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Evaluation of cardioprotective activity of *Lepidium sativum* seed powder in albino rats treated with 5-fluorouracil

Eman Taha Mohamed *, *Ghada Mohamed Safwat*

Biochemistry Department, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef, Egypt

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ABSTRACT

5-fluorouracil (5-FU) is a chemotherapeutic agent used for treatment of solid tumors. Cardiotoxicity is a major complication of 5-FU therapy. Therefore, the aim of the present study was to evaluate the possible cardioprotective potency of *Lepidium sativum* seed powder (LS) against 5-FU-induced cardiotoxicity and oxidative stress in albino rats. The rats were divided into three groups. Rats in the control group received saline daily for 8 days only. Rats in FU-treated group received saline orally for 8 days, then I.P. injected with 5-FU (150 mg\kg B.W) on the 5th day. Rats in LS-treated group were orally dosed with LS (550 mg\kg B.W\day) for 8 days, and on the 5th day rats were administrated with the same previous dose of 5-FU. 5-FU induced cardiotoxicity was assessed by a significant increase in serum concentrations of cTnI, CK-MB, lipid profile and a moderate elevation of cardiac MDA. 5-FU significantly decreased serum HDL-c and GSH concentration in cardiac homogenate. Its administration also resulted in the release of some inflammatory markers such as myeloperoxidase and Interleukin-1β (IL-1β). All 5-FU altered parameters were markedly ameliorated by LS pre-co-post-treatment. Results of the present study suggest that LS has a significant effect on the protection of the heart against 5-FU-induced cardiotoxicity through maintaining the antioxidant and anti-inflammatory activities.

1. Introduction

5-fluorouracil (5-FU) is an antimetabolite fluoropyrimidine analog of the nucleoside pyrimidine with antitumor activity. 5-FU is widely used systemically for breast, gastrointestinal, pancreatic and skin cancers (Rossi, 2013). The main mechanism of action is interfering with DNA synthesis and mRNA transcription. However, cardiotoxicity is one of the most important associated side effects resulting from its nonspecific cytotoxicity in cancer cells (Álvarez et al., 2012; Carrillo et al., 2015). Clinical cardiac toxicities associated with intravenous infusion of 5-FU covers a wide range of manifestations like angina, myocardial ischemia, congestive heart failure, myocardial infarction, pulmonary edema, and sudden death (Polk et al., 2014; Sorrentino et al., 2012; Tsavaris et al., 2002). Recent

* *Corresponding author.* Biochemistry Department, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef, Egypt. Tel.: +20 822327982; fax: +20 822327982.
E-mail address: dr_emantaha@yahoo.com (E.T. Mohamed).

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studies showed that the incidence of 5-FU-induced cardiotoxicity was in the range of 0–20% (Polk et al., 2014), and highlighted an increased risk of toxicity during the administration of higher doses of 5-FU with continuous infusion and not bolus administration. After administration, 5-FU follows different metabolic fates; more than 80% of the dose is inactivated by hepatic biotransformation, about 15–20% is excreted in the urine and only a small fraction remains available to exert its antineoplastic activity (Casale et al., 2004).

Different mechanisms of 5-FU induced cardiotoxicity are proposed, including direct drug or drug metabolite-mediated toxic action on myocytes (Mizuno et al., 1995), coronary vasospasm and thrombogenic effects (Bertolini et al., 2001). 5-FU induces the endothelial damage and extravasation of blood with the drug into cardiac tissue resulting in an inflammatory reaction and myofibril necrosis (Bertolini et al., 2001; Kumar et al., 1995). Another theory has suggested cardiotoxic impurities in the 5-FU formulation (fluoroacetaldehyde, generated in the alkaline solution of fluorouracil during storage, which may be converted to a cardiotoxic agent, fluoroacetate) (Arellano et al., 1998; Becker et al., 1999). Fluoroacetate enters the Krebs' cycle and converts into fluorocitrate, which inhibits the enzyme aconitase (Keller et al., 1996) causing citrate accumulation, disruption of the tricarboxylic acid cycle and severe impairment of energy production within the myocytes (Gradishar and Vokes, 1990). The pathogenesis of 5-FU induced cardiotoxicity may involve cellular damage due to the oxidative stress and the induction of apoptosis (Rashid et al., 2014). Accordingly, therapeutic interventions having antioxidant activity may be effective against oxidative stress associated with cardiovascular diseases.

