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RESEARCH ARTICLE

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ANTIOXIDANT STATUS OF GOAT LIVER SLICES TREATED WITH METHANOLIC LEAF EXTRACT OF *Nyctanthes arbor-tristis* EXPOSED TO OXIDATIVE STRESS

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ABSTRACT

A number of natural compounds found in plants display potential role as chemo preventive agents. Hence plant products have been used recently in treatment of several diseases. The plant selected for the present study is *Nyctanthes arbor-tristis* (Family: Oleaceae) commonly known as Pavazhamalli. Its leaves are traditionally used to treat fever, rheumatism, liver disorders and as expectorant. In the present study we estimated the antioxidant status of goat liver slices (*in vitro* model) that were exposed to oxidative stress induced by hydrogen peroxide and co-treated with methanolic leaf extract of *Nyctanthes arbor-tristis*. The enzymic antioxidants like Catalase, Glutathione Peroxidase, Superoxide Dismutase and Glutathione-S-Transferase and the non-enzymic antioxidants like Vitamin C and reduced glutathione were analyzed in the presence and absence of hydrogen peroxide. Treatment with hydrogen peroxide decreased the antioxidant status of goat liver slices and co-treatment with the extract improved the antioxidant status of liver slices

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KEY WORDS

n vitro models, Antioxidants, Nyctanthes *arbor-tristis,* Hydrogen peroxide.

INTRODUCTION

In living systems, free radicals are generated as part of the body's normal metabolic process. Antioxidants are radical scavengers which protect the human body against free radicals that may cause pathological conditions such as ischemia, anemia, asthma, arthritis, inflammation, neurodegeneration, Parkinson's diseases, mongolism, ageing process and perhaps dementias[1].

Several studies have demonstrated that plants produce potent antioxidants and represent an important source of natural antioxidants. Many minor components of foods, such as secondary plant metabolites, have been shown to alter biological processes, which may reduce the risk of chronic diseases in humans. Many of these plants are in common use even today. *Nyctanthes arbor-tristis*commonly known as night jasmine is one among them. The whole plant is used for the treatment of cancer, root for fever, anorexia; bark as expectorant; leaf for control fever, diabetes and as cholagogue, diaphoretic and anthelmintic [2]. These plant extracts have free radical scavenging activity and improved antioxidant effect. They effectively quench the free radicals generated in cells[3]. The present study focuses on free radicals generated by hydrogen peroxide in goat liver slices and treatment of these treated tissues with methanolic leaf extract of *Nyctanthes arbor-tristis.*

We should reduce the number of animals used for the experimental studies in order to reduce the sufferings of animals and pain. For that *in vitro* models are used instead of *in vivo* model. Liver was selected for the experiment due to the reasons that it is a metabolic organ and it clears many xenobiotics that enter into the body.

METHODOLOGY

PREPARATION OF METHANOLIC EXTRACT

1g of sample and 10ml of solvent were taken and ground in a mortar and pestle. The extracts were centrifuged at 2000 rpm for 5 minutes; the supernatant was transferred to a beaker and kept for evaporation at 60°C. Allowed it to dry and it was dissolved in minimal volume of DMSO (50 µl / 20 mg plant extract).

PREPARATION OF GOAT LIVER HOMOGENATE

The goat liver was freshly purchased from slaughter house. The liver was then washed with isotonic potassium chloride solution and processed. Using sterile scalpel, thin slices of liver, approximately 1mm thin were cut. This was then treated with sterile Hank's Balanced Salt Solution (HBSS) at a proportion of 0.25g/ml. This stimulates peritoneal fluid in live animal. Hydrogen peroxide (200µM) was used for inducing oxidative stress in liver slices. 20µl of plant extract is used for every 250mg of liver slices.

The treatment groups set up for the study were as follows

1) Untreated (negative) control.
2) H_2O_2 treated (positive) control.

3) Methanolic extract of *Nyctanthes arbor-tristis* leaf
4) Methanolic extract of *Nyctanthes arbor-tristis*leaf + H_2O_2
5) DMSO control

The extracts were added to tissue slices and incubated at 37° C for one hour with shaking. The tissue was homogenized in Teflon homogenizer. HBSS was used for incubation of slices.

PARAMETERS ANALYSED:

In order to check the effect of *Nyctanthes arbor-tristis*leaf extract in the *in vitro* system (goat liver slices) subjected to oxidative stress the activities of enzymic antioxidants and levels of non enzymic antioxidants were analysed.

The enzymic antioxidants assessed were Catalase(Luck *et al.*, 1974)[4], Superoxide dismutase(Misra and Fridovich, 1972)[5], Peroxidase (Reddy et al., 1995)[6]and Glutathione S- transferase (Habig et al., 1974)[7].

