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Antioxidant activity of *Grewia tiliaefolia* bark

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SUMMARY

Grewia tiliaefolia is commonly used in India and several other countries for its ethnomedical properties. The antioxidant effect of 50% methanol extract of *Grewia tiliaefolia* bark was investigated by *in-vitro* and *in-vivo* methods. The extract exhibited IC_{50} value of 51.40±3.98 μg/ml in DPPH assay. In the *in vivo* experiments, the treatment was given at 250 and 500 mg/kg body weight doses orally for seven days and catalase, SOD, TBA-RS and ascorbic acid levels were estimated. Both the doses caused a significant increase in the levels of catalase in the liver and kidney, and SOD in the liver, kidney and blood serum. A significant decrease in the level of TBA-RS was also observed. These results suggest potent antioxidant nature of the extract.

Key words: *Grewia tiliaefolia*; Tiliaceae; Antioxidant; Free radical scavengers; Lipid peroxidation

INTRODUCTION

Reactive oxygen species are important part of the defense mechanisms against infection, but excessive generation of free oxygen radicals may damage tissue. Formation of lipid peroxides by the action of free radicals on unsaturated fatty acids has been implicated in the pathogeneses of atherosclerosis, aging, cancer, diabetes, cardiovascular diseases, rheumatoid arthritis etc (Hellwell and Gutteridge, 1989). Hence, the research all over the world focused on finding naturally occurring antioxidants from plants. The possibility exists, therefore, that certain plants may possess sufficient antioxidant capacity that they can be used in the battle against cellular damage and disease.

Several members of the species *Grewia* are being used traditionally for a large number of ethnomedical properties. Among them are *Grewia subinaequalis* used as hypotensive and antidiabetic, *Grewia asiatica* for antirheumatic and cooling properties, *Grewia microsa* for indigestion, typhoid fever, small pox and ulceration of mouth, and *Grewia hirsuta* for antiviral and diuretic activities (Kirtikar and Basu, 1987; Asolkar *et al.,* 1992; Raghunathaiyar, 1996). *Grewia tiliaefolia Vahl* (Tiliaceae) is a tree upto 20 meters of height and found in India, Burma, Nepal and Africa.

Traditionally, its bark is used in vitiated conditions of pitta and kapha, burning sensation, cough, skin diseases, wounds, ulcers, diarrhoea, haemorrhage, seminal weakness, general debility (Chopra, 1933; Asolkar *et al.,* 1992; Raghunathaiyar, 1996), cardiac diseases, disorders of blood, diseases of nose, in opium poisoning (Chopra, 1933) and as aphrodisiac and tonic (Raghunathaiyar, 1996). Its hot water extract is commonly used as herbal tea in several parts of Shimoga district of Karnataka (personal communication). Except for a few biological activities (Dhawan *et al.,* 1977; Kamboj *et al.,* 1977), so far not much work was done on this bark. Three tritepenoids, betulin, friedelin and lupeol were isolated from this bark (Anjaneyulu *et al.,* 1965).

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Several of the reported ethnomedical uses may be due to antioxidant nature of *Grewia tiliaefolia* bark. Hence, the present study was undertaken to investigate the *in-vitro* and *in-vivo* antioxidant activity of the 50% methanol extract of *Grewia tiliaefolia*.

MATERIALS AND METHODS

Plant material

Grewia tiliaefolia bark was collected from Haridravati, Hosanagar, Shimoga District, Karnataka, India in the month of April 2001. The plant was authenticated by Mr. S. Rajan, Survey of Medicinal Plants and Collection Unit, Government Arts College, Ootacamund, Tamilnadu, India (Voucher No: 7346).

Extraction

The shade dried bark was powdered and extracted (170 g) with 50% methanol (750 ml) in a Soxhlet extractor for 18-20 h. The extract was concentrated to dryness under reduced pressure and controlled temperature (40-50^{o}C) to yield a dark brown solid (32 g, 18.8% w/w).

Chemicals

1,1,3,3-tetramethoxy propane, 1,1 diphenyl-2, picrylhydrazyl (DPPH) and bovine serum albumin were obtained from Sigma Chemicals Co. St. Louis, USA. Methanol and dimethyl sulphoxide were obtained from Ranbaxy Laboratories Ltd., Punjab, India. EDTA, thiobarbituric acid, trichloroacetic acid, sodium CMC were obtained from Loba Chemie, Mumbai, India. Ascorbic acid and rutin were obtained from S. D. Fine Chem., Biosar, India. Hydrogen peroxide (30%) was obtained from Qualigen Fine Chemicals, Mumbai, India.

