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ASSESSMENT OF POLYHERBAL FORMULATION FOR CNS ACTIVITIES

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ABSTRACT

The research work deals with the screening of hydroalcoholic extracts of polyherbal formulation (HAEPHF) for antidepressant and anxiolytic activity. The polyherbal formulation consists of hydro-alcoholic extract of leaves of *Butea frondosa*, roots of *Withania somnifera*, aerial parts of *Convolvulus pruricalis*, seeds of *Nigella sativa*, rhizomes of *Curcuma longa*, and leaves of *Azadirachta indica*, all of these drugs were reported to possess central nervous system (CNS) activity. Evaluations of HAEPHF of central nervous system (CNS) activities were done by Analgesic activity (Eddy's hot plate method and tail flick method), CNS depressant and muscle relaxant activity. HAEPHF was administered singly for 7 successive days and test was performed on 4th & 7th day. Recent study revealed that HAEPHF (1000 and 2000 mg/kg) produced analgesic, CNS depressant and muscle relaxant like effect in experimental animals and the efficacy of the extract was found to be comparable than that of standard drugs used diclofenac and diazepam.

Key words: Polyherbal formulation, Analgesic, Muscle relaxant, CNS depressant and Diazepam.

INTRODUCTION

Modern life stress, associated trials, life style and tribulation are responsible for the surge in incidence of variety of psychiatric disorders. Path breaking research in psychopharmacology has flooded the market place with drugs for specification. For instance, benzodiazepines (diazepam, nitrazipam, lorazepam and alprazolam) are the most frequently prescribed synthetic drugs for variety of condition particularly anxiety, tension, stress, 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 (Dhawan *et al*., 2003). Use of plant products is rising in many segment of the population (Eisenberg *et al*., 1993). At present, thousands of natural drugs are being successfully used for the treatment of variety of different diseases. According to an approximation, 80% of the world's population relied upon plants for their medication (Rakh and Chaudhari, 2010). The use of the medicinal plants is increasing in many countries where 35% of drugs contain natural products. As a result of adverse effects such as gastric lesions caused by non-steroidal anti-inflammatory drugs (NSAID), tolerance and dependence induced by opiates (Dharmasiri *et al*., 2003; Park *et al*., 2004). Moreover, synthetic drugs are very expensive to develop. It is therefore essential that efforts should be made to introduce new medicinal plants to develop cheaper drugs (Hossain *et al*., 2011).

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

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efficacy. There are a number of traditional herbal drugs used in combination as polyherbal formulation to get synergistic and desirable effects. Therefore, it is planned to carry out the evaluation of analgesic, CNS depressant and muscle relaxant activity of hydro-alcoholic extract of a polyherbal formulation (HAEPHF). The polyherbal formulation consists of hydro-alcoholic extract of leaves of *Butea frondosa* (Soman *et al*., 2004), roots of *Withania somnifera* (Bhattacharya *et al*., 2003) aerial parts of *Convolvulus pruricalis* (Sethiya *et al*., 2010), seeds of *Nigella sativa* (Paarakh *et al*., 2010), rhizomes of *Curcuma longa* (Yu ZF *et al*., 2002) and leaves of *Azadirachta indica* (Parshad *et al*., 1997), all of these drugs were reported to possess CNS activity.

MATERIALS AND METHODS

Plant Material

Polyherbal formulation consists of the leaves of *Butea frondosa*, roots of *Withania somnifera*, aerial parts of *Convolvulus pruricalis*, seeds of *Nigella sativa*, rhizomes of *Curcuma longa*, and leaves of *Azadirachta indica*. These drugs were collected from Goel ayurvedic Store, Siyana, Bulandshahr (U.P.). The plant material were identified and authenticated by the Botanist, Dr. Beena Kumari, Hindu College, Moradabad. Herbarium sheets have been preserved for future reference. The botanical nomenclature of the plants was duly identified by using standard floras and also cross-checked with Herbarium records. The plant materials were shade dried for 10 days and pulverized.

Preparation of Extract

The dried plant materials were powdered and passed through a 20-mesh sieve. The coarsely powdered materials 50 gm of each plant was taken and mixed together. The mixture was defatted with petroleum ether and then extracted with hydro-alcoholic mixture (Ethanol 95% (v/v): distilled water = 1:1) in a Soxhlet apparatus. The extracts were filtered and concentrated by distilling off the solvents and evaporated to dryness using rotary vacuum evaporator.

