Hepatoprotective activity of *Luffa cylindrica* (L.) M. J. Roem. leaf extract in paracetamol intoxicated rats

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Methanolic extract of *Luffa cylindrica* (L.) M. J. Roem. leaves (MELC) was evaluated for its hepatoprotective potential against paracetamol intoxicated wistar rats. Administration of MELC and standard drug silymarin showed significant (*P* <0.05) hepatoprotective protection in experimental animals. The study was conducted by estimating serum enzyme activities of serum glutamate oxaloacatate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT)), alkaline phosphatase (ALP) and bilirubin and by estimation of some serum antioxidant enzymes. Elevated serum marker enzymes of SGOT, SGPT, ALP and serum bilirubin were significantly (*P*<0.001) reduced to near normal level in MELC treated animals. Lipid peroxidation level was decreased significantly by MELC 250 mg/kg and 500 mg/kg doses treatment groups. In case of antioxidant enzymes SOD, GSH and catalase levels were increased significantly (*P* <0.001) after treatment with MELC. The extract showed a dose-dependent reduction of paracetamol induced elevated serum levels of enzyme activities indicating the extract could preserve the normal functional status of the liver. Results of this study demonstrated that *L. cylindrica* has good hepatoprotective potential.

**Keywords**: Hepatoprotective, *Luffa cylindrica*, Methanolic extract, Paracetamol.

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**Introduction**

Hepatic system is pivotal organ system and plays an astounding range of vital functions in the maintenance, performance and regulating homeostasis of the body by detoxification and excretion of many endogenous and exogenous compounds. Therefore, damage to the liver inflicted by hepatotoxic agents is of grave consequences1. The liver is often abused by environmental toxins, poor eating habits, alcohol and over-the-counter drug use, which can damage and weaken the liver and eventually lead to several liver disorders2,3. Unfortunately the conventional medicines used in the treatment of hepatotoxicity are inadequate. However, traditional system of medicine offers a number of medicinal preparations which play a vital role in the management of liver diseases. In the present context there is growing focus to follow systematic research methodology and to evaluate scientific basis for the traditional herbal medicines to explore the potential of the agents that are claimed to possess hepatoprotective activity.

*Luffa* [*Luffa cylindrica* (L.) Roem. syn *L. aegyptiaca* Mill.] commonly called sponge gourd, loofa, vegetable sponge, bath sponge or dish cloth gourd, is a member of Cucurbitaceous family, cultivated throughout the world and is distributed mainly in tropical to warm-temperate areas4. The plant is reported to have anti-tubercular and antiseptic properties5,6. Usually, the skin of the gourd is peeled off when it is used as vegetable. Seeds and sponge of the old fruits are used in traditional medicine as anthelmintic, stomachic and antipyretic phytomedicinal drugs7. Its seeds have also been used in the treatment of asthma, sinusitis and fever8. Its seed oil is reported to possess antioxidant9, antimicrobial10,11, anti-inflammatory12 and bronchodilator properties13. An abortifacent protein luffacin, isolated from seeds of *L. cylindrica* has ribosome inhibiting properties on the replication of HIV infected lymphocyte and phagocyte cells, explain its potential as therapeutic agent for AIDS14,15. Its fruit is used in the treatment of hyperglycaemia16, ascites, hepatotoxicity17, billiary...
and intestinal colitis and also in enlarged spleen and liver. Juice extracted from its stem has been used in the treatment of several respiratory disorders. It has been demonstrated from untimely studies that plant possesses a variety of pharmacological effects, viz. anticancer, enzyme inhibitor, etc.

However, despite such interesting health virtue of the fruit, seeds and stems of this common vegetable, literature reveals that no scientific study has been carried out to determine the pharmacological activity of leaves of this plant in terms of hepatic effects. Therefore it was considered worthwhile to explore the prospect for hepatoprotective activity of methanolic extract of *L. cylindrica* (MELC) leaves in the present investigation.

**Materials and Methods**

**Chemicals and reagents**

Paracetamol was procured from E. Merck (India) Ltd. Mumbai; Silymarin was obtained from Ranbaxy Laboratories Limited, India. Standard kit of serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP) and bilirubin was obtained from Centronic GmbH, Germany. The chemicals used for evaluation of oxidative stress parameters were obtained from Sisco Research Laboratories, India. Folin-Ciocalteu reagent was purchased from Sigma Chemicals (USA). All the other drugs and chemicals used in the study were of analytical grade.

**Plant material**

The leaves of *L. cylindrica* were procured locally from Pakbara village, District Moradabad of Uttar Pradesh, India and were authenticated by Dr. Beena Kumari, Taxonomist, Hindu College, Moradabad (India). A voucher specimen has been kept in the herbarium (HC.MBD/HAP/BK/2010/5/168) of the Department of Botany, Hindu college, Moradabad (India) for further references.

**Preparation of extract**

Leaves were washed with tap water and dried in shade. Dried leaves were ground to coarse powder and stored in an airtight container. The dried and coarsely powdered plant material was extracted with petroleum ether (60-80°) by hot percolation in soxhlet apparatus until it became colorless. The defatted plant material was also extracted with methanol until it became colorless. The extract was concentrated under reduced pressure to yield a crude semi-solid mass. The last traces of the solvent were evaporated under reduced pressure in rotatory evaporator. The methanolic extract was subjected to preliminary phytochemical screening for detection of major phytoconstituents.

**Determination of total phenolics**

100 mL of the MELC was dissolved in 250 mL of methanol/ water (60:40, v/v, 0.3% HCl) and filtered through a 0.45 μm Millipore filter. To 100 mL of filtrate, 100 mL of Folin-Ciocalteu reagent (50%, v/v) and 2.0 mL of sodium carbonate (2%, m/v) were added and mixed completely. After 2 h, the absorbance of the solution was measured at 750 nm. Quantification was based on the standard curve of gallic acid (0-1.0 mg/mL) dissolved in methanol/water (60:40 v/v, 0.3% HCl). Phenolic content was expressed as milligrams per gram of gallic acid equivalent (GAE).

**Animals**

Wistar albino male rats weighing between 120-170g were used for the hepatoprotective study. The animals were housed in a group of four in clean polypropylene cages and maintained at 22 ± 2°C under 12 h light/dark cycle and were fed *ad libitum* with standard pellet diet (Golden feed, New Delhi, India) and had free access to water. The animals were acclimatized to laboratory condition for seven days before commencement of experiments. The study protocol was approved by the Institutional Animal Ethical Committee as per the requirements of Committee for the Purpose of Control and Supervision on Animals (CPCSEA), New Delhi.

**Acute toxicity study**

Acute toxicity study of *L. cylindrica* leaf extract was performed according to OECD Guideline 423. The different doses of leaf extract solution (5, 50, 100, 300, 1000 and 2000 mg/kg b.w.) were given orally. Animals were observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 h (with special attention given during the first 4 h) and daily then after, for a total of 14 days. As there was no mortality rate found in animals, leaf extract was found to be safe in dose range of 5-2000 mg/kg. Consequently selection of dose was established as 250 mg/kg and 500 mg/kg.
Paracetamol induced hepatotoxicity

Animals were divided into five groups of six animals each. For the paracetamol induced hepatotoxicity studies, paracetamol (500 mg/kg po) suspension was prepared using 1% gum acacia and was administered to all animals except the animals of the normal control group. Silymarin (100 mg/kg po) was used as standard. Group I, which served as vehicle control received 1% gum acacia suspension only. Group II served as paracetamol treated control. 48 h after paracetamol administration, groups III, IV & V received MELC extract 250, 500 and silymarin 100 mg/kg b.w., po, respectively, once daily for 5 consecutive days.

Hepatoprotective activity

Blood was collected by retro orbital artery bleeding, 16 h after administration of last dose of drugs. Blood samples were centrifuged for 10 minutes at 3000 rpm to separate the serum. The biochemical parameters like SGOT\(^{23}\), SGPT\(^{23}\), ALP\(^{24}\) and bilirubin\(^{25}\) were estimated using respective assay kits according to the methods described by the manufacturers.

The rats were sacrificed by ether anesthesia on day 6 and liver was excised, rinsed in 0.25 M sucrose solution and 10% w/v homogenate was prepared in 0.15M KCl, centrifuged at 1000 rpm for 10 min followed by centrifugation of the supernatant at 12000 rpm for 15 min. The supernatant obtained was used for estimation of various oxidative enzymes. Lipid peroxidation (LPO) was estimated by standard method\(^{26}\) and expressed as nmol of malondialdehyde formed/min/mg protein. Superoxide dismutase (SOD) activity was assayed by method of Kakkar\(^{27}\) and the results have been expressed as unit of SOD activity/mg protein. Catalase (CAT) and reduced glutathione (GSH) were determined according to respective methods\(^{28,29}\) and the results are expressed as units of CAT activity/mg protein and GSH formed nmol/mg protein/min, respectively.

The results of hepatoprotective activity were presented as the mean ±SEM of 6 animals each group. Results were analyzed statistically using one way analysis of variance (ANOVA) followed by Dunnett’s multiple comparison test. Values of \(P <0.05\) were considered significant.

Histopathological analysis of liver

For histological studies, liver tissues were fixed with 10% phosphate-buffered neutral formalin, dehydrated in graded (50-100 %) alcohol and embedded in paraffin. Thin sections were cut and stained with hematoxylin and eosin stain for microscopic assessment. The initial examination was qualitative, with the purpose of determining histopathological lesions in liver tissue.

