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ORIGINAL ARTICLE

Identification of Phytoconstituents of *Memecylon sisparense* Gamble Leaf and Evaluation against Cisplatin-induced Oxidative Renal Damage in Mice

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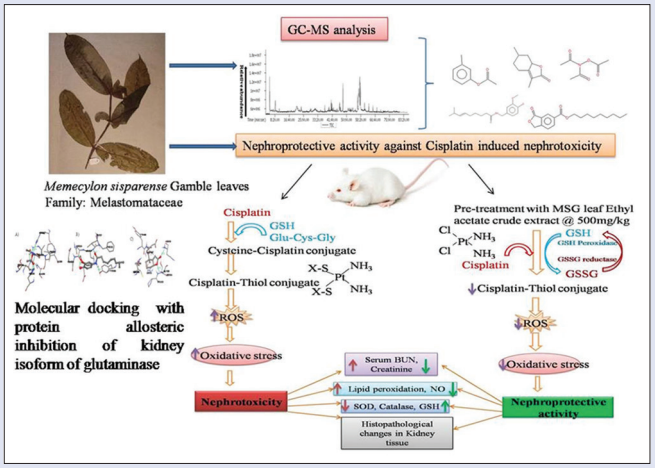
ABSTRACT

Background: *Memecylon sisparense* Gamble (MSG) belongs to *Melastomataceae* family, having a wide range of pharmacological activities such as antioxidant, hepatoprotective, and anti-inflammatory. **Objective:** The present study aimed for the first time toward the identification of biologically active compounds in MSG leaf ethyl acetate extract (MSGLEAE) by gas chromatography–mass spectrometry (GC-MS) analysis and compared with docking studies along with its nephroprotective activity against cisplatin (CP)-induced nephrotoxicity in mice. **Materials and Methods:** MSGLEAE was subjected to GC-MS analysis and molecular docking studies. Swiss albino male mice were treated with MSGLEAE (250, 500 mg/kg, PO) against CP 12 mg/kg IP evaluated for nephroprotective activity. The changes in renal tissue were assessed from serum biochemical renal toxicity, antioxidant stress markers along with histopathological studies. **Results:** Out of 41 compounds identified, 20 were found having biological activities such as nephroprotective, hepatoprotective, anticancer, antioxidant, and antimicrobial and inhibition of uric acid production. The nephroprotective active compounds (N,N,O-triacetylhydroxylamine, 2(4H)-benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, N-{(4-hydroxy-3-methoxyphenyl) methyl}-8-methyl-6-nonenamide) had shown binding energy of −5.27, −5.98, −5.27 (ΔG(Kcal/mol)), respectively, in docking studies. MSGLEAE showed a significant protective effect against CP-induced nephrotoxicity because of the identified compounds by reducing the oxidative stress in renal tissue evident by histopathological studies. **Conclusion:** This is the first ever report in terms of identification of bioactive constituents in MSGLEAE. Pretreatment has a significant therapeutic benefit during CP therapy by inhibiting oxidative stress, enhancing nephroprotective activity.

Key words: Cisplatin, docking, gas chromatography–mass spectrometry, *Memecylon*, nephrotoxicity

SUMMARY

- N,N,O-Triacetylhydroxylamine, 2(4H)-benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, N-{(4-hydroxy-3-methoxyphenyl)methyl}-8-methyl-6-nonenamide showed binding energy of −5.27, −5.98, −5.27 (ΔG (Kcal/mol)), respectively, in molecular docking studies
- Cisplatin (CP) elevated the renal biomarkers such as blood urea nitrogen, creatinine, kidney/body weight ratio, which was significantly reduced with *Memecylon sisparense* Gamble leaf ethyl acetate extract (MSGLEAE) pretreatment
- MSGLEAE increased the levels of renal glutathione, superoxide dismutase, and catalase by fostering the detoxification of free radicals and lipid peroxides to maintain the membrane integrity by inhibiting oxidative stress caused by CP, thereby enhancing the nephroprotective activity according to histological observations.



Abbreviations used: MSGLEAE: *Memecylon sisparense* Gamble leaf ethyl acetate extract; CP: Cisplatin; BUN: Blood urea nitrogen; SOD: Superoxide dismutase; CAT: Catalase; GSH: Glutathione; NO: Nitric oxide; MDA: Malondialdehyde; IAEC: Institutional Animal Ethics Committee; TCA: Trichloroacetic acid; DTNB: 5,5[1]-dithiobis-2-nitrobenzoic acid; FC: Folin–Ciocalteu reagent; SNP: Sodium nitroprusside; H and E: Hematoxylin and eosin.

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INTRODUCTION

Most of the developing countries are depending on the traditional medicine for their health care, due to the occurrence of phytochemicals, which exhibit the best sources of antioxidant, antimicrobial, and anticancer activities toward the cure of many diseases. *Memecylon* is a genus of shrubs or small trees distributed throughout the world. Up to now, 300 species were identified, out of which 30 species have been

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reported from India.[1,2] *Memecylon* species are widely used as herbal medicine with wide pharmacological activities in the cure of herpes and skin ailments along with antiviral, antimicrobial, anti-inflammatory, hepatoprotective, antidiabetic, and antioxidant activities,[3] which need to be explored scientifically. One such species is *Memecylon sisparense* Gamble (MSG) reported as a rare and endangered category in IUCN red list.[4] It belongs to *Melastomataceae* family,[5] endemic to the Western Ghats – Annamalai, South Nilgiri, and Tirupati. It needs evaluation of therapeutic activities and also needs for expanding the drugs from plant source.[6,7]

Cisplatin (CP) is a widely prescribed platinum-based antineoplastic drug.[8] It is a low molecular weight neutral compound, freely filtered in the glomerulus, penetrating the tubular cells by reaching higher concentration in the proximal tubular cells.[9] CP is cleared by the kidneys by tubular secretion and glomerular filtration.[10] CP forms platinum-reduced glutathione conjugate in the liver and then reaches the kidney where it undergoes cleavage by gamma-glutamyl transpeptidase enzyme into toxic thiol conjugate. The thiol conjugate reacts highly with macromolecules leading to free radical production, thereby causing renal cellular damage and cell death, thereby depleting antioxidant systems such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase.[9,11,12] The living cells continuously generate reactive oxygen species (ROS) whereas antioxidant defense system maintains them in proportionate in the normal physiology.[13] The increase in ROS generation apart from the range of antioxidant defense system in the cell leads to the oxidative stress in the cell, thereby causing renal tissue damage.[13] These antioxidants react with free radicals by acting as electron donors and form water-like harmless products, thereby preventing damage to the cells by protecting against the oxidative stress.[12] Due to renal insufficiency, there is an increase in the levels of serum creatinine and blood urea nitrogen (BUN).[8]

