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PHARMACODYNAMICS OF *FOENICULUM VULGARE* AGAINST ULCER, INFLAMMATION, HEPATOTOXICITY AND NEPHROTOXICITY

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ABSTRACT

The proposed study was designed to assess the phytotherapic potential of locally cultivated fennel seeds against certain lifestyle associated disarrays. Fennel seed powder was subjected to conventional solvent extraction (CSE) and supercritical fluid extraction (SFE), followed by evaluation of health improving perspectives of fennel extracted phytoconstituents. The *in vivo* phase comprised of five studies with three groups in each based upon the diets *i.e.* control (D_0), nutraceutical$_{CSE}$ (D_1), and nutraceutical$_{SFE}$ (D_2). The five studies included in the experiment were control, ulcerogenic, inflammatory, hepatotoxicity, and nephrotoxicity. Nutraceutical$_{SFE}$ showed highest decline in gastric juice volume, gastric juice acidity and ulcer index (63.54, 47.33, and 51.65%, respectively) followed by nutraceutical$_{CSE}$ (41.09, 27.63, and 38.71%, respectively) in ulcerogenic study. Likewise, the maximum decline in paw edema was observed in inflammatory study, as 30.43% and 24.54%, in D_2 and D_1, respectively. For study IV, the maximum decline in ALT, ALP, AST and bilirubin value was recorded during hepatotoxic study among all other studies *i.e.* 10.90 & 18.49%, 11.94 & 20.37%, 9.49 & 13.49% and 12.39 & 16.57% for D_1 and D_2, respectively. A maximum reduction in urea, uric acid and creatinine was found in study V *i.e.* 20.57 & 28.40%, 20.74 & 33.60% and 14.18 & 23.88%, in D1 and D2 groups, respectively, among all studies. From the findings of the current study, it can by concluded that fennel-based extracts (nutraceutical$_{CSE}$ and nutraceutical$_{SFE}$) are effectual to alleviate from the burden of lifestyle related disorders.

Keywords: Efficacy, *In Vivo*, Antiulcer, Inflammatory, Hepatotoxic, Nephrotoxic

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INTRODUCTION

Around twenty-five hundred years ago, Hippocrates coined a phrase "Let food be the medicine and medicine be the food" which is gaining a lot of popularity among food scientists as well as consumers because of the realization about health influences of certain food commodities. Such foods are mixture of ingredients which can boost particular body functions with a consequent improvement in terms of health and living (El Sohaimy, 2012). The term "nutraceutical" devised by Dr. Stephen DeFelice in 1989 is a coordinated version of two separate words *e.g.* "nutrition" and "pharmaceutical". Functional foods and nutraceutical is used interchangeably but the foods having nutritional components other than those required for basic nutrition is considered as functional, while when they are specifically subjected to extraction of specific bioactive component for prevention of a disease because of their therapeutic potentials (*e.g.* antioxidant, antiulcer, hepato-protective, anti-inflammatory and anti-carcinogenic *etc*.) those are termed as nutraceuticals (Alissa and Ferns, 2012).

Medicinal botanicals comprised of a broad range of moieties with multifaceted bio-actions considered potentially advantageous to human health. Herbs and spices have been utilized as a healthy substitute for the cure of illnesses since ancient times (Tang and Halliwell, 2010). Low side effects associated with herbal medications make them healthy alternative to chemical drugs, which is because of the incidence of antioxidants that controls the drugs harmfulness. Around five thousand out of 4.2 million flowering plants have been documented to be employed as therapeutic agents (Jamshidi *et al*., 2012). Midst, fennel botanically known as *Foeniculum vulgare* is of promising importance and is grown primarily for pharmaceutical and food segments (Abe and Ohtani, 2013). Fennel has been known to be broadly cultivated in arid as well as semi-arid zones and because of its economic importance and increased pharmaceutical use, it has acquired the rank of world's utmost utilized therapeutic herb (Sadeghpour *et al*., 2015).

Foeniculum vulgare seeds have been found to be comprised of minerals and vitamins such as iron, potassium, phosphorus, sodium, calcium, riboflavin,

niacin, thiamine and ascorbic acid, as well as various phytochemicals have been identified so far *i.e.* quercetin, coumaric acid, ferulic acid, and chlorogenic acid (Rahimi and Ardekani, 2013; Kooti *et al*., 2014; Rather *et al*., 2016; Kalleli *et al*., 2019). The essential oil of fennel is its hallmark for conferring the plants with various therapeutic potentials (Ahmed *et al*., 2019). Essential oils, like all organic compounds, are constituted by hydrocarbon molecules and are further divided into different classes on the basis of structural variations such as terpene hydrocarbons, oxygenated compounds, aldehydes and ketones *etc* (Khammassi *et al*., 2018). It has been found that fennel essential oil comprises of more than 30 types of compounds, amongst, most prevalent being anethole (50-80%), fenchone (8-10%), limonene (5%) and estragole (Kooti *et al*., 2014). The pharmaceutical potentials found to be conferred by the different parts of plant are antioxidant, anti-ulcer, anti-inflammatory, hepato-protective, antimicrobial, cardiovascular, antitumor, hypo-glycemic, hypolipidemic, and nephron defensive property (Jamwal *et al*., 2013; Badgujar *et al*., 2014; Kooti *et al*., 2014). For the practical elucidation of aforementioned perspectives of fennel, extraction of its phyto-constituents is the primary step (Azmir *et al*., 2013). The extracted fennel phytoconstituents can be further manipulated for the assessment of their therapeutic perspectives including antioxidant, antiulcer, anti-inflammatory, hepato-protective and nephron defensive potential. Keeping in view the aforementioned facts, current study was planned to extract the essential oil of fennel by conventional soxhlet extraction and supercritical fluid extraction techniques. The extracted fractions were further evaluated by various *In Vivo* assay. The diseases selected for *In Vivo* trials were ulcer, inflammation, liver toxicity and nephron toxicity.

MATERIALS AND METHODS

Procurement of reagents: The raw materials (fennel seeds) were acquired from Ayub Agriculture Research Institute, Faisalabad. For the bio-efficacy part, male Sprague Dawley rats were kept in the designated animal room situated in National Institute of Food Science and Technology (NIFSAT). To carry out the biological assay, diagnostic kits were procured from Bioassays Chemical Co. Germany (Sigma-Aldrich, Bioassay) and Cayman Europe (Cayman Chemicals).

Preparation of fennel extracts: Ethanol was employed for the preparation of fennel seed extract by following the respective method mentioned by (Goswami and Chatterjee, 2014). Afterwards, the resultant extracts were freed of solvents using rotary evaporator (Buchi, USA) which converted the extract into dense form. The thick extract prepared this way was kept for future analysis. Extract by using supercritical fluid were prepared following the protocol of (Damjanović *et al*., 2005). The supercritical fluid equipment, model SFT-150 (supercritical fluid extractor incorporation USA) was fitted with a volume extractor, separator, a syringe pump as well as a syphonated CO_2 cylinder which could be pressurized to the required level. During each experiment, 200 g of Freeze-dried fennel seed powder was put inside the extraction vessel. The working conditions adapted for the experiment were 3000 psi pressure, 60°C temperature and 4 hours extraction time.