Results

The preliminary phytochemical screening of MELC indicated the presence of glycosides, terpenoids, steroids, flavonoids and tannins. The extract was found to contain 53.78±1.01 μg/mg total polyphenolics expressed as GAE (micrograms per milligram of GAE).

Effects on serum enzymes

The results of biochemical parameters revealed the elevation of enzyme level in paracetamol treated group, indicating that paracetamol induces damage to the liver. A significant reduction (\(P <0.001\)) was observed in SGPT, SGOT, ALP and total bilirubin levels in the groups treated with silymarin and methanolic extract of \(L.\) cylindrica. The enzyme levels were almost restored to the normal after the said treatment (Table 1). It was found that the extract decreased the paracetamol induced elevated levels of the enzymes, indicating the production of structural integrity of hepatocytic cell membrane or regeneration of damaged liver cells by the extract.

<table>
<thead>
<tr>
<th>Table 1—Effect of MELC on various biochemical parameters in rats with paracetamol induced hepatotoxicity</th>
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</thead>
<tbody>
<tr>
<td><strong>SGOT (IU/L)</strong></td>
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<tr>
<td>-----------------</td>
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<tr>
<td>Vehicle control</td>
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<tr>
<td>PCM control</td>
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<tr>
<td>MELC (250 mg/kg)</td>
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<tr>
<td>MELC (500 mg/kg)</td>
</tr>
<tr>
<td>Silymarin (100 mg/kg)</td>
</tr>
</tbody>
</table>

Values represent mean ± SEM of six animals in each group; \(^a\)\(P <0.001, \)^b\(P <0.05\) compared to normal control group.
Table 2—Effect of MELC on various parameters of oxidative stress in rats with paracetamol induced hepatotoxicity

<table>
<thead>
<tr>
<th>Group</th>
<th>GSH (mM/gm)</th>
<th>Catalase (U/mg)</th>
<th>LPO (nM/mg)</th>
<th>SOD (% inhibition NBT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle control</td>
<td>35.58±1.02</td>
<td>19.57±0.11</td>
<td>3.292±0.01</td>
<td>67.19±0.48</td>
</tr>
<tr>
<td>PCM control</td>
<td>9.50±0.05</td>
<td>7.823±0.02</td>
<td>7.930±0.17</td>
<td>22.80±0.11</td>
</tr>
<tr>
<td>MELC (250 mg/kg)</td>
<td>25.09±0.46</td>
<td>14.09±0.19</td>
<td>7.930±0.17</td>
<td>22.80±0.11</td>
</tr>
<tr>
<td>MELC (500 mg/kg)</td>
<td>29.06±0.32</td>
<td>15.69±0.07</td>
<td>4.86±0.03</td>
<td>51.87±0.38</td>
</tr>
<tr>
<td>Silymarin (100 mg/kg)</td>
<td>32.25±0.19</td>
<td>16.65±0.09</td>
<td>4.212±0.02</td>
<td>58.48±0.81</td>
</tr>
</tbody>
</table>

Values represent mean ± SEM of six animals in each group; *P* <0.001, **P** <0.01 compared to normal control group.

Plate 1—Histological images of treated animals (a) Paracetamol intoxicated group (b) MELC 250 mg/kg treated group (c) MELC 500 mg/kg treated group (d) Silymarin 100 mg/kg treated group.

Effects on lipid peroxidation, antioxidant enzymes

The effects of MELC extract on GSH, LPO, SOD and catalase in rats with paracetamol induced hepatotoxicity are summarized in Table 2. There was a marked decrease in GSH level in rats treated with paracetamol, i.e. 9.50 mM/g compared to 35.58 mM/g in normal control rats. The GSH level was significantly increased to 25.09 mM/g (*P* <0.05) and 29.06 mM/g (*P* <0.001) by the treatment with MELC at 250 mg/kg and 500 mg/kg, respectively. The data obtained in the present study (Table 2) clearly shows an increase in the MDA levels of rats treated with paracetamol suggesting enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent formation of excessive free radicals. The increase in TBARS levels (7.93 nmol/mg compared to 3.29 nmol/mg in normal control) was significantly reduced by the treatment with 250 mg/kg and 500 mg/kg MELC (4.86 nmol/mg and 4.21 nmol/mg, respectively) and by the treatment with silymarin (3.717 nmol/mg). The decreased SOD level in paracetamol treated rats was also observed when compared to control. The level of SOD was increased again by the administration of MELC and silymarin.

Administration of MELC increased the CAT level in paracetamol treated rats with induced liver damage, thus preventing accumulation of excessive free radicals and protecting the liver from paracetamol intoxication (Table 2). The level of catalase (7.82 U/mg protein)
was significantly increased by the administration of 250 mg/kg MELC to 14.09 U/mg protein and by 500 mg/kg to 15.69 U/mg protein and by silymarin to a 16.65 U/mg protein value.

The decreased SOD level in paracetamol treated rats was also observed when compared to control. The level of SOD was increased again by the administration of MELC and silymarin. MELC causes a significant ($P < 0.001$) increase in hepatic SOD activity and thus suggests the reduction in reactive free radical induced oxidative damage to liver.

**Histopathological observations**

After histological examination of paracetamol treated animals, degeneration in centrilobular fatty section was revealed. Sinusoids were observed to be inflamed and flooded with inflammatory cells. Hepatocytes were observed to possess necrosis, degeneration and disarrangement. In treated groups significant protection was observed; as sign of necrosis disappeared although few of cells were found to be inflamed with sign of infiltration of macrophages. Extracts were found to be much effective in protecting hepatocytes at selected doses. Recovery against paracetamol induced necrosis in their compact arrangement of hepatic cells was observed; whereas the section of liver of animals treated with silymarin showed that extent of liver damage was lesser in magnitude as compared to the paracetamol treated animals (Plate 1).

**Discussion**

Paracetamol-induced liver injury is commonly used as models for investigation into the efficacy of hepatoprotective drugs. The toxicity of paracetamol is not due to paracetamol itself but is attributed to its reactive metabolite N-acetyl-p-benzoquinoneimine (NAPQI) which is capable of binding covalently to cellular macromolecules (proteins, DNA) to produce protein adducts. Higher doses of paracetamol and NAPQI results in oxidation and depletion of liver GSH pool and deviate it to augmented lipid peroxidation and liver damage. This also results in the alteration in the level of other oxidative enzymes. In the present investigation a significant damage to the liver tissues was observed when animals were exposed to the paracetamol.

The raised serum liver enzymes such as ALT, AST and ALP in intoxicated rats can be attributed to the damage in the histostructural integrity of the liver cells (hepatocytes). The crude extract of *L. cylindrica* leaves used in this study preserve the structural integrity of hepatocytes membrane. This was evident from the hepatoprotection provided by MELC to rats given paracetamol which reversed the rise in serum liver enzymes. The aspect of the observed alterations of serum enzymes levels to hepatic damage on health was confirmed by histopathological studies of liver which have shown that livers challenged with paracetamol have centrizonal necrosis, focal necrosis and ballooning. In animals treated with MELC no noticeable hepatocellular necrosis was observed. The preliminary phytochemical studies revealed the presence of flavonoids in methanolic extract of *L. cylindrica*. Flavonoids have been reported for their hepatoprotective activity. So the hepatoprotective effect of *L. cylindrica* may be due to its flavonoid content.

**Conclusion**

The hepatoprotective effect of *L. cylindrica* leaves may be attributed to the presence of flavonoids, however, further studies to characterize the active principles and to elucidate the mechanism is required.

**Acknowledgements**

Authors are thankful to Shri Suresh Jain, Hon’ble chancellor, Teerthanker Mahaveer University for his invariable encouragement and endowing us with facilities necessitated for successful completion of the study.

**References**


IN VITRO ANTIOXIDANT ACTIVITY OF LAGENARIA SICERARIA LEAVES

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The free radical scavenging potential of methanolic extract of Lagenaria siceraria leaves (MELS) was studied on in vitro antioxidant models. The antioxidant potential was evaluated by determining the activity of hydroxyl and hydrogen peroxide (H2O2) radicals scavenging and 1,1-diphenyl-2-picryl hydrazyl (DPPH) assay. In all these studies, a significant correlation existed between concentrations of the extract and percentage inhibition of free radicals. The extract was also shown to have high phenolic content i.e. 99.09±0.10 μg/mg. These results clearly indicated that MELS could be a potential source of natural antioxidant and effective against free radical mediated diseases.

Keywords: Antioxidant, In vitro, Lagenaria siceraria, Reactive oxygen species

INTRODUCTION

Free radicals, often called reactive oxygen species (ROS), are fundamental to any biochemical process and represent an essential part of aerobic life and metabolism (Tiwari 2001). They are generated as by-products of biological reactions or from exogenous factors. When ROS production is greater than the detoxification capacity of the cell, excessively generated ROS causes extensive damage to DNA, proteins, lipids etc. and acts as a mediator of pro-inflammatory and carcinogenic events (Kowaltowski and Vercesi 1999). Such conditions are considered to be important causative factors in the development of diseases such as diabetes, stroke, arteriosclerosis, cancer and cardiovascular diseases (Yamaguchi et al. 2000). In the treatment of such diseases, antioxidant therapy has gained an immense importance. These antioxidants interfere with the oxidative processes by reacting with free radicals, chelating catalytic metal ions and also by acting as oxygen scavengers (Buyukokuroglu, Oktay and Kufrevioglu 2001). The biological effects of dietary antioxidants have generated a lot of interests in the modern era due to their potent antioxidant activities, absence of side effects and economic viability (Auudy et al. 2003). Many scientists have tried to obtain dietary antioxidants such as ascorbate, tocopherol and carotenoids from fruits and vegetables as they could help in protecting cells from cellular damages induced by oxidative stress.

