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Effect of *Luffa echinata* on Lipid Peroxidation and Free Radical Scavenging Activity

V. PRASHANTH KUMAR, S. SHASHIDHARA, M. M. KUMAR* AND B. Y. SRIDHARA*

*Department of Pharmacognosy, Government College of Pharmacy, Bangalore, Karnataka and *Institute of Animal Health & Veterinary Biologicals, Bangalore, Karnataka, India*

Abstract

The dried alcoholic (50%) extract of the plant *Luffa echinata* was investigated for inhibition of lipid peroxidation, for hydroxyl radical scavenging activity and interaction with 1,1-diphenyl-2-picrylhydrazyl stable free radical (DPPH).

It was found that the test extract exhibited a considerable inhibition of lipid peroxidation and possessed hydroxyl radical scavenging activity. Evaluation of antiradical scavenging activity showed significant interaction with DPPH.

These properties could be considered as a useful and exploitable combination for justifying the reported activity.

Free radical-mediated cell damage and free radical attack on polyunsaturated fatty acids results in the formation of lipid radicals. These lipid radicals react readily with molecular oxygen to produce peroxy radicals responsible for initiating lipid peroxidation. The peroxidation of cellular membrane lipid can lead to cell necrosis and is considered to be implicated in a number of pathophysiological conditions as well as in the toxicity of many xenobiotics (Kappus 1985). The hydroxyl radical included among the most reactive oxygen species is thought to be a major factor responsible for oxidative injury of enzymes, lipid membranes and DNA strand breakage (Fridovich 1988).

Luffa echinata Roxb. (Cucurbitaceae), commonly known as Bindal, grows widely in the Bengal, Gujarat and Uttar Pradesh regions of India. It has been found to contain a saponin, gypsogenin, and echinatin (Bhat & Khorana 1957), cucurbitacin B (Lavie et al 1962), chrysoeriol and flavonoids along with minor amounts of two other flavones – apigenin and luteolin (Seshadri & Vydeeswaran 1971).

Therapeutic properties attributed to *Luffa echinata* by different authors are comprehensive and varied. The ethanolic extract (50%) of the plant has been reported to potentiate pentobarbitone-induced hypnosis in mice (Lauria et al 1972) and to have a hypoglycaemic action in rats (Aswal et al 1984). A Saponin isolated from *L. echinata* fruit has been reported to produce a fall in blood pressure in cats and dogs (Bhatt et al 1958a) and diuretic activity in dogs and rats (Bhatt et al 1958b). According to Kirtikar & Basu (1933) the fruits of the plant have a curative effect in chronic bronchitis and lung complaints. Nadkarni (1982) has reported that the plant has therapeutic activity in jaundice and biliary and intestinal colic, and also in cases of enlarged liver and spleen. Clinical studies have revealed significant therapeutic action against viral hepatitis (Vaidya et al 1976). Several of these properties, have been attributed to the presence of flavonoids. Flavinoids have also been found to possess various other biological properties, for example, hepatoprotective (Perrisoud & Testa 1982), antithrombotic (Gryglewski et al 1987) and antiviral (Middleton 1984) activity. Many of these actions have been correlated with their ability to scavenge oxygen-generated free radicals and to inhibit lipid peroxidation in-vitro (Younes & Siegers 1980; Robak & Gryglewski 1988).

The presence of flavonoids, as well as existing evidence concerning their hepatoprotective effect against CCl_4-induced toxicity (Lauria et al 1972), could justify an investigation into the effect of *Luffa echinata* extract on lipid peroxidation and on their ability to scavenge free radicals. The contribution of the antioxidant property could be evaluated and this may indicate an antioxidant-therapeutic activity relationship.

Correspondence: S. Shashidhara, Department of Pharmacognosy, Government College of Pharmacy, Bangalore-560 027, Karnataka, India.

