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Research Article

EFFECT OF *RHINOCANTHUS* AND SELENIUM ON VARIOUS CELLS AND CELLULAR COMPONENTS IN BLOOD SAMPLES AND HISTOLOGICAL CHANGES IN RAT LIVER

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ABSRACT: ***Rhinacanthus nasutus*** (*RN*) and selenium have been used for treatment of various illnesses, but the mechanisms of action remain largely unknown. 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 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: ***Rhinacanthus nasutus***, selenium, histopathology, methanolic extraction

INTRODUCTION

Medicinal plants, since times immemorial, have been used virtually in all cultures as a source of medicine. The widespread use of herbal remedies and health care 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 plants secondary products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment. The plants synthesize and preserve a variety of biochemical products, many of which are extractable and are used as chemical feed stocks or as raw material for various scientific investigations. Many secondary metabolites of plant are commercially important and find use in a number of pharmaceutical preparations (Joy *et al*., 2001).Although medicinal plants had been priced for their clinical qualities from centuries old, the synthetic products of the modern age surpassed their importance, for a while as stop gap arrangement. However, the blind dependence on synthetics is yet to over and people are returning to the naturals (wild) with hope of safety and security. In recent times, focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems. More than 13,000 plants have been studied during the last 5 year period (Dahanukar *et al*., 2000) and are found to possess active principles for the benefit of living organisms. Over three-quarters of the world population relies mainly on plants for food and plant extracts for health care. Isolated and purified compounds of plants, in contrast, may lose their biological activity fail to behave in the same way as in the complex matrix that the original items of plant represents (Rao *et al*, 1998 and Raveendra *et al*, 2008).

According to the World Health Organization (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.

The investigation of plants as potential sources of new drugs to treat cancer, AIDS and malaria requires the search of as many resources as possible. The discovery of phytochemical compounds with, for example, cytotoxic and/or anti-tumor activity could lead to the production of new drugs for the treatment of cancer.

Therefore, the development of appropriate extraction methods in order to obtain plant extracts with as many phytochemical compounds as possible is important. The criteria used for selecting plants from Surname for investigation were based on:

(1) Traditional medicinal information (ethno-pharmacological knowledge);
(2) Chemical composition of the plant species; and
(3) Literature reports on plant extracts' pharmacology and ethno medical claims.

The drugs are derived from the whole plant or from different parts like leaves, stem, bark, root, flower, seed, etc. More than 30% of the entire plant species, at one time or other was used for medicinal purposes. It has been estimated that in developed countries such as United States, plant drugs constitute as much as 25% of the total drugs, while in fast developing country such as India; the contribution is as much as 80% (Joy et al., 2001). Thus, the economic importance of medicinal plants is much more to countries such as India than to rest of the world.

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, KH2PO4, K2HPO4, Sucrose, and 1, 1, 3, 3-tetraethoxy propane (TEP).The male wistar 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±3ºF 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 labium).

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.

Blood Sampling and SEM analysis

The blood was collected after 14th day of initial administration of the test substance. Peripheral blood was collected from eye ball vein. The blood was spread out on slides immediately and taken for SEM analysis. The control and treated rats of liver were collected after collection of blood using syringe and were stored at -20°C until further use.

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Histopathological Analysis

Fixation and staining of control and treated samples

The tissues were isolated from control and treated livers and they were gently rinsed with physiological saline to remove blood and debris adhering to them. They were fixed in Bouie's solution until processing. Sections were cut at 6µ thickness and stained with haematoxylin (Harris, 1900) and counter stained with Eosin dissolved in 95% alcohol. After dehydration and cleaning, sections were mounted in Canada balsam. Histological examinations of the tissues were followed according to Humason, 1972 and the specimens were observed under the light microscope.

RESULTS AND DISCUSSION

The histopathology studies were conducted on liver in control and treated rats (Fig. 1 - control, fig. 2 -*Rn* treated, fig. 3 - Se treated and fig.4 - *Rn* & Se treated). Except slight modification to liver no damage was found in histology, which indicated that the PAP and Se were best antioxidants, for animal system.