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The plant, *Lagenaria siceraria* (Molina) standl. (Family: Cucurbitaceae), known as bottle gourd, is a common fruit vegetable used throughout India. Since time immemorial the fruit has been used as immunosuppressant (Sankari et al. 2010), diuretic (Ghule et al. 2007), cardio-tonic, cardio-protective (Hassanpour, Bodhankar and Dikshit 2008) and nutritive agent (Rahman et al. 2008). The fruit has been also reported to possess good source of vitamin B complex and choline along with fair source of vitamin C and β-carotene (Kirtikar and Basu 2001). It is also reported to contain cucurbitacins, fibres and polyphenol (Nadkarni 1954). Among other activities reported with the fruits of *L. siceraria* include antioxidant activity (Shirwaikar and Sreenivasan 1996) and hypolipidemic in triton-induced hyperlipidemic rats (Jiwajinda et al. 2002). HPLC analysis of methanol extract from plant showed the presence of flavones-c glycosides. Lagenin (a ribosome inactivating protein) isolated from the seeds of *L. siceraria* possessed immuno-protective, antitumor, anti-HIV and antiproliferative properties (Wang and Ng 2000). Aerial parts of the plant also have been reported to have antihyperglycemic (Saha et al. 2011a) and anticancer (Saha et al. 2011b) activities.

In view of the immense medicinal importance of the plant, the present investigator focused onto exploration of free radical scavenging activity of the leaves of this plant to determine and establish its role in various oxidative stress conditions generated by various reactive oxygen species.

**METHODS**

**Chemicals and Reagents**

All the drugs and chemicals used in the study were of analytical grade. 1,1-diphenyl-2-picryl hydrazyl (DPPH) and Folin-Ciocalteu reagent were obtained from Sigma Chemicals (St. Louis, MO, USA). Nitro blue tetrazolium (NBT), ethylene diamine tetra acetie acid (EDTA), sodium nitroprusside (SNP), trichloroacetic acid (TCA), thiobarbituric acid (TBA), 2-deoxy-2-ribose and other chemicals used for evaluation of oxidative stress parameters were obtained from Sisco Research Laboratories (Mumbai).

**Plant Material**

The leaves of *L. siceraria* were procured locally from Pakbara village, Moradabad District of Uttar Pradesh, India and were identified by Dr. Beena Kumari (taxonomist, Hindu College, Moradabad, India) as *L. siceraria* (Molina) standl. (Cucurbitaceae) leaves. A voucher specimen was preserved at the herbarium (HC.MBD/HAP/BK/2010/7/167) in the Department of Botany, Hindu College, Moradabad for further references. Leaves were washed with tap water, dried in shade and were then ground to coarse powder and stored in an airtight container.

**Preparation of Extract**

The dried and coarsely powdered plant material was extracted with petroleum ether (60°-80°) by hot percolation in soxhlet apparatus. The defatted plant material was then extracted with methanol until it became colourless. The extract was concentrated under reduced pressure to yield a crude semi-solid mass. The last traces of the solvent were
Antioxidant Activity of Lagenaria siceraria


evaporated under reduced pressure in rotatory evaporator. Standard methods were used for preliminary phytochemical screening of the extract to recognise the phytoconstituents present in the extract (Harborne 1984). It was concluded that the extract contained terpenoids, steroids, flavonoids and tannins.

In Vitro Antioxidant Activity

DPPH assay

The ability of the extracts to scavenge DPPH radicals (DPPH•) was determined according to the method prescribed (Zeyep, Muberra and Esra 2007) with minor modifications. A 50 μL aliquot of extract, in 50 mM Tris–HCl buffer (pH 7.4), was mixed with 450 μL of Tris–HCl buffer and 1.0 mL of 0.1 mM DPPH• in methanol. After 30 min incubation in darkness and at ambient temperature, the resultant absorbance was recorded at 517 nm against corresponding blanks (0.01 mM DPPH in methanol) and ascorbic acid was used as standard. All the tests were performed in triplicate and the graph was plotted with ±SEM of three observations.

Superoxide Scavenging Assay

The previously described (Liu and Ng 2000) method was used to investigate the superoxide anion radical scavenging activity of the methanolic extract of L. siceraria leaves (MELS). For the said purpose a non-enzymatic phenazine methosulfate-nicotinamide adenine dinucleotide (PMS-NADH) system was used to generate the superoxide anion through the reaction of PMS, NADH and oxygen. It was assayed by the reduction of NBT. To generate the superoxide anion, 3 mL of Tris–HCl buffer (100 mM, pH 7.4) containing 0.75 mL of NBT (300 µM) solution, 0.75 mL of NADH (936 µM) solution and 0.3 mL of different concentrations of the extract were used. The reaction was initiated by adding 0.75 mL of PMS (120 µM) to the mixture. After 5 min of incubation at room temperature, the absorbance at 560 nm was measured on spectrophotometer.

Scavenging of Hydroxyl Radicals

The competition for hydroxyl radicals generated from the Fe3+/ascorbate/EDTA/hydrogen peroxide (H2O2) system, between deoxyribose and the extracts, was measured in the study. These hydroxyl radicals attack on deoxyribose lead to the formation of thiobarbituric acid-reactive substances (TBARS) (Halliwell and Gutteridge 1981). The formed TBARS were measured by a previously described method (Ohkawa, Ohishi and Yagi 1979). The extracts were added to the reaction mixture containing 2.8 mmol/L deoxyribose, 100 μmol/L FeCl3, 104 μmol/L EDTA, 100 μmol/L ascorbic acid, 1 mmol/L H2O2 and 230 mmol/L phosphate buffer (pH 7.4), making a final volume of 1.0 mL. One mL of TBA (1%) and 1.0 mL TCA (2.8%) were added to the test tube and incubated at 100°C for 20 min. After cooling, absorbance was measured at 532 nm against a blank containing deoxyribose and buffer. The percentage inhibition of hydroxyl radical by the extract was determined by comparing the absorbance values of the control and experimental tubes.
Hydrogen Peroxide ($H_2O_2$) Radical Scavenging Activity

$H_2O_2$ scavenging activity of the extract was estimated by a previously prescribed method (Sroks and Cisowski 2003). A solution of $H_2O_2$ (20 mM) was prepared in phosphate buffer saline (pH 7.4). Different concentrations of plant extract and standard ascorbic acid solution viz. 10–100 μg/mL in methanol (1 mL) were added to $H_2O_2$ solution (2 mL). Absorbance of $H_2O_2$ at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without $H_2O_2$. For each concentration, a separate blank sample was used for back ground subtraction. The experiment was performed in triplicate.

Nitric Oxide Scavenging Activity

Nitric oxide radical scavenging activity was determined according to the reported method (Garra 1964). SNP in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be determined by the use of the Griess Illosvoy reaction. Two mL of 10 mM SNP in 0.5 mL phosphate buffer saline (pH 7.4) was mixed with 0.5 mL of extract at various concentrations and the mixture was incubated at 25°C for 150 min. From the incubated mixture 0.5 mL was taken out and added into 1.0 mL sulfanilic acid reagent (33% in 20% glacial acetic acid) and incubated at room temperature for 5 min. Finally, 1.0 mL naphthylethylenediamine dihydrochloride (0.1% w/v) was mixed and incubated at room temperature for 30 min before measuring the absorbance at 540 nm on the spectrophotometer. Consequently nitric oxide radical scavenging activity was calculated.

Estimation of Total Polyphenol Content

The total polyphenol content (μg/mg extract) was analysed using the Folin-Ciocalteu reagent method (Singleton, Orthofer and Ramuela-Raventos 1999). One hundred mg of the MELS extract was dissolved in 250 mL of methanol/water (60:40, V/V, 0.3% HCl) and filtered through a 0.45 μm Millipore filter. To 100 mL of filtrate, 100 mL of Folin-Ciocalteu reagent (50%, V/V) and 2.0 mL of sodium carbonate (2%, m/V) were added and mixed completely. After 2 hrs, the absorbance of the solution was measured at 750 nm. Quantification was based on the standard curve of gallic acid (0–1.0 mg/mL) dissolved in methanol/water (60:40, V/V, 0.3% HCl). Phenolic content was expressed as milligrams per gram of gallic acid equivalent (GAE).

Reducing Power Assay

The Fe$^{3+}$ reducing power of the extract was determined by previously described method (Oyaizu 1996). The methanolic extract (10–100 μg/mL) was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL potassium ferricyanide [K$_3$Fe(CN)$_6$] (1%), and then the mixture was incubated at 50°C for 30 min. Afterwards, 2.5 mL of TCA acid (10%) was added to the mixture and then centrifuged at 3000 rpm for 10 min. Finally, 2.5 mL of upper layer of the solution was mixed with 2.5 mL distilled water and 0.5 mL FeCl$_3$ (0.1%) and the absorbance was measured at 700 nm. Ascorbic acid was used as the reference material. All the tests were performed in triplicate and the graph was plotted with the
average of three observations. Increased absorbance of the reaction mixture indicated increased reducing power.

