WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 8, 2265-2277. Research Article ISSN 2277– 7105

PRELIMINARY SCREENING OF PSYCHOPHARMACOLOGICAL EFFECTS AND TOXICITY TESTING OF TENSNIL SYRUP IN SWISS ALBINO MICE

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Article Received on
18 June 2017,

Revised on 09 July 2017,
Accepted on 30 July 2017

DOI: 10.20959/wjpr20178-9133

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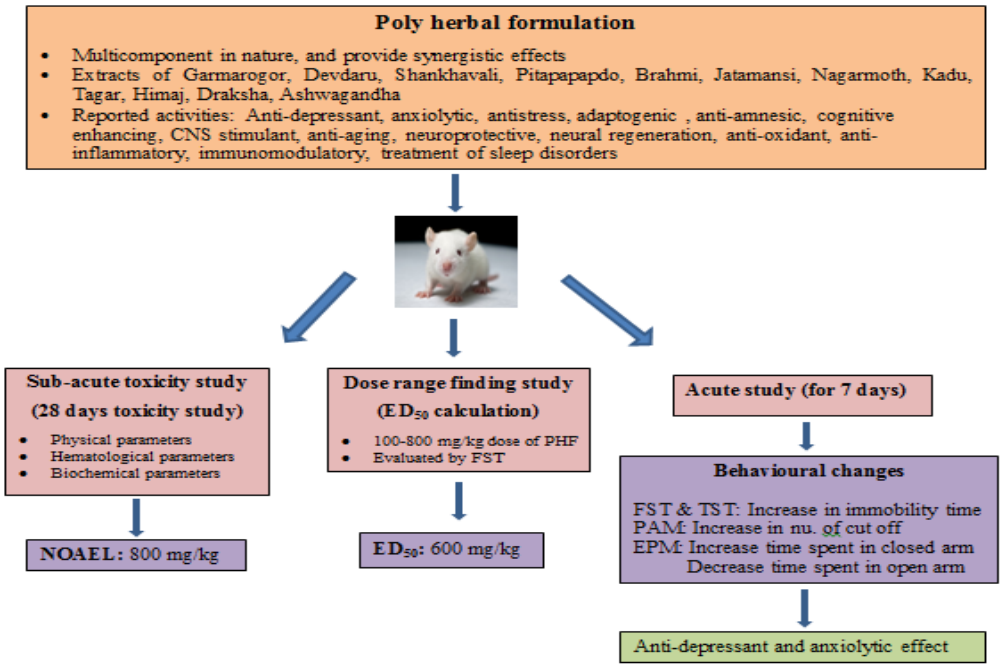
ABSTRACT

Background: Tensnil syrup is a polyherbal formulation (PHF) containing ingredients such as, extracts of garmarogor, devdaru, shankhavali, pitapapapdo, brahmi, jatamansi, nagarmoth, kadu, tagar, himaj, draksha, ashwagandha. **Aims:** This formulation was evaluated for its safety by repeated dose sub-acute oral toxicity study in swiss albino mice, dose range finding study and also for its acute antidepressant and anxiolytic effects on central nervous system (CNS) using behavioural models. **Materials and Methods:** The PHF was administered orally at a therapeutic dose range (100 - 800 mg/kg/day), for 28 days. All animals were monitored daily for their health status and signs of abnormalities. The body weight and food intake were measured once weekly. At the end of the experimental period, various haematological and biochemical parameters were estimated. For acute study, forced swim test (FST), tail suspension test (TST), elevated plus maze (EPM) and photoactometer tests were performed at doses of 400 and 800 mg/kg. Fluoxetine (20 mg/kg, p.o.) was used as standard. **Results:** Long term use of PHF did not show any remarkable change in physical, haematological and biochemical parameters. Further, single dose treatment of PHF for 7 days at the dose of 400 and 800 mg/kg, showed significant antidepressant and anxiolytic activity as evident from significant reduction of immobility time in FST and TST along with increased number of cut – offs and time spent in close arm in EPM**. Conclusions:** The study established the safety and efficacy of PHF in mice. Also this acute study reveals antidepressant and anxiolytic activity of Tensnil syrup.



Graphical abstract.

KEYWORDS: Polyherbal Formulation, Repeated Dose Oral Toxicity, Neurophyscological Effects.

