Research Paper

Free radical scavenging property and diuretic effect of triglize, a polyherbal formulation in experimental models

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ABSTRACT

Objective: To determine the *in vitro* free radical scavenging property and *in vivo* diuretic effect of Triglize™, a marketed polyherbal formulation in experimental models. **Materials and Methods:** The aqueous extract of polyherbal formulation (PHF) triglize was used for the experiment. The free radical scavenging property and antioxidant effect of PHF were studied by LPS-induced free radicals in rat macrophages cells and DPPH (2, 2-Diphenyl-1-Picrylhydrazyl) methods, respectively. The diuretic effect of a PHF was studied with Lipschitz model using male Wistar rats. **Results:** PHF significantly inhibited lipopolysaccharide-induced free radicals in rat macrophages and it showed moderate antioxidant potential in DPPH model. Polyherbal formulation at 50, 200 and 400 mg/ kg significantly increased potassium excretion in urine at 0-5 h and 5-24 h. The diuretic effect of PHF was as similar as furosemide. **Conclusion:** The PHF has significant diuretic effect and free radical scavenging properties.

Key words: Antioxidant, diuretics, polyherbal formulation

INTRODUCTION

Cardiovascular diseases have become a ubiquitous cause of morbidity and a leading contributor to mortality in most countries and this fact is well documented in developed countries. It is projected that the circulatory system disease in India would rise by 103% in man and 90% in women during the period 1985 to 2015.[1] The treatment of cardiovascular diseases with allopathic drugs causes moderate to severe adverse events which could cause death.[2] Hence the alternative systems of medicine are being explored to treat diseases. Ayurvadic system of medicine is practised in India, but the scientific evidence for treating diseases with herbs and fixed herbal formulations are poorly documented. The marketed polyherbal formulation Triglize claims that it can be used for the treatment of obesity, hypertension, palpitation, myocardial necrosis, coronary atherosclerosis, hypercholesterol, ischemic heart diseases and peripheral vascular diseases. Diuretics and antioxidants play a role in the treatment of hypertension and ischemic heart disease. So, the present study was planned to determine the free radical scavenging property of Triglize, a polyherbal formulation (PHF), and its diuretic effect using *in vitro* and *in vivo* models, respectively.

MATERIALS AND METHODS

Polyherbal formulation

Triglize™ (Batch No.: 13335002, Mfg date: Jun, 2005, Apex Laboratories Ltd., Chennai) is a marketed soft gelatin capsule and it was formulated using aqueous extracts of *Terminalia arjuna, Cissus quadrangularis, Boerhaavia diffusa,Commiphora mukul, Phyllanthus embilica, Terminalia*

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bellirica, Terminalia chebula, Tribulus terrestris, Allium sativum and Trigonella foenumgraecum.

Animals

Healthy, adult, male albino rats of Wistar strain, weighing 170 – 200 g were obtained from Animal House, J.S.S. College of Pharmacy, Ootacamund, Tamil Nadu, India. The rats were housed under 22 ± 2° C temperature, 40-60 % humidity and 12:12 ± 1 h light dark cycle. The animals were fed with water and rat pellets *ad libitum*. The rat pellets were supplied by M/s. Hindustan Lever Ltd., Bangalore, India. The study was approved by the Institute Animal Ethics Committee and all the animal experiments were carried out as per CPCSEA guidelines.

In vitro antioxidant activity of polyherbal formulation

The antioxidant activity of test compounds was assessed on the basis of the radical scavenging effects of Greiss Ilosvay reaction.[3,4] The aqueous extract of ployherbal formulation (PHF) and the known antioxidant ascorbic acid were dissolved in DMSO (Dimethyl sulfoxide) (Sigma-Aldrich) separately and used for the *in vitro* antioxidant testing by DPPH (2, 2-Diphenyl-1-Picrylhydrazyl) (Sigma-Aldrich) method. The assay was carried out in a 96 well microtiter plate. To 200 µl of DPPH solution, 10 µl of each of the test and standard solutions were added separately in wells of the 96 well microtiter plates. The final concentration of the test, standard solution used was 15.62 to 1000.00 µg/ ml. The plates were incubated at 37° C for 30 min and the absorbance of each was measured at 490 nm using ELISA reader against the corresponding test and standard blanks and the remaining DPPH was calculated. IC_{50} the concentration of the sample to scavenge 50 % of DPPH free radicals was calculated and recorded.

