EVALUATION OF ANXIOLYTIC AND MUSCLE RELAXANT EFFECT OF POLYHERBAL FORMULATION OF CURCUMA *LONGA AND BUTEA FRONDOSA* ON RATS

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Summary

The research work deals with the screening of hydroalcohlic extract of polyherbal formulation (HAEPHF) for sedative, anti-anxiety and muscle relaxant properties. HAEPHF consist of *Curcumalonga* and *buteafrondosa*. *Curcuma longa* (Family: Zingiberaceae) is a rhizomatous herbaceous perennial plant of the ginger native to tropical South Asia. It is used as cough, amenorrhea, toothache, chest pain, blood urine, hemorrhage, skin disorders, diabetes, arthritis and wounds. The main active constituents are Curcuminoids, Curcumin, Demethoxy-curcumin and Bisdemethoxy-curcumin . *Butea frondosa* (Fabaceae) popularly known as 'dhak' or 'palas' or 'Flame of forest', palash possesses appetizing, astringent, carminative, anthelmintic, aphrodisiac, tonic, anti-inflammatory and stress, anxiety and cognition in rats activities. The chemical constituents of *B. frondosa* leaves are Glucoside, Kino-oil containing oleic and linoleic acid, palmitic and lignoceric acid etc. The different activities studied like sedative antianxiety and muscle relaxation activities. The result of the study reflected that diazepam and HAEPHF (300 mg/kg *p.o*) possess sedative, antianxiety and muscle relaxant activity compare to control.

Key word: Anxiety, *Curcumalonga, Buteafrondosa*, elevated plus maze and Muscle relaxant

Progress in science and technology has contributed to an immense improvement in the feature of life of humankind. However, modern life stress, associated trials and tribulation are responsible for the surge in incidence of multiplicity of psychiatric disorders. Anxiety has been conceptualized as a frequent and serious disorder affecting the world population, independent of ethnicity and is being considered to be a cardinal symptom of many psychiatric disorders (1). Path breaking research in psychopharmacology has flooded the market place with drugs for specification. For instance, benzodiazepines (diazepam, nitrazipam lorazepam and alprazolam etc) are the most frequently prescribed synthetic drugs for variety of condition particularly anxiety, depression, epilepsy and insomnia. But these psychoneural drugs have very serious side effects like chronic use of benzodiazepines causes deterioration of cognitive function, physical dependence and tolerance. Besides addiction liabilities, benzodiazepines adversely affect the respiratory, digestive and immune system of body and the chronic treatment with benzodiazepines often prove more harmful in the longer run (2). In this context, a resurgence of interest in medicine from natural sources (mainly plant products) is seen and there is tremendous hope that drugs of plant origin will have significantly lesser side effects than that observed with synthetic drugs while having comparable efficacy. *Curcuma longa* (Family: Zingiberaceae) is a rhizomatous herbaceous perennial plant of the ginger native to tropical South Asia. It is used as cough, amenorrhea, toothache, chest pain, blood urine, hemorrhage, skin disorders, diabetes, arthritis and wounds. The main active constituents are Curcuminoids, Curcumin, Demethoxy-curcumin and Bisdemethoxy-curcumin [3, 4]. *Butea frondosa* (Fabaceae) popularly known as 'dhak' or 'palas' or 'Flame of forest', palash possesses appetizing, astringent, carminative, anthelmintic, aphrodisiac, tonic, anti-inflammatory (5) and stress, anxiety and cognition in rats activities (6). The chemical constituents of *B. frondosa* leaves are Glucoside, Kino-oil containing oleic and linoleic acid, palmitic and lignoceric acid etc (7). A literature survey reveals that no systematic approach has been made to study the anxiolytic activity of polyherbal formulation of these plants. In the present work, we have investigated of polyherbal formulation for Anxiolytic and muscle relaxant activity.

Material and Methods

Plant Material

Leaves of *Butea frondosa*, rhizomes of *Curcuma longa*, were collected from Goel ayurvedic Store, Siyana, Bulandshahr (U.P.). The plant material was identified and authenticated by the Botanist, Dr. Beena Kumari, Hindu Degree College, Moradabad. The botanical nomenclature of the plants was duly identified by using standard floras and also cross checked with Herbarium records. The plant material was shade dried for 10 days and pulverized.

Preparation of Extract

The dried plant material was powdered and passed through a 20-mesh sieve. The coarsely powdered materials (100 g) of each plant was taken and mixed together. The mixture was defatted with petroleum ether (60-80) and then extracted with hydro-alcoholic mixture (Ethanol 95%, v/v: water, 1:1) in a Soxhlet apparatus. The extract was concentrated by distilling off the solvents and evaporated to dryness using rotatory vaccum evaporator.

