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Probing the therapeutical potential of conventional and supercritical fluid extract of *Zingiber officinale* to mitigate ulcer, inflammation, hepatotoxicity and nephron toxicity

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Summary. Novel health boosting strategies of the millennium have illuminated phytoceutic as one of the promising therapeutic tool to mitigate various health related disorders. In current research work, indigenously grown ginger variety suravi was evaluated for its anti-ulcerogenic, anti-inflammatory, hepato-protective and nephron-defensive potential. For this purpose, nutraceuticalCSE T1 (ethanol extract, 90 min) and nutraceuticalSFE T2 (3300 psi, 50°C, 3 hr) were given to Sprague Dawley rats via oral gavage according to daily intake values (i.e. 3 and 0.3% respectively). In aspirin induced ulcer, ginger extracts showed 38.02% (T1) and 42.9% (T2) reduction for gastric juice volume, 8.45 (T1) and 17.87% (T2) reduction in gastric juice acidity. However, the ulcer index decreased from 2.63 to 1.97 (T1) and 1.68 (T2). In carrageenan induced inflammation, ginger extracts lessen the paw edema from 0.273 to 0.260 and 0.256 in T1 and T2. In CCl4 induced hepatotoxicity, nutraceuticalCSE showed 12.94% reduction in AST, 4.74% in ALP, 10.13% in ALT and 6.56% in bilirubin, however, the nutraceuticalSFE proved 19.99, 14.88, 18.07 and 10.18% reduction in AST, ALP, ALT and bilirubin, respectively. Similarly, in gentamicin induced nephron toxicity, the decrease rate was 8.94, 8.87 and 9.42% for urea, creatinine and uric acid content, accordingly the urea, creatinine and uric acid level lessen to 14.32, 12.10 and 15.94%, correspondingly in T1 and T2.

Key words: gingerol, nutraceuticalCSE, nutraceuticalSFE, aspirin, carrageenan, CCl4, gentamicin

Introduction

Nutraceutical diligence is one of the highest ranking industries like the increment in food and pharmaceutical industries. In future, nutraceutical companies will be more successful than the functional food product industries those are composed of food to satisfy both traditional & health assessment opinions. Consumer awareness regarding diet and health paradigm may endow with excellent prospects to exploit nutraceutical ingredients in the treatment of different ailments. The use of nutraceutical as an attempt to achieve required beneficial consequences with abridged side effects or compounds with other bioactive agents has met with great economic success (1). Antioxidants are essential for health because it provides protection against oxidation as well as against the different factors which affect lipid oxidation include the presence of oxygen and transition metal ions, moisture, heat and light (2).

The essential oil obtained from the rhizome of *Zingiber officinale* has pale yellow to light-amber hue and can be extracted within the yield range of 1.5-3% contingent on crop quality. Nonetheless, both oil and oleoresins of medicinal plants are used as a vital ingredient in food industries including beverages, drinks, and baked products along with its extensive biological investigations. In this milieu, ginger has an excellent antioxidant profile and anti-microbial status and also gaining prime position in food industries (3-5).

Prehistorically, *Zingiber officinale* rhizome has been cultivated pervasively by the means of spices as well as traditional medicinal food. The therapeutic assay of ginger include remedy of nausea, attenuating indications of arthritis, inflammation, dyspepsia, ulcer, asthma, respiratory diseases and rheumatic ailments (6). The most active ingredients of ginger are gingerol and shogaol that have been identified by numerous researchers. Shogaol is the product of gingerol that primed after the heat application. Shogaol has different structure than gingerol although has identical in therapeutical potential against physiological threats especially anti-carcinogenic probability (7-9). Among all shogaol series, 6-shogaol has shown precise chemopreventive potential against colorectal cancer *i.e.* the third most diagnosed cancer in United States (10, 11).

It is well renowned that the reactive oxygen species (ROS) formed *in vivo* including hydrogen peroxide, hydroxyl radical and superoxide anion are highly perceptive and have ephemeral damaging potential for chemical species (12). ROS are concomitant with carcinogenesis, cardiovascular disorders due to its mutilation in DNA, lipids and proteins metabolism (3). For this purpose, antioxidants are significant agents that can be hired antagonistic to disorders (13). The possible line to prevent from these disparities is to invigorate our body's revolution via antioxidant protective system, though, the recommended intake of fruits, vegetables or spices & herbs has already linked up with the reduction of such malfunctions (14).

Unanimously, *Zingiber officinale* is prominent among herbs for its anti-inflammatory, antioxidant, anti-carcinogenic, hypoglycemic, radio-protective, hypolipidemic, nephron defensive and hepato-protective evaluation. Moreover, ginger has the ability to endure inflammation damage in Alzheimer's and Parkinson's disorders. Various investigations have been conducted on the protective role of ginger bioactive entities such as oxidative stress, drug induced toxicity and inflammation damages by masses of experimental modeling. The mechanism of antioxidant and anti-inflammation action is associated with powerful radical quenching of ginger that causes vacillation in gene or protein countenance as a meddling with intracellular cell signaling pathways of inflammation (15).

Zingiber officinale is also well known for its anti-ulcerogenic perspectives owing to the presence of free as well as bound phenolics having the ability to mitigate the chances of ulcer *in vitro* specifically inhibiting of H^+, K^+-ATPase and growth of *H. pylori* (16). In another study Zaman *et al.* (17) conducted a research experiment in which oral ginger was given in chemically induced gastric damage animal model exhibiting gastro-protective perspectives against omeprazole. Ginger extract was administrated separately (200 and 400 mg/kg) and in combination with omeprazole (10 mg/kg). The estimation of percentage inhibition value was recorded as 40.91, 57.58 and 65.91%, respectively.

Various explorations have been verified health enhancing properties of ginger among them, Zaman and Mirje (18) evaluated the anti-inflammatory perspectives of ginger through rat feeding trial. In their study, they assessed the anti-inflammatory property of isolated ginger extract along with its combination with indomethacin by using carrageenan induced rat paw edema. The rats were categorized for the administration of aqueous extract of ginger (200 mg/kg or 400 mg/kg) alone and in amalgamation of 25 mg/kg of indomethacin. The paw volume was measured to compare with normal ones. They concluded that indomethacin and ginger (200 and 400 mg/kg) mounted a significant reduction in inflammation valued about 95, 89.5 and 92.6%, respectively. However, the mixture of anti-inflammatory drug (indomethacin) with both concentrations (200 and 400 mg/kg) of ginger depicted the results as 95 and 97.5%, correspondingly. These outcomes indicated analogous anti-inflammatory outline of ginger and commercially available drug as well as synergistic consequences as promising anti-inflammatory agent.

Prior investigations related to preventive properties of ginger and its derivatives have shown a considerable task in hepato-protection. Multiple experimental trials have proved its protection from CCl_4 induced hepatotoxicity (19). Likewise, another research has concluded that the augmentation of single dosed ginger extract composed of 200 and 400 mg/kg before the administration of acetaminophen was valuable to avert hepatotoxicity and also to lessen ALT, AST and ALP concentrations by improving the ability of antioxidant enzymes in liver (20). Similarly, gingerol is also responsible for hepato-protection in mancozeb induced hepatotoxic rats (21). Furthermore, a recent research also corroborated the outcomes of ginger in retrogres-

sive lead induced toxicity in liver by the means of enhancing SOD and CAT along with reduction in LPx (22). Furthermore, Li *et al.* (23) summarized the potential of gingerol in many types of disabilities such as diabetic liver, eye, kidney and neural regularity complications.

Ginger (*Zingiber officinale*) is consumed for the treatment of numerous disorders since ancient times. Moreover, oxidative stress is considered as the main reason backed up with majority of cellular and histological effects of disarrays. For the investigation of ginger as a hepato-protective agent, Mannem (24) performed a research trial in chemical induced hepatotoxic rats. By the application of chemicals, the level of liver enzymes as glutathione (GSH) and superoxide dismutase (SOD) were reduced. Epidemiological studies have confirmed that liver disclosed several histological fluctuations explicitly deprivation of hepatocytes by necrosis in addition to apoptosis, fatty alternations and inflammatory cells infiltration. Ginger in two different concentrations was augmented (200 and 300 mg/kg body weight) to attenuate the biochemical alternations in liver cells along with histological changes. The result from trial reported that *Zingiber officinale* has an exceptional control on hepatotoxicity due to its antioxidative action in rats.

The fact behind the neuron defensive mechanism of ginger is attributed to its phenolic and flavonoids. A research work carried out by Ha *et al.* (25) showed nephron-defensive ability associated with gingerol in fresh ginger and similar for shogaol in dried ginger via the reticence of microglia. Earlier, another finding suggested that ginger showed nephron protective property by enhancing antioxidant shielding mechanism of brain and reduction in MDA regulation to the regular levels in diabetic rats (26). Besides, ginger juice has also been identified to reduce the LPX level by improving the protein, GSH, SOD, CAT and GPx status in treated rats (27).

The main complication of nephrotoxicity is associated with consumption of some harmful chemicals having gentamicin persuading the renal damage by the overproduction of ROS along with the inflammation in tubular cells of nephron. Gingerol has already verified for its nephron protective mechanism by different scientists those concluded that an oral administration of ginger extract elevates the nephron-defensive impact in animals suffering with nephropathy at 6.25, 12.5 and 25 mg/kg ginger extract have momentous reduction in creatinine by increasing protein content of urine (28).

Materials and methods

Materials

Ginger variety (Suravi) was procured from the Ayub Agriculture Research Institute, Fasialabad. For efficacy trial, Male Sprague Dawley rats were housed in the Animal Room of NIFSAT. For biological assay, diagnostic kits were purchased from Sigma-Aldrich, Bioassay (Bioassays Chemical Co. Germany) and Cayman Chemicals (Cayman Europe, Estonia).

Sample preparation

Ginger was cut into small pieces in order to obtain desired size. Afterwards, slices were sun dried and ground to a fine powder using grinder. Resultant ginger powder was used for further analyses.

Preparation of ginger extract
Soxhlet extraction of ginger

Ginger extract was prepared by following the respective methods mentioned by Jalali-Nehzhad *et al.* (29) as mention Table 1. The ginger powder was successively extracted using soxhlet apparatus with ethanol as solvent. 100 g sample was extracted from 250 mL of ethanol. Afterwards the resultant extracts

Table 1. Treatments for extraction

Extraction Method	**Solvent**	**Treatment**	**Time (min)**
Soxhlet apparatus	Ethanol	T_1	90
Supercritical Fluid Extraction	Solvent	Treatment	Pressure (psi)
	CO_2	T_2	3300

were subjected to rotary evaporator (Eyela, Japan) to remove solvent and stored for further analysis.

Supercritical fluid extraction (SFE)

For comparing the efficiency of conventional and supercritical fluid extraction (SFE) technique, ginger extracts was obtained by using supercritical fluid extractor (SC-CO_2), model SFT-150 (supercritical fluid extractor incorporation USA) following the guidelines of Lim *et al.* (29) as depicted Table 1. This apparatus was equipped with a volume extractor and separator, a syringe pump and a syphonated carbon dioxide (CO_2) cylinder that was pressurized up to work pressure. In experiment, 200 g of the dried ginger rhizome was placed in the extractor vessel. The operating condition was as follows: pressure, 3300 psi, temperature, 50°C and extraction time, 3 hour.

Bioefficacy studies

To evaluate the therapeutic potential of ginger extracts against selected metabolic disorders such as ulcer, inflammation, hepatotoxicity and nephron toxicity, an efficacy trial was planned. For the purpose, 150 male Sprague Dawley rats were housed in the animal room of NIFSAT, University of Agriculture, Faisalabad. The rats were acclimatized by feeding on basal diet for a period of one week. The environmental conditions were control throughout the trial like temperature (23±2°C) and relative humidity (55±5%) along with 12 hr light-dark period (NIH Publications No. 8023, 8th edition, revised 1978). All animal procedures were approved by the local Institutional Animal Ethical Committee and performed from 8 to10 a.m. During efficacy trial, five types of studies were conducted independently by involving normal, ulcerogenic, inflammatory, hepatotoxic and renal dysfunctional rats. In Study I, rats were fed on normal diet whereas in study II, III, IV and V, high aspirin, high carrageenan, high carbon tetra chloride and high gentamicin diets (Table 2) were administrated, respectively. Each study comprised of three groups of rats five in each. Accordingly, two types of ginger extracts *i.e.* $nutraceutical_{CSE}$ and $nutraceutical_{SFE}$ were prepared considering the stability of the active ingredients and given to the representative groups against control. During two months trial, instantaneous administration of nutraceutical extracts to experimental rats was ensured to assess their therapeutic role. At the termination of the study, overnight fasted rats were decapitated and blood with organ was collected. For serum collection, blood samples were subjected to centrifugation using centrifuge machine @ 4000 rpm for 6 min. The respective sera samples were examined for various biochemical assays by using Microlab 300, Merck, Germany. Mucosa profile for anti-ulcerogenic perspectives, paw edema for inflammation test, hepato-protection and nephron-defensive assays along with antioxidant status and serum biochemistry were performed to evaluate the health boosting aspects of extracts alongside electrolyte balance, protein ratio and organ body weight for safety reasons. The entire biological trial was repeated to draw a conclusive inference.