The natural extract having potential bioactive phenolic compounds with more antioxidant capacity can be obtained by doing extraction in solvents such as ethyl acetate.[14] Hence, we selected MSG leaf ethyl acetate extract (MSGLEAE) for the first time in the present study by focusing on preliminary phytochemical screening by biochemical tests, on identification of the phytochemical constituents by gas chromatography–mass spectrometry (GC-MS) analysis, and also on capability to prevent the oxidative stress caused by CP-induced nephropathy by exploiting the nephroprotective effect of MSGLEAE.

MATERIALS AND METHODS

Chemicals

CP, trichloroacetic acid (TCA), sodium nitroprusside, 5,5[1]-dithiobis-2-nitrobenzoic acid, Folin–Ciocalteu reagent, SOD kit, and C_7 to C_{40} n-alkane mixture solution were purchased from Sigma-Aldrich Co., USA. BUN and creatinine kits were purchased from Accurex Biomedical Pvt. Ltd., Mumbai.

Plant material

The MSG leaves were collected from the forest of Tirumala hills in Chittoor district, Andhra Pradesh, India, and authentication was made by Dr. K. Madhava Chetty, Department of Botany, Sri Venkateswara University, Tirupathi, against voucher specimen number 984 deposited at the herbarium of Sri Venkateswara University.

Sample preparation

The leaves are shade-dried, powdered, and sieved, and then, 150 g of fine leaf powder was extracted in a Soxhlet apparatus with 600 ml of ethyl acetate. The extract was concentrated to dryness by a Heidolph Rotary Evaporator (Model: Hei-VAP advantage ML/HB/G3) and freeze-dried using a Verdant Scientific Lyophilizer (Model: Sub-Zero).

Phytochemical screening

Preliminary phytochemical studies such as carbohydrates, amino acids, tannins, saponins, phenols, flavonoids, triterpenoids, steroids, fixed oils, glycosides, alkaloids, anthraquinones, gums, and mucilages were carried out on MSGLEAE to highlight the major groups of phytochemical constituents by following the standard methods.[15-18]

Gas chromatography–mass spectrometry analysis

GC-MS analysis was performed using an Agilent 7890A (Agilent Technologies, USA). Gas chromatography system equipped with a time-of-flight mass spectrometer (Pegasus HT TOF, LECO Corporation, USA). Separation of analytes was carried out by a capillary column (Agilent J&W HP-5MS, 5% phenyl-methylpolysiloxane 30 m × 0.25 mm, film thickness 0.25 μm). Ultra-high pure (99.999%) helium was the carrier gas at a constant flow rate of 1 mL/min in a splitless mode.

To calculate the retention indices (RIs), C_7 to C_{40} n-alkane mixture (1 μg/μL) was run before analysis of MSGLEAE. 1 μL of the MSGLEAE was manually injected into inlet of column at 250°C operating in a splitless mode. The initial oven temperature was retained at 60°C for 5 min, and then, the initial temperature was elevated at 280°C (3°C/min) and held for 5 min. The acquisition rate of mass spectrometer was 5 spectra per second and a solvent delay of 5 min was used. The transfer line and the ion source temperature were 280°C and 230°C, respectively. The detector voltage was 1700 V, and the electron energy was −70 eV. Mass spectra were collected from 50 to 1000 m/z. RIs of each compound were calculated according to Vandendool and Kratz.[19] For qualitative analysis, LECO Chromatof software version 3.24 (LECO Corpoation, USA) was used. The parameters, such as similarity, retention time, and RI values, were matched with that of peaks and subsequently identified through a library search using the NIST/EPA/NIH Mass Spectral Library 2011 (NIST11).

Molecular docking

Molecular docking studies were accomplished to explore the binding interactions between the N-{(4-Hydroxy-3-methoxyphenyl) methyl}-8-methyl-6-nonenamide, N,N,O-triacetylhydroxylamine, and 2(4H)-benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl compounds and estimating the binding affinity of the complex with the active site of allosteric inhibition of kidney isoform of glutaminase (PDB ID: 5JYO). AutoDock 4.2 uses binding free energy assessment to assign the best binding conformation.[20] Docking studies are commonly performed for predicting binding modes to proteins and their binding energies of ligands. X, Y, Z coordinates of PDB were selected using SPDBV.[21] Binding energy and ΔG bind value exist on the basis of hydrogen bond, hydrophobic, and van der Waal interactions.

In vivo experiments

Thirty-six Swiss albino male mice weighing 20–25 g were purchased from Sainath Agencies, Hyderabad, after Institutional Animal Ethics Committee approval with the protocol number NIP/02/2013/PC/44. The animals were housed in polycarbonate cages with food and water *ad libitum* and maintained under standard conditions in accordance with the CPCSEA guidelines.

Acute oral toxicity investigation

The acute oral toxicity study was performed following the OECD guidelines.[22] A dose of 5000 mg/kg of MSGLEAE was administered to

three mice followed by 14 days observation for mortality, repeating the treatment with three more animals. After 14 days, animals were sacrificed by CO_2 euthanasia and organ macro-morphological observations were carried out.

Experimental design for cisplatin-induced nephrotoxicity

The Swiss albino male mice were randomly divided into five groups, each one comprising six animals according to the following experimental design:

- Group-I, VC – Vehicle control group, 2% sodium carboxymethyl cellulose PO for 9 days
- Group-II, HD – MSGLEAE receiving a daily dose of 500 mg/kg PO for 9 days
- Group-III, CP – Served as disease control group receiving CP 12 mg/kg single IP on day 7
- Group-IV, LD + CP – MSGLEAE receiving a daily dose of 250 mg/kg PO for 9 days with CP 12 mg/kg single IP on day 7
- Group-V, HD + CP – MSGLEAE receiving a daily dose of 500 mg/kg PO for 9 days with CP 12 mg/kg single IP on day 7.