**Statistical Analysis**

The results are expressed as mean±standard error (mean±SE) of three observations. The % inhibition of various radicals was calculated by comparing the results of the test with those of control using the formula (Shirwaikar, Rajendran and Dinesh Kumar 2004).

\[
\% \text{ inhibition} = \frac{\text{absorbance (control)} - \text{absorbance (test)}}{\text{absorbance (control)}} \times 100
\]

**RESULTS**

Several concentrations ranging from 10–100 μg/mL of the methanolic extract were compared for their antioxidant activity in different in vitro models. It was observed that free radicals were scavenged by the extracts in a concentration dependent manner (within the predetermined concentration range) in all the models.

**DPPH Radical Scavenging Activity**

Free radicals scavenging activity of DPPH has been widely used to evaluate the antioxidant activity of natural products obtained from plant and microbial sources. In DPPH scavenging activity model it was observed that MELS (10–100 μg/mL) significantly scavenged DPPH• in a concentration dependent manner. However extract showed weak scavenging activity in lower concentrations; the higher concentrations (50–100 μg/mL) exhibited promising DPPH• scavenging activity ranging from 36.26% to 65.93% (Table 1). DPPH is a relatively stable free radical and the assay determines the ability of MELS to reduce DPPH• to the corresponding hydrazine by converting the unpaired electrons to form pairs. This conversion is the action of the antioxidant.

**Superoxide Radical Scavenging Activity**

The superoxide anion radical scavenging activity of *L. siceraria* leaves extract assayed by the PMS-NADH system is shown in Table 1. The superoxide scavenging activity of MELS was increased markedly with the increase in concentrations of the extract. Thus, higher inhibitory effects of the extract on superoxide anion formation noted herein possibly rendered its promising antioxidant potential. The half inhibition concentration (IC\textsubscript{50}) of MELS was 63.49 μg/mL (Table 2). These results suggested that MELS had a potent superoxide radical scavenging effects.

**Hydroxyl Radical Scavenging Activity**

Activity of the different concentrations of MELS on hydroxyl radical had been as shown in Table 1. MELS exhibited concentration dependent scavenging activity against generated hydroxyl radical. The IC\textsubscript{50} value of extract was found to be 56.15 μg/mL (Table 2). The observed dose dependent scavenging effect could be explained by understanding the
nature and generation of radicals as well as studying different physical and chemical properties of the naturally occurring antioxidant.

Table 1: Antioxidant activity of methanolic extract of L. siceraria.

<table>
<thead>
<tr>
<th>Conc (µg/mL)</th>
<th>DPPH</th>
<th>% inhibition of radicals</th>
<th>H_2O_2</th>
<th>Superoxide</th>
<th>Nitric oxide</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>9.34±0.78</td>
<td>26.08±1.65</td>
<td>7.70±0.93</td>
<td>12.53±1.59</td>
<td>9.43±0.13</td>
</tr>
<tr>
<td>20</td>
<td>15.93±0.42</td>
<td>34.78±1.13</td>
<td>8.40±0.65</td>
<td>22.18±1.14</td>
<td>15.39±0.60</td>
</tr>
<tr>
<td>30</td>
<td>29.67±1.61</td>
<td>43.47±0.62</td>
<td>10.13±1.32</td>
<td>31.25±2.18</td>
<td>20.67±0.62</td>
</tr>
<tr>
<td>40</td>
<td>31.31±0.91</td>
<td>47.82±1.87</td>
<td>12.76±0.75</td>
<td>36.27±2.22</td>
<td>28.12±0.39</td>
</tr>
<tr>
<td>50</td>
<td>36.26±1.18</td>
<td>52.17±0.94</td>
<td>13.27±0.24</td>
<td>43.18±2.58</td>
<td>37.51±2.13</td>
</tr>
<tr>
<td>60</td>
<td>41.20±2.29</td>
<td>53.14±1.74</td>
<td>16.71±1.13</td>
<td>48.68±2.38</td>
<td>41.90±0.65</td>
</tr>
<tr>
<td>70</td>
<td>47.25±2.45</td>
<td>56.52±2.05</td>
<td>19.25±1.67</td>
<td>52.41±2.11</td>
<td>49.94±0.90</td>
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<tr>
<td>80</td>
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<td>58.72±2.04</td>
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<tr>
<td>90</td>
<td>58.79±2.27</td>
<td>60.86±2.46</td>
<td>25.43±1.70</td>
<td>67.28±2.69</td>
<td>64.24±1.99</td>
</tr>
<tr>
<td>100</td>
<td>65.93±3.13</td>
<td>65.21±1.57</td>
<td>31.81±0.47</td>
<td>72.46±3.01</td>
<td>66.13±2.05</td>
</tr>
</tbody>
</table>

Note: Data presented as ± Standard Error Mean (SEM) of each triplicate test. p<0.05 was considered significant

Table 2: Free radical scavenging ability of L. siceraria and ascorbic acid.

<table>
<thead>
<tr>
<th>Activity</th>
<th>IC_{50} (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MELS</td>
</tr>
<tr>
<td>DPPH</td>
<td>73.98</td>
</tr>
<tr>
<td>Hydroxyl radical</td>
<td>56.15</td>
</tr>
<tr>
<td>H_2O_2 scavenging</td>
<td>186.37</td>
</tr>
<tr>
<td>Superoxide scavenging</td>
<td>63.49</td>
</tr>
<tr>
<td>Nitric oxide scavenging</td>
<td>71.00</td>
</tr>
</tbody>
</table>

Hydrogen Peroxide Radical Scavenging Activity

MELS also demonstrated H_2O_2 decomposition activity in a concentration dependent manner with an IC_{50} of 186.37 µg/mL (Table 2). The decomposition of H_2O_2 by MELS might have partly resulted from its antioxidant and free radical scavenging activity.

Nitric Oxide Scavenging Activity

MELS significantly inhibited nitric oxide in a dose dependent manner (Table 1) with the IC_{50} being 71.00 µg/mL. The result indicated that the extract might contain compounds able to inhibit nitric oxide and offered scientific evidence for the use of the leaves in oxidative stress conditions.
Reducing Power Activity

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Similar to the antioxidant activity, the reducing power of MELS increased with increasing concentration. The result showed that MELS consists of hydrophilic polyphenolic compounds that might have caused the greater reducing power.

Amount of Total Phenolic Compounds

In the present study, total phenolic content present in extract was estimated using modified Folin-Ciocalteu method. The extract was found to contain $99.09 \pm 0.10 \mu g/mg$ total polyphenolics expressed as GAE (micrograms per milligram of GAE).

**DISCUSSION**

Free radicals are chemical entities that can exist separately with one or more unpaired electrons. The propagation of free radical can brings about many adverse reactions leading to extensive tissue damage. Lipid proteins are all susceptible to attack by free radical. Many plant species with antioxidant activities act as protective agents against these radicals. In the present investigation potent antioxidant activity of *L. siceraria* leaf extract was observed using different methods. However the efficacy of extract to scavenge the different radicals differed in each method depending upon the mechanism of free radical scavenging and assay methodology.

The result of DPPH scavenging activity assay in this study indicated that the plant was potently active. This suggested that the plant extract did contain compounds that could be capable of donating hydrogen to a free radical in order to remove the odd electron which is responsible for the radical's reactivity.

Hydroxyl radical is highly reactive oxygen centred radical formed from the reaction of various hydroperoxides with transition metal ions. It attacks proteins, DNA, polyunsaturated fatty acid in membranes and most biological molecules it contacts.
(Aruoma 1999) and is known to be capable of abstracting hydrogen atoms from membrane lipids (Yen and Duh 1994) and brings about peroxidic reaction of lipids. In the present study a significant correlation existed between the concentration and hydroxyl radical scavenging ability of the extract.

$\text{H}_2\text{O}_2$ is a weak oxidising agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. It can cross cell membranes rapidly. Once inside the cell, $\text{H}_2\text{O}_2$ can probably react with $\text{Fe}^{2+}$, and/or $\text{Cu}^{2+}$ ions to form hydroxyl radical and this might be the origin of many of its toxic effects (Halliwell and Gutteridge 1981). It is therefore biologically advantageous for cells to control the amount of $\text{H}_2\text{O}_2$ getting accumulated. Scavenging of $\text{H}_2\text{O}_2$ by the plant extract could be attributed to its phenolics which donate electron to $\text{H}_2\text{O}_2$, thus reducing it to water. The extract was capable of scavenging $\text{H}_2\text{O}_2$ in a concentration dependent manner.

It is well known that superoxide anions damage biomolecules directly or indirectly by forming $\text{H}_2\text{O}_2$, $\cdot\text{OH}$, peroxynitrite or singlet oxygen during aging leading to pathological events such as ischemic reperfusion injury. Superoxide has also been observed to directly initiate lipid peroxidation (Yen and Duh 1994). The scavenging activity of this radical by the plant extract compared favourably with the standard reagent suggesting that the plant could also be a potent scavenger of superoxide radical. The probable mechanism of superoxide scavenging would be attributed to the inhibitory effects of MELS towards generation of superoxide in the in vitro reaction system.