Animals

Male Wistar rats (180-200 g.) were obtained from the animal house, J. S. S. College of Pharmacy, Ootacamund, Tamilnadu, India and were maintained under standard environmental conditions and were fed standard rat feed and water *ad libitum*. The experiments were conducted as per the guidelines of CPCSEA, Chennai (approval no: JSSCP/IAEC/ Ph. Chem./01/2001-2002)

***In-vitro* antioxidant assay**

DPPH method: The antioxidant activity of the plant extract and the standards were assessed on the basis of the radical scavenging effect of the stable DPPH free radical (Gamez *et al.,* 1998). 10 µl of the ethanolic extract (from 21 mg/ml to 40 µg/ml in DMSO solution) was added to 200 µl of DPPH in methanol solution (100 µM) in a 96 well plate. After incubation at 37^{o}C for 30 min, the absorbance of each solution was determined at 490 nm using ELISA micro plate reader. The corresponding blank readings were also taken and the remaining DPPH was calculated (Hwang *et al.,* 2001). IC_{50} value is the concentration of sample required to scavenge 50% DPPH free radical.

***In-vivo* antioxidant activity**

Animals were divided into three groups comprising of 10 animals in each group. A suspension of the 50% methanol extract of *Grewia tiliaefolia* was prepared in distilled water using 0.3% sodium CMC. The first group served as control and received the vehicle (sodium CMC, 0.3% w/v) orally. The second and third groups received the 50% methanol extract of *Grewia tiliaefolia* orally at 250 and 500 mg/kg body weights, respectively. The treatment was given for 7 days and on the 8th day, blood was collected from each animal by puncturing sino ocular vein and kept at 37^{o}C in the incubator for 30 min. It was centrifuged and the serum was used for determination of catalase and SOD. Later on, all the animals were sacrificed by decapitation. The liver and kidneys were removed and weighed and homogenized immediately with Elvenjan homogenizer fitted with Teflon plunger, in ice chilled 10% KCl solution (10 ml/g of tissue). After centrifugation at 2000 rpm for 10 min, clear supernatant was used

for the determination of enzyme levels.

Catalase was estimated by following the breakdown of hydrogen peroxide according to the method of Beer and Seizer (1952). SOD was assayed according to Misra and Fridevich (1972) based on the inhibition of epinephrine auto-oxidation by the enzyme. Lipid peroxidatoin was measured in terms of malondialdehyde (MDA) content following the TBA method of Ohkawa *et al.* (1979). Ascorbic acid was measured by the method of Natelson, (1963). Statistical analysis was carried out using the Student's *t*- test and the results were judged significant, if $P < 0.05$.

RESULTS

The 50% methanol extract of *Grewia tiliaefolia* exhibited strong antioxidant activity in the DPPH radical inhibition assay as evidenced by the low IC_{50} value of 51.40 ± 3.98 µg/ml. The value for the reference standards, ascorbic acid and rutin was found to be 2.85 ± 0.20 and 9.12 ± 0.73 µg/ml, respectively (Table 1).

The administration of *Grewia tiliaefolia* crude methanolic extract to normal rats for seven days induced a dose dependent increase in the level of catalase in the liver and kidney. The increase in the levels of catalase in the treated rats at 250 and 500 mg/kg body weight in the liver was found to be 56% and 83%, respectively ($P<0.01$, when compared to control, Table 2). These results in the kidneys were found to be 20% and 36% ($P<0.05$), respectively. However, the amount of catalase in the serum was found to be less than that of control.

The administration of the extract at both the doses caused a significant increase in the level of SOD in the liver, kidney and the serum. The percentage increase in the levels of SOD in the kidney, liver and serum in the 500 mg/kg body weight treated rats was found to be 50% ($P<0.001$), 24% ($P<0.05$) and 22% ($P<0.05$), respectively. These results in the case of 250 mg/kg-body weight dose were found to be 23% ($P<0.01$), 9% and 21% ($P<0.05$), respectively.

The treatment with the methanol extract of *Grewia tiliaefolia* at both the doses caused a significant ($P<0.001$, when compared with control) and dose related decrease in the level of MDA formed in peroxidizing system. The percentage decrease in the amount of MDA in liver was found to be 69% and 81%, respectively for 250 and 500 mg/kg body weight doses. These results in case of kidney were found to be 46% and 49%, respectively. However, the treatment with the methanol extract of *Grewia tiliaefolia* does not produce any significant changes in the level of

Table 1. Effect of 50% methanol extract of *Grewia tiliaefolia* on free radical generation *in-vitro*

S. No.	Tested material	IC_{50} values (µg/ml) by DPPH Method*
1	50% Methanolic extract	51.40 ± 3.98
2	Ascorbic acid	2.85 ± 0.20
3	Rutin	9.12 ± 0.73