Lepidium sativum (LS) is locally known as 'hub arachad' belonging to family *Brassiaceae*. Chemically, the plant seeds and leaves contain flavonoids and isothiocynates glycosides, essential oils, carbohydrates, proteins, fatty acids, β-carotene and vitamins like riboflavin, niacin and ascorbic acid (Yadav et al., 2010). The seeds are consumed in salad and as a spice (Maier et al., 1998). The plant leaves and seeds have been used in traditional medicine. The plant is reported to have hypoglycemic, antihypertensive, diuretic (Jouad et al., 2001; Patel et al., 2009) hemagglutinating, fracture healing (Eddouks et al., 2005; Yadav et al., 2011), anti-inflammatory (Raval et al., 2013), hepatoprotective (Abuelgasim et al., 2008; Al-Asmari et al., 2015), antioxidant (Agarwal and Verma, 2011; Zia-Ul-Haq et al., 2012) and anti-carcinogenic activities (Maghrani et al., 2005).

The present study was designed to investigate the cardioprotective effect of LS against 5-FU-induced cardiotoxicity in rats by studying some biochemical cardiac injury markers, antioxidant defense system, serum lipid profile and inflammatory markers.

2. Materials and methods

2.1. Chemicals

5-fluorouracil was obtained from ACDIMA International (AiT) Shanghai Xudong Haipu Pharmaceutical Co., Ltd. Reduced glutathione (GSH), malondialdehyde (MDA) and nitric oxide (NO) commercial kits were purchased from Bio-diagnostic Company for research kits, Egypt. Triacylglycerol (TAG), total cholesterol (TC) and HDL-cholesterol commercial diagnostic kits were purchased from Spinreact Company, Spain. Rat Troponin I (cTnI) (Catalog number KT-639), rat Creatinine kinase-MB isoenzyme (CK-MB) (Catalog number KT-12247) and rat myeloperoxidase (Catalog number KT-60345) immunoassay kits were purchased from Kamiya Biomedical Company, USA. Rat Interleukin-1β (IL-1β) (Catalog number K 0331212) ELISA kit was purchased from Komabiotech Company, Korea.

2.2. Plant material

Lepidium sativum L. seeds (known as garden cress) (LS) were obtained from Agricultural Research Center, Egypt. Garden cress seeds were dried and ground to powder. The suspension of Lepidium seed powder was made with a sufficient quantity of distilled water by a dose of 550 mg/kg B.W (Raval and Ravishankar, 2010). It was administered through the oral route with the help of a gastric gavage. Other non-mentioned chemicals used in the present study were of the highest analytic grade and purchased from Sigma-Aldrich Company, USA.

2.3. Animals and experimental design

Thirty adult male albino Sprague–Dawley rats (120–150g) were provided by the Helwan farm of laboratory animals Cairo, Egypt. Rats were kept in standard cages at room temperature (25 ± 2 °C) with a 12: 12 h dark-light cycle and humidity (70%). All animals were allowed free access to water and fed with uniformly basal diet. All experimental procedures were conducted in accordance with the guide for the care and use of laboratory animals and in accordance with the local Animal Care and Use Committee. After acclimatization, rats were randomly divided into 3 groups (n = 10). Animals in the control group received only saline daily for 8 days by oral gavage. Animals in FU-treated group received saline orally for 8 days, then were given a single dose of 5-FU (150 mg\kg B.W\I.P injection on the 5th day) (Blijham, 1991). Animals in LS-treated group received a suspension of LS seed powder (550 mg\kg\day) (Raval and Ravishankar, 2010) orally for 8 days and were injected with a single dose of 5-FU (150 mg\kg B.W\I.P) on the 5th day.

2.4. Blood sampling and tissue preparation

Blood samples were collected 24 hours after the last dose, and all rats were sacrificed by cervical decapitation. The obtained sera were monitored for lipid profile, cTnl and CK-MB activity. Heart tissues were excised after dissection of the animals and designated for biochemical analysis. The excised heart tissue (0.5 g) was homogenized in ten volumes of ice cold phosphate buffer (pH:7) until a uniform suspension was obtained. The homogenate was then centrifuged at 20,000 × g for 10 min at 4 °C using high speed cooling centrifuge. The clear supernatant was used for the assay of GSH, MDA, NO, MPO and IL-1β.

