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Evaluation of antioxidant potential, DNA damage and hepatoprotective properties of *Lagenaria siceraria* plant against acetaminophen induced hepatotoxicity

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ABSTRACT

Introduction: *Lagenaria siceraria* has various pharmacological effects like antioxidant, anti-hyperglycemic, anti-ulcer, anti-cancer, hepatoprotective, antimicrobial, anti-hyperlipidemic, anti-inflammatory and cardioprotective activities and is used to treat various diseases like diabetes, jaundice, piles, ulcers, hypertension, colitis, insanity, and skin diseases.

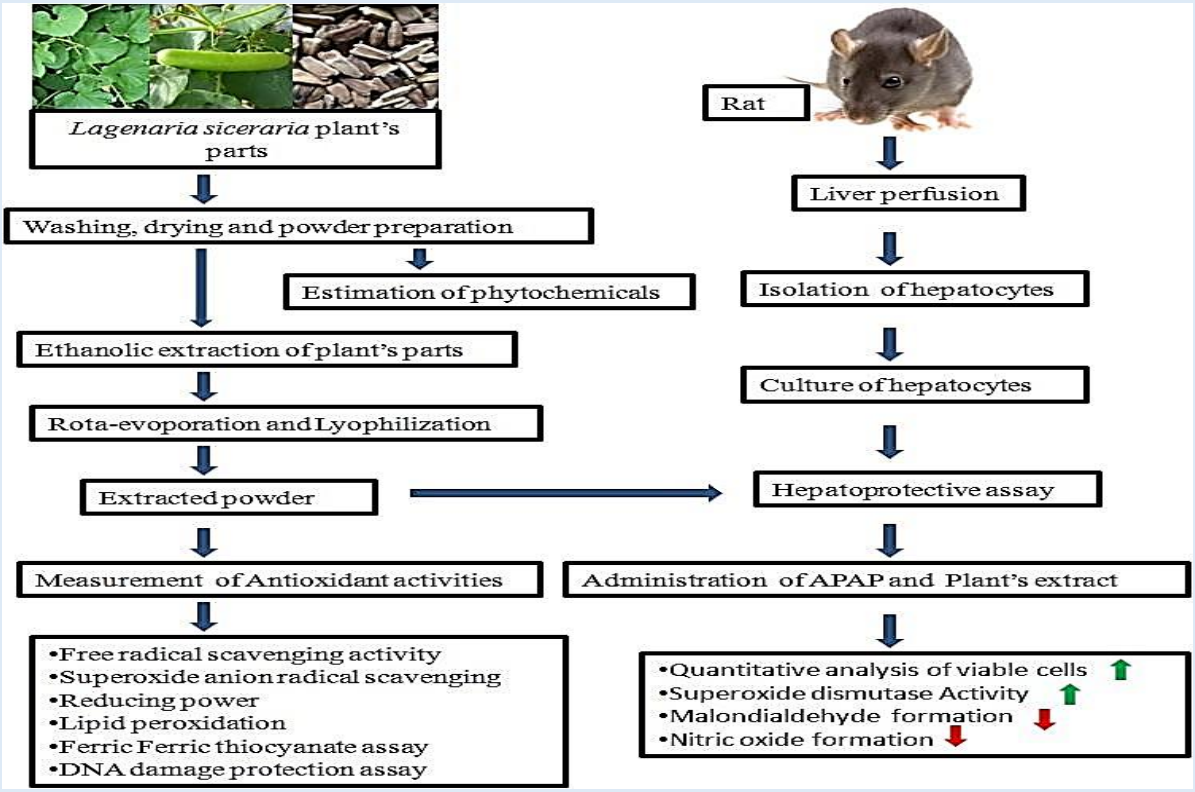
Context and purpose of the study: The aim of the present study was to evaluate the phytochemical content, antioxidant activity, DNA damage and hepatoprotective activities of *Lagenaria siceraria* (LS) plant's parts to explore their nutraceutical value.

Methods: Phytochemical content was measured by the estimation of total phenolics, carotenoids, ascorbic acid, protein and carbohydrates while antioxidant activities were investigated by assaying the parameters of free radical scavenging activity, lipid peroxidation, superoxide anion radical scavenging activity, reducing power, and DNA damage protection

assay. Hepatoprotective activity of LS leaf was measured by assaying cell viability, superoxide dismutase activity, malondialdehyde and nitric oxide formation.

Results: Among the tested plant samples, LS leaves showed high contents of total phenolics, carotenoids, ascorbic acid, and protein contents whereas LS fruits have high carbohydrate contents. Evaluation of antioxidant activities in different parts of the LS plant showed that the LS leaf has high antioxidant properties as compared to other plant parts. The *L. siceraria* leaf also showed DNA protection activities against Fenton's reagent as well as UV induced hydroxyl radical's damage. To test the hepatoprotective activity of LS leaf against acetaminophen, induced hepatocellular toxicity and leaf extract concentrations of 2.5, 5.0 and 7.5 µg/10^4 cells were administered to cultured hepatocytes before acetaminophen exposure. The experimental results showed that LS leaf extract restores superoxide dismutase activity and reduces malondialdehyde and nitric oxide formation as compared to acetaminophen alone treated cultured cells.

Conclusion: Our findings suggest that leaves of *L. siceraria* plants have high antioxidant potential and DNA damage-protective and hepatoprotective activities. Hence, the leaf part of this plant may be used in different food applications to provide nutritional and health benefits, as well as in various liver care herbal formulations.



Keywords: Antioxidant, Hepatoprotective activity, Hepatocytes, Lipid peroxidation, Total phenolics

INTRODUCTION

Herbal based medicines have recently attracted a majority of the population as alternative medicines for treating diseases/disorders due to oxidative stress [1]. Analysis of the pharmacological properties of plant products has stimulated the mass population to use herbal medicine for health benefits. The liver has vital roles in the detoxification of chemotherapeutic agents, xenobiotics and environmental pollutants. During bio-transformation of these xenobiotics, the liver faces a variety of oxidative stress and disorders. Although antioxidant activity of several plants has been examined to see its hepatoprotective role in a wide variety of liver disorders, scientists are still searching a better way to improve liver function. Antioxidants scavenge the free radicals and have a protective role against infections and degenerative diseases [2]. Hence, there is increasing demand for natural antioxidants in the therapeutic industry, food and cosmetic industry due to their high compatibility with dietary intake, low cost and less side effects on the human body. Based on the above information, natural antioxidants appear to be a more promising alternative source for reducing oxidative stress-induced diseases than synthetic medicines.

The *Lagenaria siceraria* (Molina) Standley (LS) plant belongs to the family Cucurbitaceae and is commonly known as bottle gourd, ghiya, calabash, doodhi and lauki. It is a climbing plant that bears hard, bottle shaped gourds as fruit. The *L. siceraria* plant is officially included in "The Ayurvedic Pharmacopoeia of India". In India, LS fruit is used as vegetables and in juices and the preparation of sweets. It helps with body weight loss quickly due to its low fat and cholesterol content. The bottle gourd has multiple vitamins and minerals including iron, sodium, potassium and high percentage of water (96%) that makes it ideal for quenching reactive oxygen species and thirst [3]. It has important roles in sharpening and enhancing memory due to presence of the highest content of choline, a precursor of the acetylcholine neurotransmitter [4].

The leaves also contain micro and macro-elements, and various phytochemical compounds that are beneficial to human health [5]. The fruit is used to treat diabetes, jaundice, hypertension, piles, ulcers, asthma and other bronchial disorders, pectoral cough, pain and skin disease [6]. Fruits are used as cardiotonic and as general tonics in Ayurveda. Seeds of vegetables, along with Achyranthes spp., are used to treat aching teeth and gums, boils etc. Extracts of the LS plant have been found to have various medicinal properties such as hepatoprotective, anti-oxidant, antihyperglycemic, immunomodulatory, anti-hyperlipidemic, antidiabetic, anti-cancer, antibiotic and cardiotonic properties [4, 7].

Scientific reports on functional foods showed a clear relationship between the uptake of functional foods and our health. Functional foods are the natural or processed foods that contain biologically active compounds which provide health benefits along with essential nutrients (vitamins and minerals). The most basic concept of functional foods intake is to obtain nutrients for our body and to understand the mechanisms by which foods modulate metabolism and health. *L. siceraria* is most commonly used as a vegetable in Indian houses. Vegetables are a natural source for obtaining a lot of vitamins, minerals, dietary fibre and different phytochemicals which may function as antioxidants and may participate in metabolic activities in humans. However, the scientific data available in the literature is not sufficient and systematic to explore the remedial powers of the LS plant. With rising health concerns, there is a need to explore some more prominent sources of nutraceuticals or pharmaceutical drugs to reduce the risk of oxidative stress-induced diseases. In the proposed study, an attempt has been made to obtain comprehensive information about antioxidant activity, DNA protective potential as well as hepatoprotective properties of the LS plant parts.