Table 2. Different studies conducted in efficacy trials

Studies	Groups	Diets
Study I	Control	Normal diet
Study II	Ulcerogenic	Aspirin enhanced diet
Study III	Inflammatory	Carrageenan supplemented diet
Study IV	Hepatotoxic	Carbon tetrachloride enriched diet
Study V	Nephrotoxic	Gentamicin augmented diet

The details of these studies are herein.

Study 1: Normal diet

In this study, rats were divided in to three homogeneous groups fed on normal diet along with provision of respective extract. Control diet without any ginger extract was subjected to this group. Following similar approach, four other studies were conducted to find out the impact of ginger extract against respective diets *i.e.* high aspirin, high carrageenan, high carbon tetra chloride and gentamicin enriched to correlate with the lifestyle related disorders.

Study II: Ulcerogenic rats

In study II, high aspirin diet (*i.e.* 350 mg/kg body weight) was given to the normal rats to induce ulcer. Periodic examination of rats was carried out to assess

the induction of ulcer. The ginger extracts were provided to the rats concurrently to synchronize their effect on the respective group.

Study III: Inflammatory rats

In study III, high carrageenan diet containing 5% carrageenan was given to induce inflammation in rats and determined its effect on paw swelling and edema. Besides, effect of ginger extracts on the induced syndrome was measured in each group on daily basis.

Study IV: Hepatotoxic rats

In study IV, for liver soundness 4 mL CCl_4/kg body weight was added to persuade hepatotoxicity in rats. In addition to this, ginger extracts were supplemented to respective group. The outcomes was determined at the end of study.

Study V: Nephron toxic rats

In group V, rats were administrated on high gentamicin diet @ 100 mg/kg body weight to induce nephron toxicity with simultaneous intake of respective ginger extracts to test their effect on selected serum parameters.

Anti-ulcerogenic assay

The anti-ulcerogenic assay of ginger extracts was measured including gastric secretion and gastric ulceration by following the protocol of El-metwally (31) whilst, the ulcer index was calculated by the recommendations of Zaman *et al.* (17).

Determination of Gastric Secretion

Stomachs from each rat were legated around both openings and injected by 3 mL distilled water and stored in 10% solution of formalin. The gastric juice was collected in a test tube and centrifuged at 3000 rpm for 10 minutes. The gastric juice volume was measured by graduated cylinder. The total acid content of the gastric juice was determined by titrating it with 0.01 N NaOH, using phenolphthalein as indicator and was expressed as mEq/L. The acidity of gastric juice was calculated as total acid content/gastric juice volume in mEq/L. The gastric juice decrease percentage was calculated for each group as following:

$$\text{The gastric juice decrease percentage} = \frac{\text{Volume of gastric juice of control positive} - \text{volume of gastric juice of treated group}}{\text{Volume of gastric juice of control positive}} \times 10$$

The decrease in total acidity of gastric juice percentage was calculated for each treated group as following:

$$\text{Decrease in total acidity percentage} = \frac{\text{Total acidity of gastric juice of control} - \text{total acidity of gastric juice of treated group}}{\text{Total acidity of gastric juice of control group}} \times 10$$

Determination of ulcer index

The stomachs were opened longitudinally, washed with saline and examined under dissecting microscope for gastric ulcer. The length of gastric ulcer was measured for each group to determine of ulcer index (UI) and the curative ratio. The ulcerative index was calculated by severity of gastric mucosal lesions 1mm or less, 1-2 mm and more than 2 mm and graded as 1, 2 and 3 score, respectively. Then the UI was calculated by using the formula:

UI = 1 x (number of lesions of grade 1) + 2 x (number of lesions of grade 2) + 3 x (number of lesions of grade 3) Then the overall score was divided by a factor 10, which was designated as ulcer index.

Grades of ulcer severity

0 = No ulcer

1 = Superficial ulcer

2 = Deep ulcer

3 = Perforation

Anti-inflammation perspectives

The anti-inflammation perspectives of ginger extract including paw edema was calculated according to the guidelines of Jeena *et al.* (32). In which the experimental Sprague Dawley rats were inflamed by the addition of 5% carrageenan for 60 days along with conventional and supercritical extract groups. After the induction of inflammation, the paw edema was measured on daily basis with the help of plethysmometer to determine formation of edema.

Hepato-protective probability

In hepatotoxic rats, the protective probability of ginger extract counting serum aspartate aminotransferase (AST), alkaline phosphate (ALP), alanine

aminotransferase (ALT) and Bilirubin contents was estimated by the method of Akinloye *et al.* (33). According to this protocol, plasma was separated by centrifugation at 3000 rpm for 10 minutes and used for the estimation of various biochemical parameters such as liver function tests including aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were assessed. Levels of AST and ALT were measured by the dinitrophenylhydrazene (DNPH) method using Sigma Kits 59-50 and 58-50, respectively and ALP by Alkaline Phosphates–DGKC method.

Nephron-defensive study

For the nephron-defensive study of ginger extract, creatinine, urea and uric acid was deliberated in nephron toxic rats as described by Hussein (34). The serum samples were analyzed for urea by GLDH-method, whilst creatinine by Jaffe-procedure via commercial kits to evaluate the kidney functioning.

Anti-oxidative stress

The *in vivo* anti-oxidative perspectives of ginger extract including superoxide dismutase (SOD), glutathoine peroxidase (GPX), catalase (CAT) and malondialdehyde (MDA) was evaluated by the protocol of Sani *et al*. (35). Blood obtained was collected in test tubes with heparin to prevent blood coagulation, and the plasma was separated. The blood samples were then rinsed with the same volume of 0.9% normal saline (NaCl) and centrifuged at 3000 rpm for 10min at 4°C. The upper layer was removed and the above procedure was repeated with 0.9% NaCl until it became clear. The lower layer, termed hemolysate, was then used for the antioxidant enzyme assays.

Protein analysis

Protein analysis including total protein, albumin, globulin and A/G ratio of all the studies was conducted as mentioned by Helal *et al.* (36). In this parameter, serum total protein along with serum albumin was estimated. The globulin value for each sample was obtained by subtracting the albumin value from the corresponding total protein value. The A/G ratio for each sample was obtained by dividing the albumin level to globulin level.

Organ to body ratio

In these parameters, organs *i.e.* liver, heart, kidney, spleen, lungs and pancreas were collected after dissection to determine the effect of test diets and drinks on organ weights of rats as mentioned by Sulaiman *et al*. (37). Tissues of interest (liver, kidneys, heart, spleen, lungs and pancreas) were harvested, weighed and homogenized with a Teflon homogenizer (Sigma-Aldrich Chemie GmbH, Munich, Germany) in a cold 0.25 mol/L sucrose solution (1:5, w/v). The tissue homogenates were then centrifuged at 5000 × g for 10 min to remove unbroken particulate and measured with body weight to obtain organ to body ratio.

Serum electrolyte balance

The serum electrolyte balance together with calcium, sodium and potassium was supported by Maralla (38). Indicators of electrolytes balance like Na, K and Ca of collected blood samples were also probed by their respective methods.

Hematological analyses

Hematological analysis like hemoglobin (Hb), packed cell volume (PCV), mean volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) during all the studies were performed according to Kulkarni *et al.* (39) whilst, hematocrit (Hct) was calculated by the method of Apines-Amar *et al.* (40).

Statistical analysis

The data were obtained by applying completely randomized design (CRD) and further subjected to statistical analysis using Statistical Package (Costat-2003, Co-Hort, v 6.1). Level of significance was determined (ANOVA) using 2-factor factorial CRD where applicable following the principles outlined by Montgomery, (41). The results are expressed as mean ± standard deviation (SD). A probability (P) level of < 0.05 was considered statistically significant.

Results and Discussion

Biological study was carried out to evaluate the nutraceutical potential of gingerol against selected health

related disorders using experimental Sprague Dawley rats. The trials were conducted on rodents rather than humans due to organized supervision, control diet & environmental conditions and convenient management. In the instant investigation, bioevaluation trial was comprised of five modules on the basis of different diets. In study I, normal diet was used whereas in study II, III, IV and V aspirin, carrageenan, carbon tetra chloride and gentamicin enriched diets, respectively were provided along with simultaneous intake of normal diet and ginger extracts via oral gavage (T_0, T_1 and T_2). At the initiation of trial, some rats were scarified to assess the baseline values whilst rest of the rats was killed at the termination (60th day). Mainly, gingerol was tested against ulcer, inflammation, hepatotoxicity and nephron toxicity along with oxidative stress markers. For better understanding the results of all examined parameters in different studies are discussed collectively.

Anti-ulcerogenoic assay

It is deduced from statistical analysis (F values) in Table 3 that treatments imparted non-significant variation on gastric juice volume in all studies expect study II. In studies I, III, IV and V, the gastric juice volume ranged from 1.27±0.04 to 1.29±0.4 mL however, in study II the maximum gastric juice volume was 6.97±0.22 mL in T_0 followed by T_1 (4.32±0.14) however minimum level was observed in T_2 *i.e.* 3.98±0.13 mL. Similarly in regarding to acidity, the ginger extract imparted non-momentous effect on all the studies expect study II which was enriched with aspirin along with ginger extract. In all studies, the acidity ranged from 2.04±0.07 to 2.08±0.08% whilst, in study II the maximum acidity was observed in control (4.14±0.13%) followed by nutraceutical$_{CSE}$ (3.79±0.12%) and nutraceutical$_{SFE}$ (3.40±0.11%). Likewise the results regarding to ulcer index proved the ulcer index only in study II maximum in T_0 (2.63±0.08) followed by T_1 (1.97±0.06) however minimum level (1.68±0.05) was observed in T_2. It is depicted from figures that the maximum reduction was observed in nutraceutical$_{SFE}$ as compared to nutraceutical$_{CSE}$.

The reduction of gastric volume in ulcerogenic study was 42.9% for nutraceutical$_{SFE}$ and 28.02% for

Table 3. Effect of ginger extracts on ulcer in different studies

Parameters	Studies	Treatments			F-value
		T_0	T_1	T_2	
Gastric Juice Volume (mL)					
	Study I	1.28±0.04	1.27±0.04	1.28±0.04	0.10^{NS}
	Study II	6.97±0.22	4.32±0.14	3.98±0.13	437**
	Study III	1.27±0.04	1.29±0.04	1.28±0.04	0.29^{NS}
	Study IV	1.29±0.05	1.29±0.04	1.27±0.05	0.39^{NS}
	Study V	1.27±0.05	1.28±0.05	1.28±0.05	0.29^{NS}
Gastric Juice Acidity (%)					
	Study I	2.09±0.07	2.06±0.07	2.07±0.07	0.26^{NS}
	Study II	4.14±0.13	3.79±0.12	3.40±0.11	42.4**
	Study III	2.05±0.06	2.06±0.06	2.05±0.06	0.04^{NS}
	Study IV	2.08±0.08	2.06±0.08	2.05±0.08	0.26^{NS}
	Study V	2.06±0.07	2.04±0.07	2.04±0.07	0.15^{NS}
*Ulcer Index					
	Study I	0.00±0.00	0.00±0.00	0.00±0.00	0.00^{NS}
	Study II	2.63±0.08	1.97±0.06	1.68±0.05	219**

*Study I= Control; Study II= Ulcerogenic; Study III= Inflammatory; Study IV= Hepatotoxic; Study V= Nephrontoxic; T_0= water; T_1= 3% nutraceutical$_{CSE}$; T_2= 0.3% nutraceutical$_{SFE}$; *No ulcer was observed in study III, IV, V; NS= Non-significant; *= Significant; **= Highly Significant*

nutraceutical$_{CSE}$ (Figure 1). Similarly, supercritical fluid extract showed 17.87% deduction in gastric juice acidity whilst, it was 8.45% for conventional solvent system (Figure 2). Furthermore, the ulcer index decreased 36.12 and 25.1% in T_1 and T_2, respectively (Figure 3).

The findings of current research work were in line with the finding of Khalil, (42) concluded that after the utilization of 400 mg/kg body weight of aspirin can induce ulcer in rats and the ulcer index score elevated to 2.83±0.4 that decreased to 1.17±0.4 with addition of 200 mg/kg of ginger powder along with 400 mg/kg body weight of aspirin in diet. Lastly, Liju *et al.* (43) reported the consumption of alcohol (ethanol @ 5 mL/day) induced ulcer index at a level of 4.77±0.56 in wistar rats that reduced to 1.88±0.58 (60.6%), 1.47±0.78 (69.2%) and 0.71±0.28 (85.1%) scores after addition of 100, 500 and 1000 mg/kg of ginger per body weight, respectively.