Formulation

Suspension of hydro alcoholic extract of polyherbal drug was prepared by using 2 % Tween 80.

Animals

Experiments were performed on either sex of Swiss albino rats (60-150 g) and Swiss albino mice (25-30g). Animals were procured from the animal house of the IFTM University, Moradabad and maintained on a natural day–night cycle (12h dark: 12h 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 animals were divided into three groups.

Animal Treatment

I. Control group- Vehicle (5 ml/kg p.o) was administered singly for 7 successive days and test was performed on 4^{th} & 7^{th} day.

II. Test groups-

i. Hydroalcoholic extract of poly herbal formulation (HAEPHF) (500 mg/kg p.o) was administered singly for 7 successive days and test was performed on 4^{th} & 7^{th} day after 60 minutes (min) administration of the test drugs.

ii. The HAEPHF (1000 mg/kg p.o) was administered singly for 7 successive days and test was performed on 4^{th} & 7^{th} day after 60 min administration of the test drugs.

iii. The HAEPHF (2000 mg/kg p.o) was administered singly for 7 successive days and test was performed on 4^{th} & 7^{th} day after 60 min administration of the test drugs.

III- Standard groups-

Diazepam (2 mg/kg i.p.) was administered singly for 7 successive days and test was performed on 4^{th} and 7th day after 30 min administration of the drug.

Diclofinac sodium (6 mg/kg i.p.) was administered for 7 successive days and test was performed on 4^{th} and 7th day after 60 min administration of the drug.

The study was approved by Institute Animal Ethical Committee, department of pharmacology and clinical research, college of pharmacy, IFTM University, Moradabad. All animals care and experimental protocols were in compliance with the NIH guidelines for the care and use of the Laboratory Animals (NIH 1985).

Acute Oral Toxicity Study

The acute oral toxicity study was performed according to the OPPTS (Office of Prevention, Pesticides and Toxic Substance) guidelines following Up and Down procedure (URL http://www. epa. Gov/opts/home/ guideline. 1994).

The CNS activities were carried out by following way:

Analgesic activity

Eddy's hot plate test (EHPT)

The Hot plate test evaluates thermal pain reflexes due to foot pad contact with a heated surface (Kulkarni 1987). Rats of either sex weighing 130-140 gm were divided into five groups of six animals each. Group I- is the control group, treated with vehicle (5 ml/kg p.o), Group II, III and IV are the test groups, treated with HAEPHF (500, 1000 and 2000 mg/kg p.o) were administered singly for 7 successive days and test was performed on 4^{th} & 7^{th} day after 60 min administration of the test drugs, group V is the standard group, treated with Diclofenac (6 mg/kg) was administered singly for 7 successive days and test was performed on 4^{th} and 7th day

after 30 min administration of the drug. The animals were placed on the hot plate maintained at constant temperature of 55 ^{0}C and reaction time (in seconds) jumping of paw responses was noted. This was repeated at 30, 60, 90, 120 minute intervals. A cut off period of 15 sec was observed to avoid damage to the paw.

Tail Immersion test

Prior to the analgesic experiments, the animals were screened for a sensitivity test by immersing the tip of tail (5 cm) gently in hot water (55^{0}C). Within few seconds the rats react by withdrawing tail. The reaction time was recorded by stopwatch (Vogel *et al*., 1997). Swiss albino rats (120-150gm) were divided into five groups, each group comprised of 6 animals. Group I- is the control group, treated with vehicle (5 ml/kg p.o), Group II, III and IV were the test groups, treated with HAEPHF (500, 1000 and 2000 mg/kg p.o) were administered singly for 7 successive days and test was performed on 4th & 7th day after 60 min administration of the test drugs, group V was the standard group, treated with Diclofenac (6 mg/kg) was administered singly for 7 successive days and test was performed on 4th and 7th day after 60 min administration of the drug. The reaction time was determined periodically after treatment to all of the groups.

Acetic acid induced Writhing test

Acetic acid (1% v/v) was administered intraperitoneally to all the groups at the dose of 1 ml/kg body weight 60 min after the administration of vehicle in control group, test compounds in test groups and diclofinac in standard group. Anti-nociception was recorded by counting the number of writhes after the injection of acetic acid for a period of 10 min. A writhe is indicated by abdominal constriction and full extension of hind limb (Koster 1959). Swiss albino rats (120-150gm) were divided into five groups, each group comprised of 6 animals. Group I- is the control group, treated with vehicle (5 ml/kg p.o), Group II, III and IV are the test groups, treated with HAEPHF (500, 1000 and 2000 mg/kg p.o) and diclofinac (6 mg/kg p.o) has taken as standard drug were administered singly for 7 successive days and test was performed on 4th & 7th day of experiment.