Body weight and organ weight

The body weight of all the animals was recorded bi-weekly during the experimentation, and percent increase in body weight was estimated based on initial weight. Animals were sacrificed and liver, kidney, and spleen were excised. The organs washed with cold saline were dry blotted and weighted. The relative kidney weight of each animal was determined.

Serum analyses for the assessment of the renal function

After 72 h of CP administration, blood was collected in nonheparinized tubes from retro-orbital plexus using isoflurane anesthesia and then centrifuged at 1500 × *g* for 10 min. Then, serum was collected toward biochemical parameters estimation of BUN and creatinine levels.

Preparation of kidney homogenate and estimation of oxidative stress markers

One kidney of each animal was used for histopathological study and the other one was homogenized in ice-cold phosphate-buffered saline, pH 7.4 to obtain 1:9 (w/v) whole homogenate. Kidney homogenate was centrifuged at 17,000 × *g* for 60 min at 4°C (Make: Thermo Scientific, Model: Heraeus Multifuge X3R) and supernatant was used for the CAT, SOD, and nitric oxide (NO) estimation. 200 µL of kidney tissue homogenate was taken and mixed with the same volume of 10% TCA and centrifuged at 2900 × *g* for 10 min (Make: Thermo Scientific, Model: Heraeus Multifuge X3R). Supernatant was collected and used for the estimation of glutathione (GSH) according to Ellman method[23] and malondialdehyde (MDA) according to Ohkawa and Yagi method,[24] both with slight modifications. The protein concentration in the supernatant was assessed using crystalline bovine serum albumin as reference standard.[25] CAT was measured according to Aebi method with slight modification,[26] SOD activity (cytosolic and mitochondrial) was determined using a SOD assay kit following the specifications of manufacturer, and the NO was determined according to the Garratt method with slight modification.[27]

Statistical analysis

The variation between various groups and inter-group was measured by one-way analysis of variance (ANOVA) followed by Bonferroni multiple comparison test, compared VC with CP-treated groups, CP-treated group with MSGLEAE 250 mg/kg LD + CP, and CP-treated group with MSGLEAE 500 mg/kg HD + CP, respectively. Results with $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively, were considered statistically significant.

Histopathological examination

One kidney of each animal was perfused in 10% neutral formalin solution and embedded in paraffin block. Four-micrometer thickness sections using a rotary microtome were obtained and stained by hematoxylin and eosin (H and E). Histological changes by light microscopy using a Nikon Inverted Trinocular Microscope (Model: Ti-U) were evaluated.

RESULTS

Phytochemical screening

The preliminary studies of MSGLEAE revealed the presence of phenols, flavonoids, steroids, triterpenoids, alkaloids, tannins, saponins, glycosides, carbohydrates, amino acids, and fixed oils [Table 1].

Gas chromatography–mass spectrometry analysis

In GC-MS analysis, 41 compounds were identified using NIST11 Library based on retention time, RI, molecular formula, and molecular weight, listed in Table 2 and corresponding GC-chromatagram is in Figure 1. Area percentages were determined from the peak areas. The phytochemicals identified in GC-MS corresponds to alkaloids (1H-imidazole, 1-acetyl-), alkalonamine family (diglycolamine), phenols (m-cresyl acetate, phenol, 2,4-bis(1,1-dimethylethyl)-), triterpenes (2(4H)-benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-), fatty acids (pentadecanoic acid, heptadecane). Among the 41 compounds, 20 compounds are found to have various biological activities which were reported from Dr. Duke's Phytochemical and Ethnobotanical Databases-USDA[28] and are mentioned in Table 3.

Molecular docking studies against nephroprotective activity

Experimental activities and predicted values by Lamarckian genetic algorithm dockings of the N-{(4-Hydroxy-3-methoxyphenyl) methyl}-8-methyl-6-nonenamide, N,N,O-triacetylhydroxylamine, and 2(4H)-benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl- having nephroprotective activity are summarized in Table 4. The compounds selected for molecular docking have some collective structural features. All the lead compounds showed good binding energy and exhibited interactions with lower free energy

Table 1: Phytochemical constituents detected in *Memecylon sisparense* Gamble leaf ethyl acetate extract

Phytochemical constituent	Result
Carbohydrates	+
Glycosides	+
Amino acids	+
Tannins	+
Saponins	+
Phenols	+
Flavonoids	+
Triterpenoids	+
Steroids	+
Fixed oils	+
Gum and mucilages	-
Anthraquinones	-
Alkaloids	+

+: Positive result; -: Negative result

Table 2: List of phytochemical constituents identified by gas chromatography-mass spectrometry in *Memecylon sisparense* Gamble leaf ethyl acetate extract*