MATERIALS AND METHODS

Chemicals and Reagents: Bovine serum albumin (BSA), gallic acid, silymarin and quercetin were procured from Sigma-Aldrich, St. Louis, MO USA. Phenazine metho-sulphate (PMS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), nitro blue tetrazolium (NBT), reduced nicotinamide adenine dinucleotide (NADH), trichloro-acetic acid (TCA), potassium ferricyanide, thiobarbituric acid (TBA), ferric chloride and sodium dodecyl sulphate were purchased from SRL, India, whereas agarose was obtained from GE Healthcare (USA). Ascorbic acid, Folin Ciocalteau's phenol reagents, β-Carotene were the product of E. Merk, Mumbai, India. Calf thymus DNA was purchased from Himedia Co. Ltd (India). All the other chemicals used throughout the study were of analytical grade.

Samples Collection: Samples were collected from the vegetable cultivated region of Ayodhya, Uttar Pradesh and washed, dried, powdered and kept at 4°C. Plant was identified at National Botanical Research Institute (NBRI), Lucknow, India (Accession No: 96510).

Extraction: Twenty-gram plant samples were extracted with 70% ethanol and filtered. This process repeated till the disappearance of color from the solvent and pool filtrate. The filtrates were evaporated to 10 ml on a rotary evaporator (BUCHI Rotavapor R-114, Switzerland) followed by lyophilization till dryness and stored at −4 °C.

Animals: Albino Wistar male rats (*Rattus norvegicus*) of 150±20 g were collected from Indian Institute of Toxicology Research (IITR) animal house. Animals were kept under at 12 h light/dark cycle, temperature (25±2 °C) and humidity (60–70%) standard controlled conditions. All the protocols were approved by the institutional animal ethics committee of the IITR (ITRC/IAEC/01/2010).

Estimation of Phytochemicals: Ascorbic acid content of samples was evaluated by Association of Official Analytical Chemists (AOAC) method [8] and values denoted in mg/100g of fresh weight (FW) whereas carotenoids quantity was evaluated by Jensen method [9] and values mentioned as µg /g of FW. Total phenolic content (TPC) was evaluated based on method by the Ragazzi and Veronese [10] and values obtained were mentioned as mg of gallic acid equivalent (GAE)/g of dry weight (DW). Protein content was estimated with the Lowry method [11], whereas the Anthrone method [12] was used for carbohydrate content assay and reported as mg/g of DW.

Antioxidant studies: DPPH (1,1-diphenyl-2-picrylhydra-zyl) dye was used for free radical scavenging activity of samples based on method described by Yen and Duh [13]. DPPH• is one of the few stable and commercially available organic nitrogen radicals, and it is widely used in the evaluation of antioxidant activity. This assay is based on the hydrogen donating capacity of an antioxidant containing plant extract. The purple colour gradually decreases or disappears when plant extracts containing an antioxidant compound are added to the medium. Superoxide anion radical scavenging potential was assayed with the method of Nishikimi et al. [14] which was based on the reduction of nitro blue tetrazolium (NBT). Plant extract's reducing power (RP) was determined by Apati et al. method [15]. Lipid peroxidation assay was carried out according to Ohkawa et al. [16] methodology by using egg homogenates as lipid rich media. Ferric thiocyanate assay (FTC) was determined by testing the inhibitory capacity of extracts against the oxidation of linoleic acid by Tsuda et al. [17].

Estimation of DNA damage: To evaluate the DNA damage protective potential of the extracts, plasmid nicking assay was performed using calf thymus DNA according to the method of Lee et al. [18] with some modifications. The nicking of DNA was introduced by two different methods, Fenton's reagent induced damage and UV rays induced damage. In the case of Fenton's

reagent induced damage, Catalase and Silymarin (2.5 µg/ml) were used as positive controls whereas in the case of UV rays induced damage, Catechin and Silymarin were used as positive controls.

Hepatoprotective assay

Isolation of hepatocytes: Rat liver perfusion was performed according to the method of Seglen [19]. 400 ml of Hepes buffer was prepared with 130 µM NaCl, 3 mM KCl, 0.8 mM NaH_2PO_4 and 10 mM Glucose and 10 mM Hepes (pH 7.4; 37°C). After anaesthetization, 200 ml of pre-wash solution (Hepes buffer + 0.5 mM EDTA) was perfused through the portal vein for 15 min, followed by 100 ml of collagenase solution (Hepes buffer + 5 mM $CaCl_2$ + 30 mg collagenase (Type IV) for 10 min. 100 ml of post wash solution (Hepes buffer + 5 mM $CaCl_2$) was perfused for washing after collagenase solution. The liver was mechanically disintegrated and filtered through a 100-mesh filter and then resuspended in RPMI (Roswell Park Memorial Institute) medium with 10% FBS, which was maintained during the experiments. Cell viability was confirmed by the MTT reduction test within 1h of cell isolation. The viability of hepatocytes was at least 95%.

Primary cell culture: RPMI-1640 media were used to maintain hepatocyte cells along with fetal bovine serum, antibiotics streptomycin and penicillin, sodium pyruvate, Amphotericin B and glutamine in an incubator (Thermoforma) with controlled humidity at 37^0C. 96 well plates, pre-coated with 0.1% collagen were used for cell seeding with count $1.0x10^4$ cells/well (counted on hemocytometer) and then drug exposure experiments were performed after the cells had been cultured for 24 h. The viability of cells was determined by the MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) reduction test.

Treatment of cells: Acetaminophen (APAP) was dissolved in 1% dimethyl sulfoxide (DMSO) and filtered with a 0.22 µM filter for subsequent treatment. Isolated cells were pre-exposed with plant extracts as well as with standard silymarin (5µg/10^4 cells). Cells were treated with plant extract (45 min) before treatment with APAP (30 min). In this experiment, 0.1% DMSO given to hepatocytes was considered as control. Silymarin was used as positive control.

Quantitative analysis of viable cells: The cell viability of hepatocytes against APAP was determined by MTT assay as described by Mosmann [20]. Various concentrations of acetaminophen and extracts were exposed to 24h old hepatocytes. After the incubation period was completed, culture medium was removed and MTT (10 µl from a stock of 5 mg/ml) was added to each well. The culture medium was removed after 4 h of incubation and in each well, 0.2 ml DMSO was added. The optical density (OD) was measured at 530 nm using a Spectramax PLUS 384 microplate reader. MTT is a water soluble tetrazolium salt, which is converted to an insoluble purple formazan by the succinate dehydrogenase enzyme. This formazan product is water-insoluble and hence accumulates within the cell. The colour produced due to formazan complex quantified by spectrophotometer. Higher colour intensity indicates high cell viability and high mitochondrial membranal integrity.

Superoxide dismutase (SOD) activity: SOD activity was determined spectrophotometrically by measuring inhibition of the NADH-PMS-NBT reaction system by the method of Kakkar et al. [21]. 10 µl of cell lysate, 40 µl of double distilled water (DDW), 10 µl of 0.96 mM PMS, 90 µl of 50 mM sodium tetra pyrophosphate buffer (pH 8.3) and 30 µl of 0.3 mM nitro blue tetrazolium were added to the wells of the plates. The reaction was initiated with 20 µl 0.72 mM NADH. The reaction was terminated by adding 50 µl of glacial acetic acid after incubation for 90s at 30°C. NBT reduction leads to the formation of blue formazan, which was read at 560 nm. 1 unit activity/min was considered as 50% inhibition of formazan formation in 1 min.

Malondialdehyde (MDA) determination: MDA, a product of lipid peroxidation, was estimated by using the method of Wallin et al [22]. In this method, oxidation of phospholipids was estimated by measuring the amount of MDA at 530 nm. The oxidised lipid products were isolated by precipitating the cell lysate with trichloroacetic acid (TCA) and MDA was measured with thiobarbituric acid (TBA) reaction which forms a colourful product. Silymarin was used as a positive control. Lipid peroxiation was expressed as moles of MDA formation.

Determination of nitric oxide (NO): Nitric oxide radical formation was measured by assaying nitrite in all treated and control hepatocytes. In hepatocytes, nitrite concentration was measured by using the Griess reagent [1% sulfanilamide in 5% phosphoric acid (sulfanilamide solution) and 0.1% N-1-naphthylethylenediamine dihydrochloride in distilled water (NED solution)] as described by Feelisch et al [23].

