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Antiasthmatic Role of "Pentapala -04" A Herbal Formulation Against Ova Albumin and Aluminium Hydroxide Induced Lung Damage in Rats.

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ABSTRACT:

Bronchial asthma is a clinical syndrome characterized by proximal dysphasia and wheeze due to increased resistance to the flow of air through the narrowed bronchi. Asthma has become the most common chronic disease in the world and epidemiological studies suggest that its prevalence, severity and mortality are rising at a time when mortality from other common treatable conditions is falling. The reasons for the above statistics are environmental factors such as increased exposure to allergens and atmospheric pollutants. Antiasthmatic treatment includes corticosteroids, which are very effective in the treatment of asthma. But corticosteroids are costly and if given systemically, have many severe adverse effects. Hence, the present research work involves the use of a herbal compound formulation Pentapala -04 prepared from five medicinal plants namely, *Adhatoda vasica Need, Ocimum sanctum Linn, Coleus aromaticus Benth, Glycyrrhiza glabra Linn* and *Alpiania galangal Sw.*

The effect of "Pentapala-04" on ova albumin and aluminium hydroxide induced lung damage in albino wistar rats was investigated. The rats were divided into three groups of four animals each. Group I, II and III serves as control, toxic and post treatment group respectively. Our results showed that their was increased level of lipid peroxidation and secreased level of antioxidants in toxic group animals. But the levels of antioxidant enzymes were restored in post-treated groups of animals, which might be due to the ability of "ability of "Pentapala-04 to scavenge the reactive oxygen species.

Key words:- Ova albumin, alumininum hydroxide and Pentapala-04

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years, and an impressive number of modern drugs have been isolated from natural source many based on their use in traditional medicine[1]. These plant-based traditional medicine system continue to play an essential role in health care, with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care[2].

The lung is directly exposed to higher levels of oxygen than most other tissue[3]. Normal human lung is efficiently protected and "buffered" against exogenous free radicals4. Toxic free radicals have been implicated as important pathologic factors in pulmonary

diseases, cardiovascular diseases, autoimmune diseases, inherited metabolic disorders, cancer and aging[5]. Oxidative stress rises when the balance between pro-oxidants and antioxidants is shifted to wards the pro-oxidants[6]. in normal individuals, the level of lipid peroxidation in the lungs is very low because of the powerful antioxidant system. Under certain conditions the antioxidant reserve can be depleted[7]. the lung antioxidant levels are decreased in non-malignant lung disorders such as in asthma and chronic obstructive lung disease[8].

It has long been recognized that naturally occurring substances in higher plants have antioxidant activity. Recently, there is a growing interest in oxygen containing free radicals in biological system and their implied roles as causative agents in the etiology of variety of chromic disorders[9].

Hence, the present study involves the use of a herbal formulation "Pentapala 04" prepared form various parts of five medicinal plants namely *Adathoda vasica, Ocimum sanctum, Coleus aromaticus, Glycyrrhiza glabra, Alpinia galangal* in the prevention and cure of ova albumin ans aluminium hydroxide induced lung damage in rats.

Materials and method

Plant Materials

Apolyherbal drug formulation consisting of five different plants viz.,

Plant names	Part Used	Percentage
Adhatoda vasica;	Nees.Leaves	30%
Ocimum sactum:Linn	Leaves	15%
Coleus aromaticus; Benth	Leaves	15%
Glycyrrhiza glabra; Linn.	Rhizome	20%
Alpinia galangal; Sw	Rhizome	20%

Young, fresh, leaves and rhizomes were collected from Palani hills, Tamilnadu, (India).

Preparation of Extracts

Aqueous extract was prepared under boiling water (<100oC) at normal pressure[10]. These aqueous extract was subjected to studies in laboratory animals.

Animals

Wistar starin of albino rats (160-200g) obtained from P.S.G Institute of Medical Sciences, Coimbatore, were used in these experiments. The animals were keep under standard laboratory conditions and feed with standard pellet diet form Hindustan Lever Ltd., (Mumbai) and water *ad libitum*. The pellet composition was found to be similar to R.D.A for laboratory animals as described earlier[11]. All the studies were conducted in accordance with national Institute of Health "Guide for the care and use of laboratory animals" and the experiments were carried out as per the Institution Ethic committee.