Nitric oxide is an essential bioregulatory molecule required for several physiological processes like neural signal transmission, immune response, control of vasodilation and control of blood pressure (Rees, Palmer and Moncada 1989; Palmer, Ferrige and Moncada 1987) etc. However, the elevation of the $\text{NO}^\bullet$ results in several pathological conditions including cancer. Moreover in the pathological conditions, nitric oxide reacts with superoxide anion and form potentially cytotoxic molecules, peroxynitrite. Nitric oxide inhibitors have been shown to have beneficial effects on some aspects of inflammation and tissue damage seen in inflammatory diseases. The level of nitric oxide was significantly reduced in this study by the crude extract. Since $\text{NO}^\bullet$ plays a crucial role in the pathogenesis of inflammation, this may explain the use of L. siceraria for the treatment of inflammation.

For the measurements of the reducing ability, the $\text{Fe}^{3+}$-$\text{Fe}^{2+}$ transformation was investigated in the presence of MELS. Such reducing capacity of a compound might serve as a significant indicator of its potential antioxidant activity. However, the activity of antioxidants would have been assigned to various mechanisms such as prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging (Yildirim et al. 2000).

Phenolics are the most wide spread secondary metabolite in plant kingdom. These diverse groups of compounds have received much attention as potential natural antioxidant in terms of their ability to act as both efficient radical scavengers and metal chelator. It has been reported that the antioxidant activity of phenol is mainly due to their redox properties, hydrogen donors and singlet oxygen quenchers (Rice-Evans et al. 1995). The preliminary photochemical analysis suggested the presence of terpenoids, steroids, flavonoids and tannins in the methanolic extract; hence the observed antioxidant activity could be due to the presence of any of these constituents in the extract. However, the confirmation of activity by these constituents requires further analysis and would be the matter of investigation in our group.
CONCLUSION

The results obtained in the present study indicated that *L. siceraria* leaves extract exhibited free radical scavenging activity against hydroxyl, peroxide and DPPH•. The overall antioxidant activity of MELS might be attributed to its polyphenolic content and other phytochemical constituents. The findings of the present study suggested that *L. siceraria* leaves could be a potential source of natural antioxidant that would have great importance as therapeutic agents in preventing or slowing the progress of reactive oxygen species and associated oxidative stress related degenerative diseases.

ACKNOWLEDGEMENT

Authors are very thankful to Shri Suresh Jain, Honourable Chancellor of the Teerthanker Mahaveer University for his invariable encouragement and endowing us with facilities necessitated for successful completion of the study. Authors are also thankful to Dr. Beena Kumari (botanist, Department of Botany, Hindu College, Moradabad, India) for authentication of plant material.

REFERENCES


Free radical scavenging activity of methanolic extract of *Luffa cylindrica* leaves

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**Context:** Free radicals contribute to more than one hundred disorders in humans including atherosclerosis, hypertension, arthritis, ischemia, gastritis, central nervous system injury, reperfusion injury of many tissues, cancer, Alzheimer's disease, Parkinsonism, diabetes mellitus and AIDS. There is considerable evidence that antioxidants could help to prevent these diseases because they have the capacity to quench free radicals. **Aim:** Free radical scavenging activity of methanolic extract of the leaves of *Luffa cylindrica* (MELC) was evaluated in various *in vitro* systems. **Materials and Methods:** The methods were extensively reviewed and free radical scavenging activity was performed by employing various *in vitro* assay methods like DPPH, hydroxyl radical, superoxide and nitric oxide scavenging activities. **Statistical Analysis:** Results were analyzed statistically using one way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test and were expressed as mean ± SE of three observations. Values of *P* < 0.05 were considered significant. **Results:** In all the studies, a significant correlation existed between concentrations of the extract and percentage inhibition of free radicals. The preliminary phytochemical screening of MELC indicated the presence of terpenoids, steroids, flavonoids and glycosides. The extract was found to contain 53.78 ± 1.01 µg/mg total polyphenolics expressed as GAE (micrograms per milligram of GAE). **Conclusion:** The results of the study suggested that the methanolic extract of the leaves of *Luffa cylindrica* possessed a significant scavenging effect with increasing concentrations probably due to its antioxidant potential and could serve as a potential source of natural antioxidants effective in treatments against free radical mediated diseases.

**Key words:** Antioxidant, *Luffa cylindrica*, methanolic extract, phenolic compounds

**INTRODUCTION**

Oxidation is well known to be major cause of foods and other material degradation.[¹] Oxidation reactions can produce free radicals which start chain reactions damaging the host cells.[²] Free radicals are natural by-products of our own metabolism and cause lipid peroxidation in foods which leads to their deterioration.[³] These are electrically charged entities that attack our cells tearing through cellular membranes and react with the nucleic acids, proteins, enzymes etc. present in the body.[²]

It is widely appreciated now that antioxidant activity elicited by the inhibition of generation of free radicals plays a crucial role in providing protection against variety of tissue damages. Several herbs and herbal products are known to possess antioxidant principles and may be useful as organ protective agents.[⁴,⁵] Herbs belonging to various families have been reported to possess antioxidant principles like flavonoids and show organ protective properties. In the past few years, there existed an increased preference for antioxidants from natural sources rather than from synthetic sources because of the health risks and toxicity of synthetic antioxidants.

*Luffa* [*L. cylindrica* (L.) Roem syn *L. aegyptiaca* Mill] commonly called sponge gourd, loofa, vegetable sponge, bath sponge or dish cloth gourd, is a member of cucurbitaceous family. The plant is widely distributed throughout India. Its fruit is used in the traditional medicine as an anthelmintic, carminative, laxative, deputrative, emollient, expectorant, diuretic and lactagogue and are also employed in fever, syphilis, tumors, bronchitis, splenopathy and leprosy.[⁶]

It is applied as a vegetable either prepared like squash or eaten raw like cucumbers.[⁶,⁷] Its seeds have been used in the treatment of asthma, sinusitis and fever.[⁸] The seed oil has been reported to be used for skin infections in the form of tincture.[¹⁰,¹¹] The fruit is used in the treatment of ascites, jaundice, and biliary and intestinal colitis and also in enlarged spleen and liver.[¹²] The plant is reputed...
to have anti tubercular and antiseptic properties.\textsuperscript{[13,14]} The extract of leaves has been used in snake-bites. Although extensive studies have been carried out on fruits and seeds, the pharmacology of the leaves of \textit{L. cylindrica} has remained unexplored as yet. The present investigation was therefore carried out to evaluate \textit{in vitro} antioxidant potential of methanolic extract of \textit{Luffa cylindrica} leaves.

\section*{MATERIALS AND METHODS}

\subsection*{Plant Material}
The leaves of \textit{L. cylindrica} were collected locally from village Pakbara, District Moradabad, Uttar Pradesh, India and were authenticated by Dr. Beena Kumari, Taxonomist, Hindu College, Moradabad (India) as \textit{L. cylindrica} (Cucurbitaceae) leaves. A voucher specimen has been kept in the herbarium (HC.MBD/HAP/BK/2010/5/168) of the Department of Botany, Hindu college, Moradabad (India).

\subsection*{Chemicals and Reagents}
All the drugs and chemicals, used in the study, were of analytical grade. 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and Folin-Ciocalteu reagent were obtained from Sigma chemicals USA. Nitro blue tetrazolium (NBT), ethylene diamine tetra acetic acid (EDTA), sodium nitroprusside (SNP), trichloroacetic acid (TCA), thiobarbituric acid (TBA), 2-deoxy-2-ribose and other chemicals used for evaluation of oxidative stress parameters, were obtained from Sisco Research Laboratories, India.

\subsection*{Preparation of Extract}
The dried and coarsely powdered plant material (leaves) was extracted with petroleum ether (60-80\textdegree) by hot percolation in soxhlet apparatus. The defatted plant material was then extracted with methanol until it became colorless. The extract was concentrated under reduced pressure to yield a crude semi-solid mass. The last traces of the solvent were evaporated under reduced pressure in rotatory evaporator. Standard methods were used for preliminary phytochemical screening of the extract to recognize the phytoconstituents present therein.\textsuperscript{[15]} It was found that the extract contained terpenoids, steroids, flavonoids and glycosides.

\subsection*{Superoxide Scavenging Assay}
The scavenging activity of the MELC towards superoxide anion radicals was measured by the method of Liu \textit{et al.}\textsuperscript{[16]} The study was carried out in a non-enzymatic phenazine methosulfate-nicotinamide adenine dinucleotide (PMS-NADH) system to generate the superoxide anion. It was assayed by the reduction of nitroblue tetrazolium (NBT). For the said purpose 0.75 ml of each of NBT (300 \textmu M) and NADH (936 \textmu M) solutions were mixed with 3 ml of Tris-HCl buffer (100 mM, pH 7.4) to generate the superoxide anion. The mixture was added with 0.3 ml of different concentrations of the extract and standard compound i.e., ascorbic acid. The reaction was initiated by adding 0.75 ml of PMS (120 \textmu M) to the mixture. After 5 min of incubation at room temperature, the absorbance at 560 nm was measured in spectrophotometer. Corresponding blanks were taken and % inhibition was calculated. All the tests were performed in triplicate and the graph was plotted with ± SEM of three observations.

