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Advance *In-Vivo* Activities of Polyherbal Formulations (*Foeniculum Vulgare, Emblica Officinalis* and *Ocimum Sanctum*) with Different Pharmacological Studies

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Abstract

Herbal products with potential therapeutic and nutritional values are gaining importance among people around the world. Herbal products are typically regarded as safe for use on humans. The preparation and marketing of extracts from various herbal items or purified bioactive components takes many different forms. Products with more than two herbal extracts are called polyherbal products. They are frequently regarded as prophylactically or therapeutically useful due to their complimentary and/or potentiating functions as a result of the advantages of one another. The phytochemical screening was performed for presence of various phytoconstituents. Our interest was to study the effect of polyherbal formulation (PHF), composed of Foeniculum vulgare, Emblica officinalis and aerial part of Ocimum sanctum. The anxiolytic activity of polyherbal formulations were examined by using the elevated plus maze test (EPMT), open field test (OFT), and motor coordination test assessed by Rota rod test (RT) and hole board test (HST). The present study is designed to evaluate the Acute toxicity studies, anti-stress effect and anti-anxiety effect of extract of Foeniculum vulgare, Emblica officinalis and aerial part of Ocimum sanctum using various experimental models in rodents. A current investigation concluded that PHF exhibited a strong anti-stress effect and anti-anxiety effect.

Keywords: *Polyherbal Formulations (PHFs), Acute toxicity, Anti-anxiety, Anti-stress*

1. Introduction

The active phytochemical constituents of individual plants are inadequate to attain the desirable therapeutic effects. When numerous herbs are combined in careful ratios in polyherbal and herbo-mineral formulations, the medicinal benefit is boosted and the toxicity is reduced. [1]. The active constituents used from individual plant are inadequate to provide attractive pharmacological action. There are evidences that crude plant extracts often have greater potency rather than isolated constituents [2-4]. In traditional medicine whole plants or mixtures of plants are used rather than isolated compounds [5].

PHFs are known to communicate high viability in countless illnesses. As previously mentioned, the restorative impact of homegrown med is applied because of the presence of various phytoconstituents and the impacts are additionally potentiated when viable herbals are formed together in PHFs [6]. Asthma, dermatitis, premenstrual illness, rheumatoid joint discomfort, headache, menopausal side effects, chronic tiredness, and touchy intestine condition are only a few of the conditions that cultivators cure. Natural arrangements should ideally be made with the guidance of a trained expert [7]. It has additionally perceived a polyherbal treatment or spice blend. The dynamic phytochemical constituents of individual plants are lacking to accomplish the helpful restorative impacts. When polyherbal and herbs mineral definitions consolidate the various spices in a careful proportion, it will give an improved helpful impact and lessen the harmfulness. The dynamic constituents utilized from the individual plants are insufficient to give alluring pharmacological activity. There are confirmations that unrefined plant removes frequently have more noteworthy intensity as opposed to disengaged constituents. In customary medication, entire plants or combinations of plants are utilized as opposed to disconnected

compounds [8]. In the larger part of customary frameworks, diabetes is better overseen by the spices blend (Polyherbal) rather than a single spice given synergism and fewer side effects. Diabetic injury cream ready by utilizing polyherbal detailing was viewed as effective as well as protected in mending diabetic foot ulcers like the standard silver sulphadiazine cream [9].

For the manufacture of Kasaya, specific medications or medication combinations are ground into a coarse powder (Yavakuta). Kwath Churna is the name for these powders. Kwath Churna will maintain its potency for a year and needs to be stored in an airtight container. Other names for them include srta, Niryuha, and Kasaya. Kasaya, Hima, Phanta, and other foods can be made with Kwath Churna. The churna powder has a minimum sieving density of 80 mesh. It must not stick to one another or get wet. The powder's medicinal efficacy increases with increasing fineness. They should be kept in airtight containers and have a one-year shelf life.

