

**SALVIA OFFICINALIS OIL IMPROVES THE ATHEROGENIC INDEX AND
CARDIOTOXICITY IN ALBINO RATS TREATED WITH 5- FLUOROURACIL****GHADA MOHAMED SAFWAT* AND EMAN TAHA MOHAMMED***Biochemistry Department, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef, Egypt.***ABSTRACT**

5-Fluorouracil is reported as the second most common chemotherapeutic agent after anthracyclines causing cardiotoxicity. Thirty male albino rats were divided into three groups. Rats in group I received saline daily for 8 days only and served as the control. Rats in group II received saline orally for 8 days, then administrated with 5-Fluorouracil (150mg\ kg B.W\once I.P injection at the 5th day). Rats in group III were orally dosed with *Salvia officinalis oil* (0.4ml\ kg B.W\day) for 8 days and at the 5th day, were administrated with the same previous dose of 5-Fluorouracil. 5-Fluorouracil induced a significant increase in serum concentrations of cTnI, CK-MB and MDA concentration in cardiac homogenate. 5-Fluorouracil significantly decreased GSH concentration in cardiac homogenate and altered serum lipid profile. Its administration also resulted in release of some inflammatory markers such as IL-1 β and myeloperoxidase. *Salvia officinalis oil* pre-co-post-treatment ameliorated the altered parameters caused by 5-Fluorouracil induced cardiotoxicity in rats.

KEYWORDS: 5- Fluorouracil, *Salvia officinalis oil*, Troponin, oxidative stress, heart, Rat.

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INTRODUCTION

The synthetic pyrimidine 5-Fluorouracil is widely used as an anti-tumor agent for treatment of solid tumor as colorectal, breast, gastric, pancreatic, prostate, and bladder cancers. Cardiotoxicity is a well-known side effect of 5-Fluorouracil¹. The reported incidence of cardiotoxicity related to 5-Fluorouracil ranges from 1.2% to 18%². Few laboratory or animal studies have been done to understand the effect of 5-Fluorouracil on the heart or vascular endothelium. The pathophysiology of 5-Fluorouracil associated cardiotoxicity might be arisen from vasospasms leading to ischemia, direct toxicity on the myocardium and endothelial damage leading to extravasation of the drug containing blood into myocardium resulting in myofibril necrosis and inflammatory reaction, activation of coagulation system, coronary artery thrombosis^{3,4}. Some investigators have concluded that 5-Fluorouracil effect on the myocardium is due to disruption of the Tricarboxylic acid cycle within the myocytes⁵. Studies of myocardial metabolism in guinea pigs showed that 5-Fluorouracil induced a decrease in myocardial high energy phosphate levels⁶. *Salvia officinalis*L. is one of the widest-spread members of the family Lamiaceae, have been used as a traditional herbal medicine against a variety of diseases. The plant is reported to have multiple pharmacological effects, including anti-inflammatory⁷ and antioxidative⁸ effects. Considering the potential protective role of herbs and spices as antioxidant agents, we have great interest in investigating whether treatment with *Salvia officinalis*L oil has any protective effect against 5-Fluorouracil-induced cardiotoxicity in rats by studying the biochemical markers, antioxidant defense system and lipid profile.

MATERIALS AND METHODS

(i) Chemicals

5-Fluorouracil was obtained from ACDIMA International (AiT) Shanghai Xudong Haipu Pharmaceutical Co., Ltd. Malondialdehyde (MDA), reduced glutathione (GSH) and nitric oxide (NO) commercial kits were purchased from Bio-diagnostic Company for research kits, Egypt. TAC, TC and HDL-C commercial diagnostic kits were purchased from Spinreact Company, Spain. Rat cTnl (Catalog number KT-639), rat CK-MB isoenzyme (Catalog numberKT-12247) and rat myeloperoxidase (Catalog number KT-60345) immunoassay kits were purchased from Kamiya Biomedical Company, USA. Rat IL-1 β (Catalog number K 0331212) ELISA kit was purchased from Komabiotech Company, Korea. *Salvia officinalis* oil (SO) was purchased from Cap-pharm for extracting natural oils, Egypt. Other non-mentioned chemicals used in the present experiment were of the highest analytic grade and purchased from Sigma-Aldrich Company.

