

Original Article

Antioxidant activity of Quercetin 3-O-alpha-L-arabinopyranoside isolated from *Hibiscus vitifolius* (Linn.) in mercury chloride induced toxicity

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A B S T R A C T

The fresh flowers of *Hibiscus vitifolius* Linn. were screened to determine antioxidant activity. A methanol extract of flower was prepared and partitioned sequentially with petrol, ether and ethyl acetate. The active compounds were identified by UV, ¹H NMR (Proton Nuclear Magnetic Resonance) and ¹³C NMR (Carbon-13 Nuclear Magnetic Resonance) as quercetin and quercetin 3-0-arabinopyranoside. The antioxidant activity was also studied in mercury chloride (HgCl₂) toxicity through biochemical parameters such as SOD, CAT, GSH, GP_x, AST, LPO, vitamin C, vitamin E and urea. Quercetin 3-0-arabinopyranoside treated rats showed decrease in lipid peroxidation. Statistically significant increased in enzymes compared to HgCl₂ induced group was observed. Which indicates that quercetin 3-0-arabinopyranoside isolated from the flowers of *Hibiscus vitifolius* act as a potent antioxidant.

Key Words:

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1. INTRODUCTION

Hibiscus vitifolius Linn. (Manjal Thutti) belongs to the Malvacea (Hooker JD 1954). The flowers of this plant are yellow with a purple base. The yellow petals of *Hibiscus vitifolius* are rich in flavonols with high tinctorial properties (Matthews 1947). Isolation of gossypin and quercetin have been reported earlier from the flowers (Roa and Seshadri 1935).

Free radicals are continuously produced in our body. However, these are vigorously controlled by antioxidants. When this precarious balance is broken, in favor of free radicals, it causes an oxidative stress. This oxidative stress can attack lipids, which constitute the cellular membranes, bases of the DNA, and amino acids of the proteins. Free radicals scavengers (antioxidants) are key elements in the defense system, which the body uses in order to neutralize the activity of these dangerous and, over the long term, free radical enemies (Barry 1991).

Mercury is a widespread environmental and industrial pollutant, which induces several alteration in the tissue of both animals and men (Lund *et al* 1993; Mahboob *et al* 2001; Staley and Kappas 1982). Various mechanisms, including lipid peroxidation have been proposed for the biological toxicity of HgCl₂ and it has been demonstrated that lipid peroxidation occurs in the liver, kidney and other tissues of the rats and mice following parenteral administration of mercuric

chloride (Huang *et al* 1996 and Mahboob *et al* 2001). In the present study, the antioxidant potential of quercetin 3-0-arabino pyranoside isolated from the flowers of *Hibiscus vitifolius* and oxidative stress HgCl₂ has been investigated.

2. MATERIALS AND METHODS

Isolation of flavone

Fresh flowers (1 kg) of *Hibiscus vitifolius* were collected from the banks of Cauvery in Kumbakonam, Tamil Nadu, India. The yellow portion of the petals along were carefully separated and extracted with boiling methanol (85%) under reflux. The alcoholic extract was concentrated in vacuum and the aqueous concentrated successively fractionated with petrol (60-80°C), peroxide free diethyl ether and ethyl acetate. The petrol fraction did not yield any isolated material. The diethyl ether fraction yield aglycone and ethyl acetate fraction yield the glycoside. This active compounds were identified by UV and ¹H and ¹³C NMR as quercetin and quercetin 3-0-arabinopyranoside. The ¹H and ¹³C NMR values of aglycone and glycoside were appended in table 1 and table 2.

Experimental Design

Adult male albino wistar rats weighing 120-180gm were obtained from the Indian Institute of Science, Bangalore. The animals were housed in polypropylene cages and maintained under standard conditions (12 h light and dark cycles, at 25 \pm 3°C and 35-60 % humidity). Standard pelletized feed and tap water were provided *ad libitum*.

Animals were grouped as follows and each group contains 6 rats. **Group I:** Normal animals received standard feed and water *ad libitum*. No other treatment. **Group II:** received single intraperitoneal injection of mercury chloride (5 mg/kg.bwt). **Group III:** received single intraperitoneal injection of mercury chloride (5 mg/kg.bwt) as like group II then by oral administration of quercetin (500mg/kg bwt) in aqueous suspension. **Group IV:** animals received oral administration of quercetin (500mg/kg bwt) in aqueous suspension alone.

The body weight of the animals was recorded throughout the experimental period starting from Day 0. After the experimental regimen, the rats were fasted overnight and were sacrificed by cervical dislocation under light ether anesthesia, and the blood was collected on decapitation. Serum was separated by centrifugation (20 min at 2000 rpm) and stored at -20°C for biochemical assays. Plasma was separated from heparinized blood by centrifugation at 1000g for 15 min.

Biochemical estimations

Biochemical estimations were carried out in blood samples of normal and experimental animals in each group. LPO (Beuge and Aust, 1978) Glutathione (Moron., 1979), superoxide dismutase (SOD) (Kakkar et al., 1984), catalase (Beers and Sizer, 1952), Glutathione peroxidase (Rotruk et al., 1973), Aspartate transaminase (Reitman and Frankel, 1957), Vitamin E (Baker et al., 1980) and Vitamin C (Omaye et al., 1979) were estimated..

Statistical analysis

Statistical analysis of data were done using students 't' test, and data were expressed as means \pm SD. The values at p<0.05 were considered statistically significant.