RT (min)	Name	Empirical formula	Exact mass	RI_{Exp}	RI_{Lib}	Area percentage
05:11	3,3-Dimethyl-4-methylamino-butan-2-one	$C_7H_{15}NO$	129.1154	968	967	2.28
05:14	Methyl glyoxal	$C_3H_4O_2$	72.0211	972	970	2.59
05:17	Diglycolamine	$C_4H_{11}NO_2$	105.079	974	980	4.17
08:34	1H-Imidazole, 1-acetyl-	$C_5H_6N_2O$	110.048	1069	1054	3.76
09:32	1-Amino-2,6-dimethylpiperidine	$C_7H_{16}N_2$	128.1313	1095	1098	0.01
09:39	N,N,O-Triacetylhydroxylamine	$C_6H_9NO_4$	159.0532	1099	1122	0.24
10:50	m-Cresyl acetate	C_7H_8O	108.0575	1126	1136	0.97
13:48	Phenol, 2,4-dimethyl-, acetate	$C_{10}H_{12}O_2$	164.0837	1194	1217	0.11
17:39	1H-Pyrrole-2,5-dione, 3-ethyl-4-methyl-	$C_7H_9NO_2$	139.0633	1281	1265	0.36
17:57	Benzene, 1,3-bis (1,1-dimethylethyl)-	$C_{14}H_{22}$	190.1722	1287	1249	0.11
19:01	Phenol, 4-ethyl-2-methoxy-	$C_9H_{12}O_2$	152.0837	1312	1303	0.02
29:01	Cyclohexane carboxylic acid, 3-fluorophenyl ester	$C_{13}H_{15}FO_2$	222.1056	1554	1596	0.13
29:05	Phenol, 2,4-bis (1,1-dimethylethyl)-	$C_{14}H_{22}O$	206.1671	1557	1539	0.51
29:14	2(4H)-benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	$C_{11}H_{16}O_2$	180.115	1560	1532	1.69
29:16	Naphthalene, 1,6,7-trimethyl-	$C_{13}H_{14}$	170.1096	1561	1552	0.22
32:44	Pentadecane, 2,6,10-trimethyl-	$C_{18}H_{38}$	254.2974	1653	1654	0.45
34:45	Azulene, 1,4-dimethyl-7-(1-methylethyl)-	$C_{15}H_{18}$	198.1409	1709	1734	0.49
35:58	Chamazulene	$C_{14}H_{16}$	184.1252	1743	1728	0.18
36:24	Heptadecane, 2,3-dimethyl-	$C_{19}H_{40}$	268.313	1756	1782	0.06
37:43	3-Octadecene, (E)-	$C_{18}H_{36}$	252.2817	1794	1795	1.43
38:55	Pentadecanoic acid	$C_{15}H_{30}O_2$	242.2246	1829	1823	0.20
39:39	9-Nonadecene	$C_{19}H_{38}$	266.2974	1851	1880	1.53
41:05	7-Octadecyne, 2-methyl-	$C_{19}H_{36}$	264.2817	1895	1863	2.17
41:14	2-Heptadecanone	$C_{17}H_{34}O$	198.1984	1899	1878	1.31
41:18	9-Nonadecene	$C_{19}H_{38}$	266.2974	1901	1880	0.52
41:19	1,2-Dioctylcyclopropene	$C_{19}H_{36}$	264.2817	1902	1913	1.61
42:04.	1H-Indene, 5-decyloctahydro-	$C_{19}H_{36}$	264.2817	1925	1937	1.42
44:00	Hexadecanoic acid, 15-methyl-, methyl ester	$C_{18}H_{36}O_2$	284.2715	1986	1975.6	0.42
44:39	Butyric acid, 4-pentadecyl ester	$C_{19}H_{38}O_2$	298.2872	2007	2013	0.91
45:58	10-Undecenoic acid, octyl ester	$C_{19}H_{36}O_2$	296.2715	2050	2067	1.23
46:26	Eicosane, 10-methyl-	$C_{21}H_{44}$	296.3443	2065	2041.6	1.81
48:44	Oxalic acid, allyl tridecyl ester	$C_{18}H_{32}O_4$	312.2301	2143	2135	0.13
52:11	5-Eicosene, (E)-	$C_{20}H_{40}$	280.313	2265	2285	0.36
52:23	Docosane, 2,21-dimethyl-	$C_{24}H_{50}$	338.3913	2271	2279	1.48
55:47	5-Isobenzofurancarboxylic acid, 1,3-dihydro-3-oxo-, nonyl ester	$C_{18}H_{24}O_4$	304.1675	2397	2447	10.71
57:56	Heptadecane, 9-octyl-	$C_{25}H_{52}$	352.4069	2481	2442	13.87
58:48	3-Hexadecylaminopyridine	$C_{21}H_{38}N_2$	318.3035	2515	2477	0.24
59:21	N-{(4-Hydroxy-3-methoxyphenyl) methyl}-8-methyl-6-nonenamide	$C_{18}H_{27}NO_3$	305.1991	2537	2541	2.10
60:29	Docosane, 11-butyl-	$C_{26}H_{54}$	366.4226	2582	2542	1.33
72:05	Hentriacontane	$C_{31}H_{64}$	436.5008	3096	3100	0.32
74:06	dl-α-Tocopherol	$C_{29}H_{50}O_2$	430.3811	3194	3149	0.16

RI_{Exp}: Retention index of experimental value; RI_{Lib}: Retention index as per NIST11 Library; RT: Retention time

values, indicating more thermodynamically favored interaction. N-{(4-Hydroxy-3-methoxyphenyl) methyl}-8-methyl-6-nonenamide exhibited −5.98 Kcal/mol with interacting Leu323, Thr394, and Asn324. The compounds N,N,O-triacetylhydroxylamine and 2(4H)-benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl show the same binding energy of −5.27 Kcal/mol interacting with His330, Asn335, Arg387 and Gly315, Asn319, respectively, and are represented in Figure 2.

Effect of *Memecylon sisparense* Gamble leaf ethyl acetate extract on acute oral toxicity

No mortality was observed in Swiss albino mice treated with 5000 mg/kg PO. The animal body weight was found increased. On the 14th day, animals were sacrificed by CO_2 euthanasia and there was no change in gross organ observation for morphological changes. After initial screening of higher doses of extract for toxicity at 5000 mg/kg body weight, hence, one-tenth (500 mg/kg) and one-twentieth (250 mg/kg) were selected for the evaluation of nephroprotective activities.

Effect of *Memecylon sisparense* Gamble leaf ethyl acetate extract on change in body weight and kidneys weight

CP enhanced the renal toxicity markers such as loss in body weight and proportionate kidney weight. Upon 9 days continuous treatment with MSGLEAE (250 mg/kg, 500 mg/kg), body weights and relative kidney weights were found improved in comparison to CP group as shown in Figure 3. There is no change in weight of liver and spleen in CP-treated animals compared to VC group.