CNS depressant/Sedative/Stimulatory activity by Actophotometer

The spontaneous locomotor activity of each mouse was recorded individually by actophotometer for 10 min (Tatarezyynska et al., 2004). The actophotometer (24 cm in diameter) illuminated by two beams, which were connected to a counter for recording of light beam interruptions. The animals (20-25gm) were divided into five groups, each group comprised of 6 animals. Group I- is the control group, treated with vehicle (5 ml/kg p.o), Group II, III and IV are the test groups, treated with HAEPHF (500, 1000 and 2000 mg/kg p.o) were administered singly for 7 successive days and test was performed on 4th & 7th day after 60 minutes (min) administration of the test drugs. Group V is the standard group, administered Diazepam (2 mg/kg i.p) was administered singly for 7 successive days and test was performed on 4th and 7th day after 30 min administration of the drug.

Muscle co-ordination activity

Inclined plane

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 set at 65 degrees. The animals are placed at the upper part of the inclined plane and are given 30 sec to hang on or to fall off (Allmark *et al*., 1949). The animals (20-25gm) were divided into four groups, each group comprised of 6 animals. Group I- is the control group, treated with vehicle (5 ml/kg p.o), Group II, III and IV are the test groups, treated with HAEPHF (500, 1000 and 2000 mg/kg p.o) were administered singly for 7 successive days and test was performed on 4th & 7th day after 60 minutes (min) administration of the test drugs. Group V is the standard group, treated with Diazepam (2 mg/kg i.p) was administered singly for 7 successive days and test was performed on 4th and 7th day after 30 min administration of the drug.

Rota rod

The apparatus consisted of a horizontal wooden rod or metal rod coated with rubber 3 cm diameter 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 disc, thereby 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. The animals were placed on the rotating rod and time spent by the animals on the rod was recorded (Rakotonirina *et al*., 2001). Swiss albino rats (120-150gm) were divided into five groups, each group comprised of 6 animals. Group I- is the control group, treated with vehicle (5 ml/kg p.o), Group II, III and IV are the test groups, treated with HAEPHF (500, 1000 and 2000 mg/kg p.o) were administered singly for 7 successive days and test was performed on 4th & 7th day after 60 minutes (min) administration of the test drugs. Group V is the standard group, treated with Diazepam (2

mg/kg i.p) was administered singly for 7 successive days and test was performed on 4th and 7th day after 30 min administration of the drug.

RESULTS

Acute oral toxicity study

In acute oral toxicity study, all the animals were observed for any signs of toxicity or mortality for 48 h. All the animals were subjected for sharp observation for a period of 48 days. Hydro-alcoholic extract of polyherbal formulation (HAEPHF) showed no mortality and toxic effect up to dose, 5000 mg/kg body weight in mice. So, HAEPHF was found to be safe at a dose of 5000 mg/kg. Hence three doses 1/2.5th, 1/5th and 1/10th of the doses 2000, 1000 and 500 mg/kg body weight of HAEPHF were chosen for CNS activities.

Eddy's hot plat method

On 4th day, after 60, 90 and 120 min of administration of HAEPHF (1000 and 2000 mg/kg) significantly increased the reaction time, HAEPHF (2000 mg/kg) more significantly increased the reaction time only after 90 min of drug administration, and standard drug Diclofinac (6 mg/kg) more significantly increased the reaction time. Whereas HAEPHF (500mg/kg) did not produce any significant effect as results are shown in table 1.

On 7th day, after 60, 90 and 120 min of administration of HAEPHF (2000 mg/kg) and standard drug Diclofinac (6 mg/kg) more significantly increased the reaction time and HAEPHF (1000 mg/kg) and significantly increased the reaction time. Whereas, HAEPHF (500mg/kg) did not produce any significant effect as the results are shown in table 2.

Tail immersion test

On 4th and 7th day of treatment, after 60, 90 and 120 min of administration of HAEPHF (2000 mg/kg) and standard drug diclofinac (6 mg/kg) more significantly increased the reaction time and HAEPHF (1000 mg/kg) and significantly increased the reaction time. Whereas HAEPHF (500mg/kg) did not produce any significant effect as results are shown in table 3 and 4.