Statistical analysis: Results were expressed as the mean ± SD (standard deviation). Data was analysed on PRISM software using student's t-test and one way ANOVA. * p≤0.05; ** p≤0.01 and *** p≤0.001 were used as levels of significance.

RESULTS

Phytochemicals assay: In the tested plant samples, the highest concentration of ascorbic acid and carotenoids was present in the *L. siceraria* leaf followed by *L. siceraria* seed and fruit. The leaf had highest total phenolics and protein content followed by the fruit and seed. Carbohydrate content was present in the following order: *L. siceraria* fruit >*L. siceraria* leaf > *L. siceraria* seed (Table 1).

Table 1. Estimation of phytochemicals in fruit, seed and leaf extracts of LS plant parts

Biochemical parameter	LS Fruit	LS Seed	LS Leaf
AA (mg/100g of FW)	17.33±0.34	21.17±0.85	165.82±2.45
Carotenoid (µg /g of FW)	3.58±0.14	3.64±0.13	5.10±0.17
TPC (mg of GAE/g of DW)	48.82±1.61	43.42±0.34	61.06±1.67
Protein (mg/g of DW)	135.01±1.25	124.84±2.09	173.81±4.21
Carbohydrate (mg/g of DW)	161.48±7.22	53.58±1.87	91.85±3.39

Total phenolic content (TPC), Ascorbic acid (AA), Fresh Weight (FW), Dry Weight (DW). The data is presented as the mean ±SD of three replications.

Antioxidant activities assay

Free radical scavenging assay (FRSA): The FRSA assay was performed using DPPH free radicals and represented in terms of IC_{50} (inhibitory concentration). Phyto-chemicals present in plant extracts having antioxidant properties reduce the free radical DPPH (deep violet colour) into 1,1-diphenyl-2-picryl hydrazine with decoloration. *L. siceraria* leaf extract (LSLE) scavenges the radicals by 25.09, 38.76, 47.06 and 52.71%, respectively, when 50, 100, 150 and 200 µg/ml plant extract were added to the reaction mixture. DPPH scavenging activity of the *L. siceraria* leaf showed the lowest IC_{50} value (175.65µg/ml) among the tested samples, followed by *L. siceraria* fruit extract (267.55 µg/ml) and *L. siceraria* seed extract (325.45 µg/ml) (Table 2).

Superoxide anion radical scavenging activity (SARSA): Antioxidant potential of plant extracts was further estimated by assessing the superoxide radical quenching ability of the extract, depicted in Table 2. Among the tested plant samples, SARSA was higher in the *L. siceraria* leaf (IC_{50}: 36.47±1.36 µg/ml) as compared to seed (56.75±3.65 µg/ml) and fruit (65.45±4.85 µg/ml).

Reducing power (RP): Reducing power assay is used to determine the reducing potential of plant extracts by assaying the transformation of ferric ions to ferrous ions. The reducing potential of plant extracts is a significant indicator of its high antioxidant activity. Among the

tested samples, the *L. siceraria* leaf showed high reducing power 8.90±1.18 ascorbic acid equivalents (ASE)/ml) as compared to LS Fruit 15.10±1.65 ASE/ml and LS Seed 21.40±1.45 ASE/ml, but lower than standard quercetin 0.14±0.18 ASE/ml (Table 2).

Lipid peroxidation: The antioxidant potential of plant parts was further explored by lipid peroxidation assay (Table 2). In this study, the *L. siceraria* leaf extract showed 46.71, 57.99, 62.89 and 67.88 % inhibition when 125, 250, 375 and 500 µg/ml *L. siceraria* leaf extracts were added to the reaction mixture. Hence, *L. siceraria* leaf showed minimum inhibitory concentration (IC_{50}: 194.06±5.13 µg/ml) followed by fruit (246.48±7.89) and seed (284.86±6.44). The above results showed that leaf extract had greater reduction in MDA formation as compared to fruit and seed extracts.

Table 2: Estimation of antioxidant activity (AOA) in different parts of *L. Siceraria* Plant

Antioxidant activity	LS Fruit	LS Seed	LS Leaf	Quercetin
FRSA (IC_{50} in µg/ml)	267.55±8.78	325.45±7.56	175.65±7.47	5.34±0.44
SARSA (IC_{50} in µg/ml)	65.45±4.85	56.75±3.65	36.47±1.36	11.58±0.51
RP (ASE/ml)	15.10±1.65	21.40±1.45	8.90±1.18	0.14±0.18
LPO (IC_{50} in µg/ml)	246.48±7.89	284.86±6.44	194.06±5.13	106.79±1.09
FTC (IC_{50} in µg/ml)	48.38±1.52	95.25±3.56	65.03±0.97	17.07±1.56

Data is presented as mean ±SD (n =3). FRSA: Free radical scavenging activity; SARSA: Superoxide anion radical scavenging activity; RP: Reducing power; LPO: Lipid peroxidation; FTC: Ferric thiocyanate chelating assay; IC: Inhibitory concentration; ASE: Ascorbic acid equivalents.

Ferric thiocyanate chelating (FTC) assay: An FTC assay was performed to determine the peroxide level at the initial stage of linoleic acid oxidation. Peroxides formed during oxidation were determined by the quantification of complex compounds formed between Fe3+ and SCN^- ions. Among the tested extracts, maximum FTC activity was observed in *L. siceraria* fruit extract (48.38±1.52 mg/ml). Based on IC_{50} values, the order of FTC activities of the plant extracts was as follows: *L. Siceraria* fruit > *L. Siceraria* leaf > *L. Siceraria* seed. Plant extracts inhibited the conversion of ferrous ions to ferric ions. So, a low quantity of ferric thiocyanates formed in the reaction mixture. Lower inhibitory concentrations indicate a higher level of antioxidant activity. Table 2 showed that the inhibitory effect on oxidation of linoleic acid depends on plant's part. Table 2 suggested that the inhibitory effect of plant extracts on linoleic acid oxidation varies in different parts.

Among the tested plant samples, the *L. siceraria* leaf extract showed higher biochemical content and antioxidant activities as compared to other tested samples (Table 1 & 2). So, the *L. siceraria* leaf was selected for further investigation of DNA protection as well as hepatoprotective assay.

DNA damage protective assay: DNA damage protective assay of LS leaf extract was studied *in vitro* against UV rays and Fenton's reagent induced DNA damage. Fenton's reagent and UV light produce highly reactive hydroxyl radicals which cause damage in calf thymus DNA. LS leaf extract showed protective activity and neutralized the impact of the hydroxyl radical in a

concentration dependent manner. Results were analysed through horizontal gel electrophoresis, which indicated that the LS leaf extract significantly protected DNA damage against Fenton's reagent (Figure 1) and UV rays (Figure 2). In the absence of LS leaf extract, Fenton's reagent as well as UV rays induced hydroxyl radicals cause complete fragmentation of DNA (Figure 1: Lane 4; Figure 2: Lane 3). However, with supplementation of leaf extract of *L. siceraria* at concentrations of 1, 2.5, and 5.0 µg/ml, protective activity was observed against Fenton's reagent as well as UV rays induced DNA damage (Figure 1 & 2). In case of Fenton's regent induced DNA damage, standard catalase (2.5 µg/ml) and silymarin (2.5 µg/ml) showed better protection as compared to LS leaf extract (2.5µg/ml), whereas in the case of UV induced DNA damage, standard silymarin (2.5 µg/ml) and catechin (2.5µg/ml) showed better protection as compared to LS leaf extract (2.5µg/ml).

