Research Article

PHARMACOLOGICAL SCREENING OF ANTIHYPERLIPIDEMIC, ANTI-STRESS AND ANXIOLYTIC ACTIVITY OF POLYHERBAL FORMULATION IN RODENTS

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Abstract: **Background and objectives:** DivyaMedoharVati is a polyherbal formulation comprising of *Commiphorawightii, Shilajita Sat, Terminaliachebula, Terminaliabellerica, Emblicaofficinalis, Picrorrhizakurro, Boerrhaaviadiffusa, Operculinatrupethum* and many other related plant extracts, all of which in Ayurveda, are reported to promote physical and mental health, and improve defence mechanism of body. The present study was undertaken to investigate the anti-hyperlipidemic, anti-stress and anxiolytic activity of this polyherbal formulation. **Methods:** The extent of Anti-hyperlipidemic activity of the polyherbal formulation was assessed after drug treatment by the lipid profile evaluation in Triton-X induced obesity in adult male rats.Atorvastatin was used as the reference standard. The anti-stress activity was observed in anoxic stress tolerance and swimming endurance test models of Swiss albino mice. Aswagandha was used as the reference standard. The anxiolytic activity was estimated in Swiss albino mice using Elevated Plus Maze, and Light and Dark Box Model. Diazepam was used as the reference standard. **Results:** The polyherbal formulation showed significant Anti-hyperlipidemic activity by decreasing the serum Triglycerides and cholesterol levels as well as showed significant increase in HDL levels. The stress and anxiety levels were also significantly reduced. **Interpretation and Conclusion:** The polyherbal formulation showed significant antihyperlipidemic, anti-stress and anxiolytic activity.
Keywords: Hyperlipidaemia, DivyaMedoharVati, Polyherbal formulation, Stress, Anxiety, Diazepam, Clofibrate, Aswagandha

INTRODUCTION

Hyperlipidaemia is a condition characterised by an increase in the amount of fat (such as cholesterol and triglycerides) in the blood. Hyperlipidaemia includes several conditions, but it usually indicates high cholesterol and high triglyceride levels. Stress is a common phenomenon that is experienced by every individual.Stress is involved in the pathogenesis of a variety of diseases that includes psychiatric disorders such as obesity, depression and anxiety, immunosuppression, endocrine disorders including diabetes mellitus, male impotence, cognitive dysfunction, peptic ulcer, hypertension and ulcerative colitis.Anxiety is an unpleasant emotional experience of daily living characterized by a sense of apprehension, uneasiness or impending distress; this feeling is usually associated with changes in the autonomic nervous system and behaviour.

While hyperlipidaemia – may seem to be unrelated to anxiety, depression and other behavioural disorders, these conditions are often thoroughly intertwined, particularly in patients with multiple comorbidities. The most common behavioural disorders linked to hyperlipidaemia are anxiety and depression. Anxiety disorders have been found to increase the risk of high cholesterol. Though the relation between these disorders is unclear, it may impair the quality of life of a person suffering with hyperlipidaemia. The presently used treatments for behavioural disorders are

effective but variety of autonomic, endocrine, allergic, hematopoietic and neurological side effects, on prolonged use. Therefore, there is need for a drug that can treat the hyperlipidaemia as well as its complications, without causing side-effects.

MedoharVati is an indigenous polyherbal formulation containing the *Commiphorawightii, Shilajita Sat, Terminaliachebula, Terminaliabellerica, Emblicaofficinalis, Picrorrhizakurro, Boerrhaaviadiffusa, Operculinatrupethum.*

The aim of the present study is to evaluate the antihyperlipidemic, anti-stress and anxiolytic activity of MedoharVati in rodents.

MATERIALS AND METHODS:
Experimental Animals:

Albino Wistar rats weighing 150 ± 25 gand Swiss albino mice weighing 20 ± 5 g of either sex were used for the study in different disease models. The animals were procured from College Of Veterinary Sciences, Hyderabad at least 2 weeks prior to the study, so that animals could acclimatize to the new environment.