Animals

Experiments were performed on either sex of Swiss albino rats (125–150g). Animals were procured from the animal house of the I.F.T.M. University, Moradabad and maintained on a natural day–night cycle (12hr dark: 12hrs light) at room temperature of about 24-26°C, with free access to standard food pellets and water *ad libitum*. Animals were acclimatized for at least ten days before exposure to behavioral experiments. Experiments were carried out between 10:00-17:00 hours. The experimental protocol was approved by the Institutional Animal Ethics Committee, I.F.T.M. University, Moradabad.

Drugs and Chemicals

The standard drugs and related chemicals used in research work are listed below-

- Diazepam (Cipla, Ahmedabad:India)
- Petroleum ether (S.D. Fine chemicals, Mumbai: India)
- Tween 80 (S.D. Fine chemicals, Mumbai: India)

Formulation of Extract:

Suspension was prepared by using 2% w/v Tween 80 in distilled water.

Experimental Protocols

The animals were divided into three groups and each group contained six animals.

1. Control group- After 60 min administration of vehicle (6 mg/ml p.o.) for 7 successive days and test was performed on 7th day.
2. Test groups-

i. After 60 min administration of HAEPHF (100 mg/kg p.o.) for 7 successive days and test was performed on 7th day.

ii. After 60 min administration of HAEPHF (200 mg/kg p.o.) for 7 successive days and test was performed on 7th day.

iii. After 60 min administration of HAEPHF (300 mg/kg p.o.) for 7 successive days and test was performed on 7th day.

3. Standard group- After 60 min administration of Diazepam (2 mg/kg i.p.) for 7 successive days and test was performed on 7th day.

Anti-Anxiety Activity

The anti-anxiety activity was evaluated using staircase test and elevated plus maize test

Staircase Test

Staircase consists of five identical steps 2.5 cm high, 10 cm wide and 7.5 cm deep. The internal height of the walls is constant along whole length of the staircase. The drugs and treatments were same as mentioned under inclined plane. The animals were placed on the floor of the box with its back to the staircase. The number of steps climbed and the number of rears are counted over a 3 min period. A step is considered to be climbed only if the mouse had placed all four paws on the step. In order to simplify the observation, the numbers of steps descended were not taken into account. After each step the box was cleaned in order to eliminate any olfactory cues, which might modify the behavior of the next animal (8).

Elevated plus maze test

The apparatus consist of two open arms (5x10cm) and two closed arms (5x10x15cm) radiating from a platform (5x5cm) to form a plus-sign figure. The apparatus was situated 40 cm above the floor. The open-arms edges were 0.5 cm in height to keep the mice from falling and the closed-arms edges were 15 cm in height. The drugs and treatments were same as mentioned under inclined plane. The animal was placed at the center of the maze, facing one of the closed arms. And test was performed for five min. Arm entries were counted when the animal had placed all of its four paws on it. The procedure was conducted in a sound attenuated room, with observations made from an adjacent room (9).

Muscle Relaxant Activity

Rotarod

The rotarod apparatus consists of a metal rod (3 cm diameter) coated with rubber attached to a motor with the speed adjusted to 2 rotations per minute. The rod is 75 cm in length and is divided into 6 sections by metallic discs, allowing the simultaneous testing of 6 mice. The rod is in a height of about 50 cm above the tabletop in order to discourage the animals from jumping off the roller. Cages below the section serve to restrict the movements of the animals when they fall from the roller. Swiss albino rats underwent a pretest on the apparatus. Only those animals, which had demonstrated their ability to remain on the revolving rod (20 rpm) for 2 min, were used for the test. (10).

Inclined Plan Test

The plane consists of two rectangular plywood boards connected at one end by a hinge. One board is the base; the other is the movable inclined plane. Two plywood side panels with degrees marked on their surface are fixed on the base. A rubber mate with ridges 0.2 cm in height is fixed to the inclined plane, which was set at 65 degrees. Swiss albino mice were taken and divided into four groups, each group comprised of 6 animals. The animals are placed at the upper part of the inclined plane and were given 30 sec to hang on or to fall off (11).

Statistical analysis

The results of these experiments are expressed as means±sem of six animals in each group. The data was subjected to one-way ANOWA followed by Dunetts test and the values of $p \leq 0.05$ were considered statistically significant.

Results

Staircase Test

Table 1 presents statistical summary of the rearing and number of steps climbed. A reduction in anxiety-linked behavior was indicated by a reduction in number of rearing and sedation that was evaluated by number of steps climbed, which reflect locomotion capacity. High dose of HAEPHF (300 mg/kg) and standard drug (diazepam 2 mg/kg, i.p) both were more significantly reduced the number of rearing as well as the number of steps climbed. HAEPHF (200 mg/kg, p.o) produced a significant effect and HAEPHF (100 mg/kg, p.o) did not produce a significant decrease in the number of rearing and number of steps climbed.

Table 1: Effects of HAEPHF and diazepam in stair case test.

