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Evaluation of Anti-Inflammatory and Antioxidant Activities of Polyherbal Extract by *In Vitro* Methods

N Deattu[1*], N Narayanan[2] and L Suseela[3]

[1]Department of Pharmaceutics, College of Pharmacy, Madras Medical College, Chennai-03.
[2]Jaya College of Pharmacy, Tiruninravur, Chennai.
[3]Jamia Salafia College of Pharmacy, Calicut, Kerala

ABSTRACT

This study reports the *in vitro* anti-inflammatory activity and *in vitro* antioxidant activity of a polyherbal extract (PHE). The PHE was prepared from the the following plants viz, *Saraca indica, Symplocos racemosa, Hemidesmus indicus, Aloe vera, Asteracantha longifolia, Erythrina indica* and *Tribulus terrestris*. In the *in vitro* tests for anti-inflammatory activity, the membrane stabilizing activity and thermally induced protein denaturation were studied. The PHE exhibited significant membrane stabilizing property. Thermally induced protein denaturation was significantly inhibited by the PHE. The antioxidant capacity of the PHE was studied *in vitro* using total antioxidant capacity and reducing power. The results revealed that the PHE showed very potent antioxidant capacity.
Keywords: Polyherbal extract, membrane stabilization, protein denaturation, total antioxidant activity, reducing power.

Corresponding author

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INTRODUCTION

Herbal medicine, sometimes referred to as Herbalism or Botanical Medicine, is the oldest form of healthcare known to mankind. Herbs had been used by all cultures throughout history for their therapeutic or medicinal value. An herb is a plant or plant part valued for its medicinal, aromatic or savoury qualities. Herbal plants contain therapeutically active chemical substances that act upon the body [1]. Keeping all these facts in mind a polyherbal extract has been made from various plants, viz, *Saraca indica, Symplocos racemosa, Hemidesmus indicus, Aloe vera, Asteracantha longifolia, Erythrina indica* and *Tribulus terrestris*. This study reports the *in vitro* anti-inflammatory activity and *in vitro* antioxidant activity of the polyherbal extract.

The *in vitro* anti-inflammatory studies were carried out by the methods of membrane stabilization and inhibition of thermally induced protein denaturation. Inflammation is the reaction of living tissues to injury, infection or irritation. Lysosomal enzymes released during inflammation produce a variety of disorders which leads to the tissue injury by damaging the macromolecules and lipid peroxidation of membranes which are assumed to be responsible for certain pathological conditions as heart attacks, septic shocks and rheumatoid arthritis etc. The extra cellular activity of these enzymes is said to be related to acute or chronic inflammation. Stabilization of lysosomal membrane is important in limiting the inflammatory response by inhibiting the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extra cellular release or by stabilizing the lysosomal membrane. HRBC or erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the extract may as well stabilize lysosomal membranes. Stabilization of human red blood cell membrane (HRBC) by hypo tonicity induced membrane lysis can be taken as an *in vitro* measure of anti inflammatory activity of the polyherbal extract.

Protein denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most biological proteins lose their biological function when denatured. Denaturation of proteins is a well documented cause of inflammation. As part of the investigation on the mechanism of the anti-inflammation activity, ability of polyherbal extract to inhibit protein denaturation was studied.

The present study also reports the *in vitro* antioxidant activity of the PHE. The antioxidant activity was studied in terms of total antioxidant capacity and by determination of reducing power of the PHE.

MATERIALS AND METHODS

The plants were collected from Palode, Kerala and Papanasam, Tamil Nadu. The plants were authenticated by Botanical Survey of India, Coimbatore.

Preparation of the polyherbal extract

The collected plant parts were cleaned and shade dried. The dried plant parts were coarsely powdered and subjected to soxhlet extraction using ethanol. The extract was evaporated in rotary evaporator to get a powdery mass. A solution of the extract was prepared freshly in distilled water to make an appropriate concentration for the studies.

Determination of *in vitro* anti-inflammatory activity

1 Membrane stabilizing activity [2-5]

Whole human blood was obtained from a healthy human volunteer and transferred to heparinized centrifuge tube. The blood was washed three times with isotonic buffered solution (154 mM NaCl) in 10 mM sodium phosphate buffer (pH 7.4) for 10 minutes at 3000 rpm. The test sample consisted of stock erythrocyte (RBC) suspension (0.5 ml) mixed with 5 ml of hypotonic solution (50 mM NaCl) in 10 mM sodium phosphate buffered saline (pH 7.4) containing the extract (10-50 µg) or indomethacin (0.1 mg/ml). The control sample consisted of 0.5ml of RBC mixed with hypotonic-buffered saline solution alone. The mixtures were incubated for 10 min at room temperature and centrifuged for 10 min at 3000 rpm and the absorbance of the supernatant was measured at 540 nm. Each experiment was carried out in triplicate and the average was taken. The percentage inhibition of haemolysis or membrane stabilization was calculated by following equation.

$$\% \text{ Inhibition of haemolysis} = 100 \times (A_1 - A_2/A_1)$$

Where, A_1 = Absorption of hypotonic buffered saline solution alone
A_2 = Absorption of test sample in hypotonic solution

Statistical Analysis

Data obtained from this study were expressed as mean ± SEM. Statistical analysis was performed using Student's t-test and p values less than 0.001 were considered statistically significant.