The animals were maintained on a standard diet and water ad libitum. All animals were housed at ambient temperature ($21 \pm 1°C$) and relative humidity ($55 \pm 5\%$)

with fixed 12h/12h light/dark cycle. Animals had free access to standard pellet diet and water given ad libitum. The experimental protocol was approved by Institutional Animal Ethical Committee as per the guidance of committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Drug and Dosage:

The lower and higher dose of PHF was calculated according to daily human dose using conversion factor based on body surface area. The aqueous extract of dry powder of PHF was administered at doses of 100 mg/kg p.oand 200 mg/kg p.oin rats for hyperlipidemic activity and in mice for anti-stress and anxiolytic activity. The solution was prepared freshly before use.

SCREENING OF ANTIHYPERLIPIDEMIC ACTIVITY:

Antihyperlipidemic activity of the Medoharvati was evaluated by comparing the reduction in total cholestral, triglyceride, low density lipoprotein level and significant increase in high density lipoprotien level.

Treatment:
Method:

Hyperlipidemia was induced in Wistar albino rats by single intraperitoneal injection of freshly prepared solution of Triton-X-100 (100 mg/kg) in physiological saline solution after overnight fasting for 18 hrs in groups II-IV. These groups were given a 100mg/kg, i.p single dose of triton. After 72 hours of triton injection, this group received a daily dose of Medoharvati (p.o) for 7 days. On the 8th day, blood was collected by retro orbital sinus puncture, under mild ether anaesthesia. The collected samples were centrifuged for 10 minutes. Then serum samples were collected and used for various biochemical experiments.

Albino Wistar rats were divided into five groups of 6 animals each. The different groups were assigned as described below:

Group I: Animals were administered distilled water (Normal Control)

Group II: Animals were administered Triton X-100 (Positive control)

Group III: Animals were administered Atorvasatin (10mg/kg, p.o.) + Triton X-100 (Standard)

Group IV: Animals were administered Medoharvati (200 mg/kg, oral) + Triton X-100

Group V: Animals were administered Medoharvati (100 mg/kg, oral) + Triton X-100

Estimation of Biochemical Parameters:

Animal were starved for 18hrs after last treatment. Exactly after 18 hrs blood samples were collected through retro orbital puncture under mild ether anaesthesia from rats. Serum obtained by immediate centrifugation of blood at 3000 rpm/10 min for estimation of biochemical parameters such as Serum Total Cholesterol (TC), Serum Triglycerides (TG), Serum HDL Cholesterol (HDL-C), Serum LDL Cholesterol (LDL-C), Serum VLDL Cholesterol(VLDL-C), Serum blood glucose. Triglycerides, cholesterol and HDL were measured with enzymatic kits.

The VLDL- and LDL-cholesterol concentrations were calculated from the Friedewald equation:

LDL cholesterol = Total cholesterol - (HDL cholesterol + VLDL-cholesterol)

VLDL cholesterol = Triglycerides/5

Atherogenic Index was calculated by using the formula of Schulpis:

Atherogenic Index (AI) = Total Cholesterol – HDL/HDL

SCREENING OF ANTI STRESS ACTIVITY:

a) **Anoxic stress tolerance test:**
Grouping of Animals

Hermetic glass vessels of 1 litre air capacity were used in this study. Each vessel was blackened completely except for a small area, which was used as an observation window. These vessels could be made air-tight at the start of the experiments. Mice of the same age and of equal weight were used in these experiments. Each mouse served as its own control.

Four groups of 6 mice each were used.

The different groups were assigned as described below:

Group I: Animals were administered distilled water (Normal Control)

Group II: Animals were administered Aswagandha 50mg/kg p.o.(Standard)

Group III: Animals were administered Medoharvati (200 mg/kg, oral)

Group IV: Animals were administered Medoharvati (100 mg/kg, oral)

Experimental Procedure

Each animal was kept in the hermetic vessel and time was noted by a stopwatch. The moment the animal showed the first convulsion it was immediately removed from the vessel and resuscitated if needed. The time duration from the entry of the animal in the hermetic vessels to the appearance of the first convulsion was taken as time of 'Anoxic Tolerance'. The appearance of convulsion was a very sharp end-point as delay of even 1 minute in removal of the animals killed them.