Table 1. Treatments for preparing fennel extracts.

Extraction technique	Solvent	Treatments	Time (min) Pressure (psi)
Conventional Solvent Extraction (CSE)	**Ethanol**	$Nutraceutical_{CSE}$	90 minutes
Supercritical Fluid Extraction (SFE)	**CO_2**	$nutraceutical_{SFE}$	3000 psi

Efficacy trials: For the determination of preventive potential of fennel extracted essential oil against selected disorders such as ulcer, inflammation, liver toxicity and nephrotoxicity, bio-efficacy trial was planned. The study was approved by Institutional Biosafety Committee (No 6426, Dated 30-11-2018). For the study, seventy-five rats (age around 8 weeks, weight 150-200gm, male rats) were purchased and kept in the animal room located in National Institute of Food Science and Technology (NIFSAT), Faculty of Food, Nutrition and Home Sciences (FFNHS), University of Agriculture, Faisalabad (UAF). The environmental conditions for instance, temperature (23±2°C) and relative humidity (55±5%) as well as twelve hour light-dark cycle were maintained during the study period (NIH Publication No. 8023, revised 1978). At the start, few rats were slaughtered to get the baseline values for the studies. All the rats (seventy five) were further divided randomly into five different studies with three groups in each study constituting a total of 15 groups with five rats in each. The detail description of five studies with three groups each is given in figure 1. These five studies were categorized as normal, ulcerogenic, inflammatory, nephrotoxic and hepatotoxic. Study I (normal) included rats which were fed on normal diet, while in all other studies *i.e.* II, III, IV and V, different agents were used to induce ulcer, inflammation, hepatotoxicity and nephrotoxicity. Ulcer was induced by feeding rats on

aspirin rich diet in study II. Likewise, Carrageenan, Carbon Tetrachloride and Gentamicin was used to induce inflammation, hepatotoxicity and nephrotoxicity in study III, study IV and study V respectively (Table 2). Each study was further divided into three groups (D_0, D_1, and D_2) based on the fennel essential oil obtained by two extraction methods *i.e.* $nutraceutical_{CSE}$ and $nutraceutical_{SFE.}$ During trial period, the administration of nutraceutical diet to the experimental rats via oral gavage was ensured to study their therapeutic potential. At the end of bio-efficacy, all night fasted rats were slaughtered. Blood samples of the slaughtered animals were amassed via cardiac puncture. For the serum collection, the blood samples were subjected to centrifugation at 4000 rpm for 6 minutes. The obtained sera samples were subjected to various biochemical assay through Microlab-300, (Merck, Germany).

Table 2. Scheme of studies conducted during *In Vivo* trials.

Studies	Groups	Diets	
Study I	Control	Normal diet	
Study II	Ulcerogenic	Aspirin enhanced diet	(350 mg/kg body weight)
Study III	Inflammatory	Carrageenan supplemented diet	(5%)
Study IV	Hepatotoxic	Carbon tetrachloride enriched diet	(4 mL/kg body weight)
Study V	Nephrotoxic	Gentamicin augmented diet	(100 mg/kg body weight)

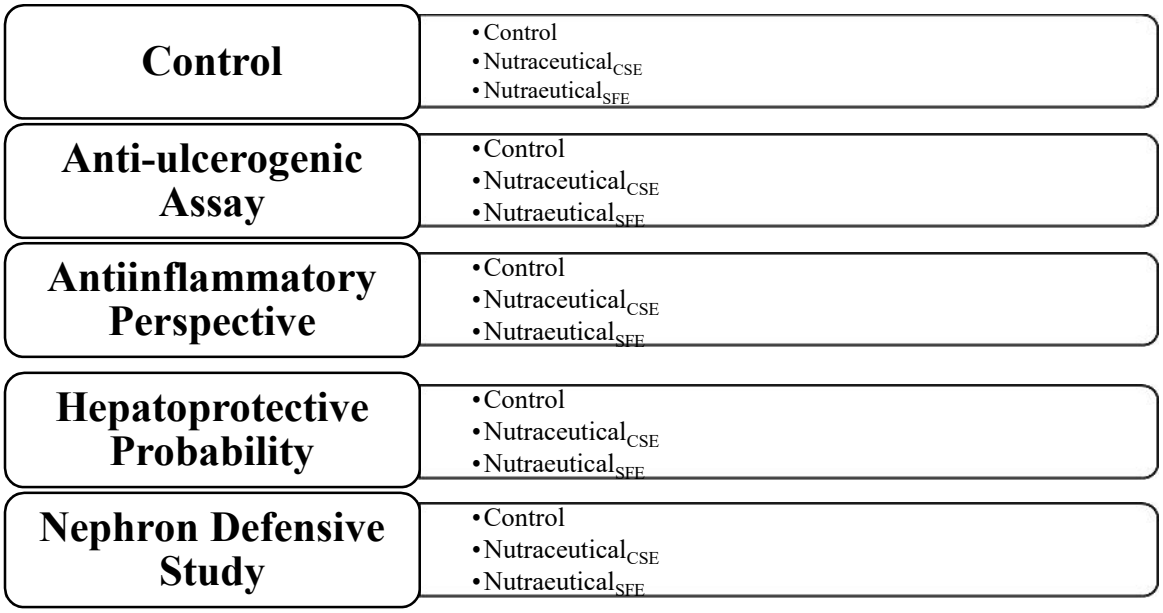

Figure 1. Efficacy plan

Anti-ulcerogenic assay: The anti-ulcerogenic potential of fennel extracts was estimated by measuring gastric secretion, gastric ulceration and ulcer index by following the methods of (Birdane *et al*., 2007, El-Metwally, 2014).

