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Effect of *Rhinacanthus nasutus* extracts on the non-enzymic antioxidant status of oxidant induced goat liver slices

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The present study was carried out to evaluate the antioxidant status of three different extracts of *Rhinacanthus nasutus* leaves on the oxidant induced goat liver slices under *in vitro* conditions. The antioxidant potential of all the extracts were assessed by analysing the non-enzymic antioxidants. The extracts were also analysed for the extent of inhibition of lipid peroxidation in H_2O_2 induced goat liver slices. The results of the present study showed that the methanolic extract was found to contain highest amount of non-enzymic antioxidants followed by the aqueous extract and the chloroform extract. It is evident that *Rhinacanthus nasutus* leaf extracts offered efficient antioxidant defense in the goat liver slices, an *in vitro* model which simulates *in vivo* condition, when exposed to H_2O_2. Health benefits can be obtained from the leaves with decreased risk of disease as the leaves could prevent or protect the oxidative damage caused by environmentally benign oxidant hydrogen peroxide.

Key words : Vitamin C, Vitamin E, Vitamin A and Reduced glutathione, free radicals, Methanolic extract, Lipid Peroxidation, *Rhinacanthus nasutus*

INTRODUCTION

Natural products have served as a major source of drugs for centuries, and about half of the pharmaceuticals in use today are derived from natural products. The use of natural substances particularly those derived from plants, to control diseases is a centuries old practice that has led to the discovery of more than half of all modern pharmaceuticals. A growing worldwide interest in the use of phytopharmaceutical as complimentary or alternative medicine either to prevent or ameliorate many diseases have been noted in recent years (Krishna, 2008).

Biological compounds with antioxidant properties contributed to the protection of cells and tissues against deleterious effects of Reactive Oxygen Species (ROS) and other free radicals. Protective agents from plant origin with antiperoxidative and antioxidant properties play an important role in protecting the liver against toxicity. Traditional medicines are effective in certain disorders and are based on experience in the use of plant products in amelioration of common diseases (Jayaprakash and Chinnaswamy, 2007).

Free radicals have been implicated in the causation of several diseases such as liver cirrhosis, atherosclerosis, cancer and diabetes. The compounds that can scavenge free radicals have great potential in ameliorating these disease processes. Antioxidants thus play an important role to protect the human body against damage by Reactive Oxygen Species. An antioxidant is a molecule stable enough to donate an electron to a rampaging free radical and neutralize it, thus reducing its capacity to damage. The mechanism involves significant inhibition or delay in the oxidative process. As per biochemists and epidemiologists, antioxidant neutralizes free radicals by binding their lone pair of electrons and rendering them harmless (Shivaprasad *et al.*, 2008).

Reactive oxygen species are generated continuously in the body by both endogenous and exogenous factors like normal aerobic respiration by stimulated polymorphonuclear leucocytes macrophage and exposure to various pollutants like tobacco smoke, ionizing radiation, organic solvents, pesticides and various lipid peroxides. These species causes the cellular damage by reacting with various biomolecules such as membrane lipids, nucleic acids, proteins and enzymes (Mishra and Lavhale, 2007).

Antioxidant principles from natural resources are multi faceted in their magnitude of activities and provide enormous scope in correcting the imbalance through regular intake of proper diet. Therefore, in the recent years the interest is centered on antioxidants derived from herbal medicine in view of their medicinal benefits. Commonly available phytoantioxidants are less toxic, serving food and medicinal components have been suggested to reduce the threat of wide range of ROS and its associated diseases. (Tripathi and Kamat, 2007).

Rhinacanthus nasutus is a small shrub, its trunk is edge shaped. The short twigs and young leaves are covered with hair. The blooms in bunch at the lane of twigs. Herbal tea preparation using Rhinacathus nasutus lowers blood pressure. Leaves and roots of the plant have antifungal activity (Gotoh *et al.*, 2004).

Various parts of *Rhinacanthus nasutus* plants have also been used for the treatment in many other diseases such as eczema, pulmonary tuberculosis, herpes, hepatitis, diabetes, hypertension, and various skin diseases, and the active components of this plant have been widely investigated (Shimizu *et al.*, 2006).

The candidate plant chosen for the study is *Rhinacanthus nasutus* and the common name for *Rhinacanthus nasutus* is nagamalli and it belongs to acanthaceae family. Earlier studies conducted in our laboratory reveal that the leaf part of *Rhinacanthus nasutus* was found to be rich in both enzymic and non-enzymic antioxidants (Bindu, 2005). Therefore, the objective set for the present study was to analyse the effect of *Rhinacanthus nasutus* leaf extracts on the antioxidant status in goat liver slices exposed *in vitro* to a standard oxidant, hydrogen peroxide, to compare the efficiency of the components of the leaves extracted into solvents of differing polarities (water, methanol, chloroform) and to study and compare the effect of *Rhinacanthus nasutus* leaf extracts on biological end points of oxidative damage like lipid peroxidation.