Materials and Methods

Materials

Luffa echinata fruits were obtained from Natural Remedies Pvt. Ltd, Bangalore and were freed from all extraneous matter. The plant material was authenticated morphologically as well as microscopically (Shah & Mody 1971). The fruits were ground and extracted with 50% alcohol in a Soxhlet apparatus until extraction was completed, and the solvent was removed under reduced pressure in a Rotavapor. Completely dried material was stored at room temperature in a dessicator until use.

Male albino Wistar rats (SVE-experimental animal house, Bangalore) 160–190 g, were housed in clean acrylic cages and allowed free access to standard laboratory chow and water ad libitum. The rats not fed for 12 h before experiments. All chemicals used were of analytical grade. Solvents were distilled before use.

All experiments were performed in triplicate.

Inhibition of lipid peroxidation in rat liver homogenate

For preparing liver homogenate (10%), Wistar rats were killed by cardiac puncture followed by perfusion of the liver with saline. The liver was separated and then homogenised in 10 volumes of 0·15 M KCl (Ohkawa et al 1979).

The level of lipid peroxidation in the rat liver homogenate was measured in-vitro as thiobar-bituric acid reactive substances (TBARS), as des-cribed previously (Buege & Aust 1978). Briefly, 0·2 mL suspension of fresh liver homogenate was incubated with Tris HCl buffer (pH 7·5), 0·1 mL of 0·15 M KCl and 2 μM adenosine diphosphate with different concentrations of the test extract separately (10 μg mL^{-1}, 100 μg mL^{-1}, 1000 μg mL^{-1} of final volume). After 5 min, 0·1 mL each of 10 μM $FeSO_4$ and 0·1 μM ascorbic acid were added and incubated at 37°C for 1 h. The reaction was terminated by addition of 2 mL thiobarbituric acid reagent, boiled for 15 min at 95°C, cooled, centrifuged and absorbance was read at 535 nm. Malondialdehyde thus formed was quantified using a molar extinction coefficient of $1{\cdot}56 \times 10^{-5}$ mol^{-1} cm^{-1} and expressed as U mg^{-1} of protein.

The protein content of the rat liver homogenate was determined by the method of Lowry et al (1951) using bovine serum albumin as a standard.

In-vitro hydroxyl radical scavenging potential of the test extract

The formaldehyde formed during the oxidation of the dimethyl sulphoxide by the Fe^{3+}–ascorbic acid system was used to detect hydroxyl radicals (Klein et al 1981). The reaction mixture contained EDTA, 0.1 mM Fe^{3+} (as a 1 : 2 with EDTA) 167 μM DMSO (33 mM) in phosphate buffer (50 mM, pH 7·4). The test extract was added at three different concentrations (10, 100 and 1000 μg mL^{-1} of the final volume) separately. The reaction was started by the addition of 2 mM ascorbic acid. The mixture was incubated at 37°C for 30 min and then the reaction was stopped by the addition of 125 μL trichloroacetic acid (17·5% w/v). The formaldehyde formed was assayed spectrophotometrically by the method of Nash (1953).

Interaction of test extract with 1,1-diphenyl-2-phenylhydrazyl free radical (DPPH)

The antiradical scavenging potential of the test extract was assessed by adding to the ethanolic solution of DPPH (final concentration 200 μM) an equal volume of the test extract dissolved in ethanol at various concentrations separately (10 μg mL^{-1}, 100 μg mL^{-1}, 1000 μg mL^{-1}). Appropriate controls were maintained. The absorbance was read at 517 nm after 20 min at room temperature (Kato et al 1988).

Results

We examined the role of *Luffa echinata* extract with respect to anti-lipid peroxidation and free-radical-scavenging activity. *Luffa echinata*, at different concentrations, inhibited the in-vitro lipid peroxidation of rat liver homogenate in a dose-dependent manner. The anti-lipid peroxidation was found to be 26·31%, 43·85% and 61·4% with 10 μg mL^{-1}, 100 μg mL^{-1} and 1000 μg mL^{-1} of final volume, respectively, as shown in Table 1.