First observations were made with each animal of each different group and 'Anoxia Tolerance' time duration noted. These animals were then treated with different doses of PHF as described earlier and were exposed to 'Anoxic Stress' after one, two and three weeks of drug treatment and the time duration of anoxia tolerance was noted.

a) **Forced swimming endurance test**
Grouping of Animals

The different groups were assigned as described below:

Group I: Animals were administered distilled water (Normal Control)

Group II: Animals were administered Aswagandha 50mg/kg p.o.(Standard)

Group III: Animals were administered Medoharvati (200 mg/kg, oral)

Group IV: Animals were administered Medoharvati (100 mg/kg, oral)

The animals were divided into four groups of six animals each. In case of swimming endurance test, Group I was administered with distilled water and was not subjected to stress daily for 7 days. Group II, III, IV were subjected to stress for 7 days.

Experimental Procedure

The animals treated with MedoharVati 100 mg/kg and 200 mg/kg were made to swim in a propylene water tank (140x60x45 cm) maintained at room temperature (30±2°C) until they sank. This was recorded as the swimming time. The animals were removed and allowed to recover and dry for about 5 min.

SCREENING OF ANXIOLYIC ACTIVITY:
Grouping of Animals
Male albino mice weighing between 18-25g were divided into four groups, each comprising of six animals and were treated as follows:

Group I: Animals were administered Distilled Water (Normal Control)

Group II: Animals were administered Standard drug

Group III: Animals were administered Medoharvati (200 mg/kg, oral)

Group IV: Animals were administered Medoharvati (100 mg/kg, oral)

Experimental Procedure
a) Elevated Plus Maze Model

The plus-maze apparatus consisting of two open arms (30 x5 x 0.2cm) and two closed arms (30cm x 5cm x 15cm) extending from central platform and was elevated to a height of 45cm above the floor. The entire maze was made up of clear plexiglass.

Prior to the test, animals were treated with vehicle, Diazepam (2 mg/kg, p.o.), MedoharVati (100 and 200 mg/kg, p.o). One hour after the treatments, each mouse was individually placed on the centre of the elevated plus maze with its head facing the open arm. During the entire experiment, mice were allowed to socialize. Every precaution was taken to ensure that no external stimuli, other than the height of the plus-maze could invoke maze anxiety. During the 5 min experiment, following behaviour of the mouse was recorded;

➢ Number of entries into open arm
➢ Number of entries into closed arm
➢ Time spent in the open arm
➢ Time spent in the closed arm and
➢ Time spent in neutral zone
➢ Rearing

Every time before placing each animal, the arena was washed with 5% alcohol to eliminate the possible bias due the odour left by the previous animal.

b) Light And Dark Box Model

The experiment was conducted in a sound attenuated room. A two-compartment chamber (40cm×60cm×20cm) comprising of a brightly illuminated area (40cm×40 cm) and a dark area (40cm×20cm) were separated by a wall with a round hole (7cm diameter) was used.

At the start of the experiment, the mouse was placed in the illuminated area of the chamber. The following parameters were recorded during the test session of 5 min:

• Latency to the first crossing to the dark compartment;
• Number of crossings between the light and dark area;
• Total time spent in the illuminated part of the cage.
• Total locomotion

• Rearing
• Every time before placing each animal, the arena was washed with 5% alcohol to eliminate the possible bias due the odour left by the previous animal.

Statistical Analysis:
Values were expressed as mean ± SEM from 6 animals. Statistical differences in mean were calculated using one way ANOVA (Analysis Of Variance) followed by Tukey's test/ Dunnet's Test and compared with respective control groups. $P<0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION:
ANTIHYPERLIPIDEMIC ACTIVITY
Effect on TC, TG, LDL-C, VLDL-C and glucose level

The experimental results demonstrated a significant increase in serum TC, TG, LDL-C, VLDL-C and glucose level in animals treated with triton X-100 alone as compared to normal control. Whereas prophylactically treated groups like Medoharvati at a dose of 100 mg/kg, 200 mg/kg and Atorvastatin at a dose of 10 mg/kg showed significant reduction in TC, TG, LDL-C, VLDL-C and glucose level compare to hyperlipidemic control group.

