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Protective effect of flavonoid and saponin-rich fractions against renal toxicity induced by mercuric chloride in rodent model

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ABSTRACT

The present study was carried out to evaluate the ameliorating potential of the flavonoid-rich fraction of *Phyllanthus emblica* (FRFPE) and saponin-rich fraction of *Tribulus terrestris* (SRFTT) against mercuric chloride ($HgCl_2$)-induced renal toxicity in rats. Forty-two male SD rats were divided into seven different groups, namely normal control (C1), toxicity control (C2), vehicle control (C3), standard control (C4), Flavonoid rich fraction of *Phyllanthus emblica* (FRFPE; T1), Saponin rich fraction of *Tribulus terrestris* (SRFTT; T2) and FRFPE + SRFTT (T3). Serum biochemical markers and oxidative stress indicators were measured. Histopathological examination of kidney sections was also carried out. Our data revealed that BUN and creatinine levels in rats' serum were significantly higher, whereas serum total protein, albumin, and globulin levels were significantly lower in the toxicity group. $HgCl_2$ administration reduced the activity of superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) and an elevated MDA in kidney tissue when compared with the control. The treatment with FRFPE and SRFTT markedly attenuated $HgCl_2$-induced oxidative stress in kidney. Further, oxidative stress-related alteration in biochemical markers was confirmed by histopathological changes in rats of different treatment groups. According to histopathology of the kidney, the treatment of FRFPE and SRFTT considerably reduced the damage produced by $HgCl_2$ in rats. LC-QTOF-MS analysis of FRFPE and SRFTT showed the presence of tannins, triterpenoids, alkaloids, gallic acid, steroid derivatives, quinoline derivatives and flavonoids. According to the findings, the flavonoid-rich fraction of *P. emblica* and the saponin-rich fraction of *T. terrestris* showed an antioxidant activity and protected the rat kidney from mercury-induced oxidative damage.

Keywords: Mercuric chloride, *Phyllanthus emblica,* Rats, *Tribulus terrestris*

Mercuric chloride ($HgCl_2$) is one of the most dangerous metals having no biological purpose according to the Agency for Toxic Substance and Disease Registry (ATSDR) (Emsley 2001). It is one of the most common environmental pollutants which causes serious changes in both human and animal body systems (Sener *et al.* 2003). Mercuric chloride is widely distributed throughout the body, although it tends to accumulate in the kidneys, resulting in acute renal failure. $HgCl_2$ can change the structure and activity of proteins by covalently attaching to sulfhydryl groups, causing an increase in reactive oxygen species (ROS). Increased ROS causes antioxidant enzymes to be depleted, resulting in cell membrane damage and cell death (Pillai and Gupta 2005).

Tribulus terrestris fruit is well-known in Indian traditional medicine and has antioxidant properties (Kamboj *et al.* 2011). Terrestribisamide, tribulusterine, terrestriamide, hecogenin, aurantiamide acetate, xanthosine, fatty acid ester, ferulic acid, vanillin, and hydroxybenzoic acid identified from *T. terrestris* fruits scavenge ROS, DPPH radicals, and hydrogen peroxide (H_2O_2) (Lakshmi *et al.* 2012). *T. terrestris* has also been proven to prevent lipid peroxidation and oxidative stress in rats (Kamboj *et al.* 2011). *Phyllanthus emblica* L. generally known as Amla or Indian Gooseberry, grows in India's tropical and subtropical areas. *P. emblica* fruit contains high quantity of acorbic acid, gallic acid, phenolic chemicals, and tannin components such as emblicanin A and B, which have strong antioxidant activity *in vitro* (Mandal and Reddy 2017).

The research suggests that a variety of crude herbal extracts are a rich source of potentially beneficial novel chemicals to treat kidney issues (Liwa *et al.* 2016). It may have additive, potentiative, or antagonistic effects. We chose two plants that are said to play a role in kidney protection as per mythology. We hypothesised that the flavonoid-rich fraction of *P. emblica* (FRFPE) and the saponin-rich fraction of *T. terrestris* (SRFTT) have good phytochemicals, but this study alone with a combination of extract plants has not been evaluated for having an antioxidant-mediated protective role against nephrotoxicity. Therefore, both

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plants were selected to prepare FRFPE and SRFTT, which were then tested for their effect on the biochemical profile with other antioxidant parameters and histopathological changes in the mercuric chloride-induced renal toxicity rodent model.

MATERIALS AND METHODS

Chemicals and reagents: Mercuric chloride was purchased from Himedia, Mumbai. Vitamin E was procured from Merck, India. Chemicals like KH_2PO_4 and $Na_2HPO_4.2H_2O$ were purchased from S.D. Fine Chemicals, Mumbai. Other analytical grade chemicals were acquired from Sigma Aldrich, including RBC lysis buffer, pyrogallol, dTNB, acetylthiocholine iodide, and Bradford reagent. Merck Ltd., Mumbai, provided EDTA and H_2O_2. All the substances used in the experiment were analytical grade.