To examine the hydroxyl-radical-scavenging activity of test extracts at different concentrations, the competition between them and DMSO for HO$^{\bullet}$

Table 1. Effect of *Luffa echinata* extract on lipid peroxidation in rat liver homogenate.

Treatment	*Luffa Echinata*	
	Concn of drug (μg mL^{-1} in final volume)	Lipid peroxidation (μM (mg protein^{-1})
Control		73·07 ± 0·007
Luffa echinata	10	53·84 ± 0·006
	100	41·02 ± 0·007
	1000	28·20 ± 0·006

Values are represented as mean ± s.e.m., n = 3. $P < 0{\cdot}0001$.

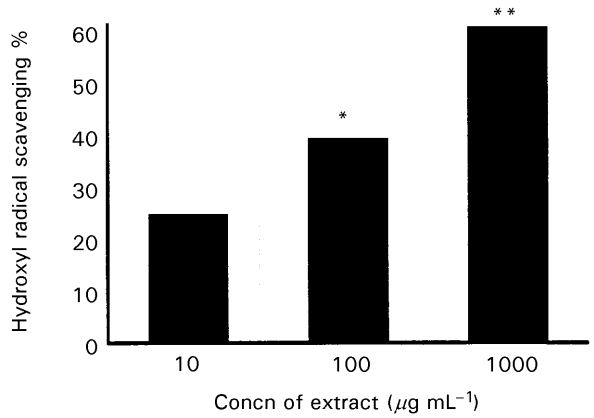

Figure 1. Hydroxyl radical scavenging potential of *Luffa echinata* extract. Values are represented as mean ± s.e.m., n = 3. $*P = 0{\cdot}003$, $**P < 0{\cdot}0001$, compared with control, Student's *t*-test.

Table 2. Interaction of *Luffa echinata* extract with DPPH stable free radical (200 μM).

Concn of *Luffa echinata* (μg mL^{-1} in final volume)	% Interaction
10	41·80
100	67·02
1000	84·05

generated from the ascorbic Fe^{3+} ascorbic–acid system, expressed as percentage inhibition of formaldehyde production. Results are shown in Figure 1.

Anti-radical scavenging activity of the *Luffa echinata* extract was evaluated by the interaction of the extract with the stable free radical DPPH (200 μM) at three different concentrations (10 μg mL^{-1}, 100 μg mL^{-1}, 1000 μg mL^{-1}).

Discussion

Oxygen-generated free radicals have been shown to be implicated in many pathophysiological conditions (Marx 1987). The peroxidation of membrane lipids initiated by oxygen removal can lead to cell injury. The HO· radical produced in the body is very reactive and one of the strongest oxidizing agents, and is involved in many pathophysiological processes including DNA strand breakage (Imlay & Linn 1988) and K^+ loss from the cell membrane (Maridoneau Parinin et al 1986). Antioxidant treatment seems to offer protection against free radical-induced injury. Although many of the synthetic antioxidants are more efficient than biological antioxidants in-vitro, they produce side reactions unrelated to their biological functions (Scott 1985).

Investigation of the effects of dried alcoholic extract of *Luffa echinata* on lipid peroxidation reveals that it exerts a dose-dependent anti-lipid peroxidation. The HO·-radical-scavenging activity of the test extract was also investigated. From the DMSO results it can be seen that at all the doses tested, oxidation of DMSO was significantly inhibited. This inhibition was principally due to HO·-scavenging ability. The interaction with the stable free radical DPPH expresses the reducing activity of the test extract and indicates its ability to scavenge free radicals (Ratty et al 1988).

A direct relationship between HO· radical scavenging and anti-lipid peroxidative potential of the test extract with about the same order, constitutes an exploitable and apparently useful combination in preventing free radical-induced pathophysiological abnormalities.

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