Antioxidant and Hepatoprotective Activity of *Lagenaria siceraria* Aerial parts

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ABSTRACT

Introduction: *Lagenaria siceraria* is traditionally used in liver disorders and various free radicals induced diseases. The present study was carried out to evaluate the antioxidant and hepatoprotective activities of the aerial parts of *L. siceraria* methanol extract (MELS). **Methods:** DPPH, nitric oxide, superoxide, hydrogen peroxide, lipid peroxide free radical scavenging activity, reductive ability and total phenolic and flavonoid content of MELS were determined. Hepatoprotective activity of the extract was investigated against carbon tetrachloride induced hepatotoxicity in rats. **Results:** The results explored significant *in vitro* antioxidant activity of MELS. It also showed potent hepatoprotective activity in rat, which was evident from its significant effect on the levels of serum biomarker enzymes and total protein & bilirubin. Significant improvement of the endogenous antioxidant status by the treatment of MELS further reflects its hepatoprotective potential, which was finally substantiated by the histological studies of the liver tissues. **Conclusion:** The results reveal potent hepatoprotective activity of MELS which is probably attributed to its significant free radical scavenging activity and high polyphenolic and flavonoid contents.

Key words: Antioxidant, Cucurbitaceae, Carbon tetrachloride, DPPH, Free radical, Hepatoprotective, *Lagenaria siceraria*.

INTRODUCTION

Free radicals generated either exogenously or endogenously in our body have been implicated in the pathophysiology of various clinical disorders, such as liver cirrhosis, inflammation, atherosclerosis, diabetes, cancer, neurodegenerative diseases, nephrotoxicity and also the process of aging.[1-5] The link between free radicals and diseases has led to considerable research into nontoxic drug that can scavange the free radicals. These radicals react with cell membrane, induce lipid peroxidation and are responsible for various deleterious effects in cells and tissues where they are generated.[6] The inhibition of free radical generation can serve as facile model for evaluating the activity of hepatoprotective agents. Several plant extracts and plant products have been found to possess significant *in vitro* and *in vivo* antioxidant property.[7-11]

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Lagenaria siceraria (Mol.) Standley, commonly known as bottle-gourd (in English), belongs to cucurbitaceae family. The plant is widely available throughout India as an edible vegetable. It is a climbing or trailing herb, with bottle or dumb-bell shaped fruits. Both of its aerial parts and fruits are commonly consumed as vegetable. Traditionally the plant is used as medicine in India, China, European countries, Brazil, Hawaiian island etc. for its cardiotonic, general tonic, hepatoprotective, diuretic properties.[12] Further, antihepatotoxic, analgesic and anti-inflammatory, hypolipidemic, antihyperglycemic, immunomodulatory and antioxidant activities of its fruit extract have been evaluated.[13-17] *Lagenaria siceraria* fruits are good source of vitamin B complex, ascorbic acid, fibers, proteins, cucurbitacins, saponins, fucosterols and compesterols, polyphenolics, flavones-C-glycoside.[13,14,18-20] Methanol extract of its leaves showed the presence of sterols, polyphenolics, flavonoids, saponin, protein and carbohydrates.[21] A novel protein, Lagenin has also been isolated from its seeds and it possesses antitumor, immunoprotective and antiproliferative properties.[22] Although extensive studies have been carried out on its fruits and seeds, pharmacology of the aerial parts of *L. siceraria* however has not been explored yet. The present investigation was therefore carried out to evaluate the antioxidant and hepatoprotective potential of methanol extract of *L. siceraria* aerial part (MELS).

MATERIALS AND METHODS

Plant Material

The aerial parts of *L. siceraria* was collected in November 2008, from Madanpur, West Bengal, India and identified by the Botanical Survey of India, Howrah, India. A voucher specimen (P/LS/1/08) was retained in our laboratory for further reference.