\subsection*{Nitric Oxide Scavenging Activity}
Griess Illosvoy reaction\textsuperscript{[17]} was used to determine the nitrite oxide ions generated by interaction of oxygen and nitric oxide at physiological pH. Sodium nitroprusside was used as a source of nitric oxide. 10 mM solution of sodium nitroprusside (2 ml) was added in 0.5 ml phosphate buffer saline (pH 7.4) followed by the addition of 0.5 ml of extract of various concentrations. The resulting mixture was incubated at 25\textdegree C for 150 min. From the incubated mixture, 0.5 ml was taken out and added into 1.0 ml sulfanilic acid reagent (33% in 20% glacial acetic acid) and incubated at room temperature for 5 min. Finally, 1.0 ml naphthyl ethylenediamine dihydrochloride (0.1% w/v) was mixed and incubated at room temperature for 30 min before measuring the absorbance at 540 nm with a spectrophotometer. The nitric oxide radicals scavenging activity was calculated.\textsuperscript{[17]}

\subsection*{DPPH Assay}
The method prescribed by Zeyep \textit{et al.}\textsuperscript{[18]} was used, with minor modifications, for the assay. One ml of 0.1 mM DPPH’ in methanol, 450 \mu l of Tris-HCl buffer and 50 \mu l aliquot of extract in 50 mM Tris-HCl buffer (pH 7.4) were mixed and incubated in darkness for 30 min. The resultant absorbance was recorded at 517 nm against corresponding blanks (0.01 mM DPPH in methanol). The percentage scavenging was determined and compared with that of ascorbic acid used as the standard.

\subsection*{Scavenging of Hydroxyl Radicals (’OH)}
The competition between deoxyribose and the extracts for hydroxyl radicals was measured as the determinant for hydroxyl ion scavenging activity. The formed thiobarbituric acid-reactive substances (TBARS)\textsuperscript{[19]} due to attack of the hydroxyl radical on deoxyribose were measured by the method given by Ohkawa \textit{et al.}\textsuperscript{[20]} Briefly, the extracts were added to the reaction mixture containing 2.8 mmol/l deoxyribose, 100 \mu mol/l FeCl\textsubscript{3}, 104 \mu mol/l EDTA, 100 \mu mol/l ascorbic acid, 1 \mu mol/l H\textsubscript{2}O\textsubscript{2} and 230 mmol/l phosphate buffer (pH 7.4), making a final volume of 1.0 ml. One milliliter of thiobarbituric acid (TBA, 1\%) and 1.0 ml trichloroacetic acid (TCA, 2.8\%) were added to the test tube and incubated at 100\textdegree C for 20 min. After cooling, absorbance was measured at 532 nm against a blank
containing deoxyribose and buffer. Reactions were carried out in triplicate.

**Hydrogen Peroxide Radical Scavenging Activity**
Hydrogen peroxide scavenging activity of the extract was estimated by method prescribed.[23] A solution of hydrogen peroxide (20 mM) was prepared in phosphate buffer saline (pH 7.4). Different concentrations of plant extract and standard ascorbic acid solution viz. 10-100 µg/ml in methanol (1 ml) were added to hydrogen peroxide solution (2 ml). Absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. For each concentration, a separate blank sample was used for background subtraction. The experiment was performed in triplicate.

**Reducing Power Assay**
The Fe³⁺ reducing power of the extract was determined by the method.[23] Extracts of different concentration (10-100 µg/ml) were mixed with 2.5 ml of phosphate buffer (0.2M, pH 6.6) and 2.5 ml potassium ferricyanide [K₃Fe(CN)₆] (1%), and then the mixture was incubated at 50°C for 30 min. Afterwards, 2.5 ml of trichloroacetic acid (10%) was added to the mixture which was then centrifuged at 3000 rpm for 10 min. Finally, 2.5 ml of upper layer solution was mixed with 2.5 ml distilled water and 0.5 ml FeCl₃ (0.1%) and the absorbance was measured at 700 nm. Ascorbic acid was used as the reference material. All the tests were performed in triplicate and the graph was plotted with the average of three observations. Increased absorbance of the reaction mixture indicated increased reducing power.

**Estimation of Total Polyphenol Content**
The total polyphenol content (µg/mg extract) was analyzed using the Folin-Ciocalteu reagent method.[23] One hundred milligrams of the MELC extract was dissolved in 250 ml of methanol/water (60:40, V/V, 0.3 % HCl) and filtered through a 0.45µm millipore filter. To 100 ml of filtrate, 100 ml of Folin-Ciocalteu reagent (50%, V/V) and 2.0 ml of sodium carbonate (2%, m/V) were added and mixed completely. After 2 hours, the absorbance of the solution was measured at 750 nm. Quantification was based on the standard curve of gallic acid (0-1.0 mg/ml) dissolved in methanol/water (60:40, V/V, 0.3 % HCl). Phenolic content was expressed as milligrams per gram of gallic acid equivalent (GAE).

**Statistical Analysis**
Results were analyzed statistically using one way analysis of variance (ANOVA) followed by Dunnett’s multiple comparison test and expressed as mean ± SE of three observations. Values of P < 0.05 were considered significant. The statistical analysis was performed on Graphpad Prism software of version 4. The % inhibition of various radicals was calculated by comparing the results of the test with those of control using the formula.

\[
\% \text{ inhibition } = \frac{\text{Abs (Control)} - \text{Abs (Test)}}{\text{Abs (Control)}} \times 100
\]

**RESULTS**
Phytochemical screening of the crude methanolic extract of the leaves of *Luffa cylindrica* revealed the presence of steroids, flavonoids, terpenoids, glycosides, alkaloids and phenolic compounds. The total phenolic content of the leaf extract was 53.78 ± 1.01 µg/mg of GAE. These phytochemical compounds are known to support biological activities in medicinal plants and thus might be responsible for the antioxidant activities of this plant extract used in this study.

The results of superoxide anion radical scavenging activity of the leaf extract of *Luffa cylindrica* assayed by the PMS-NADH system were as shown in the Table 1. In Figure 1, MELC demonstrated superoxide decomposition activity in a concentration dependent manner with an IC₅₀ of 61.02 µg/ml. Higher inhibitory effects of the leaf extracts on superoxide anion formation shown herein possibly rendered them as a promising antioxidant characteristics.

![Figure 1: Superoxide scavenging activity of L. cylindrica at different concentrations](image)

**Table 1: Antioxidant activity of methanolic extract of L. cylindrica**

<table>
<thead>
<tr>
<th>Conc (µg/ml)</th>
<th>DPPH</th>
<th>Hydroxyl radical</th>
<th>Hydrogen peroxide</th>
<th>Superoxide</th>
<th>Nitric oxide</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>39.81±0.04</td>
<td>14.28±0.06</td>
<td>10.99±0.07</td>
<td>11.31±0.14</td>
<td>8.57±0.18</td>
</tr>
<tr>
<td>20</td>
<td>46.29±0.05</td>
<td>21.42±0.13</td>
<td>12.88±0.05</td>
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<td>13.74±0.19</td>
</tr>
<tr>
<td>30</td>
<td>52.77±0.07</td>
<td>25.00±0.11</td>
<td>16.32±0.08</td>
<td>29.6±0.26</td>
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</tr>
<tr>
<td>40</td>
<td>57.4±0.07</td>
<td>31.42±0.17</td>
<td>20.83±0.09</td>
<td>36.2±0.19</td>
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<tr>
<td>50</td>
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<td>35.71±0.09</td>
<td>23.68±0.08</td>
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</tr>
<tr>
<td>60</td>
<td>68.51±0.11</td>
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<td>30.97±0.07</td>
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</tr>
<tr>
<td>70</td>
<td>70.37±0.09</td>
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<td>58.3±0.14</td>
<td>54.32±0.20</td>
</tr>
<tr>
<td>80</td>
<td>74.67±0.08</td>
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<td>40.03±0.05</td>
<td>63.82±0.14</td>
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</tr>
<tr>
<td>90</td>
<td>79.62±0.09</td>
<td>60.08±0.11</td>
<td>48.92±0.03</td>
<td>68.09±0.21</td>
<td>65.75±0.22</td>
</tr>
<tr>
<td>100</td>
<td>85.18±0.12</td>
<td>63.18±0.07</td>
<td>52.63±0.10</td>
<td>73.17±0.18</td>
<td>68.03±0.21</td>
</tr>
</tbody>
</table>

Data presented as±SEM of each triplicate test. P<0.05 was considered significant.
The results suggested that the plant extract had a potent superoxide radical scavenging effect.

Nitric oxide inhibitors have been shown to have beneficial effects on some aspect of inflammation and tissue damage seen in inflammatory diseases. MELC significantly inhibited [Figure 2] nitric oxide in a dose dependent manner [Table 1] with the IC_{50} being 69.49 µg/ml. The results indicated that the extract might contain compounds capable of inhibiting nitric oxide and offered scientific evidence for the use of the leaves in inflammatory conditions.

The decrease in absorbance of the DPPH radical caused by antioxidant was due to the scavenging of the radical by hydrogen donation. It was visually noticeable by a color change from purple to yellow. The methanolic extract of L. cylindrica and ascorbic acid showed DPPH radical scavenging activity in a concentration–dependent manner [Figure 3]. MELC exhibited maximum scavenging activity of 85.18% whereas that of the ascorbic acid was 76.61 %. The IC_{50} values were as shown in Table 2.

