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Antioxidant Activity of the Methanolic Extract of *Ricinus communis* Leaves

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Plants containing flavonoids have been reported to possess strong properties. The 50 % methanolic extract of *Ricinus communis* leaves was screened for *in vitro* and *in vivo* antioxidant properties using standard procedures. The methanol extract exhibited IC_{50} values of 41.40 ± 3.98 and 46.75 ± 08.73 µg/mL, respectively in DPPH and nitric oxide radical inhibition assays. These values were less than those obtained for ascorbic acid and quercetin, used as standards. In the *in vivo* experiments the extract treatment at 250 and 500 mg/kg body weight dose caused a significant increase in the level of the catalase in the liver and the kidneys. A significant increase in the level of SOD in the liver was observed. The treatment also caused a significant decrease in the TBA-RS and increase in the ascorbic acid levels. These results suggest strong antioxidant potential of the methanolic extract of *Ricinus communis* leaves.

Key Words: *Ricinus communis*, Antioxidant, DPPH, Nitric oxide, Peroxidation, Free radical scavenging.

INTRODUCTION

Ricinus communis leaves belong to the family Euphorbiaceae. It is a small tree, which grows to 6 meters or more in height and found in India, South Africa, Brazil, Russia, *etc*. Leaves are used in the form of a poultice or fermentation on sores, boils and swelling. Leaves coated with oil and warms, are commonly applied over the abdomen to give relief in flatulence in children. An infusion of leaves is used for stomach ache and as a lotion for the eye. Fresh juice of the leaves is used as an emetic in poisoning by narcotics like opium[1]. They are useful as laxative, antiinflammatory in GIT disorders and skin disease[2,3]. Except for these studies, so far, no other chemical and biological investigations have been carried out on this plant.

Lipid peroxidation has gained more importance nowadays because of its involvement in pathogenesis of many diseases like atherosclerosis,

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cancer, diabetes mellitus, myocardial infarction and also in ageing. Free radicals or reactive oxygen species (ROS) are produced *in vivo* from various biochemical reactions and also from the respiratory chain as a result of occasional leakage. These free radicals are the main culprits in lipid peroxidation[4]. Plants containing flavonoids have been reported to possess strong oxidant properties[5]. Hence, in the present study, the methanolic extract of *Ricinus communis* was screened for *in vitro* and *in vivo* antioxidant properties using standard procedures.

EXPERIMENTAL

The plant was collected from Jhansi, India in the month of April 2004 and authenticated by Dr. Gaurav Nigam, Department of Botany, Institute of Basic Sciences, Bundelkhand University, Jhansi, India.

Preparation of extracts and standards: The methanolic extract of the shade-dried powdered whole plant of *Ricinus communis* was obtained. The suspension of this extract was prepared in sodium carboxy methyl cellulose (CMC, 0.3 %) using distilled water in the case of *in vivo* experiments, whereas, for *in vitro* experiments, a weighed quantity of the extract was dissolved in distilled DMSO and used. Solution of ascorbic acid and quercetin used as standards for *in vitro* studies were prepared in distilled DSMO.

Test animals: Male wister strain rats (170-200 g) were obtained from the animal house, Institute of Pharmacy, Bundelkhand University, Jhansi, India and were maintained under standard environmental conditions and fed with Raman Dairy Vikas Udhyog, Pashu Aahar Kendra, Vanarasi, India and water *ad libitum*.

In vitro assays

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[6]. A total of 200 µL of the methanolic extract (from 21 to 40 µg/mL in DMSO solution) or standard was added to 4 mL of DPPH in methanol solution (100 µM). After incubation at 37°C for 0.5 h, the absorbance of each solution was determined at 490 nm. The corresponding blank readings were also taken and remaining DPPH was calculated. IC_{50} value is the concentration of sample required to scavenge 50 % DPPH free radical.

Nitric oxide radical inhibition assay: Sodium nitroprusside in aqueous solution at physiological pH, spontaneously generates nitric oxide, which can be estimated with oxygen to produce nitrite ions and can be estimated by the use of Griess IIIosvoy reaction[7]. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxides[8]. The reaction mixture (3 mL) containing sodium nitroprusside (10

mM, 2 mL), phosphate buffer saline (0.5) and extract or standard solution (0.5 mL) was incubated at 25°C for 2.5 h. After incubation, 0.5 mL of the reaction mixture containing nitrite was pipette and mixed with 1 mL of sulphanilic acid reagent (0.33 % in 20 % glacial acetic acid) and allowed to stand for 5 min for completing diazotization. Then, 1 mL of 1-naphthylamine (5 %) was added, mixed and allowed standing for 0.5 h a pink coloured chromophore was formed in diffused light. The absorbance of these solutions was measured at 540 nm against the corresponding blank solutions. The IC_{50} value is the concentration of sample required to inhibit 50 % of nitric oxide radical.

***In vivo* antioxidant activity**

Animal were divided into three groups comprising of six rats in each group. Group-1 served as control and was given the vehicle alone (sodium CMC, 0.3 %). The second and third groups received methanolic extract of *Ricinus communis* leaves orally at 250 and 500 mg/kg body weight, respectively. The treatments were given for 7 d and on the 8th day of the experiment, all the animals were sacrificed by decapitation. The liver and kidneys were removed, weighed and homogenized immediately with Elvenjan homogenizer fitted with Teflon plunger, in ice chilled 10 % KCl solution (10 mg/g of tissue). The suspension was centrifuged at 2000 rpm at 4°C for 10 min and the clear supernatant was used for the following estimations.