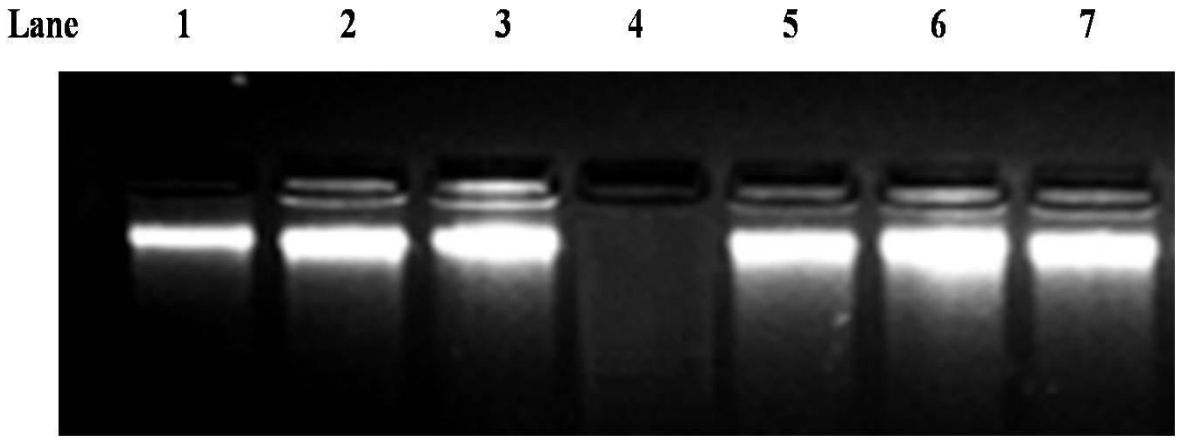


Figure 1. Protective effects of *L. siceraria* leaf extract (LSLE) on DNA damage caused by Fenton's reagent. Lane 1: DNA (500 ng) + Phosphate Buffer Saline; Lane 2: DNA + Fenton's reagent (60 min) + Catalase (2.5 µg/ml); Lane 3: DNA + Fenton's reagent (60 min) + Silymarin (2.5 µg/ml); Lane 4: DNA + Fenton's reagent (60 min); Lane 5-7: DNA + Fenton's reagent (60 min) + LSLE (1.0, 2.5, 5.0 µg/ml).

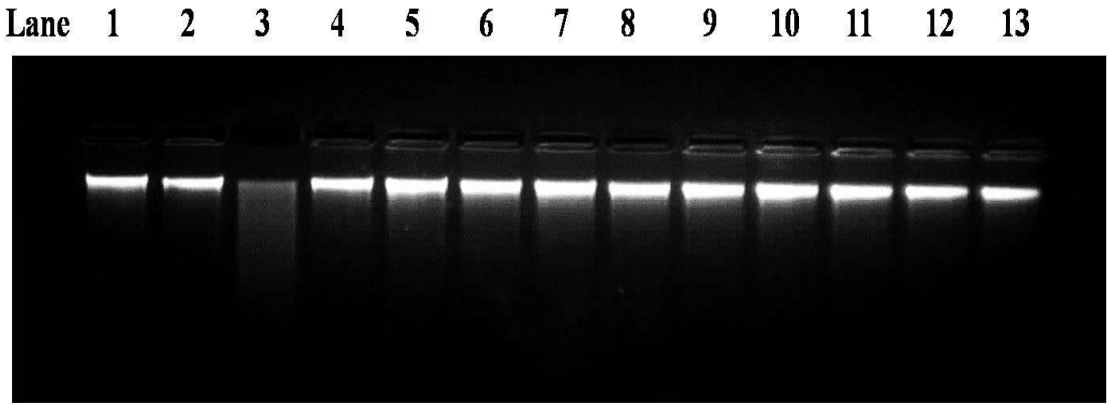


Figure 2. Protective effects of *L. siceraria* leaf extract (LSLE) on nicked DNA caused by Ultraviolet rays induced hydroxyl radicals. Lane 1: DNA (500 ng) + Phosphate Buffer Saline; Lane 2: DNA + Hydrogen peroxide (20.5 mM); Lane 3: DNA + Hydrogen peroxide (20.5 mM) + Ultra Violet rays (8 min); Lane 4-7: DNA + Hydrogen peroxide (20.5 mM) + Ultra Violet rays (8 min) + LSLE (1, 1.5, 2.5, & 5.0 µg/ml); Lane 8-11: DNA + Hydrogen peroxide (20.5 mM) + Ultra Violet rays (8 min) + Silymarin (1, 1.5, 2.5 & 5.0 µg/ml); Lane 12-13: DNA + Hydrogen peroxide (20.5 mM) + Ultra Violet rays (8 min) + Catechin (2.0 & 2.5 µg/ml)

Hepatoprotective assay

LS leaf extracts increased the survival of acetaminophen stressed primary hepato-cytes: Acetaminophen is an antipyretic and analgesic agent that is widely used to lower the fever and pain. When N-acetyl-p-benzo quinamine (NAPQI), a toxic by-product of acetaminophen metabolism, covalently binds to cysteine groups on proteins to form 3-(cysteine-S-yl) acetaminophen adducts, hepatic glutathione levels are depleted. According to experimental results, aceta-minophen (APAP) at 650 µM concentration reduces 51.90%±1.22 cell survival, implying that the inhibitory concentration (IC_{50}) of APAP was 675 µM (Figure 3). This concentration of APAP was used for further experiments.

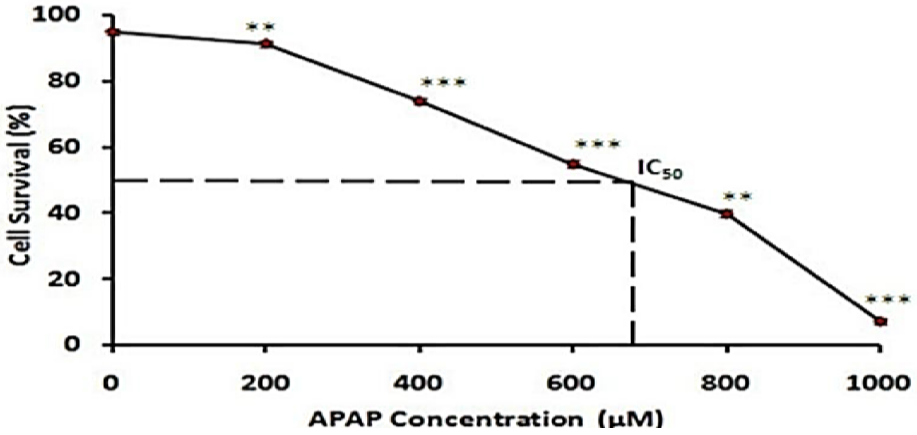


Figure 3. Concentration dependent effect of APAP toxicity on cell survival.

Cell viability assay: Quantitative analysis of viable cells was assayed by using MTT dye and the absorbance of solubilized formazan complex was measured at 530 nm. To explore the impact of plant extracts on cell viability, hepatocyte cells were treated with varying concentrations (1-10 µg/ml/10^4 cells) of plant extract (Figure 4). Results showed that no cytotoxicity was observed in hepatocyte cells with plant extract treatment with respect to control. To see the cytoprotective effects of LSLE extract against APAP induced hepatocellular toxicity, plant extract concentrations 2.5, 5.0 and 7.5 µg/10^4 cells were chosen for further testing. Similar concentrations of silymarin (Sil) were used as a positive control. The treatment with silymarin at a concentration 5.0 µg/10^4 cells significantly increased the cell survival rate up to 44.45%±4.33 of hepatocytes ($p \leq 0.001$). Selected concentrations of extract were added to hepatocytes 45 min before exposure to APAP for 30 min. The results revealed direct correlation between leaf extract dose-response and cell viability. The cells that were pre-incubated with LSLE extract showed a significant increase in cell survival rate up to 19.07% ($p \leq 0.001$), as compared to cells treated with APAP (Figure 5).

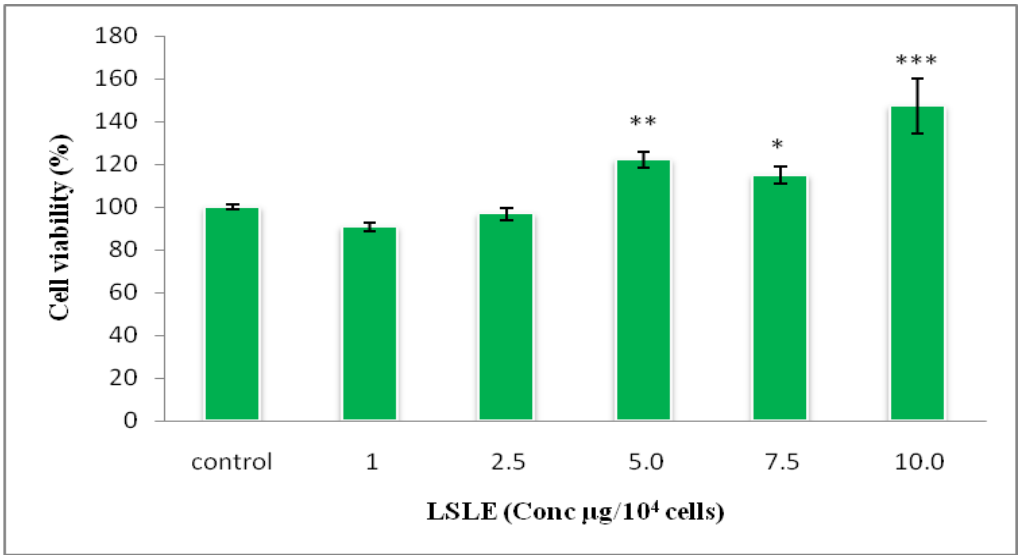


Figure 4. Measurement of cell viability of primary hepatocytes to see the cytotoxic role of LSLE extract at different concentrations. Data are presented in mean ±SD (n=3). *p≤0.05,** p≤ 0.01 and ***p≤0.001 are levels of significance compared with control values.