2. Materials And Methods

Plant Material:

The fruit of *Foeniculum vulgare, Emblica officinalis* and aerial part of *Ocimum sanctum* were obtained from Haridwar city and authenticated by Department of Botany, IFTM University, Moradabad, U.P. and Deendayal Research Institute, Arogyadham, Chitrakoot, Satna (M.P.) Ref.: AD/AS/Consult/-2019-20/09.

Preparation of formulation: The plant parts were separated, dried under shade and coarsely powdered. They are formulated for Kwath & Churna formulation in equal ratio (1:1:1) of each plant. Lack of standardization of these preparations, access to well-controlled trials, and little knowledge of the chemical makeup of the goods all reduce the effectiveness of these formulations [10-14].

Kwath: Kwath is a polyherbal formulation which is consist of several herbal plants boiled together with 4, 8, 16 times of water quantity under reduced flame till the volume get reduced to 1/4th of its initial volume.

Method of preparation: Plant materials are cleaned and dried. They are coarsely powdered (Yavakuta), weighed as per formula (1:1:1), and then mixed well [15].

Churna: The term "churna" refers to a fine powdered substance. The plants specified have been adequately cleaned and dried. They are sieved and ground to a fine powder. When there are many medications present, each drug is sieved and powdered separately. Each one (of the powders) is weighed individually and thoroughly combined (1:1:1). But in the industrial setting, disintegrators are used to clean, dry, and powder all the medications at once. There are other mechanical sifters [16].

Pharmacological studies:

For Pharmacological studies, the animals were taken from animal house, Indian Veterinary Research Institute, Izatnagar, Bareilly. The experimental protocol has been approved by the Institutional Animal Ethics Committee and by the Animal Regulatory Body of the Government (Regd. No. 1867/Po/Re/S/16/CPCSEA). The Pharmacological activities of Churna formulation were evaluated as follows:

Preparation of test doses:

The concentrated extract was first dried, then powdered, and the resulting powder was then suspended in the vehicle. From a stock solution of 100 mg/ml, several strengths were created. The oral administration of the freshly made suspensions.

Experimental Animals:

Healthy adult swiss albino mice were used in the study. They were provided with standard diet and water ad libitum. They were housed in plastic cages (4 in each) at room temperature of 24±10C and humidity of 55±5%, with 12 hours light cycle.

Acute oral toxicity Method:

The acute toxicities of the formulation were determined in swiss albino mice under standard conditions. The animals were fasted overnight prior to the experiment. Both the formulations of churna (aqueous and hydroalcoholic extract) at dose levels of 100, 200, 300, 400, 500, 750 and 1000 mg/kg body weight were adopted for toxicity studies. Animals were observed for 14 days to study their behavioral neurological toxicity. All animals in the toxicity study shall normally be subjected to careful examination of the external surface of the body, all orifices, and the cranial, thoracic and abdominal cavities and their contents [17].

Screening for antianxiety activity:

Treatment schedule

The elevated plus maze test (EPMT), open field test (OFT), motor coordination test (RT) and hole board test (HST) were used to determine the anxiolytic efficacy of polyherbal formulation churna [18-19].

- ❖ **Animal:** Swiss albino mice of either sex
- **Grouping of animals:** Animals were divided in 6 groups (n=4)
- **Group 1 (Normal Control):** Received saline (1 ml/kg)
- **Group 2 (Positive Control):** Received diazepam (Standard drug 1 mg/kg bw p.o.)
- **Group 3 (Test Control I):** Received test substance at low dose (100 mg/kg) (hydroalcoholic formulation)
- **Group 4 (Test Control II):** Received test substance at high dose (200 mg/kg) (hydroalcoholic formulation)
- **Group 5 (Test Control III):** Received test substance at low dose (100 mg/kg) (Aqueous formulation)
- **Group 6 (Test Control IV):** Received test substance at high dose (200 mg/kg) (Aqueous formulation)

a. Elevated plus Maze test

Two closed arms and two open arms (each measuring 50 x 10 x 40 cm) are elevated to a height of 50 cm to make up the plus maze. Groups were given extract plus diazepam (1 mg/kg). Each mouse was placed in the maze's centre, facing one of the closed arms, 30 minutes after the treatment. For five minutes, the total amount of time each mouse spent in the open and closed arms of the maze was recorded.