Writhing test

In this test on 4th and 7th day of treatment HAEPHF (500 and 1000 mg/kg) did not significantly reduced the number of writhing, HAEPHF (2000mg/kg) produced significant effect, whereas Standard drug diclofenac (6 mg/kg) more significantly reduced the number of writhing on 4th and 7th day of experiments as the results can seen in table 5.

CNS depressant/sedative activity

In this test, on 4th and 7th day of treatment, animals treated with HAEPHF (500 mg/kg) did not show significant decrease in locomotor activity while those treated with HAEPHF (1000 & 2000 mg/kg) showed more significant decrease in locomotor activity indicating the sedative like effect. Animals treated with diazepam (2 mg/kg) showed a more significant decrease in locomotor activity. The test drug was found to be almost equally effective with the standard drugs. The test drug produced better effect on 7th day of treatment compared to the 4th day of treatment as shown in Table 6.

Muscle relaxant activity

Inclined plane

In this test, on 4th and 7th day of treatment, animals treated with HAEPHF (500 mg/kg) did not show significant effect that is number of animal falling down within (30sec) from inclined plane while those treated with HAEPHF (1000 & 2000 mg/kg) and standard drug diazepam (2 mg/kg) showed more significant effect. The test drug was found to be comparable to the standard drugs. The test drug produced better effect on 7th day of treatment compared to the 4th day of treatment as shown in Table 7.

Rota rod

In this test, on 4th and 7th day of treatment, animals treated with HAEPHF (500 mg/kg) did not show significant decrease in time spent on revolving rod while those treated with HAEPHF (1000 & 2000 mg/kg) and diazepam (2 mg/kg) showed more significant decrease in time spent on revolving rod indicating the muscle relaxant activity. The test drug was found to be comparable to the standard drugs. The test drug produced better effect on 7th day of treatment compared to the 4th day of treatment as shown in Table 8.

Table 1. Analgesic effect of HAEPHF on "Eddy's hot plate induced pain on 4th day

S.No.	Groups	Reaction times in minutes on 4th day		
		60 min	90 min	120 min
1	Control (vehicle 5ml/kg)	4.66 ± 0.49	4.50± 0.42	4.83 ± 0.40
2	HAEPHF (500mg/kg)	5.23 ± 0.12	5.345 ± 0.32	6.01 ± 0.45
3	HAEPHF (1000mg/kg)	7.03±0.603*	8.53 ± 0.41*	7.5 ± 0.76*
4	HAEPHF (2000mg/kg)	8.603±0.60*	9.16±0.47**	8.66±0.71*
5	Diclofinac (6 mg/kg)	9.33± 0.71**	11.02 ±1.03**	10.16±0.79**

Data are expressed as Mean±S.E.M. Test employed ANOVA one way followed by Dunett's test. (n=6) animal in each group. ** (p<0.01),*(p<0.05), ns (non-significant) compared to control group.

Table 2. Analgesic effect of Test Drug on "Eddy's Hot Plate Induced Pain" on 7th day

S.No.	Groups	Reaction times in minutes 7th day		
		60 min	90 min	120 min
1	Control (vehicle 5ml/kg)	3.60± 0.49	3.10± 0.02	3.03 ± 0.20
2	HAEPHF (500mg/kg)	5.23 ± 0.12	5.345 ± 0.32	6.01 ± 0.45
3	HAEPHF (1000mg/kg)	6.53±0.02*	7.63 ± 1.31*	6.05 ± 0.76*
4	HAEPHF (2000mg/kg)	8.87±0.60**	10.06±0.42**	9.66±0.71**
5	Diclofinac (6 mg/kg)	9.63± 0.71**	11.07 ±1.03**	10.86±0.79**

Data are expressed as Mean±S.E.M. Test employed ANOVA one way followed by Dunett's test. (n=6) animal in each group. ** (p<0.01),*(p<0.05), ns (non-significant) compared to control group.

Table 3. Analgesic effect of Test Drug on "Tail immersions test" on 4th day

S.No.	Groups	Reaction times in minutes on 4th day		
		60 min	90 min	120 min
1	Control (vehicle 5ml/kg)	2.60± 0.33	2.88± 0.42	2.83 ± 0.20
2	HAEPHF (500mg/kg)	4.23 ± 0.10	4.34 ± 0.35	3.01 ± 0.44
3	HAEPHF (1000mg/kg)	7.53±0.02*	6.06 ± 1.31*	5.05 ± 0.46*
4	HAEPHF (2000mg/kg)	10.66±0.60**	7.06±0.42**	8.56±0.22**
5	Diclofinac (6 mg/kg)	12.10± 0.71**	11.07 ±1.04**	11.00±0.19**

Data are expressed as Mean±S.E.M. Test employed ANOVA one way followed by Dunett's test. (n=6) animal in each group. ** (p<0.01),*(p<0.05), ns (non-significant) compared to control group.