(ii) Animals and experimental design

Thirty adult male Wister albino rats weighing 120-150g were obtained from Helwan farm of laboratory animals Cairo; Egypt. Rats were kept at optimum temperature (25 ± 5 °C) and humidity (75%). Rats were given uniformly basal diet and water ad libitum. 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 one week of acclimatization, rats were randomly divided into 3 equal groups (n= 10). Group I (Control) was kept as a control and received only saline daily for 8 days by oral gavage. Rats in group II (F-treated) received saline orally for 8 days then were given Fluorouracil (150mg/kg B.W \ I.P injection on the 5th day) as a single dose⁹. Rats in groupsIII (SO-treated) received *salvia officinalis* oil (0.4ml/kg B.W/day)¹⁰ orally for 8 days and were injected with 5-Fluorouracil (150mg/kg B.W \ I.P) single dose on the 5th day.

(iii) Sampling and 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.

Fresh heart tissue sample, 0.5 g was homogenized in ten volumes of ice cold phosphate buffer (pH 7) until a uniform suspension was obtained. The homogenate was centrifuged at 20000×g for 10 min at 4 °C. The supernatant was collected and stored at -20 C° for biochemical determination of MDA, NO, GSH, IL-1 β , MPO.

(iv) Serum analysis

Serum cTnl and CK-MB were measured according to instruction of diagnostic kits. Serum lipid profile includes triacylglycerol; total cholesterol and HDL-cholesterol were estimated with enzymatic colorimetric method described by authors ^{11, 12} and ¹³ respectively. VLDL-cholesterol was calculated as TG/5 while LDL- cholesterol was calculated by the formula [LDL-C = total cholesterol-HDL-C-VLDL-C] described by authors ¹⁴. Atherogenic index was calculated by the equation (LDL-C /HDL-C) described by authors ¹⁵.

(v) Heart tissue

Oxidant-antioxidant status in heart tissue includes GSH concentration was determined according to the method of ¹⁶. Lipid peroxidation as malondialdehyde (MDA) concentration was measured according to the method of ¹⁷. Nitric oxide (NO) was determined by using of biochemical method of ¹⁸. Pro-inflammatory cytokines in cardiac tissue includes MPO and IL-1 β was estimated according to instruction of diagnostic kits respectively.

(vi) 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 comparisons post test were used. The values are expressed as mean±standard error (SE). The *p* values below 0.05 were considered statistically significant.

RESULTS

1. Serum TAG, TC, HDL-C, VLDL-C, LDL-C concentrations in different rats groups.

Results in table 1 showed a significant increase in serum concentration of TAG and TC in F-treated group in comparison to control group indicating hypertriglyceridemia and hypercholesterolemia. Serum concentrations of some lipoprotein fractions like LDL-C and VLDL-C are significantly increased in F-treated group while serum concentration of HDL-C is significantly decreased in the same group in comparison to control group. Treatment with *salvia officinalis* oil significantly decreases serum concentration of the previous parameters and significantly increases serum concentration of HDL-C to be somewhat similar to normal control level.

Table 1
Serum TAG, TC, HDL-C, VLDL-C, LDL-C concentrations in different rats groups.

	TG (mg/dl)	Cholesterol (mg/dl)	VLDL-C (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)
Control	62.10± 5.08	92.90±5.004	12.42±1.02	45.47±2.49	35.01±4.38
F-treated	143.0±12.24 ^a	212.0±18.84 ^a	28.61±2.45 ^a	23.29±2.05 ^a	160.1±16.34 ^a
SO-treated	82.24±7.75 ^b	124.8±6.51 ^b	16.45±1.55 ^b	44.31±3.21 ^b	64.02±5.61 ^b

The data are presented as means± S.E and those with dissimilar superscript letters (significantly differ at *p* <0.001): (a) letter is significantly differing from control group; (b) letter is significantly differing from SO-treated group.

2. The mean ratio of atherogenic index LDL-C /HDL-C in different treated groups.

The LDL/HDL-cholesterol ratio has become recognized as a stronger risk predictor of cardiovascular. Results in table 2 showed a significant increase in this ratio in the F-treated group in comparison to control group. *Salvia officinalis* oil treatment (SO-treated group) significantly decreases this atherogenic index.

Table 2
The mean ratio of atherogenic index LDL-C /HDL-C in different treated groups

Ratio of atherogenic index LDL-C /HDL-C		
Control	F-treated	SO-treated
0.896±0.084	7.563±0.703 ^a	1.521±0.132 ^b

The data are presented as means± S.E and those with dissimilar superscript letters (significantly differ at $p < 0.001$): (a) letter is significantly differing from control group; (b) letter is significantly differing from SO-treated group.

3. Serum Ck-MB and cTnl concentrations in different rats groups.

Results of cardiac biomarkers are reported in table 3 and showed a significant increase in serum concentrations of CK-MB and cTnl in F-treated group in comparison to control group. This elevation is significantly decreased in SO group indicating the cardioprotective role of *salvia officinalis* oil.