INTRODUCTION

The World Health Organization (WHO) claims that the safety of herbal medicines is a critical element in the quality control of healthcare products.[1] An extract of herbal plants encompass compounds which do not directly affect the pathophysiological processes, but may modify the absorption, distribution, metabolism and excretion of bioactive constituents, or reduce the side-effects.[2] Poly herbal formulation (PHF) possesses some advantages such as reduction in dose, and ease of administration.[3-5] The multi target responses of herbal drugs are proven to be useful in chronic conditions and also in returning the health status.[6]

Tensnil Syrup, a PHF, contains extracts of Garmarogor, Devdaru, Shankhavali, Pitapapapdo, Brahmi, Jatamansi, Nagarmoth, Kadu, Tagar, Himaj, Draksha, Ashwagandha. Some of these plants have been reported for use in nervous system disorders as they calm down the brain, produce quality sleep[7], and remove toxins from brain.[8] Based on this hypothesis, we aim to evaluate their effects in anxiety and sleep disorders. The safety studies of Tensnil Syrup have not been established so far. Also, the safety profile of the Tensnil Syrup has been studied by a sub-acute oral toxicity study in swiss albino mice, in order to optimize its safe use.

MATERIALS AND METHODS

Drugs and biochemistry kits

Tensnil Syrup was supplied by the manufacturer, Cadila Pharmaceutical Private Limited. Clinical chemistry kits for serum biochemistry parameters, namely, triglycerides, total protein, uric acid, albumin, glucose, Creatinine, urea, total bilirubin, direct bilirubin, asparatae amino - tranferase (AST), alkaline phosphatase (ALP), alanine amino - tranferase (ALT) and cholesterol were purchased from span diagnostics.

Animals and housing

An approval for the study was obtained from Institutional Animal Ethics Committee (IAEC) (LMCP/COLOGY/16/09). A total of 36 swiss albino mice of both sex with body weight ranging from 25 to 35 g were used for toxicity study, and total 30 male swiss albino mice were used for psychopharmacological effects. Mice were acclimatized for 7 days and provided with chaw diet. Temperature and relative humidity were maintained at 25 ± 1°C and 40-70%, respectively and illumination were controlled to give approximately a sequence of 12 hours light and 12 hours dark. Mice were individually housed in polypropylene cages (27 × 19 × 14 cm) with lids and rice husk bedding.

Experimental design

Selection of dose

The human clinical dose of Tensnil syrup is 10 ml for two to three times / day. The therapeutic doses of Tensnil syrup for mice were selected as 100, 200, 400, 600, 800 mg/kg by calculating from the human clinical dose (1000 - 1500 mg/day/70 kg). It was calculated based on the total body surface area of the mice, using 0.0026 as the conversion factor.[9] The animals were divided into six groups, each having three animals. The drug was administered in a volume of 2, 4, 8, 12, 16 ml/kg.

Toxicity study

Tensnil Syrup was administered orally at five dose levels i.e. 100 mg/kg, 200 mg/kg, 400 mg/kg, 600 mg/kg and 800 mg/kg body weight for twenty eight days. Normal saline was administered to the animals of control group.

ED_{50} calculation

The animals were divided into six groups, each having six animals. Formulation was administered orally at five dose levels i.e. 100 mg/kg, 200 mg/kg, 400 mg/kg, 600 mg/kg and

800 mg/kg body weight. Normal saline was administered to the animals of control group. ED_{50} doses of formulation was calculated in forced swim test (FST) using dose response curve with different doses in geometrical progression versus immobility time in seconds.

Psychopharmacological effects

(1) Forced swim test[10]

The mice were taken to a separate room and were immediately placed in a cylinder (45 cm high, 20 cm diameter) filled to 30 cm depth and maintained at 25 ± 1°C. Mice were examined for the duration of 5 minutes. They were dried and returned to their respective home cages later. The oral treatments in the various groups were carried out 2 hour prior to forced swim test in the second session. The cylinder used had been freshly cleaned and disinfected prior to the forced swim test. Clean water was used for each behavioural trial.

(2) Tail suspension test[11]

TST was implemented based on the previous method that the mouse was hung 25 cm above the floor by the tip of the tail (1 cm) tied up to the level. The immobility time was counted during a test period of 6 min (prior 1 min to adapt and recorded the last 5 min). And only when the mouse hung passively and completely motionless, it could be regarded as immobile.