*Average of 10 determinations

Table 2. Effect of 50% methanol extract of *Grewia tiliaefolia* bark on antioxidant enzymes in rat liver

Treatment	Dose (mg/kg body wt)	Catalase (IU/min/mg of tissue)	SOD (Unit/min/mg of tissue)	TBA-RS (nM/mg of tissue)	Ascorbic acid (µg/mg of tissue)
Control	-	0.644 ± 0.024	0.086 ± 0.008	0.247 ± 0.017	4.510 ± 0.380
Methanol (50%) extract	250	1.005 ± 0.066**	0.106 ± 0.019**	0.075 ± 0.002***	5.660 ± 0.510
	500	1.179 ± 0.167**	0.123 ± 0.015***	0.0459 ± 0.004***	5.860 ± 0.560

Results are mean ± SE (n=10). **$P<0.01$, ***$P<0.001$, when compared with control (Student's *t*-test).

Table 3. Effect of 50% methanol extract of *Grewia tiliaefolia* bark on antioxidant enzymes in rat kidney

Treatment	Dose (mg/kg body wt)	Catalase (IU/min/mg of tissue)	SOD (Unit/min/mg of tissue)	TBA-RS (nM/mg of tissue)	Ascorbic acid (μg/mg of tissue)
Control	-	1.556 ± 0.149	0.105 ± 0.004	0.223 ± 0.004	4.060 ± 0.230
Methanol (50%)	250	1.868 ± 0.120	0.115 ± 0.003	0.120 ± 0.003**	4.160 ± 0.310
extract	500	2.119 ± 0.146*	0.131 ± 0.015*	0.114 ± 0.012**	4.240 ± 0.350

Results are mean ± SE (n=10). *P<0.05, **P<0.001, when compared with control (Student's t-test).

Table 4. Effect of 50% methanol extract of *Grewia tiliaefolia* on antioxidant enzymes in rat blood serum

Treatment	Dose (mg/kg body wt)	Catalase (IU/min/ml)	SOD (Unit/min/ml)
Control	-	0.813 ± 0.144	0.107 ± 0.01
Methanol (50%)	250	0.613 ± 0.019*	0.1304 ± 0.070*
extract	500	0.559 ± 0.056*	0.1308 ± 0.012*

Results are mean ± SE (n=10). *P<0.05, when compared with control (Student's t-test).

ascorbic acid in both kidney and liver of treated rats (Tables 2-4).

DISCUSSION

Ethnomedical literature contains a large number of plants that can be used against diseases, in which reactive oxygen species and free radicals plays a major role. A large number of such plants are known in the literature (Badami *et al*., 2003a; 2003b). In the present study, one such plant *Grewia tiliaefolia* is screened for the *in vitro* and *in vivo* antioxidant potentials.

The most abundant oxidative free radicals generated in living cells are superoxide anions and derivatives, which are known to induce cellular damage and have been shown to be responsible for the oxidative free radical induced human diseases (Hellwell and Gutteridge, 1989). The superoxide is inactivated by SOD, the only enzyme known to use superoxide anion as a substrate. However, the free radical scavenging activity of the SOD is effective only, when it is followed up by increase in the activity of catalase and/or glutathione peroxidase. SOD generates hydrogen peroxide metabolite, which is more tissue toxic than oxygen radicals and has to be scavenged by catalase or glutathione peroxidase. Thus, a concomitant increase in the activity of catalase or glutathione peroxidase is essential, if beneficial effect from increase in SOD activity is to be expected (Harman, 1991).

From the present studies, a significant increase in the level of catalase in the liver and kidney and a significant increase in the level of SOD in the liver, kidney and the serum of the treated animals is observed. This supports the antioxidant potential of *Grewia tiliaefolia* bark. The significant decrease in the level of TBA-RS in the liver and kidney of the treated rats also support the antioxidant nature of the extract. The depletion in the ascorbic acid level in the biological system is correlated to the loss of antioxidant activity mechanism. A non-significant slight increase in the ascorbic acid was observed with the extract treatments, but based on other parameters, it can be concluded that the methanol (50%) extract of *Grewia tiliaefolia* possess potent antioxidant properties. The low IC_{50} value in the *in vitro* studies also confirms the same. These results support the ethnomedical uses of the bark in several parts of India. The preliminary phytochemical studies of the extract indicated the presence of triterpenoids, phenolics, glycosides, tannins and saponins. Several of these constituents are known to possess antioxidant activity (Raj and Shalini, 1999; Badami *et al*., 2003a; 2003b). Hence, the observed antioxidant activity may be due to the presence of

any of these constituents and the plant merits further investigation to isolate its active constituents.

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