b. Open field test

An acrylic cage measuring 50 × 50 × 10 centimeters held each animal. Nine core squares were placed in the centre of the open field's arena, and 16 squares were placed along the walls' periphery to create a total of 25 squares. Animals were placed one at a time in a corner square of the experimental room, which was sound-attenuated and dark, and for five minutes, the number of rearings, aided rearings, and squares crossed were recorded. Oral administration of the vehicle, diazepam, and plant extract was given to the animals for one hour.

c. Rota Rod

A rota-rod apparatus was used to measure the impact on motor coordination. An iron rod measuring 3 cm in diameter and 30 cm in length, with a non-slip surface, made up the Rota rod apparatus. This rod was divided into four equal portions by three discs, which allowed four mice to go down it at once at a speed of 22 rpm for periods of 15, 30, 45, 75, and 90 minutes, respectively. The duration of the performance was measured as the time between the animal being mounted on the rod and falling off of it. After that, four mice were chosen at random

d. Hole board test:

The hole board is a 40 cm x 40 cm hardwood board that has been painted white and has four evenly spaced holes that are 1 cm in diameter and 2 cm deep. The board was divided into four equal sections squares of 20 cm x 20 cm using two broad-coloured lines that connect in the middle. Each mouse was placed in a different corner of the board, travelling around and dipping its head into the holes as it went. Each mouse's head dips and sectional crossings over the course of five minutes were counted. [17].

Screening for anti-stress activity

Treatment schedule

The anti-stress activity polyherbal formulations (churna) was examined by using the Forced swim test (FST) and Tail suspension test (TST).

Animal: Swiss albino mice of either sex

- **Grouping of animals: Animals were divided in 6 groups (n=4)**
- **Group 1 (Normal Control):** Received saline (1 ml/kg)

- **Group 2 (Positive Control):** Received diazepam (Standard drug1 mg/kg bw p.o.)
- **Group 3 (Test Control I):** Received test substance at low dose (100 mg/kg) (hydroalcoholic formulation)
- **Group 4 (Test Control II):** Received test substance at high dose (200 mg/kg) (hydroalcoholic formulation)
- **Group 5 (Test Control III):** Received test substance at low dose (100 mg/kg) (Aqueous formulation)
- **Group 6 (Test Control IV):** Received test substance at high dose (200 mg/kg) (Aqueous formulation)

a. Forced swim test (FST)

A total number of mice were administered the doses for a total of 7 days. On day 6, all the mice were allowed to swim individually for 6 min for adaptation. On day 7, the mice were allowed to swim individually for 6 min and the duration of immobility (period during which the mice only float in the upright position with minimum movement to keep their heads above water) was scored 4 min after placement into the water [20].

b. **Tail suspension test (TST)**

Animals were suspended individually by the end of tail with micropore adhesive tape (approximately 1 cm) with the head 50 cm from the bottom. Mice were suspended for a total of 6 min. During the final 4 min interval of the test, duration of immobility was recorded. Mice were considered immobile only when they will be hung passively and completely motionless [21].

3. Results and Discussion

Acute oral toxicity Method:

Neither toxicity symptoms nor mortality were observed up to a dose level of 1000 mg/kg body weight. LD50 was considered as more than 1000 mg/kg. Hence 1/10th (100 mg/kg of body weight) and 1/5th (200 mg/kg of body weight) were selected for pharmacological studies of polyherbal formulation (Kwath). Results of AOT are shown in Table no. 1:

Table 1: Acute toxicity testing of aqueous and hydroalcoholic churna

1. Loss of reflex	–
Righting reflex	–
Pinna reflex	–
Corneal reflex	–
2. Changes in the bw, skin, and fur	–
3. Any clinical abnormalities	
Tremors	–
Convulsions	–
Salivation	–
Diarrhea	–
Lethargy	–
Sleep	–
Coma	–
4. Death within	
24 h	+
1–14 days	+

Antianxiety activity:

Elevated plus maze

The amount of time spent in open arms, the quantity of rearings in open arms, and the number of open arm entry are all dramatically increased by the administration of diazepam (0.5 m/kg). They indicated a decrease in the amount of time spent in closed arms. Mice treated with PHF extracts showed a substantial increase in open arm entrances. As indicated in table no. 2 and figure no. 1, the number of arm entrances increases but the amount of time spent in closed arms decreases.