Anti-inflammatory

The F value regarding to anti-inflammatory perspective of ginger extract (Table 4) for paw edema in different studies showed non-momentous effect in study I whereas, significant effect was proved in all rest of studies. In study I, the paw width ranged from 0.239±0.01 to 0.248±0.01 cm. Means regarding study II, showed maximum edema in control (0.273±0.01 cm), 0.260±0.01 in conventional solvent system and 0.256±0.01 cm in supercritical extract group. Similarly, in study III, the maximum edema was observed in

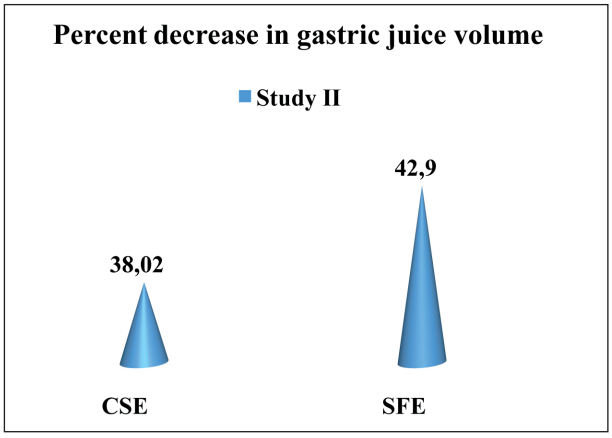

Figure 1. Percent decrease in gastric juice volume
CSE= Conventional solvent extraction
SFE= Supercritical fluid extraction

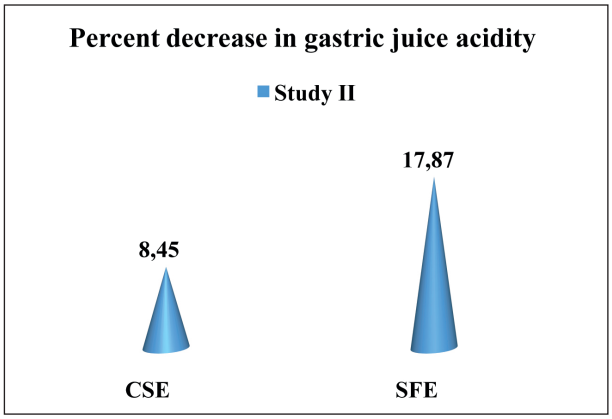

Figure 2. Percent decrease in gastric juice acidity
CSE= Conventional solvent extraction
SFE= Supercritical fluid extraction

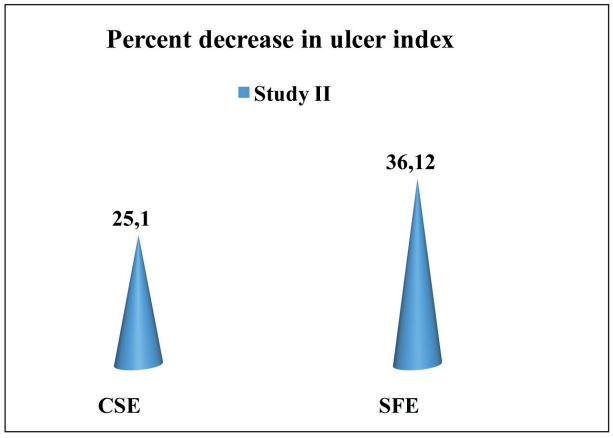

Figure 3. Percent decrease in ulcer index
CSE= Conventional solvent extraction
SFE= Supercritical fluid extraction

Table 4. Effect of ginger extracts on paw edema (cm) in different studies

Studies	Treatments			F-value
	T_0	T_1	T_2	
Study I	0.248±0.01	0.242±0.01	0.239±0.01	1.54^{NS}
Study II	0.273±0.01	0.260±0.01	0.256±0.01	5.25*
Study III	0.369±0.01	0.294±0.01	0.278±0.01	106**
Study IV	0.310±0.01	0.288±0.01	0.271±0.01	20.1**
Study V	0.305±0.01	0.282±0.01	0.267±0.01	20.1**

*Study I= Controi; Study II= Ulcerogenic; Study III= Inflammatory; Study IV= Hepatotoxic; Study V= Nephrontoxic; T_0= water; T_1= 3% nutraceutical$_{CSE}$; T_2= 0.3% nutraceutical$_{SFE}$; NS= Non-significant; *= Significant; **= Highly Significant*

T_0 (0.369±0.01 cm) abide by T_1 (0.260±0.01 cm) and minimum in T_2 (0.256±0.01 cm). Likewise in study IV, the means related to treatment effect showed maximum reduction in supercritical fluid extract (0.271±0.01 cm) followed by conventional extract (0.288±0.01 cm) against control (0.310±0.01 cm). Moreover, in study V means related to treatment showed the reduction in paw width from 0.305±0.01 cm (control) to 0.282±0.01 cm (nutraceutical$_{CSE}$) and 0.267±0.01 cm (nutraceutical$_{SFE}$). It is clear from figure 4 that nutraceutical$_{SFE}$ decreased maximum paw edema in study III i.e. 24.66%, however it was 20.33% by nutraceutical$_{CSE}$ in same study.

The results of current investigation were in line with the outcomes of Zaman and Mirje, (18) reported about the anti-inflammatory effect of ginger on carrageenan induced inflammation. They concluded that ginger aqueous extract has the ability to reduce inflammation equally as compared to anti-inflammatory drugs. To prove this, they divided rats into six groups i.e. control, indomethacin drug treated (25 mg/kg), ginger (200 mg/kg), ginger (400 mg/kg), indomethacin + ginger (25 +200 mg/kg) and indomethacin + ginger (25 + 400 mg/kg) and observed for four hours. They concluded that after carrageenan induced inflammation the inhibition level was 4.5, 25, 74 and 95% for indomethacin drug in 1st, 2nd, 3rd and 4th hour however, the inhibition in inflammation was 12.7, 26.66, 64.5 and 89.5% for ginger @ 200 mg/kg and 23.4, 29.1, 74.1 and 92.6% for ginger at the dose rate of 400 mg/kg for 1st to 4th hour. When ginger (200 mg/kg) was used along with indomethacin (25 mg/kg) the reduction percentage gradually decreased from first to fourth hour as 17.39, 36.66, 64.5 and 95%, respectively. Furthermore, the best reduction level was observed with combination of indomethacin (25mg/ kg) and ginger (400 mg/kg) that proved 8.6, 37.4, 77.4 and 97.5% inhibition in inflammation after four hours of induction.

Hepato-protective probability

The hepato-protective analyses comprised of aspartate transaminase (AST), alkaline phosphatase (ALP), alanine transaminase (ALT) and bilirubin.

Aspartate aminotransferase (AST)

The F value in Table 5 reported that AST was affected non-significantly in first and second study

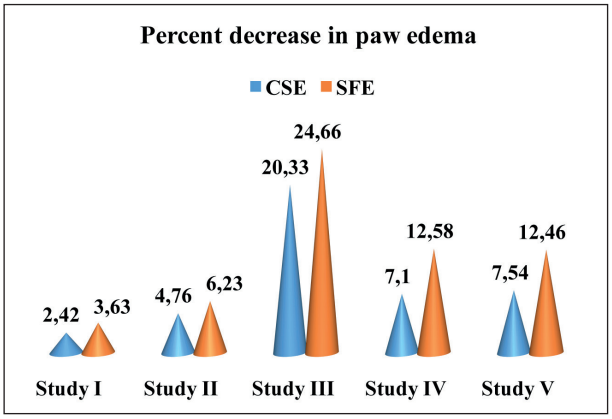

Figure 4. Percent decrease in paw edema
CSE= Conventional solvent extraction
SFE= Supercritical fluid extraction

whereas this parameter was significantly effected in rest of studies. Mean AST values of T_0, T_1 and T_2 in study I were 107.61±3.66, 104.96±3.57 and 103.58±3.52 IU/L, respectively. However, the mean AST values in study II was highest in control (106.45±3.41 IU/L) followed by T_1 and T_2 (105.02±3.36 and 103.27±3.30 IU/L, correspondingly). On the other hand in study III, means for AST indicated maximum value in T_0 (118.32±3.55 IU/L) than that of T_1 (114.87±3.45 IU/L) and minimum for T_2 (110.16 IU/L). In hepatotoxic study (study IV) the nutraceutical$_{SFE}$ reduced the AST maximum (154.88±5.89 IU/L) than the nutraceutical$_{CSE}$ (168.53±6.40 IU/L) as compared to control (193.57±7.36 IU/L). Besides in study V, T_0 showed maximum AST level (108.92±3.92 IU/L) that reduced in T_1 (104.33±3.76 IU/L) and T_2 (101.74±3.66 IU/L). It is depicted from figure 5 that maximum reduction was observed in study IV in which T_2 decreased up to 19.99% however, T_1 decreased AST level 12.94%.

Alkaline Phosphatase (ALP)

It is obvious from F values that ginger extract treatments affected serum ALP level non-momentously in study I and II however in other studies it proved substantial effects (Table 5). In study I, mean ALP values for T_0, T_1 and T_2 were 154.83±5.26, 152.61±5.19 and 151.46±5.15 IU/L, accordingly. Similarly, in study II, this trait was highest in T0 (158.19±5.06 IU/L) that reduced in T_1 (155.30±4.97 IU/L) and T_2 (153.27±4.90 IU/L). In study III, means regarding treatments were

Table 5. Effect of ginger extracts on hepatotoxicity in different studies

Parameters	Studies	Treatments			F-value
		T_0	T_1	T_2	
AST (IU/L)	Study I	107.61±3.66	104.96±3.57	103.58±3.52	1.70^{NS}
	Study II	106.45±3.41	105.02±3.36	103.72±3.30	1.04^{NS}
	Study III	118.32±3.55	114.87±3.45	110.16±3.30	5.76*
	Study IV	193.57±7.36	168.53±6.40	154.88±5.89	57.8**
	Study V	108.92±3.92	104.33±3.76	101.74±3.66	5.39*
ALP (IU/L)	Study I	154.83±5.26	152.61±5.19	151.46±5.15	1.63^{NS}
	Study II	158.19±5.06	155.30±4.97	153.27±4.90	1.31^{NS}
	Study III	163.72±4.91	156.89±4.71	152.52±4.58	5.61*
	Study IV	239.48±9.10	218.13±8.67	203.85±7.75	44.6**
	Study V	197.51±7.11	189.28±6.81	184.30±6.63	6.93*
ALT (IU/L)	Study I	51.68±1.76	50.26±1.71	49.84±1.69	0.56NS
	Study II	52.37±1.68	51.72±1.66	50.63±1.62	1.47NS
	Study III	55.41±1.66	52.99±1.59	51.69±1.55	5.75*
	Study IV	79.60±3.02	71.54±2.72	65.22±2.48	29.6**
	Study V	53.02±1.91	50.38±1.81	49.10±1.77	5.52*
Bilirubin (mg/dL)	Study I	0.593±0.02	0.587±0.02	0.570±0.02	1.87NS
	Study II	0.548±0.02	0.531±0.02	0.522±0.02	2.75NS
	Study III	0.619±0.02	0.595±0.02	0.583±0.02	4.19*
	Study IV	1.463±0.06	1.367±0.05	1.314±0.05	13.2**
	Study V	0.584±0.02	0.546±0.02	0.529±0.02	11.6**

Study I= Control; Study II= Ulcerogenic; Study III= Inflammatory; Study IV= Hepatotoxic; Study V= Nephrontoxic;
*T_0= water; T_1= 3% nutraceutical$_{CSE}$; T_2= 0.3% nutraceutical$_{SFE}$; NS= Non-significant *= Significant **= Highly Significant*

163.72±4.91 IU/L in control, 156.89±4.71 IU/L in nutraceutical$_{CSE}$ and 152.52±4.58 IU/L in nutraceutical$_{SFE}$ groups. Nonetheless, in study IV, the parameters was higher in control (239.48±9.10 IU/L) that significantly reduced in T_1 (218.13±8.67 IU/L) and T_2 (203.85±7.75 IU/L). In study V, ALP value in T_0 was 197.51±7.11 IU/L trailed by T_1 and T_2 groups with mean values 189.28±6.81 and 184.30±6.63 IU/L. the maximum reduction was depicted in study IV in which nutraceutical$_{SFE}$ decreased ALP level 14.88% although, nutraceutical$_{CSE}$ showed 8.92% reduction (Figure 6).

Alanine transaminase (ALT)

The F value regarding means of ALT (Table 5) depicted that ALT level was non-momentously effected in study I and II whilst, in study III, IV and V this trait was significantly reduced. Means for ALT in study I were 51.68±1.76, 50.26±1.71 and 49.84±1.69 IU/L for T_0, T_1 and T_2, respectively. However the same trends was observed in study II in which means for T_0, T_1 and T_2 were 52.37±1.68, 51.72±1.66 and 50.63±1.62 IU/L, correspondingly. Although in inflammatory study (study III) the ALT reduced to 52.99±1.59 IU/L in T_1 and 51.69±1.55 IU/L in T_2 that was 55.41±1.66 IU/L in T_0. Nevertheless, in study IV (hepato-toxic) the maximum ALT was observed in control (79.60±3.02 IU/L) that decreased in nutraceutical$_{CSE}$ (71.54±2.72 IU/L) and nutraceutical$_{SFE}$ (65.22±2.48 IU/L). In study V, the ALT level in T_0 (53.02±1.91 IU/L) was higher than that of T_1 (50.38±1.81 IU/L) and T_2 (49.10±1.77 IU/L). The maximum reduction was observed in hepatotoxic study *i.e.* 18.07 and 10.13% in T_1 and T_2 (Figure 7).

