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In-Vivo Antioxidant Activity of *Caesalpinia mimosoides,* LAMK.

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ABSTRACT

Plants are reservoirs of potent and safe anti-oxidant molecules. The isolation and identification of such molecules may lead to the development of new drugs that can be used to control or prevent many diseases. *Caesalpinia mimosoides* Lamk (Caesalpiniaceae) is a climbing shrub found throughout India. In the present study the ethanolic extract of aerial parts of *C.mimosoides* was investigated for its in-vivo anti-oxidant activities by conducting assay of superoxide dismutase, catalase, glutathione, glutathione peroxidase, glutathione reductase. The analysis of the result showed that the plant possesses significant anti-oxidant activity.

Keywords: free radical scavenging, antioxidant, anti-inflammatory, scavenging enzymes

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INTRODUCTION

The role of oxygen radicals have been implicated in the causation of several diseases such as liver cirrhosis, atherosclerosis, cancer, diabetes, pre-mature ageing etc. the drugs that can scavenge oxygen radicals have great potential in ameliorating these disease processes. Supplementations of non-toxic antioxidants may have a chemo protective role in the body in these conditions [1]. It has been determined that active oxygen molecules such as superoxide play an important role in the inflammation process after intoxication by ethanol, carbon tetra chloride or carrageenan.

In-vivo anti-oxidant activity was screened by assay of superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and also by quantitative determination of glutathione and lipid peroxidation.

MATERIALS AND METHODS

Plant material

Aerial parts of the plant *C. mimosoides* were collected from Thrissur district during the month of December and was authenticated by botanist of Kerala Forest Research Institute, Thrissur. A specimen was deposited in the herbarium with voucher number 11092.

Preparation of the extract

The air-dried and coarsely powdered material was extracted with ethanol (70%) for 16 hours. The extract was filtered and concentrated using rotary vacuum drier. The dried extract is stored in a refrigerator until it is being used.

In-vivo antioxidant activities

Activity of Scavenging Enzymes

Assay of Superoxide dismutase (SOD)

SOD activity was determined by the method of Kakkar et al [2]. The rat liver tissue was homogenized in 0.25M sucrose and differentially centrifuged at 10,000 rpm under cold conditions to get the cytosol fraction. The initial purification was done by precipitating the protein from the supernatant with 90% ammonium sulphate and after dialysis against 0.0025M Tris-HCl buffer, pH 7.4. The supernatant was used as the enzyme source.

The assay mixture contained 1.2ml Sod.pyrophosphate buffer (0.052M, pH8.3), 0.1ml 186□M phenazine methosulphate (PMS), 0.3ml 300□m nitroblue tetrazolium (NBT) 0.2ml 780□M NADH, appropriately diluted enzyme preparation and water in a total volume of 3ml. Reaction was started by the addition of NADH. After incubation at 30^0C for 90 seconds, reaction was stopped by the addition of 1ml glacial acetic acid. The reaction mixture was stirred vigorously and shaken with 4ml of n-butanol. Mixture was then allowed to stand for ten minutes. Centrifuged and butanol layer was taken out. Colour intensity of the chromogen in the butanol fraction was measured at 560nm against butanol. A system devoid of enzyme served as control.

One unit of enzyme activity is defined as the enzyme concentration required inhibiting chromogen production (OD of 560 nm) 50% in one minute under the assay conditions. The specific activity is expressed in units/ mg protein. Unit is defined as the velocity constant per second.

Assay of Catalase

The catalase activity was assayed by the method of Maehly and Chance [3].The tissue was homogenized with 0.91M-phosphate buffer (pH 7.0) at 1- 4^0C and centrifuged at 5000 rpm. The estimation was done spectrophotometrically following the decrease in absorbance at 240nm. The reaction mixture contained 0.91 M phosphate buffer pH 7.0, 2mM H_2O_2 (diluted 0.1ml H_2O_2 to 100ml using buffer) and 50□l

enzyme extract. Specific activity is expressed in terms of units/ mg protein. Unit is defined as the velocity constant per second.

