ISSN - 2250-1991 | IF : 5.761 | IC Value : 79.96

Original Research Paper Biological Science

EVALUATION OF THE ANTIOXIDANT ACTIVITY OF *Cassia alata* LEAVES USING PRECION-CUT GOAT LIVER SLICES AS AN *in vitro* MODEL.

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ABSTRACT

Liver, the largest organ in vertebrate body, has a pivotal role in regulation of physiological processes. It is involved in several vital functions such as metabolism, secretion and storage.The body cells continuously produce Reactive Oxygen Species(ROS) and free radicals as part of their metabolic processes.These free adicals can be reduced by an elaborate antioxidant defence system consisting of Enzymic and non-enzymic antioxidants. This study was done to prove the antioxidant status of the leaves of Cassia alata using precision-cut goat liver slices as an in vitro model. The activity of enzymic and non-enzymic antioxidants were found to increase upon treatment with the methanolic extract of the leaves of Cassia alata.

KEYWORDS

INTRODUCTION.

Medicinal plants are natural sources, yielding valuable herbal products, which are often used in the treatment of various ailments [1]. Medicinal plants have been traditionally used in the treatment of several human diseases and their pharmacologic and therapeutic properties have been attributed to different chemical constituents isolated from crude extracts. Of particular importance, chemical constituents with antioxidant activity can be found at high concentrations in plants and can be responsible for their preventive effects in various degenerative diseases including cancer, neurological and cardiovascular diseases. Thus the antioxidant properties of plants have a full range of perspective applications in human healthcare [2].

The interest in medicinal plants has increased together with the number of investigations into their biological effects on human beings and animals [3]. The medicinal plants are used in traditional treatments to cure variety of diseases for thousands of years [4]. The production and processing of medicinal plants offers the possibility of fundamentally upgrading the lives and well-being of people in rural regions [5]

In the human body, reactive Oxygen species(ROS) can be neutralized by antioxidant enzymes and antioxidant compounds [6].Oxidative stress occurs when the production of ROS overrides the antioxidant capacity in the target cell resulting in the damage of macromolecules such as lipids, nucleic acids and proteins causing alterations in the target cell function and leading to cell death[7]. Oxidative stress significantly impacts multiple cellular pathways that can lead to the initiation and progression of varied disorders throughout the body [8].

Precision-cut liver slices have proven useful for several pharmacological and toxicological investigations. Liver was the organ of choice because it is the metabolic organ and is responsible for the metabolic clearance of many xenobiotics. Liver slices are a microcosm of the intact liver and therefore it is an *in vitro* technique that offers the advantages of *in vivo* situation and hence is a more suitable model for the experimental analysis of antioxidant protection studies [9]. Precision-cut liver slices are described as a valuable tool for *in vitro* metabolism studies of potential drug candidates. Some papers have demonstrated successful cryopreservation conditions for liver slices, facilitating a broader and more efficient use of the tissue [10].

MATERIALS AND METHODS

PLANT MATERIAL

The plant sample *Cassia alata* was collected from Tiruchengode, Tamilnadu.The leaves were thoroughly washed with tap water followed by distilled water and to rid them of any surface debris

PREPARATION OF PLANT EXTRACT

Cassia alata leaves (20.0g) were homogenized in approximately 200ml of the solvent (methanol). The supernatant was collected and dried at 60°C well protected from light. The residue obtained after drying the methanol extracts was weighed and dissolved in a known amount of DMSO to yield a concentration of 20mg/5µl, DMSO was maintained at a minimum level to avoid DMSO-induced events, if any.

PREPARATION OF GOAT LIVER SLICES.

The *in vitro* model system used in the study was precision-cut goat liver slice. Fresh goat liver was obtained from a local slaughterhouse and transported to the laboratory on ice. The liver was washed with isotonic KCl and processed for the assays. Very thin slices (1mm thick) were cut from the liver using a sterile scalpel. The slices were taken in sterile Phosphate Saline Buffer (PBS) at a proportion of 0.25g in 1ml. Hydrogen peroxide was used as the oxidant to induce oxidative stress in the liver slices. H_2O_2 was used at a final concentrationof 500µM, which is the dose used in *in vivo* studies for intraperitoneal administration. 20µl of the plant extract corresponding to 20mg of plant extract was used to study the antioxidant effect on the cells.

TREATMENT GROUPS.

Liver slices
Liver slices + H_2O_2
Liver slices + H_2O_2 + Plant Extract
Liver slices + Plant Extract

Determination of the activity of enzymic antioxidants

Assay of Superoxide dismutase (SOD)

The incubation medium contained a final volume of 3ml, 50mM potassium phosphate buffer, 45µM Methionine, 5.3µM Riboflavin, 84µM NBT and 20mM potassium cyanide and enzyme source. The tubes were placed in an aluminium foil-lined box maintained at 25°C and equipped with 15W fluorescent lamps. Reduced NBT was measured spectrophotometrically at 600nm

after exposure to light for 10 minutes. The maximum reduction was evaluated in the absence of the amount of SOD giving 50% inhibition of the reduction of NBT [11].