2.5. Biochemical assays

2.5.1. Serum analysis

Serum cardiac markers (CK-MB and cTnI) were measured according to instruction of diagnostic kits. Serum lipid profile (TAG,

TC and HDL-cholesterol) were assayed spectrophotometrically with the enzymatic colorimetric method described by authors (Burstein et al., 1970; Fossati and Principe, 1982; Richmond, 1973), respectively. VLDL-cholesterol was calculated as TG/5 while LDL-cholesterol was calculated by the formula [LDL-c = total cholesterol-HDL-c-VLDL-c] according to Fruchart (1982). Atherogenic index was calculated by the equation (LDL-c/HDL-c) described by Castelli and Levitar (1977).

2.5.2. *Heart tissue analysis*

Oxidant\antioxidant status in heart tissues includes GSH concentration was determined according to the method of Beutler et al. (1963). Lipid peroxides as MDA concentration were measured according to the method of Satoh (1978). NO concentration was determined by using biochemical method of Montgomery and Dymock (1961). Pro-inflammatory cytokines in cardiac tissue (MPO and IL-1B) were estimated according to instruction of diagnostic kits.

2.6. *Statistical analysis*

Statistical analysis was carried out using Graph Pad In stat software (version 3, ISS-Rome, Italy). One way analysis of variance (ANOVA) test followed by Tukey–Kramer (TK) multiple comparison post test were used. The values are expressed as mean ± standard error (SE). The p values below 0.05 were considered statistically significant.

3. Results

Results of cardiac biomarkers were reported in Figs. 1 and 2 and showed a significant increase in serum concentrations of cTnl (Fig. 1) and CK-MB (Fig. 2) in FU-treated group in comparison to control group. This elevation was significantly decreased in LS-treated group, indicating the cardioprotective role of the plant.

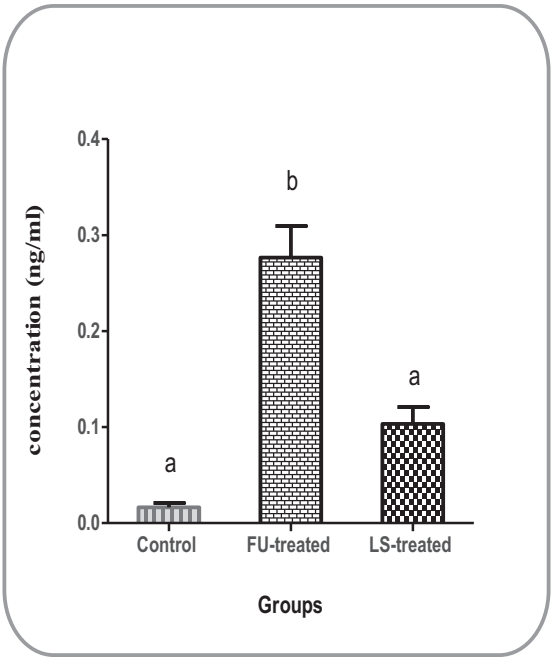

Fig. 1 – Serum troponin I (cTnI) concentration in different treated groups.

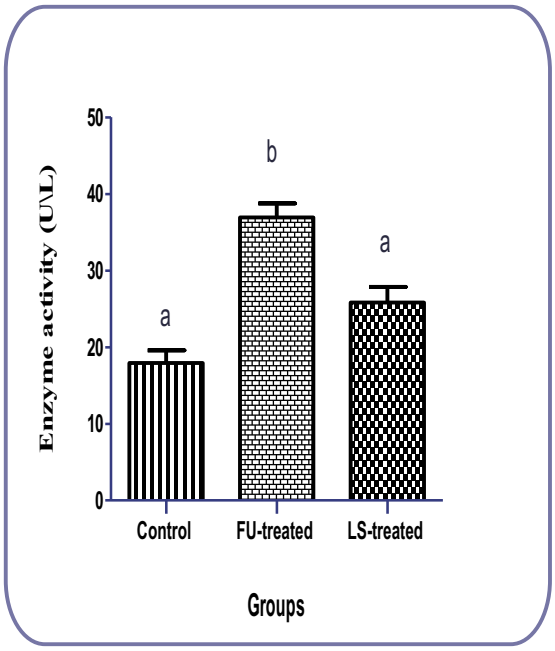

Fig. 2 – Serum CK-MB activity in different treated groups.