Experimental animals and environment: Total 42 male albino rats (8-10 weeks old, weighing 230-250 g) were obtained from Cadila Healthcare Ltd., Ahmedabad, Gujarat, and housed at the Institute's Laboratory Animal House Facility. Four animals were placed in each polycarbonate cage, which was kept at a constant temperature of (25±2°C) and humidity (50–55%) with a 12-hour light/dark cycle. All the animals had unlimited access to food and water. Prior to the trial, the animals were given a week of acclimatisation. The Institutional Animal Ethics Committee (IAEC) of the College of Veterinary Science and Animal Husbandry, Junagadh, approved the experimental protocol (No.: JAU/JVC/IAEC/SA/30/2018), which included the number of animals and procedures (Gujarat, India).

Plant material collection and authentication: The fruits of *Tribulus terrestris* and *Phyllanthus emblica* were collected from area of Porbandar district and campus of Junagadh Agricultural University, Junagadh of Gujarat state (India). Authentication of plant materials was done by Prof. R. C. Viradiya, Department of Biology, Bhakta Kavi Narsinh Mehta University, Junagadh, India. The voucher specimens have been kept at the Herbarium, College of Veterinary Science and Animal Husbandry, Junagadh, Gujarat.

Extraction and fraction procedure: To remove dust and pollutants, the fruits were carefully washed in running water. The material was chopped into pieces after drying in the shade. The powdered plant material was then extracted three times using the maceration procedure with a hydro-alcoholic solvent. It was left to stand at room temperature for 7 days, with occasional vigorous shaking. The extracts were then heated for 1.5 hours at 45°C (Su *et al.* 2009). The extract was evaporated until it was completely dry, and the residue was dissolved in a methanol:water solution (5:95). The saponin-rich fraction from *T. terrestris* was extracted using n-hexane, chloroform, ethyl acetate, and n-butanol in that order (SRFTT). For flavonoid rich fraction from *P. emblica*, the extract solution was shaken with n-hexane, chloroform, and ethyl acetate (50 ml each time) (FRFPE). The extraction was carried out until the organic phase turned colourless. These fractions were concentrated in a vacuum and then reconstituted in water prior to use.

Determination of total phenolic content: The total phenolic content (TPC) was determined using the Folin–Ciocalteu method with some modification, employing gallic acid as standard (Encarnacao *et al.* 2016). The total phenolic content of the samples was expressed as gallic acid equivalents (GAE), which reflected the phenolic content as amount of gallic acid in sample. Experiments were performed in triplicate.

Determination of total flavonoid content: A modified colorimetric method was used to assess the total flavonoids content (Encarnacao *et al.* 2016). The content of total flavonoids was measured in milligrammes of quercetin.

Determination of DPPH scavenging activity: The radical scavenging ability of individual plant extract was evaluated by using 1, 1- diphenyl – 2 – picrylhydrazyl hydrate (DPHP) assay (Brand-Williams *et al.* 1995).

LC-QTOF-MS/MS analysis of the SRFTT and FRFPE: The metabolite profiles of FRFPE and SRFTT were achieved by the non-targeted LC-QToF-MS method with the Agilent 6540 UHD accurate-mass quadrupole time-of-flight (Q-TOF) was applied in a negative mode. The MassHunter Workstation Software (Agilent Technologies, Santa Clara, CA, USA) was used for the extraction and identification of compounds. METLIN Compound Database with an instrument having an accurate mass was used as the reference.

Experimental design: At the commencement of the study, the weight variation of animals did not exceed ± 10% of average weight of each group. The animals were divided into seven groups (6 rats in each group) as follows: C1 - Control group received RO water by oral gavage for 28 days; C2 - Toxicity group received a single dose of mercuric chloride @ 2 mg/kg, orally, for 28 days; C3 - Vehicle group received corn oil at dose of 100 mg/kg, followed by $HgCl_2$ administration orally; C4 - Standard group received vitamin E at dose of 100 mg/kg, followed by $HgCl_2$ administration orally; T1- FRFPE group received flavonoid rich fraction of *P. emblica* extract at dose of 100 mg/kg, followed by $HgCl_2$ administration orally for 28 days; T2 - SRFTT group received saponin rich fraction of *T. terrestris* extract at dose of 100 mg/kg, followed by $HgCl_2$ administration orally for 28 days; T3 - FRFPE + SRFTT group received combination of *P. emblica* and *T. terrestris* extract at dose of 100 mg/kg, followed by $HgCl_2$ administration orally for 28 days.

Blood sampling and tissue collection: Blood samples were withdrawn from retro-orbital venous plexus on 28 days of experiments using a glass capillary collector. One portion of each blood sample was collected in test tubes without anticoagulant and allowed to clot by standing at room temperature for 15 min. The serum was separated by centrifugation (Eppendorf 5430 R, Germany) at 3000 × g for 15 min at 4°C, and then stored at −20°C for further determination of biochemical analysis. After the completion of the experiment, all rats were euthanized by CO_2 asphyxiation. The autopsy was carried out in the

confined disinfected laboratory. The major organs of rats were collected, trimmed off and weighed. Detailed gross pathological lesions from all rats were observed and recorded by systemic approach. The tissues were fixed with 10% formalin solution for histopathological examinations.

Weight of body and kidney weight: At end of the experiment, the body weight was measured which was used to evaluate the change in the body weight from the initial body weight. The kidneys were excised, weighed using analytical balance and the result was expressed as change in kidney weight among the experimental groups.