Effect of *Memecylon sisparense* Gamble leaf ethyl acetate extract on serum biochemical parameters

CP-treated mice showed significant levels ($P < 0.001$) of BUN and creatinine levels in serum compared to VC group animals. Pretreatment with MSGLEAE 250 mg/kg for 9 consecutive days along with CP induction showed significant decrease ($P < 0.05$) for serum creatinine

Table 3: Biological activity of phytochemical constituents identified by gas chromatography-mass spectrometry in *Memecylon sisparense* Gamble leaf ethyl acetate extract

Name of the compound	Activity*
Methylglyoxal	Increase glyoxalate transamination, catechol-O-methyl-transferase inhibitor, methyl donor, methyl-Guanidine inhibitor
1H-Imidazole, 1-acetyl-	Acetyl-choline antagonist, 5-HT inhibitor, antidote, hallucinogenic, inhibit acetyl coenzyme A, hepatoprotective
1-Amino-2,6-dimethylpiperidine	Increase aromatic amino acid decarboxylase activity
N,N,O-Triacetylhydroxylamine	Inhibit production of tumor necrosis factor, NO synthase inhibitor, antitumor (nasopharynx), nephroprotective, neuromuscular blocker, NF-kB inhibitor, NO scavenger, nociceptive, inhibit production of uric acid, oxidant
m-Cresyl acetate	Anticancer (mammary), antidote, inhibit microtubule formation, MAPK inhibitor, mast cell stabilizer, microphagocytogenic, mitogen, regulate calcium metabolism
1H-Pyrrole-2,5-dione, 3-ethyl-4-methyl-	Methyl-guanidine inhibitor, anti-5-HT, antidote, hallucinogenic, hepatoprotective, increase T-helper
Cyclohexane carboxylic acid, 3-fluorophenyl ester	Arachidonic acid inhibitor, inhibit production of uric acid
2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	Histamine inhibitor, HIV-RT inhibitor, hyperglycemic, increase T-helper, ACE inhibitor, analgesic, antiarthritic, antacid, antibiotic, anticancer, anticoagulant, antidiuretic, antimicrobial, antioxidant, GABA antagonist
Pentadecanoic acid	Arachidonic acid inhibitor, inhibit production of uric acid
7-Octadecyne, 2-methyl-	Methyl-guanidine inhibitor, methyl donor
1H-Indene, 5-decyl octahydro-	Anti-5-HT, hepatoprotective, HIV-RT inhibitor, increase T-helper
Hexadecanoic acid, 15-methyl-, methyl ester	Methyl-guanidine inhibitor, methyl donor, arachidonic acid inhibitor, inhibit production of uric acid
Butyric acid, 4-pentadecyl ester	Arachidonic acid inhibitor, inhibit production of uric acid
10-Undecenoic acid, octyl ester	Arachidonic acid inhibitor, inhibit production of uric acid
Eicosane, 10-methyl-	Methyl-guanidine inhibitor, methyl donor
Oxalic acid, allyl tridecyl ester	Arachidonic acid inhibitor, inhibit production of uric acid
5-Eicosene, (E)-	Anticancer (esophagus), decrease epinephrine production, decrease oxalate excretion, ER-beta binder
N-{(4-Hydroxy-3-methoxyphenyl) methyl}-8-methyl-6-nonenamide	Antitumor, nephroprotective, NO scavenger, tumor necrosis factor inhibitor
5-Isobenzofuran carboxylic acid, 1,3-dihydro-3-oxo-, nonyl ester	Arachidonic acid inhibitor, inhibit production of uric acid
dl-α-Tocopherol	Tocopherol synergist

*Reported from Dr. Duke's Phytochemical and Ethnobotanical Databases-USDA. 5-HT: 5-hydroxytryptamine; NF-kB: Nuclear factor-kB; MAPK: Mitogen-activated protein kinase; ACE: Angiotensin-converting enzyme; GABA: γ-aminobutyric acid; ER: Estrogen receptor; RT: Reverse transcriptase; NO: Nitric oxide

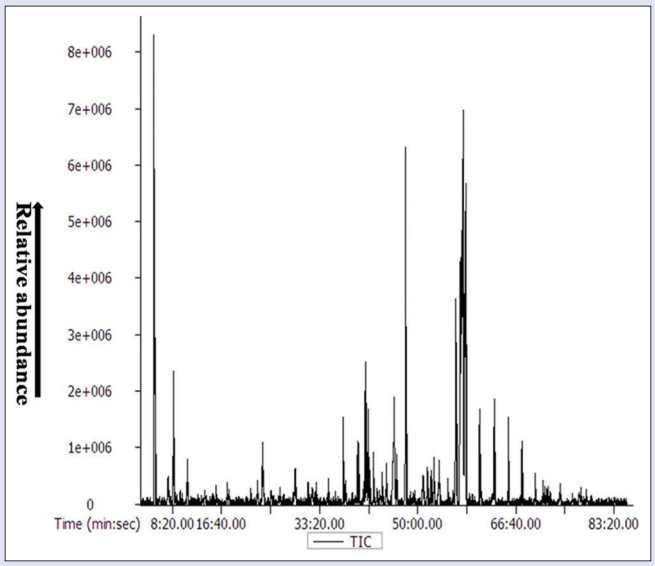

Figure 1: Gas chromatography–mass spectrometry chromatogram of *Memecylon sisparense* Gamble leaf ethyl acetate extract

when compared to the CP group, whereas MSGLEAE 500 mg/kg for 9 consecutive days along with CP induction showed significant decrease ($P < 0.001$) for serum creatinine when compared to the CP group. Pretreatment with MSGLEAE 250 mg/kg and 500 mg/kg for 9 consecutive days along with CP induction showed significant decrease ($P < 0.001$) for serum BUN when compared to the CP group. The serum biochemical parameters in CP-induced mice are shown in Figure 3.

Effect on *Memecylon sisparense* Gamble leaf ethyl acetate extract on oxidative stress markers of kidney tissue

The MSGLEAE 500 mg/kg treatment for 9 days showed significant change ($P < 0.001$) in the levels of GSH, SOD, and CAT in mice kidney tissue as shown in Figure 4. Compared to VC group, CP-treated mice showed lower levels of GSH, SOD, and CAT ($P < 0.01$, $P < 0.001$, $P < 0.001$, respectively). The elevated levels of MDA indicate the lipid peroxidation in the CP group compared with the VC group ($P < 0.001$) and MSGLEAE of 250 mg/kg and 500 mg/kg significantly decreased the MDA levels in the kidney tissue ($P < 0.01$, $P < 0.001$, respectively) as shown in Figure 3. The CP-intoxicated mice showed higher levels of NO as compared with VC group mice ($P < 0.001$), whereas the pretreatment with MSGLEAE 250 mg/kg, 500 mg/kg significantly decreased the levels of NO ($P < 0.05$, $P < 0.001$, respectively) in kidney tissue as shown in Figure 4.