MATERIALS AND METHODS

Preparation of Plant extracts:

The leaves of *Rhinacanthus nasutus* were collected freshly from Coimbatore district, Tamil Nadu. The collected leaves were washed and dried. Then 1gm of the leaves were weighed and the homogenate was prepared using 10ml of appropriate solvent. The homogenate was then centrifuged to clarify the debris and the supernatant was used for all the experimental analyses. Three different extracts were prepared from the leaves of Rhinacanthus nastus in the order of decreasing polarity (aqueous, chloroform and methanol). The organic solvents were allowed to dry and the residue was dissolved in Dimethyl sulfoxide (DMSO) known amount to yield a concentration of 20mg/5µl. DMSO was maintained at minimum level to avoid DMSO induced events, if any.

Experimental design:

Earlier studies done in our laboratory validates the use of goat liver slices as an *in vitro* model, which stimulates *in vivo* condition. The goat liver was obtained from the slaughterhouse. The liver was washed with isotonic KCl and processed for the assays. The liver was cut in to thin slices and taken in sterile Hank's Balanced Salt Solution. Hydrogen peroxide at 0.1 M concentration was used an oxidant for the induction of oxidative stress in the liver slices.

The following groups were set for the study

- Untreated tissue slices (Control).
- Tissues slices treated with H_2O_2 (Oxidant).
- Tissues slices treated with aqueous extract.
- Tissues slices treated with chloroform extract.
- Tissues slices treated with methanol extract.
- Tissues slices treated with aqueous extract + H_2O_2.
- Tissues slices treated with chloroform extract + H_2O_2
- Tissues slices treated with methanol extract + H_2O_2.

Parameters analysed

The biochemical parameters were analysed in order to evaluate the antioxidants status of leaf samples of the selected plant *Rhinacanthus nasutus* under *in vitro* conditions.Vitamin C, a scavenger of oxy radicals was estimated by the method of Roe and Keuther (1953) Vitamin E, an antioxidant and scavenger of free radicals was determined by the method of Varley *et al.* (1981) Vitamin A is reported to play a vital role in suppressing carcinogenesis by increasing immunity to tumours. Vitamin A was assayed by the technique of Bayfield and Cole (1980). The amount of Reduced glutathione which plays a crucial function on detoxification and cellular protein was estimated by the method of Moron *et al.* (1979). Free radicals in the presence of oxygen molecules attack the biomembrane of the cells and a chain of peroxidative reaction occurs. It leads to the formation of hydrocarbon gases and aldehyde like malonaldehyde (MDA). The extent of LPO was assayed by Okhawa *et al.* (1979)

RESULTS AND DISCUSSION

Plants have evolved protective enzymatic and non-enzymic mechanisms to scavenge reactive oxygen species and minimize their harmful effects. The control of oxidant levels is achieved by inducing the antioxidative defense mechanism (Ali *et al.*, 2006).

The levels of Vitamin C and Vitamin E are depicted in Table 1. There was a significant ($P<0.05$) decrease in the Vitamin C and Vitamin E content upon exposure to

the standard oxidant H_2O_2. It is evident from the values that *Rhinacanthus nasutus* leaf extracts brought a significant increase in the levels of ascorbate and tocopherol even in the presence of hydrogen peroxide. An increase in the vitamin C and Vitamin E levels revealed the ability of the leaf extracts to counteract the oxidant-induced changes. Among the three extracts (aqueous, methanol and chloroform) the aqueous extract showed the highest antioxidant activity.

Present results are in agreement with *Rhinacanthus nasutus* leaves on the oxidant induced goat liver slices under *in vitro* conditions study as the level of vitamin C and vitamin E decreased significantly when compared to respective plant control groups. This reduction reflects exploitation of vitamin C and vitamin E against oxidative damage through GSH depletion.

Table 2 presents the levels of vitamin A and Reduced glutathione in the oxidant induced goat liver slices. A decline in glutathione content was observed when the goat liver slices exposed to the standard oxidant (H_2O_2). Administration of the oxidant H_2O_2 and the plant extracts could revert the damage caused to the cells as indicated by the increased levels of antioxidant vitamin in all the H_2O_2 treated groups. An increased vitamin A and reduced glutathione level in the methanolic extract of *Rhinacanthus nasutus* leaves revealed its ability to counteract the oxidant-induced changes. The *Rhinacanthus nasutus* leaf extracts evoked a significant increase in the depleted Vitamin A and reduced glutathione content even in the presence of H_2O_2.

