Antioxidant activity of *Gloriosa superba* against paracetamol induced toxicity in experimental rats.

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Abstract:

Paracetamol was chosen to induce hepatotoxicity in rats. *Gloriosa superba* tubers were extracted and used for the treatment against paracetamol induced toxicity. In this study, the animals were divided into three groups comprising of six animals each. Group I served as a control, group II animals were administered with paracetamol(200 µg/kg) orally for 10 days, group III animals were received *Gloriosa superba* tubers aqueous extract (500 mg/kg/bw/po) for 5 days followed by paracetamol(200 µg/kg) orally for 2 days. Level of LPO was found to be increased during paracetamol intoxication with concomitant decrease in the activity of enzymic and non- enzymic antioxidants. The antioxidant enzyme levels were reverted to near normal in Group III rats which results the antioxidant activity of *Gloriosa superba.*

Key words: *Gloriosa superba,* Antioxidant, Lipid per oxidation, Paracetamol

INTRODUCTION:

Gloriosa superba is one of the oldest species from ancient time. Being native form Indian specially Southern India it is known as glory lily and climbing lily- in English; Karihari- in Hindi; Langli- in Sanskrit. Antimicrobial and *in vitro* antioxidant activities of the plants were reported. Analgesic and anti-inflammatory properties of *Gloriosa superba* were determined [1]. *Gloriosa superba* L. is a medicinal plant belonging to the family Liliaceae. Seeds and tubers contain alkaloids such as colchicine and colchicoside, which are used to treat gout and rheumatism[2]. In the Indian systems of medicine, the tubers are used as tonic, antiperiodic, antihelmenthic, and also against snake bites [3]. The plant is known as 'Kalihari' in Hindi, 'Manthori khizangu' in Malayalam, and 'Kazhappai kizhangu' in Tamil. The species has been domesticated more recently following its overexploitation in the natural habitats [4].

Paracetamol is a common antipyretic agent, which is safe in therapeutic doses but can produce fatal hepatic necrosis in man, rats and mice with toxic doses [5].protection against paracetamol – induced toxicity has been used as a test for potential hepatoprotective activity by several investigators[6]. Based on the beneficial properties of *Gloriosa superba* tuber extract as a hepatoprotectant against paracetamol induced hepatotoxicity in experimental rats and delineate the mechanism of action by studying the alteration in enzymic activities and antioxidant status.

MATERIALS AND METHODS:

Collection of plant material:

The tubers of *Gloriosa superba* were collected from ayurveda shop of Tharapuram district of Tamilnadu, India.

Preparation of Aqueous extract:

The tubers were shade dried at room temperature and powdered, weighed 10gm of plant powder and dissolved in 100ml of distilled water and mixed well and the extract was filtered through Whatmann No. 1 filter paper. This extract was used for further analysis.

Preliminary Phytochemical analysis of aqueous extract of *Gloriosa superba*

Qualitative phytochemical analysis of aqueous extract of *Gloriosa superba* tubers was performed by the method of Peach and Tracy, 1955.

Animals used:

The female Wistar strains albino rats weighing between 100-150 gm were obtained from Small Animal Breeding Center, Kerala Agricultural University, Trissur.

Experimental setup:

Rats were divided into three groups comprising of six animals each.

Group I – Normal healthy rats were served as Control.

Group II – Administered paracatamol (200µg/kg/) orally for 2 days.

Group III – Received Gloriosa *superba* (500mg/kg/ bw /po) for 5 days followed by paracetamol (200 µg/kg/).orally for 2 days.

Preparation of Tissue Homogenate

At the end of experimental period, the animals were killed by cervical decapitation. Blood was collected, Serum separated and used for determination of biochemical constituents. Liver was removed and washed with ice-cold saline. A 10 % homogenate of the washed kidney tissues were prepared in 0.01 M Tris-HCL buffer, pH 7.4. The homogenate was centrifuged for 30min and the supernatant was used for the assay of enzymes.

Assay of Enzymic and Non-Enzymic Antioxidants

Enzymic antioxidants such as Catalase (Sinha, 1972.), Glutathione peroxidase (Rotruck *et al.*, 1973), Non-Enzymic antioxidants such as Ascorbic acid (Omaye *et al.*, 1979)**,** Reduced Glutathione (GSH) (Moron *et al*., 1979) and Lipid Per oxidation (Uchiyama and Mahara, 1978) were measured.

RESULTS AND DISCUSSION

I. **Preliminary phytochemical analysis of *Gloriosa superba* tubers**

The phytochemicals present in *Gloriosa superba* tubers is presented in table 1.This shows that aqueous extract contains Alkaloids, Carbohydrates, Proteins, Thiols.

II. Assay of Enzymic and Non-enzymic antioxidants

1. Assay of Lipid per oxidation

Lipid per oxidation has been implicated in a number of pathological status. It can be use as a measure of oxidative damage. Peroxidation causes damage in the structure, fluidity and permeability of membrane, inactivates membrane bound enzymes and protein-receptors, induces swelling and alterations in respiratory functions, causes loss of –SH groups from membrane bound proteins, mediates DNA damage, carcinogenesis is also related to lipid peroxidation [7].