Table 3
Serum Ck-MB and cTnl concentrations in different rats groups.

	Serum Ck-MB (ng/ml)	Serum cTnl (ng/ml)
Control	13.267±1.955	0.013±0.004
F-treated	32.266±2.162 ^a	0.243±0.024 ^a
SO-treated	19.067±2.99 ^b	0.053±0.038 ^b

The data are presented as means± S.E and those with dissimilar superscript letters (significantly differ at $p < 0.001$): (a) letter is significantly differing from control group; (b) letter is significantly differing from SO-treated group

4. Cardiac homogenate concentrations of MPO, IL-1B, GSH, MDA and NO in different rats groups.

Table 4 showed a significant increase in cardiac homogenate concentrations of MPO and IL-1 β in the F-treated group and a significant decrease in GSH concentration in comparison to control group. Treatment with *salvia officinalis* oil significantly increases the GSH concentration and significantly decreases the concentrations of MPO and IL-1 β in cardiac homogenate. MDA and NO concentrations of cardiac homogenate are non-significantly increased in the F-treated group in comparison to control group and are non-significantly decreased in the SO-treated group.

Table 4
Cardiac homogenate concentrations of MPO, IL-1B, GSH, MDA and NO in different rats groups.

	MPO (ng/ml)	IL-1 β (pg/ml)	GSH (mg/g tissue)	MDA (umol/g tissue)	NO (umol/g tissue)
Control	0.310±0.064	34.60±2.108	38.81±3.07	88.27±6.50	39.72±4.11
F-treated	1.403±0.194 ^a	72.90±1.963 ^a	21.69±1.64 ^a	102.8±0.655	47.07±3.14
SO-treated	0.563±0.081 ^b	39.13±1.940 ^b	32.34±1.55 ^b	96.21±2.14	43.77±3.90

The data are presented as means± S.E and those with dissimilar superscript letters (significantly differ at $p < 0.001$): (a) letter is significantly differing from control group; (b) letter is significantly differing from SO-treated group.

DISCUSSION

Due to its structure, 5-Fluorouracil interferes with nucleoside metabolism and can be incorporated into ribonucleic acid (RNA) and deoxyribonucleic acid (DNA), leading to cytotoxicity and cell death¹⁹. 5-Fluorouracil has diverse adverse effects such as cardiotoxicity, nephrotoxicity and hepatotoxicity which restrict its wide and extensive clinical usage. It causes marked organ toxicity