Table 1 represented the effects of 5-FU and LS treatment on serum lipid profile, including TAG, TC, HDL-c, VLDL-c LDL-c concentrations in different rat groups. 5-FU treatment significantly increased the serum TAG and TC levels in FU-treated group in comparison to control group indicating hypertriglyceridemia and hypercholesterolemia. Serum LDL-c and VLDL-c concentrations are significantly increased while the serum HDL-c concentration is significantly decreased in FU-treated group in comparison to control group. Pre-co-post-treatment with *LS* significantly improved the tested parameters. Fig. 3 illustrated the mean ratio of the atherogenic index ($LDL\text{-}_C/HDL\text{-}_C$) in different treated groups. Atherogenic index is a stronger risk predictor of cardiovascular. Results showed a significant increase in this ratio in the FU-treated group which was significantly decreased by *LS* treatment.

Table 2 represented the concentrations of cardiac homogenate MPO, IL-1β, GSH, MDA and NO in different groups. It showed a significant increase in inflammatory markers such as myocardial IL-1β and MPO activity and a significant decrease in GSH concentration in the FU-treated group when compared to control rats. LS treatment reversed the results of these tested parameters. However, cardiac MDA and NO concentrations were non-significantly increased in the FU-treated group in comparison to control group and were non-significantly decreased in the LS-treated group.

4. Discussion

5-fluorouracil is a commonly prescribed chemotherapy for a wide range of solid tumors. When elemental fluorine is reacted

Table 1 – Serum TAG, cholesterol, HDL-c, VLDL-c, LDL-c concentrations in different treated groups.

	TAG (mg/dl)	Cholesterol (mg/dl)	VLDL-c (mg/dl)	HDL-c (mg/dl)	LDL-c (mg/dl)
Control	64.39 ± 4.99^{a}	95.05 ± 5.09^{a}	12.88 ± 1.01^{a}	47.62 ± 2.60^{a}	34.55 ± 2.64^{a}
FU-treated	145.2 ± 8.59^{b}	214.1 ± 17.12^{b}	29.04 ± 1.72^{b}	25.43 ± 1.65^{b}	159.7 ± 13.15^{b}
LS-treated	97.14 ± 4.54^{c}	136.2 ± 8.68^{a}	19.43 ± 0.91^{c}	41.77 ± 2.51^{a}	75.01 ± 6.27^{c}

Means with different superscript letters are significantly different at $p < 0.05$. The data are presented as means ± S.E.

Table 2 – Cardiac concentrations of MPO, IL-1 β, GSH, MDA and NO in different treated groups.

	MPO (u/100 mg tissue)	IL-1β (pg/mg)	GSH (mg/g tissue)	MDA (nmol/g tissue)	NO (μ mol/L)
Control	0.410 ± 0.064^{a}	44.60 ± 0.95^{a}	40.24 ± 3.10^{a}	89.70 ± 6.54	41.15 ± 4.27
FU-treated	1.503 ± 0.169^{b}	82.90 ± 0.81^{b}	23.12 ± 2.04^{b}	104.3 ± 6.74	48.50 ± 3.66
LS-treated	0.817 ± 0.023^{a}	50.40 ± 2.15^{a}	33.27 ± 1.50^{a}	93.40 ± 3.81	42.18 ± 3.61

Means with superscript letters are significantly different at $p < 0.05$. The data are presented as means ± S.E.