Determination of gastric secretion: For the determination of anti-ulcerogenic potential of fennel oil, stomach from each rat was ligated around both openings followed by the injection of 3 mL of distilled water. The obtained gastric juice was assembled in a test tube and subjected to centrifuge for 10 minutes at 3000 rpm. Graduated cylinder was used for measuring the volume of gastric juice. The total acid percentage of gastric juice was measured by titrating it against 0.01 N NaOH and using phenolphthalein as indicator and the results were expressed as mEq/L. The decrease in gastric juice percentage was computed by using the subsequent formula

$$\text{D ii g ju v(\%} = \frac{(A - B)}{A} \text{ x } 100$$

A = Gastric juice volume of control group

B = Gastric juice volume of induced and fennel treated group

The decline in gastric juice acidity was estimated for all treated groups by using subsequent formula.

$$\text{D ii t(a \%a} = \frac{a - b}{a} \text{x } 100$$

a = Total acidity of control group gastric juice

b = Total acidity of induced group gastric juice

Determination of gastric ulceration: For the determination of gastric ulceration, stomach from each animal were examined. For the purpose, stomach were opened longitudinally followed by a wash with saline and observed beneath the microscope for the presence of gastric ulcer. The gastric ulcer length was calculated for each group for the determination of ulcer index (UI). The ulcer index was estimated by analyzing severity of gastric mucosal lesions and graded as 1, (if the lesions are 1 mm or less), 2 (1-2 mm) and 32 (>2 mm), respectively. Then the UI was calculated by applying the subsequent formula;

$$U = [1\text{x (number of lesions of grade 1)} + 2\text{ x (number of lesions of grade 2)} + 3\text{ x}\frac{\text{(number of lesions of grade 3]}}{10}$$

Anti-inflammatory perspectives: The anti-inflammation perspectives of fennel extract such as paw edema was measured according to the protocol of (Ozbek, 2005). Carrageenan @ 5% was fed to the Sprague Dawley rats for a duration of 60 days with subsequent administration of both extracts. After the inflammation induction, paw edema was calculated on daily basis with displacement technique using plethysmometer.

Hepato-protective probability: In hepatotoxic study group, the protecting prospect of fennel extracts was determined in terms of liver safety biomarkers such as serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphate (ALP) and bilirubin contents according to the scheme described by (El Baz *et al*., 2014).

Nephron-defensive potential: For the evaluation of nephron-defensive potential among toxicity induced groups, creatinine, uric acid and urea were estimated as explained by (Shaheen *et al*., 2014). The GLDH method was adapted for serum urea estimation while creatinine was measured by Jaffe procedure.

Anti-oxidative stress: The *in vivo* anti-oxidative status of all studies was determined by antioxidant defense system of different enzyme assays such as catalase (CAT), glutathoine peroxidase (GPX), malondialdehyde (MDA), and superoxide dismutase (SOD) by the protocol described by (Sadeghpour *et al*., 2015). Blood samples obtained were collected in anti-coagulant coated test tubes to prevent blood coagulation and plasma was separated. The blood samples were washed with equal volume of 0.9% normal saline (NaCl) trailed by centrifugation at 3000 rpm at 4°C for 10 minutes. The lower layer (hemolysate) was then employed for the antioxidant enzyme assays.

Protein analysis: Protein analysis was conducted by calculating total protein, globulin, albumin and A/G ratio (albumin to globulin) of all the studies by methods as explained by (Al-Masri and Waffa, 2013). The globulin value was estimated by subtracting the albumin content from total protein. A/G ratio was estimated by diving the albumin level to the globulin content.

Organ to body weight ratio: Organ to body weight ratio such as kidney, liver, heart, lungs and spleen of all the studies was carried out by the guidelines of (Mohammed and Abbas, 2009). For this, the rats were cut apart and the organs such as kidneys, heart, liver, lungs, and spleen were instantly removed, washed, dried with sanitary tissue paper and assessed to find out the relative organ weights.

Serum electrolyte balance: The serum electrolyte balance was measured by estimating the sodium (Na), calcium (Ca) and potassium (K) content by the procedure defined by (Gulec *et al*., 2013).

Hematological analysis: Hematological analysis comprised of hemoglobin (Hb), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), packed cell volume (PCV), mean corpuscular volume (MCV) for all studies were accomplished according to the method of (Mansouri *et al*., 2015).

Statistical analysis: Experiment was conducted under CRD (completely randomized design) with treatment as one factor. All the analyses were performed in triplicates and repeated three times. Data obtained in all studies was analyzed using analysis of variance (ANOVA) and expressed as mean value ± standard deviation. Tukey's honest significant difference test was conducted to assess significant differences among experimental mean values (α=0.05) (Montgomery, 2019).

RESULTS AND DISCUSSION

It is evident on the basis of statistical analysis that fennel seed extracts imparted a momentous effect on gastric juice volume, gastric juice acidity and ulcer index in study II (ulcerogenic study) while on paw edema in study III (anti-inflammatory study). The results regarding anti-ulcerogenic assays are elaborated in figures 2-4. For paw edema, maximum decline was observed for supercritical fluid extract (30.43%) followed by conventional solvent extract (24.54%) (Figure 5). The results obtained for the alanine transaminase (ALT), alkaline phosphatase (ALP), Aspartate transaminase (AST), and bilirubin are presented in Table 3. The maximum decline in ALT, ALP, AST and bilirubin value was recorded during hepatotoxic study among all other studies as indicated in Figures 6-9. ALT, ALP, AST and bilirubin was reduced by 10.90 and 18.49%, 11.94 and 20.37%, 9.49 and 13.49%, and 12.39 and 16.57%, for D_1 and D_2, respectively.

The F values presented in Table 4 pointed out non-momentous effect of fennel seed extracts on serum urea in control and inflammatory studies, while uric acid and bilirubin were also non-significantly altered in study I and II. A reduction of 20.57% in D_1 and 28.40% in D_2 of urea level was found in kidney toxicity induced study (Figure 10). For uric acid and creatinine, maximum decline was found in study V, as 20.74 and 33.60%, and 23.88 and 14.18% in nutraceutical$_{CSE}$ and nutraceutical$_{SFE}$, respectively (Figure 11-12). The *in vivo* anti-oxidative perspectives of fennel extracts comprised of superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT) and malondialdehyde (MDA). The statistical examination presented in Table 5 explicated momentous differences due to treatments on superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase, and malondialdehyde (MDA) value. Superoxide dismutase enzyme is capable of converting the superoxide radicals into normal oxygen or hydrogen peroxide (H_2O_2) molecule. Glutathione is capable of protecting the organisms from oxidative damage by reducing the lipid peroxide and free hydrogen peroxide to their respective alcohol and water, respectively. Catalase (CAT) catalyzes the conversion of H_2O_2 to H_2O and O_2 molecules. Malondialdehyde (MDA) is an oxidative stress marker formed as a result of the lipid peroxidation of poly unsaturated fatty acids. The level of MDA in serum is an indicator of lipid peroxidation. The results regarding elevation in SOD, GPx and CAT level, whilst a reduction in MDA level are presented in Table 5. The statistical exploration highlighted noteworthy impact of treatments on total protein, albumin, globulin levels, whilst A/G ratio was non-momentously altered during the studies (Table 6).

The F value related to organ/body weight ratio, exhibited a non-momentous effect of treatments during the bio-efficacy trial period. Liver weight in all studies ranged from 4.09±0.14 to 4.17±0.14 g/100 BW. Right and left kidney weights ranged from 0.36±0.01 to 0.41±0.02 g/100 g BW and 0.36±0.01 to 0.42±0.02 g/100 g BW, respectively, during all studies. Similarly, the lungs to body weight changed non-significantly from 1.05±0.02 to 1.09±0.03 g/100g BW. Moreover, the fennel extracts did not influence weight of the heart and spleen to body weight ratio during all the studies which ranged from 0.28±0.01 to 0.34±0.01 and 0.26±0.01 to 0.32±0.01 g/100g body weight, respectively. Statistical analysis (F value) for the effect of treatments on calcium, sodium and potassium levels depicted a non-significant effect. The F value in Table 7 depicted significant effect of fennel seed extracts based treatments on hemoglobin and packed cell volume (PCV) during all studies, whilst mean corpuscle volume (MCV), mean corpuscle concentration (MCH), and mean corpuscle hemoglobin concentration (MCHC) was unaltered during all studies.