Bilirubin

It is realized from F value (Table 5) that ginger extract showed non-significant impact on bilirubin

content in study I and II however it proved significant reduction in inflammatory, hepatotoxic and nephron toxic studies. The mean values of bilirubin in study I were 0.593±0.02 mg/dL in T_0, 0.587±0.02 in T_1 and 0.570±0.02 mg/dL in T_2. Similarly, in study the mean values of bilirubin were 0.548±0.02, 0.531±0.02 and 0.522±0.02 mg/dL in T_0, T_1 and T_2 groups, accordingly. Even so, in study III the bilirubin content gradually lowered in T_1 (0.595±0.02 mg/dL) and T_2 (0.595±0.02 mg/dL) than the T_0 (0.619±0.02 mg/dL). The highest means were observed in carbon tetrachloride enriched food group in which the bilirubin content decreased to 1.367±0.05 mg/dL by the oral administration of nutraceutical$_{CSE}$ and 1.314±0.05 mg/dL by nutraceutical$_{SFE}$ against control (1.463±0.06 mg/dL). In study V, the means were 0.584±0.02, 0.546±0.02 and 0.529±0.02 mg/dL in T_0, T_1 and T_2, respectively. It is cleared from figure 8 that maximum decrease was observed by supercritical fluid extract (10.18%) as compared to conventional solvent extract (6.56%) in study IV.

The outcomes of this research work are in according with the finding of Kalaiselvi *et al.* (44) determined the hepato-protective effect of ginger against aluminum chloride induced toxicity and they reported that the AST level was 130.6±2.37 IU/L in control group that increased to 146.5±6.77 IU/L in aluminum chloride induced toxic group. However, when ginger augmented diet was given to toxic group AST level decreased to 145.1±8.16 IU/L whilst, ginger alone de-

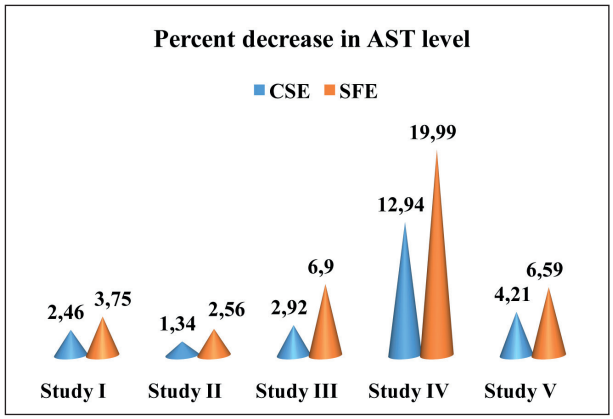

Figure 5. Percent decrease in AST level
CSE= Conventional solvent extraction
SFE= Supercritical fluid extraction

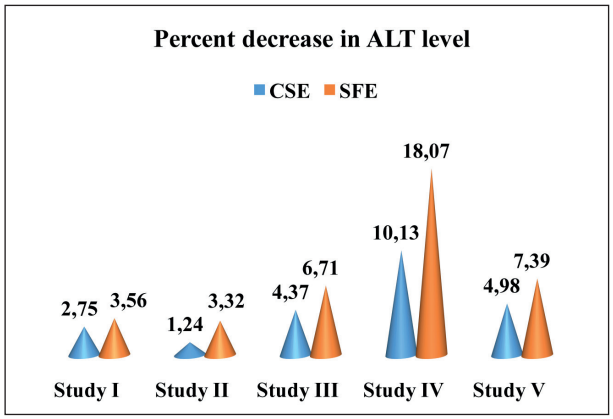

Figure 7. Percent decrease in ALT level
CSE= Conventional solvent extraction
SFE= Supercritical fluid extraction

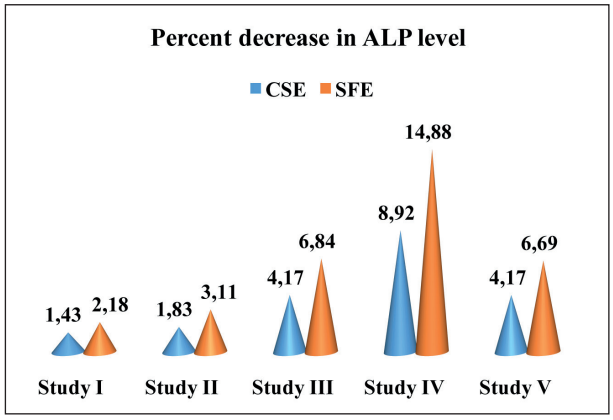

Figure 6. Percent decrease in ALP level
CSE= Conventional solvent extraction
SFE= Supercritical fluid extraction

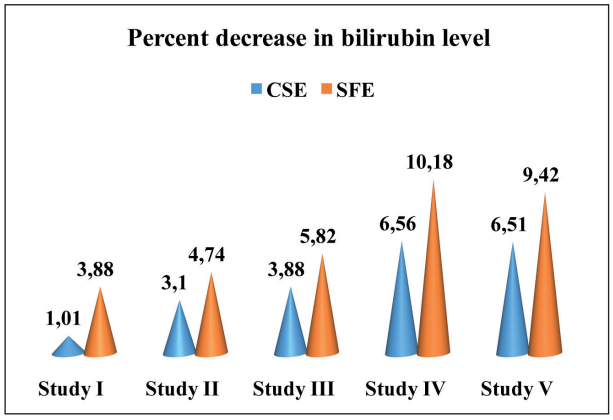

Figure 8. Percent decrease in bilirubin level
CSE= Conventional solvent extraction
SFE= Supercritical fluid extraction

creased AST content up to 136.6±6.07 IU/L. similarly, ALT level were 50.0±2.01, 60.3±3.22, 57.8±3.31 and 51.6±3.82 IU/L in normal control group, toxic group, toxic group with simultaneously ginger diet and ginger alone group, accordingly.

Nephron defensive property

To evaluate the nephron defensive property of bioactive moieties in ginger extract, different tests such as urea, creatinine and uric acid content were carried out against gentamicin induced nephron toxicity along with all other studies.

Urea

The F values indicated non-significant effect of ginger extracts on serum urea in study I and IV however, momentous effect were noticed in study II, III and V (Table 6). In study I, mean serum urea values were 31.45±1.07, 31.09±1.06 and 30.58±1.04 mg/dL in T_0, T_1 and T_2 accordingly. Although, in study II the serum urea values decreased from 34.18±1.09 mg/dL (control) to 33.56±1.07 ($nutraceutical_{CSE}$) and 32.04±1.03 mg/dL ($nutraceutical_{SFE}$). Nonetheless in study III, the carrageenan enriched diets showed momentous decrease in serum urea from 36.70±1.10 mg/dL (T_0) to 34.87±1.05 (T_1) and 33.41±1.00 mg/dL (T_2). In case of study IV the ginger extracts showed minimum reduction in serum urea and mean values were 32.33±1.23, 31.45±1.20 and 30.96±1.18 mg/dL in T_0, T_1 and T_2. In gentamicin enriched diets the serum urea level elevated to 43.51±1.57 mg/dL in control and then lowered to 39.62±1.43 in conventional ginger extract and 37.28±1.34 mg/dL in supercritical ginger extract based group. Figure 9 depicted that the maximum urea reduction was observed in study V by $nutraceutical_{SFE}$ (14.32%) and $nutraceutical_{CSE}$ (8.94%).

Creatinine

The statistical analysis (F value) concluded non-substantial effect of extracts on creatinine level in study I and IV however, significant trend was observed in study II, III and V (Table 6). In study I. T_0 showed

Table 6. Effect of ginger extracts on nephron toxicity (mg/dL) in different studies

Parameters	Studies	Treatments			F-value
		T0	T1	T2	
Urea	Study I	31.45±1.07	31.09±1.06	30.58±1.04	0.38NS
	Study II	34.18±1.09	33.56±1.07	32.04±1.03	4.93*
	Study III	36.70±1.10	34.87±1.05	33.41±1.00	9.94**
	Study IV	32.33±1.23	31.45±1.20	30.96±1.18	2.17NS
	Study V	43.51±1.57	39.62±1.43	37.28±1.34	27.6**
Creatinine	Study I	0.83±0.03	0.81±0.03	0.80±0.03	1.79NS
	Study II	0.91±0.03	0.87±0.03	0.85±0.03	6.09*
	Study III	0.96±0.03	0.90±0.03	0.88±0.03	8.67**
	Study IV	0.87±0.03	0.85±0.03	0.84±0.03	1.79NS
	Study V	1.24±0.04	1.13±0.04	1.09±0.04	19.5**
Uric acid	Study I	0.96±0.03	0.94±0.03	0.93±0.03	1.17NS
	Study II	1.01±0.03	0.96±0.03	0.94±0.03	6.00*
	Study III	1.16±0.03	1.07±0.03	1.02±0.03	18.0**
	Study IV	1.02±0.04	0.99±0.04	0.98±0.04	2.00NS
	Study V	1.38±0.05	1.25±0.05	1.16±0.04	32.5**

*Study I= Control; Study II= Ulcerogenic; Study III= Inflammatory; Study IV= Hepatotoxic; Study V= Nephrontoxic; T0= water; T1= 3% nutraceuticalCSE; T2= 0.3% nutraceuticalSFE; NS= Non-significant *= Significant **= Highly Significant*

maximum creatinine mean value 0.83±0.03 mg/dL whereas, T_1 and T_2 showed reduced values as 0.81±0.03 and 0.80±0.03 mg/dL, respectively. However, in study II, the creatinine level was 0.91±0.03mg/dL in T_0 that subsequently reduce to 0.87±0.03 and 0.85±0.03 mg/dL in T_1 and T_2, correspondingly. Similarly, in study III the maximum creatinine level was observed in control (0.96±0.03 mg/dL) that gradually lowered to 0.90±0.03 and 0.88±0.03 mg/dL in conventional extract and supercritical group, accordingly. Besides this, in study IV, the creatinine level non-momentously reduced to 0.85±0.03 and 0.84±0.03 mg/dL in T_1 and T_2, respectively against T_0 (0.87±0.03 mg/dL). In nephron toxic study (study V) highest reduction was observed in nutraceutical$_{SFE}$ (1.09±0.04 mg/dL) followed by nutraceutical$_{CSE}$ (1.13±0.04 mg/dL) as compared to control (1.24±0.04 mg/dL). It was observed in study V, the reduction in creatinine level was 12.1 and 8.87% by nutraceutical$_{SFE}$ and nutraceutical$_{CSE}$, correspondingly (Figure 10).

Uric acid

The F values deduced non-significant effect of ginger extract on uric acid level in study I and IV however momentous effect was noticed in all other studies (Table 6). Means regarding the effect of ginger extract in study I showed maximum uric acid in T_0 (0.96±0.03 mg/dL) followed by T_1 (0.94±0.03 mg/dL) and T_2 (0.93±0.03 mg/dL). Although. In study II, the uric acid was reduced to 0.96±0.03 in T_1 and 0.94±0.3 mg/dL in T_2 against T_0 (1.01±0.03 mg/dL). Similarly, in study III the highest uric acid content was observed in T_0 (1.16±0.03 mg/dL) that bit by bit reduced in T_1 (1.07±0.03 mg/dL) and T_2 (1.02±0.03 mg/dL). Nonetheless, in study IV the uric acid content decreased non-momentously from T_0 (1.02±0.014 mg/dL) to T_1 (0.99±0.04 mg/dL) and T_2 (0.98±0.04 mg/dL). However, in study V, elevated uric acid content, 1.38±0.05 mg/dL was noticed in gentamicin enriched diet (T_0) that reduced to 1.25±0.05 mg/dL in gentamicin and conventional ginger extract group (T_1) and 1.16±0.04 mg/dL in gentamicin with supercritical fluid ginger extract (T_2). Figure 11 declared that maximum uric acid reduction was observed in nephron toxic *i.e.* 15.94 and 9.42% via nutraceutical$_{SFE}$ and nutraceutical$_{CSE}$, accordingly.