Effect on *Memecylon sisparense* Gamble leaf ethyl acetate extract on histopathological findings

The microscopic examination of kidney sections after H and E staining in VC group mice showed the normal histology with no change in

Table 4: Interacting amino acids, binding energy and dissociation constant of compounds with protein allosteric inhibition of kidney isoform of glutaminase

Name of the compound	Interacting amino acids	Binding Energy, ΔG (Kcal/mol)	Dissociation constant (KI) (μM)
N,N,O-Triacetylhydroxylamine	His330, Asn335, Arg387	−5.27	138.10
N-{(4-Hydroxy-3-methoxyphenyl) methyl}-8-methyl-6-nonenamide	Leu323, Thr394, Asn324	−5.98	41.05
2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	Gly315, Asn319	−5.27	136.49

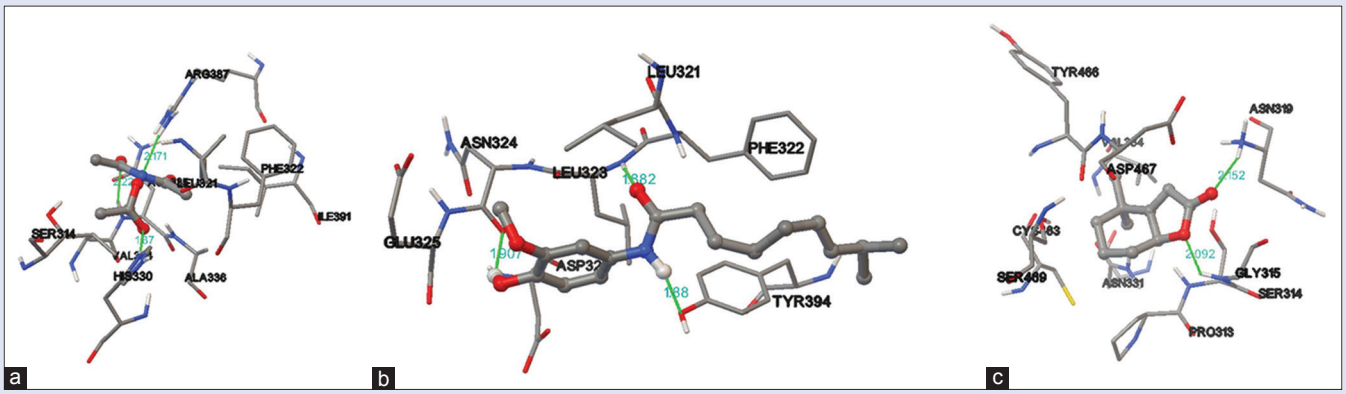

Figure 2: Interacting amino acids of compounds (a) N,N,O-Triacetylhydroxylamine, (b) N-{(4-Hydroxy-3-methoxyphenyl) methyl}-8-methyl-6-nonenamide, (c) 2(4H)-benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl with protein allosteric inhibition of kidney isoform of glutaminase

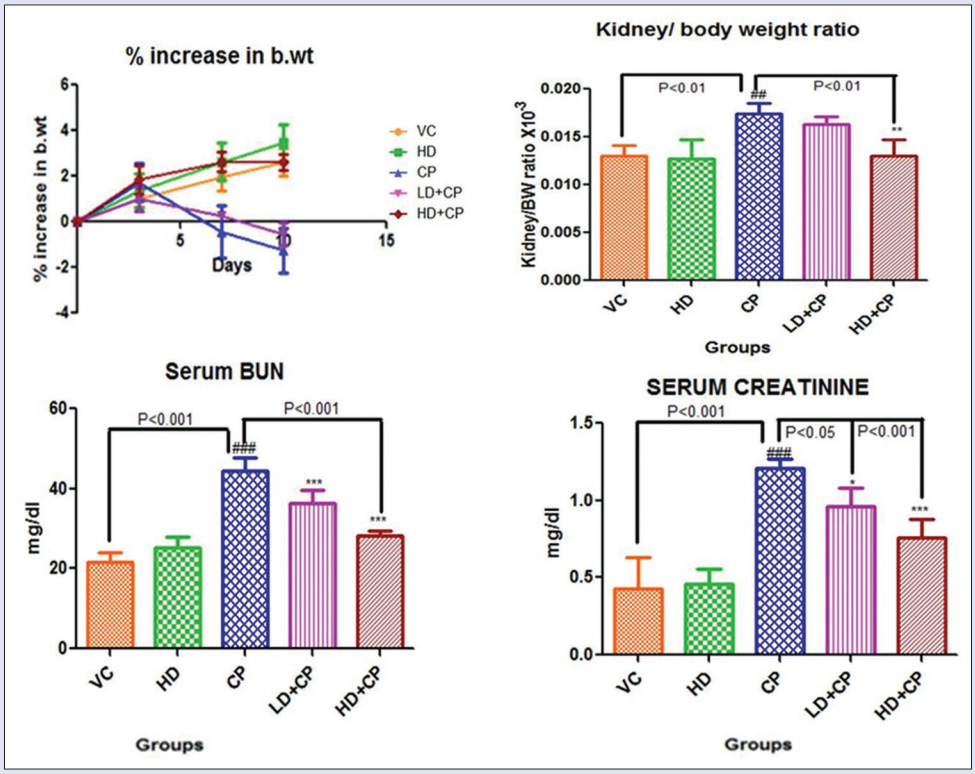

Figure 3: Percentage increase in body weight, kidney/body weight ratio, and serum biochemical parameters. VC: Vehicle control group; HD: *Memecylon sisparense* Gamble leaf ethyl acetate extract at 500 mg/kg; CP: Cisplatin at 12 mg/kg; LD + CP: *Memecylon sisparense* Gamble leaf ethyl acetate extract at 250 mg/kg with cisplatin; HD + CP: *Memecylon sisparense* Gamble leaf ethyl acetate extract at 500 mg/kg with cisplatin

glomerulus and tubules whereas in CP-intoxicated group mice showed degeneration of tubules. The microscopic examination of kidney sections of MSGLEAE pretreated 250 mg/kg (LD + CP) showed glomeruli and degeneration of tubules whereas mice MSGLEAE pretreated 500 mg/kg (HD + CP) showed glomeruli with tubules, thereby showing good protective effect. The microscopic examination of kidney sections