Effect on HDL-C level

HDL-C levels showed a significant reduction by animals treated with triton X-100 alone compare to normal control whereas the results of Medoharvati in both doses and atorvastatin expressed a significant increase in HDL-C level compare to hyperlipidemic control group.

ANTI-STRESS ACTIVITY
a) Anoxic Stress Tolerance Test:

PHF treatment enhanced the duration of anoxic tolerance in all the dosages used. In the anoxic tolerance test, the extract (100 mg/kg and 200mg/kg) statistically produced a significant (p<0.05) increase in mean time to convulsion in mice subjected to anoxic stress.

b) Swimming Endurance Test:

PHF treatment enhanced the duration of swimming endurance in all the dosages used. In the anoxic tolerance test, the extract (100 mg/kg and 200mg/kg) statistically produced a significant (p<0.05) increase in mean time to exhaustion in mice subjected to stress due to Swimming Endurance Test.

ANXIOLYTIC ACTIVITY
a) Elevated Plus Maze Test:

Insignificant effect was recorded with number of rearing when compared to control. The aqueous solution of PHF at dose 200 mg/kg showed significant anxiolytic activity by increasing the number of entries in open arms along with time spent in open arms and significant reduction in time spent in closed arms and neutral zone. Lower dose (100 mg/kg) of the PHF showed significant increase in time spent in open arms but the effect on number of entries in open arms and other parameters was insignificant. The effect of both doses of PHF on number of entries in closed arms and rearing was insignificant.

b) Light and Dark Box Model:

100mg/kg and 200mg/kg doses of the PHF were screened for anxiolytic activity by using light-dark model. Both doses increased the time spent in light zone, total locomotion in light zone and number of crossings between the light and dark zone along with decreased time spent in dark zone compared to control. Both the doses of PHF also increased the latency which showed decrease in fear of animal indicating significant anxiolytic activity.

Table 1: table 1 showing effects of Medoharvati and atorvastatin on serum TC, TG, LDL-C, VLDL-C and glucose level.

Treatments	Serum HDL-C	Serum TC	Serum TG	Serum LDL-C	Serum VLDL-C	Serum Glucose
Normal Control	19± 0.31	19.28±0.03	22±0.23	4.8± 0.39	4.25±0.04	61.12± 0.34
Positive ControL	4.2± 0.19***	52.3± 0.04***	35.88± 0.30***	54± 1.7***	12± 0.06***	139.89± 0.24***
Atorvastatin (10mg/kg)	18± 0.04	22.34± 0.3	23± 0.24**	12.1± 1.07	4.4± 0.04**	101.5± 1.11**
Medoharvati (200mg/kg)	16.2± 0.07	22.8±0.35***	24±0.15	13.23± 0.43**	5.2± 0.03	83.1± 0.82
Medoharvati (100mg/kg)	15.8± 0.14**	24.1± 0.23**	26.9± 0.20	15.67± 0.49	7± 0.04	101.9± 0.70*

All values are expressed as mean ± SEM (n=6).
*p<0.05, **p<0.01, ***p<0.001 when compared to control

Table2: table showing effects of Medoharvati and Aswagandha on mean convulsion time

Treatments	Mean Time Before Convulsions (min)
Control	17.62±0.36
Standard	70.81±0.21***
MedoharVati 100mg/kg	35.96±0.57
MedoharVati 200mg/kg	67.11±0.38***

All values are expressed as mean ± SEM (n=6).
*p<0.05, **p<0.01, ***p<0.001 when compared to control

Table3: table showing effects of Medoharvati and Aswagandha on Swimming endurance

Treatments	Swimming Time (sec)
Control	313.8 ± 18.24
Standard	614.2± 5.32***
MedoharVati 100mg/kg	386.2±15.81
MedoharVati 200mg/kg	434.2 ± 20.50***