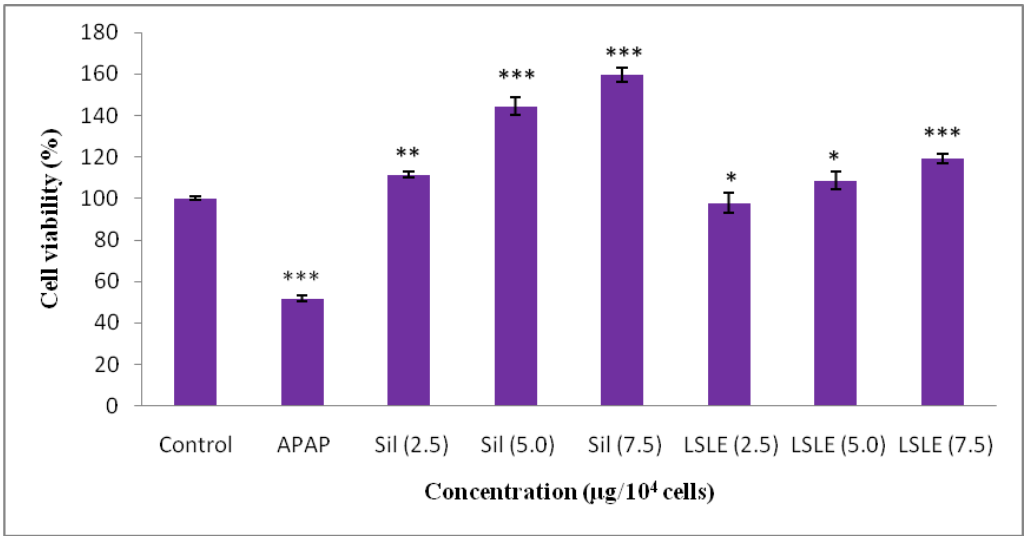


Figure 5. Measurement of cell viability to see the cytoprotective role of LSLE extract against acetaminophen induced toxicity in primary hepatocytes. Silymarin (Sil) at different concentrations was used as a positive control. The data shown are mean ± SD (n=3). APAP was compared with control and treatment with plant extract and silymarin were compared with APAP. Results with *p≤0.05, ** p≤ 0.01 and ***p≤0.001 were considered as levels of significance.

Effect of LS leaf extract on SOD: Hepatocytes subjected to APAP stress had 2.3-fold lower SOD activities as compared to untreated cells (control) and SOD activity was found 4.80±0.45 units/min/10^4 cells in APAP exposed cells (Figure 6). Cells that were treated with extracts (2.5 to 7.5 µg/10^4 cells) showed a significant increase in SOD activity (6.81±0.30, 7.57±0.34 and 8.93±0.24 Units/min/10^4 cells) whereas cells treated with standard silymarin (5.0 µg/10^4 cells) also showed restoration of SOD activity (12.82±0.52 Units/min/10^4 cells) as compared to cells treated with APAP hepatocytes.

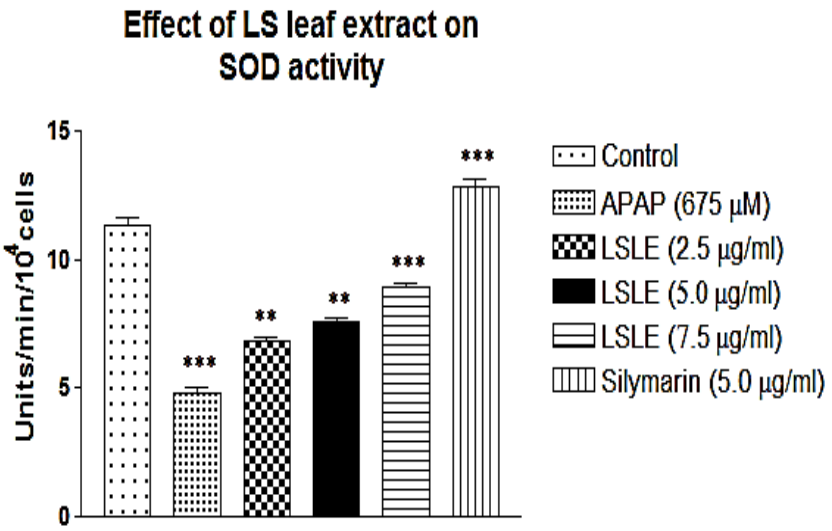


Figure 6. Impact of LS leaf extract on SOD enzyme restoration in APAP treated cells. Cells treated without plant extract and APAP were considered as negative control. APAP alone treated cells compared with control cells, whereas LS leaf extracts treated cells followed by APAP compared with the APAP treated cells. Data are mean ± SD of three replicates in each case. **P<0.01, ***P<0.001 were considered as levels of significance.

Effect of LS leaf extract on MDA formation: Lipid peroxidation was measured in terms of MDA formation in primary hepatocytes induced by the APAP compound. Results of this experiment showed that cells treated with APAP along with different concentrations (2.5, 5.0, 7.5 µg/ml) of leaf extract reduced the MDA formation from 0.57±0.024 to 0.48±0.018 nM MDA formation /10^4 cells than the APAP treated cells, which showed 0.63±0.015 nM MDA formation/10^4cells. The cells pre-exposed with silymarin (5 µg/10^4 cells) showed a significant decrease in MDA concentration (0.24±0.014 nM MDA formation /10^4 cells) than the APAP treated cells (Figure 7).

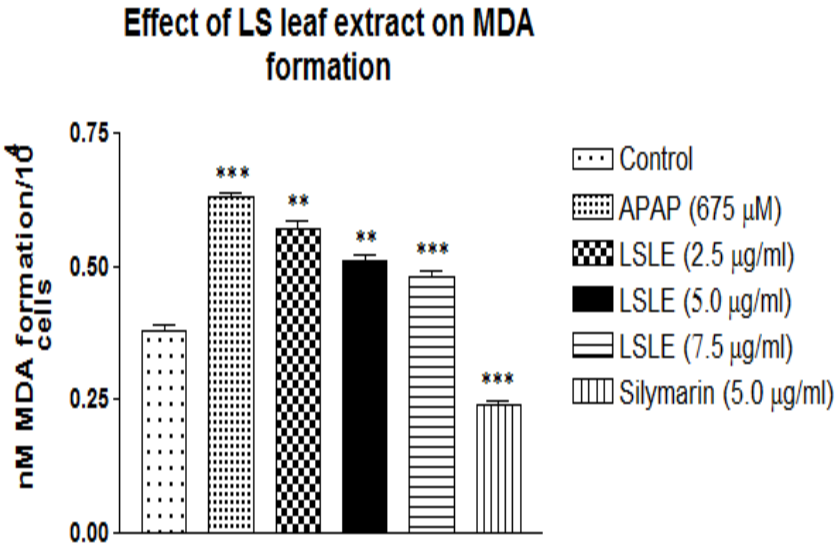


Figure 7. Anti-lipid peroxidation effect of ethanolic LS leaf extracts in APAP treated cells. Cells treated without plant extract and APAP were considered as negative control. APAP alone treated cells compared with control cells, whereas LS leaf extracts treated cells followed by APAP compared with the APAP treated cells. Data are mean ± SD of three replicates in each case. **P<0.01, ***P<0.001 were considered as levels of significance.

Effect of LS leaf extract on nitric oxide formation: Cultured hepatocytes subjected to APAP stress showed the formation of 125.74±4.42 pg NO /10^4cells which had 1.26-fold higher than the control cells. Administration of plant extract showed concentration dependent scavenging of $NO^{\cdot}$ which ranged from 81.51±1.67 to 64.46±2.09 pg NO /10^4cells (1.54-1.95 fold lower than APAP treated cells) (Figure 8). The cells pre-exposed with silymarin (5 µg/10^4 cells) had a 2.08-fold (60.45±2.06 pg NO /10^4cells) quenching of NO higher than APAP treated cells.