Table2 shows that the level of lipid per oxidation was significantly increased ($p<0.01$) in serum of Paracetamol treated Group II rats. After the treatment with plant extract the value showed near normal in group III rats, which proved the medicinal value of the plant. Thus from the above result it was evident that the activity of LPO was brought back to normal range on treatment with tuber extract in Paracetamol treated rats

2. Assay of Reduced Glutathione (GSH) and Vitamin C

Table 3 represents the levels of non-enzymic antioxidants, glutathione (GSH) and vitamin-C in control and experimental rats. The activity of reduced Glutathione enzyme and ascorbic acid were significantly decreased ($p<0.01$) in serum of Paracetamol treated hepatic damaged rats. After the treatment with plant extract the value showed near normal in group III rats, which proved the medicinal value of the plant.

Thus from the above result it was evident that the activity of GSH&VIT C was brought back to normal range on treatment with tuber extract in Paracetamol treated rats.

3. Assay of Catalase and Glutathione Peroxidase (GPx)

Catalase is found in both cytosol (70%) and mitochondria (30%) of various tissues [8]. Table 4 shows that the activity of enzymes were significantly decreased ($p<0.01$) in serum of Paracetamol treated rats. After the treatment with plant extract the value showed near normal in group III rats, which proved the medicinal value of the plant. Thus from the above result it was evident that the activity of CAT & GPx were brought back to normal range on treatment with tuber extract in Paracetamol treated rats

Decreased activity of, CAT and GPx were observed after the administration of Paracetamol to experimental rats, suggesting an increased concentration of peroxides, thereby a balance between the pro oxidant-antioxidant status is diminished.

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TABLE 1: PHYTOCHEMICAL STUDIES ON AQUEOUS EXTRACT OF Gloriosa superba tubers

TEST	OBSERVATION
	Gloriosa superba
	AQUEOUS EXTRACT
ALKALOIDS Meyer's test	 +
FLAVANOIDS Dilute HCI	 _
SAPONINS Sodium bicarbonate	 -
CARBOHYDRATES Fehling's test Benedict's test Molisch's test	 + +
PROTEINS Millon's	 +
PHENOLS Ferric chloride test Lead acetate test Libermann's test	 - - -
GLYCOSIDES	_
THIOLS	+
TANNINS Ferric Chloride test Lead Acetate test	 _ _

(+) …… PRESENCE **(-) ……. ABSENCE**

TABLE 2: LIPID PEROXIDATIVE STATUS IN THE LIVER OF CONTROL AND EXPERIMENTAL RATS

(Values are mean ± SD for six animals in each group)

Particulars	Group I C	Group II C +PC	Group III PC + *Gs*
LPO	0.86 ± 0.21	2.46± 1.32 [a*]	0.56 ± 0.16[b*]

C - Control, PC – Paracetamol,*Gs – Gloriosa superba*
Treatment Groups are as
Group I : Control
Group II : Control +Paractamol 200 mg/ Kg bw/)orally
Group III : Paractamol + *Gloriosa superba* 500 mg/ Kg bw/)orally
Enzyme units are expressed as: LPO : µ mole of MDA liberated/ min
Comparison made between the groups are as
a- Group I and Group II
b- Group II and Group III
The symbol represents statistical significance: *p<0.01 – Significant at 1 % level

TABLE 3:NON-ENZYMIC ANTIOXIDANTS IN THE LIVER OF EXPERIMENTAL AND CONTROL RATS

(Values are mean ± SD for six animals in each group)

Particulars	Group I C	Group II C +PC	Group III PC + *Gs*
VITAMIN - C	1.45 ± 0.8	0.4 ± 0.02[a*]	1.38 ± 0.13[b*]
GSH	2.02 ± 0.15	0.3 ± 0.01[a*]	1.34 ± 0.12[b*]

C - Control, PC – Paracetamol,*Gs – Gloriosa superba*
Treatment Groups are as TABLE 1
Enzyme units are expressed as:Vit-C : µg of Vit-C liberated/ min
GSH : µg of GSH liberated/ min
Comparison made between the groups are as in TABLE 1
The symbol represents statistical significance:*p<0.01 – Significant at 1 % level
ns – Non Significant

TABLE 4: ACTIVITIES OF ENZYMIC ANTIOXIDANTS IN THE LIVER OF CONTROL AND EXPERIMENTAL RATS

(Values are mean ± SD for six animals in each group)

Particulars	Group I C	Group II C +PC	Group III PC + *Gs*
CATALASE	28.92± 2.5	6.81± 0.2[a*]	21.10 ± 10[b*]
GPx	8.33 ± 1.66	2.54 ± 0.44[a*]	6.31 ± 0.92[b*]

C - Control, PC – Paracetamol, *Gs – Gloriosa superba*
Treatment Groups are as TABLE 1
Enzyme units are expressed as: CAT : µ mole of H_2O_2 consumed/ min
GPx : µg of GSH utilized/ min
Comparison made between the groups are as in TABLE 1
The symbol represents statistical significance:*p<0.01 – Significant at 1 % level