All values are expressed as mean ± SEM (n=6).
*p<0.05, **p<0.01, ***p<0.001 when compared to control

Table 4: table showing effects of Medoharvati and Diazepam in Elevated Plus Maze Test

TREATMENTS	Elevated plus maze model					
	NUMBER OF ENTRIES (COUNTS/5MIN)		TIME SPENT IN (SEC/5MIN)		TIME SPENT IN NEUTRAL ZONE (COUNTS/5MIN)	REARING (Counts/5min)
	OPEN ARM	CLOSED ARM	OPEN ARM	CLOSED ARM		
Control	3.66±0.55	13.16±1.22	29.16±7.94	218.16±14.28	52.66±7.67	4.83±0.47
Diazepam (2 mg/kg)	12.16±1.04***	15.16±1.04	137.16±9.33***	142.83±7.71***	20.0±3.49**	7.33±0.55
MedoharVati 100mg/kg	5.66±1.02	13.16±1.01	76.5±7.89*	183.83±12.39	39.33±7.11	5.16±0.94
MedoharVati 200mg/kg	10.33±1.14**	14.16±1.16	129.5±11.00***	148.66±8.60***	21.83±5.16*	5.83±1.01

Values are expressed as mean±SEM from 6 mice
P<0.05 *, <0.01 ** and <0.001 *** as compared to control group

Table5: table showing effect of PHF on Light and Dark Box Model

Treatments	Latency (sec)	Time spent in dark zone (sec/5 min)	Time spent in light zone (sec/5 min)	No. of crossings (Counts/5min)	Total locomotion time in light zone (sec/5min)	Rearing (Counts/5min)
Control	10.16±1.86	206.83±9.30	93.16±9.30	4.83±0.70	60.33±6.31	7.66±1.40
Diazepam (100 mg/kg)	19.16±3.13	107.83±8.91[***]	192.16±8.91[***]	11.33±0.98[*]	174.33±10.51[***]	9.66±0.95
PHF (100 mg/kg)	13.33±1.94	170.16±16.22	129.83±16.22	7.16±1.37	106.33±16.59	8.83±1.30
PHF (200 mg/kg)	18.33±2.98	141.16±18.60[*]	158.83±18.60[*]	9.16±1.62	141.83±19.22[**]	8.83±0.87

Values are expressed as mean±SEM from 6 mice
$P<0.05$ *, <0.01 ** and <0.001 *** as compared to control group

CONCLUSION:

This polyherbal formulation contains the extracts which have properties useful in ameliorating stress and anxiety and hence was also screened for its anti-stress and anxiolytic activities in mice. The potent antihyperlipidemic, anti-stress and anxiolytic activity was believed to be because of the active constituents of the MedoharVati.

The findings in this study suggest that the PHF possesses antihyperlipidemic, anti-stress and anxiolytic activity since it decreased the elevated serum cholesterol, triglyceride, LDL and VLDL, as well as glucose levels, corrected the biochemical parameters, increased the HLD-cholesterol levels and decreased anxiety and stress levels. Hence, it can be stated that this polyherbal formulation may be an ideal alternative to the existing synthetic formulation.

Though the ingredients present in the formulation have been reported to haveantihyperlipidemic, anti-stress and anxiolytic activity, the formulation was not previously evaluated preclinically or clinically for the claimed activity. In our study, we have made an attempt to prove its efficacy in experimental animals.

Polyherbal Formulation was found to be safe and effective pre-clinically and hence our results suggest that the polyherbal formulation may be an active and promising formulation for treating hyperlipidemia, stress and anxiety. Future research may be conducted to evaluate the polyherbal formulation for other lipid disorders. As this formulation reduces stress and anxiety levels, it can be evaluated for treating the symptoms associated with the lipid disorders, like stress, anxiety and depression. Patients administered with this polyherbal formulation can be beneficial as there are no side effects and effective in treating in treating hyperlipidemia, stress and anxiety.

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