Preparation of tissue homogenates: At the end of experiment, the kidney tissue samples were isolated immediately and kept at –80°C in ice-cold (2 ml) phosphate buffer saline (0.1M, *p*H 7.4) until analysis. Using a teflon homogenizer, 25% (w/v) of tissue homogenate was prepared in ice cold phosphate buffer saline and centrifuged at 10,000 × g for 10 min at 4°C; the supernatant was then taken and stored at –80°C until needed. Each sample's supernatant was utilised to determine antioxidant markers, except for SOD, which was centrifuged at 12000 × g for 40 min in Tris-EDTA buffer (8.5 *p*H). The bradford assay was used to determine the total protein content in the tissue homogenate using bovine serum albumin as a reference.

Evaluation of biochemical parameters: The biochemical parameters such as blood urea nitrogen, creatinine, total protein, and albumin were determined using a semiautomatic biochemistry analyser (Diatek Healthcare Pvt. Ltd., India) with standard kits. By subtracting the value of albumin from the total protein level, the globulin concentration was obtained.

Evaluation of oxidative stress parameters: The activity of superoxide dismutase (SOD) was determined using the method of Marklund and Marklund (1974) and expressed as U/mg tissue, whereas the activity of catalase (CAT) was determined using the method of Aebi *et al.* (1974). The catalase activity was measured in units per mg of protein (one unit of catalase equals 1 mole of H_2O_2 decomposition per min). The reduced glutathione (GSH) level was calculated using Ellman's (1959) standard method and expressed as g/mg tissue. Following the manufacturer's protocol, the lipid peroxidation was evaluated using a standard kit (Sigma). The malondialdehyde (MDA) level was expressed as µM/mg tissue.

Histopathologic examinations: The isolated organs were transferred into 10% buffered formalin. The formalin-fixed tissues were subjected to paraffin wax embedding for tissue sectioning. A semi-automated rotary microtome (Leica Biosystems, Germany) was used to cut 5-micron thick slices, which were then stained with hematoxylin and eosin (H & E) (Luna 1968). Pathological lesions were noted when the stained slides were examined under a microscope.

Statistical analysis: All the numerical data are expressed as the mean±standard error of mean (SEM). Data were analyzed by one-way analysis of variance (ANOVA), followed by Duncan's multiple range tests (DMRT). Statistical significance was considered if $p<0.05$.

RESULTS AND DISCUSSION

Mercuric chloride has a high affinity for sulfhydryl groups (–SH), which increases the amount of reactive oxygen species in the cell (Rooney 2007). The primary location of cellular synthesis of reactive oxidants such as superoxide (O^{2-}) and hydrogen peroxide is the mitochondrial electron transport chain (H_2O_2). Mercury induces hydrogen peroxide to develop primarily in the ubiquinone-cytochrome b section of the mitochondrial respiratory chain, causing oxidative stress and reducing reactive species neutralisation capacity. It was observed that such processes occur *in vivo* after exposure to $HgCl_2$, and that enhanced H_2O_2 production may be accompanied by increased mitochondrial lipid peroxidation (Lund *et al.* 1993).

The FRFPE, SRFTT, and FRFPE+SRFTT may be fractionated into different amounts of total phenolic and total flavonoid content. Our results demonstrated a higher amount of phenolic (360.78±25.19 mg of gallic acid equivalents per gram) and total flavonoid content (118.00±4.12 mg of quercetin equivalents per gram) in FRFPE when compared to the SRFTT and FRFPE+SRFTT fractions.

Our results of an *in vitro* scavenging activity assay suggest that all extracts exhibited inhibition of DPPH free radicals. The reduction in DPPH radical was determined by the decrease in its absorbance at 517 nm by antioxidants. The radical scavenging activity of the FRFPE and SRFTT and their combination showed higher activity (41.63, 30.07 and 55.24%, respectively) at concentrations of 9 µg/mL. Per cent inhibition of DPPH by fractions of herbal plants was found to increase with increase in concentration. Natural active constituents such as flavonoids, gallic acid, and saponin have been detected in extracts. Hence, the probable mechanism of nephroprotection by FRFPE and SRFTT may be attributed to their antioxidant and free radical scavenging properties. It protects the radical scavenging activity of peroxide, hydroperoxide, or lipid peroxyl, thereby inhibiting oxidative stress and preventing disease (Mishra *et al.* 2010).

The metabolite profiles of FRFPE and SRFTT were assessed by applying non-targeted LC-QToF-MS using ESI in negative ionisation mode. Compound name, retention time (Rt), experimental mass (m/z), height of the peak and area covered by the individual peak of the identified compounds is given in Tables 1 and 2. The LC-QTOF-MS analysis of the FRFPE and SRFTT revealed the presence of various diverse primary and secondary metabolites. Some important phytochemicals were identified, which might be responsible for the observed anti-oxidant activity. Flavonoids are polyphenolics compounds that possess antioxidant properties due to their reducing and chelating capabilities. It can prevent injury caused by free radicals by its significant free radical scavenging activity (Robert *et al.* 2001). Steroids are of great interest to