Table no. 2: Observations of Elevated plus maze test

Treatment	Dose mg/kg	Time spent in open arms (sec)	Enteries in open arms (sec)
Saline	1ml	44.6±1.208304597	3.2±0.374166
Standard	1	84.8±1.562049935	7.6±0.509902*
Hydroalcoholic	100	76±0.577350269	6.6±0.50990195*
Hydroalcoholic	200	55.4±1.503329638	6.2±0.37416574
Aqueous	100	42.2±0.734846923	3.6±0.50990195
Aqueous	200	41.8±4.487761134	2.8±0.374165739

All values are mean ±SEM (n=6); *p< 0.1 when compared to control.

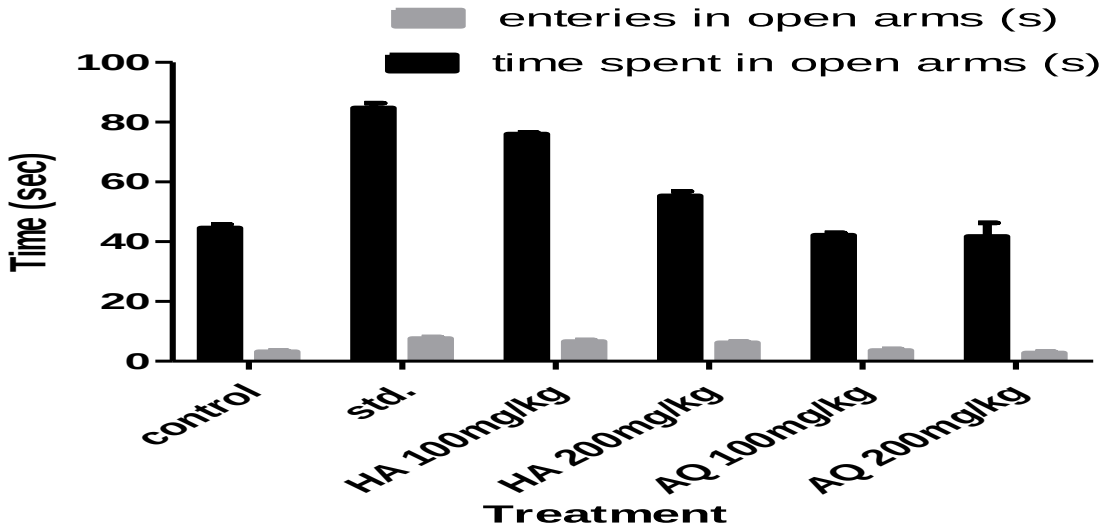

Fig. no. 1: Graph represent the effect of PHF (churna) on Elevated plus maze in swiss albino mice

Open field test

Comparing diazepam plant extracts to the control revealed considerable anxiolytic activity. PHF extract significantly increased the number of rearings, squares crossed, and assisted rearings during 5-minute test intervals in the open field test when compared to the control, as shown in table 3 and fig. no. 2.

Table no. 3: Observations of Open field test

Treatment	Dose mg/kg	No. of square crossed	No. of rearings
Saline	1ml	14.2±1.319090596	10.6±0.927362
Standard	1	38±1	14.8±1.68523
Hydroalcoholic	100	25.8±1.392838828*	12.8±1.3190906*
Hydroalcoholic	200	21.6±1.077032961	11.2±1.28062485
Aqueous	100	14.8±1.496662955	9.6±1.07703296
Aqueous	200	13.8±1.655294536	6.8±0.860232527

All values are mean ±SEM (n=6); *p< 0.1 when compared to control.