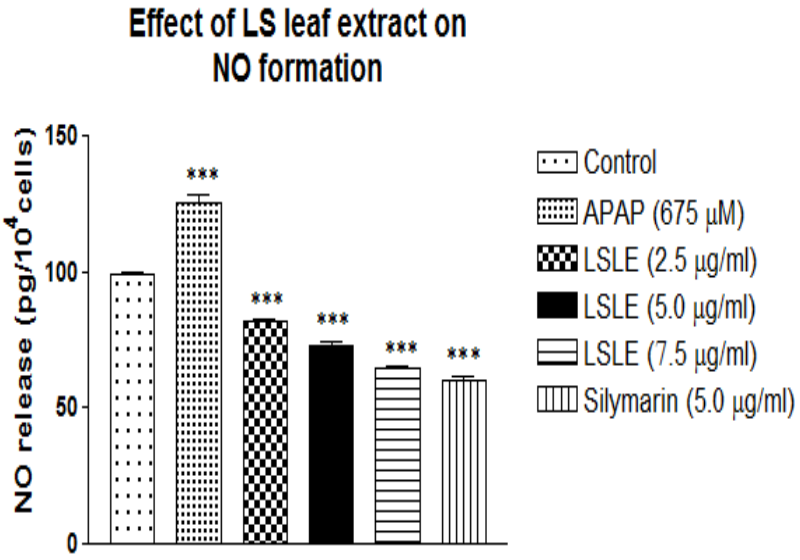


Figure 8. Impact of ethanolic LS leaf extracts on nitric oxide formation in APAP treated cells. Cells treated without plant extract and APAP were considered as negative control. APAP alone treated cells compared with control cells, whereas LS leaf extracts treated cells followed by APAP compared with the APAP treated cells. Data are mean ± SD of three replicates in each case. ***P<0.001 was considered as levels of significance.

DISCUSSION

Most plants have a good number of phenolic compounds with high antioxidant activities which contribute to their hepatoprotective properties. Hence, plants with high phenolic compounds could be a prominent source of antioxidants [24 -25]. The discovery of new natural products may be good source of therapeutics for treating different types of chronic and infectious diseases. Herbal-based therapeutics drugs have many significant advantages due to their wide availability, pharma-cological activity, less toxicity, and low side effects compared to synthetic drugs [26]. The LS leaf possesses a good number of carotenoids, proteins, ascorbic acid, total phenolics and carbohydrate contents. There has been increasing interest about the pharmacological properties of these phytochemicals in the prevention of oxidative damage to human body tissues. Ascorbic acid is a powerful antioxidant that has the capacity to neutralize harmful reactive oxygen species/reactive nitrogen species. It is a water-soluble vitamin and can control free radical induced damage in both extracellular as well as intracellular level of cells. It helps to synthesize collagen, the most abundant extracellular protein which is needed for healthy bones, teeth, gums and blood vessels. The

phytochemicals having reducing potential reduces the DPPH radical into a colourless α-α-diphenyl-β-picryl hydrazine [27]. DPPH radical scavenging activity of the ethanolic vegetable extracts was measured along with standard quercetin. Vegetable extracts were found to have significant free radical scavenging activities.

The liver has some important roles in our body like vascular, metabolic, secretory, immunological, and excretory functions [28-29]. Living cells utilise oxygen for respiratory purposes, but some part of the available oxygen is converted into several harmful reactive oxygen species and starts a chain reaction leading to the formation of more and more free radicals. Among the reactive oxygen species, the superoxide anion radical is one of the strongest free radicals that are generated due to the loss of one electron in molecular oxygen. Later, this reactive superoxide anion forms other harmful reactive oxygen species such as the hydroxyl radical and hydrogen peroxide. Superoxide dismutase is an antioxidant enzyme that catalyses the reduction of the highly reactive superoxide anion to the less reactive species H_2O_2, which is then converted into water by catalase or glutathione peroxidase [30]. The SOD enzyme is also capable of preventing the oxidation of a substrate by $O_2^{\cdot-}$.

In the reducing power assay, the presence of antioxidants in the samples would result in reducing the ferric ion to a ferrous ion by donating an electron which is an important mechanism of action of phenolic anti-oxidants [31]. The reducing power of plants is believed to not only react directly with peroxide free radicals but also prevent its formation. Results of the present study also indicate that the *L. siceraria* leaf is a ferric ion quencher and possesses good antioxidant properties. Therefore, these extracts have the capability to donate electrons and that will reduce free radicals or will terminate chain reactions induced by free radicals.

Lipid peroxidation is an indicator of cellular injury and oxidative stress in cells and tissues of both animals and plants. LPO is a free radical chain reaction process in which a peroxidation reaction is initiated by hydroxyl radical and causes destruction of polyunsaturated fatty acids of membrane's lipid. Lipid peroxidation produces a number of stable end products, the most common of which are 4-hydroxy-2-nonenal, 2-propenal (acrolein), α, β-unsaturated reactive aldehydes such as malon-dialdehyde, and isoprostanes, which can be ascertained in plasma and urine [32-33]. Decreased lipid peroxidation, which can be detected with thiobarbituric acid reactive substances assay and FTC methods, can be used to assess the antioxidant potential of plant extracts. IC_{50} values show the inhibitory concentration of plant extract required to inhibit 50% reaction and its lower value indicates a higher antioxidant capacity of plant extracts. The lower IC_{50} value of the *L. siceraria* leaf extract showed strong antioxidant activity and inhibition of lipid peroxidation (LPO) by FTC and TBA radical scavenging methods. Jaeschke and Ramachandran [34] reported that administration of acetaminophen causes oxidation of lipids and may initiate a destructive process in the liver. To explore the hepatoprotective properties of the LS leaf, primary hepatocyte cells were treated with extracts and standard silymarin along with APAP. Results showed that the LS leaf extract has the potential to reduce the formation of the lipid peroxidation end product (i.e. malondialdehyde), as compared to primary hepatocyte cells treated with APAP alone. The findings indicate that the LS leaf extract has hepatoprotective

activity and can be used in various herbal formulations for the treatment of liver disorders.

LS leaf extract provided concentration dependent protection (1.0-5.0 µg/ml) against DNA damage caused by Fenton's reagent / Ultraviolet rays. Oxidative DNA damage was found to be reduced in the presence of the standard antioxidants silymarin and catalase (Figure 1), and silymarin and catechin (Figure 2). Results from the experiment revealed that LS leaf extract showed DNA damage protective properties in calf thymus DNA against Fenton reagent and UV rays.

In the animal system, the liver is the main organ for the biotransformation of the toxic compound, acetaminophen. Acetaminophen has antipyretic properties and is widely used during fever. An accidental over dosage of acetaminophen produces severe hepatic damage. It is converted into the highly toxic metabolite N–acetyl–p–benzo quinamine (NAPQI) via the cytochrome P450-dependent oxidation in the liver that causes damage to liver cells. Studies on antioxidant enzyme activity, lipid peroxidation and nitric oxide concentration were observed during liver cell damage. Reduced concentration of antioxidant enzymes (SOD, CAT and GPx) and increased formation of MDA and NO is marker of cellular hepatotoxicity [35]. The concentration of these biomarkers was significantly restored to normal values with LS leaf extract treated hepatocytes as compared to cells treated with APAP alone. Plant extracts have a high content of polyphenolic compounds and can minimize the damage caused by acetaminophen.

Acetaminophen (APAP) is most commonly used as an antipyretic and pain reliever drug but at the same time, APAP can also cause dose-dependent hepatocellular necrosis [36]. The toxic effect of APAP on cellular integrity was shown by the MTT assay. MTT is taken up by live cells and converted into purple formazan product by mitochondrial enzymes [37]. A decrease in cell viability was observed in APAP treated hepatocytes as compared to normal cells. Administration of LS leaf extract along with APAP reduces the impact of APAP toxic effects on cell viability in a dose dependent manner.

The treatment of hepatocyte cells with APAP resulted in a significant decrease in the SOD level, which was restored to normal level with plant extract. Superoxide dismutase enzyme scavenges the superoxide anion and converts it into hydrogen peroxide, which is less toxic [38]. If these radicals are not neutralized, they can cause cellular dysfunction and ultimately organ failure. SOD enzyme controls the levels of a variety of reactive nitrogen species (RNS) and reactive oxygen species (ROS), and thus reduces the deleterious effects of the superoxide radical on cells. In this study, LS leaf extract showed protection against the toxic effects of APAP by enhancing the activity of the SOD enzyme as compared to APAP treated cells. Among the three concentrations, LS leaf extract showed dose dependent effective protection that was comparable to the known hepatoprotective antioxidant silymarin.