Table 1. Metabolite profiling of FRFPE by LC-QToF-MS in negative ion mode

Class	Compound name (Cpd#)	Molecular formula	m/z value (mass)	Retention time (min)	Peak height	Peak area	METLIN ID
Tannins	1,2-Digalloyl-beta-D-glucopyranose	$C_{20}H_{20}O_{14}$	484.0849	2.261	11437	144141	93772
L-phenylalanine derivative	N-(1-Deoxy-1-fructosyl) phenylalanine	$C_{15}H_{21}NO_7$	327.1319	8.371	11818	106460	92654
Isoprenoids	Bacteriorubixanthinal	$C_{41}H_{56}O_3$	596.4241	8.519	7344	51071	41400
13-Desmethylspirolide C	13-Demethylspirolide C	$C4_2H_{61}NO_7$	691.4471	13.7	3189	21337	93063
Triterpenoids	Tangeraxanthin	$C_{34}H_{44}O_2$	484.335	13.873	13132	62404	93621
Alkaloids derivatives	Perlolyrine	$C_{16}H_{12}N_2O_2$	264.0899	9.107	4640	29395	67538
Flavouring and aroma agent	Anethole	$C_{10}H_{12}O$	148.0883	14.101	6954	28990	43903
Autoinducers	N-cis-tetradec-9Z-enoyl-L-Homoserine lactone	$C_{18}H_{31}NO_3$	309.2302	13.575	4316	23887	64725
Benzene derivatives	5-Methoxy-galloyl-1,4-galactarolactone	$C_{14}H_{14}O_{11}$	358.0534	2.075	20616	125594	92170
Tannins	Ellagic acid	$C_{14}H_6O_8$	302.0067	8.091	11805	131341	-
Gallic acid	Beta-Glucogallin	$C_{13}H_{16}O_{10}$	332.0745	2.008	14737	86983	93381
Benzene derivatives	3-O-Galloyl-1,4-galactarolactone	$C_{13}H_{12}O_{11}$	344.0386	2.081	39590	693103	92168

Cpd#, Compound numbers as matched with the library of the agilent mass hunter software of the modal QTOF/LCMS 6540.

Table 2. Metabolite profiling of SRFTT by LC-QToF-MS in negative ion mode

Class	Compound name (Cpd#)	Molecular formula	m/z value (mass)	Retention time (min)	Peak height	Peak area	METLIN ID
Flavonoids	Robinetin 3-rutinoside	$C_{27}H_{30}O_{16}$	610.1535	8.181	111222	953613	49992
Flavonoids	Luteolin 7-rhamnosyl(1->6) galactoside	$C_{27}H_{30}O_{15}$	594.158	8.9	46295	301335	49141
Flavonoids	6-C-Rhamnopyranosylrhamnetin 3-O-glucopyranoside	$C_{28}H_{32}O_{16}$	624.1686	9.076	65727	464446	49464
	Stigmatellin Y	$C_{29}H_{40}O_6$	484.282	14.101	220495	1766435	69325
Spermidine alkaloid	Lunarine	$C_{25}H_{31}N_3O_4$	437.2307	7.871	29419	240999	43760
Steroid derivatives	Melongoside O	$C_{45}H_{74}O_{19}$	918.481	10.531	76916	386050	86682
Steroid derivatives	(25S)-5beta-spirostan-3beta-yl beta-D-glucoside	$C_{33}H_{54}O_8$	578.3807	10.731	28784	154556	57759
Quinoline derivatives	2-Undecyl-4(1H)-quinolinone N-oxide	$C_{20}H_{28}NO_2$	314.2111	14.098	9621	59127	88891
Flavonoids	Kaempferol 3-lathyroside-7-rhamnoside	$C_{32}H_{38}O_{19}$	726.1991	8.271	13875	86934	50096
Flavonoids	Kaempferol 3-(2G-glucosylrutinoside)	$C_{33}H_{40}O_{20}$	756.2094	8.387	20333	138977	50169
Steroids and steroid derivatives	Tuberoside E	$C_{45}H_{74}O_{18}$	902.4851	10.931	24752	197529	89835
Steroids and steroid derivatives	Alliospiroside C	$C_{38}H_{60}O_{13}$	724.4031	10.681	9100	50583	87282
Steroids and steroid derivatives	(25S)-Spirostane-3b,5b,6a-triol 3-[4''-rhamnosylglucoside]	$C_{39}H_{64}O_{14}$	756.427	10.528	21432	197424	90770
Steroids and steroid derivatives	Torvoside C	$C_{39}H_{64}O_{13}$	740.4315	10.924	9109	80926	86383
	Scopoloside I	$C_{45}H_{72}O_{20}$	932.4583	8.797	6544	34804	71946
Imidazo pyrimidines	Xanthine	$C_5H_4N_4O_2$	152.0336	2.011	24880	157724	82
Fatty Acyls	Ethyl (S)-3-hydroxybutyrate glucoside	$C_{12}H_{22}O_8$	294.1313	2.144	12977	43482	87869

Cpd#, Compound numbers as matched with the library of the agilent mass hunter software of the modal QTOF/LCMS 6540.

the pharmaceutical industry because of their varied pharmacological properties and strong ability to permeate the lipid bilayer of cells (Julianada *et al.* 2018). Khanduja *et al.* (1999) reported that ellagic acid possesses antimutagenic, antioxidant and anti-inflammatory activity in bacterial and mammalian systems. β-glucogallin, a major component from the fruit of the gooseberry that are used in the development of novel therapies to treat diabetic complications such as cataract (Puppala *et al.* 2012). Gallic acid is a well-known natural antioxidant that is basically a secondary polyphenolic metabolite (Niloofar *et al.* 2019). In evidence of this, the polyphenolic compounds of FRFPE and SRFTT could contribute to nephron protection through their antioxidant activity. Yet, the precise mechanism is not known, but its protective effect can be linked with the presence of phytoconstituents.