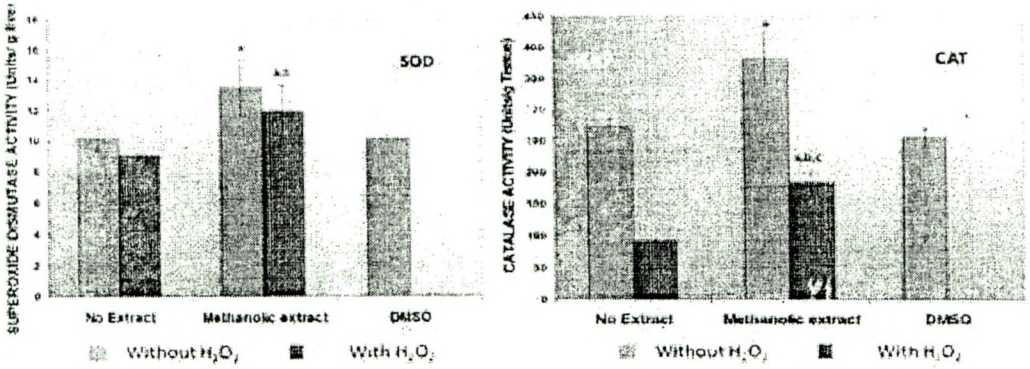
The non-enzymic antioxidants assessed were ascorbic acid and reduced glutathione. Ascorbic acid was assayed by method proposed by Roe and Keuther, (1950) [8]. The reduced glutathione was estimated according to the method of Moron et al., (1979)[9].

RESULTS

ENZYMIC ANTIOXIDANTS

Enzymic antioxidants may be useful in preventing the deleterious consequences of oxidative stress and there is increasing interest in the protective biochemical functions of natural antioxidants contained in species. Hence it was felt imperative to study the antioxidant status of tissue slices exposed to oxidant assault and co-treated with leaf of *Nyctanthes arbor-tristis*. The results obtained are depicted in Figure 1. From the results it is clear that the leaf extract improved

Figure 1: EFFECT OF *Nyctanthes arbor-tristis* LEAF EXTRACT ON THE ACTIVITIES OF ENZYMIC ANTIOXIDANTS IN GOAT LIVER SLICES EXPOSED TO OXIDATIVE STRESS



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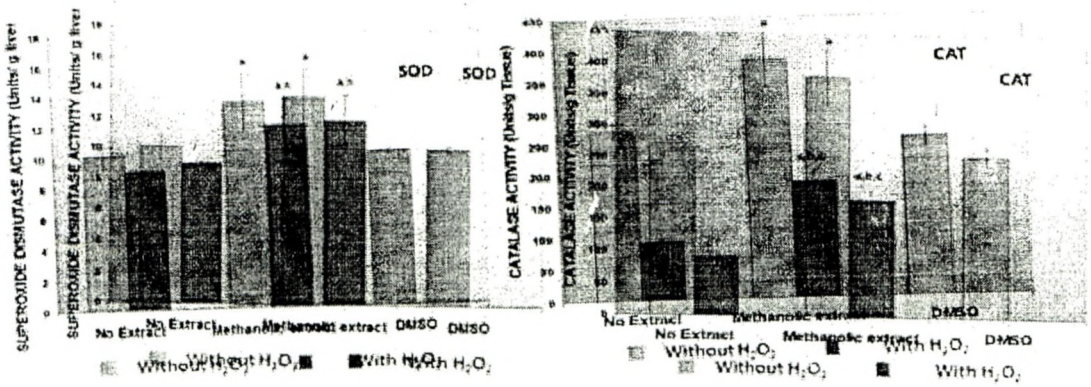

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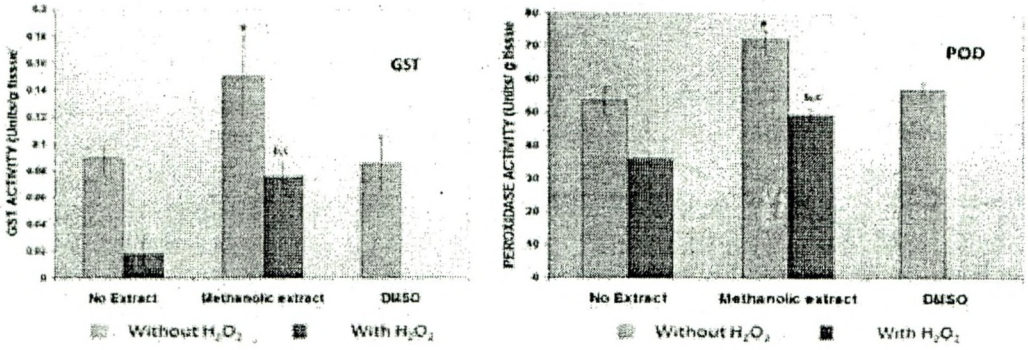
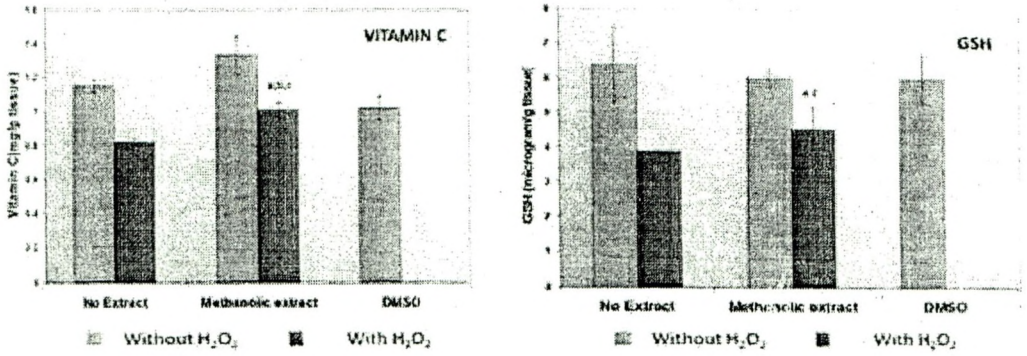