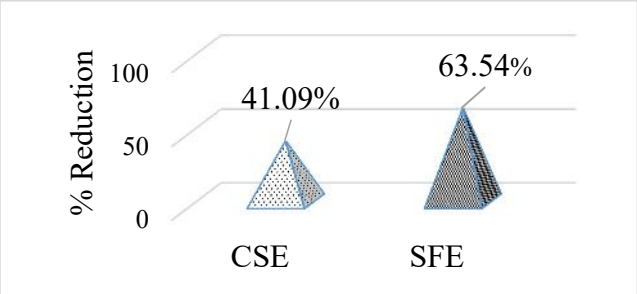

Figure 2. Decrease in gastric juice volume (%)

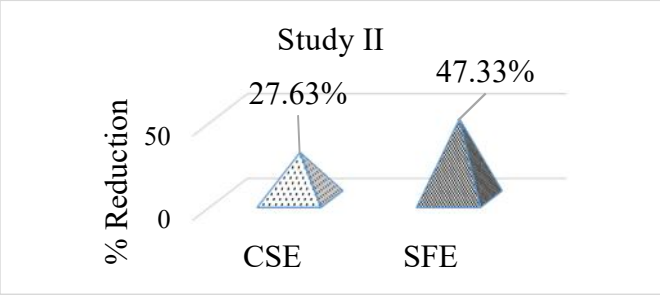

Figure 3. Decrease in gastric juice acidity (%)

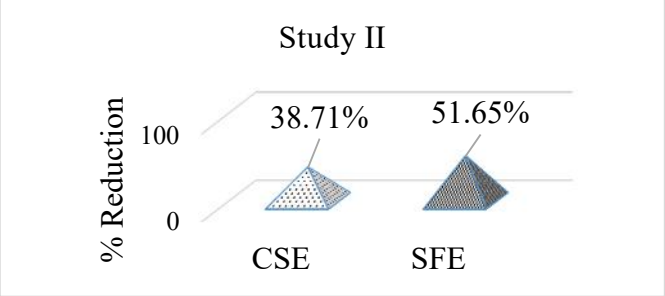

Figure 4. Decrease in gastric ulcer index (%)

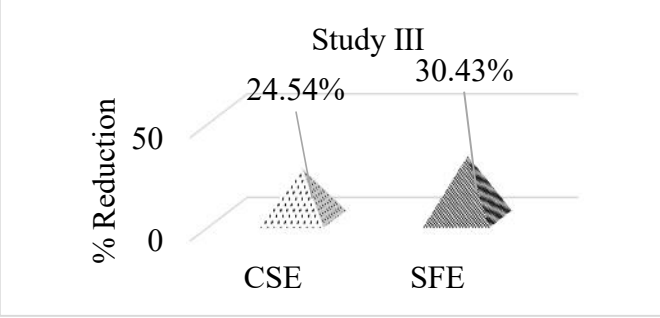

Figure 5. Decrease in paw edema (%)

Table 3. Effect of fennel seed extracts on hepatoxicity in different studies.

Parameters	Studies	Treatments			F Value
		D_0	D_1	D_2	
ALT (IU/L)	Study I	38.3±1.42	39.5±1.83	37.8±1.53	1.89^{NS}
	Study II	40.2±1.39	38.7±1.12	39.8±1.29	2.26^{NS}
	Study III	$44.9±1.66^{a}$	$42.8±1.83^{ab}$	$41.9±1.84^{b}$	4.81^{*}
	Study IV	$59.2±1.37^{a}$	$52.8±1.89^{b}$	$48.3±1.67^{c}$	73.6^{**}
	Study V	$43.6±1.92^{a}$	$42.0±1.70^{ab}$	$40.1±1.62^{b}$	6.60^{*}
ALP (IU/L)	Study I	124.6±3.60	123.4±4.28	126.3±4.81	0.73^{NS}
	Study II	128.3±4.45	131.5±3.80	125.8±3.63	3.39^{NS}
	Study III	$143.5±4.97^{a}$	$139.8±4.04^{ab}$	$135.1±5.78^{b}$	4.68^{*}
	Study IV	$214.7±5.21^{a}$	$189.1±4.37^{b}$	$171.0±4.94^{c}$	137^{**}
	Study V	$138.8±5.61^{a}$	$131.1±5.30^{ab}$	$128.6±5.20^{b}$	6.60^{*}
AST (IU/L)	Study I	76.8±2.22	74.3±2.15	77.1±2.23	3.29^{NS}
	Study II	71.1±2.47	73.6±2.55	74.4±2.58	3.01^{NS}
	Study III	$86.2±2.99^{a}$	$82.3±2.85^{ab}$	$81.7±2.83^{b}$	4.75^{*}
	Study IV	$109.5±2.66^{a}$	$99.1±2.40^{b}$	$94.7±2.30^{c}$	63.6^{**}
	Study V	$83.5±1.45^{a}$	$81.2±2.35^{ab}$	$79.6±2.76^{b}$	5.12^{*}
Bilirubin (mg/dL)	Study I	0.54±0.02	0.54±0.07	0.53±0.06	0.56^{NS}
	Study II	0.54±0.02	0.53±0.02	0.52±0.09	2.24^{NS}
	Study III	$0.68±0.02^{a}$	$0.66±0.02^{a}$	$0.62±0.02^{b}$	11.0^{**}
	Study IV	$1.32±0.05^{a}$	$1.15±0.03^{b}$	$1.10±0.03^{c}$	63.7^{**}
	Study V	$0.67±0.02^{a}$	$0.64±0.02^{ab}$	$0.63±0.02^{b}$	6.54^{*}

Means having same letter are not significantly different from each other
IU/L = International unit/Liter Study I = Control Study II = Ulcerogenic Study III = Inflammatory
Study IV = Hepatotoxic Study V = Nephrotoxic * = Significant ** = Highly significant
NS = Non-significant ALT= Alanine transaminase ALP = alkaline phosphatase AST = Aspartate transaminase

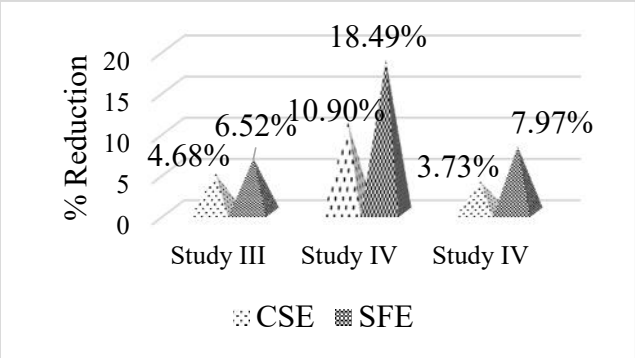

Figure 6. Decrease in ALT level (%)