Table 4. Analgesic effect of HAEPHF on "Tail immertion test" on 7th day

S.No.	Groups	Reaction times in minutes on 7th day		
		60 min	90 min	120 min
1	Control (vehicle5ml/kg)	2.70± 0.33	2.48± 0.42	3.83 ± 0.40
2	HAEPHF (500mg/kg)	4.63 ± 0.30	4.04 ± 0.35	4.01 ± 0.01
3	HAEPHF (1000mg/kg)	7.56±0.03*	7.10 ± 1.41*	6.75 ± 0.43*
4	HAEPHF (2000mg/kg)	10.86±0.63**	7.67±0.42**	9.56±0.02**
5	Diclofinac (6 mg/kg)	12.00± 0.71**	12.23±1.04**	12.00±0.09**

Data are expressed as Mean±S.E.M. Test employed ANOVA one way followed by Dunett's test. (n=6) animal in each group. ** (p<0.01),*(p<0.05), ns (non-significant) compared to control group.

Table 5. Analgesic effect of HAEPHF and diclofenac on writhing test

S.No.	Groups	No. of Writhing in 20 minutes	
		On 4th day	On 7th day
1	Control (vehicle 5ml/kg)	25.16±0.60	26.16±0.50
2	HAEPHF (500mg/kg)	23.16±0.21	20.16±0.41
3	HAEPHF (1000mg/kg)	22.32.16±0.21	21.16±0.21
4	HAEPHF (2000mg/kg)	14.16±0.21*	13.16±0.21*
5	Diclofinac (6 mg/kg)	10.16±0.21**	09.16±0.25**

Data are expressed as Mean±S.E.M. Test employed ANOVA one way followed by Dunett's test. (n=6) animal in each group. ** (p<0.01),*(p<0.05), ns (non-significant) compared to control group.

Table 6. The effect of HAEPHF on locomotor activity

S.No.	Groups	Locomotor activity on 4th day	Locomotor activity on 7th day
1.	Control vehicle (5ml/kg)	137.67±21.93	137.67±21.93
2.	HAEPHF(500mg/kg)	155.50±11.70 ns	154.83±11.75 ns
3.	HAEPHF(1000mg/kg)	98.167±7.77**	94.50±8.44**
4.	HAEPHF(2000mg/kg)	89.167±10.34**	75.00±8.77**
5.	Diazepam(2mg/kg)	95.67±14.28**	76.67±14.28**

Values are in mean (s) ±S.E.Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's test. ns= not significant, $^{*}p<0.05$, $^{**}p<0.01$.

Table 7. The Effects of HAEPHF and diazepam on Rota rod

S.No.	Groups	No of animal falling down within (30sec) from inclined plane	
		on 4th day	on 7th day
1	Control (Vehicle, 6 mg/ml)	323±24.85	343±24.85
2	HAEPHF (500 mg/kg)	219±32.47	229±32.27
3	HAEPHF (1000 mg/kg)	155.5±41.59*	145.5±31.50*
4	HAEPHF (2000 mg/kg)	107.6±2.41**	100.6±4.71**
5	Diazepam (2 mg/kg)	97.6±2.81**	95.6±2.81**

Values are in mean (s) ±S.E. Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's test. Ns = not significant, $^{*}p<0.05$, $^{**}p<0.01$.

Table 8. The Effects of HAEPHF and diazepam on inclined plane

S.No.	Groups	Time spent on revolving rod (Sec)	
		on 4th day	on 7th day
1	Control (Vehicle, 6 mg/ml)	0±0	0±0
2	HAEPHF (500 mg/kg)	0±0	0±0
3	HAEPHF (1000 mg/kg)	0.34±0.11*	0.44±0.11*
4	HAEPHF (2000 mg/kg)	0.52±0.14**	0.62±0.15**
5	Diazepam (2 mg/kg)	0.81±0.12**	0.88±0.12**

Values are in mean (s) ±S.E.Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's test. ns= not significant, $^{*}p<0.05$, $^{**}p<0.01$.