(3) Locomotor activity

Each mouse was placed in a closed square (30 cm) area equipped with infrared light-sensitive photocells using a digital photoactometer. The mice was observed for a period of 5 min and the values were expressed as counts per 5 min. The apparatus was placed in a darkened, light- and sound-attenuated and ventilated test room.[12]

(4) Elevated plus maze

Elevated plus maze (EPM) assesses unconditioned anxiety like behaviour in mice. EPM consisted of two open arms (30×5 cm), two enclosed arms (30×5 cm), and a connecting central platform (5×5 cm). The maze was elevated 38.5 cm above the ground. At the beginning of the 5-min session, each mouse was placed in the central neutral zone, facing one of the close arms. Time in the open and number of entries into open arms were recorded. An arm entry was defined as a mouse having entered an arm of the maze with all four legs.[13]

Physical Parameters: Physical parameters (body weight and food intake), and local injury were observed throughout the treatment. Mortality if any, in all the groups, during the course

of treatment was also recorded. At the end of treatment, haematological and biochemical parameters were analysed.

Haematological Parameters

RBC, WBC, lymphocytes (%), monocytes (%), eosinophils (%), basophils (%), MCV (Mean Corpuscular Volume) (%), MCH (Mean Corpuscular Hemoglobin) (%), MCHC (Mean Corpuscular Hemoglobin Concentration), PLT (Platelet) (*10^9/L), and HGB (Hemoglobin) (%) were estimated.

Biochemical Parameters

Serum triglycerides, total protein, uric acid, albumin, glucose, Creatinine, urea, total bilirubin, direct bilirubin, asparatae amino - tranferase (AST), alkaline phosphatase (ALP), alanine amino - tranferase (ALT) and cholesterol were estimated.

Statistical analysis

The data were expressed as Mean ± SEM. Statistical analysis was performed by one way ANOVA followed by Tukey's multiple comparison test. The values were considered statistically significant when $P < 0.05$.

RESULTS

Sub-acute oral toxicity of Tensnil syrup

Effects of Tensnil syrup on body weight and food intake.

Body weight and food intake of all the animals were measured every week throughout the study as shown in table 1and table 2 respectively. There was no remarkable change in body weight and food intake of the mice in any of the study groups.

Table. 1: Body weight (g).

Study day	Body weight (g)					
	Control	100 mg/kg	200 mg/kg	400 mg/kg	600 mg/kg	800 mg/kg
Day 0	28.33 ± 0.84	28.00 ± 0.45	28.67 ± 0.56	29.17 ± 0.48	29.33 ± 0.42	28.33 ± 0.84
Day 7	25.83 ± 0.82	26.50 ± 0.43	27.33 ± 0.42	27.83 ± 0.54	28.33 ± 0.42	25.83 ± 0.82
Day 14	30.00 ± 0.701	29.83 ± 1.58	30.33 ± 1.58	30.83 ± 1.47	30.83 ± 1.51	30.00 ± 0.701
Day 21	30.67 ± 0.71	30.00 ± 1.39	30.17 ± 1.45	30.83 ± 1.45	31.17 ± 1.28	30.67 ± 0.71
Day 28	30.83 ± 0.00	28.50 ± 0.89	29.17 ± 0.79	29.50 ± 0.89	29.83 ± 0.75	30.83 ± 0.00

Values are expressed as mean ± SEM, n = 6, $p < 0.05$ compared between different groups, where no significant difference observed.

Table. 2: Food intake (g).

Study Group	Food Intake (g)
Control	3.1 ± 0.07
100 mg/kg	2.9 ± 0.08
200 mg/kg	3.1 ± 0.06
400 mg/kg	3.0 ± 0.09
600 mg/kg	3.3 ± 0.05
800 mg/kg	3.2 ± 0.09

Values are expressed as mean ± SEM, n = 6, $p < 0.05$ compared between different groups, where no significant difference observed.

Effects of Tensnil syrup on haematological parameters

Our observations of the study for the period of 28 days did not reveal any significant change in any of the haematological parameters (table 3). The formulation was considered safe at the 800 mg/kg dose level.

