Original Article

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Pharmacology Section

Studies on the in Vitro Anti-Oxidant Properties of a Polyherbal Formulation: Rumalaya Forte

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ABSTRACT

The Polyherbal Formulation (PHF), Rumalaya Forte that has a combination of medicinal herbs such as the powders of Boswellia serrata, Commiphora wightii, Alpinia galanga and Glycyrrhiza glabra and the extracts of Tribulus terrestris and Tinospora cordifolia, was tested for its antioxidant and free radical scavenging activity in vitro. This PHF has been traditionally used by the ayurvedic practioners in India, for the treatment of various inflammatory disorders. The in vitro scavenging of the NO radical activity, Lipid Peroxidation (LPO) inhibition and the 1-Diphenyl-2-Picrylhydrazil (DPPH) inhibition of PHF were tested by using a spectrophotometer at absorbance levels of 546nm, 532 and 517nm respectively. The experiments were performed in triplicates with different concentrations of PHF (1.95-500 g/ml).The percentage inhibition IC50 values were 203.57%, 315.92% and 10.37% g/ml for LPO, DPPH and NO respectively. The polyherbal formulation, Rumalaya Forte demonstrated a significant NO free radical scavenging activity.

Key Words: Nitric oxide, Poly herbal formulation, Free radical scavenging, Spectrophotometer, Ayurveda, Inflammation

INTRODUCTION

Today, herbal medicines are manufactured similarly as the different dosage forms of the modern system. Though the plants have been proven for their efficacy since centuries, the documentation of the evidences are not available for every practice. Hence, herbal medicines need to be revalidated at present for the evidence based medicine practice.

In the advent of the internationally accepted protocol for the evaluation of a drug and with due consideration to the established understanding of the plants, as is available in the literature of ayurveda, a better utilization may be achieved.

Oxidative stress results from an imbalance between the generation of the oxygen derived radicals and the organism's endogenous antioxidant potential to counteract. Such an imbalance plays an important role in many chronic diseases [1]. There is an increasing evidence which has shown the involvements of free radicals and Reactive Oxygen Species (ROS) in a variety of diseases, that can cause damage to the cellular biomolecules such as nucleic acids, protein, lipids and carbohydrates and this consequently may adversely affect the immune functions [2]. The efficacy of a plant extract as an antioxidant is best evaluated, based on the results which are obtained by commonly accepted assays, taking into account the different oxidative conditions, system compositions and the antioxidant mechanism [3]. It is believed that medicinal plants are a potential source of antioxidants and ROS scavenger molecules [2]. The natural antioxidants tend to be safer and they possess anti-viral, anti-tumour and hepatoprotective properties [4]. The free radicals and ROS are well-known inducers of cellular and tissue pathogenesis which leads to several chronic human ailments such as rheumatoid arthritis and cancer, as well as the ageing processes. Many anti-inflammatory, digestive, neuroprotective, hepatoprotective and anti-ulcer herbal drugs have been recently evaluated and they have been shown to possess anti oxidant and/or free radical scavenging mechanisms as a part of their activity [5].

Hence, this Polyherbal Formulation (PHF), Rumalaya Forte (RF) which contains medicinal herbs such as the powders of Boswellia serrata, Commiphora wightii, Alpinia galangal and Glycyrrhiza glabra and the extracts of Tribulus terrestris and Tinospora cordifolia, which is being currently widely used by ayurvedic practioners for the treatment of inflammatory conditions, was screened for its anti-oxidant activity by checking for DPPH, LPO and NO radical inhibition by doing in vitro assays. Though the individual herbs which had been studies previously had shown anti-inflammatory activities, this study was done to explore the antioxidant potential of the PHF –RF by in vitro studies.

MATERIALS AND METHODS

Plant material

PHF contains medicinal herbs such as the powders of *Boswellia serrata* 240mg, *Commiphora wightii* 200mg, *Alpinia galanga* 70mg and Glycyrrhiza glabra 70mg and the extracts of *Tribulus terrestris* and *Tinospora cordifolia*.

Use in traditional medicine

Boswellia serrata- anti-inflammatory, antiarrthritic, expectorant [6].

Commiphora wightii – It has astringent, antiseptic and antisuppurative properties and it is used in the treatment of haemorrhoids [7].

Alpinia galanga - Anti-inflammatory agent which is used for rheumatism and rheumatoid arthritis [8].