DISCUSSION AND CONCLUSION

In this study, HAEPHF consists of hydro-alcoholic extract of leaves of *Butea frondosa*, roots of *Withania somnifera* aerial parts of *Convolvulus pruricalis*, seeds of *Nigella sativa*, rhizomes of *Curcuma longa*, and leaves of *Azadirachta indic* . It was planned to carry out the pharmacological assessment of polyherbal formulation for CNS activities in rats by using following methods e.g. analgesic activity by *hot* plat method, *t*ail immersion test and acetic acid induced writhing method, CNS depressant/Sedative activity by actophotometer and muscle relaxant activity by inclined plane and rota rod method. The results of these studies reflected that HAEPHF possessed analgesic, CNS depressant/sedative and muscle relaxant activities.

In acute oral toxicity study, all the animals did not observe for any signs of toxicity or mortality for 48 h. HAEPHF 200, 300, 750, 1000, 2000 and 5000 mg/kg body weight were administered to the animal kept for observation for 48 hr. HAEPHF 200, 300, 750, 1000, 2000 and 5000 mg/kg body weight showed no mortality and toxic effect in mice. So, HAEPHF was found to be safe at a dose of 5000 mg/kg. Hence three doses $1/2.5^{th}$, $1/5^{th}$ and $1/10^{th}$ of the doses of 5000mg/kg body weight that is 2000, 1000 and 500 mg/kg of HAEPHF were chosen for recent activities.

The anti-nociceptive activity of HAEPHF was evaluated using both chemical and thermal methods of nociception in mice. These methods are used to detect central and peripheral analgesics. Acetic acid induced writhing test was used for detecting both central and peripheral analgesia, whereas hot plate and tail flick tests are most sensitive to centrally acting analgesics. Thermal induced nociception indicates narcotic involvement (Besra *et al*., 1996). Thermal nociceptive tests are more sensitive to opioid μ receptors and non-thermal tests are to opioid κ receptors (Abbott 1988 and Furst *et al*., 1988). From this experiment it may be assumed that HAEPHF might have some chemical constituents that are responsible to inhibit prostaglandin synthesis or to block pain sensation or might exert other specific mechanism to counteract the pain. HAEPHF was reported to possess the flavonoids (Singh, 2000; Burits, 2000; Kraus *et al*., 1995), were known to inhibits the prostaglandins synthetase (Ramaswamy *et al*., 1985). This was indicating that HAEPHF possessed analgesics activity.

In this test, on 4^{th} and 7^{th} day of treatment, animals treated with HAEPHF (500 mg/kg) did not show significant decrease in locomotor activity while those treated with HAEPHF (1000 & 2000 mg/kg) and diazepam (2 mg/kg) showed more significant decrease in locomotor activity as compared to the control, indicating the sedative like effect. The CNS depressant/sedative effects of HAEPHF could be due to the interaction of flavonoids chemical constituent of HAEPHF (Singh 2000, Burits 2000 and Kraus *et al*., 1995) with the GABA/benzodiazepine receptor complex in brain (Trofimiuk *et al*., 2005). The test drug was found to be more effective than the standard drugs. The test drug

produced better effect on 7th day of treatment compared to the 4th day of treatment.

In muscle relaxant activity, on 4th and 7th day of treatment, animals treated with HAEPHF (500 mg/kg) did not show significant effect while those treated with HAEPHF (1000 & 2000 mg/kg) showed more significant effect, while in inclined plane test, HAEPHF (1000 & 2000 mg/kg) showed significant effect and the standard drug diazepam (2 mg/kg) drug showed more significant effect in both the experiment (rota rod and inclined plane) indicating the muscle relaxant activity. 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 (Allmark *et al*., 1949), HAEPHF (1000 & 2000 mg/kg) also reduced the time spent on the revolving rod by animal in the rotarod test, a test mainly used to screen centrally acting muscle relaxants (Rakotonirina *et al*., 2001). This represented that HAEPHF may have muscle relaxant activity. The muscle relaxant effect of HAEPHF could be due to the interaction of flavonoids chemical constituent of HAEPHF (Singh, 2000; Burits *et al*., 2000; Kraus *et al*., 1995) with the GABA/benzodiazepine receptor complex in brain (Trofimiuk *et al*., 2005).

The above study showed that HAEPHF possessed CNS activities, On 7th day of treatment singly, produced more significant effect in animals compare to the 4th day treatment, if this poly herbal formulation administer for more longer period of time and as twice or thrice daily, this would be produced better effect. Further studies would be necessary to evaluate precise mechanism related to the contribution of active chemical constituents for these activities.

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