Preliminary Phytochemical Screening

Preliminary phytochemical screening was carried out by following the standard procedures.[23]

Preparation of Plant Extract

The aerial parts were dried under shade and powdered in a mechanical grinder. The powdered material was extracted with methanol using soxhlet apparatus. This extract was filtered and concentrated in *vacuo* in a Buchi evaporator, R-114 and kept in vacuum desiccators until use. The yield was 18.13% w/w with respect to dried powder. Various concentrations of methanol solution of MELS was used for *in vitro* antioxidant studies, while, aqueous suspension of MELS was prepared using 2% (v/v) Tween-80 for oral administration.

Animals

Healthy Wistar albino rats (180 g ± 20) were used for the present study. They were maintained at standard laboratory conditions and fed with commercial pellet diet (Hindustan Lever, Kolkata, India) and water *ad libitum*. The animals were acclimatized to laboratory condition for one week before commencement of experiment. The experiments were performed following the animal ethics guidelines of Institutional Animals Ethics Committee.

Acute Toxicity Study

Healthy rats were starved overnight and then were divided into five groups ($n = 4$). Group I-IV animals were orally fed with MELS in increasing dose levels of 0.5, 1.0, 1.5 and 2.0 g/kg b.wt, while group V (untreated) served as control. The animals were observed continuously for first 2 h for any gross change in behavioral, neurological and autonomic profiles or any other symptoms of toxicity and mortality if any, and intermittently for the next 6 h and then again after 24 h, 48 h and 72 h for any lethality or death. One-tenth and one-fifth of the maximum safe dose of the extract tested for acute toxicity, were selected for the experiment.[24]

In vitro Antioxidant Activity Study

Various concentrations of MELS (10-160 µg/ml in methanol) was used for *in vitro* antioxidant studies on different models, DPPH, NO, SO, H_2O_2 and LPO. For reductive ability study, 100-800 µg/ml concentration of the extract was used. Butylated hydroxyl toluene (BHT) was used as standard.

Determination of DPPH Radical Scavenging Activity

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was measured using the method of Cotelle *et al*[25] with some modifications. 3 ml of reaction mixture containing 0.2 ml of DPPH (100 µM in methanol) and 2.8 ml of test or standard solution of various concentrations was incubated at 37 °C for 30 min and absorbance of the resulting solution was measured at 517 nm using Beckman model DU-40 spectrophotometer. The percentage inhibition of DPPH radical was calculated by comparing the results of the test with those of the control (not treated with extract) using the formula:

$$\text{Percentage inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of test})}{\text{Absorbance of control}} \times 100$$

Determination of Nitric oxide Scavenging Activity Assay

At physiological pH, sodium nitroprusside generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be measured by the Griess reaction.[26] 1 ml of 10 mM sodium nitroprusside was mixed with 1 ml of test or standard solution of different concentrations in phosphate buffer (pH 7.4) and the mixture was incubated at 25 ºC for 150 min. From the incubated mixture, 1 ml was taken out and 1 ml of Griess' reagent (1% sulphanilamide, 2% o-phosphoric acid and 0.1% naphthyl ethylene diamine dihydrochloride) was added to it. Absorbance of the chromophore formed by the diazotization of nitrite with sulfanilamide and subsequent coupling with naphthyl ethylene diamine dihydrochloride was read at 546 nm and percentage inhibition was calculated by comparing the results of the test with those of the control using the above formula.

Determination of Superoxide Radical Scavenging Activity

Superoxide anion scavenging activity was measured according to the method of Robak and Gryglewski[27] with some modifications. All the solutions were prepared in 100 mM phosphate buffer (pH 7.4). 1 ml of nitroblue tetrazolium (NBT, 156 µM), 1 ml of reduced nicotinamide adenine dinucleotide (NADH, 468 µM) and 3 ml of test/standard solution were mixed. The reaction was initiated by adding 100 µl of phenazine methosulphate (PMS, 60 µM). The reaction mixture was incubated at 25 ºC for 5 min, followed by measurement of absorbance at 560 nm. The percentage inhibition was calculated from the above formula.

Determination of Hydrogen peroxide Scavenging Activity

The hydrogen peroxide scavenging ability of the extract was determined according to the method of Ruch *et al*.[28] A solution