Effect of PHF on lipopolysaccharide (LPS)-induced free radicals in rat peritoneal macrophage cells

Male Wistar rat (200 g) peritoneal macrophages cells were induced using 3% w/v sodium thioglycolate (i.p.). After 3 days, the macrophages cells were collected, washed with phosphate buffer saline and resuspended with RPMI 1640 medium (Fisher chemicals), supplemented with $NaHCO_3$ (2 g/I), penicillin (100 IU/L), streptomycin (100 µg/ml), gentamicin (20 µg/ml) and fetal calf serum (10%) and plated in 24 wells microtiter plate. Equal amounts of the macrophages cells (2.6×10^6/well) were incubated in humidified 5% CO_2 at 37° C for 24 h to allow macrophages adherence and the plate was washed 3-4 times with serum free fresh medium to dislodge the non-adherent cells. The macrophages were cultured for 3 h and incubated with LPS 25 ng/ml along with five different concentrations of PHF at the concentration of 3 µg - 1000 µg. The aqueous extract of PHF (dissolved in DMSO) was added 2-5 min to the cells before incubation of cell with LPS 25 ng/ml. Finally free nitrites were estimated using greiss reagents and the cell viability was calculated using the MTP (Microculture Tetrazolium) assay method.[5]

Diuretic effect of PHF

Thirty-six male Wistar rats (170-190 g) were divided into six groups containing six animals and grouped as follows:

Group I - Control (0.5% w/v CMC)
Group II - Standard drug treatment (Furosemide- 5mg/kg)
Group III - PHF- 12.5 mg/kg
Group IV - PHF- 50 mg/kg
Group V - PHF- 200 mg/kg
Group VI - PHF- 400 mg/kg

Seventeen to twenty-four hours prior to the experiment, food and water were withdrawn and animals were placed individually in a metabolic cage. The aqueous extract of PHF at various concentrations like 12.5, 50, 200, and 400 mg/kg and reference standard furosemide (5 mg/kg) made as suspension with 0.5% w/v carboxymethyl cellulose (CMC) and administered orally as a single dose. Additionally, 5 ml of 0.9% w/v NaCl solution per 100 g body weight was given by oral gauge. Urine was collected 0- 5^{th} and 5-24^{th} h of dosing and urine volume, acidity (pH) was measured immediately. The electrolyte contents of urine samples were analyzed within 24 h of collection. The sodium, potassium content of urine was determined by flame photometry and concentration of chloride was determined by argentometric titration.[6,7]

Statistical analysis

The mean ± SEM values were calculated for each group. Significant difference between groups was determined using one-way ANOVA followed by Tukey's multiple comparison test. A *P* value less than 0.05 was considered to be significant.

RESULTS

The aqueous extract of PHF showed a moderate antioxidant activity by DPPH method with an IC_{50} value of 219.22 ± 1.64 µg/ml when compared to standard ascorbic acid (IC_{50} = 64.33 ± 0.91). *In vitro* results indicated that thioglycolate activated macrophages were hypersensitive to LPS and produced NO between 55-59 µM/ 2.6×10^6 cells, whereas normal cells produced NO between 17-22 µM/2.6×10^6 cells under similar conditions. NO production was significantly inhibited by simultaneous and pre-incubation with PHF aqueous extract in a dose dependent manner [Table 1].

A summary of the diuretic activity is presented in Tables 2 and 3. The results showed significant increase in urine volume in 0-5 h and 5-24 h. The percentage differences in urine volume in treatment group at the dose levels of 50, 200 and 400 mg/kg b.wt were 115.87%, 129.03% and 151.61% for 0-5 h and 106.4%, 108.0% and 146.4% for 5-24 h when compared to control.

Excretion of potassium ion in urine significantly increased with furosemide 5 mg/kg and PHF 50, 200 and 400 mg/kg

Table 1: Effect of PHF on LPS induced NO production and cell viability on thioglycolate induced rat macrophages

Parameter→ Group↓		NO (µM/well)	Cell viability (MTT Assay values)
Normal		19 ± 1.08	83.13 ± 1.89$^{###}$
LPS (25 ng/ml)		57.5 ± 3.90***	32.39 ± 3.15
LPS (25 ng/ml) + Different concentration of poly herbal formulation (µg)	3.75	52.75 ± 4.70***	39.73 ± 4.36
	7.5	47 ± 4.70***	50.93 ± 1.47$^{#}$
	15	41.75 ± 3.56**	55.94 ± 1.01$^{###}$
	31	34.5 ± 2.59**	57.19 ± 0.54$^{##}$
	62.5	25 ± 5.67*	56.94 ± 1.57$^{##}$
	125	22.75 ± 3.81*	57.14 ± 2.37$^{###}$
	250	22.25 ± 4.21	69.09 ± 3.43$^{###}$
	500	19.73 ± 4.26	67.86 ± 3.88$^{###}$
	1000	21 ± 3.56	81.73 ± 1.81$^{###}$

(Values are mean ± SEM of 4 experiments) : NO estimation as $NO^{\cdot}$ µM /2.6x10^6 macropages cells. IC_{50} concentration: Not fall in the ranges of 3.75µg - 1000 µg. *P* value***<0.001, **<0.01, *<0.05 as compared to control. *P* value $^{###}$<0.001, $^{##}$<0.01, $^{#}$<0.05 as compared to LPS(25ng/ml) control.

