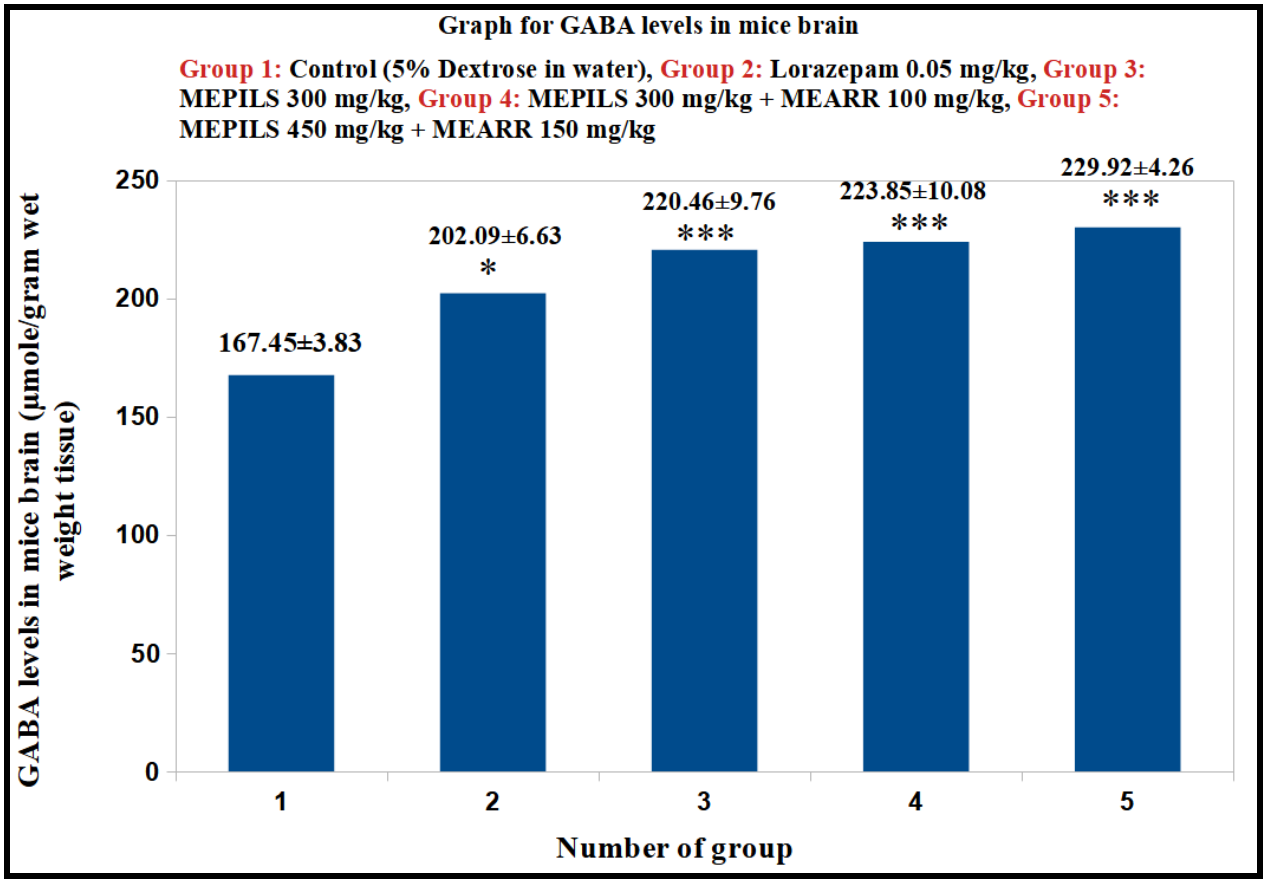

Fig 6: Graph for effect of drugs on GABA levels in mice brain (µmoles/gram wet weight tissue) using UV-Vis Spectrophotometer, (Mean±SEM)

Discussion

Anxiety disorders are known to be one of the most common type of psychiatric disorders. Anxiety disorders affect person's daily activities. Person suffering from anxiety disorder experiences common symptoms like disturbance in sleep, loss of appetite, unable focus on work, poor coordination, excess worry or fear about anything. Anxiety disorders can also affect persons immune and cardiovascular system [4, 8, 9].

Medicines like benzodiazepines, Azapirones, monoamine oxidase inhibitors, tricyclic antidepressants, selective serotonin reuptake inhibitors (SSRIs) and serotonin-norepinephrine reuptake inhibitors (SNRIs) are used for the treatment of anxiety disorders [55]. Herbal medicines like Passiflora incarnata has been used in the treatment of anxiety disorders from long time. Herbal medicines are known for their safety, also herbal drugs are knows to be suitable for long term treatment.

In this study anxiolytic activity of Methanolic extract of Passiflora incarnata leaf-stem (P.O.) was evaluated. Also, anxiolytic activity of methanolic extract of Passiflora incarnata leaf-stem with treatment of methanolic extract of Asparagus racemosus roots (P.O.) was evaluated to find out whether the use of this two herbal agents together produces synergistic anxiolytic activity or not. Lorazepam 0.05 mg/kg was used as standard drug.