Table. 3: Effect of Tensnil Syrup on the haematological parameters in mice.

	Control	100 mg/kg	200 mg/kg	400 mg/kg	600 mg/kg	800 mg/kg
RBC	10.90 ± 2.22	11.84 ± 1.59	12.55 ± 2.04	12.01 ± 2.17	12.01 ± 1.86	11.45 ± 1.55
WBC	6.95 ± 0.54	10.19 ± 1.08	8.33 ± 0.50	8.35 ± 0.99	7.83 ± 0.36	9.24 ± 1.70
Lympho(%)	18.03 ± 0.95	26.53 ± 1.82*	23.23 ± 1.72	20.60 ± 2.26	17.80 ± 0.48	24.70 ± 2.67
Monocy (%)	81.82 ± 2.78	76.63 ± 4.12	76.05 ± 3.78	76.30 ± 4.53	79.27 ± 2.72	80.05 ± 2.14
Eosin (%)	2.27 ± 0.44	2.25 ± 0.20	2.50 ± 1.08	1.45 ± 0.33	2.10 ± 0.38	2.73 ± 0.57
Baso(%)	1.67 ± 0.35	1.83 ± 0.49	1.87 ± 0.40	1.60 ± 0.47	1.97 ± 0.41	2.02 ± 0.37
MCV(%)	0.12 ± 0.05	0.08 ± 0.04	0.08 ± 0.04	0.10 ± 0.05	0.07 ± 0.03	0.07 ± 0.03
MCH (%)	43.37 ± 0.40	43.80 ± 0.51	42.40 ± 0.95	41.13 ± 1.17	40.77 ± 0.50	44.40 ± 0.59
MCHC	53.28 ± 1.06	53.63 ± 1.03	54.33 ± 1.22	53.18 ± 0.52	53.10 ± 0.76	53.12 ± 0.44
PLT (*10^9/L)	17.12 ± 0.41	17.20 ± 0.61	17.25 ± 0.45	17.17 ± 0.49	16.13 ± 1.25	16.88 ± 0.56
HGB (%)	32.17 ± 0.64	32.05 ± 0.81	31.77 ± 0.87	31.68 ± 1.12	30.28 ± 2.37	31.78 ± 0.92

Values are expressed as mean ± SEM, n = 6, * $p < 0.05$ indicates significant difference between 100 mg/kg and control group.

Effects of Tensnil syrup on the biochemical parameters

The repeated oral dose treatment for 28 days did not cause significant changes in hepatic functional transaminases viz. ALT, AST and ALP levels. The renal function was evaluated in terms of serum urea and creatinine. Other biochemical parameters like triglyceride, total protein, uric acid, albumin, glucose, total bilirubin, direct bilirubin, globulin, cholesterol levels also did not change remarkably (table 4).

Table. 4: Effect of Tensnil Syrup on the biochemical parameters in mice.

	Control	100 mg/kg	200 mg/kg	400 mg/kg	600 mg/kg	800 mg/kg
TG	178.07 ± 9.83	179.32 ± 11.64	178.07 ± 12.37	171.15 ± 10.55	170.13 ± 14.12	170.52 ± 12.14
Total Protein	6.01 ± 0.66	5.78 ± 0.41	6.18 ± 0.58	6.68 ± 0.91	6.27 ± 0.74	6.18 ± 0.57
Uric Acid	2.13 ± 0.13	2.35 ± 0.21	2.30 ± 0.17	2.43 ± 0.23	2.48 ± 0.18	2.31 ± 0.20
Albumin	3.54 ± 0.17	3.78 ± 0.11	3.42 ± 0.06	3.26 ± 0.18	3.39 ± 0.09	3.40 ± 0.15
Glucose	112.53 ± 4.71	103.79 ± 5.34	102.52 ± 3.99	97.15 ± 5.90	95.48 ± 3.96	100.86 ± 6.08
Creatinine	0.39 ± 0.01	0.43 ± 0.01	0.43 ± 0.01	0.36 ± 0.01	0.43 ± 0.03	0.41 ± 0.01
Urea	40.94 ± 3.41	38.35 ± 4.30	45.05 ± 5.94	47.20 ± 8.22	45.92 ± 7.69	46.87 ± 7.09
Total Bilirubin	0.50 ± 0.08	0.64 ± 0.11	0.60 ± 0.09	0.50 ± 0.08	0.64 ± 0.11	0.60 ± 0.09
Direct Bilirubin	0.12 ± 0.02	0.11 ± 0.03	0.12 ± 0.03	0.14 ± 0.03	0.08 ± 0.02	0.14 ± 0.03
Globulin	2.47 ± 0.64	2.00 ± 0.51	2.77 ± 0.56	3.42 ± 0.97	2.88 ± 0.82	2.78 ± 0.65
AST	106.82 ± 3.21	103.28 ± 2.66	105.05 ± 4.78	106.37 ± 4.44	106.08 ± 2.83	99.89 ± 7.26
ALP	87.69 ± 2.84	90.40 ± 2.44	84.75 ± 2.64	87.91 ± 2.41	89.72 ± 1.13	90.85 ± 2.55
ALT	43.91 ± 2.12	42.73 ± 1.53	42.14 ± 1.58	42.28 ± 2.11	40.37 ± 2.45	41.11 ± 1.53
Cholesterol	106.03 ± 4.44	104.65 ± 3.61	101.32 ± 5.54	103.23 ± 5.38	99.57 ± 6.30	99.27 ± 4.23