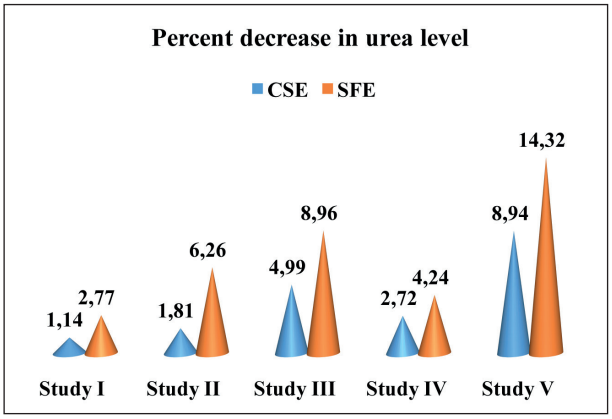

Figure 9. Percent decrease in urea level
CSE= Conventional solvent extraction
SFE= Supercritical fluid extraction

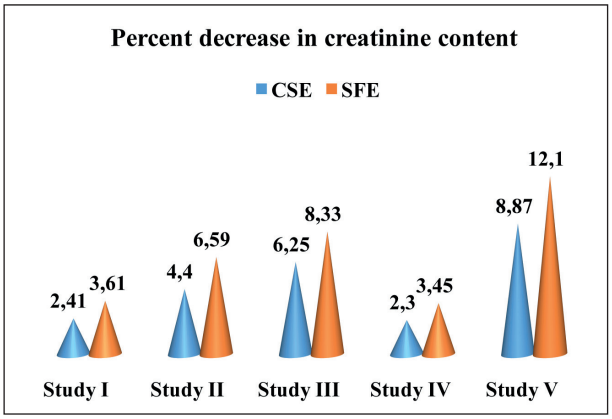

Figure 10. Percent decrease in creatinine content
CSE= Conventional solvent extraction
SFE= Supercritical fluid extraction

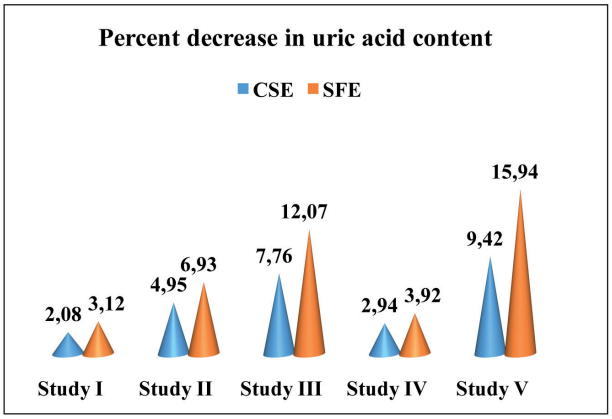

Figure 11. Percent decrease in uric acid content
CSE= Conventional solvent extraction
SFE= Supercritical fluid extraction

The results of current investigation were in harmony with the suggestions of Rodrigues *et al.* (28) assessed the reduction level of ginger on gentamicin induced nephron toxicity and reported that the urea level in control and gentamicin induced toxicity group were 47.3±2.61 and 80.6±7.77 mg/dL. After the supplementation of ginger @ 6.25, 12.5 and 25 mg/kg the urea level was 62.5±6.7, 53.8±6.9 and 41.7±1.8 mg/dL, however when the normal rats were fed on ginger diet (25 mg/kg) the urea level was 37.9±2.8 mg/dL. Similarly, in case of creatinine, the control and nephrotoxic group had the creatinine level 0.62±0.03 and 1.05±0.08 mg/dL that decreased to 0.87±0.06 (6.25 mg/kg), 0.78±0.07 (12.5 mg/kg) and 0.63±1.66 mg/dL (25 mg/kg) in nephrotoxic group whilst, the creatinine level was 0.51±0.01 mg/dL (25 mg/kg) in normal rats. Likewise, the uric acid content were 1.33±0.21 mg/dL in control group that increased to 3.74±0.52 mg/dL due to nephrotoxicity induced by gentamicin, the uric acid level decreased to 2.5±0.5 mg/dL after supplementation of diet with 6.25 mg/kg of ginger powder, 2.33±0.71 mg/dL for 12.5 mg/kg and 1.66±0.33 mg/dL for 25 mg/kg supplementation of ginger although, 25 mg/kg ginger in normal rats reduced the uric acid level up to 1.57±0.21 mg/dL.

Oxidative stress

The *in vivo* anti-oxidative perspectives of ginger extract based treatments included superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT) and malonaldehyde (MDA).

Superoxide dismutase (SOD)

The statistical analysis (F value) regarding SOD in Table 7 revealed significant differences due to treatments in different studies. In study I maximum SOD

Table 7. Effect of ginger extracts on oxidative stress in different studies

Parameters	Studies	Treatments			F-value
		T0	T1	T2	
SOD (µg/mg)	Study I	17.54±0.60	16.80±0.57	16.37±0.56	5.53*
	Study II	17.98±0.58	17.05±0.55	16.76±0.54	6.11*
	Study III	17.73±0.53	17.19±0.52	16.51±0.50	5.71*
	Study IV	18.07±0.69	17.40±0.66	16.29±0.62	12.2**
	Study V	17.69±0.64	16.94±0.61	16.48±0.59	5.77*
GPX (µg/mg)	Study I	54.02±1.84	55.96±1.90	57.86±1.97	5.29*
	Study II	58.74±1.88	60.84±1.95	63.05±2.02	5.63*
	Study III	53.15±1.59	54.91±1.65	57.04±1.71	5.62*
	Study IV	57.92±2.20	59.33±2.25	64.52±2.43	14.8**
	Study V	57.65±2.08	58.78±2.12	61.90±2.23	6.15*
CAT (µg/mg)	Study I	7.04±0.24	7.32±0.25	7.58±0.26	6.19*
	Study II	7.51±0.24	7.86±0.25	8.10±0.26	6.53*
	Study III	9.43±0.28	9.69±0.29	10.17±0.31	6.58*
	Study IV	8.76±0.33	9.12±0.35	9.81±0.37	15.0**
	Study V	8.19±0.29	8.48±0.31	8.83±0.32	6.43*
MDA (nmol/g)	Study I	15.59±0.53	15.02±0.51	14.63±0.50	4.58*
	Study II	16.30±0.52	15.85±0.51	15.29±0.49	4.62*
	Study III	16.07±0.48	15.34±0.46	15.09±0.45	4.89*
	Study IV	15.71±0.60	15.18±0.58	14.56±0.55	6.49*
	Study V	15.84±0.57	15.29±0.55	14.87±0.54	4.56*

*Study I= Control; Study II= Ulcerogenic; Study III= Inflammatory; Study IV= Hepatotoxic; Study V= Nephrontoxic; T0= water; T1= 3% nutraceuticalCSE; T2= 0.3% nutraceuticalSFE; NS= Non-significant *= Significant **= Highly Significant*

level was observed in T_0 (17.54±0.60 µ/mg) that significantly decreased in T_1 (16.80±0.57 µ/mg) and T_2 (16.37±0.56 µ/mg). Similarly, in study II, the SOD level gradually decreased to 17.05±0.55 and 16.76±0.54 µ/mg in T_1 and T_2 as compared to 17.98±0.58 µ/mg in T_0. Nonetheless, in study III, SOD level was observed as 17.73±0.53, 17.19±0.52 and 16.51±0.50 µ/mg in T_0, T_1 and T_2, respectively. Besides, in study IV the maximum reduction was observed in T_2 (16.29±0.62 µ/mg) followed by T_1 (17.40±0.66 µ/mg) as compared to T_0 (8.07±0.69 µ/mg). In the same way study V showed substantial reduction in SOD level 16.48±0.59 µ/mg in nutraceutical$_{SFE}$ group, 16.94±0.61 µ/mg in nutraceutical$_{CSE}$ group that was 17.69±0.64 µ/mg in control group.

Glutathione peroxidase (GPX)

The F value in Table indicated that GPX level was significant affected by ginger extract treatments in all studies (Table 7). Means in study I, showed minimum GPX level in T_0 as 54.02±1.84 µ/mg that substantially elevated in T_1 55.96±1.90 µ/mg trailed by T_2 57.86±1.97 µ/mg, respectively. In study II, GPX level was increased from 58.74±1.86 µ/mg (T_0) to 60.84±1.95 µ/mg (T_1) and 63.05±2.02 µ/mg (T_2). Similarly, in study III, the GPX level was minimum in control (53.15±1.59 µ/mg) that gradually increased in nutraceutical$_{CSE}$ (54.91±1.65 µ/mg) and nutraceutical$_{SFE}$ (57.04±1.41 µ/mg). In the same way, study IV, showed lowest GPX level in T_0 (57.92±2.20 µ/mg) that significantly increased in T_1 (59.33±2.25 µ/mg) and T_2 (64.52±2.45 µ/mg). Besides, in study V, maximum increase was observed in T_2 (61.90±2.23 µ/mg) as compared to T_1 (58.78±2.12 µ/mg) and T_0 (57.65±2.08 µ/mg).

Catalase (CAT)

It is obvious from F value that treatments of ginger extract impart momentous impact on CAT level in all studies (Table 7). In study I, the CAT level was 7.04±0.24, 7.32±0.25 and 7.58±0.26 µ/mg in T_0, T_1 and T_2, accordingly. Likewise, in study II, the lowest CAT level was noticed in T_0 (7.51±0.24 µ/mg) that significantly increased in T_1 (7.86±0.25 µ/mg) and T_2 (8.10±0.26 µ/mg). The same trend was observed in study III, the lowest CAT was noticed in control group as 9.43±0.28 µ/mg trailed by conventional solvent extracted group (9.69±0.29 µ/mg) and supercritical extracted group (10.17±0.31 µ/mg). Similarly, in study IV, maximum CAT was observed in T_2 (9.81±0.37 µ/mg) as compared to T_1 (9.12±0.35 µ/mg) and T_0 (8.76±0.33 µ/mg). Nonetheless, in study V, the observed CAT levels in T_0, T_1 and T_2 were 8.19±0.29, 8.48±0.31 and 8.83±0.32 µ/mg, respectively.

Malonaldehyde (MDA)

It is observed from F value that MDA level significantly affected due to treatments in all studies (Table 7). In study I, the recorded MDA levels in T_0, T_1 and T_2 were 15.59±0.53, 15.02±0.51 and 14.63±0.50 nmol/g, correspondingly. Whilst, in study II the MDA level significantly decreased from 16.30±0.52 nmol/g (T_0) to 15.85±0.51 nmol/g (T_1) and 15.29±0.49 nmol/g (T_2). Alongside, in study III, the maximum MDA level was observed in T_0 (16.07±0.48 nmol/g) trailed by T_1 (15.34±0.46 nmol/g) and T_2 (15.09±0.45 nmol/g). Similarly, MDA level in study IV, proved maximum reduction in T_2 (14.56±0.55 nmol/g) as compare to T_1 (15.18±0.58 nmol/g) and T_0 (15.71±0.60 nmol/g). Likewise, I study V, the maximum MDA level was observed in control group as 15.84±0.57 nmol/g that gradually lowered to 15.29±0.55 and 14.87±0.54 nmol/g in nutraceutical$_{CSE}$ and nutraceutical$_{SFE}$ treatment group.
The results were in hormonally with the outlines of Liju *et al.* (43) who reported that the CAT level increased gradually by the addition of ginger essential oil in diet of wistar rats. The CAT level was 6.01 U/mg in control that reduced to 3.57 U/mg after consumption of 5 mL of ethanol on daily basis. After the addition of ginger essential oil @ 100, 500 and 1000 mg/kg body weight the CAT level elevated to 4.83, 5.40 and 5.94 U/mg, respectively. Likewise, the GPX content were 35.00 U/mg in control and 20.41 U/mg in alcoholic (ethanol 5 mL/day) group however, GPX level was 28.59, 33.42 and 39.78 U/mg after consumption of ginger essential oil (100, 500 and 1000 mg/kg body weight).

Protein analysis

Total protein

The F value indicated significant effect of treatments on total protein content in all studies (Table 8). Means in study I, regarding total protein were 6.28±0.21 g/L in T_0 that significantly increased to 6.51±0.21 g/L in T_1

and 6.73±0.20 g/L in T_2. Whereas in study II, means regarding to total protein were 7.20±0.27, 6.91±0.25 and 7.34±0.25 g/L in T_0, T_1 and T_2 groups. However, in study III the mean total protein content were in T_0 (6.76±0.22 g/L) than that of T_1 (6.97±0.21 g/L) and T_2 (7.19±0.27 g/L). Similar trend was noticed in study IV in which the minimum total protein were observed in control group (6.81±0.25 g/L) that significantly increased in nutraceutical$_{CSE}$ (7.03±0.24 g/L) and nutraceutical$_{SFE}$ (7.27±0.23 g/L). Likewise, in study V the same pattern regarding total protein were noticed 7.18±0.22, 7.41±0.28 and 7.66±0.28 g/L in control and ginger extract treatments groups T_1 and T_2, respectively.

Albumin

The F value indicated significant effect of ginger extracts on albumin level in all studies (Table 8). Means (study I) showed minimum albumin value 2.67±0.09 g/L in T_0 that substantially increased to 2.78±0.09 and 2.92±0.09 g/L in T_1 and T_2 groups, accordingly. Similarly, in study II, T_0 exhibited lowest albumin value (3.32±0.13 g/L) that increased momentously in T_1 (3.49±0.13 g/L) and T_2 (3.68±0.13 g/L). Whereas in study III, albumin value 3.19±0.10 g/L in T_0 group was significantly increased to 3.35±0.10 g/L (T_1) and 3.52±0.13 g/L (T_2). Likewise in study IV, mean albumin value for T_0, T_1 and T_2 differed momentously *i.e.* 2.98±0.11, 3.15±0.11 and 3.36±0.11 g/L, correspondingly. Likewise, in study V, the respective values for T_0 were 3.32±0.10 g/L that increased substantially in T_1 and T_2 groups as 3.51±0.13 and 3.64±0.13 g/L, respectively.