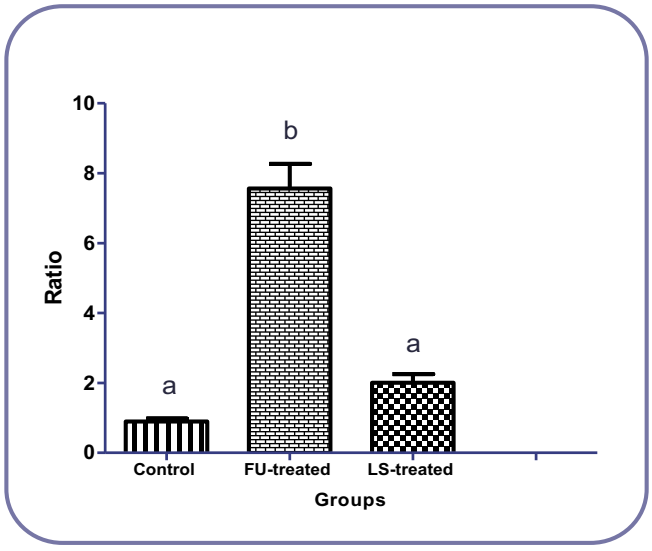

Fig. 3 – The mean ratio of the atherogenic index (LDL-c/HDL-c) in different treated groups.

with uracil, 5-fluorouracil is produced. Fluorouracil and its metabolites have a number of various mechanisms of action. It acts principally as a thymidylate synthase (TS) inhibitor. Following its administration, 5-FU is rapidly metabolized to 5-fluordeoxyuridine 5'monophosphate (FdUMP) which competitively binds to TS and blocks the methylation of uracil toward thymine which is required for DNA replication. So the cancerous cells undergo cell death via thymine-less death (Longley et al., 2003). Moreover, 5-FU is phosphorylated to triphosphate (F-UTP) and incorporated into RNA instead of uracil, thus blocking their transcription and stopping the growth of cancerous cells. Cytotoxic effects are also deriving from incorporation of FdUTP into DNA as well as F-UTP and 5-fluorocytosine into RNA. These metabolites are thought to influence calcium channel dependent membrane function, to interfere with mitochondrial phosphate metabolism, to alter contractile proteins, to cause oxidative damage and release of vasoactive substances like histamine and catecholamines, and lead to autoimmune mechanisms (Cianci et al., 2003).

The mechanisms of 5-FU-induced cardiotoxicity are hemorrhagic infarction, myocardial inflammatory reaction with interstitial fibrosis; arterial endothelial injury followed by thrombosis (Bertolini et al., 2001); increased metabolism leading to depletion of ATP, increased levels of superoxide anion and a decreased antioxidant capacity; arterial vasoconstriction and altered plasma levels of substances involved in coagulation and fibrinolysis (Dechant et al., 2012). Oxidative stress causes cellular damage, coronary artery spasm and the decreased affinity of RBCs to transfer oxygen, resulting in myocardial ischemia, cardiac arrest and sudden death (Polk et al., 2014).

Myocardium contains high concentrations of diagnostic markers for myocardial infarction and once metabolically damaged, it releases its content into the extracellular fluid (Farvin et al., 2004; Upaganlawar et al., 2009). Serum creatinine kinase (CK) and troponins are some of these markers. Assay of the CK-MB isoenzyme activity in serum is an important diagnostic indicator owing to its marked excess in myocardial tissue and its consequent sensitivity. The increased activity of serum CK-MB isoenzyme reflects the alterations in the plasma membrane integrity and permeability (Farvin et al., 2004). Cardiac troponin I (cTnI) is a cell-structural protein specific to myocardial tissue. cTnI is sensitive and specific biochemical marker of myocardial cell necrosis and has been considered as the gold standard marker for acute myocardial infarction and drug-induced cardiotoxicity (Gaze and Collinson, 2005). In the present study, 5-FU treated rats showed a significant elevation in the activity of serum CK-MB and cTnI level (Figs. 1 and 2), which indicated 5-FU induced myocardial necrotic damage and the leakiness of the plasma membrane. LS treatment resulted in lower activity of the CK-MB and cTnI level in serum. It was demonstrated that LS could maintain membrane integrity, thereby limiting the leakage of these biomarkers.