Activity of the different concentrations of MELC on hydroxyl radical has been shown in Table 1. MELC exhibited concentration dependent scavenging activity against hydroxyl radical generated [Figure 4]. The IC_{50} value of the extract was found to be 72.33 µg/ml. The observed dose dependent scavenging effect could be explained by understanding the nature and generation of radicals as well as studying different physical and chemical properties of the naturally occurring antioxidant.

As shown in Figure 5, MELC also demonstrated hydrogen peroxide decomposition activity in a concentration dependent manner with an IC_{50} of 97.50 µg/ml. The decomposition of H_{2}O_{2} by MELC might have resulted from its antioxidant and free radical scavenging activity.

Table 1 showed the reducing power of the Luffa cylindrica methanolic extracts as a function of their concentration. In this assay, the yellow color of the test solution changed to various shades of green and blue depending on the reducing power of each compound. Presence of reducers caused the conversion of the Fe^{3+}/ferricyanide complex used in this method to the ferrous form. By measuring the formation of Perl's Prussian blue at 700 nm, it was possible to determine the IC_{50} values of ascorbic acid and methanolic extract of L. cylindrica

### Table 2: IC_{50} of ascorbic acid and methanolic extract of L. cylindrica

<table>
<thead>
<tr>
<th>Activity</th>
<th>IC_{50} (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MELC</td>
<td>Ascorbic acid</td>
</tr>
<tr>
<td>DPPH</td>
<td>26.46</td>
</tr>
<tr>
<td>Hydroxyl radical</td>
<td>72.33</td>
</tr>
<tr>
<td>H_{2}O_{2} scavenging</td>
<td>97.50</td>
</tr>
<tr>
<td>Superoxide scavenging</td>
<td>61.02</td>
</tr>
<tr>
<td>Nitric oxide scavenging</td>
<td>69.49</td>
</tr>
</tbody>
</table>
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the Fe²⁺ concentration. The reducing power of the MELC increased with their concentrations [Figure 6]. The result revealed that MELC did consist of polyphenolic compounds inducing the greater reducing power.

DISCUSSION

In the present investigation potent antioxidant activity of L. cylindrica leaf extract was observed using different methods. However the efficacy of extract to scavenge the different radicals differed in each method depending upon the involved mechanism of free radical scavenging and adopted assay methodology.

Superoxide anion is an oxygen-centered radical with selective reactivity. This species is produced by a number of enzyme systems in auto-oxidation reactions and by nonenzymatic electron transfers. Its univalent function reduces molecular oxygen. It can also reduce certain iron complexes such as cytochrome. The present study showed potent superoxide radical scavenging activity of L. cylindrica leaf extract. The scavenging activity of such radical by the plant extract when compared favorably with the standard reagents such as ascorbic acid suggested that the plant might also serve as a potent scavenger of superoxide radical.

Hydrogen peroxide is an important reactive oxygen species because of its ability to penetrate biological membranes. However, it may be toxic if converted to hydroxyl radical in the cell. Scavenging of H₂O₂ by the plant extracts may be attributed to their phenolic contents, which donate electron to H₂O₂, thus reducing it to water. The extract was capable of scavenging hydrogen peroxide in a concentration dependent manner.

Nitric oxide is a free radical product in mammalian cells involved in the regulation of various physiological processes. However, excess production of nitric oxide radical is associated with several diseases. In the present study the nitrite produced by the incubation of solutions of sodium nitroprusside in standard phosphate buffer at 25°C was reduced by the methanolic extract of L. cylindrica. This might be due to the antioxidant principles present in the extract which competed with oxygen to react with nitric oxide thereby inhibiting the generation of nitrite.

The result of DPPH scavenging activity assay in this study indicated that the plant was potently active. This suggested that the plant extract contained compounds that were capable of donating hydrogen to a free radical in order to remove odd electron which is responsible for radical’s reactivity. The scavenging activity of DPPH radical by the plant extract was found to be appreciable; this implied that the plant extract might be useful for treating radical related pathological damages especially at higher concentration.

Hydroxyl radical is highly reactive oxygen centered radical formed from the reaction of various hydroperoxides with transition metal ions. It attacks proteins, DNA, polyunsaturated fatty acid in membranes and most biological molecules it contacts and is known to be capable of abstracting hydrogen atoms from membrane lipids that brings about peroxidation reaction of lipids. In the present study a significant correlation existed between the concentration and hydroxyl radical scavenging ability of extract.

CONCLUSION

The results obtained in the present investigation indicated that the methanolic extract of leaves of L. cylindrica exhibited free radical scavenging activity. The overall antioxidant activity of the leaves extract might be attributed to the presence of secondary metabolites. The findings of the present study suggested that L. cylindrica leaves could be a potential source of natural antioxidant having great importance as therapeutic agent in preventing or slowing the oxidative stress related degenerative diseases.

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ANTIHEPATOTOXIC EFFECTS OF METHANOLIC EXTRACT OF LAGENARIA SICERARIA LEAVES IN RATS

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Summary

Treatment of rats with paracetamol turned out in significant elevation in the levels of serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP) and total bilirubin. Rats treated with methanolic extract of leaves of Lagenaria siceraria (MELS) in dose of 250 mg/kg and 500 mg/kg body weight, reported significant ($p<0.05$) collapse in the biochemical enzymes. The protective effect of MELS extract was compared with the standard drug silymarin.
Various biochemical parameters such as glutathione (GSH), lipid peroxide (LPO), superoxide dismutase (SOD) and catalase (CAT) were also evaluated. The antioxidant parameters GSH, SOD and catalase levels were increased considerably in a dose dependant manner compared to their levels in paracetamol control groups. The results revealed that MELS possesses a potential antihepatotoxic activity.

**Keywords:** Hepatoprotective, Lagenaria siceraria, Biochemical parameters, Paracetamol

**Introduction**

Liver diseases are the most serious ailment and are mainly caused by toxic chemicals (Excess consumption of alcohol, high doses of paracetamol, carbon tetrachloride, chemotherapeutic agents, peroxidised oil, etc). Liver damage is associated with cellular necrosis, increase in tissue lipid peroxidation and depletion in the tissue GSH levels. In addition serum levels of many biochemical markers like SGOT, SGPT, triglycerides, cholesterol, bilirubin, alkaline phosphatase are elevated.

Management of liver disease is still a challenge to the modern medicine [1]. In spite of remarkable strides in allopatic medicine, no effective hepatoprotective medicine is available that stimulate liver function, offer protection to the liver from damage or help regeneration of hepatic cell. Plant drugs are known to play a vital role in the management of liver diseases. There are numerous plants and polyherbal formulations claimed to have hepatoprotective activities. Some of these plants have already been reported to possess strong antioxidant activity [2-3].

*Lagenaria siceraria* (Molina) Standley syn. *L. leucantha* Rusby; *L. vulgaris* Ser. (Family: Cucurbitaceae) commonly known as Bottle gourd, is a reputed vegetable plant used in traditional system of medicine for the treatment of various oxidative stress conditions. Its fruit has a composition of all the essential constituents that are required for normal and good health of humans [4].
Since time immemorial the fruit is used as immunosuppressant [5], diuretic [6], cardio-tonic, cardio-protective [7] and nutritive agent [8]. The fruit is also reported to have good source of vitamin-B complex and choline along with fair source of vitamin-C and β-carotene [9]. It is also reported to contain Cucurbitacins, fibres, and polyphenol [10]. Two sterols namely campesterol and sitosterol have been identified and isolated from the petroleum ether fraction of methanol extract of *Lagenaria siceraria* fruits, which is reported to possess antihepatotoxic activity [11]. The fruit has been reported to possess antioxidant activity [12], hypolipidemic and triton-induced hyperlipidemic rats [13]. Lagenin, a ribosome inactivating protein (RIP) isolated from the seeds of *lagenaria siceraria* possesses immunoprotective, antitumor, anti HIV and antiproliferative properties [14]. The present study was aimed to explore the prospect for hepatoprotective activity of methanolic extract of *L. siceraria* (MELS) leaves in the experimental rodents.

**Materials and methods**

**Plant material**

The leaves of *L. siceraria* were procured locally from Moradabad district of Uttar Pradesh in India and were authentified by Dr. Beena Kumari, Taxonomist, Hindu College, Moradabad (India) as *Lagenaria Siceraria* (Molina) standl. (Cucurbitaceae) leaves. Voucher specimens are kept in the herbarium (HC.MBD/HAP/BK/2010/7/167) of the Institute for further references. Leaves were washed with tap water and dried in shade. Dried leaves were ground to coarse powder and stored in an airtight container. The dried leaves were defatted with petroleum ether (60-80°) by hot percolation in soxhlet apparatus and then extracted (250 g) with methanol in a soxhlet extractor for 18–20 h. The extract was concentrated to dryness under reduced pressure and controlled temperature (40–50 °C). Preliminary phytochemical screening [15] was carried out on the MELS to assess the presence of terpenoids, steroids, flavonoids and tannins in the extract.
Animals
Studies were carried out using male Wistar albino rats (120–170g). They were obtained from the animal house of the Teerthanker Mahaveer College of Pharmacy, Moradabad, India. The animals were grouped and housed in polyacrylic cages with not more than six animals per cage and maintained under standard laboratory conditions (temperature 22 ± 2 °C) with light and dark cycles of 12 and 12 h, respectively. They were allowed free access to the standard dry pellet diet and water ad libitum. All procedures described were reviewed and approved by the Institutional Animal Ethical Committee.