Nitric oxide (NO) is an unconventional gaseous neurotransmitter and is not stored in synaptic vesicles, but synthesized at the time of need. Nitric oxide is an important free radical of RNS family and is a precursor for the formation of all other RNS. It is neither intrinsically more reactive nor highly toxic but when it reacts with superoxide, it forms more toxic peroxynitrite ($ONOO^-$), which is a strong nitrating agent and oxidant for a wide range of biomacromolecules. It can cause protein nitrosylation and nitrotyrosination, excitotoxicity, inhi-

bition of the mitochondrial respiratory complex and organelle fragmentation. Although nitric oxide plays an important role in our body such as in vasodilation, neurotransmission, immune response, and is involved in modulation of diverse physiological processes, high concentrations of nitric oxide are related to various pathophysiological conditions such as Parkinson's disease, Alzheimer's disease, ischemic brain injury, amyotrophic lateral sclerosis and Huntington's disease. The emerging strategies to reduce NO production are an important therapeutic strategy for the treatment of neurodegenerative disorders. In the present study, the treatment of cells with LS leaf extract along with APAP and APAP alone showed a significant reduction in NO concentration (64.46 pg/10^4cells at 7.5 µg/ml) against APAP induced oxidative stress (125.74 pg/10^4cells) and protection that is comparable to standard Silymarin (60.45 pg/10^4cells at 5.0 µg/ml).

It is a well-known fact that prevention is a more effective strategy than treatment, especially for chronic diseases. A healthy diet rich in fruits, vegetables, whole grain cereals, legumes, and nuts can reduce the risk of non-communicable diseases [39]. Many countries have accepted the nutraceutical role of functional foods [40]. The constant use of phytochemicals and functional food products having bioactive compounds is essential for improving health, prevention, treatment and management of chronic as well as viral diseases [41-42]. Food and food products have several plant polyphenols which reduces DNA damage, tumor growth and cancer, cholesterol levels, cataracts, muscular degeneration and killing viruses like herpes [43-44]. Phytochemicals from vegetables such as tannins, quercetin, anthocyanin and catechin are known to inhibit lipid peroxidation and scavenge free radicals. These compounds may play the primary and most important role in DNA damage protection. Due to the beneficial impact of dietary polyphenols on human health, food scientists and consumers are receiving tremendous attention among nutritionists.

CONCLUSION

Analysis of the *L. siceraria* plant's parts revealed that the plant had varied levels of bioactive constituents among the tested samples which may be responsible for its antioxidant and hepatoprotective activities. The present study suggested that leaf extract of LS had better antioxidant potential as compared to other plant parts and it may be due to the presence of various phytochemicals such as total phenolics, ascorbic acid, carotenoids etc. The DNA damage protective assay also revealed that leaf extract of the LS plant showed maximum protection in a dose-dependent manner against damage induced by Fenton's reagent and Ultraviolet rays. The hepatoprotective activity of LS leaf extract was observed under *in vitro* conditions against APAP induced cell death. Results obtained from the *in vitro* experiments showed that cells treated with the plant's leaf extract have higher cell viability, high SOD activity and lower nitric oxide formation and lipid peroxidation. Restoration of an altered antioxidant enzyme, NO and MDA concentration with administration of leaf extract suggest that LS leaf extract has the potential to reduce the effect of APAP on hepatocytes and hence have hepatoprotective properties. The different parts of the LS plant have a high quantity of bioactive components such as vitamin C, carotenoids and phenolic compounds which have been found to be of

great importance to human's health. The findings of our study concluded that the LS leaf is a rich source of many antioxidants' bioactive compounds and has the potential for use as a nutritional supplement or in herbal liver care formulation and functional food products.

List of abbreviations: AA: Ascorbic acid, AOAC: Association of Official Analytical Chemists, ASE: Ascorbic acid equivalents, BPB: Bromophenol blue, BSA: Bovine serum albumin, DMSO: Dimethyl sulphoxide, DPPH: 1,1-diphenyl-2-picrylhydrazyl, DW: Dry weight, FRSA: Free radical scavenging activity, FTC: Ferric thiocyanate chelating assay, FW: Fresh weight, IC: Inhibitory concentration, IITR: Indian Institute of Toxicology Research, LPO: Lipid peroxidation, LS: *Lagenaria siceraria,* LSLE*: L. siceraria* leaf extract, MDA: Malondialdehyde, MTT: (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide), NADH: Reduced nicotinamide adenine dinucleotide, NAPQI: N–acetyl–p–benzo quinamine, NBT: Nitro blue tetrazolium, NO: Nitric oxide, PBS: Phosphate-buffer saline, PMS: Phenazine methosulphate, RNS: Reactive nitrogen species, ROS: Reactive oxygen species, RP: Reducing Power, RPMI: Roswell Park Memorial Institute, SARSA: Superoxide anion radical scavenging activity, SD: Standard deviation, SOD: Superoxide dismutase, TBA: Thiobarbituric acid, TBA: Thiobarbituric acid, TBARS: Thiobarbituric acid reactive substance, TCA: Trichloroacetic acid, TPC: Total phenolic content

Conflict of interest: The authors confirm that this article content has no conflicts of interest.

Authors' contributions: US, PS, RLS and SM: conceptualization, writing original draft, reviewing, editing, drawing figures and finalize the manuscript. PKS and SS: reviewing, editing, and drawing figures.

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REFERENCES

1. Singh P, Vishwakarma SP, Singh RL: Evaluation of antioxidant, oxidative DNA damage protective and antimicrobial activities of *Foeniculum vulgare* plant. J Med Plant Res 2013, 7(35):2551-2563. DOI: https://doi.org/10.5897/JMPR2013.5120
2. Singh P, Vishwakarma SP, Singh RL: Antioxidant, oxidative DNA damage protective and antimicrobial activities of *Trigonella foenum-graecum* plant. J Sci Food Agri 2014, 94(12):2497-2504. DOI: https://doi.org/10.1002/jsfa.6585
3. Modgil M, Modgil R, Kumar R: Carbohydrate and mineral content of chyote (*Sechium edule*) and bottle gourd (*Lagenaria Siceraria*). J Hum Ecol 2004, 15(2):157-159. DOI: https://doi.org/10.1080/09709274.2004.11905683
4. Milind P, Satbir K: Is Bottle Gourd a Natural Guard? Int Res J Pharm 2011, 2(6):13-17. DOI: https://doi.org/10.7897/2230-8407
5. Mkhize P, Mashilo J, Shimelis H: Progress on genetic improvement and analysis of bottle gourd [*Lagenaria siceraria* (Molina) Standl.] for agronomic traits, nutrient compositions, and stress tolerance: a review. Front Sustain Food Syst 2021, 5:683635. DOI: https://doi.org/10.3389/fsufs.2021.683635
6. Mehboob M, Naureen I, Saleem A, Amanat A: Medicinal and nutritional importance of *Lagenaria siceraria* (Lauki). Saudi J Biomed Res 2022, 7(2):67-73. DOI: https://doi.org/10.36348/sjbr.2022.v07i02.001
7. Saeed MG, Ali SA, Ali R, Sayeed SA, Mobin L: Exploring the potential of bottle gourd (*Lagenaria siceraria*) flour as a fat mimetic in biscuits with improved physicochemical and nutritional characteristics and anti-diabetic properties. Ital J Food Sci 2022, 34(2):50–66.