Hyperlipidemia plays an important role in cardiovascular diseases and the development of atherosclerosis (Hassarajani et al., 2007; Neil et al., 1990). A significant elevation in the serum TC, TAG, VLDL-c and LDL-c fractions along with a decrease in HDL-c were observed in 5-FU treated rats compared to control rats (Table 1). These observed changes concerning lipid profile come in agreement with Abdel-Hamid et al. (2011) and could be attributed to the enhanced lipid synthesis via cardiac cyclic

adenosine monophosphate (Paritha and Devi, 1997). Hypercholesterolemia and hypertriglyceridemia are mainly related to cardiac ischemia (Jackson and Beaglehole, 1995). The atherogenic effect of 5-FU as the mean ratio of LDL-c/HDL-c was significantly increased in FU-treated rats (Fig. 3). The higher atherogenic index, the higher is the risk of the cardiovascular disease (Karthikeyan et al., 2007). Atherogenic index indicates the deposition of foam cells or fatty infiltration of lipids in heart, coronaries, aorta, liver and kidneys.

The treatment with LS successfully restored the altered serum parameters as shown in Table 1. Halaby et al. (2015) reported that a diet supplemented with 5% and 10% LS seed powder improved the altered lipid profile in cisplatin-injected rats. Moreover, treatment with flavonoid and sapogenin extracts of LS lowered TC, TAG, LDL-c and AI values in hyperlipidemic rats (Shukla et al., 2015). Also, hypolipidemic and hypocholesterolemic effects of LS were mentioned by Umesha and Naidu (2012) and Althnaian (2014). The hypolipidemic effect of *LS* might be due to inhibition of absorption and enhanced excretion of lipids through the gastrointestinal tract (Chauhan et al., 2012). The hypocholesterolemic effect of LS might be attributed to inhibition of cholesterol biosynthesis via inhibition of HMG-CoA reductase, the rate-limiting enzyme that mediates the first step in cholesterol biosynthesis (Althnaian, 2014). The increased levels of the cardio protective lipoprotein; HDL-c after administration of LS concluded that the plant has a potent cardioprotective effect, and this effect may be due to the increased activity of lecithin: cholesterol acyl transferase (LCAT), an enzyme which plays a key role in incorporating the free cholesterol into HDL-c which is then catabolized by the hepatocytes (Shukla et al., 2015).

Oxidative stress is an overproduction of reactive oxygen species (ROS) with a deficiency of enzymatic and non-enzymatic antioxidants (Valko et al., 2007). Recent studies have suggested that the 5-FU-induced cytotoxicity is linked to the enhanced ROS formation as 5-FU increased intracellular levels of superoxide anion (O^{-}_{2}) (Afzal et al., 2012). As a consequence, the triggering of apoptotic program and cardiomyocyte damage were occurring (Lamberti et al., 2012). However, the exact molecular mechanisms of 5-FU cardiotoxicity have not yet been completely understood. These ROS may attack any type of molecules, but their main target appears to be polyenoic fatty acids within membranes forming peroxyl radicals. These radicals then attack adjacent fatty acids within membranes, causing a chain reaction of lipid peroxidation (Priscilla and Prince, 2009) and myocardial necrosis (Rajadurai and Prince, 2006). MDA is used as a marker of lipid peroxidation (Nielsen et al., 1997). It was elevated in the hearts of guinea pig (Durak et al., 2000) and rat (Eskandari et al., 2014) after 5-FU-treatment. Moreover, Kinhult et al. (2003) suggested that the arterial endothelial damage resulted from 5-FU therapy may be due to the generation of ROS. These findings agreed with our results as shown in Table 2 and indicate the occurrence of some degrees of oxidative stress and myocardial damage during 5-FU treatment.

Glutathione depletion is an indicator of oxidative stress. GSH, a tripeptide, is one of the cellular non-enzymatic antioxidant biomolecules. GSH has a direct antioxidant action by reacting with superoxide radicals, peroxy radicals and singlet oxygen followed by the formation of oxidized GSSG and other disulfides. In addition, it has a role in drug detoxification (Meister, 1988). When isolated cardiomyocytes were incubated with 5-FU, glutathione depletion was progressing as a consequence of ROS formation (Eskandari et al., 2014). In our experiment, the cardiac GSH level was significantly decreased as shown in Table 2 after 5-FU administration. Hence, it is confirmed that the cytotoxic mechanism of 5-FU is mediated via oxidative stress. LS has an antioxidant effect (Halaby et al., 2015; Olorunnisola et al., 2012) so it caused a significant elevation of the GSH level and a non-significant decrease of the MDA concentration in cardiac homogenate of LS-treated group. These results were parallel with the data obtained by Behrouzian et al. (2014) and Doke and Guha (2014).