Values are expressed as mean ± SEM, n = 6, $p < 0.05$ compared between different groups, where no significant difference observed.

ED_{50} calculation: The ED_{50} value of different doses of the formulation obtained from the FST was 600 mg/kg p.o., in mice (table 5).

Table. 5: ED_{50} of PHF calculated in forced swim test model of depression in mice.

% Inhibition of duration of Immobility		
Groups	**Male mice**	**Female mice**
Control	100	100
PHF(100 mg/kg)	99.853	93.282
PHF(200 mg/kg)	74.444	71.543
PHF(400 mg/kg)	57.206	53.761
PHF(600 mg/kg)	48.888	45.143
PHF(800 mg/kg)	44.577	42.27

Psychopharmacological Effects of Tensnil syrup

(1) Forced swim test: In the forced swim test, the duration of immobility was significantly ($p < 0.001$) increased in the disease control groupon day 7 when compared with the results of day 0. Fluoxetine (reference standard) and PHF – 400 mg/kg and 800 mg/kg treatment from day 7 to day 14 resulted in significant reduction in duration of immobility time as compared to data of disease control group on day 7 (fig. 1).

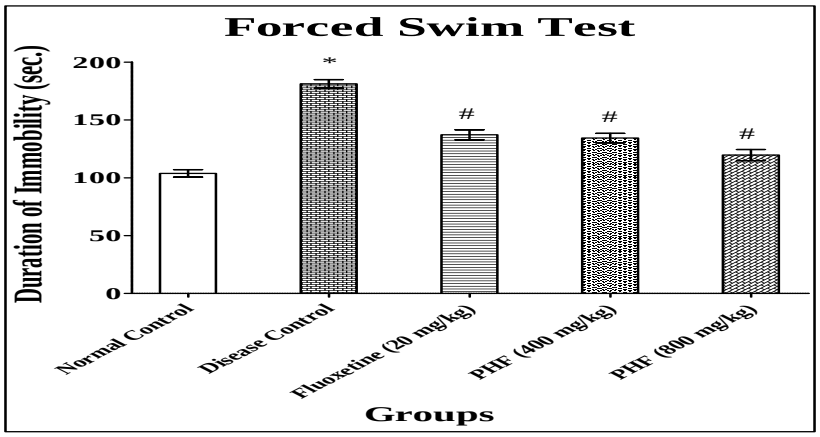


Fig. 1: Forced swim test.

*$p < 0.001$, when compared with normal control.

#$p < 0.001$, when compared with disease control.

(2) Tail suspension test: There was significant reduction in duration of immobility in fluoxetine and PHF (400 and 800 mg/kg) treated mice when compared with the disease control group on day 14 (fig. 2).