2 Effect on protein denaturation [6,7]

Test solution(1ml) containing different concentrations of herbal extract (20 – 100µg) or indomethacin (100µg/ml) was mixed with 1ml of egg albumin solution (1mM) and incubated at 27 ± 1° C for 15 min. Denaturation was induced by keeping the reaction mixture at 70° C in a water bath for 10 min. After cooling, the turbidity was measured spectrophotometricaly at 660 nm. Percentage inhibition of denaturation was calculated from control where no drug was added. Each experiment was carried out in triplicate and the average was taken.

Statistical analysis

Data was represented as mean ±SEM, which was statistically analyzed by Student's t-test and p values less than 0.001 were considered to be significant when compared with control.

Determination of *in vitro* antioxidant activity

Total antioxidant activity [8,9]

The total antioxidant activity was evaluated by the method of Prieto *et al*. An aliquot of 0.1 ml of extract was combined with 1 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4mM ammonium molybdate mixture). The tubes were incubated at 95^{o} C for 90 min. The mixture was cooled to room temperature and the absorbance was measured at 695 nm against blank using a spectrophotometer. The antioxidant activity was expressed as the number of gram equivalents of vitamin E.

Reducing power [10]

Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity The reducing power of the polyherbal extract was evaluated by the method of Oyaizu. To 1 ml of extract 0.1ml of DTC reagent (0.4g of thiourea, 0.05g of copper sulphate and 3g of dinitrophenyl hydrazine) were added and incubated at 37º C for 3 hours. After incubation, 0.75ml of 85% HCl was added and incubated at room temperature for 30minutes. The absorbance of the resulting solution was measured at 540nm using a spectrophotometer. The reducing power was expressed as the number of gram equivalents of vitamin C.

Statistical Analysis

All experiments were carried out in triplicate, and the results are expressed as mean ± SEM. The significance between the results was assessed using the Student's t-test and significance was accepted for p-values< 0.01 and 0.05.

RESULTS AND DISCUSSION

The results of the study showed that the PHE possesses significant anti-inflammatory and antioxidant properties. The anti-inflammatory activity of the PHE may be related to the membrane stabilizing property and inhibition of thermally induced protein denaturation. The *in vitro* antioxidant activity was assessed by the determination of total antioxidant activity and reducing power.

The PHE exhibited membrane stabilization effect by inhibiting hypotonicity induced lyses of erythrocyte membrane. In the study of membrane stabilization activity the PHE at

concentration range of 10-50µg /ml protect significantly ($p<0.001$) the erythrocyte membrane against lysis induced by the hypotonic solution. At a concentration of 50µg /ml the PHE showed 63.76 % inhibition of haemolysis whereas indomethacin at 0.1mg/ml showed 55.50 % inhibition of RBC haemolysis when compared with control. The results are shown in Table 1.

Table 1: Effect of polyherbal extract in erythrocyte haemolysis

Concentration		Absorbance at 540nm Average ± SEM	% Inhibition of haemolysis
Control		0.218 ± 0.0014	-
Indomethacin (0.1 mg/ml)		0.097 ± 0.001	55.50
Polyherbal extract	10 µg /ml	0.199 ± 0.002***	8.72
	20 µg /ml	0.157 ± 0.001***	27.98
	30 µg /ml	0.128 ± 0.001***	41.28
	40 µg /ml	0.079 ± 0.002***	63.76
	50 µg /ml	0.079 ± 0.001***	63.76

Each value represents the mean ± SEM of 6 experiments.
*** $p<0.001$ when compared to control

Denaturation of proteins is a well documented cause of inflammation. Several anti inflammatory drugs have shown dose dependent ability to inhibit thermally induced protein denaturation. Ability of the PHE to inhibit thermal denaturation of protein is possibly a contributing factor for its anti inflammatory activity. The inhibitory effect on protein denaturation is shown in Table 2. The PHE (20-100µg/ml) showed significant ($p<0.001$) inhibition of denaturation of egg albumin in a concentration dependent manner. The PHE at concentration of 100µg /ml and indomethacin at concentration of 100µg /ml showed significant inhibition of protein denaturation when compared with control.

Table 2: Effect of polyherbal extract in protein denaturation

Concentration		Absorbance at 660nm Average ± SEM	% Inhibition of denaturation
Control		1.36 ± 0.012	-
Indomethacin (100 µg/ml)		0.38 ± 0.026	72.06
Polyherbal extract	20 µg /ml	1.09 ± 0.015***	19.85
	40 µg /ml	0.86 ± 0.006***	36.76
	60 µg /ml	0.72 ± 0.018***	47.06
	80 µg /ml	0.49 ± 0.012	63.97
	100 µg /ml	0.37 ± 0.006^{ns}	72.79

Each value represents the mean ± SEM, n=3 ***$p<0.001$compared with control

The results of total antioxidant activity and reducing power are shown in Table 3.The PHE showed very potent antioxidant activity.Total antioxidant capacity by Phosphomolybdenum method assay is based on the reduction of Mo(VI) to Mo(V) by the sample and the subsequent formation of green phosphate / Mo (V) complex at acidic pH. The total antioxidant activity is expressed as the number of gram equivalents of vitamin E. Reducing power is associated with antioxidant activity and may serve as a significant reflection

of the antioxidant activity. The reducing power is expressed as the number of gram equivalents of vitamin C.

Table 3: Total antioxidant activity and reducing power

S.No.	Total antioxidant activity	Reducing power
1.	0.7012 ± 0.01**	0.7041 ± 0.02*

Values are expressed as mean ± SEM, n=3
** & * p value of 0.01 & 0.05 respectively

CONCLUSION

The present study concludes that the PHE exhibits promising *in vitro* anti-inflammatory activity and *in vitro* antioxidant activity.

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