Table 3. Effect of FRFPE and SRFTT on body weight and kidney weight in mercuric chloride-induced renal toxicity in rats

Treatment group	Body weight		Kidney weight (g)
	0 day (g)	28 day (g)	
NC	256.78±14.51^{a}	353.02±19.70^{a}	2.68±0.17^{a}
TC	244.41±7.70^{a}	340.68±15.56^{a}	2.89±0.17^{a}
VC	236.91±16.15^{a}	321.78±17.23^{a}	2.88±0.24^{a}
SC	251.43±10.57^{a}	337.13±7.80^{a}	3.00±0.08^{a}
FRFPE	246.25±11.44^{a}	333.80±12.11^{a}	2.83±0.13^{a}
SRFTT	238.53±13.13^{a}	336.88±10.12^{a}	2.93±0.17^{a}
FRFPE + SRFTT	244.85±9.58^{a}	337.20±14.85^{a}	2.75±0.34^{a}

Data are expressed as mean±SEM (n=6).Values with different superscript in a column differ significantly (p<0.05). NC, Normal control group; TC, Toxicity control group; VC, Vehicle control group; SC, Standard control group; FRFPE, $HgCl_2$ + flavonoid rich fraction of *P. emblica* group (100 mg/kg); SRFTT, $HgCl_2$ + saponin rich fraction of *T. terrestris* group (100 mg/kg); FRFPE + SRFTT, $HgCl_2$ + flavonoid rich fraction of *P. emblica* + saponin rich fraction of *T. terrestris* group (100 mg/kg).

The changes in body and kidney weight of the rats in different treatments are shown in Table 3. There was a non-significant difference in body weights in all experimental groups when compared with the mercuric chloride-treated group. The non-significant and progressive weight loss in mercuric chloride treated rats might be due to injury of renal tubules and the subsequent loss of the tubular cells' ability to reabsorb water, leading to dehydration and loss of body weight. Dieter *et al.* (1992) recorded a reduction in the body weights of rats after mercuric chloride treatment. Oral administration of doses of the FRFPE and SRFTT has a protective role against weight loss induced by mercuric chloride. It is reasonable to conclude that the antioxidant effect was responsible for the prevention of weight loss recorded in this study. The weight of the kidney was found to be non-significantly increased in rats treated with mercuric chloride, and the administration of FRFPE and SRFTT failed to decrease the normalised kidney weight induced by mercuric chloride. All treatment groups of rats had higher kidney weights than the normal control group. There are very few agents that inhibit acute renal failure in the mercuric chloride-induced model of nephrotoxicity. One of them is FRFPE and SRFTT, which showed to afford protection against $HgCl_2$-induced nephrotoxicity.

One of the traditional screening indices for function and renal structural integrity is serum creatinine concentration. The levels of creatinine and BUN in different treatment groups were significantly reduced (p<0.05) as compared to toxicity group and at par with standard treatment group (Table 4). The elevated serum creatinine level in the mercuric chloride treatment group in this study could be attributable to a lower glomerular filtration rate. In addition, Oriquat *et al.* (2012) reported higher creatinine levels as well as glomerular function and tubular damage in rats, supporting our findings. Renal failure and glomerular injury are indicated by elevated blood urea nitrogen levels (Wang *et al.* 2012). When compared to the control groups, the level of BUN in the mercuric-chloride-treated group was considerably higher. The results of this investigation clearly show that FRFPE, SRFTT, and their combination supplementation prevented the mercuric chloride-induced renal injury, as shown by normal BUN and creatinine levels. These findings are like those of a study that combined *E. officinalis* dried fruit extract with cisplatin (Purena *et al.* 2018). Sugunavaman *et al.* (2010) also found that giving *T. terrestris* at a dose of 50 mg/kg to mercury-treated mice lowered the high level of blood creatinine. Yokozawa *et al.* (2007) also found that a 10 mg/kg dose of ethyl acetate extract of *E. officinalis* lowered the increased level of BUN in aged rats.