Catalase was estimated by following the breakdown of hydrogen peroxide according to the method of Beer and Seizer[9]. Superoxide dismutase (SOD) was assayed according to Misra and Fridovich[10] based on the inhibition of epinephrine auto-oxidation by the enzyme. Lipid peroxidation was measured in term of MDA content following the thiobarbituric acid (TBA) method of Buege and Aust[11]. Ascorbic acid was measured by the method of Natelson[12].

RESULTS AND DISCUSSION

***In vitro* assay:** The methanolic extract of *Ricinus communis* leaves exhibited antioxidant activity in the DPPH and the nitric oxide radical inhibition assay as evidenced by the low IC_{50} values (Table-1). The IC_{50} values obtained are 41.40 ± 3.98 and 46.75 ± 8.73 μg/mL, respectively assays. These values were found to be less than those obtained for the reference standards, ascorbic acid and quercetin.

***In vivo* assays:** The administration of 50 % methanolic extract of *Ricinus communis* leaves to normal rats for 7 d induced a dose dependent increase in the level of catalase in the liver and kidneys. The results are significant at 250 and 500 mg/kg body weight dose of the treatment (respectively for liver and kidney when compared with control; Tables 2

and 3). The treatment caused a significant increase in the level of SOD in the liver and kidney (when compare with control). The treatment with 50 % methanol extract also caused a significant and dose related decrease in the level of malonodialdehyde (MDA, when compare with control) formed in peroxidizing system and a significant increase in the level of ascorbic acid (when compare with control) in the liver and kidney.

TABLE-1
EFFECT OF THE 50 % METHANOLIC EXTRACTION OF *Ricinus communis* LEAVES ON FREE RADICAL GENERATION *In vitro*

Tested material	IC_{50} (µg/mL) ± SE[a]	
	DPPH	Nitric oxide radical inhibition assay
50 % Methanolic extrac	41.40 ± 3.98	46.75 ± 8.73
Ascorbic acid	78.17 ± 4.05	20.50 ± 1.16
Quercetin	53.60 ± 1.79	19.50 ± 1.85

[a]Average of 10 determinations.

Free radical oxidative stress has been implicated in the pathogenesis of a wide variety of clinical disorders, resulting usually from deficient natural antioxidant defenses[13]. Potential antioxidant therapy should, therefore include either natural free radical scavenging enzyme or agents, which are capable of augmenting the activity of these enzymes, which include SOD and catalase[14]. The administration of the 50 % methanolic extract of *Ricinus communis* leaves at 250 and 500 mg/kg body weight significantly increased the level of catalase in liver and kidneys and the level of SOD in liver and kidney. This shows the antioxidant nature of the extract. The present study also showed the depletion in the lipid peroxidation as observed by the significant decrease in the MDA content of the liver and kidneys in the treated groups. Vitamin C is regarded as the first line natural antioxidant defense in plasma and powerful inhibitor of lipid peroxidation. It also regenerates the major antioxidant tocopherol in lipoproteins and cell membrane. Intracellular mechanisms exist which can regenerates ascorbate from its dehydroascorbate by reduced glutathione[15]. The treatment with the methanolic extract also caused a significant increase in the ascorbic acid level of the treated rats in the liver and kidneys. Fries[16] has reported the ability of vitamin C to preserve the level of other antioxidants in human plasma. Depletion of the ascorbic acid level in a biological system has been found to correlate to a loss in antioxidant capacity[17]. It can be concluded that the 50 % methanolic extract of *Ricinus communis* leaves possess antioxidant properties as evidenced by the significant increase in the levels of catalase, SOD and ascorbic acid and decrease in the level of

TABLE-2

EFFECT OF THE METHANOLIC EXTRACT (50 %) OF *Ricinus communis* LEAVES ON 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 ± 0.0244	0.086 ± 0.0081	0.2570 ± 0.0173	4.61 ± 0.38
Ricinus communis	250	1.015 ± 0.0667	0.116 ± 0.0210	0.1750 ± 0.0030	5.33 ± 0.71
Ricinus communis	500	1.130 ± 0.1140	0.143 ± 0.0123	0.0550 ± 0.0400	5.99 ± 0.90

Results are mean ± SE (n=6).

TABLE-3

EFFECT OF THE METHANOLIC EXTRACT (50 %) OF *Ricinus communis* LEAVES ON RATE 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 ± 0.249	0.105 ± 0.004	0.223 ± 0.004	4.06 ± 0.23
Ricinus communis	250	1.868 ± 0.200	0.115 ± 0.003	0.120 ± 0.003	4.16 ± 0.91
Ricinus communis	500	2.119 ± 0.246	0.131 ± 0.015	0.114 ± 0.012	4.94 ± 0.65

Results are mean ± SE (n=6).

TBA-RS. The *in vitro* studies confirm the same. The methanolic extract of *Ricinus communis* leaves is found to contain flavonoids and tannins. A large number of flavonoids including these are known to possess strong antioxidant properties. Hence the antioxidant activity of *Ricinus communis* leaves is probably due the presence of flavonoids and tannins in the 50 % methanolic extract.

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