Assay of Catalase

3ml of H_2O_2 in phosphate buffer (0.067M, pH 7.0) was taken in a quartz cuvette and the baseline was adjusted at 240nm. 20µl of homogenate was added rapidly and mixed thoroughly. The time interval required for decrease in absorbance by 0.05 units was recorded at 240nm. The concentration of H_2O_2 was calculated using the extinction coefficient 0.036 per µM/cm. One unit is the amount of enzyme activity required to decrease the absorbance at 240nm by 0.05 units [12]

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Assay of Peroxidase

3ml of pyrogallol solution (0.05M in 0.1M phosphate buffer) and 0.2ml of the homogenate were pipetted out into cuvette. Adjusted the spectrum to read zero at 430nm. 0.5ml of H_2O_2 was added in the test cuvette. The change in absorbance was recorded every 30 seconds up to 3 minutes. One unit of peroxidase activity is defined as the change in absorbance/ min at 430nm [13]

Assay of Glutathione S-transferase

The substrates for GST (1mM GSH and 1mM CDNB, 0.1ml each) were taken in a test cuvette along with 0.1M phosphate buffer (pH 6.5) to make a volume of 2.9ml. The reaction was started by the addition of 0.1ml of the enzyme source to this mixture. The readings were recorded against distilled water blank for a minimum of 3 minutes. The complete assay mixture without the enzyme source served as the control. The enzyme activity was determined by recording the changes in absorbance at 340nm. One unit of GST activity is defined as the nM of CDNB conjugated/minute [14]

Determination of the level of non-enzymic antioxidants
Estimation of Tocopherol

The liver homogenate (1.5ml), 1.5ml standard (10ng/l alcohol) and water (1.5ml) were pipetted out into three centrifuge tubes namely test, standard and blank respectively. To all the tubes, xylene (1.5ml) was added, stoppered, mixed well and centrifuged. The xylene layer (1.0ml) was taken and transferred to another set of stoppered tubes, 1.0ml of 2,2' dipyridyl (1.2g/l n-propanol) was added to each and mixed. The reaction mixture (1.5ml) was taken in a spectrophotometric cuvette and the extinction of test and standard were read against the blank at 460nm. 0.33ml Ferric chloride solution (1.2g/l ethanol) was added and after exactly 15 minutes, the absorbance of the red colour was read against blank at 520nm. The amount of Tocopherol in the sample was calculated using the formula,

Tocopherol (µg) = [(Reading of standard at 520nm- Reading at 450nm)/ (Reading at 520nm)]X 0.29 x 0.15
The results are expressed as µg tocopherol/g sample [15]

RESULTS AND DISCUSSION

Table1. Effect of *C. alata* leaf extracts on the activities of enzymic antioxidants in goat liver slices exposed to H_2O_2 *in vitro*

Parameters	Without H_2O_2		With H_2O_2	
	Liver slices	Liver slices + P.E	Liver slices	Liver slices + P.E
SOD	3.52	4.60	2.70	3.18
CAT	267	279	233	314
POD	9.5	10.1	7.3	17.7
GST	0.44	0.47	0.26	0.38

Table2. Effect of *C.alata* leaf extracts on the levels of non-enzymic antioxidants in goat liver slices exposed to H_2O_2 *in vitro*

Parameters	Without H_2O_2		With H_2O_2	
	Liver slices	Liver slices + P.E	Liver slices	Liver slices + P.E
Vitamin E	0.17	0.43	0.08	0.16

SOD activity of the liver slices decreased drastically on exposure to H_2O_2. Treatment with leaf extract significantly improved the activity compared to control. Previous studies revealed that *Ososma armeniacum* root extract exhibited antioxidant mechanism by increasing SOD level and inhibited oxidant mechanism in ethanol induced rat stomach tissue [16]. 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 [17].

Previous studies have shown that the methanolic extract of *Nelumbo nucifera* inhibits H_2O_2 induced damage on fatty acid peroxidation and plasmid DNA damage in Swiss albino mice [18]

These supportive studies are in accordance with our reports indicating the protective effect of *Cassia alata* leaf extracts against oxidant induced damage.

The goat liver slices treated with the methanolic extract of *Cassia alata* leaves showed a significant increase in catalase activity, compared to untreated control. Hydrogen peroxide caused a considerable decrease in the SOD activity. The co-treatment with the methanolic extract resulted in a significant increase compared to untreated control.

The peroxidase activity in the H_2O_2 treated liver slices decreased compared to the untreated control. The toxic effect of H_2O_2 negated by the concordant treatment with *Cassia alata* leaf extracts. The leaf extracts, by themselves, significantly increased the peroxidase activity compared to control. Previous study revealed that powder of selenium enriched green tea was able to enhance peroxidase and superoxide dismutase activity in blood serum and liver [19].

The activities of GST decreased upon exposure to H_2O_2. Treatment with the methanolic extract of *Cassia alata* leaves caused an increase in GST activity over the control. It also caused the reversal of the decrease caused by H_2O_2 in GST activity, to a considerable extent. Previous studies indicate that the GSTs are active in the detoxification of numerous products, including reactive oxidant damage to DNA and lipids, such as organic epoxides, lipid hydroperoxides and unsaturated aldehydes [20].

The goat liver homogenate when treated with hydrogen peroxide showed a slight decrease in the levels of vitamin E. This depleting effect was counteracted by the co-treatment with the leaf extracts. The vitamin E levels showed a significant increase in the methanolic extract treated groups. It is, thus, conceivable from the results, that the treatment with the leaf extracts of *Cassia alata* can improve the vitamin E status in the liver, thereby preventing H_2O_2 induced damages.It has been reported that that pre-treatment with vitamin E to NDEA induced rats provide protection against oxidative stress in liver caused by the carcinogen [21]. In line with these reports; it is conceivable that the *Cassia alata* leaf extracts can render protection to the membranes by increasing the levels of vitamin E, the major antioxidant present in the membrane

CONCLUSION

Thus, the results of the study using precision-cut goat liver slices revealed that the methanolic extract of leaves and rhizomes of *Cassia alata* possess antioxidant principles that can fight against free radical mediated disorders.

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