In the phytochemical tests of plant extract, methanolic extract of Passiflora incarnata leaves-stem (MEPILS) shows presence of alkaloids, flavonoids, steroids, glycosides and saponins. While methanolic extract of Asparagus racemosus roots (MEARR) shows presence of alkaloids, flavonoids, steroids, glycosides, saponins and mucilage.

Marble bury test is used for evaluation anxiolytic drugs. As neophobia (fear of strange or novel objects) is seen in rodents, they bury marbles due to anxiety or fear responses. Decrease in the number of marble buried by mice indicates anxiolytic activity.

In marble bury test, Significant decrease in number of marble bury was observed with the treatment of lorazepam 0.05 mg/kg (group 2), MEPILS 300 mg/kg (group 3), MEPILS 300 mg/kg + MEARR 100 mg/kg (group 4) and MEPILS 450 mg/kg + MEARR 150 mg/kg (group 5) treated groups.

Lowest number of marble bury was observed by mice treated with Lorazepam 0.05mg/kg (group 2), $P \leq 0.01$ when compared with control. Hence highest significant anxiolytic activity was observed with this group.

Number of marbles buried by mice treated with MEPILS 300 mg/kg was almost equivalent with number of marble buried by mice treated with MEPILS 300 mg/kg + MEARR 100 mg/kg. Hence no synergistic activity was observed by the treatment of MEPILS 300 mg/kg + MEARR 100 mg/kg.

Mice treated with MEPILS 450 mg/kg + MEARR 150 (group 5) buries significantly less number of marbles ($P \leq 0.05$) than control and found to be less than MEPILS 300 mg/kg, hence synergistic anxiolytic activity was observed with the treatment of MEPILS 450 mg/kg + MEARR 150 mg/kg.

Elevated plus maze model is useful for study of anxiolytic drugs. Anxiolytic agent increases number of entries and % time spent in open arm by mice.

In elevated plus maze, for number of entries in open arm, Mice treated with lorazepam 0.05 mg/kg and MEPILS 300 mg/kg showed significant increase in number of open arm entries, hence significant anxiolytic activity was observed.

Significant synergistic anxiolytic activity was observed with the treatment of MEPILS 300 mg/kg + MEARR 100 mg/kg (group 4) and MEPILS 450 mg/kg + MEARR 150 mg/kg (group 5) as significantly increased number of open arm entries was observed (6.66±0.55 and 6.83±0.94 respectively), $P \leq 0.01$ when compared with control.

In the evaluation of % time spent in open arm, mice treated with MEPILS 450 mg/kg + MEARR 150 mg/kg (group 5) showed significantly more % of time spent in open arm (20.15±1.34), Significant synergistic anxiolytic activity was observed with the treatment of MEPILS 450 mg/kg + MEARR 150 mg/kg , $P \leq 0.001$, when compared with control. Significant increase in % time spent in open arm was observed with the oral treatment of lorazepam 0.05 mg/kg ($P \leq 0.05$), MEPILS 300 mg/kg ($P \leq 0.01$), MEPILS 300 mg/kg + MEARR 100 mg/kg ($P \leq 0.01$), significant anxiolytic activity was observed in this groups.

GABA is an important inhibitory neurotransmitter of the CNS. Its important functions include modulation of synaptic transmission, promotion of neuronal development, relaxation and prevention of sleeplessness and depression. Many drugs used for treatment of anxiety disorders work by acting on GABA receptors [36]. Current data suggests that disturbance in GABAergic transmission may leads to anxiety disorders [4, 5].

GABA levels in the brain of mice treated with MEPILS 450 mg/kg + MEARR 150 mg/kg (Group 5) found to be significantly highest (229.92±4.26 µmoles/gm of wet weight tissue). Also, significant increase in brain GABA levels was observed from the treatment of MEPILS 300 mg/kg + MEARR 100 mg/kg (group 4). Significant synergistic activity was observed with the treatment of MEPILS 300 mg/kg + MEARR 100 mg/kg (group 4) and MEPILS 450 mg/kg + MEARR 150 mg/kg (group 5), $P \leq 0.001$ when compared with control. Significant increase in brain GABA levels was observed in mice treated with Lorazepam 0.05 mg/kg ($P \leq 0.05$), MEPILS 300 mg/kg ($P \leq 0.001$) when compared with control. Increased GABA levels may improve GABAergic transmission in the brain and may reduce anxiety. From the results of this study, it was observed that use of methanolic extract of asparagus racemosus roots increases anxiolytic activity of methanolic extract of Passiflora incarnata leaves stem. From the results of this study, synergistic anxiolytic activity was observed from the treatment of MEPILS with MEARR.

Conclusion

This study observed significant synergistic anxiolytic activity from the oral treatment of methanolic extract of Passiflora incarnata leaves and stem (MEPILS) with methanolic extract of Asparagus racemosus roots (MEARR) in mice.

This study suggests that use of methanolic extract of Passiflora incarnata leaves and stem with methanolic extract of Asparagus racemosus roots may produce synergistic anxiolytic activity in the treatment of anxiety. However more study is necessary on these plants to better understand their safety, efficacy and best possible way to use them in the treatment of anxiety and related disorders.

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