Globulin

The F value indicated substantial differences due to treatments on globulin level in all studies (Table 8). Means regarding globulin in study I depicted lowest

Table 8. Effect of ginger extracts on protein analyses in different studies

Parameters	Studies	Treatments			F-value
		T0	T1	T2	
Total Protein (g/L)	Study I	6.28±0.21	6.51±0.21	6.73±0.20	4.76*
	Study II	7.20±0.27	6.91±0.25	7.34±0.25	4.39*
	Study III	6.78±0.22	6.97±0.21	7.19±0.27	4.48*
	Study IV	6.81±0.25	7.03±0.24	7.27±0.23	5.63*
	Study V	7.18±0.22	7.41±0.28	7.66±0.28	5.36*
Albumin (g/L)	Study I	2.67±0.09	2.78±0.09	2.92±0.09	5.92*
	Study II	3.32±0.13	3.49±0.13	3.68±0.13	5.08*
	Study III	3.19±0.10	3.35±0.10	3.52±0.13	4.71*
	Study IV	2.98±0.11	3.15±0.11	3.36±0.11	6.94*
	Study V	3.32±0.10	3.51±0.13	3.64±0.13	4.06*
Globulin (g/L)	Study I	3.32±0.11	3.45±0.11	3.59±0.11	6.20*
	Study II	4.10±0.16	4.32±0.16	4.46±0.15	4.33*
	Study III	4.20±0.13	4.31±0.13	4.56±0.17	4.33*
	Study IV	3.48±0.13	3.69±0.13	3.89±0.12	5.92*
	Study V	3.88±0.12	4.10±0.16	4.16±0.15	5.29*
A/G Ratio	Study I	0.80±0.03	0.81±0.03	0.81±0.02	0.10NS
	Study II	0.81±0.03	0.81±0.03	0.83±0.03	0.39NS
	Study III	0.76±0.02	0.78±0.02	0.77±0.03	0.29NS
	Study IV	0.86±0.03	0.85±0.03	0.86±0.03	0.10NS
	Study V	0.86±0.03	0.86±0.03	0.88±0.03	0.37NS

*Study I= Control; Study II= Ulcerogenic; Study III= Inflammatory; Study IV= Hepatotoxic; Study V= Nephrontoxic; T0= water; T1= 3% nutraceuticalCSE; T2= 0.3% nutraceuticalSFE; NS= Non-significant *= Significant **= Highly Significant*

level in T_0 (3.32±0.11 g/L) that increased significantly in T_1 (3.45±0.11 g/L) and T_2 (3.59±0.11 g/L). Nonetheless, in study II, globulin value elevated to 4.32±0.16 g/L (T_1) and 4.46±0.15 g/L (T_2) that was 4.10±0.16 g/L in T_0. Similarly, in study III, globulin value increased significantly from 4.20±0.13 g/L (control) to 4.31±0.13 g/L ($nutraceutical_{CSE}$) and 4.56±0.17 g/L ($nutraceutical_{SFE}$). Likewise in study IV, globulin level were 3.69±0.13 and 3.89±0.12 g/L in T_1 and T_2, respectively as compared to 3.48±0.13 g/L in T_0. However, in study V, mean values for T_0, T_1 and T_2 differed significantly *i.e.* 3.88±0.12, 4.10±0.16 and 4.16±0.15 g/L, correspondingly.

A/G ratio

The F value showed that A/G value in different groups was non-significantly affected by treatments in all studies (Table 8). In study mean A/G ratio in T_0, T_1 and T_2 group were 0.80±0.03, 0.81±0.03 and 0.81±0.02, respectively. Whereas, in study II, T_0 group illustrated 0.81±0.03 value for A/G ratio that remained constant in T_1 (0.81±0.03) and increased non-momentously to 0.83±0.03 in T_2. Likewise, in study III, the lowest A/G value was observed in T_0 (0.76±0.02) that gradually increased to 0.77±0.03 (T_2) and 0.78±0.02 (T_1). Besides study IV indicated non-momentous effect of treatments in T_0, T_1 and T_2 as 0.86±0.03, 0.85±0.03 and 0.86±0.03, accordingly. In the same way study V showed non-substantial results for control, $nutraceutical_{CSE}$ and $nutraceutical_{SFE}$ ginger extract groups a 0.86±0.03, 0.86±0.03 and 0.88±0.03, correspondingly. The results of present study were in line with the findings of Hamouda *et al.* (45) who concluded that the bilirubin level can be reduced by the utilization of ginger ethanol extract after the toxicity induced by carbon tetra chloride. They depicted that before toxicity was 0.66±0.82 mg/dL that elevated to 1.59±0.06 mg/dL after liver injury induced by carbon tetra chloride. When ginger ethanol extract was given simultaneously with CCl_4 the bilirubin level lessen to 1.31±1.34 mg/dL however, the content was 0.66±0.86 mg/dL when ginger was given to control group.

Organs to body weight ratio

The F value concerned organ to body weight ration proved non-substantial effect of treatments during the efficacy trials (Table 9). Means related to liver to body weight ratio in different studies varied from 4.10±0.12 to 4.18±0.13 g/100g body weight. Likewise, right kidney weight regarding to different studies varied non-significantly from 0.39±0.01 to 0.41±0.01 g/100g body weight. Similarly, left kidney ranged non-momentously from 0.37±0.01 to 0.41±0.01 g/100g body weight in different studies. Moreover the weight of heart to body weight ratio varied non-substantial from 0.29±0.01 to 0.33±0.01 g/100g body weight however, the same pattern was observed in spleen that varied in different studies from 0.29±0.01 to 0.33±0.01g/100g body weight body weight in different studies. The ginger extract didn't impart any effect on the weight of lungs and pancreas that ranged from 1.02±0.04 to 1.09±0.04 g/100g body weight and 0.51±0.02 to 0.57±0.02 g/100g body weight in different studies, respectively. The conclusions of present research work were in line with the findings of Hegazy *et al.* (46) in their research work they determined the effect of 6-gingerol on gentamicin induced renal cortex and concluded that the kidney weight of normal rats was 0.70±0.020 g although after addition of ginger the kidney weight was 0.69±0.014 g. After renal cortex induction by gentamicin, the kidney weight was 0.58±0.032 g, however, after supplementation of 6-gingerol in toxic group, the kidney weight was 0.69±0.029 g.

Electrolyte balance

Calcium (Ca)

The F value in Table 10 showed non-significant effect of treatments on calcium content in entire experiment. In preliminary study, means for calcium in T_0, T_1 and T_2 were 13.84±0.47, 14.26±0.48 and 14.69±0.50 mEq/L, respectively. However, the calcium electrolyte in study II was non-significantly increased from 12.25±0.39 mEq/L (T_0) to 12.67±0.41 mEq/L (T_1) and 13.04±0.42 mEq/L (T_2). Similarly, in study III, T0 showed 12.06±0.36 mEq/L of Ca that was raised non-significantly in T_1 and T_2 groups *i.e.* 12.53±0.38 and 12.98±0.39 mEq/L, accordingly. Likewise in study IV, means for Ca in T_0 was 12.32±0.47 mEq/L that uplifted to 12.79±0.49 and 13.26±0.50 in T_1 and T_2, respectively. The same pattern was observed in study V in which the minimum Ca content were observed in T_0 (12.58±0.45 mEq/L) that gradually increased in T_1 (12.95±0.47 mEq/L) and T_2 (13.45±0.48 mEq/L).

Table 9. Effect of ginger extracts on organ to body weight ratio (g/100 g body weight) in different studies

Parameters	Studies	Treatments			F-value
		T0	T1	T2	
Liver	Study I	4.15±0.14	4.10±0.14	4.12±0.14	0.33NS
	Study II	4.11±0.13	4.11±0.13	4.13±0.13	0.07NS
	Study III	4.10±0.12	4.15±0.12	4.18±0.13	1.08NS
	Study IV	4.14±0.16	4.14±0.16	4.17±0.16	0.26NS
	Study V	4.18±0.15	4.08±0.15	4.11±0.15	1.96NS
Right Kidney	Study I	0.39±0.01	0.40±0.01	0.38±0.01	0.36NS
	Study II	0.40±0.01	0.39±0.01	0.39±0.01	0.27NS
	Study III	0.41±0.01	0.41±0.01	0.39±0.01	1.15NS
	Study IV	0.39±0.01	0.40±0.01	0.40±0.01	1.18NS
	Study V	0.41±0.01	0.40±0.01	0.41±0.01	0.47NS
Left Kidney	Study I	0.38±0.01	0.39±0.01	0.40±0.01	0.34NS
	Study II	0.38±0.01	0.39±0.01	0.40±0.01	1.00NS
	Study III	0.40±0.01	0.40±0.01	0.41±0.01	0.49NS
	Study IV	0.37±0.01	0.39±0.01	0.40±0.01	2.97NS
	Study V	0.38±0.01	0.38±0.01	0.39±0.01	0.17NS
Heart	Study I	0.29±0.01	0.30±0.01	0.31±0.01	1.14NS
	Study II	0.33±0.01	0.33±0.01	0.31±0.01	2.97NS
	Study III	0.30±0.01	0.29±0.01	0.29±0.01	0.24NS
	Study IV	0.32±0.01	0.31±0.01	0.32±0.01	0.46NS
	Study V	0.33±0.01	0.32±0.01	0.31±0.01	075NS
Spleen	Study I	0.30±0.01	0.31±0.01	0.32±0.01	1.03NS
	Study II	0.29±0.01	0.33±0.01	0.30±0.01	3.16NS
	Study III	0.32±0.01	0.32±0.01	0.30±0.01	1.49NS
	Study IV	0.31±0.01	0.29±0.01	0.31±0.01	2.19NS
	Study V	0.31±0.01	0.28±0.01	0.29±0.01	0.71NS
Lungs	Study I	1.05±0.04	1.05±0.04	1.07±0.04	0.36NS
	Study II	1.07±0.03	1.06±0.03	1.08±0.03	0.51NS
	Study III	1.06±0.03	1.07±0.03	1.07±0.03	0.36NS
	Study IV	1.06±0.04	1.09±0.04	1.07±0.03	0.91NS
	Study V	1.05±0.04	1.02±0.04	1.05±0.04	1.23NS
Pancreas	Study I	0.54±0.02	0.55±0.02	0.57±0.02	1.28NS
	Study II	0.55±0.02	0.54±0.02	0.54±0.02	0.14NS
	Study III	0.54±0.02	0.56±0.02	0.55±0.02	0.85NS
	Study IV	0.57±0.02	0.56±0.02	0.56±0.02	0.78NS
	Study V	0.53±0.02	0.55±0.02	0.51±0.02	2.60NS

*Study I= Control; Study II= Ulcerogenic; Study III= Inflammatory; Study IV= Hepatotoxic; Study V= Nephrontoxic; T0= water; T1= 3% nutraceuticalCSE; T2= 0.3% nutraceuticalSFE; NS= Non-significant *= Significant **= Highly Significant*

Table 10. Effect of ginger extracts on electrolyte (mEq/L) in different studies

Parameters	Studies	Treatments			F-value
		T0	T1	T2	
Calcium	Study I	13.84±0.47	14.26±0.48	14.69±0.30	1.70NS
	Study II	12.25±0.39	12.67±0.41	13.04±0.42	1.87NS
	Study III	12.06±0.36	12.53±0.38	12.98±0.39	2.60NS
	Study IV	12.32±0.47	12.79±0.49	13.26±0.50	2.59NS
	Study V	12.58±0.45	12.95±0.47	13.45±0.48	2.17NS
Sodium	Study I	118.97±4.04	119.64±4.07	120.97±4.11	0.14NS
	Study II	115.28±3.69	119.35±3.82	124.10±3.97	5.89NS
	Study III	117.56±3.53	122.08±3.66	125.56±3.77	6.42NS
	Study IV	110.99±4.22	111.73±4.25	112.98±4.29	0.16NS
	Study V	114.31±4.12	114.90±4.14	115.65±4.16	0.07NS
Potassium	Study I	7.79±0.26	8.03±0.27	8.34±0.28	2.26NS
	Study II	6.81±0.22	7.01±0.22	7.25±0.23	1.91NS
	Study III	6.49±0.19	6.92±0.21	7.33±0.22	4.21NS
	Study IV	6.57±0.25	6.98±0.27	7.21±0.27	4.24NS
	Study V	6.48±0.23	6.87±0.25	7.14±0.26	4.53NS

*Study I= Control; Study II= Ulcerogenic; Study III= Inflammatory; Study IV= Hepatotoxic; Study V= Nephrontoxic; T0= water; T1= 3% nutraceuticalCSE; T2= 0.3% nutraceuticalSFE; NS= Non-significant *= Significant **= Highly Significant*

Sodium (Na)

Statistical analysis (F value) indicated non-significant effect on treatment on sodium in all studies (Table 10). In study I, means for Na value in T_0, T_1 and T_2 were 118.97±4.04, 119.64±4.07 and 120.97±4.11 mEq/L, accordingly. In the same way, study II, the mean value for Na electrolyte in T_0 was 115.28±3.69 mEq/L that non-significantly increased in T_1 and T_2 groups as 119.35±3.82 and 124.10±3.97 mEq/L. However, T_0 in study III, showed lowest Na level (117.56±3.53 mEq/L) that bit by bit increased in T_1 (122.08±3.66 mEq/L) and T_2 (125.56±3.77 mEq/L). Likewise, the minimum Na content were 110.99±4.22 (T_0) that increased in T_1 and T_2 *i.e.* 111.73±4.25 and 112.98±4.29 mEq/L, correspondingly. Mean for Na value (study V) in T_0 was 114.31±4.12 mEq/L that differed non-substantially in T_1 and T_2 as 114.90±4.14 and 115.65±4.16 mEq/L, respectively.