Glycyrrhiza glabra - Antitioxidant, immunostimulant and mild anti-

inflammatory activities [9].

Tribulus terrestris – Used for urolithiasis, dysurea, impotence and kidney dysfunction [10].

Tinospora cordifolia- Which is used for dyspepsia, fevers and urinary diseases [11].

The previously isolated classes of the constituents

Commiphora wightii - The principal constituent is Guggulusterones Z and E, gugulsterols I-V and two diterperoids – a terpene hydrocarbon which is named cembrane-A [7].

Glycyrrhiza glabra- The principle constituent is glycyrrhizin [9].

Tribulus terrestris- The principle constituent is harmine [10].

Tested material

The PHF, Rumalaya Forte tablet which contains the powders of *Boswellia serrata, Commiphora wightii, Alpinia galangal* and *Glycyrrhiza glabra* and the extracts of *Tribulus terrestris* and Tinospora cordifolia, was obtained from the local medical store. The experimental protocol was subjected to the scrutiny of the institutional animal ethical committee and it was approved (IAEC-XII/SRU/74/2008).This study was done on February 2009 at the Department of Biotechnology, Sri Ramachandra Medical College and Research Institute, Sri Ramachandra University, Chennai, India.

Studied activity

1. *In vitro* lipid peroxidation inhibition assay [12]:

A 10% liver homogenate (chicken liver homogenate which was purchased from a local butcher's shop) was prepared by using ice-cold KCl (0.15M) in a Teflon tissue homogenizer and the protein content was adjusted to 500 g/ml. In the control system (1 ml of the tissue homogenate), lipid peroxidation was initiated by the addition of FeSO4 (25M), ascorbate (100 M) and KH2PO4 (10mM) and the volume was made up to 3ml with distilled water. This mixture was incubated at 37^{0}C for 30 minutes. In the test system, the homogenate was incubated with different concentrations of PHF (1.95-5000g/ml). The extent of inhibition of the lipid peroxidation was evaluated by the estimation of the Thiobarbituric Acid Reactive Substances (TBARS) levels by measuring the absorbance at 532 nm [13]. The percentage inhibition of the lipid peroxidation was calculated by the formula.

(%) Inhibition = [(control-test)/control)]/100

2. Scavenging of the nitric oxide radical activity [14]:

Aqueous sodium nitroprusside at a physiological pH, spontaneously generates Nitric Oxide (NO) which interacts with oxygen to produce nitrite, which can be estimated by the use of the Greiss reagent. Sodium nitroprusside (5 mM) in phosphate buffered saline was mixed with 3 ml of different concentrations (1.95-500 µg/ml) of the PHF which was dissolved in methanol and incubated at 25^{0}C for 150 minutes. The samples from the above were allowed to react with the Greiss reagent. The absorbance of the chromophore which was formed during the diazotization of nitrite with sulphanilamide and its subsequent coupling with naphthylethylenediamine was read at 546nm [15]. The experiments were repeated in triplicates. The percentage scavenging of the nitric oxide radical activity was calculated by the formula which has been given below and the results were computed.

(%) NO scavenged = [(control-test)/control)]/100

[3]. Determination of the DPPH (one, 1-diphenyl-2-picrylhydrazil) radical scavenging activity [4]:

The DPPH assay was performed as has been described. About 10 µL of each PHF test sample solution of different concentrations (1.95-500 µg/ml) was added to 190 µL DPPH (150 µM) in ethanol solution. After vortexing, the mixture was incubated for 20 minutes at 37°C. The control blank contained the solvent without the PHF. The decrease in the absorbance of the test mixture (due to quenching of the DPPH free radicals) was measured at 517 nm and the percentage inhibition was calculated. The IC 50 (Inhibition Concentration) values were determined as the concentrations of the PHF test mixtures that gave a 50% reduction in the absorbance from a control blank.

Statistical analysis

All the in vitro experiments were performed in triplicate. The IC50 values were calculated by linear regression analysis.

RESULTS

In the present study, the percentage inhibition of the PHF, Rumalaya Forte was assayed by the in vitro Lipid Peroxidation inhibition assay (LPO) , by the evaluation of the nitric oxide free radical scavenging activity and by the determination of the DPPH (one, 1-diphenyl-2-picrylhydrazil) radical scavenging activity in various concentrations which ranged from 1.95 g/ml to 500 g/mg in a geometric progression.