Induction of asthma in rats

Lung damage was induced by the subcutaneous injection of 1ml of saline containing 1mg of ova albumin and 200 mg aluminum hydroxide. At the same time 1ml

of *Bordetella pertussis* (from Canada) vaccine containing $6x10^9$ heat killed organisms was given intraperitoneally as adjuvant for 21 days twice per day[12].

Experimental design

After the induction of lung damage, the rats were divided into two groups of six animals each and the whole experiment was done twice. Rats in the control group received normal diet.

Group I	Control group fed with normal diet.
Group II	Toxic group animals, asthma, asthma induced by Administrating ova albumin and aluminum hydroxide.
Group III	Post treatment group, asthma induced and simultaneously followed by treatment with polyherbal drug pentapala – 04 (0.2mg/kg body weight) from second day after sensitization.

Preparation of tissue extracts

After the experimental regimen, the animals were sacrificed under mild chloroform anesthesia. Lung, liver and kidney were excised immediately and washed with cold saline and 10% homogenate of these organs were prepared with Tris HCl buffer (pH7.4). The tissues homogenates were assayed.

Biochemical analysis of lung, liver and kidney

The antioxidants like superoxide dismutase, catalase, peroxidase, glutathione peroxidase, glutathione peroxidase, vitamin E and lipid peroxidation were assayed according to the method described by Dos et al., (2002)[13], Sinha (1972)[14], Moran et al., (1979)[15], Rotruck et al., (1979)[16], Varley (1976)[17] and Buege et al., (1978)[18] respectively.

Statistical analysis

Values were represented as mean ±SD. Data were analysed using one-way analysis of variance (ANOVA) and group means were compared using Duncan's multiple range tests. P. values<0.05 were considered significant.

Results and Discussion

The lung is an active metabolic organ[19] and is line with a highly surface active material that maintains alveolar stability at low lung volume[20]. The lungs and the pulmonary vasculature are potentially at high risk of injury mediated by oxygen-derived free radicals and lipid peroxidation free radical[21]. The lungs are particularly susceptible to lesions by free radicals and pulmonary antioxidant defenses are extensively distributed and include both enzymatic and non-enzymatic systems[22].

Based on this fact, our investigation was aimed to study the pathological effect of (Reactive Oxygen Species) in lug diseases and the role of "Pentapal -04" in treating the lung diseases.

Lipid peroxidation in lung, liver and kidney

Basal, ferrous sulphate and ascorbate induced lipid peroxidation in lung, liver and kidney homogenate of control and experimental rats are depicted in Table I.

Oxidative damage induced by ova albumin and aluminum hydroxide resulted in the

formation of highly reactive hydroxyl radicals, which stimulated lipid peroxidation, which causes destruction of the cell membrane. Oxygen free radicals and H_2O_2 are closely involved in the pathogenesis of asthma. Oxygen free radicals are responsible for a wide range pf tissue injury such as atherosclerosis and inflammation[23]. In the present study, rats induced with ova albumin and aluminum hydroxide showed significant ($P<0.05$) increase of MDA release both under basal and induced conditions when compared to control (Group I) rats.

After the simultaneous treatment with the herbal formulation "Pentapala-04" along with ova albumin and aluminum hydroxide (group III) resulted in significant ($P<0.01$) fall in the levels of MDA as compared to toxic (group II) rats, which might be due to the potential free radical scavenger in "pentapala-04". Formation of ROS, oxidative stress and lung injury have been implicated to respiratory diseases. It has been documented that lung cells are exposed to more oxygen than any other cells in the body and are major sources of ROS during ova albumin and aluminum hydroxide consumption, and these are primed and activated for enhanced formation of pro-inflammatory factors. Moreover, ova albumin and aluminum hydroxide induced lung damage has been associated with increased amount of lipid Peroxidation. Pentapala – 04 supplementation in our study was potentially effective in blunting lipid peroxidation, suggesting that it possibly has antioxidant property to reduce ova albumin and aluminum hydroxide induced membrane lipid peroxidation and there buy to preserve membrane structure. The present investigation is in agreement with results of Ernst (1998)[24] and Bielory et al., (1999)[25] who showed that the lipid peroxidation level was decreased following the administration of polyherbal extract.

Enzymatic and non-enzymatic antioxidant in lung

The results of the changes in the levels of enzymatic and non-enzymatic antioxidants like superoxide Dismutase (SOD), Catalase (CAT), Reduced glutathione (GSH), Peroxidase (GP_x) and Vit-E (â tocopherol) in the lung of normal are experimental rats and illustrated in Table-II.

