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Original Research Article

PHARMACOLOGICAL EVALUATION OF *PLUMBAGO ZEYLANCIA* LEAF EXTRACTS FOR ANXIOLYTIC ACTIVITY BY USING OPEN FIELD TEST

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Abstract: Treatment for various problems through herbal medicines is a traditional system being practiced for thousands of years. As all can obey the practice due to fewer side effects, considerable research on pharmacognosy, phytochemistry, pharmacology and clinical therapeutics has been carried out tremendously. Coming to the current research, on anti-anxiety or anxiolytic activity, most of the people in now a day have got feared in the present society due to many circumstances like stress, inferiority, backward in the hype areas in their own field. For this our basic research started with safe herbal extracts by using mice as experimental animals which are exposed on open field apparatus for evaluating anxiolytic activity. The current research got a good response as this procedure was very easy and fast for the evaluation.

Introduction: Herbal products are extensively used globally for the treatment of many diseases where allopathic fails or has severe side effects. Psycho neural drugs are also have very serious side effects like physical dependence, tolerance, deterioration of cognitive function and affect on respiratory, digestive and immune system. So in this contest the treatment through natural source is seen with the hope that they have the lesser side effects than that observed with synthetic drugs[1-4].

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Plumbago zeylanica L. is a multipurpose medicinal herb of family Plumbaginaceae. *P. zeylanica* is the most common plant used in Indian traditional system of medicine. A native of South Asia, the species is distributed throughout most of the tropics and subtropics; growing in deciduous woodland, savannas' and scrub lands from sea level up to 2000 m altitude. The root is used as laxative, expectorant, astringent, abortifacient and in dysentery[5]. Tincture of root bark is used as antiperiodic. The leaves are caustic and used in treatment of scabies. Plumbago is chemically characterized by the presence of naphthoquinones, flavonoids, terpenoids and steroids, many of them being responsible for several biodynamic activities. Popular name of *Plumbago zeylanica* is lead wort. This plant is

also known by several names in different parts of the world[6-9]. In India its common name is "Chitrak".

Experimental Animals:

All experimental protocols and procedures were approved by the Institutional Animal Ethics Committee of Chalapathi Institute of Pharmaceutical Sciences. Male swiss albino mice between 8 and 10 weeks old, weighing 20–25 g, were used throughout the study. The animals were housed in standard laboratory conditions (12-h light/dark cycle, 21 ± 1°C, and relative humidity of 55 ± 5%) with free access to food and water prior to the experiments. After 7 days of acclimatization to laboratory conditions, the animals were randomly assigned to experimental groups, each consisting of 5 mice. Each animal was used only once in the experimental procedures. All experiments were carried out between 9 a.m. and 3 p.m.

Materials and Methods: Treatment Groups:

Group-1 - Control group (0.9% normal saline 1ml/ kg orally)

Group-2 – Standard group (Diazepam 2 mg/kg i.p)

Group-3 - leaf extracts (mg/kg i.p)

Procedure:Open field test:

Mice were carried into the test room in their home cages and were handled by the base of their tails at all times. Mice were placed in the centre or one of the four corners of the open field and allowed to explore the apparatus for 5 minutes. After the 5 minutes test, mice were returned in their home cages and the open field was cleaned with 70% ethyl alcohol and permitted to dry between tests. To assess the process of habituation to the novelty of the arena, mice were exposed to the apparatus for 5 minutes on 2 consecutive days[10-13].

Behaviours scored included:

1. **Line crossing:** Frequency with which the mice crossed one of the grid lines with all four paws.
2. **Center square entries:** Frequency with which the mice crossed one of the lines with all four paws into the central square.
3. **Center square duration**: Duration of time the mice spent in the center square.
4. **Rearing**: Frequency with which the mice stood with the hind legs in the maze.
5. **Stretches attend postures**: Frequency with which the animal demonstrated forward elongation of the head and shoulders followed by retraction to the original position.
6. **Grooming**: Duration of time the animal spent licking or scratching itself while stationary.
7. **Freezing**: Duration with which the mouse was completely stationary
8. **Urination**: Number of puddles or streaks of urine.
9. **Defection**: Number of fecal boli produced.