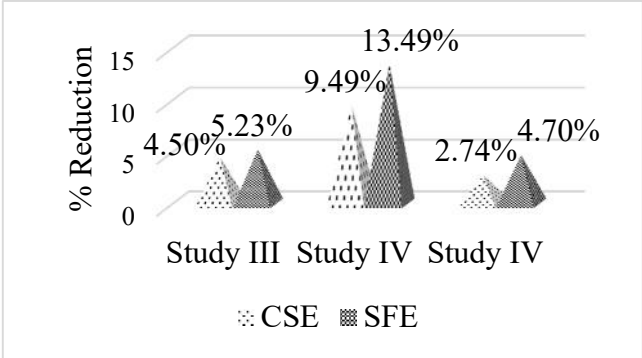

Figure 8. Decrease in AST level (%)

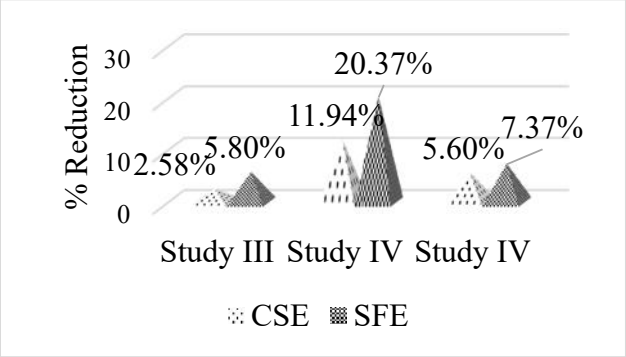

Figure 7. Decrease in ALP level (%)

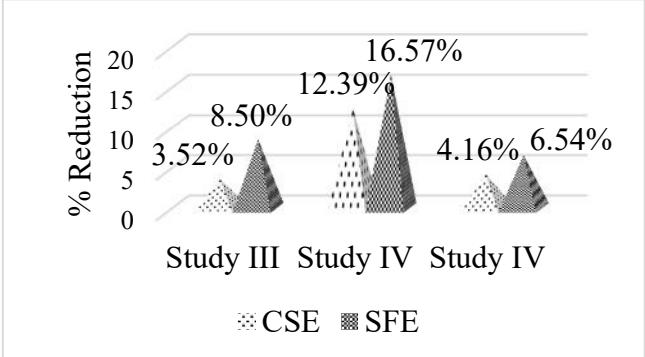

Figure 9. Decrease in bilirubin level (%)

Table 4. Effect of fennel extracts on nephrotoxicity in different studies.

Parameters	Studies	Treatments			F Value
		D_0	D_1	D_2	
Urea (mg/dL)	Study I	39.2±1.13	38.1±1.10	38.0±1.10	2.53^{NS}
	Study II	41.8±1.45[a]	40.2±1.40[ab]	39.4±1.37[b]	5.13^{*}
	Study III	35.4±1.23	34.6±1.20	33.9±1.18	2.47^{NS}
	Study IV	35.1±1.42[a]	34.0±1.22[ab]	33.1±1.23[b]	4.05^{*}
	Study V	65.0±2.10[a]	51.6±1.49[b]	46.5±1.61[c]	197^{**}
Uric Acid (mg/dL)	Study I	4.23±0.19	4.12±0.18	3.98±0.18	3.11^{NS}
	Study II	4.09±0.17	4.01±0.16	3.87±0.16	3.11^{NS}
	Study III	3.57±0.12[a]	3.46±0.12[ab]	3.35±0.12[b]	5.61^{*}
	Study IV	3.71±0.15[a]	3.57±0.14[ab]	3.49±0.14[b]	3.92^{*}
	Study V	6.22±0.20[a]	4.93±0.16[b]	4.13±0.13[c]	266^{**}
Creatinine (mg/dL)	Study I	0.75±0.03	0.73±0.03	0.71±0.03	2.47^{NS}
	Study II	0.77±0.04	0.74±0.03	0.73±0.03	2.68^{NS}
	Study III	0.85±0.03[a]	0.81±0.03[ab]	0.79±0.03[b]	7.77^{**}
	Study IV	0.83±0.03[a]	0.80±0.03[ab]	0.78±0.03[b]	4.00^{*}
	Study V	1.34±0.04[a]	1.15±0.04[b]	1.02±0.03[c]	119^{**}

Means having same letter are not significantly different from each other

Study I = Control Study II = Ulcerogenic Study III = Inflammatory Study IV = Hepatotoxic

Study V = Nephrotoxic* = Significant ** = Highly significant ^{NS} = Non-significant

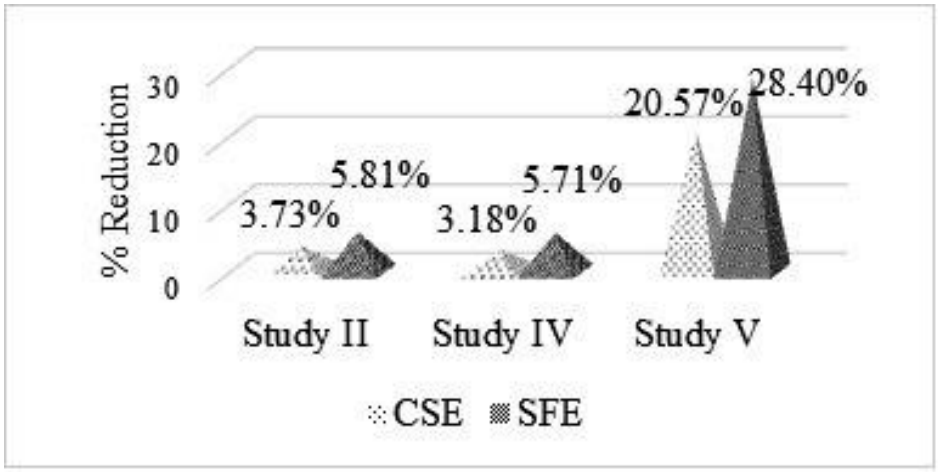

Figure 10. Decrease in urea level (%)