Paracetamol induced hepatotoxicity
Animals were divided into five groups of six animals each. Group I served as vehicle control and received 1% gum acacia suspension only. All other Groups received paracetamol once (500 mg/kg, p.o., aqueous solution) with Group II serving as paracetamol treated control. 48 h after paracetamol administration, Groups III, IV & V received MELS extract 250 mg/kg, 500 mg/kg and silymarin 100 mg/kg b.w., p.o. respectively, once daily for 5 consecutive days. 16 h after administration of last dose of drugs, the blood was collected by retro orbital artery bleeding. Blood samples were centrifuged for 10 minutes at 3000 rpm to separate the serum. ALP, SGOT, SGPT, and Bilirubin levels were estimated from the serum by using standard kits. The rats were sacrificed by ether anaesthesia on day 6 and liver was excised, rinsed in 0.25 M sucrose solution and 10% w/v homogenate was prepared in 0.15M KCl, centrifuged at 800 g for 10 min at 4°C. The supernatant obtained was used for estimation of catalase and lipid peroxidation. Further the homogenate was centrifuged at 1000 g for 20 min at 4°C and the supernatant was used for the estimation of SOD and glutathione.

Biochemical analyses
The SGOT and SGPT levels in the serum were estimated using commercially available kits. The reduced glutathione (GSH) level in the liver was determined according to the method of Ellman [16]; hepatic superoxide dismutase (SOD) activity by the method of Kakkar et al. [17] and catalase by the method of Aebi [18]. The hepatic TBARS level, an index of malonyldialdehyde (MDA) production, was determined by the method of Ohkawa et al. [19].
**Histopathological analysis of liver**
For histological studies, liver tissues were fixed with 10% phosphate-buffered neutral formalin, dehydrated in graded (50–100%) alcohol and embedded in paraffin. Thin sections were cut and stained with hematoxylin and eosin stain for microscopic assessment. The initial examination was qualitative, with the purpose of determining histopathological lesions in liver tissue.

**Statistical analysis**
The results are expressed as mean±SE and the difference between the groups were analyzed by one-way ANOVA. P<0.05 was considered significant. The post hoc analysis was carried out by Dunnet’s multiple comparison test.

**Results and discussion**
The preliminary phytochemical screening of MELS indicated the presence of terpenoids, steroids, flavonoids and tannins. The extract was found to contain 98.986 ± 0.1789 μg/mg total polyphenolics expressed as GAE (micrograms per milligram of GAE). There was a significant increase in the SGPT, SGOT and ALP levels in rats treated with paracetamol compared to normal control. In groups treated with 250 and 500 mg/kg of MELS, above activities of enzymes were found to be significantly (P<0.05) decreased. Maximum protection against paracetamol generated hepatic damage was offered at dose of 500 mg/kg of MELS.

Silymarin, which was used for comparative evaluation, produced a highly significant fall in the enzyme levels at 100 mg/kg dose level. Alleviated bilirubin levels were also reduced in MELS treated animals from 11.66 mg/dl to 3.42 mg/dl, when compared to paracetamol intoxicated animals.
Table 1. Effect of MELS on various biochemical parameters in rats with paracetamol induced hepatotoxicity

<table>
<thead>
<tr>
<th>Group</th>
<th>SGPT (IU/L)</th>
<th>SGOT (IU/L)</th>
<th>ALP (IU/L)</th>
<th>Total Bilirubin (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle control</td>
<td>32.71±1.56</td>
<td>41.14±2.07</td>
<td>51.03±2.39</td>
<td>2.1±0.42</td>
</tr>
<tr>
<td>PCM control</td>
<td>84.32±1.71</td>
<td>118.02±2.60</td>
<td>127.89±3.76</td>
<td>11.66±0.82</td>
</tr>
<tr>
<td>MELS (250 mg/kg)</td>
<td>58.83±2.74</td>
<td>79.26±3.28</td>
<td>77.01±3.62</td>
<td>4.59±0.73</td>
</tr>
<tr>
<td>MELS (500 mg/kg)</td>
<td>43.01±1.81</td>
<td>68.95±4.81</td>
<td>61.49±4.08</td>
<td>3.42±0.61</td>
</tr>
<tr>
<td>Silymarin (100 mg/kg)</td>
<td>35.69±2.10</td>
<td>54.06±3.03</td>
<td>54.32±3.91</td>
<td>2.52±0.53</td>
</tr>
</tbody>
</table>

Values represent mean ± SEM of six animals in each group. P<0.05

N-acetyl-p-benzoquinoneimine (NAPQI) is the highly reactive metabolite of paracetamol, capable of binding covalently to cellular macromolecules (proteins, DNA) to produce protein adducts. Higher doses of paracetamol and N-acetyl-p-benzoquinoneimine can alkylate and oxidise intracellular GSH, which results in the depletion of liver GSH pool subsequently leads to increased lipid peroxidation and liver damage [20]. In our experiments it is observed that the lipid peroxidation levels in the paracetamol group is increased. This clearly indicates that there is a significant hepatic damage due to paracetamol and this is further evident from the fact that there is elevation in the levels of various markers of hepatic damage like SGOT, SGPT, ALP and total bilirubin. Treatment with MELS has decreased the levels of lipid peroxidation and the elevated levels of above mentioned biochemical markers to the near normal levels.
The data obtained in the present study (Table 2) clearly shows an increase in the MDA levels of rats treated with paracetamol suggesting enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent formation of excessive free radicals. The increase in TBARS levels was significantly reduced by the treatment with 250 mg/kg and 500 mg/kg MELS (4.02 nmol/mg and 3.42 nmol/mg respectively) and by the treatment with silymarin (3.23 nmol/mg).

SOD has been reported as one of the most important enzymes in the antioxidant defense system. It scavenges the superoxide anion to form hydrogen peroxide and thus diminishing the toxic effect caused by this radical. Decrease in enzyme activity of superoxide dismutase (SOD) is a sensitive index in hepatocellular damage. The decreased SOD level in paracetamol treated rats was also observed when compared to control. The level of SOD was increased again by the administration of MELS and Silymarin. MELS causes a significant increase in hepatic SOD activity and thus reduces reactive free radical induced oxidative damage to liver.

Table 2. Effect of MELS on various parameters of oxidative stress in rats with paracetamol induced hepatotoxicity

<table>
<thead>
<tr>
<th>Group</th>
<th>LPO (nM/mg)</th>
<th>GSH (mM/gm)</th>
<th>Catalase (U/mg)</th>
<th>SOD (% inhibition)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle control</td>
<td>2.99±0.76</td>
<td>33.78±1.69</td>
<td>18.74±0.9</td>
<td>68.79±1.77</td>
</tr>
<tr>
<td>PCM control</td>
<td>7.27±0.74</td>
<td>9.51±0.802</td>
<td>7.37±0.93</td>
<td>22.67±2.03</td>
</tr>
<tr>
<td>MELS (250 mg/kg)</td>
<td>4.02±0.98</td>
<td>26.79±1.84</td>
<td>13.83±0.85</td>
<td>55.57±3.14</td>
</tr>
<tr>
<td>MELS (500 mg/kg)</td>
<td>3.42±0.67</td>
<td>28.14±2.68</td>
<td>14.14±1.12</td>
<td>61.33±2.58</td>
</tr>
<tr>
<td>Silymarin (100 mg/kg)</td>
<td>3.23±0.83</td>
<td>30.85±1.51</td>
<td>16.66±1.18</td>
<td>64.19±2.01</td>
</tr>
</tbody>
</table>

Values represent mean ± SEM of six animals in each group. P<0.05
Catalase (CAT); an enzymatic antioxidant, decomposes hydrogen peroxide and protects the tissues from highly reactive hydroxyl radicals [21]. Therefore reduction in the activity of CAT may result in a number of deleterious effects due to the assimilation of superoxide radical and hydrogen peroxide. Administration of MELS increased the CAT level in paracetamol treated rats with induced liver damage, thus preventing accumulation of excessive free radicals and protecting the liver from paracetamol intoxication.

Fig. 1. Section of the liver of (A) rat treated with PCM, (B) rat treated with MELS (250 mg/kg) (C) rat treated with MELS (500 mg/kg) (D) rat treated with silymarin (100 mg/kg).

Histological observations basically supported the results obtained from serum enzyme assays. The liver of paracetamol-intoxicated rats showed massive fatty changes, gross necrosis and broad infiltration of lymphocytes and loss of cellular boundaries. Histopathological observations of the liver of rats treated with MELS showed a more or less normal architecture of the liver, having reversed to a large extent the hepatic lesions produced by the toxin, almost comparable to the normal control and the silymarin group (Fig. 1a-d).
Conclusion

Based on the results obtained, it may be concluded that the *Lagenaria siceraria* leaves extract has a significant effect on liver injuries as well as on oxidative stress, resulting in reduced lipid peroxidation and improved serum biochemical parameters such as AST and ALT. The reduced levels of parameters of SOD, CAT, and GSH in paracetamol-treated rats were significantly increased by treatment with *Lagenaria siceraria* leaves extract. However the exact hepatoprotective mechanism of MELS is unknown, the extract may either inhibit the formation of the toxic paracetamol metabolite or stimulate the hepatic regeneration. This type of stimulation is known to cause the liver to become more resistant to damage by toxins. Apart from all these antioxidant effect of MELS can be the root cause for its hepatoprotective activity.

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