In the current investigation, treatment groups had considerably higher total protein levels than the toxicity

Table 4. Effect of FRFPE and SRFTT on biochemical parameters following simultaneous administration of mercuric chloride in rats

Parameter	Treatment group						
	NC	TC	VC	SC	FRFPE	SRFTT	FRFPE+SRFTT
BUN (mg/dL)	23.44±0.54^{a}	28.18±0.80^{c}	27.20±0.43^{bc}	23.63±1.50^{ab}	25.66±1.35^{abc}	24.88±1.82^{abc}	23.69±0.72^{ab}
Creatinine (mg/dL)	0.35±0.05^{a}	0.53±0.03^{b}	0.46±0.03^{ab}	0.37±0.02^{a}	0.40±0.04^{a}	0.43±0.04^{ab}	0.37±0.04^{a}
Total protein (g/dL)	5.24±0.05^{b}	4.72±0.12^{a}	4.76±0.15^{a}	5.26±0.05^{b}	5.18±0.07^{b}	5.15±0.04^{b}	5.06±0.03^{b}
Albumin (g/dL)	2.96±0.04^{b}	2.78±0.05^{a}	2.79±0.03^{a}	2.89±0.03^{ab}	2.88±0.06^{ab}	2.81±0.06^{ab}	2.86±0.05^{ab}
Globulin (g/dL)	2.28±0.04^{c}	1.94±0.10^{a}	1.97±0.16^{ab}	2.38±0.04^{c}	2.30±0.06^{c}	2.34±0.05^{c}	2.20±0.04^{bc}

Data are expressed as the mean±SEM (n=6). Values with different superscript in a row differ significantly (p<0.05). NC, Normal control group; TC, Toxicity control group; VC, Vehicle control group; SC, Standard control group; FRFPE, $HgCl_2$ + flavonoid rich fraction of *P. emblica* group (100 mg/kg); SRFTT, $HgCl_2$ + saponin rich fraction of *T. terrestris* group (100 mg/kg); FRFPE + SRFTT, $HgCl_2$ + flavonoid rich fraction of *P. emblica* + saponin rich fraction of *T. terrestris* group (100 mg/kg).

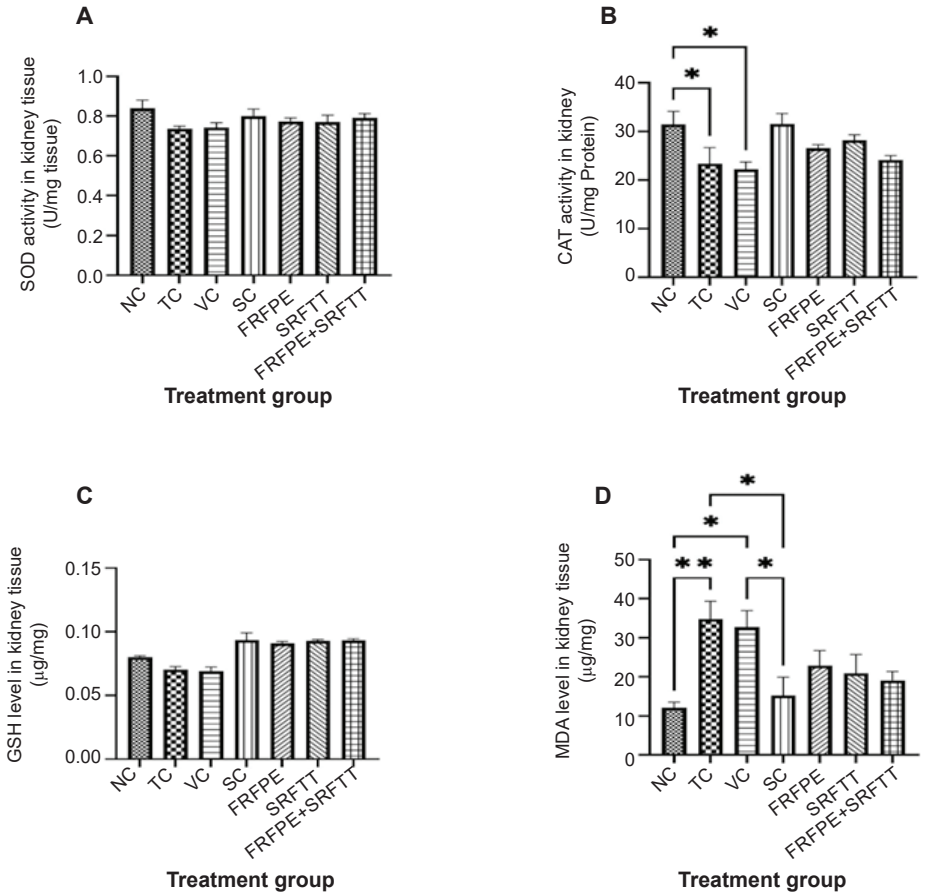

Fig. 1. Effects of FRFPE and SRFTT on oxidative stress markers in kidney tissue induced by mercuric chloride. **A**) SOD activity, **B**) CAT activity, **C**) GSH level, **D**) MDA level. Data were analyzed by one way ANOVA followed by DMRT. * indicates $p < .05$, ** indicates $p < .01$. NC, Normal control group; TC, Toxicity control group; VC, Vehicle control group; SC, Standard control group; FRFPE, $HgCl_2$ + flavonoid rich fraction of *P. emblica* group (100 mg/kg); SRFTT, $HgCl_2$ + saponin rich fraction of *T. terrestris* group (100 mg/kg); FRFPE + SRFTT, $HgCl_2$ + flavonoid rich fraction of *P. emblica* + saponin rich fraction of *T. terrestris* group (100 mg/kg).

group. However, albumin and globulin were significantly lower in toxicity group compared to the normal control group and the other treatment groups. The decrease in serum total proteins, albumin, and globulin due to $HgCl_2$ might be related to a decrease in protein synthesis by hepatic cells and an increase in excretion rate due to renal injury. Inorganic mercury has a strong affinity for thiol groups, interferring with the antioxidant system's activity and protein synthesis. The decrease in protein content could be attributed to mercuric chloride-induced loss of albumin producing cellular metrics. The findings were consistent with those of Samipillai *et al.* (2009), who found that mercury intoxication resulted in a substantial decrease in total protein in albino wistar rats. Agran oil and sodium selenite supplementation have been shown to reduce mercuric chloride-induced toxicity, which supports our findings (Youcef *et al.* 2013).