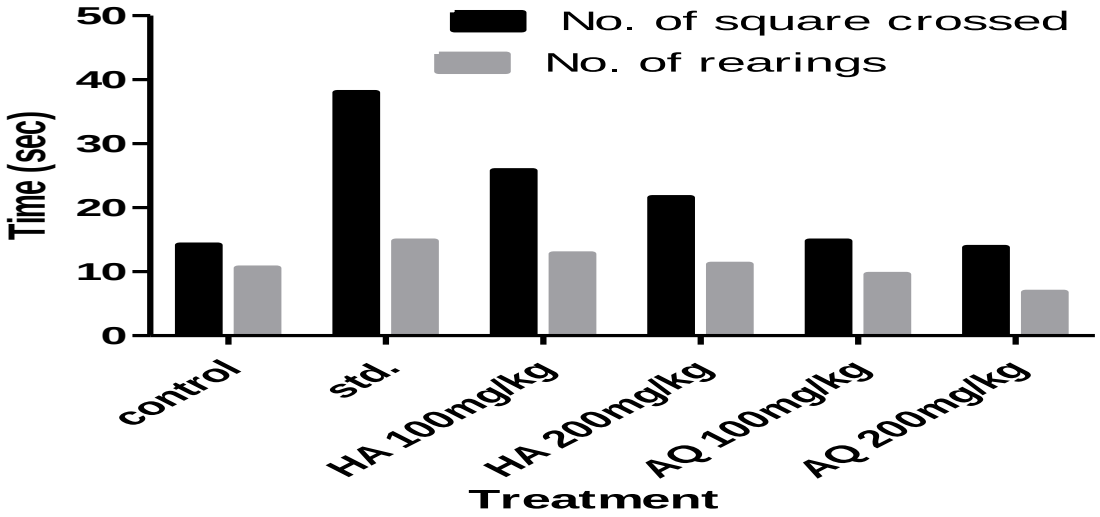

Fig.no. 2: Graph represent the effect of PHF (churna) on Open field test in swiss albino mice

Rota rod test

When compared to the control animals, diazepam caused a considerable drop in the locomotor score. According to table no. 3 and figure no. 3, both doses of PHF extract significantly reduced the locomotory score compared to control animals.

Table no. 4: Observations of Rota rod test

	Time (sec) of animals remained without falling from rod						
Treatment	**Dose mg/kg**	**15 min**	**30 min**	**45 min**	**60 min**	**75 min**	**90 min**
Saline	1ml	144.6±1.63	137.2±1.68	125.2±1.28	92.8±1.42	64.8±1.56	56.6±3.04
Standard	1	209.4±3.10*	189.2±2.83*	160.4±3.18	137.2±5.37	120.4±3.37	92.6±1.69
Hydroalcoholic	100	184.4±1.72*	173.8±4.58*	143.2±5.43	115.8±1.82	96.4±1.6	71.8±1.93
Hydroalcoholic	200	134.6±1.53	122.2±3.05	100.2±2.74	84.8±1.56	70.4±3.65	64.2±4.11
Aqueous	100	122.6±0.50	97.6±1.07	77.6±1.07	65.4±1.80	58.2±3.35	54.8±1.59
Aqueous	200	113.6±1.07	85.2±1.28	64.2±1.62	54.4±1.43	56.4±2.11	46.6±2.11

All values are mean ±SEM (n=6); *p< 0.1 when compared to control.