Each animal was then given a score for total locomotor activity that was calculated as the sum of line crosses and number of rears.

The open field apparatus was constructed of white plywood and measured 72 x 72 cm with 36 cm walls. One of the walls was clear Plexiglas, so mice could be visible in the apparatus. Blue lines were drawn on the floor with a marker and were visible through the clear Plexiglas floor. The lines divided the floor into sixteen 18 x 18 cm squares. A central square (18 cm x 18 cm) was drawn in the middle of the open field[14]. The central square is used because some mouse strains have high locomotor activity and cross the lines of the test chamber many times during a test session[15]. Also, the central square has sufficient space surrounding it to give meaning to the central location as being distinct from the outer locations.

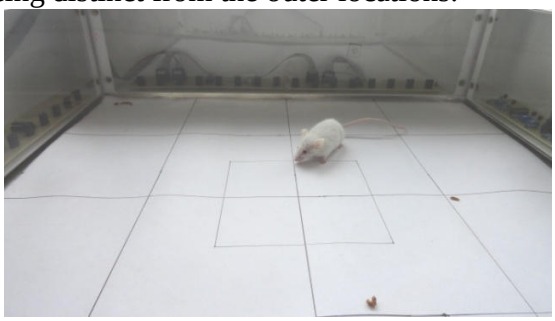

Figure 1: Open field test apparatus

Results: The leaf extracts has shown significant anxiolytic activity along with the standard treatment depicted in Table 1 & Figure 2.

Table 1: Evaluation of anti-anxiety for control, standard and test compounds

S.No	Treatment	Evaluation of parameters for 5min			
		No.of Rearings	Central square entries	No.of line crossings	Freezing time (Sec)
1	Control	16	2±0.03	82±0.02	27
2	Standard Diazepam (2mg/kg)	13	4±0.06	59±0.04	40
3	PZ [Aqueous extract] (mg/kg)	15	5±0.04	57±0.03	35

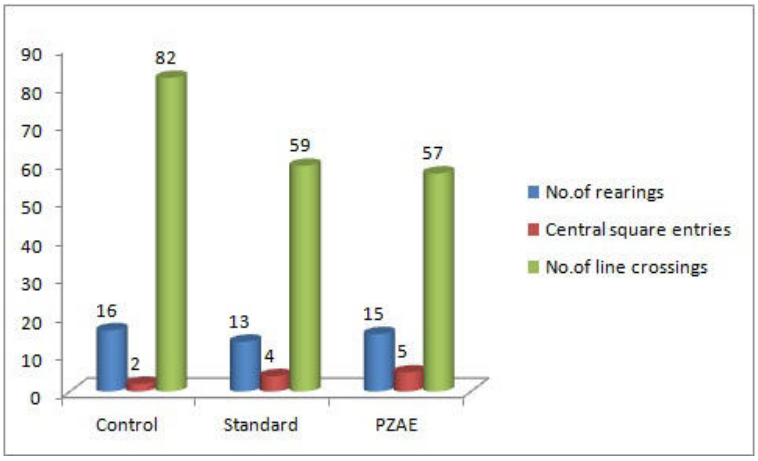

Figure 2: Evaluation of anxiolytic activity by different treatment groups

Conclusion: The *in-vitro* anxiolytic activity of the *plumbago zeylancia* was compared with the standard and found to be very effective along with the standard anxiolytic drug. The research was carried out by open field apparatus as it gives very easy and basic output for evaluation of anxiolytic activity. Scientific research on this plant reported the antibacterial, antifungal, antiviral, antiplasmodial, leishmanicidal, trypanocidal and anticariogenic activity of various parts of this plant in the literature. Unfortunately, most of the compounds have not properly been evaluated for the exploitation of new lead molecules. Moreover, mechanisms of action of a few bioactive compounds have been identified so far. Hence, extensive research is required to find out the mechanisms of action as well as bioactivity of the various phytochemicals and efficacy of the medicinal values of *P. zeylanica*.

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