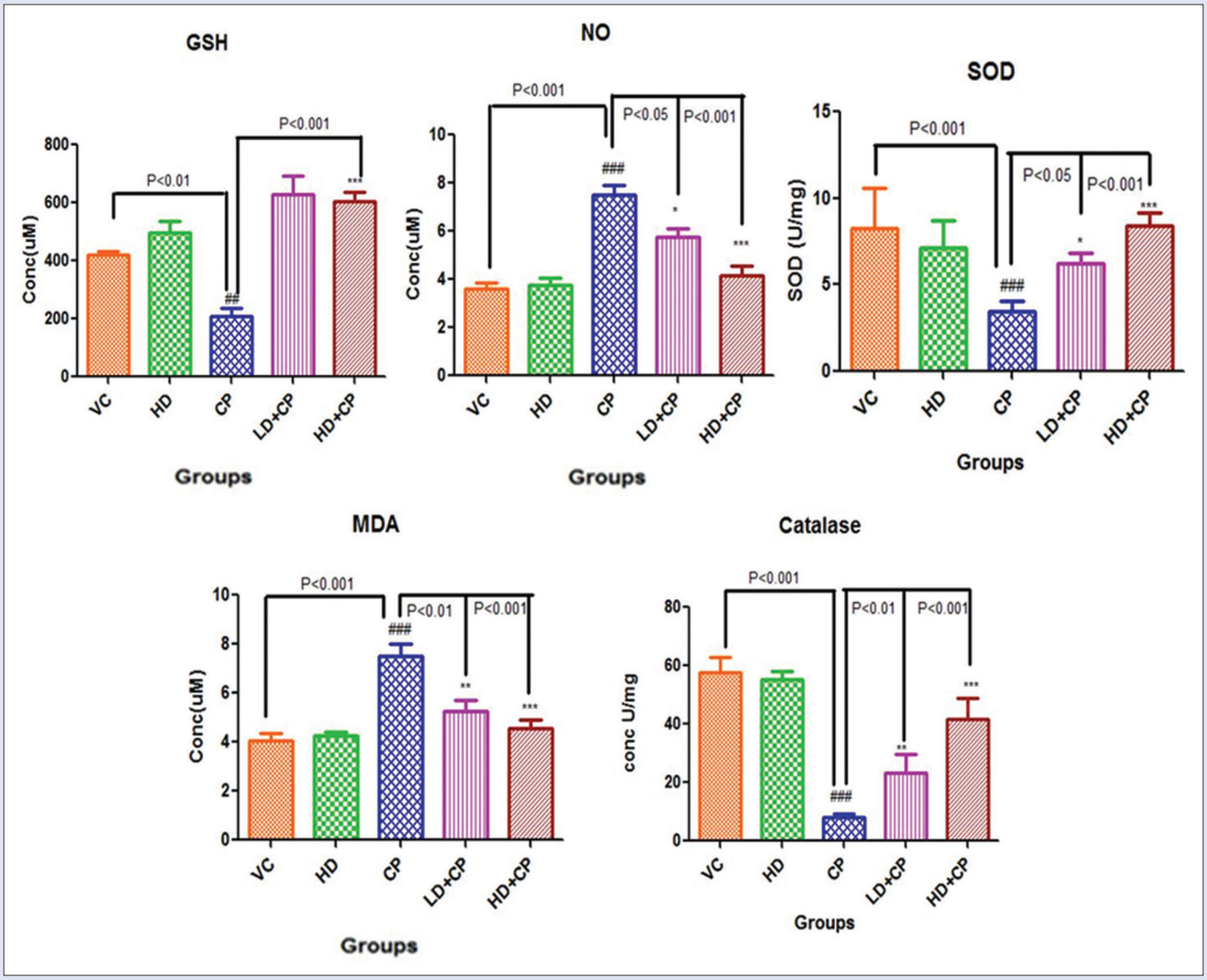

Figure 4: Assessment of antioxidant stress parameters in kidney tissue. VC: Vehicle control group; HD: *Memecylon sisparense* Gamble leaf ethyl acetate extract at 500 mg/kg; CP: Cisplatin at 12 mg/kg; LD + CP: *Memecylon sisparense* Gamble leaf ethyl acetate extract at 250 mg/kg with cisplatin; HD + CP: *Memecylon sisparense* Gamble leaf ethyl acetate extract at 500 mg/kg with cisplatin

of MSGLEAE 500 mg/kg treated showed glomerulus and tubules as shown in Figure 5.

DISCUSSION

Kidneys play an important role in the elimination of various endogenous and exogenous substances such as drugs by accumulating large amounts in the proximal tubules.[29] There are several studies which demonstrated the acute and chronic kidney injuries, renal failures caused by CP treatment.[30] Hence, CP-induced nephrotoxicity became a well-reputable experimental model of drug-induced renal toxicity. In the present study, we studied the effect of MSGLEAE on CP-intoxicated mice by observing the activity of the kidney function which can be examined by serum analysis and focusing on renal antioxidant enzymes such as SOD, CAT, GSH, NO, and lipid peroxidation.

