

EFFECT OF *RHINOCANTHUS NASUTUS* AND SELENIUM EXPOSURE ON LIPID
PEROXIDATION IN RATS LIVERKhajapeer, K.V*, Muniswamy, G. Suman, B and ^aJohn.K

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ABSTRACT: *Rhinacanthus nasutus* (RN) and selenium have been used for treatment of various illnesses, but the mechanisms of action remain largely unknown. This study focused on the influence of RN and selenium extracts on lipid peroxidation RN (water and ethanol extracts). 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. It is evident that *Rhinacanthus nasutus* leaf extracts offered efficient antioxidant defense in the rat liver an *in vitro* model which simulates *in vivo* condition, when exposed to H₂O₂. 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: *Rhinacanthus nasutus*, selenium, lipid peroxidation, methanolic extraction

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 widespread use of herbal remedies and healthcare preparations, as those described in ancient texts such as Bible, Vedas, and obtained from commonly used traditional herbs and medicinal plants, has been traced to the occurrence of natural products with medicinal properties. Medicinal plants are staging a comeback and 'renaissance' is happening all over the globe. 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. 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 anti peroxidative and antioxidant properties play an important role in protecting the liver against toxicity. 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. Many secondary metabolites of plant are commercially important and find use in a number of pharmaceutical preparations (Joy et al., 2001). 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 (Shiva Prasad *et al.*, 2008). Reactive oxygen species are generated continuously in the body by both endogenous and exogenous factors like normal aerobic respiration by stimulated polymorph nuclear 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.

According to the World Health Organization (WHO, De Silva, 1997), approximately 80% of population in many of developing countries is still use traditional medicine (e.g., medicinal plants) for their primary health care, due to poverty and lack of access to modern medicine. Since about 80 % of the 6.1 billion people of the world live in less developed countries, this means that more than 3.9 billion people will likely use medicinal plants on a frequent basis. Therefore, there is a need to study medicinal plants for their efficacy, safety and quality, and also to search for potentially valuable medicinal material from which novel curative agents may be created for the benefit of all humankind. While in fast developing country such as India; the contribution is as much as 80% (Joy et al., 2001).

MATERIALS AND METHODS

In order to conduct the experiments the following materials and methods were procured and conducted based on the available literature and methodology. The materials needed for my study were procured from local dealers. The following chemicals were of pure quality and where ever is necessary made pure crystals in our laboratory. The chemicals purchased are Potassium Chloride (KCl), Methanol, Sodium (dodesyl) lauryl sulphate (SDS), Thiobarbituric acid, n-butanol-pyridine mixture (15:1 v/v), formaldehyde, NaCl, EDTA, KH_2PO_4 , K_2HPO_4 , Sucrose, and 1, 1, 3, 3-tetraethoxy propane (TEP). The male Wister rats weighing about 150-200g and 3 months old were purchased from Sri Venkateswara Enterprises, (Animal agency), Bangalore.

Collection of plants and preparation of extract

The fresh leaves of *Rhinacanthus nasutus* were collected from Tirumala Hills and Tirupati, Chittoor district of Andhra Pradesh. Fresh leaves of *Rhinacanthus nasutus* (L) were shade dried and milled to fine powder using a mechanical grinder. The powdered plant material was macerated with methanol. The extract was then filtered with filter paper (What man No. 1) under reduced pressure using rota evaporator at 40°C. The concentrate is to obtain a dark molten mass then layered on aluminum foil and freeze dried for further use (Chattopadhyay, 2003).

Treatment

These rats were acclimatized for seven days after arrival from the supplier. Control and treatment groups consisted of six animals each. Temperature was maintained at $71 \pm 3^\circ\text{F}$ with relative humidity of 30-70% on 12:12hr (5am-5pm) light: dark cycle. Animals were housed individually in polycarbonate cages and provided food (Purina certified Rodent Chow 5002 and tap water ad libitum).

Selenium treatment

The rats were treated with oral administration of selenium with constant at 0.05ppm/ liter through water and normal diet for 2 weeks continuously and the rats were sacrificed on 15th day of treatment.

Plant active principles (PAP) treatment

The rats were treated with oral administration of *Rhinacanthus nasutus* methanol extract with constant at 100gm /1gm extract with mixed with normal diet for 2 weeks continually and the rats were sacrificed on 15th day of treatment.

Treatment of mixture of Selenium and PAP

The rats were treated with oral administration of selenium with constant at 0.05ppm/liter through water or 5mg/ liter (5ppm) and *Rhinacanthus nasutus* methanol extract with constant at 100gm /1gm extract with mixed with normal diet for 2 weeks continually and the rats were sacrificed on 15th day of treatment.

Measurement of Tissue Lipid Peroxides

The levels of lipid peroxides in terms of MDA levels were determined by the method of Okhawa et al., 1979. A 10% tissue homogenate was prepared in 1.15% of KCl for lipid peroxidation. To 0.1ml of the tissue homogenate, added 0.2ml of 8.1% SDS and 1.5ml of 0.8% TBA. The total volume was made up to 4ml with distilled water and the tubes were kept at 95°C for 60min, and then cooled. To this added 1ml of distilled water along with 5ml of n-butanol-pyridine mixture (15:1 v/v) and the contents were mixed vigorously. Then the tubes were centrifuged at 4000 rpm for 10 minutes and the color of the organic layer was measured at 532 nm. A standard curve was plotted taking 1, 1, 3, 3-tetraethoxy propane as standard and the values of the samples were obtained from the standard curve.