From the table II, it is evident that all the estimated enzymatic and non-enzymatic antioxidants decreased in toxic (groupie) animals. Subsequently they increased after the treatment with our prepared herbal formulation "Pentapala-04".

The SOD, CAT, GP_x, GSH and Vit E activity were significantly ($P<0.01$) higher in control (Group I) as compared to toxic (Group II). After the treatment with herbal drug, the activities significantly came to normal ($P<0.01$). SOD protects tissue against oxygen free radicals by catalyzing the removal of superoxide radical (O_2), which damages the membrane and biological structure[26,27]. Catalase represents a H_2O_2 scavenging enzyme with optimal activity at high H_2O_2 concentrations. In the lung, catalase is localized mainly in alveolar macrophages and alveolar epithelium[28]. Catalase has been shown to be responsible for the detoxidation of significant amounts of H_2O_2[29] . GPx catalyses the reduction of H_2O_2 to H_2O and O_2 at the expense of GSH[30]. Rat lung GP_x activity is due to both a seleno enzyme and to a non selenium dependent enzyme[31]. Therefore it seems logical to infer that "Pentapala-04" because of it antioxidant property, might be capable of protecting lung tissue from ova albumin and aluminum hydroxide induced injury and

inflammatory changes in the lungs. This was found to be similar to the earlier observation that herbal extract have antioxidant properties[32].

COUNCLUSION

To conclude, we demonstrate that 'pentapala-04' prevents ova albumin and aluminum hydroxide induced oxidative stress, lung injury and inflammatory changes. Based on the above findings, we infer that herbal formulation "pentapala-04" is effective against lipid peroxidation and could be used as an antiasthmatic drug.

Table I

Effect of pentapala 04 on the levels of lipid peroxidation in lung, liver and kidney of control and experimental rats.

Group	Control (Group I)	Toxic (Group II)	Treatment (Group III)
Basal			
Lung	0.30 ± 0.14	1.49 ± 0.56a**	0.29 ± 0.08b**
Liver	0.49 ± 0.23	1.74 ± 0.30a**	0.37 ± 0.05b**
Kidney	0.77 ± 0.24	2.39 ± 0.25a**	0.74 ± 0.26b**
Fe SO4	0.52 ± 0.17	1.73 ± 0.20a**	0.43 ± 0.03b**
Lung	0.35 ± 0.08	2.45 ± 0.24a**	0.24 ± 0.06b**
Liver	0.35 ± 0.25	1.45 ± 0.45a**	0.24 ± 0.10b**
Kidney			
Ascorbate	0.39 ± 14	0.76 ± 1.2a**	0.39 ± 1.14b**
Lung	0.27 ± 0.06	0.85 ± 0.11a**	0.22 ± 0.08b**
Liver	1.13 ± 0.10	2.48 ± 0.32a**	1.18 ± 0.19b**
Kidney			

Values are expressed mean ± S.D (n=4)

Statistical Comparison

A: Group II Compared with Group I

B: Group III Compared with Group II

Units:

LPO – nmoles of MDA formed/min mg of protein. **P<0.01

Table –II
Effect of pentapala-04 on the levels of enzymatic and non-enzymatic antioxidants in lung of control and experimental rats.

Group	Control (Group I)	Toxic (Group II)	Treatment (Group III)
SOD	15.38 ± 1.65	9.93 ± 1.2a***	11.92 ± 1.2 b**
Catalase	55.28 ± 2.29	37.83 ± 1.00 a**	52.93 ± 2.50b**
GPX	2.40 ± 0.74	0.71 ± 0.36a**	2.24 ± 0.75b**
GSH	1.21 ± 0.17	0.50 ± 0.21a**	1.36 ± 0.21b**
Vit E	1.98±0.10	0.49± 0.34a**	1.72 ± 0.20b**

Value are expressed as mean ± SD (n=4)

Statistical Comparison

A: Group II Compared with Group I
B: Group III Compared with Group II

Units:

SOD – 50% inhibition of nitrate/min/mg protein
CAT - ỉ moles of H2O2 decomposed/min/mg protein
GPX - ỉ of GSH utilized /min/mg protein
GSH - ỉ of GSH consumed /min/mg protein
Vit E - ỉ g/mg protein
**P<0.01

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