Body weight is considered one of the important parameters in toxicity studies. In our present study, the percentage body weight decrease was observed in CP-treated group compared to VC control group and significantly increased with MSGLEAE-pretreated groups. This may be because of reduction in food ingestion with a concomitant loss of animal appetite, loss of salts, proteins, water resulted with subsequent weight loss in body weight of CP-treated animals.[30] Kidney to bodyweight ratio is increased in CP-intoxicated animals which significantly decreased in the pretreatment groups because of reduction of renal tissue function by renal tissue damage.[30] Our analysis showed that the presence of high levels of creatinine and BUN in the serum indicated the kidney injury caused by CP, showing a sign of nephrotoxicity,[8] whereas MSGLEAE pretreatment significantly decreased the levels of creatinine and BUN compared to CP-intoxicated group animals. After CP administration, the renal cells take up the formed platinum-sulfhydryl group for intracellular GSH depletion causing the renal toxicity through reactive metabolites upon rapid transformation, thereby increasing lipid peroxidation. The free radicals generated by the CP binds to the lipids present in the plasma membrane which then interacts with polyunsaturated fatty acids in the renal cells, thereby resulting in the lipid peroxidation.[29] The results showed that MSGLEAE pretreatment improved the levels of antioxidant enzymes and also showed as a suppressor against the renal-induced dysfunction by a decrease in lipid peroxidation compared to the CP-intoxicated group. The concentration of MDA was increased

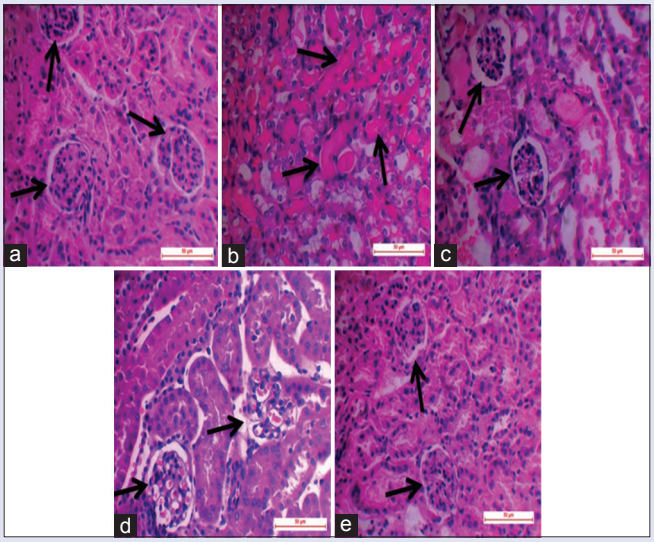

Figure 5: Photomicrographs of kidney sections stained with hematoxylin and eosin. (a) VC: Vehicle control group showing normal glomerulus and tubules; (b) CP: Cisplatin-treated group mice showing degeneration of tubules; (c) LD + CP: *Memecylon sisparense* Gamble leaf ethyl acetate extract at 250 mg/kg with cisplatin showing glomeruli and degeneration of tubules; (d) HD + CP: *Memecylon sisparense* Gamble leaf ethyl acetate extract at 500 mg/kg with cisplatin showing glomeruli and tubules; (e) HD: *Memecylon sisparense* Gamble leaf ethyl acetate extract at 500 mg/kg showing glomerulus and tubules

in CP-intoxicated group compared to the VC group, which significantly decreased with MSGLEAE pretreatment groups. MSGLEAE pretreatment showed the nephroprotective effect against the CP toxicity by directly relating to the antioxidant activity by decrease in lipid peroxidation.

In kidneys, due to CP administration, there was a loss of copper and zinc, essential for SOD enzyme activity responsible for scavenging of superoxide anion released in our normal metabolic process.[31] MSGLEAE pretreatment enhanced the SOD enzyme activity, thereby decreasing the superoxide anion, which is responsible for lipid peroxidation.

After CP administration, there was a decreased ability of the kidney toward the scavenging of toxic hydrogen peroxide and lipid peroxides, which results in the decrease of CAT activity.[31] MSGLEAE pretreatment elevated the CAT enzyme activity by comparing with CP-toxicated group. It was significantly decreased in CP-toxicated group by comparing to the VC group.

In CP-induced nephrotoxicity, NO also plays an important role which is a precursor in L-arginine metabolism.[32] NO reacts with the superoxide anion forming peroxynitrite anion, which rapidly dissociates to hydroxyl radical under normal physiological pH, which is responsible for the tissue damage.[33] MSGLEAE pretreatment significantly decreased the NO, thereby decreasing the superoxide anion.

The antioxidant markers such as SOD, CAT, and GSH were decreased in the CP-intoxicated group compared to the VC group whereas MSGLEAE pretreatment enhanced the CP-induced renal dysfunction by an increase in SOD, CAT, and GSH compared to the CP-intoxicated group, thereby showing the protective mechanism. However, only MSGLEAE-treated group maintained the antioxidant MDA, NO, CAT, GSH, and SOD levels near to the VC group.

The GC-MS analysis of *M. sisparense* leaf ethyl acetate extract revealed the presence of phytochemicals such as hexadecanoic acid, 15-methyl-, methyl ester, and phenol, 2,4-bis(1,1-dimethylethyl), which were also identified in *Memecylon umbellatum* leaf petroleum ether extract,[34] whereas 2(4H)-benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl were identified in chloroform extract of *M. umbellatum* leaves.[34]

The bioactive compounds N,N,O-triacetylhydroxylamine, N-{(4-Hydroxy-3-methoxyphenyl)methyl}-8-methyl-6-nonenamide are commonly known as capsaicin, an active ingredient in hot peppers,[35] whereas 2(4H)-benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl compound was also identified in *Alpinia purpurata* (Vieill) n-hexane leaf extract.[36] These three compounds identified in GC-MS analysis had also shown a good binding energy against allosteric inhibition of kidney isoform of glutaminase in molecular docking studies, thereby showing renal protective activity. Molecular docking studies also supported our present study hypothesis by correlating the results toward the protective activity of MSGLEAE against CP-induced nephrotoxicity in mice.

Furthermore, results showed that NO and lipid peroxidation were significantly increased in CP-intoxicated group by comparing to the VC group, which supports our hypothesis that nephrotoxicity is caused by the depletion of renal antioxidant defense mechanism. The MSGLEAE 500 mg/kg body weight PO pretreatment before administration of CP restored the antioxidant defense system in the renal tissue, thereby showing the protective mechanism.

CONCLUSION

The present investigation revealed that MSGLEAE possess bioactive phytoconstituents, which are responsible for its nephroprotective activity and oral administration of the extract at 500 mg/kg has produced a significant protective effect against CP-induced nephrotoxicity, thereby attenuating oxidative stress and lipid peroxidation. Therefore, MSG leaf may find a role in preventing the CP-induced complications of nephrotoxicity in cancer chemotherapy.

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Conflicts of interest

There are no conflicts of interest.

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