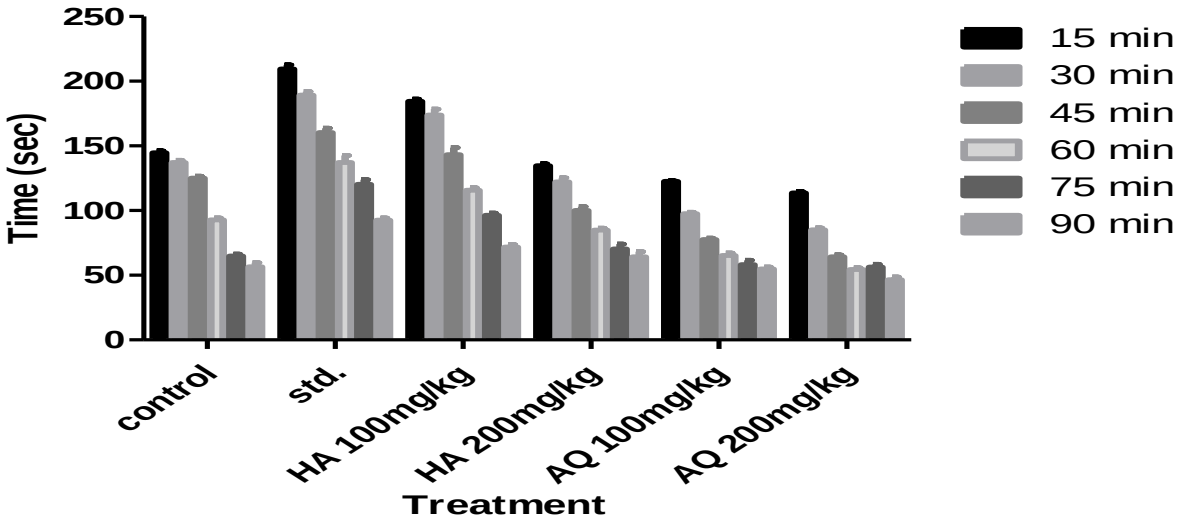

Fig. no. 3: Graph represent the effect of PHF (churna) on Rota rod test in swiss albino mice

Hole board test

When compared to control animals, the number of line crossings and head dipping was considerably higher in diazepam-treated animals. As indicated in table no.5 and fig. no.4, the PHF extracts considerably increased the number of lines crossing and head dipping as compared to control animals.

Table no. 5: Observations of Hole board test

Treatment	Dose mg/kg	Number of head dipping	Number of line crossing
Saline	1ml	11.4±1.029563014	28.4±1.029563
Standard	1	17.6±0.92736185*	43.2±2.477902
Hydroalcoholic	100	15.2±1.428285686*	31.8±1.06770783
Hydroalcoholic	200	13.2±1.319090596	17.8±1.77200451
Aqueous	100	8.8±2.154065923	22.4±3.74966665
Aqueous	200	7.2±2.154065923	25.8±2.083266666

All values are mean ±SEM (n=6); *p< 0.1 when compared to control.

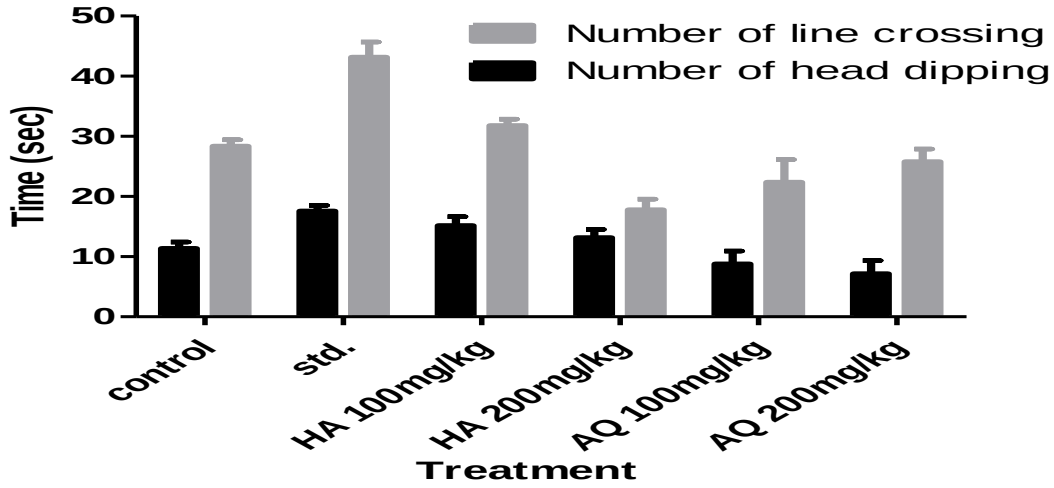

Fig. no. 4: Graph represent the effect of PHF (churna) on Hole board test in swiss albino mice

Statistical analysis:

The experimental results were expressed as the mean ± standard error of the mean (S.E.M.). Data were evaluated by using one-way analysis of variance (ANOVA) followed student's t-test and means were compared using Graph pad prism5 software t- test at $p \leq 0.001$ and will be considered statistically significant.