Potassium (K)

The F value (Table 10) elucidated non-significant effect of treatments on potassium level in all studies. Means for this trait in T_0, T_1 and T_2 were 7.79±0.26, 8.03±0.27 and 8.34±0.28 mEq/L, correspondingly. Likewise in study II, K level in T_0 (6.81±0.22 mEq/L) differed non-significantly from T_1 (7.01±0.22 mEq/L) and T_2 (7.25±0.23 mEq/L). Similarly, in study III, the K content were 6.49±0.19 mEq/L in T_0 that increased to 6.92±0.21 mEq/L in T_1 and 7.33±0.22 mEq/L in T_2. In study IV, values for this parameter were 6.57±0.25, 6.98±0.27 and 7.21±0.27 mEq/L for T_0, T_1 and T_2, respectively. The same trend was noticed in study V, in minimum K level was in T_0 (6.48±0.23 mEq/L) that non-substantially increased in T_1 and T_2 as 6.87±0.25 and 7.14±0.26 mEq/L, accordingly.

The findings of current research work were in harmony with the outcomes of Maralla, (38) performed a research work to determine the effect of ginger on alcohol induced renal toxicity and concluded that the calcium level in control group was 9.664±0.12 mg/dL that changed to 8.656±0.08 mg/dL by addition of alcohol however, this calcium content 8.264±0.09 mg/dL with supplementation of ginger. Likewise, the potassium level was 17.511±1.34, 13.572±0.43 and 15.522±0.14 mg/dL in control, alcohol induced nephron toxic and toxic group fed on ginger diet, correspondingly. Simi-

larly, all different groups *i.e.* control, alcohol induced toxic and toxic group along with ginger diet have calcium content about 320.620±1.17, 356.70±2.18 and 331.96±4.26 mg/dL, respectively.

Hematological aspects

Red blood cells (RBC)

The F value in Table 11 showed significant impact of ginger extract in all studies. Mean RBC values for study I were 5.90±0.20, 6.13±0.20 and 6.29±0.19 cells/pL in T_0, T_1 and T_2 group, accordingly. Besides this in study II, the lowest value for RBC was observed in T_0 (6.59±0.25 cells/pL) that substantially increased to 6.68±0.24 and 6.97±0.24 cells/pL in T_1 and T_2 group, respectively. Similarly, in study III this trait showed maximum value in T_2 (6.85±0.26 cells/pL) than that of T_1 (6.64±0.20 cells/pL) as compared to T_0 (6.42±0.21 cells/pL). Likewise, in study IV, the highest mean value for RBC was observed in nutraceutical$_{SFE}$ extract group (7.32±0.23 cells/pL) followed by nutraceutical$_{CSE}$ group (6.96±0.24 cells/pL) and control group (6.86±0.25 cells/pL). Moreover, in study V, the lowest RBC mean value was 6.93±0.21 cells/pL (T_0) that momentously increased to 7.08±0.27 (T_1) and 7.40±0.27 cells/pL (T_2).

Hemoglobin (Hb)

It is depicted from F value that ginger extract treatments showed significant effect on all studies (Table 11). The means regarding different treatment inferred lowest Hb value in T_0 (10.20±0.35 g/dL) followed by T_1 (10.53±0.34 g/dL) and T_2 (10.87±0.33). Similarly, in study II the value of Hb in T_0 was 11.98±0.46g/dL that significantly increased to 12.05±0.43 g/dL in T_1 and 12.83±0.44 g/dL in T_2. Nonetheless, in study III, the value for this trait was maximum for T_2 (11.36±0.43 g/dL) followed by T_1 (11.04±0.33 g/dL) and T_0 (10.61±0.34 g/dL). The group T_0 in study IV showed minimum Hb level (12.43±0.45 g/dL) that momentously increased in T_1 (12.88±0.44 g/dL) and T_2 (13.28±0.42 g/dL). In the same way, the mean values of Hb in study V were observed as 12.30±0.37, 12.52±0.48 and 13.19±0.47 g/dL in T_0, T_1 and T_2 groups, harmoniously.

Hematocrit (HCT)

The F value in Table 11 inferred significant effect of ginger extract in all studies. In study I, the means values for HCT were 34.96±1.106 in T_0, 36.24±1.16% in T_1 and 37.12±1.11 in T_2. Similarly, in study II, the minimum value for this trait was 38.79±1.47% (T_0) followed by 39.29±1.41% (T_1) and 40.90±1.39% (T_2). However, in study III the lowest HCT value was observed in T_0 (37.85±1.21%) that increased in T_1 (39.07±1.17%) and T_2 (42.24±1.53). Means regarding different treatments in study IV depicted highest HCT value in nutraceutical$_{SFE}$ extract group (42.85±1.37%) followed by nutraceutical$_{CSE}$ group (40.85±1.39%) as compared to control (40.29±1.45%). Likewise in study V, the maximum increase was observed in T_2 (43.29±1.56%) than that of T_1 (41.51±1.58%) and T_0 (40.68±1.22%).

Mean corpuscular volume (MCV)

F value in Table showed that ginger extract showed substantial effect in all studies (Table 11). Means in study I indicated that all the treatments momentously altered this attribute from 59.25±2.01 fl (T_0) to 59.11±1.89 fl (T_1) and 59.02±1.77 fl (T_2). Similarly, in study II, MCV values were recorded as 58.82±2.24, 58.86±2.12 and 58.68±2.00 fl in T_0, T_1 and T_2, respectively. The MCV means in study III were 58.95±1.89 fl (T_0), 58.84±1.77 fl (T_1) and 58.74±2.23 fl (T_2). Likewise, in study IV maximum MCV was observe in control group (58.73±2.11 fl) that slowly decreased in conventional extract group (58.69±2.00 fl) and supercritical fluid extract group (58.53±1.87 fl). According to study V, mean MCV values for T_0, T_1 and T_2 were 58.70±1.76, 58.63±2.23 and 58.50±2.11 fl, accordingly.

Packed cell volume (PCV)

Statistical analysis (F value) indicated that ginger extracts imparted significant different on PCV values in all studies (Table 11). In study I, mean PCV in T_0 (32.78±1.11%) increased substantially in T_1 (34.06±1.09%) and T_2 (34.94±1.05%) groups. Similarly, in study II, this trait was significantly higher in T_2 (38.72±1.32%) as compared to T_1 (37.11±1.34%) and T_0 (36.61±1.39%). In study III, the recorded values for PCV were 35.67±1.14, 36.89±1.11 and 38.06±1.45% in T_0, T_1 and T_2, correspondingly. Accordingly, in study IV, the maximum mean value for PCV was recorded in nutraceutical$_{SFE}$ group (40.67±1.30%) followed by nutraceutical$_{CSE}$ (38.11±1.37%) and control (38.11±1.37%). Likewise in study V, mean of this trait

Table 11. Effect of ginger extracts on hematological analyses in different studies

Parameters	Studies	Treatments			F-value
		T0	T1	T2	
RBC (cells/pL)	Study I	5.90±0.20	6.13±0.20	6.29±0.19	5.05*
	Study II	6.59±0.25	6.68±0.24	6.97±0.24	5.61*
	Study III	6.42±0.21	6.64±0.20	6.85±0.26	5.96*
	Study IV	6.86±0.25	6.96±0.24	7.32±0.23	5.91*
	Study V	6.93±0.21	7.08±0.27	7.40±0.27	5.85*
Hb (g/dL)	Study I	10.20±0.35	10.53±0.34	10.87±0.33	4.02*
	Study II	11.98±0.46	12.05±0.43	12.83±0.44	5.94*
	Study III	10.61±0.34	11.04±0.33	11.36±0.43	4.72*
	Study IV	12.43±0.45	12.88±0.44	13.28±0.42	4.34*
	Study V	12.30±0.37	12.52±0.48	13.19±0.47	5.32*
PCV (%)	Study I	32.78±1.11	34.06±1.09	34.94±1.05	5.15*
	Study II	36.61±1.39	37.11±1.34	38.72±1.32	5.68*
	Study III	35.67±1.14	36.89±1.11	38.06±1.45	6.02*
	Study IV	38.11±1.37	38.67±1.31	40.67±1.30	5.89*
	Study V	38.50±1.16	39.33±1.49	41.11±1.48	5.88*
MCV (fl)	Study I	59.25±2.01	59.11±1.89	59.02±1.77	5.32*
	Study II	58.82±2.24	58.86±2.12	58.68±2.00	5.58*
	Study III	58.95±1.81	58.84±1.77	58.74±2.23	6.09*
	Study IV	58.73±2.11	58.69±2.00	58.53±1.87	5.73*
	Study V	58.70±1.76	58.63±2.23	58.50±2.11	5.67*
MCH (pg)	Study I	17.29±0.59	17.18±0.55	17.28±0.52	1.25NS
	Study II	18.04±0.69	18.18±0.65	18.41±0.63	4.29*
	Study III	16.53±0.53	16.63±0.50	16.58±0.63	0.21NS
	Study IV	18.12±0.65	18.51±0.63	18.14±0.58	14.3**
	Study V	17.75±0.53	17.68±0.67	17.82±0.64	4.98*
MCHC (%)	Study I	29.18±0.99	29.06±0.93	29.28±0.88	1.08*
	Study II	30.67±1.17	30.88±1.10	31.37±1.07	4.78*
	Study III	28.03±0.90	28.26±0.85	28.23±1.09	0.26NS
	Study IV	30.85±1.11	31.53±1.07	30.99±1.00	10.5**
	Study V	30.24±0.91	30.16±1.15	30.47±1.10	4.88*
HCT (%)	Study I	34.96±1.19	36.24±1.16	37.12±1.11	5.15*
	Study II	38.79±1.47	39.29±1.41	40.90±1.39	5.68*
	Study III	37.85±1.21	39.07±1.17	40.24±1.53	6.02*
	Study IV	40.29±1.45	40.85±1.39	42.85±1.37	5.89*
	Study V	40.68±1.22	41.51±1.58	43.29±1.56	5.88*

Study I= Control; Study II= Ulcerogenic; Study III= Inflammatory; Study IV= Hepatotoxic; Study V= Nephrontoxic; T0= water; T1= 3% nutraceuticalCSE; T2= 0.3% nutraceuticalSFE; NS= Non-significant *= Significant **= Highly Significant

in T_0 were 38.50±1.16% that significantly increased to 39.33±1.49 and 41.11±1.485 in ginger extract tested groups.

Mean corpuscular hemoglobin (MCH)

The F value in Table 11 elucidated that treatments imparted non-significant effect on MCH in study I and IV however, this trait was affected momentously in study II, IV and V. means for MCH values in study I were 17.29±0.59, 17.18±0.55 and 17.28±0.52 pg in T_0, T_1 and T_2 groups, respectively. Nonetheless in study II, lowest MCH value was recorded in T_0 (18.04±0.65 pg) followed by T_1 (18.18±0.69 pg) and T_2 (18.41±0.63 pg). According to means of study III, T_0 exhibited lowest MCH value (16.53±0.53 pg) that significantly uplifted in T_1 (16.63±0.50 pg) and T_2 (16.58±0.63 pg). Similarly in study IV, the means for MCH were recorded as 18.12±0.65 pg (T_0), 18.51±0.63 pg (T_1) and 18.14±0.58 pg (T_2) however in study V, the means of this trait were 17.75±0.53, 17.68±0.67 and 17.82±0.64 pg in control, conventional extract and supercritical extract treated group.

Mean corpuscular emoglobin concentration (MCHC)

It is deduced from the statistical analysis (F value) that MCHC contents were affected non-momentously in study I and III by the ginger extract treatments in contrary momentous differences were noticed in study II, IV and V (Table 11). In study I, mean values for this trait were 29.18±0.99, 29.06±0.93 and 29.28±0.38% in T_0, T_1 and T_2, respectively. Whereas in study II, MCHC in T_0 (30.67±1.17%) significantly enhanced I T_1 (30.88±1.10%) and T_2 (31.37±1.07%). Similarly, in study III, T_0 exhibited lower value (28.03±0.90%) than that of T_2 (28.23±1.07) and T_1 (28.26±0.85%). In study IV, means for this attribute in T_0, T_1 and T_2 were 30.85±1.11, 31.53±1.07 and 30.99±1.00% in T_0, T_1 and T_2. The same trend was observed in study V in which T_2 showed maximum content of MCHC (30.47±1.10%) followed by T_0 (30.24±0.91%) and T_1 (30.16±1.15%).