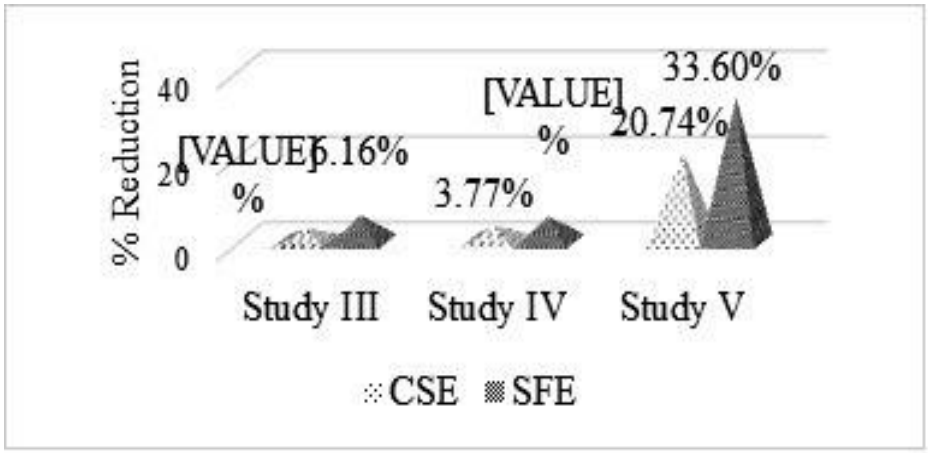

Figure 11. Decrease in uric acid level (%)

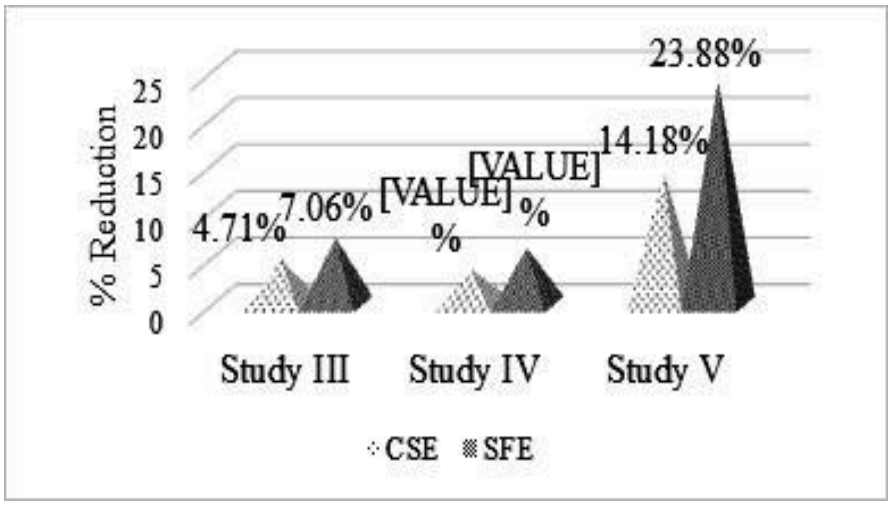

Figure 12. Decrease in creatinine level (%)

Table 5. Effect of fennel seed extracts on oxidative stress markers in different studies.

Parameters	Studies	Treatments			F Value
		D_0	D_1	D_2	
SOD (U/mL)	Study I	2.93±0.13^{b}	3.06±0.14^{ab}	3.18±0.15^{a}	5.22*
	Study II	2.85±0.13^{b}	2.97±0.14^{ab}	3.13±0.14^{a}	6.92*
	Study III	2.37±0.12^{b}	2.51±0.12^{ab}	2.62±0.14^{a}	6.60*
	Study IV	1.89±0.10^{b}	2.11±0.12^{a}	2.29±0.15^{a}	16.7**
	Study V	1.96±0.10^{b}	2.28±0.13^{a}	2.47±0.16^{a}	24.2**
GPX (mmol/dL)	Study I	16.6±0.96^{b}	17.4±1.01^{ab}	18.79±1.30^{a}	6.42*
	Study II	16.4±1.14^{b}	17.1±0.99^{ab}	18.28±0.95^{a}	5.27*
	Study III	17.9±1.24^{b}	18.9±0.98^{ab}	20.07±1.39^{a}	5.15*
	Study IV	14.2±0.99^{c}	16.1±0.85^{b}	18.46±1.28^{a}	26.6**
	Study V	13.7±0.71^{c}	16.0±1.11^{b}	18.19±0.95^{a}	38.0**
CAT (U/mL)	Study I	273.2±9.46^{b}	281.3±9.73^{ab}	289.0±6.67^{a}	5.60*
	Study II	285.2±9.87^{b}	297.1±10.3^{ab}	304.2±11.2^{a}	5.60*
	Study III	298.6±11.0^{b}	311.4±10.8^{ab}	319.3±11.8^{a}	5.97*
	Study IV	242.7±8.94^{c}	269.7±9.94^{b}	286.6±10.6^{a}	33.9**
	Study V	186.5±6.87^{c}	212.8±7.83^{b}	237.4±8.76^{a}	104**
MDA (nmol/mL)	Study I	7.48±0.34^{a}	7.21±0.25^{ab}	6.93±0.40^{b}	4.43*
	Study II	8.65±0.60^{a}	7.82±0.55^{b}	7.15±0.55^{b}	13.0**
	Study III	8.37±0.40^{a}	8.01±0.46^{ab}	7.46±0.43^{b}	5.78*
	Study IV	10.5±0.67^{a}	9.46±0.67^{b}	8.12±0.56^{c}	26.1**
	Study V	10.9±0.89^{a}	9.72±0.60^{b}	8.54±0.59^{c}	22.5**

Means having same letter are not significantly different from each other
Study I = Control; Study II = Ulcerogenic; Study III = Inflammatory; Study IV = Hepatotoxic
Study V = Nephrotoxic; * = Significant; ** = Highly significant; ^{NS} = Non-significant
SOD = Superoxide dismutase; GPX = Glutathione peroxidase; CAT = Catalase; MDA = Malondialdehyde

Table 6. Effect of fennel seed extracts on protein analysis in different studies.

Parameters	Studies	Treatments			F Value
		D_0	D_1	D_2	
Total Protein (g/dL)	Study I	7.03±0.24^{b}	7.28±0.21^{ab}	7.42±0.21^{a}	5.23*
	Study II	6.85±0.13^{b}	6.97±0.19^{ab}	7.14±0.14^{a}	5.99*
	Study III	6.67±0.12^{b}	6.89±0.20^{a}	6.96±0.12^{a}	6.79*
	Study IV	5.81±0.27^{b}	6.23±0.29^{a}	6.47±0.26^{a}	9.95**
	Study V	5.54±0.19^{b}	6.07±0.27^{a}	6.28±0.29^{a}	15.1**

Albumin (g/dL)	Study I	3.21±0.11^{b}	3.32±0.12^{ab}	3.41±0.12^{a}	5.07*
	Study II	3.15±0.11^{b}	3.22±0.11^{ab}	3.37±0.12^{a}	6.65*
	Study III	3.38±0.08^{b}	3.47±0.12^{ab}	3.56±0.12^{a}	4.53*
	Study IV	2.94±0.10^{b}	3.12±0.11^{a}	3.19±0.13^{a}	8.60**
	Study V	3.09±0.07^{b}	3.16±0.09^{ab}	3.27±0.11^{a}	6.27*
Globulin (g/dL)	Study I	3.35±0.12^{b}	3.47±0.12^{ab}	3.59±0.12^{a}	6.64*
	Study II	3.23±0.13^{b}	3.35±0.15^{ab}	3.48±0.12^{a}	5.63*
	Study III	3.57±0.08^{b}	3.68±0.08^{ab}	3.76±0.17^{a}	4.12*
	Study IV	2.99±0.13^{b}	3.14±0.13^{ab}	3.23±0.11^{a}	6.41*
	Study V	3.31±0.13^{b}	3.46±0.14^{ab}	3.57±0.16^{a}	5.27*

Means having same letter are not significantly different from each other
Study I = Control Study II = Ulcerogenic Study III = Inflammatory Study IV = Hepatotoxic
Study V = Nephrotoxic * = Significant ** = Highly significant ^{NS} = Non-significant

Table 7. Effect of fennel seed extracts on hematological analysis in different studies.