In the renal tissues of rats from various groups, SOD and CAT activity, as well as GSH and MDA levels, are shown in Fig. 1. Lipid peroxidation is triggered by ROS-induced hydrogen abstraction in cellular membranes, resulting in the creation of relatively stable molecules like MDA. Endogenous antioxidant systems such as TAC, SOD, and CAT enzymes, which are the first line of cellular defence, prevent the generation of free radicals (Kongkham *et al.* 2013). Increasing MDA and decreased GSH levels, as well as lower SOD and CAT activity in mercury-exposed renal tissue, imply that these enzymes are consumed as a result of increased oxidative stress. Mercuric chloride causes oxidative stress and lipid peroxides by increasing the generation of numerous reactive oxygen species (ROS) (Mendez-Armenta *et al.* 2011). Renal injury would be indicated by an increase in MDA levels in renal tissue caused by $HgCl_2$. The reduction in catalase activity and GSH levels in mercuric chloride-treated rats could be related to the creation of H_2O_2 and superoxide radicals, which produced acute renal damage. Due to an excess of ROS, mercury inhibited SOD activity by diminishing the –SH group reaction (Renugadevi and Prabu 2010). The treatment of rats with $HgCl_2$ resulted in considerable increases in MDA levels and CAT activity, as well as decreases in SOD activity and GSH levels, according to our findings.

As a mercury carrier and antioxidant, glutathione plays a critical function in protecting the body against mercury intoxication. Mercury binds to GSH, forming a compound

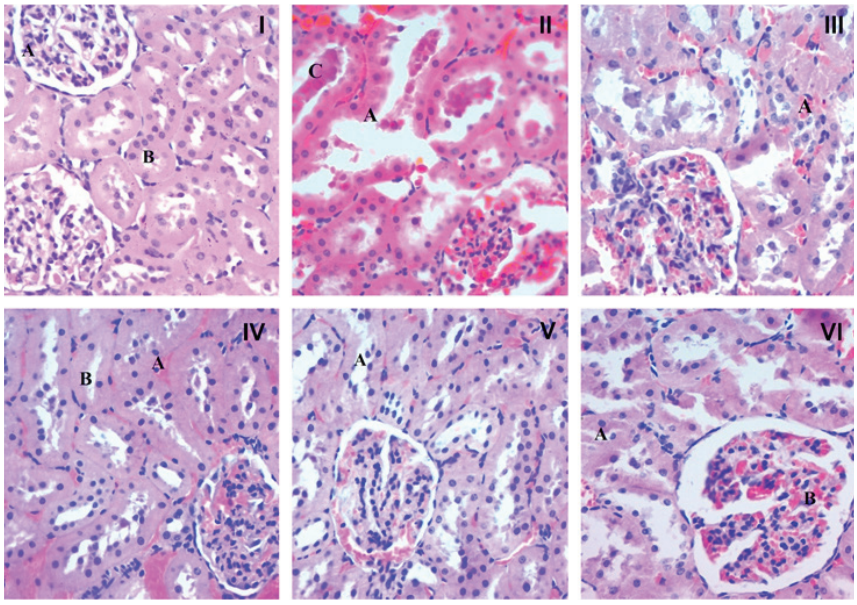

Fig. 2. Protective effect of FRFPE and SRFTT on histopathological changes in kidney induced by mercuric chloride. **I**) Normal control group – normal arrangement of histoarchitecutre of renal tubules (A) and distal and proximal convoluted tables (B); **II**) Toxic control group – congestion, degeneration of tubular epithelium and presence of necrotic debris in the lumen of tubules, and shrunken glomeruli; **III**) Standard control group – mild congestion (A), degeneration and necrosis of tubular epithelium (B); **IV**) FRFPE group – mild congestion (A), degeneration and necrosis of tubular epithelium (B); **V**) SRFTT group – almost normal architecture of tubules (A) and degenerative changes along with mild interstitial infiltration of inflammatory cells (B); **VI**) FRFPE + SRFTT group – mild tubular degeneration and necrosis (A) along with normal architecture of glomeruli (B) (400×, H and E).

that inhibits mercury from attaching to cellular proteins and causes harm to enzymes and tissue (Kromidas *et al.* 1990). The availability of non-critical nucleophile for inactivation of electrophiles is increased by metal GSH conjugation, which may play a key role in metallo protection. Hg ions accumulated in excess in renal tissue, disrupting GSH metabolism and damaging renal cells. The formation of glutathione-mercury complexes also diminishes the amount of intracellular glutathione that is eventually eliminated, lowering the cell's antioxidant capability. Glutathione levels in renal tissues were shown to be lower in our investigation due to $HgCl_2$ toxicity. Thus, it appears that the suppression of a range of pro-inflammatory mediators produced by leukocytes and macrophages is involved in the alleviation of $HgCl_2$-induced oxidative tissue damage observed by *P. emblica* and *T. terrestris* treatment. Previous research has suggested that the presence of secondary metabolites from *P. emblica* and *T. terrestris* may be responsible for the increased GSH levels (Kavitha and Jagadeesan 2006, Sugunavarman *et al.* 2010). In the current study, SRFTT and FRFPE treatment improve the GSH level as compared to that of $HgCl_2$-treated rats, indicating that SRFTT and FRFPE ameliorate oxidative stress via antioxidant components present in it like phycocyanin and β-carotene (Guan *et al.* 2009).