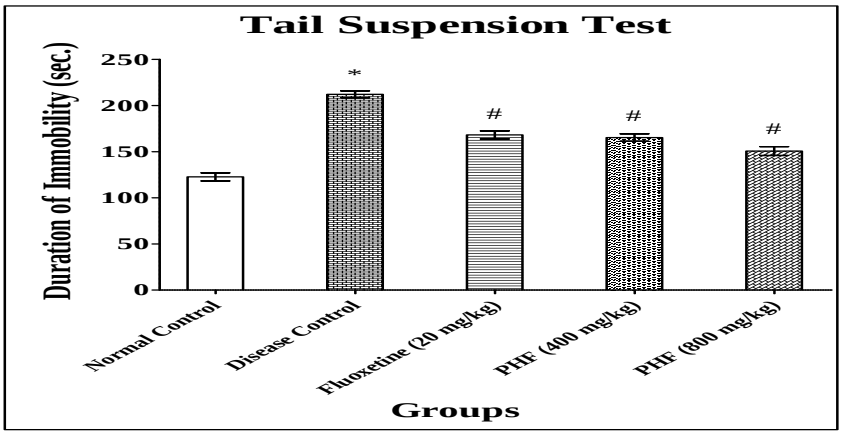


Fig. 2: Tail suspension test.

*$p < 0.001$, when compared with normal control.

#$p < 0.001$, when compared with disease control.

(3) Locomotor activity: The locomotor activity observed using photoactometer was significantly ($p < 0.05$) reduced in the disease control group when compared with the normal control group on day 7. Treatment with fluoxetine and PHF from day 7 to day 14 significantly increased locomotor activity as matched to the disease control group (fig. 3).

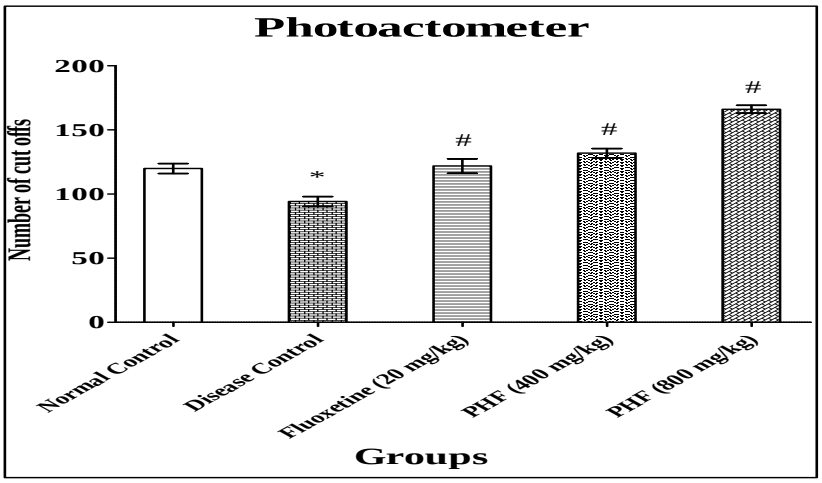


Fig. 3 Locomotor activity.

*$p < 0.01$, when compared with normal control.

#$p < 0.05$, when compared with disease control.

(4) Elevated plus maze: Time spent in open arm for fluoxetine and PHF groups (400, 800 mg/kg) was found statistically significant ($p < 0.05$) when compared with the disease control group on day 14 (fig. 4).

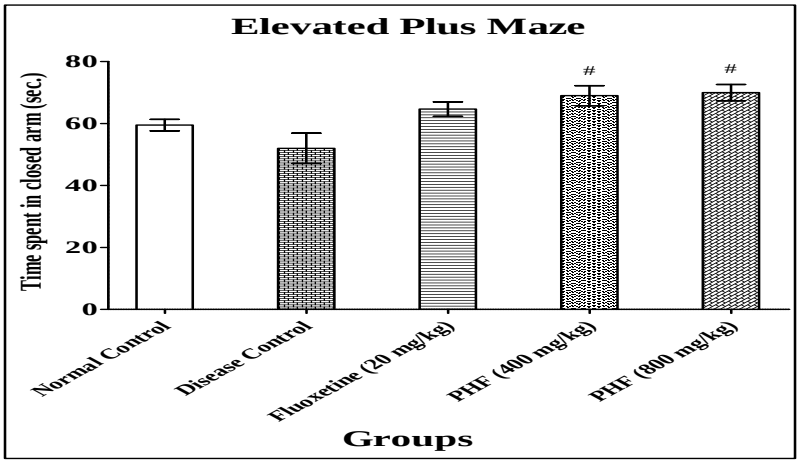


Fig. 4 Elevated plus maze.