RESULT AND DISCUSSION

Effect (Se) on lipid peroxidation in liver of *R.nasutus* (PAP) and selenium of rats was represented in Table 1 and Fig 1. The MDA levels were observed to be decreased in treated liver as compare to control as 1.18, 1.25 and 1.90 fold upon treatment with PAP, Se and mixture of PAP and Se respectively. Therefore the PAP and Se can serve as potential antioxidant for liver activities. These results were almost similar to the earlier reports Raveendra *et al* (2008) in rats and chick embryos respectively.

Table1. Effect of *R.nasutus* and selenium on LPO in rat liver and lung

S.No	Treatment	Liver
1	Control	0.95 ± 0.35
2	R.nasutus	0.80 ± 0.26
3	Selenium	0.76 ± 0.15
4	R.nasutus +Selenium	0.50 ± 0.10

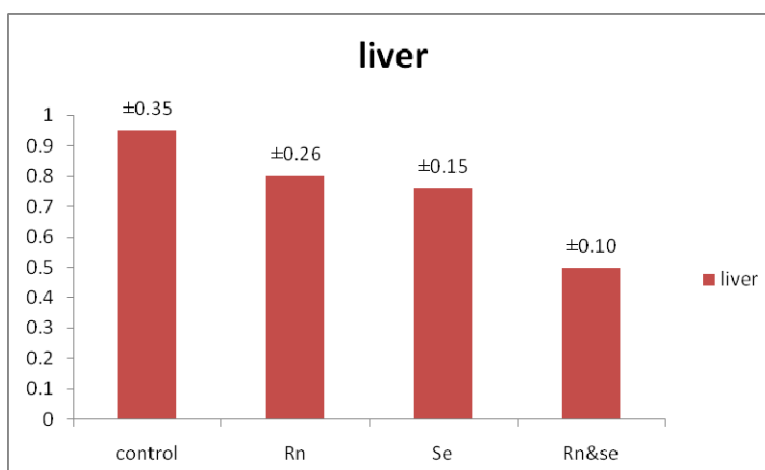


Fig 1. Effect of *R.nasutus* and selenium on LPO in rat liver.

The effect of PAP and Se, the Lox inhibition assays were conducted using spectrophotometric analysis. The results were represented in Table 2 and Fig. 2.

Effect of *R.nasutus* (PAP) and Selenium on Lipoxygenase activity:

Table 2. UV Visible Spectrophotometer absorbance maxima at 234 nm in liver.

S.No	Contents	LOX activity
1	Control	2.153 ± 0.035
2	RN	1.813 ± 0.017
3	Se	1.679 ± 0.008

S = Substrate (EPA) RN = Rhinacanthus *nasutus* (PAP) Se = Selenium

Effect of *R.nasutus* (PAP) and Selenium (Se) on lipoxygenase activity was represented in table 2. Data represent that lipoxygenase activities were decreased in RN and Se as compare to control (E+S) as 1.18 and 1.28 fold respectively. Hence *R.nasutus* (PAP) and Selenium (Se) can serve as inhibitors of lipoxygenase enzymes of animal system. The above results have suggested that PAP and Se were effective antioxidants to rat liver lipoxygenases (Table 2).

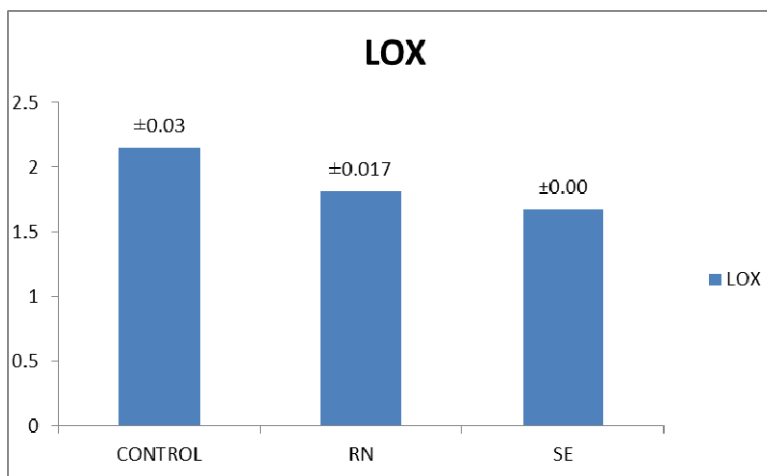


Fig. 2: Effect of *rn* & *se* on *lox* in rat liver

CONCLUSION

Free radicals are continuously produced in vivo and there are number of protective antioxidant enzymes (Superoxide, dismutase, catalase, glutathione S-transferase, glutathione peroxidase, glutathione reeducates and antioxidant reduced glutathione) and non enzymatic antioxidants (GSH, Ascorbic acid, tocopherol, Epinherin and taurine) for dealing with toxic xenobiotic substances.

R.nasutus is reported to have antioxidant activity. The mechanism is to remove superoxide from the human body. *R. nasutus* has previously been shown to protect skin cells against INF- α and TNF- α induced apoptosis, potentially through an antioxidant mechanism (Thongrakard *et al.*, 2010), furthermore other studies have shown *R. nasutus* to have free radical scavaging capabilities (Upendra *et al.*, 2010) and nitric oxide (NO) modulating activities when added to mouse macrophages in conjunction with LPS used H2DCFDA staining and showed that *R. nasutus* reduce reactive oxygen species production in HT-22 cells.

Selenium is essential trace element, sulphur analogue with high chemical activity Selenium is active immune modulator, much more potent anti-oxidant than vitamins E, C and A, beta-carotene, but much more toxic. As food component selenium is an exceptional agent of protection from atherosclerosis, coronary ischemic disease and cancer.

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