After Histopathological analysis to observe the modifications of cells and their surfaces, the Collected Blood Samples were analyzed by using SEM. The SEM studies table.5 -control, table.6- *Rn* treated, table.7- Se treated and table.8 - mixture of *Rn* & Se treated has revealed that the *RN*(PAP) can stimulate the surface proteins and their extension systems in protecting the more of platelets and extend protein systems observed were less compare to PAP upon Se treatment therefore the LPO, Histopathology and SEM studies of my work suggest that PAP of ***R.nasutus*** and minerals and Se can serve as best antioxidants for hepatocytes of liver and surface proteins blood platelets.

Free radicals are continuously produced in vivo and there are number of protective antioxidant enzymes (Superoxide dismutase, catalase, glutathione S-transferase, glutathione peroxidase, glutathione reductase and antioxidant reduced glutathione) and non enzymatic antioxidants (GSH, Ascorbic acid, tocopherol, Epinherin and taurine) for dealing with toxic xenobiotic substances. *Rn* & Se treated except slight modification to liver no damage was found in histology, which indicated that the PAP and Se were best antioxidants, for animal system. *R.nasutus* (PAP) has stimulated the extension of surface proteins of platelets which is a defensive mechanism for the host system.

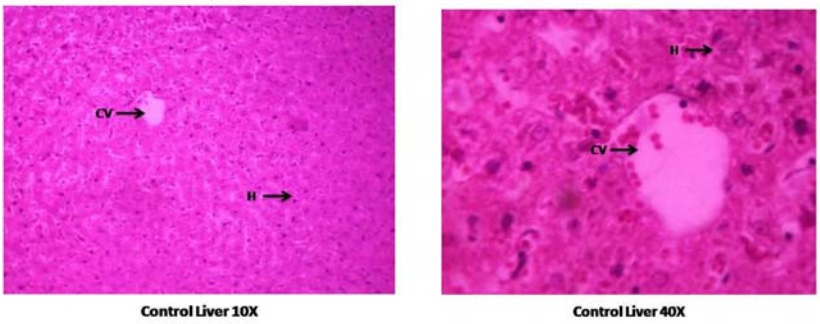

Fig: 1. Control Liver

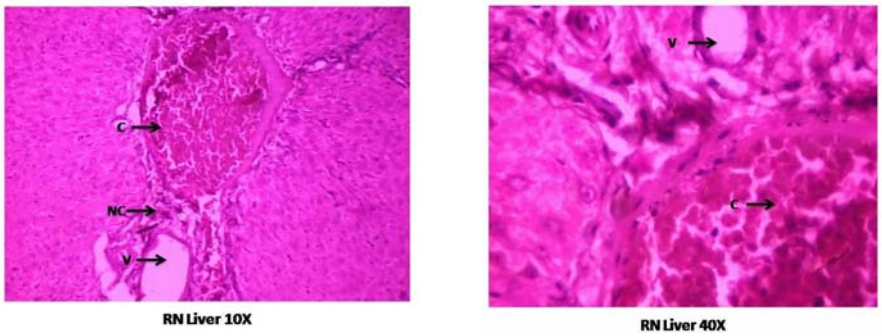

Fig: 2 R. nasutus treated liver

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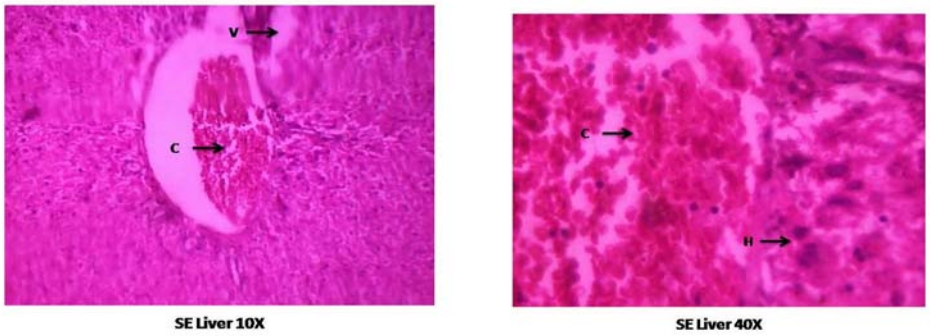
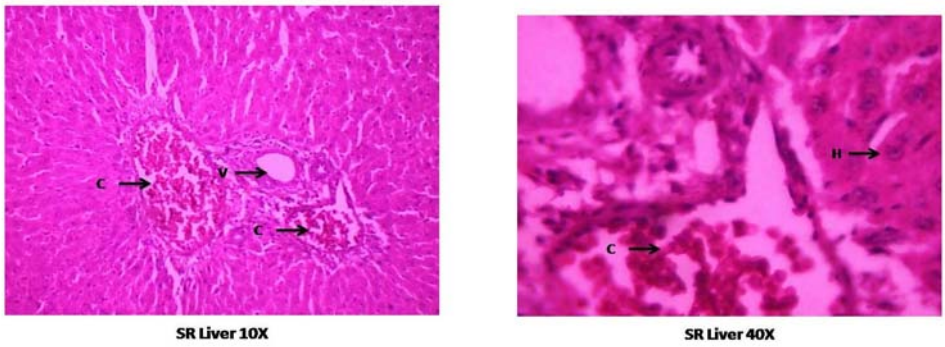