coupled with increased oxidative stress and apoptosis²⁰. Life- cardiac side effects by 5-Fluorouracil are still speculative and based upon cardiac symptoms. There is no evidence for a single mechanism responsible for 5-Fluorouracil induced cardiotoxicity, and the underlying mechanisms might be multifactorial. Several investigators postulated its direct toxic effects on cardiomyocytes and vascular endothelial cells. Others like^{21,22} thought that the severe impairment of energy production in tissues especially heart is the cause of death due to the cardiotoxicity of the Fluorouracil. Fluoroacetate is the metabolite of Fluorouracil enters the Krebs cycle and is then transformed into Fluorocitrate, which inhibits the enzyme aconitase. Inhibition of aconitase leads to a build-up of citrate in animal tissue and serum, and the production of ATP is severely limited. Hyperlipidemia has been ranked as one of the greatest risk factors contributing to the prevalence and severity of coronary heart diseases²³ and atherosclerosis. Hyperlipidemia is characterized by elevated serum total cholesterol (TC), low density lipoprotein (LDL), and very low density lipoprotein (VLDL) and decreased high density lipoprotein (HDL) levels. Among these, hypercholesterolemia and hypertriglyceridemia are closely related to ischemic heart disease²⁴. That was approved in our experiment as shown in table (1) due to 5-Fluorouracil administration. These changes could be attributed to enhanced lipid biosynthesis by cardiac cyclic adenosine monophosphate²⁵. High levels of LDL cholesterol show a positive correlation with myocardial infarction, whereas high levels of HDL cholesterol have a negative correlation²⁶, our results in table (2) reported the atherogenic effect of 5-Fluorouracil as mean ratio of LDL-c/HDL-c was significantly increased in F-treated rat group. *Salvia officinalis* oil succeeded to improve and significantly decreased the previous results tables (1&2) due to its antioxidant effects as oxidative stress also has been related to the pathogenesis of atherosclerosis. Elevated blood concentrations of cardiac biomarkers are a sign of cardiac injury which could be due to supply-demand imbalance, toxic effects, or hemodynamic stress²⁷. Serum Creatinine kinase (CK) and troponins are some of these markers. CK-MB isoenzyme activity is useful not only as an index of early diagnosis of myocardial infarction, but also any type of myocardial injury. The concentrations of these cellular markers in serum reflect the alterations in plasma membrane integrity and/or permeability²⁸. Because of its high sensitivity and specificity, elevated levels of troponin indicate myocardial damage but not the mechanism of damage²⁹. Troponin I (TnI) is a cell-structural protein specific to myocardial tissue. An increase of its level in serum is an early, sensitive and specific marker of myocardial injury, including minor myocardial damage, acute coronary syndrome, after coronary angioplasty, in heart failure, acute myocarditis and other clinical situations in which conventional markers of myocyte necrosis are often negative³⁰. That explained the cardiotoxicity of 5-Fluorouracil which achieved in our experiment as showed in table (3) as serum concentration of Ck-MB and c-TnI were significantly increased in F-treated group and significantly decreased in SO-treated group. This indicates the cardioprotective effect of *salvia officinalis* oil. One mechanism of the pathogenesis of 5-Fluorouracil induced cardiotoxicity may involve oxidative stress³¹. Reactive oxygen species (ROS) are under normal physiological conditions cleared by antioxidant defense systems. Increased ROS levels inside cells lead to oxidation of macromolecules, including lipids, nucleic acids, and proteins, thereby disturbing cellular functions³². MDA is a frequently used marker of lipid peroxidation³³, and MDA levels were elevated in guinea pig hearts after 5-Fluorouracil treatment³⁴, and slightly elevated (but not significantly) in isolated rat hearts after 5-Fluorouracil treatment³⁵. These findings agreed with our results as in table (4) indicate that some degree of oxidative stress and cellular damage takes place in animal hearts during 5-Fluorouracil treatment. GSH plays a crucial role in both scavenging reactive oxygen species and the detoxification of the drugs. GSH with its -SH group functions as a catalyst for disulfide exchange reactions, and plays a major role in H₂O₂ detoxification. In our experiment GSH level in cardiac homogenate was significantly decreased as shown in table (4) after 5-Fluorouracil administration. GSH depletion results in impaired cell defense and tissue injury³⁶ augmenting the cardiotoxicity of 5-Fluorouracil. *Salvia officinalis* oil has an antioxidant effect⁸; so it significantly increased the GSH concentration in cardiac homogenate in SO-treated group and non-significantly decreased the MDA concentration in the same group. Nitric oxide (NO) is a short-life molecule produced by the enzyme known as NO synthase (NOS), in a reaction that converts arginine and oxygen into citrulline and NO. Excess NO

may contribute to inflammation through nitrosation, oxidative damage, and enhanced inflammatory cytokines³⁷. Several publications have also shown effects of 5- Fluorouracil on the production of nitric oxide³⁸ but that not achieved in our results as shown in table (4) as its concentration in cardiac homogenate was not increased as a result of Fluorouracil administration. Myeloperoxidase (MPO) is an enzyme linked to both inflammation and oxidative stress. It is abundantly expressed in most leukocyte subspecies, including neutrophils and monocytes. The MPO has been implicated the initiation and propagation of atherosclerosis. MPO generates numerous reactive oxidants and diffusible radical species³⁹ that are capable of both initiating lipid peroxidation⁴⁰ and promoting an array of post-translational modifications to target proteins, including halogenation, nitration, and oxidative cross-linking^{41,42}. Interleukin-1 β is an important pro-inflammatory cytokine with a relevant role in the inflammatory disorders. IL-1 β is produced by a variety of cells types, including monocytes, macrophages, fibroblasts and endothelial cells⁴³. Our results in table (4) reported a significant increase in MPO and IL-1 β concentrations in cardiac homogenate in F-treated group in comparison to control one indicating the inflammatory disorder of 5- Fluorouracil^{44,45}. Administration of *Salvia officinalis* oil ameliorated that effect as it significantly decreased the concentration of MPO and IL-1 β in the SO-treated group due to its anti-inflammatory effect⁷.

CONCLUSION

Our results revealed the cardiotoxicity of 5- Fluorouracil which was indicated by an elevation of serum cardiac biomarkers, altered lipid profile and atherogenic index. *Salvia officinalis* oil was able to improve the oxidative and inflammatory effects of 5- Fluorouracil. This proved the antioxidant and anti-inflammatory effects of *Salvia officinalis* oil.

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