Anti-stress activity: Neurobehavioral and motor performances were measured and statistically correlated. Signs of intoxication were not observed 24 h post-treatment as dose did not produce any significant changes in behavioral pattern and failed to elicit any clinical abnormality.

Forced swim test (FST)

The entire four test groups of the aqueous and hydroalcoholic extract showed dose-dependent decrease in immobility time when compared against control as well as against diazepam which was used as a standard. Results of FST are shown in Table no.6 and Fig. no.5.

Table no. 6: Observations of Forced swim test

Treatment	Dose mg/kg	Immobility (s)	Mobility (s)
Saline	1ml	104.6±1.435270009	118.6±0.927362
Standard	1	84±1.516575089	164.2±1.593738*
Hydroalcoholic	100	62±0.707106781	162.4±0.92736185*
Hydroalcoholic	200	33.2±1.772004515	161.8±4.7581509
Aqueous	100	44±1.870828693	123.8±1.59373775
Aqueous	200	52±0.707106781	117±0.707106781

All values are mean ±SEM (n=6); *p< 0.1 when compared to control.

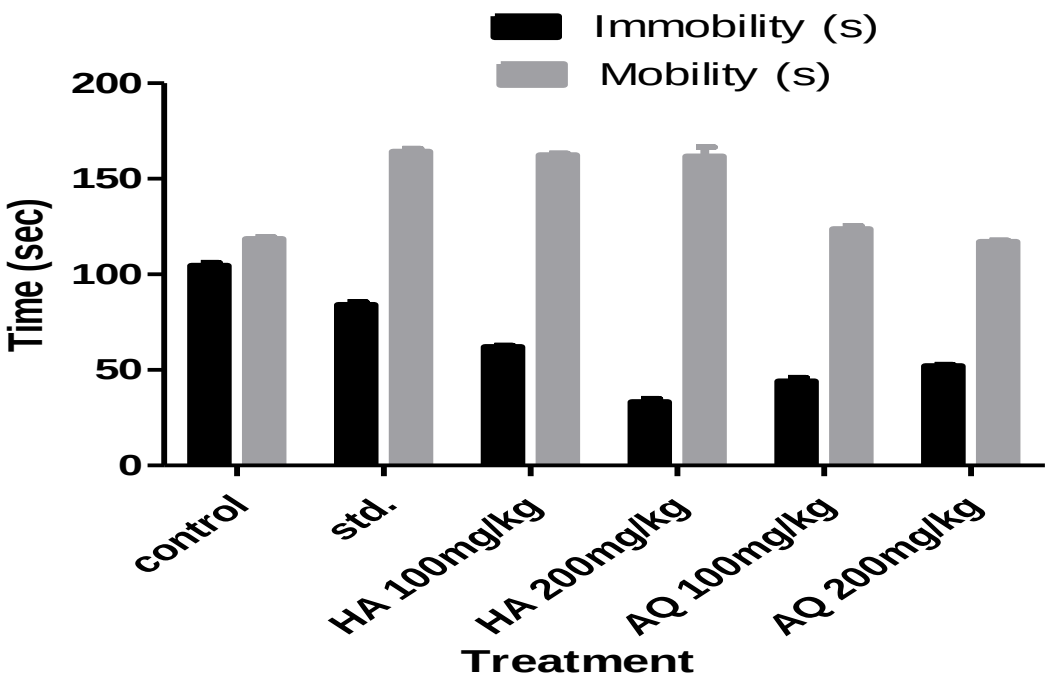

Fig. 5: Graph represents the effect of PHF (churna) on mobility and immobility time of forced swim test in swiss albino mice

Tail suspension test (TST)

The entire four test groups of the aqueous and hydroalcoholic extract showed dose-dependent decrease in immobility time when it was compared against control as well as against diazepam which was used as a standard. Results of TST are shown in Table no. 7 and Fig. no. 6.