#$p < 0.01$, when compared with disease control.

DISCUSSION

Numerous herbal preparations have been shown to value in treating various neurological and psychological aliments through different mechanisms, but the toxicity related data for many of these has not been well-known. Tensnil Syrup contain many ingredients of herbal origin, which have been used traditionally as brain tonics in treating many neuropsychological disorders like depression, anxiety, sleep disorders. Herbal formulations are multicomponent in nature, and provide synergistic effects and there is an increasing claim for these medicines among the public, for chronic use.

In the present study, the results obtained from the safety profile of orally administered Tensnil syrup, up to a therapeutic dose of 800 mg/kg have been reported. No mortality or abnormal behaviour was seen in the animals treated with Tensnil syrup, up to the dose of 800 mg/kg. The formulation did not have any significant impact on the body weight and food intake indicating that treatment with Tensnil syrup did not affect the common health status of the animals.

The haematopoietic structure is one of the most sensitive targets for toxic compounds and an important index of physiological and pathological status in human and animal.[14] The treatment with Tensnil syrup did not have significant impact on the haematological study. The levels of glucose, cholesterol, and triglyceride remained unaffected, which indicated that the formulation did not interfere with the carbohydrate and lipid metabolism in mice.[15] Treatment with Tensnil syrup in mice also did not alter the hepatic and renal function, as evident from the hepatic enzyme AST and ALT levels and renal serum biomarkers of creatinine. It further, confirmed the normal functioning of hepatocytes and nephrons during treatment period. Based on these findings, the safety of the formulation is confirmed at the therapeutic dose level under the study. In addition to this, no observed adverse effect level (NOAEL) of Tensnil syrup was observed up to the dose of 800 mg/kg. ED_{50} was also calculated for this PHF for a dose range 100 - 800 mg/kg and was found to be 600 mg/kg.

Mice treated with acute stress produced an increase in immobility time in FST and TST while decreased number off cut offs in photoactometer and reduced the time spent in open arm. The behavioural changes seen in the animals treated with stress were replicated as described in previous study. Our study showed parallel results with those of previous studies in which exposure to stress increased immobility time.[16] In FST, mice were forced to swim in a restricted space, which induced a characteristic behaviour of immobility. In TST, mice were

suspended by their tip of the tail from a metal rod which also induced a state of immobility in animals like that in FST. This immobility reflects a state of despair in animals and is claimed to reproduce a condition similar to depression in humans. PHF produced marked decrease into the duration of immobility when compared with the disease control group and there by produced anti-depressant activity. Locomotor activity is measured as an index of alertness and a decrease in it is indicative of sedative activity. However, none of the doses of PHF were found to have any sedative effect activity. The Elevated plus maze is used to estimate the anxiety state in animals. It is simple, less time consuming, and does not use any sophisticated equipment or prior training. In the EPM, acquisition can be considered as transfer latency on the first-day trials and the retention/consolidation later. The animals treated with PHF (400, 800mg/kg) showed significant increase in time spent in close arm indicating anxiolytic activity of the drug under investigation.

CONCLUSION

Our data suggest that there was no observable finding of serious signs and significant changes in the physical, haematological, and biochemical parameters that resulted from the 28 - day's administration of Tensnil syrup. Also, no mortality was observed in experimental animals treated with this drug.Therefore, in the present study, Tensnil syrup reflected the innocuous nature of this formulation on hepatic, renal and hematopoietic system even at high dose level of daily administration indicating safety of the formulation and devoid of any neurotoxicity effect.

In addition to above, psychopharmacological findings revealed significant improvement in depression and anxiety in mice. These findings have scientifically validated the traditional claim and suggested its valuable role in the treatment neurological disorders. As the present study is based on the behavioural models without any associated neurochemical estimations, it becomes necessary to carry out specific binding studies and estimations of the neurotransmitter levels in the brain, to understand the exact mechanism of action and extend these results further.

ACKNOWLEDGEMENTS

This research was financially supported by GUJCOST: Minor Research Project. We are gratefully acknowledging Cadila Pharmaceutical Private Limitedfor providing the drug sample (Tensnil Syrup).

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