3. RESULTS AND DISCUSSION

Isolation of Flavone

The ¹H and ¹³C NMR of the aglycone appended in Table 1 and 2. It was identified as quercetin and same was confirmed by compared with an authentic sample of quercetin from *physalis minma*. (Sethuraman and Sulochana, 1988). In addition the ¹H and ¹³C NMR of the glycoside are appended in Table 1 and 2. It was

identified as quercetin 3-0- arabinopyranoside (Guajaverin) and confirmed by with an authentic sample isolated from *Psidium guajava* (Khadam and Mohammed, 1958).

Antioxidant activity

The levels of antioxidant enzymes in the serum of the control and experimental groups are shown in table 3. It is now generally accepted that lipid peroxidation and its product play an important role in liver, kidney, heart and brain toxicity (Lakshmi *et al* 2005). In this context a marked increase in the concentration of LPO was observed in mercury chloride intoxicated rats when compared to control rats, which is in agreement with the previous studies, where lipid peroxidation products were increased from 40% to 12% above basal values (Company *et al* 2004). Administration of quercetin 3-0-arabinopyranoside significantly decreased the level of LPO in HgCl₂ intoxicated rats as compared to control rats (Sener *et al* (2007).

Mercury is a transition metal and has high efficient with GSH and causes the irreversible excretion of up to two GSH tri peptide (Zalups and Lash, 1996). The present study indicated the administration of quercetin 3-0-arabino pyranoside to HgCl₂ intoxicated rats, significantly elevated GSH content and decline lipid peroxidation and reduces the mercury toxicity and reduces liver damage. Activities of SOD and CAT to scavenge superoxide ions and hydroxyl ions respectively were significantly lower in HgCl₂ intoxicated rats as compared with control rats. Lowered activities of SOD and CAT will result in the accumulation of these highly reactive free radicals leading to deleterious effects such as loss of cell membrane integrity and membrane function (Reedy and Lokesh, 1992). The observed restoration of the SOD and CAT activities in the study on treatment with quercetin 3-0-arabinopyranoside may be due to the direct stimulatory effect of quercetin 3-0-arabinopyranoside on SOD and CAT.

In the present study, decreased activity of GPx suggests an increased production of H₂O₂ and decreased level of GSH in HgCl₂ intoxicated rats. Administration of quercetin 3-0-arabinopyranoside to HgCl₂ intoxicated rats restored the activity of GPX. The restored activity of GPX may increase the level of GSH in HgCl₂ intoxicated rat. In addition, significant increase was shown in the activities of liver markers (AST) in the serum of HgCl₂ intoxicated rats, which is consistent with earlier reports (Sener *et al.*, 2007). Administration of quercetin 3-0-arabinopyranoside was significantly prevented the HgCl₂ induced elevation in the levels of diagnostic marker enzymes in serum, indicating the cytoprotective activity of quercetin 3-0-arabinopyranoside in Group III rats.

Serum levels of Vitamin E and Vitamin C in our study were significantly reduced in HgCl₂ intoxicated rats (Group II) than in the experimental control rats (Group I). Supplementation with quercetin 3-0-arabinopyranoside to HgCl₂ intoxicated rats resulted in near normal serum levels of Vitamin E and Vitamin C (p<0.001).

Animals treated with quercetin 3-0-arabinopyranoside isolated from *Hibiscus vitifolius* retained the activity of the antioxidant enzymes to near normal levels. The above findings showed that quercetin 3-0-arabinopyranoside isolated from the fresh flowers of *Hibiscus vitifolius* possess significant antioxidant activity.

Table 1: ¹H NMR spectral Data of the flavone complex

Compound	-NCH ₂ (s)	Aromatic Protons		
		2	3	4
Aglycone	3.51	7.78 (d)	7.41 (dd)	7.58 (d)
Glycoside	3.56	7.93 (d)	7.23 (dd)	7.63 (d)

Table 2: ¹³C NMR spectral Data of the flavone complex

Compound	Chemical Shift Values			
	>C=O	>C=N	-CH	C=S
Aglycone	176.71	158.72	32.23	-
Glycoside	-	158.72	32.21	194.71

Table 3: Effect of quercetin 3-0-arabinopyranoside on the antioxidant enzymes in normal and experimental rats.

Parameters	Group I	Group II	Group III	Group III
LPO (mg/dl)	5.7 ± 1.6	11.5 ± 1.2 [#]	8.5 ± 1.10 [*]	5.87 ± 1.06 [*]
SOD (u/dl)	0.41 ± 0.07	0.21 ± 0.04 [#]	0.29 ± 0.06 [*]	0.43 ± 0.08 [*]
CAT (u/dl)	56.8 ± 6.5	36.8 ± 5.9 [#]	52.5 ± 5.8 [*]	57.21 ± 5.9 [*]
GPx (u/dl)	8.2 ± 1.6	5.3 ± 1.9 [#]	8.5 ± 2.4 [*]	8.12 ± 1.8 [*]
GSH (mg/dl)	48.56 ± 9.2	37.6 ± 9.12 [#]	48.98 ± 6.2 [*]	47.58 ± 8.12 [*]
Vitamin C (mg/dl)	56.5 ± 4.1	34.7 ± 4.09 [#]	42.15 ± 5.6 [*]	57.40 ± 3.91 [*]
Vitamin E (mg/dl)	3.00 ± 0.17	2.30 ± 0.24 [#]	3.00 ± 0.15 [*]	2.97 ± 0.18 [*]

Values expressed as mean ±SD
^{*} Significantly different from group II (p<0.05)
[#] Significantly different from group I (p<0.05)

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