Table no. 7: Observations of Tail suspension test

Treatment	Dose mg/kg	Immobility (s)	Mobility (s)
Saline	1ml	111.4±0.92736185	122±0.707107
Standard	1	154±1.870828693*	174±1.414214
Hydroalcoholic	100	133.8±1.15758369*	120.4±0.678233
Hydroalcoholic	200	123.6±1.630950643	104.2±1.77200451
Aqueous	100	105.4±2.731300057	96.4±0.92736185
Aqueous	200	117.2±1.428285686	97.2±1.392838828

Every value is mean ± SEM (n=6); *p <0.1 against control.

Statistical analysis:

The mean and ± standard error of the mean (S.E.M.) were used to express the experimental results. The one-way analysis of variance (ANOVA) was used to examine the data, followed by the student t-test, and the means were compared using the Graph Pad Prism 5 software t-test at $p \leq 0.001$ and will be regarded as statistically significant.

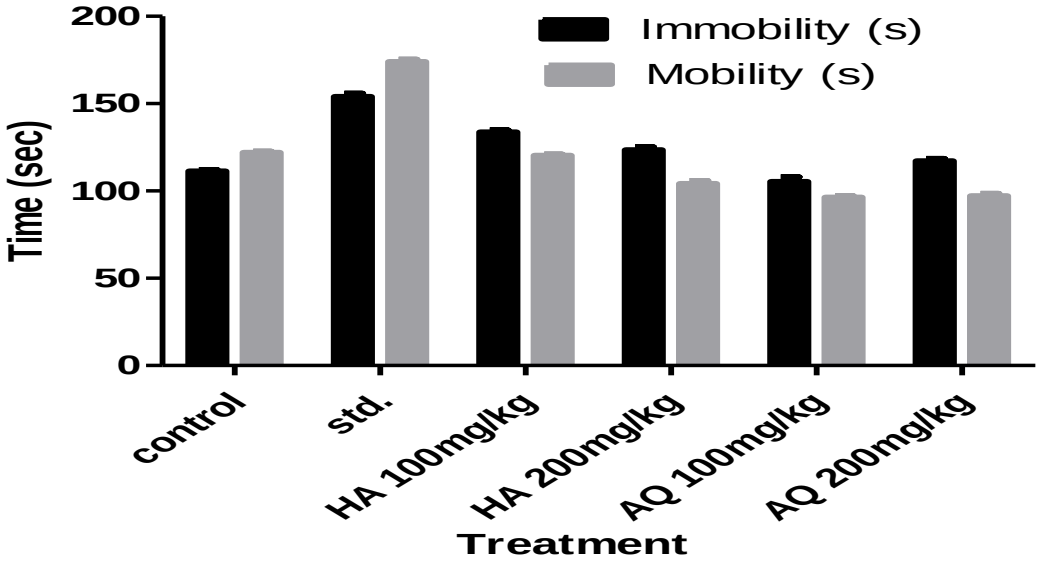

Fig. 6: Graph represents the effect of PHF (churna) on mobility and immobility time of Tail immersion test in swiss albino mice

4. Conclusion

The current research work concluded that churna which is polyherbal formulation extracted from fruit of *Foeniculum vulgare, Emblica officinalis* and aerial part of *Ocimum sanctum* were showed the anti-stress and anti-anxiety effect. All *in-vivo* studies showed that polyherbal formulations were non-toxic and safe after evaluating the acute toxicity studies. Polyherbal details have plant-based pharmacological specialists who might apply synergistic, potentiation, agonistic opposing activities by ideals of its assorted dynamic standards inside themselves. These pharmacological standards cooperate deeply to create the greatest helpful effectiveness with the least after effects. Therapeutic plants existed even before a person showed up on the earth. The advancement strategies of natural medications for overall use need to be different structure of engineered drugs. Here is a need of time to assess polyherbal detailing utilizing scientific strategies like clinical preliminary, conceivable bioactive mixtures, and systems of activity for the future world.

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Conflicts Of Interest

All authors declare that they have no conflicts of interest.

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