The outcomes of present research work were in favor of findings of Osama *et al.* (47) performed various hematological analysis of aluminum toxic rats to evaluate the effect of ginger extract on chemistry and biochemistry of blood. They concluded that the RBC of control group was 6.75±0.49 10^6/μL that decreased to 5.24±0.36 10^6/μL by addition of aluminum. By the supplementation of ginger in the diet of toxic group, the RBC level moved to 6.57±0.32 10^6/μL however, the RBC content was 6.13±0.46 10^6/μL after the augmentation of ginger in the diet of normal rats. Similarly, the Hb level was 18.29±0.43, 11.47±0.36, 17.07±0.49 and 15.06±0.37 g/dL in control, aluminum toxic, aluminum along with ginger and ginger alone group, respectively. Moreover, the PCV content in control, toxic group, toxicity induced group treated with ginger and ginger alone was 52.20±0.80, 35.70±0.53, 50.01±1.26 and 46.0±0.70%, correspondingly. For MCV content, values for control group was 18.99±6.17 fl, for aluminum toxic group, 70.67±3.94 fl, for toxic group treated with ginger 77.20±5.26 and for ginger alone group 76.81±0.04 fl whilst, in case of MCH, the values were 27.77±2.42, 22.42±2.04, 26.16±1.19 and 25.84±2.34 for control, toxic, toxic with ginger and ginger alone group, hormonally. Lastly, the content of MCHC in control, aluminum group, aluminum with ginger and ginger alone group were 35.09±1.15, 32.18±1.2, 34.21±0.57 and 32.73±0.57, individually.

Conclusion

Novel health boosting strategies of the millennium have illuminated phytoceutic as one of the promising therapeutic tool to mitigate various health related disorders. Health claims are different statements that imply the link between upgraded health outcomes and food ingredients. Contemporary, there is a great interest of all industries in healthy effects of plants that have impact on the maintenance of better health via prevention of disorders. Herbs and spices derived phytoceuitcs are of noteworthy important to curtail many health related disorders via diverse pathways. Amid herbs and spices, ginger is gaining attention of the scientists owing to the availability of persuasive antioxidants. It has proven antioxidative, anti-ulcerogenic, anti-inflammatory, hypocholesterolemic, hepato-protective, hypoglycemic, nephron defensive and antioncogenic potencies. Considering the facts, present exploration was an attempt to assess the health boosting role of ginger against selected metabolic ailments.

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References

1. Radhika PR, Brij R, Sivakumar T, Singh M. Nutraceuticals: An area of tremendous scope. Int J Res Ayur Pharma 2011; 2: 410-415.
2. Amorati R, Foti MC, Valgimigli L. Antioxidant activity of essential oils. J Agric Food Chem 2013; 61: 10835-10847.
3. Sasidharan I, Menon AN. Comparative chemical composition and antimicrobial activity fresh & dry ginger oils (Zingiber Officinale Roscoe). Int J Curr Pharm Res 2010; 2:40-43.
4. Takahashi M, Inouye S, Abe S. Anti-Candida and radical scavenging activities of essential oils and oleoresins of Zingiber officinale Roscoe and essential oils of other plants belonging to the family Zingiberaceae. Drug Disccov Ther 2011; 5:238-245.
5. Bellik Y, Boukraâ L, Alzahrani HA, Bakhotmah BA, Abdellah F, Hammoudi SM. Molecular mechanism underlying anti-inflammatory and anti-allergic activities of phytochemicals: an update. Mol 2013; 18:322-353.
6. Kubra IR, Ramalakshmi K, Pao LJM. Antioxidant enriched fractions from Zingiber officinale Roscoe. E-J Chem 2011; 8:721-726.
7. Dugasani S, Pichika MR, Nadarajah VD, Balijepalli MK, Tandra S, Korlakunta JN. Comparative antioxidant and anti-inflammatory effects of (6)-gingerol, (8)-gingerol, (10)-gingerol and (6)-shogaol. J Ethnopharmacol. 2010; 127: 515-520.
8. Wu H, Hsieh MC, Lo CY, Liu CB, Sang S, Ho CT, Pan MH. 6-Shogaol is more effective than 6-gingerol and curcumin in inhibiting 12-O-tetradecanoylphorbol 13-acetate-induced tumor promotion in mice. Mol Nutr Food Res 2010; 54: 1296-1306.
9. Bak MJ, Ok S, Jun M, Jeong WS. 6-Shogaol-rich extract from ginger up-regulates the antioxidant defense systems in cells and mice. Mol 2012; 17: 8037-8055.
10. Siegel R, Ward E, Brawley O, Jemal A. The impact of eliminating socioeconomic and racial disparities on premature cancer deaths. Cancer J Clin 2011; 61: 212-236.
11. Tan BS, Kang O, Mai CW, Tiong KH, Khoo AS, Pichika MR, Bradshaw TD, Leong CO. 6-Shogaol inhibits breast and colon cancer cell proliferation through activation of peroxisomal proliferator activated receptor gamma (PPARgamma). Cancer Lett 2013; 336: 127-139.
12. Nain P, Kumar A, Sharma S, Nain J. In vitro evaluation of antimicrobial and antioxidant activities of methanolic extract of Jaminum humile leaves. Asian Pac J Trop Med 2011; 4: 804-807.
13. Motawi TK, Hamed MA, Shabana MH, Hashem RM, Naser AAF. Zingiber officinale acts as a nutraceutical agent against liver fibrosis. Nutr Metabol. 2011; 8: 40
14. Bajpai VK, Yoon JI, Kang SC. Antioxidant and antidermatophytic activities of essential oil and extracts of Metasequoiq glyptostroboides. Food Chem Toxicol 2009; 47: 1355-1361.
15. Kumar L, Harjai K, Chhibber S. Recent update on multiple pharmacological benefits of zingerone: A quick review. Am J Phytomed Clin Therap 2014; 2: 693-704.
16. Darbar S. Antiulcer effect of livina, a herbal formulation against ethanol induced acute gastric ulcer in mice. Int J Perid Restor Dent 2010; 2: 37-45.
17. Zaman SU, Mirje MM. Evaluation of the anti-inflammatory effect of Zingiber officinale (ginger) root in rats. Int J Biotechnol Pharm Res 2014; 1: 292-298.
18. Zaman SU, Mirje MM, Ramabhimaiah S. Evaluation of anti-ulcerogenic effects of Zingiber officinale (ginger) root in rats. Int J Curr Microbiol App Sci 2014; 3: 347-354.
19. Patrick-Iwuanyanwu KC, Wegwu MO, Ayalogu EO. Prevention of CCl4 induced liver damage by ginger, garlic and vitamin E. Pak J Biol Sci 2007; 10: 617-621.
20. Ajith TA, Hema U, Aswathy MS. Zingiber officinale Roscoe prevents acetaminophen-induced acute hepatotoxicity by enhancing hepatic antioxidant status. Food Chem Toxicol 2007; 45: 2267-2272
21. Sakr SA. Ameliorative effect of ginger (Zingiber officinale) on mancozeb fungicide induced liver injury in albino rats. Aus J Basic Appl Sci 2007; 1: 650-656.
22. Khaki AA, Khaki A. Antioxidant effect of ginger to prevent lead-induced liver tissue apoptosis in rat. J Med Plants Res 2010; 4: 1492-1495.
23. Li Y, Tran VH, Duke CC, Roufogalis BD. Preventive and protective properties of Zingiber officinale (ginger) in diabetes mellitus, diabetic complications and associated lipids and other metabolic disorders: A brief review. Evid Comp Alt Med 2012; 51: 68-70.
24. Mannem P. Lead toxicity on hematological changes and amelioration with ginger (Zingiber officinale) extract in male albino rats. Int J Adv Res 2014; 2: 23-28.
25. Ha SK, Moon E, Ju MS, Kim DH, Ryu JH, Kim SY. 6-shogaol, a ginger product, modulates neuro inflammation: a new approach to neuroprotection. Neuropharmacol 2012; 63: 211-223.
26. Shanmugam KR, Mallikaarjuna K, Kesireddy N, Reddy SK. Neuroprotective effect of ginger on antioxidation enzymes in stretozotocin-induced diabetic rats. Food Chem Toxicol 2011; 49: 893-897.
27. Sharma P. Singh R. Neuroprotective effect of ginger juice against dchlorvos and lindane induced toxicity in wistar rats. Plant Med 2011; 77: 122.
28. Rodrigues FAP, Prata MMG, Oliveira CM, Alves NTQ, Freitas REM, Monteiro HSA, Silva JA, Vieira PC, Viana DA, Liborio AB, Havt A. Gingerol fraction from Zingiber officinale protects against gentamicin-induced nephrotox-

icity. Antimicrob Agents Chemotherap 2014; 58: 1872-1878.
29. Jalali-Nehzhad AA, Farajian-Mashhadi F, Komeili G, Barkhordari-Amadi F. The effect of ginger hydroalcoholic extract on rat ileal concentration in vitro. Zahedan J Res Med Sci 2015; 15: 29-33.
30. Lim S, Moon M, Oh H, Kim HG, Kim SY, Oh MS. Ginger improves cognitive function via NGF-induced ERK/CREB activation in the hippocampus of the mouse. J Nutr Biochem 2014; 25: 1058-1065.
31. El-metwally EM. Evaluation of antiulcer activity of ginger, clove and castor oils against aspirin induced gastric ulcers in rats. World Appl Sci J 2014; 29: 815-824.
32. Jeena K, Liju VYB, Kuttan R. Antioxidant, anti-inflammatory and antinociceptive activities of essential oil from ginger. Indian J Physiol Pharmacol 2013; 57: 51-62.
33. Akinloye OA, Somade OT, Akindele AS, Adelabu KB, Elijah FT, Adewumi OJ. Anticlastogenic and hepatoprotective properties of ginger (Zingiber officinale) extract against nitrobenzene-induced toxicity in rats. Rom J Biochem 2014; 51: 3-15.
34. Hussein MAA. The effect of ginger (Zingiber officinale) aqueous extract on some biochemical parameters a kidney function in male mice. Kufa Med J 2012; 15: 273-278.
35. Sani NFA, Belani LK, Sin CP, Rahman NAA, Das S, Chi TZ, Makpol S, Yousfi YAM. Effect of combination of gelam honey and ginger on oxidative stress and metabolic profile in streptozotocin-induced diabetic Sprague-dawley rats. Biomed Res Int 2014; 1: 1-9.
36. Helal EGE, El-Wahab SMA, Sharaf AMM, Zedan GA. Effect of Zingiber officinale on fatty liver induced by oxytetracycline in albino rats. Egyptian J Hosp Med 2012; 46: 26-42.
37. Sulaiman FA, Kazeem MO, Waheed AM, Temowo SO, Azeez IO, Zubair FI, Adeyemi TA, Nyang A, Adeyemi OS. Antimicrobial and toxic potential of Allium sativum, Hibiscus sabdariffa and Zingiber officinale in wistar rats. J Taibha Univ Sci 2014; 8: 315-322.
38. Maralla S. Effect of ginger extract consumption on renal function during ethanol withdrawal induced-stress. Int J Innov Res Sci Eng Technol 2013; 2: 6412-6418.
39. Kulkarni, R, Deshpande A, Saxena K, Varma M, Sinha ARS. Ginger supplementary therapy for iron absorption in iron deficiency anemia. Indian J Trad Knowledge 2012; 11: 78-80.
40. Apines-Amar MJS, Amar EC, Jr JPF, Jr RVP, Satoh S. Dietary onion and ginger enhance growth, hemato-immunological response and disease resistance in brown-marbled grouper, Epinephelus fuscoguttatus. Aqua Conser Legis Int J Bioflux Soci 2012; 5: 231-239.
41. Montgomery DC. Design and Analysis of Experiments. 7th Ed. John Wiley & Sons Inc., Hoboken, NJ, USA, 2008, pp. 162-264.
42. Khalil MS. The postulated mechanism of the protective effect of ginger on the aspirin induced gastric ulcer: Histological and immunohistochemical studies. Histol Histopathol 2015; 30: 1-10.
43. Liju VB, Jeena K, Kuttan R. Gastroprotective activity of essential oils from turmeric and ginger. J Basic Clin Physiol Pharmacol 2015; 26: 95-103.
44. Kalaiselvi A., Reddy G.A., Ramalingam V., Ameliorative effect of ginger extract (Zingiber officinale Roscoe) in liver marker enzymes, lipid profile in aluminium chloride induced male rats. Int. J. Pharma. Sci. Drug Res., 2015, 7: 52-58.
45. Hamouda AF, Sameeh MY, Shrourou RM. Effect of avocado (Persea Americana), cabbage (Brassica oleracea) and ginger (Zingiber officinale) on rat liver and thyroid injuries induced by CCl4 (carbon tetra chloride). J Pharma Pharmacol 2016; 4: 108-118.
46. Hegazy AMS, Mosaed MM, Elshafey SH, Bayomy NA. 6-gingerol ameliorates gentamicin induced renal cortex oxidative stress and apoptosis in adult male albino rats. Tissue Cell 2016; 48: 208-216.
47. Osama A, Fatma A, El-Boshy M, Huda S. Studies on the protective effect of ginger extract and in combination with ascorbic acid against aluminum toxicity induced hematological disorders, oxidative stress and hepatorenal damage in rats. Ann Vet Anim Sci 2014; 1: 137-150.

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