DOI: https://doi.org/10.15586/ijfs.v34i2.1954

8. Association of Official Analytical Chemists (AOAC): Official methods of analysis. 14th eds. Edited by Williams S. AOAC, Virginia; 1984:844-846.
9. Jenssen A: Chlorophyll and Carotenoids. In: Handbook of Phycological Methods. Physiological & Biochemical Methods (Ed. By Hellebust JA & Craigie JS). Cam-bridge University Press, Cambridge, UK; 1978:59–70.
10. Ragazzi E, Veronese G: Quantitative analysis of phenolic compounds after thin layer chromatographic separation. J Chromatogr 1973, 77(2):369–375. DOI: https://doi.org/10.1016/s0021-9673(00)92204-0
11. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ: Protein measurement with the Folin phenol reagent. J Biol Chem 1951, 193(1):265-275. DOI: https://doi.org/10.1016/S0021-9258(19)52451-6
12. Thomas G, Ludwig Hyman JVG: The anthrone method for the determination of carbohydrates in foods and in oral ringing. J Dent Res 1956, 35(1):90-94. DOI: https://doi.org/10.1177/00220345560350012301
13. Yen GC, Duh PD: Scavenging effect of methanolic extracts of peanut hulls on free radical and active oxygen. J Agric Food Chem 1994, 42(3):629-632. DOI: https://doi.org/10.1021/jf00039a005
14. Nishikimi M, Appaji N, Yagi K: The occurrence of superoxide anion in the reaction of reduced phenazine methosulphate and molecular oxygen. Biochem Biophys Res Commun 1972, 46(2):849-854. DOI: https://doi.org/10.1016/s0006-291x(72)80218-3
15. Apati P, Szentmihalyi K, Kristo ST, Papp I, Vinkler P, Szoke E. et al: Herbal remedies of soli-dago-correlation of phytochemical characteristics and antioxidative properties. J Pharm Biomed Anal 2003, 32(4-5):1045-1053. DOI: https://doi.org/10.1016/S0731-7085(03)00207-3
16. Ohkawa M, Ohisi N, Yagi K: Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979, 95(2):351-358. DOI: https://doi.org/10.1016/0003-2697(79)90738-3
17. Tsuda T, Watanabe M, Ohshima K, Yamamoto A, Kawakishi S, Osawa T: Antioxidative components isolated from the seed of tamarind (*Tamarindus indica* L.). J Agric Food Chem 1994, 42(12):2671-2674. DOI: https://doi.org/10.1021/jf00048a004
18. Lee JC, Kim HR, Kim J, Yang YS: Antioxidant Property of an ethanol extract of optunia ficus-indica saboten. J Agric Food Chem 2002, 50(22):6490–6496. DOI: https://doi.org/10.1021/jf020388c
19. Seglen PO: Preparation of isolated rat liver cells. Methods Cell Biol 1976, 13:29-83. DOI: https://doi.org/10.1016/s0091-679x(08)61797-5
20. Mosmann T: Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 1983, 65(1-2):55-63. DOI: https://doi.org/10.1016/0022-1759(83)90303-4
21. Kakkar P, Das B, Viswanath PN: A modified spectrophotometer assay of superoxide dismutase. Indian J Biochem Biophys 1984, 21:130-132. DOI: http://nopr.niscpr.res.in/handle/123456789/19932
22. Wallin B, Rosengren B, Shertzer HG, Camejo G: Lipoprotein oxidation and measurement of thiobarbituric acid reacting substances formation in a single microtiter plate: its use for evaluation of antioxidants. Anal Biochem 1993, 208(1):10-15. DOI: https://doi.org/10.1006/abio.1993.1002
23. Feelisch M, Noack E: Nitric oxide (NO) formation from nitrovasodilators occurs independently of hemoglobin or non-heme iron. Eur J Pharmacol 1987, 142(3):465-469. DOI: https://doi.org/10.1016/0014-2999(87)90090-2
24. Singh P, Kakkar, P, Singh RL: Antioxidant, DNA damage protective and hepatoprotective activities of *Amor-phophyllus campanulatus*. Int J Pharm Pharm Sci 2016, 8(3):330-338. https://innovareacademics.in/journals/index.php/ijpps/article/view/10150
25. Yu M, Gouvinhas I, Rocha J, Barros AIRNA: Phytochemical and antioxidant analysis of medicinal and food plants towards bioactive food and pharmaceutical resources. Sci Rep 2021, 11:10041. DOI: https://doi.org/10.1038/s41598-021-89437-4
26. Antona M.E., González P.M., RamosC., CabreraJ., Olano C., MoralesC., Zago V. et al. Curcumin exerts a protective effect against obesity and liver injury induced by an atherogenic diet. *Functional Foods in Health and Disease* 2021; 11(12): 673-689. DOI: https://www.doi.org/10.31989/ffhd.v11i12.862
27. Singh P, Vishwakarma SP, Singh U, Shukla M, Singh R, Singh RK et al: Quantification and evaluation of antioxidant activity of some bioactive phytochemicals in different medicinal plants. Open Nutraceutical J 2012, 5:179-186. DOI: https://doi.org/10.2174/1876396001205010179
28. Shali K. S., Soumya N. P., Mondal S., Mini S. Hepatoprotective effect of morin via regulating the oxidative stress and carbohydrate metabolism in STZ

induced diabetic rats. *Bioactive Compounds in Health and Disease* 2022; 5(3): 53-66.
DOI: https://www.doi.org/10.31989/bchd.v5i3.893

29. Teibo J. O., Ayinde K.S., Olaoba O.T., Adelusi T.I., Teibo T. K. A., Bamikunle M.V., Jimoh Y. A., et al. Functional foods' bioactive components and their chemoprevention mechanism in cervical, breast, and liver cancers: A systematic review. *Functional Foods in Health and Disease* 2021;11(11): 559-585.
DOI: https://www.doi.org/10.31989/ffhd.v11i11.818
30. Krishnamurthy P, Wadhwani A: Antioxidant enzymes and human health. In: El-Missiry MA, Ed. Antioxidant Enzyme. Intech Open, London; 2012.
31. Nabavi SM, Ebrahimzadeh MA, Nabavi SF, Fazelian M, Eslami B: In vitro antioxidant and free radical scavenging activity of *diospyros lotus* and *pyrus boissieriana* growing in Iran. Phcog Mag 2009, 5(18):122-126. DOI: http://www.phcog.com/text.asp?2009/5/18/122/57969
32. Kurutas EB: The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. Nutr J 2016, 15(1):71.
DOI: https://doi.org/10.1186/s12937-016-0186-5
33. Kalpana V.S., Mary J., Mini S., Soumya N.P., Mondal S. Hibiscus rosa sinensis L. anthocyanins prevent lipid peroxidation and improve antioxidant status in the liver of streptozotocin-induced diabetic rats. *Bioactive Compounds in Health and Disease* 2021; 4(10): 240-255. DOI: https://www.doi.org/10.31989/bchd.v4i10.842
34. Jaeschke H, Ramachandran A: Oxidant stress and lipid peroxidation in acetaminophen hepatotoxicity. React Oxyg Species 2018, 5(15):145-158.
DOI: https://doi.org/10.1089/ars.2021.0102
35. Tripathi M, Singh BK, Kakkar P: Glycyrrhizic acid modulates t-BHP induced apoptosis in primary rat hepatocytes. Food Chem Toxicol 2009, 47(2):339-347.
DOI: https://doi.org/10.1016/j.fct.2008.11.028
36. Lee MW: Acetaminophen (APAP) hepatotoxicity-isn't it time for APAP to go away? J Hepatol 2017; 67(6):1324-1331. DOI: https://doi.org/10.1016/j.jhep.2017.07.005
37. Ghasemi M, Turnbull T, Sebastian S, Kempson I: The MTT assay: Utility, limitations, pitfalls, and interpretation in bulk and single-cell analysis. Int J Mol Sci 2021, 22(23): 12827. DOI: https://doi.org/10.3390/ijms222312827
38. Wang Y, Branicky R, Noe A, Hekimi S: Superoxide dismutases: Dual roles in controlling ros damage and regulating ros signaling. J Cell Biol 2018, 217(6):1915-1928. DOI: https://doi.org/10.1083/jcb.201708007
39. Iriti M., Varoni E.M., Vitalini S. Phytochemical diversity behind health-promoting effects of traditional Mediterranean foods. *Functional Food Science* 2021; 1(11): 67-81. DOI: https://www.doi.org/10.31989/ffs.v1i11.849
40. Martirosyan D., von Brugger J., Bialow S. Functional food science: Differences and similarities with food science. *Functional Foods in Health and Disease* 2021. 11(9): 408-430.
DOI: https://www.doi.org/10.31989/ffhd.v11i9.831
41. Martirosyan D., Kanya H., Nadalet C. Can functional foods reduce the risk of disease? Advancement of functional food definition and steps to create functional food products. *Functional Foods in Health and Disease* 2021; 11(5): 213-221. DOI: https://www.doi.org/10.31989/ffhd.v11i5.788
42. Adany, A. Kanya, H. Martirosyan, D. Japan's health food industry: An analysis of the efficacy of the FOSHU system. *Bioactive Compounds in Health and Disease* 2021; 4(4):63-78. DOI: https://www.doi.org/10.31989/bchd.v4i4.795
43. Singh P., Singh R., Pathak N., Singh K. P., Tripathi M., Mondal S. Phytochemistry and Nutraceutical Properties of Carica papaya (Linn.): A Review. *Dietary Supplements and Nutraceuticals* 2022; 1(9):1-15. DOI: https://www.doi.org/10.31989/dsn.v1i9.991
44. Soumya N.P., Mini S., Sivan S., Mondal S. Bioactive compounds in functional foods and their role as therapeutics. *Bioactive Compounds in Health and Disease* 2021. 4(3); 24-39.
DOI: https://www.doi.org/10.31989/bchd.v4i3.786