Fig: 3. Selenium treated liver



V = Vacuolization
C = Congestion
CV = Central Vein
H = Hepatocytes
NC = Necrotic changes

Fig: 4 R.nasutus and Selenium treated liver

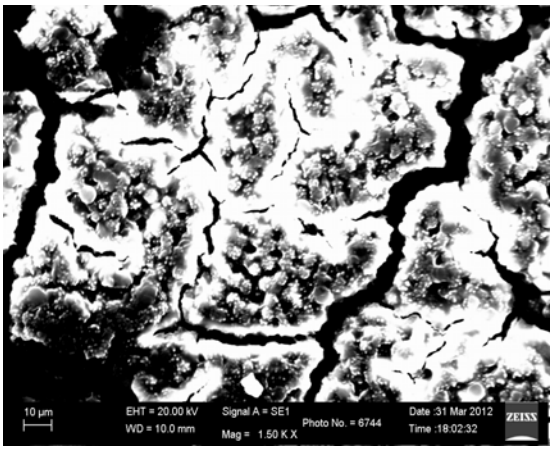


Fig - 5: Blood sample of control rat

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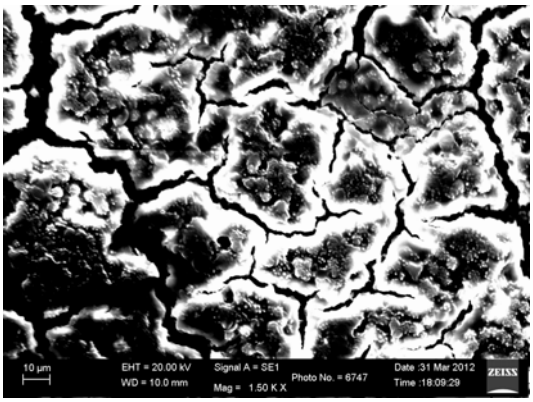

Fig – 6 Blood sample of R.naustus treated rat

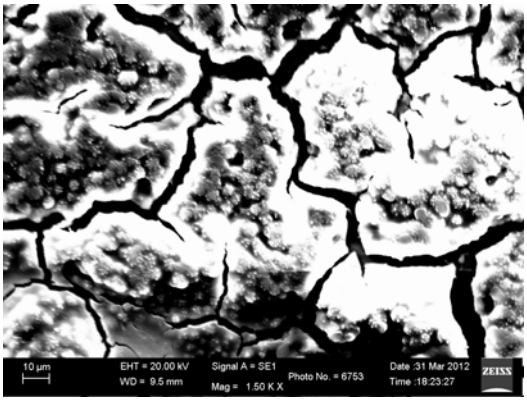

Fig –7: Blood sample of selenium treated rat

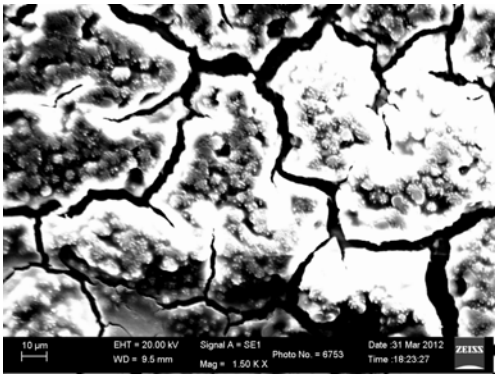

Fig - 8. Blood sample of mixture containing R.nasutus + selenium treated

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