Assay of Glutathione Peroxidase

The activity of glutathione peroxidase was determined by the method of Lawrence and Burk [4] as modified by Agerguard and Jense [5]. Tissue homogenate (10%) was prepared in 0.25M sucrose, centrifuged at 10,000 rpm for 30 minutes and the supernatant fraction was used for the assay. Activity was determined in phosphate buffer (50mM pH7.0) containing EDTA (1.5mM), sodium azide (1.0mM), reduced glutathione (1.0mM), NADPH (0.1mM) and glutathione reductase (1.0µM/ml). Absorbance was measured at 340nm at 20 seconds interval. Enzyme activity is defined as µM of NADPH oxidized/min /mg protein using 0.25mM H_2O_2 as substrate.

Assay of Glutathione reductase

Glutathione reductase activity was determined by the method described by Bergmeyer [6]. Tissue homogenate (10%) was prepared in 0.25 M sucrose, centrifuged at 10,000 rpm for 30 minutes and supernatant fraction was used for the enzyme assay. The assay system contained 1ml phosphate buffer (0.12M, PH 7.2) 0.1ml EDTA, 0.1ml sodium azide (10mM/l), 0.1ml oxidized glutathione (6.3mM) and 0.1 ml enzyme source. It was kept for 3 minutes. Then 0.1 ml NADPH (9.6 mM/l) was added. The absorbance at 340 nm was measured at an interval of 15 seconds for 2 minutes. The activity is expressed as µM NADPH oxidized per minute / mg protein.

Estimation of Antioxidants

Estimation of Glutathione Content

Glutathione content was estimated by the method of Benke et al [7].

20% tissue homogenate prepared in 5% TCA containing 0.001M EDTA, was centrifuged at 2000 rpm for 5 minutes; 0.2ml aliquot of each supernatant fraction was transferred to another tube containing 4.75ml of 0.1M sodium phosphate buffer (pH 8) and to it 0.05ml of 0.01m 5,5 dithiobis-2-nitrobenzoic acid (DTNB) was added. The absorbance was read at 412nm within 4 minutes.

Estimation of Malondialdehyde

Malondialdehyde was estimated by the assay method of Nichans and Samuelson [8]. Weighed quantity of tissue was homogenised in 0.1M Tris-HCl buffer, pH 7.5, and allowed to stand for 5 minutes. The supernatant was used for the determination of lipid peroxide level. One ml of the tissue homogenate was combined with 2ml of TCA-TBA-HCl reagent and mixed thoroughly. The solution was heated for 15 minutes in a boiling water bath. After cooling, the flocculent precipitate was removed by centrifugation at 1000 rpm for 10minutes. The absorbance of the sample was read at 535nm against a blank that contained no tissue homogenate. The extinction co-efficient of malondialdehyde is $1.56 \times 10^5 M^{-1} cm^{-1}$.

RESULTS AND DISCUSSION

Effect of *C.mimosoides* on Antioxidant Enzymes, Glutathione and Lipid Peroxidation in CCl_4 treated Albino Rats

Concentration of Malondialdehyde (MDA) In Liver

The treatment of rats with CCl_4 produced very significant elevation in the levels of MDA. In the test group (testII), compared to group II, the reduction was 51.6%. A similar result was observed in test II where drug was given along with the toxin with reduction of 56.8%.

Activity of Glutathione Content (GSH)

Unlike lipid peroxidation, the activity of reduced glutathione (GSH) was decreased when treated with CCl_4 as observed in group II - 3.93 to 2.96 mg/g. Again in the protective group (test-I) when the drug was given along with the toxin the activity was increased to 3.46 mg/g. In the last group (test II) a further enhanced value was obtained (3.59mg/g) proving its effectiveness.

Activity of Glutathione Reductase and Peroxidase

Normal values of GR and GPx were observed in the control group of animals (group I). But after treatment with CCl_4 (group II) they were reduced by 26% and 48.7% respectively. Protective treatment produced a better result in the GR and GPx level.

Activity of Catalase

The activity of catalase was very significantly decreased in the toxin treated group. But a very significant result was noticed in the test groups. Compared to control group, 62.48% increase in the level of catalase was observed in test II.