Groups	No. of climbing in 3 min	No. of rearing in 3 min
Control (Vehicle, 6 mg/ml, p.o)	20.33±1.38	17.83±0.60
HAEPHF (100 mg/kg p.o)	14.66±0.80	13.18±0.48
HAEPHF (200 mg/kg p.o)	11.15±6.23*	8.24±0.34*
HAEPHF (300 mg/kg p.o)	9.15±0.23*	7.11±0.32**
Diazepam (2 mg/kg, i.p)	6.20±0.68**	6.10±0.60**

n=6 in each group, **p<0.05, **p<0.001 compared against control group.

HAEPHF (200 and 300 mg/kg p.o) significantly increased the time spent and number of entries into in open arms and decrease time spent and number of entries into closed arm when compared with control. The standard drug (diazepam 2 mg/kg, i.p) showed a significant decrease in the number of entries into closed arms and also more significantly increase the time spent and number of entries into in open arms. HAEPHF (100 mg/kg p.o) did not show any differences in activity compared to control (Table 2).

Table 2: Effects of HAEPHF **and diazepam in elevated plus-maze test.**

Groups	No of entries into		Time spent in open arms (sec)
	Closed arms (sec)	Open arms (sec)	
Control(Vehicle, 6 mg/ml,p.o)	15.83±1.35	10.66±0.80	97.16±5.89
HAEPHF (100 mg/kg p.o)	13.66 ±1.20	8.83±1.66	128.33±7.97*
HAEPHF (200 mg/kg p.o)	12.16 ±0.95	15.83±1.35*	132.51±13.84*
HAEPHF (300 mg/kg p.o)	9.34 ±0.95*	18.83±1.35*	143.51±15.84*
Diazepam (2 mg/kg, i.p)	7.16 ±0.95**	21.83±1.35*	193.51±15.84**

n=6 in each group, **p<0.05, **p<0.001 compared against control group.

Rota rod and Inclined plan tests

In rotarod test, HAEPHF (200 and 300 mg/kg) significantly reduced the time spent by the animals on revolving rod. The standard drug (diazepam 2 mg/kg) showed highly significant effect when compared with control. HAEPHF (100 mg/kg) did not show significant effect.

(Table 3) In inclined plane test, from all the groups, HAEPHF (200 mg/kg) showed significant differences compare to control, HAEPHF (300 mg/kg) and standard drug (diazepam 2 mg/kg, i, p) treated group, showed more significant differences compared to control. HAEPHF (100 mg/kg) did not show any significant effect. (Table 3)

Table 3: Effects of HAEPHF **and diazepam in rota rod and inclined plan tests.**

Groups	Time spent on revolving rod (in sec)	No of animal falling down within 30sec from inclined plane
Control(Vehicle, 6 mg/ml, p.o)	323±24.85	0±0
HAEPHF (100 mg/kg p.o)	219±32.47	0±0
HAEPHF (200 mg/kg p.o)	155.5±41.59*	0.34±0.11*
HAEPHF (300 mg/kg p.o)	167.6±2.81*	0.62±0.14*
Diazepam (2 mg/kg, i.p)	97.6±2.81**	0.88±0.12**

n=6 in each group, **p<0.05, **p<0.001 compared against control group.

Discussion

The study reflected that HAEPHF (200 and 300 mg/kg p.o) possess antianxiety and muscle relaxant activity. The staircase was used for the assessment of anxiety (number of rearing) and sedation (number of steps ascended). Greater number of rear indicates anxiety like behavior and lesser number of steps ascended indicated increased sedation (8). The present investigation successfully detected the anxiolytic-like effects of HAEPHF and diazepam; both significantly decreased the number of rearing and number of steps ascended compare to control. This showed that HAEPHF has both anxiolytic and sedative properties.

The sedative and anxiolytic effects of HAEPHF could be due to the interaction of chemical constituents of the plant with the GABA/benzodiazepine receptor complex in brain (12).

Elevated plus-maze test is used to evaluate psychomotor performance and emotional aspects of rodents (13). The results showed that HAEPHF significantly increased the time spent on the open arms and decreased the number of entries closed arms. This types of effect is observed with the drugs that act on GABA/benzodiazepine receptor complex (12) and inhibit reuptake of serotonin. The present study suggests the importance of 5-HT in the etiology of anxiety and cognitive function by its modulatory effects on the locus coeruleus (14).

Inclined plane method was originally developed for testing curare-like agents. Later on, it has been used by many authors for testing compounds for muscle relaxing activity of both centrally acting and peripheral acting muscle relaxants (11). HAEPHF (300 mg/kg p.o) and diazepam (2

mg/kg.); both significantly made the animals unable to stay on inclined plane during 30 sec period and also reduced significantly the time spent on the revolving rod by rats in the rotarod test, a test mainly used to screen centrally acting muscle relaxants (15). This represented that HAEPHF may have muscle relaxant activity.

Conclusions

To conclude, HAEPHF possess sedative, anti-anxiety and muscle relaxant properties. The result of the present study substantiates the traditional use of HAEPHF for the treatment of insomnia and anti-anxiety like CNS disorders.

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