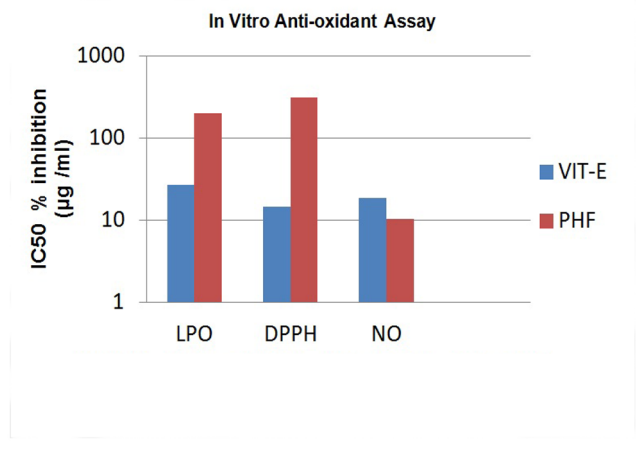
Lipid peroxidation inhibition

The PHF, Rumalaya Forte showed free radical scavenging by LPO in a concentration dependent manner. The IC50 value of the formulation was found to be 203.0 µg /ml, which indicated mild activity. This was not significant as compared to the positive control, Vitamin E, where the IC50 value was 27 µg /ml.

Inhibition of the DPPH radical.

The potential to scavenge the DPPH free radical of the PHF, Rumalaya Forte was not significant with the various assayed concentrations of 1.95-500 µg /ml. The inhibitory concentration (IC50) of the PHF is found to be 315.92 µg/ml, which was not significant as compared to that of the positive control, Vitamin E, which was 14.4 µg/ml.

Scavenging of the nitric oxide radical activity



[Table/Fig-1]: Effect of PHF on LPO, DPPH and NO In Vitro anti-oxidant assay, Values are µg /ml

The PHF, Rumalaya Forte revealed that a potential significant inhibition of the nitric oxide free radical scavenging activity, which was mainly concentration dependent with the inhibitory concentration (IC50), at which there was a 50% free radical inhibition, was found to be 10.37 µg /ml as compared to that of the positive control, Vitamin E, which was 18.5 µg /ml.

DISCUSSION

The present investigation was performed to test the hypothesis "Does the PHF, Rumalaya Forte have any antioxidant activity by which it controls inflammatory conditions?"

The alterations in the oxidant and the antioxidant profile are known to be involved in the pathophysiology, thus affecting the cell and its components, causing damage to them and releasing their products as markers [16]. Many earlier studies have shown the influence of lipid peroxidation, oxidative stress and the antioxidant status on carcinogenesis [17,18]. In an inflammatory setting or in the presence of endotoxins and cytotoxines, the inducible calcium independent Nitric Oxide Synthase (iNOS) is expressed in numerous cell types which include endothelial cells, smooth muscle cells and macrophages [19].

NO is an important physiologic messenger and effector molecule in many biological systems which include immunological, neuronal and cardiovascular tissues [20]. NO is an important signaling and effector molecule in inflammation and immunity, as it is known to couple with superoxides to form peroxynitrite. These, in turn, induce the production of prostaglandin endoperoxide synthase from the monocytes/macrophages, resulting in an enhanced synthesis of prostaglandins which are the established mediators of inflammation [21]. In the chronic synovial inflammation of arthritis, macrophages play a central role in the inflammation. The nitric oxide in the macrophages is produced as a free radical by iNOS by the catalyzation of the oxidation of the guanidino nitrogen of L-arginine, thereby converting L-arginine to L-citrulline [22].

The above data has shown that this PHF, Rumalaya forte contains medicinal herbs such as the powders of *Boswellia serrata, Commiphora wightii, Alpinia galangal* and *Glycyrrhiza glabra* and the extracts of *Tribulus terrestris and Tinospora cordifolia*, which have a high NO free radical scavenging activity with an IC50 10.37 g/ml. This NO free radial scavenging activity of this PHF provides a different level of inflammation control and it thus provides a synergistic effect in controlling the inflammatory conditions.

CONCLUSION

The PHF, Rumalaya Forte formulation which was screened for its antioxidant effects, showed an NO free radical scavenging activity. However, it did not exhibit any appreciable LPO inhibitory activity, nor any DPPH radical scavenging activity.

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