Figure 2: EFFECT OF *Nyctanthes arbor-tristis* LEAF EXTRACT ON THE LEVELS OF NON-ENZYMIC ANTIOXIDANTS IN GOAT LIVER SLICES EXPOSED TO OXIDATIVE STRESS



In figure 1 and 2 ,each bar represents, Mean ± Standard Deviation of each group.
(CAT- Catalase, SOD - Superoxide dismutase, POD - Peroxidase, GST - Glutathione-S-Transferase, GSH - Reduced Glutathione, Vitamin C - Ascorbic acid)

a - Statistically significant ($p<0.05$) compared with untreated control. b - Statistically significant ($p<0.05$) compared with Hydrogen Peroxide alone treated control. c - Statistically significant ($p<0.05$) compared to respective plant control.

the superoxide dismutase, catalase,glutathiones transferase and peroxidase activities in the liver slices exposed to H_2O_2.

NON ENZYMIC ANTIOXIDANTS

Having assessed the enzymic antioxidant, the level of non enzymic antioxidants namely Vitamin C, and reduced glutathione were assessed in goat liver slices exposed to H_2O_2 and the results are depicited in Figure 2.From the results it can be revealed that the leaf extract improved the vitamin C and reduced glutathione level in the liver slices exposed to H_2O_2.

DISCUSSION

The activities of all the enzymic antioxidants tested decreased drastically on exposure to H_2O_2. Treatment with leaf extract significantly improved the activity compared to H_2O_2 control. DMSO treatment did not alter the

activity and was comparable to the control levels. Previous studies revealed that *Ososmaarmeniacum*root extract exhibited antioxidant mechanism by increasing SOD level and inhibited oxidant mechanism in ethanol induced rat stomach tissue[10]. Similarly green tea extract ameliorates gentamycin elicited nephrotoxicity and oxidative damage in rat renal tissue by improving activity of enzymic antioxidants like superoxide dismutase and catalase[11]. It has been reported that saffron, a plant derivative suppresses oxidative stress induced by 7, 12 dimethyl benz (a)anthrazene (DMBA) induced skin carcinoma in mice by elevating the activities of enzymic antioxidants like catalase and superoxide dismutase[12]. Earlier studies revealed that berberine, berbamine and palmatine, constituents of *Berberisaristata*root have anti hyperglycemic and antioxidant effect and this increases catalase activity significantly in the liver of diabetic rats[13]. Similarly the leaf extract of the medicinal plant *Cassia fistula* Linn. showed increased activity of GST against diethylnitrosamine induced liver injury in ethanol pretreated rats[14]. Similar studies revealed that extract of *Bacopamonneri*modulates enzymic antioxidants like Peroxidase, Catalase etc., by increasing its activity and enhances the defense against ROS generated damage in brain and kidney of diabetic rat[15]. Previous study revealed that powder of selenium enriched green tea was able to enhance peroxidase and superoxide dismutase activity in blood serum and liver [16].

The statistical analysis showed that leaf extract treated group showed significantly higher peroxidase activity in goat liver slices compared to both H_2O_2 treated and untreated control . All these studies support our findings.

Treatment of goat liver slices with H_2O_2 significantly depleted levels of Vitamin C which was effectively counter acted by the leaf extract. It showed a significant increase in the levels of Vitamin C. Earlier studies revealed that administration of phenol rich wild blue berry extract in mice increased ascorbic acid level in the brain [17]. Similar studies revealed thatantioxidant vitamins ascorbic acid, retinol and b-carotene play an important acute and chronic role in reducing or eliminating the oxidative damage produced by ROS [18]. GSH level was drastically decreased on exposure to H_2O_2. Treatment with leaf extract increased the GSH level. Studies have reported that the administration of *Amaranthusspinosus* increased the level of reduced glutathione which counter acted the CCL_4 treatment in rat liver [19]. It has been reported that the GSH levels in plasma and in lungs were significantly elevated in bleomycin-induced rats treated with EGCG [20].

CONCLUSION

In the present study treatment with hydrogen peroxide decreased the antioxidant status of goat liver tissues. The addition of the leaf extract to the hydrogen peroxide treated liver slices increased the antioxidant status significantly. Thus it can be concluded from the present study that the leaf extract of *Nyctanthes arbor-tristis* rendered protection against the oxidative stress induced by hydrogen peroxide by improving the antioxidant status.

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