Table 2: Effect of PHF on urine volume, pH and electrolytes (0-5th hour urine biochemical estimation)

Treatment	Urine volume (ml)	Urine pH	Concentration of Na ion (µg/ml)	Concentration of K+ ion (µg/ml)	Concentration of Cl- ion (µg/ml)
Control	3.15 ± 0.17	6.70 ± 0.11	0.25 ± 0.01	0.19 ± 0.00	0.20 ± 0.00
Furosemide 5 mg/kg	8.29 ± 0.24***	6.43 ± 0.17	0.26 ± 0.01	0.36 ± 0.01**	0.19 ± 0.00
PHF 12.5 mg/kg	3.30 ± 0.26	6.75 ± 0.11	0.26 ± 0.01	0.19 ± 0.01	0.21 ± 0.00
PHF 50 mg/kg	6.83 ± 0.21***	6.58 ± 0.15	0.16 ± 0.01	0.29 ± 0.01**	0.17 ± 0.01*
PHF 200 mg/kg	7.21 ± 0.15***	6.83 ± 0.11	0.19 ± 0.00	0.28 ± 0.01**	0.19 ± 0.01
PHF 400 mg/kg	7.83 ± 0.21***	6.83 ± 0.11	0.17 ± 0.01	0.30 ± 0.01**	0.17 ± 0.01*

(Values are mean ± SEM of six animals) $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ as compared to control. One-way ANOVA by Tukey's multiple comparison test.

Table 3: Effect of PHF on urine volume, pH and electrolytes (5th-24th hour urine biochemical estimation)

Treatment	Urine volume (ml)	Urine pH	Concentration of Na^+ ion (µg/ml)	Concentration of K^+ ion (µg/ml)	Concentration of Cl^- ion (µg/ml)
Control	6.20 ± 0.27	6.80 ± 0.11	0.29 ± 0.01	0.22 ± 0.01	0.22 ± 0.01
Furosemide 5 mg/kg	14.93 ± 0.61***	6.64 ± 0.15	0.22 ± 0.01	0.32 ± 0.02**	0.18 ± 0.02
PHF 12.5 mg/kg	6.55 ± 0.29	6.75 ± 0.11	0.29 ± 0.01	0.21 ± 0.01	0.23 ± 0.01
PHF 50 mg/kg	12.83 ± 0.29***	6.67 ± 0.17	0.23 ± 0.01	0.31 ± 0.01**	0.23 ± 0.01
PHF 200 mg/kg	12.92 ± 0.68***	6.75 ± 0.11	0.25 ± 0.01	0.32 ± 0.01**	0.22 ± 0.01
PHF 400 mg/kg	15.52 ± 0.64***	6.75 ± 0.11	0.25 ± 0.01	0.34 ± 0.01**	0.21 ± 0.01

(Values are mean ± SEM of six animals) $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ as compared to control. One-way ANOVA by Tukey's multiple comparison test.

($P < 0.01$). The percentage changes in excretion of potassium ion with PHF 50, 200 and 400 mg/kg were 52.63%, 47.36% and 57.87% for 0-5 h urine sample and 40.90%, 45.45%, and 54.45% for 5-24 h urine sample as compared to control.

PHF showed 15% significant reduction in chloride ion excretion at dose levels of 50 and 400 mg/kg at 0-5 h urine sample ($P < 0.05$), but there is no significant reduction of chloride ion concentration in PHF treated animals at 5-24th h urine sample.

DISCUSSION

The results of present study suggest that PHF has free radical scavenging property and diuretic activity. However, the free radical scavenging property or antioxidant property of PHF is moderate when compared to ascorbic acid while the diuretic activity is comparable to frusemide.

Free radicals are formed in the cells as a consequence of both oxidative biochemical reaction and have been implicated in the pathogenesis of wide variety of clinical disorders, resulting from deficient natural antioxidant defences.[8] PHF possesses a series of substituted phenolic compounds and variety of flavones which have known antioxidant property.[9,10] This is the reason why it significantly inhibited NO production in a dose-dependent manner in rat peritoneal macrophage cells in our study.

PHF exhibits diuretic activity at the dose levels of 50, 200 and 400 mg/kg and the effect is comparable to furosemide (10 mg/kg). The mechanism of diuretic activity of PHF is unknown. Diuretics play a key role in the management of congestive heart failure and hypertension.[11,12] Since PHF exhibits significant diuretic activity, it may be useful in the management of vascular and cardiac diseases. Though the *in vitro* results can be extrapolated to *in vivo* system, in the present study, the free radical scavenging property of PHF was studied only for NO free radical. In a living system, the oxidative reaction can occur through many mediators/ toxins and their inducing superoxide and hydroxyl free radicals. Hence PHF should be ideally tested in an *in vivo* system to confirm its free radical scavenging property and what mediators are involved.

As there are no published reports on PHF, the results of our study could not be compared. It has been shown that some of the ingredients in the PHF have antioxidant and diuretic properties.[13-15]

CONCLUSION

PHF exhibits significant antioxidant effect *in vitro* and diuretic effect *in vivo*. Since it is currently approved for use in humans (as an ayurvedic medicine), it is worthwhile to explore the usefulness of the PHF in the treatment of diseases of cardiovascular system such as hypertension and cardiac failure.

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