Parameters	Studies	Treatments			F Value
		D_0	D_1	D_2	
Hb (g/dL)	Study I	11.90±0.86^{b}	12.5±0.87^{ab}	13.4±0.70^{a}	5.75*
	Study II	11.83±0.82^{b}	12.8±0.59^{ab}	13.3±0.92^{a}	5.91*
	Study III	11.70±0.81^{b}	12.7±0.66^{ab}	13.2±0.84^{a}	6.45*
	Study IV	11.74±0.74^{b}	12.2±0.56^{ab}	12.8±0.67^{a}	4.57*
	Study V	11.49±0.73^{b}	11.9±0.50^{ab}	12.8±0.74^{a}	6.38*
PCV (%)	Study I	35.8±1.45^{b}	37.5±1.78^{ab}	38.9±2.70^{a}	3.98*
	Study II	34.8±1.65^{b}	36.4±2.53^{ab}	38.2±1.55^{a}	5.15*
	Study III	34.1±1.62^{b}	35.9±2.11^{ab}	37.4±1.34^{a}	5.98*
	Study IV	35.6±1.68^{b}	37.0±1.67^{ab}	39.1±2.30^{a}	5.68*
	Study V	33.9±1.96^{b}	35.5±2.09^{ab}	37.6±1.78^{a}	6.29*
MCV (fl)	Study I	58.6±1.01	58.7±2.03	58.3±1.41	0.08 ^{NS}
	Study II	56.3±0.98	56.1±0.95	56.0±1.36	0.11 ^{NS}
	Study III	55.9±0.97	55.7±0.93	56.9±1.38	1.18 ^{NS}
	Study IV	57.4±0.99	56.9±1.97	56.9±1.38	0.29 ^{NS}
	Study V	56.8±0.98	56.3±1.95	55.8±1.35	0.77^{NS}
MCH (pg)	Study I	17.9±0.85	17.3±0.90	18.0±0.94	1.06 ^{NS}
	Study II	18.6±0.88	17.9±0.93	18.3±0.95	1.02 ^{NS}
	Study III	17.5±0.83	17.3±0.90	18.1±0.94	1.38 ^{NS}
	Study IV	18.1±0.86	18.4±0.96	17.8±0.93	0.84 ^{NS}
	Study V	18.0±0.85	17.8±0.93	18.3±0.95	0.59 ^{NS}
MCHC (%)	Study I	30.9±1.11	29.1±1.18	30.1±1.39	3.38 ^{NS}
	Study II	29.7±0.72	30.8±1.24	30.6±1.42	1.68^{NS}
	Study III	30.4±1.09	30.7±0.89	29.8±1.03	1.17^{NS}
	Study IV	28.9±0.70	29.5±0.85	29.7±1.37	1.05^{NS}
	Study V	30.4±1.09	30.8±1.25	30.2±1.04	0.53^{NS}

Means having same letter are not significantly different from each other
Study I = Control Study II = Ulcerogenic Study III = Inflammatory Study IV = Hepatotoxic
Study V = Nephrotoxic * = Significant ** = Highly significant ^{NS} = Non-significant
Hb = Hemoglobin PCV = Packed cell volume MCV = Mean corpuscle volume MCH = Mean corpuscle hemoglobin
MCHC = Mean corpuscle hemoglobin concentration

An imbalance between gastric mucosal defense system and offensive factors leads to the incidence of gastrointestinal ulcer (Birdane *et al*., 2007). Different mechanisms are associated with the gastric protection

potential of fennel seed essential oil depending upon the method of ulcer induction. Anti-secretory potential of certain medicinal plants including fennel is the chief characteristics for the prevention of gastric lesions caused by increased gastric acid content. The hyper acid secretion is induced by the stimulation of antral gastric mucosal receptors followed by vagus-vagal activation via pylorus ligation (Baggio *et al*., 2003). The outcomes from previous studies revealed that fennel suspension owns the aptitude to prevent the basal gastric acid secretion and ulceration. Such outcomes let fennel be used either alone or in a blend with other medicinal plants for the prevention of hyperacidity in pharmaceutical industry. Fennel supplementation also reduced elevated MDA (malondialdehyde) level, end product of lipid peroxidation, formed due to oxidative damage of the gastric tissues confirming that the plant possess antioxidant activity (Al-Mofleh *et al*., 2013). Non-steroidal anti-inflammatory drugs are responsible for ulceration either by topical damage to the mucosal berries or the decline in the mucosal prostaglandin contents (Firulescu *et al*., 2010). Fennel administration causes a momentous increase in gastric wall mucus leading to gastric protection. The gastric mucus lining is believed to be significant for the prevention as well as repair of the gastric epithelium (Odabasoglu *et al*., 2006). Another group of scientists lead by Massimiliano conducted experiment for protection of gastric mucosa against chemically-induced lesions through fennel administration. Significant gastro-protective potential of fennel essential oil and its chief component anethole was found at dose level of 50 and 100 mg/kg in ethanol induced rat models. Ethanol causes vaso-congestion with vascular stasis and hyper release of ROS leading to mucosal damage. The antiulcer potential of the fennel can be attributed to the antiplatelet and vaso-relaxant effect through maintenance of adequate blood supply in the gastric mucosa (Tognolini *et al*., 2007). Similar evidences have been found for the gastro-protective effect of fennel against ulcer at different dose levels (Al-Mofleh *et al*., 2013; Coelho-de-Souza *et al*., 2013; Marinov and Valcheva-Kuzmanova, 2015).

Kataoka *et al*. (2002) demonstrated the anti-inflammatory potential of fennel. The conclusions of the experiment depicted that the fennel seed extract obtained with methanol as a solvent prevent from inflammation by lipoxygenase and cyclooxygenase pathways. (Nassar *et al*., 2010) measured the anti-inflammatory potential of fennel extracts and it depicted that the methanol extract acquires significant activity. A dose level of 1500 and 2000 mg/kg decreased the weight of carrageenan induced edema by providing a protective effect of 28 and 47%, respectively, while ibuprofen as a control demonstrated a protective effect of 52.23%. Anethole as well as estragole, monoterpene position isomers, are components of volatile oils of many fragrant plants, were studied during a project conducted by Ponte with his colleagues, with the intention to assess their anti-inflammatory potential. Both were fed to the mice models at three dosage levels *i.e*. 3, 10 and 30, mg/kg body weight. Anti-inflammatory activity was assessed after 60 and 240 minutes among carrageenan induced edema mice models and it was revealed that anethole inhibited edema at all dose levels while estragole confer inhibition from 60 to 120 minutes after induction at two higher doses *i.e.* 10 and 30 mg/kg BW (Ponte *et al*., 2012).