Nitric oxide (NO) is an important signaling messenger known to play important roles in many physiological (such as host defense and homeostasis) and pathological (such as inflammation) conditions (Zhang et al., 2008). In mammalian cells, NO is generated in the cells by the NADPH-dependent oxidation of arginine to citrulline by the enzyme nitric oxide synthase (NOS) (Laskin et al., 1994). Overflow of NO may contribute to inflammatory reaction through nitrosation, oxidative damage, and enhanced release of inflammatory cytokines (Kanwar et al., 2009). Several studies have shown action of 5-FU on the production of NO (Matthews et al., 2001). Jung et al. (2002) suggested the efficacy of 5-FU to block NO production through the inactivation of IκB kinase in stomach cancer cells. While, Leitão et al. (2007) suggested an important role of NO in the pathogenesis of oral mucositis induced by 5-FU. In the present study, the 5-FU administration showed no significant change of NO level, which may be attributed to the formation of peroxynitrite by the reaction of NO with generated superoxide radicals (Gandhi et al., 2009).

Myeloperoxidase, an enzyme that is associated with the neutrophils, has been studied to form an index of the neutrophils infiltration into inflamed tissue. Myeloperoxidase, a green hemoprotein enzyme can use H_2O_2 generated by NADPH oxidase to oxidize halides (Cl^-, Br^- and I^-) to their corresponding hypohalous acids (an additional class of active oxygen metabolite) (Kettle et al., 1997). MPO generates different reactive oxidants and diffusible radical species that are capable of both initiating lipid peroxidation (Zhang et al., 2002) and stimulating post-translational modifications to target proteins, including halogenation, nitration, and oxidative cross-linking (Heinecke, 2003; Podrez et al., 2000). Consequently, it is linked to both inflammation and oxidative stress. In the present study, the significant increase in myocardial MPO activity is an indicative of 5-FU-induced inflammation of myocardial tissue and neutrophil infiltration. Restored levels of myocardial inflammatory markers in LS-treated group, indicated that the LS suppressed the neutrophil infiltration and inflammatory cytokine release to the injured myocardium.

Interleukin-1β (IL-1β) is an important pro-inflammatory cytokines with a relevant role in the inflammatory disorders. IL-1β is produced by monocytes, macrophages, endothelial cells and fibroblasts (Dung et al., 2009). Okamoto et al. (1998) and Curra et al. (2013) indicated that 5-FU is a potent inducer of several types of cytokines, including IL-1β. This was achieved in our results reported in Table 2 that showed a significant increase in IL-1β concentrations in cardiac homogenate of FU-treated rats in comparison to control ones. The increased MPO

and IL-1β concentrations in cardiac homogenate indicate the inflammatory effect of 5-FU (Soares et al., 2011). LS ameliorated that effect as it significantly decreased the concentration of MPO and IL-1β in the LS-treated group due to its anti-inflammatory activity.

Restored levels of myocardial MDA, GSH, MPO and IL-1β by LS Administration may be attributed to its high content in antioxidants (vitamin C, E, carotenoids, polyphenols and flavonoids) (Donno et al., 2013; Lee et al., 2013) and its anti-inflammatory activities (Calder, 2006; Lopez-Garcia et al., 2004). In this respect, gas chromatography–mass spectrometry analysis of LS showed the presence of alpha-linolenic acid (C18:3) which has an anti-inflammatory activity (Al-Asmari et al., 2015). The role of alpha linolenic acid is to downregulate the gene expression of inflammatory cytokines such as IL-6, IL-1β and TNF-α (Zhao et al., 2007).

5. Conclusion

Our results confirmed the existence of cardiotoxicity due to 5-FU therapy, which was indicated by an elevation of serum cardiac cTnI, CK-MB, altered lipid profile and atherogenic index with enhanced oxidative stress and the release of some inflammatory markers. It can be concluded that LS seed exerts cardioprotective activity that could be partly contributed by its antioxidant and anti-inflammatory activities. So, *Lepidium sativum* can be considered a candidate to protect against cardiotoxicity commonly encountered with 5-FU treatment.

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