Effect of Rhinacanthus nasutus leaf extracts on the extent of inhibition of lipid peroxidation in the H_2O_2 induced goat liver slices under in vitro conditions:

Fig. 1 shows the elevated level of TBARS formation observed in H_2O_2 treated goat liver slices which indicated the excessive formation of free radicals and activation of lipid peroxidation system resulting in oxidative stress. The significant decline in the concentration of these constituents in the liver slices treated with both the oxidant and the plant leaf extracts indicates anti-lipid peroxidation effect of *Rhinacanthus nasutus* leaves.

Thus the present study showed the strong free radical scavenging activity and antioxidant potential of *Rhinacanthus nasutus* leaves and thus validates the use of these leaves in medicinal preparations for disorders and disease caused by oxidative stress.

Summary and conclusion:

The present study assesses the non-enzymic antioxidant status and also the extent of inhibition of lipid peroxidation rendered by the leaf extract of three different

Table 1 : Effect of *Rhinacanthus nasutus* leaf extracts on the levels of vitamin C and vitamin E in the H_2O_2 induced goat liver slices under *in vitro* conditions

Samples	Vitamin C level (mg/g tissue)		Vitamin E level (mg/g tissue)	
	Without H_2O_2	Without H_2O_2	Without H_2O_2	With H_2O_2
No extract	24.21 ± 0.070	10.68 ± 0.240	11.67 ± 0.231	14.95 ± 0.23[a]
Aqueous extract	36.10 ± 0.318[a]	18.65 ± 0.07[abc]	12.65 ± 0.403[a]	16.80 ± 0.33[abc]
Methanol extract	27.00 ± 0.141[a]	14.50 ± 0.014[abc]	22.00 ± 0.283[a]	18.15 ± 0.24[abc]
Chloroform extract	16.85 ± 0.001[a]	15.10 ± 0.156[abc]	18.30 ± 0.001[a]	18.10 ± 0.23[ab]

The values are mean ± SD of duplicates
a - Statistically significant (P<0.05) compared to untreated control Statistically
b - Significant (P<0.05) compared to H_2O_2 alone treated group
c - Statistically significant (P<0.05) compared to the respective plant control

Table 2 : Effect of *Rhinacanthus nasutus* leaf extracts on the levels of vitamin A and Reduced glutathione in the H_2O_2 induced goat liver slices under *in vitro* conditions

Samples	Vitamin A level (mg/g tissue)		GSH level (nm/g tissue)	
	Without H_2O_2	Without H_2O_2	Without H_2O_2	With H_2O_2
No extract	161.230 ± 0.933	111.93 ± 0.16	182.260 ± 1.004	111.28 ± 0.244[a]
Aqueous extract	211.50 ± 1.30[a]	209.16 ± 0.28[abc]	221.380 ± 0.707[a]	206.79 ± 0.998[abc]
Methanol extract	235.43 ± 1.80[a]	211.64 ± 1.05[abc]	236.965 ± 0.997[a]	226.84± 0.997[abc]
Chloroform extract	113.38 ± 1.11[a]	154.42± 0.25[abc]	178.206 ± 0.998[a]	112.71± 0.156[abc]

The values are mean ±SD of duplicates
a - Statistically significant (P<0.05) compared to untreated control Statistically
b - Significant (P<0.05) compared to H_2O_2 alone treated group
c - Statistically significant (P<0.05) compared to the respective plant control

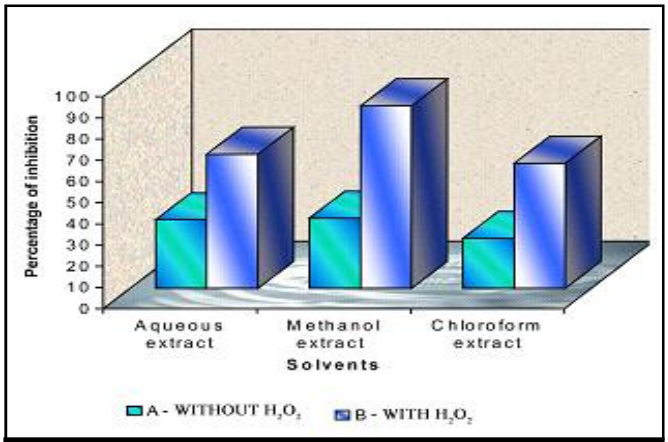

Fig. 1. Extent of inhibition of lipid peroxide

extract of *Rhinacanthus nasutus* (aqueous, methanol and chloroform) leaves and were analysed for the non-enzymic antioxidant status. Parameters analysed include vitamin C, vitamin E, and vitamin A and reduced glutathione. The results revealed that the methanolic extract was found to contain maximum amount of non-enzymic antioxidants followed by the aqueous extract and the chloroform extract. *Rhinacanthus nasutus* leaves possessed sufficient antioxidant efficacy and because of its potential medical value it can be effectively used for therapeutic purposes.

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