Recently a study was planned with the objective to ass the anti-inflammatory impact of fennel main constituent *i.e.* anethole against acute lung injury induced by lipopolysaccharide (LPS). It was concluded that anethole possess the anti-inflammatory potential through regulation of Th17/Treg responses. Hence it can be inferred that this plant could attribute healing potential on disorders involving inflammation (Zhang *et al*., 2018).

The uplift in liver biomarker enzymes from the hepatocytes into the blood circulation because of administering CCl_4 is due to the membrane rupture and cellular damage. Liver protective potential of fennel against liver toxicity is probably due to the inhibition of lipid peroxidation as well as to the enhancement of antioxidant and detoxification enzymes levels (El Baz *et al*., 2014). The data obtained in the present project is found to be in harmony with the previous work done from time by time (Ozbek, 2005; Qiang *et al*., 2011; El Baz *et al*., 2014; Rabeh *et al*., 2014). They worked for the assessment of liver protective probability of fennel essential oil (FEO) in rats with injured liver induced by carbon tetrachloride (CCl_4). The liver toxicity was observed to be repressed by administration of fennel oil as evidenced by reduced ALT, AST, ALP and bilirubin content. Administration of CCl_4 caused an uplift in AST, ALT, ALP and bilirubin from 103.33±026.41 to 1392.50±300.50 U/L, 77.33±019.79 to 1269.66±231.39 U/L, 361.50±15.20 to 757.66±70.94 U/L and 0.125±0.015 to 0.153±0.035 mg/dL, respectively, which was further lowered down to 512.60±108.32, 552.00±119.18, 640.40±25.50 U/L and 0.128±0.016 mg/dL, respectively, upon fennel administration.

In another study, liver protective potential of fennel plants with different genotypes were assessed on male wister rats. Among different liver functioning tests, bilirubin and ALP were most prominently reduced. Bilirubin was declined to as low as 0.38 mg/dL, while ALP reduced to 229.15 U/L upon fennel administration (Agarwal *et al*., 2018).

Nephrotoxicity, a chronic impediment, is characterized by functional modifications in kidney function such as decreased glutathione depletion, lipid peroxidation, hindrance in protein synthesis, and mitochondrial impairment. The living cells on coming in contact with chemicals such as sodium oxalate, carbon tetra chloride (CCL_4), ethylene glycol and heavy metals

i.e. mercury, lead, and arsenic, induced nephrotoxicity (Pydi *et al*., 2011). Most of the therapeutic plants have few organic components that are responsible for conferring physiological functions such as alkaloids, tannins, flavonoids, glycosides and carbohydrates *etc*. The nephron protective potential of medicinal plants is due to the presence of these bioactive moieties. Fennel is a source of phenolics, essential oil, and ascorbic acid that help boosting health status. Flavonoids in fennel are oxidized by free radicals resulting in the formation of more stable and less reactive radical. These compounds can also inhibit the catalytic activity of several enzymes such as peroxidase, xanthine oxidase, and nitric oxide synthase, which are thought to be responsible in free radical generation, thus causing reduced oxidative damage to macromolecules (Shaheen *et al*., 2014). The data enumerated in the present project is found to be in agreement with the earlier work of (Al-Okbi *et al*., 2014; Shaheen *et al*., 2014) who worked in collaboration with their teams for the assessment of potential of fennel seed powder, oil or extracts on rat models with gentamicin induced kidney toxicity. The marked rise in levels of urea uric acid, and creatinine was significantly reduced by fennel administration.

Rezq (2012), determined the MDA, SOD and GPX level of male rats administered with high fat diet to elucidate the effect of fennel-based diet on oxidative markers. The MDA level momentously increased for group fed on high fat diet (positive control) (2.79±0.003 µmol/dL) compared with the negative control rats (1.25±0.002 µmol/dL). However, groups fed on high fat di*et al*ong with fennel supplementation in three percentages *i.e.* 5, 10 and 15%, significantly declined serum MDA levels (1.68 ±0.003, 1.48±0.004 and 1.41 ±0.004 µmol/dL, respectively). Similarly, the SOD level in negative control (95.30±0.14 U/dL) was momentously lowered down in positive control (55.95±0.17 U/dL). Feeding fennel-based diets markedly increased SOD activity as 68.98±0.17, 75.99±0.04, 87.88±0.04 U/dL, for 5, 10 and 15% supplementation, respectively). For GPX, positive control rats fed on high fat-diet experienced significant decline in the enzyme activity (8.09±0.16mmol/dL) in comparison with the negative control rats group fed on the basal normal diet (18.67±0.16 mmol/dL). GPX activity was increased significantly (9.28±0.03, 10.74±0.04 and 15.65±0.16 mmol/dL) by feeding rats with high fat-diet supplemented along with fennel seeds at different levels (5, 10 and 15%, respectively). Similarly, a decline in CAT activity in positive control as compared to negative control was markedly improved by fennel administration at three ratios *i.e.* 68.56±0.49, 42.19±0.27, 52.43±0.23, 56.53±0.17, and 63.04±0.23 mmol/dL, respectively. Similar trend was obtained by the work of (Al-Okbi *et al*., 2014; El Baz *et al*., 2014; Rabeh *et al*., 2014; Shaheen *et al*., 2014; Sadeghpour *et al*., 2015).

Conclusion: The current research unveiled the data regarding the pharmacological potential of fennel (*Foeniculum vulgare*) against ulcer, inflammation, hepatotoxicity, and nephron toxicity. During the *In Vivo* phase, a reduction of 51.65, 30.43, 18.49, 20.37, 13.49, 16.57, 28.40, 33.60 and 23.88% was found in ulcer index, paw edema, ALT, ALP, AST, bilirubin, urea, uric acid and creatinine by administration of supercritical fluid extract. From the research, it was concluded that fennel-based extracts (nutraceutical$_{CSE}$ and nutraceutical$_{SFE}$) are effective in alleviating various lifestyle related disorders. Furthermore, it is suggested that dietary supplementations with fennel should be adapted to take advantage of its pharmacological potential.

Future Directions: Fennel essential oil investigated in the present project offers a promising alternative to pharmaceutical drugs. Still there is a need to identify the exact mechanism of action of fennel's major constituents at cellular level that resulted in marked decline in different disease biomarkers. In this stance, future studies could be conducted to elucidate the molecular mechanisms lying behind the anti-ulcer, anti-inflammatory, hepato-protective and nephron defensive potential of fennel essential oil constituents.

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