Increased SOD activity in the renal tissues of intoxicated rats treated with SRFTT and FRFPE was observed in this study, which is comparable to Rajkumar *et al.* (2011), who found a restoration of decreased SOD activity in lead-intoxicated rats treated with *E. emblica* extracts. *T. terrestris* extract increased the activity of SOD in the renal tissue of mercuric-intoxicated mice (Kavitha and jagadeesan 2006). The CAT protects cells from hydrogen peroxide produced within them and aids in the development of oxidative stress tolerance in the adaptive response of cells (Mates *et al.* 1999). The decrease in CAT enzyme activity in this study could be related to hydrogen peroxide produced by mercury chloride (Rao and Chhunchha 2010). When administered with *T. terrestris* fruit extract at a dose rate of 6 mg/kg b. wt. for 7 days, Kavitha and Jagadeesan (2006) showed similar effects in kidney tissue of mercuric chloride-intoxicated mice. The activity of catalase was improved to normal levels in the rats treated with SRFTT and FRFPE and their combination in the current investigation.

Lipid peroxidation causes an increase in MDA levels. Kavitha and Jagadeesan (2004) found that acute mercury exposure caused a substantial increase in reactive oxygen species (ROS) to accumulate in mouse liver tissue, leading to lipid peroxidation, protein breakdown, and cell death. We found a significant increase in MDA concentration due to mercury toxicity in the current investigation, which is consistent with earlier studies that found increased lipid peroxidation products (Jagadeesan and pillai 2007, Samipillai *et al.* 2009). Gallic acid, quercetin, and rutin (polyphenolic molecule with numerous hydroxyl groups) are found in the extract of *P. emblica* and can donate their proton to break the chain reaction of free radicals, acting as a lipid peroxidation inhibitor (Lu *et al.* 2006). As a result, antioxidant properties have been linked to reducing oxidative stress injury after extract treatment. *In vitro* and *in vivo* investigations showed that *P. emblica* has an antioxidant effect by increasing the activity of antioxidant

enzymes (SOD and CAT) and decreasing MDA levels (Sai Ram *et al.* 2002).The antioxidant activity of the SRFTT and FRFPE was shown to enhance antioxidant enzyme SOD, CAT activities, and GSH levels in renal tissues, as well as lower MDA levels.

Fig. 2 depicts histopathological alterations in the kidneys of rats from various treatment groups. In addition to oxidative stress, mercuric chloride exposure resulted in histological alterations in the kidney tissue. These changes could be connected to mitochondrial dysfunction caused by mercuric chloride, as well as the increased production of reactive oxygen species and impairment of antioxidant enzymes such as glutathione peroxidase and glutathione transferase. As a result of lipid peroxidation, oxygen radicals attack the cell membrane, causing destabilisation and disintegration of the cell membrane of renal tubules and glomeruli (Stajn *et al.* 1997). In the vitamin E plus mercuric chloride-treated group, these histological alterations were minor. The link between tissue damage and elevated levels of key oxidative enzymes has been thoroughly established (Dhu *et al.* 2004). This backs up our reports on mercury-induced histological changes in the kidney. *P. emblica* and *T. terrestris* were shown to inhibit mercury from binding to thiol groups in this investigation. Furthermore, thiol content, which is a component of structural proteins and nonprotein molecules, is vital in cellular functions including enzyme activity and the restoration of cellular pathways and mechanisms in cell detoxification (Ziegler 1985).The administration of FRFPE and SRFTT decreased lipid peroxidation and boosted renal antioxidant capacity, preventing mercuric chloride-induced tissue damage. Many studies have shown the antioxidant effects of *P. emblica* and *T. terrestris*, which are compatible with these findings (Kavitha and jagadeesan 2006, Abdel-Kader *et al.* 2016). The free radical scavenging activity of flavonoid components is commonly linked to the protective function of *T. terrestris* against tissue injury (Lakshmi *et al.* 2012). Our findings clearly showed that *P. emblica* and *T. terrestris* extracts have radical scavenging action, inhibiting lipid peroxidation damage and hence improving tissue functional parameters. Furthermore, these effects were linked to a reduction in histological damage.

In conclusion, an increase in reactive oxidative species was responsible for mercury-induced nephrotoxicity, which was reduced by the flavonoid rich fraction of *Phyllanthus emblica* (FRFPE) and the saponin rich fraction of *Tribulus terrestris* (SRFTT), as evidenced by improved antioxidant parameters and histopathological changes in the kidney. The antioxidant activity of active phytochemicals like flavonoids and saponin may be responsible for the nephroprotective action of *P. emblica* and *T. terrestris*.

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