Figure-I In-vivo antioxidant activity of *C.mimosoides*

Groups	Treatment	GR nmol of NADPH oxidized/min/mg protein Mean±SEM	GPx nmol of NADPH oxidized/min/mg protein Mean±SEM	Catalase mol of H_2O_2 decomposed/min/ mg protein Mean±SEM
Normal	vehicle	59 ± 0.402	187.92 ±1.245	69.36 ± 0.48
Control	CCl_4+vehicle	43.64 ± 0.28	96.38 ±1.12	38.76 ± 0.37
Test- I(C.m)	CCl_4+200mg/kg	54.48 ± 0.371*	134.42 ±1.012*	58.72 ± 0.71
Test-II(C.m)	CCl_4+400mg/kg	56.58 ± 0.407*	158.86 ±0.389*	62.98 ± 0.561

Figure-II In-vivo antioxidant activity of *C.mimosoides*

Groups	Treatment	Lipid peroxidation(MDA) nmols/ g wet tissue Mean ±SEM	GSH mg/g Mean ±SEM	SOD Units/mg Mean ±SEM
Normal	vehicle	0.74 ±0.001	3.93 ±0.024	7.3 ±.031
Control	CCl_4+vehicle	3.06 ±0.012	2.96 ±0.002	2.28 ±0.002
Test-I(C.m)	CCl_4+200mg/kg	1.48 ±0.0138*	3.46 ±0.042*	4.81 ±0.09
Test-II(C.m)	CCl_4+400mg/kg	1.32 ±0.0076	3.59 ±0.038	5.63 ±0.0345

The results (table-1 and II) indicate that the alcoholic extract of *C.mimosoides* possesses significant antioxidant activity. Plants which contain flavonoids and polyphenols are reported to have several biological properties including protective effects through several mechanisms such as antioxidant, anti-inflammatory effects etc. Free radicals have been demonstrated to be involved in the triggering of several diseases such as atherosclerosis; cancer etc. Superoxide anion radical regulates metabolites capable of signaling and communicating important information to the cellular genetic machinery. Over production of $O_2^{\cdot}$ takes place in various chronic inflammatory cases, induced by drugs, toxin, stress, tissue injury and heavy exercise. Hydroxyl radical is produced from H_2O_2 and $O_2^{\cdot}$. H_2O_2 is relatively stable, poorly reactive non-radical oxygen species, which easily cross cell membrane and attack different sites by converting to $HO^{\cdot}$. H_2O_2 is also involved in the generation of free radicals in presence of transitional ions. The inflammatory response is loosely interwined with the process of repair and is fundamentally protective response, the ultimate goal of which is to rid the organism of both the initial cause of cell injury. ODFR (Osteoclast Differentiation Factor Receptor) may be released extracellularly from leucocytes after exposure to chemo tactic agents, immune complexes or a phagocytic challenge. Their production is dependent on superoxide anion, hydrogen peroxide and hydroxyl are the major species produced within cell and these metabolites combines with nitric oxide to form other reactive nitrogen intermediates. Extracellular release of low levels of these potent mediators can increase the

expression of chemokines (eg. IL-8) cytokines and endothelial leucocyte adhesion molecules, amplifying the cascade that elicits the inflammatory response [9]. It is important to understand what inflammation is and how it contributes to physiological processes such as wound healing and infection. In response to injury, a multifactorial network of chemical signals initiates and maintains a host response designed to 'heal' the afflicted tissue. This involves activation and directed migration of leucocytes (neutrophils, monocytes, and eosinophils) from the venous system to sites of damage and tissue mast cells also have significant role. For neutrophils, a four step mechanism is believed to co-ordinate recruitment of these inflammatory cells to sites of tissue injury and to the provisional extracellular matrix that forms scaffolding upon which fibroblast and endothelial cells proliferate and migrate, thus providing a nidus for reconstitution of the normal microenvironment. Chemical features derived from plasma or cells and triggered by the stimulus mediate the vascular and cellular response of both the acute and chronic inflammation.

CONCLUSION

Ethanolic extract of *Caesalpinia mimosoides* was screened for the *in- vivo* anti-oxidant activity. *In-vivo* anti-oxidant activity was screened by assay of superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and also by quantitative determination of glutathione and lipid peroxidation. The extract showed significant antioxidant activity.

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