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Oxidative Response Associated with Treatment of Male Albino Rats with *Eruca sativa* Mill Leaves Extract and Correlations with Complete Blood Picture

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Abstract

The present study investigates the effect of the methanolic extract of E. sativa leaves on some oxidative stress markers as reactive oxygen species, total antioxidant capacity, metallothionein, cytochrome P450 (CYP1A1) and acetylcholinesterase concentrations and their correlations with hematological parameters (CBC profile) in male albino rats. Three experimental groups were used in this study which was 250 and 500 mg/kg body weight of 20 % methanolic of E. sativa leaves extract which given orally for 40 consecutive days and the third group treated with distilled water as a placebo-treated control group. The data of this study showed no significant differences in reactive oxygen species and cytochrome P450 concentrations between groups treated with E. sativa leaves extract and control group. Both dose 250 and 500 mg/ kg of E. sativa leaves extract could cause significant increasing in MT concentration while only 500 mg/ kg of E. sativa caused a significant increasing in acetylcholinesterase concentration compared with control group. In addition, there were non-significant differences in red blood cells count and indices while there were some significant differences in total and differential white blood cells count in treated male rats.

Key word: Eruca sativa, Hematological parameters, Oxidative response, Male rats

1- INTRODUCTION

Eruca sativa Mill. which is locally known as Jarjeer in Arabic is used in this study and is immensely used as vegetable and spice. All civilization used plants as sources of food because of their essential nutritional value, physiological effect, and as pharmaceutical materials (Okasha *et al*., 2008). So they considered as complementary and alternative medicine (Kamel, 2014). Medicinal plants constitute the main source of new pharmaceuticals and healthcare products (Ivanova *et al*., 2005).

E. sativa Mill, a member of Brassicaceae (Cruciferae) family (Al-Shehbaz *et al*., 2006; Lamy *et al*., 2008). It was used as a garden crop, spice and as a medicinal plant (Yaniv *et al*., 1998**)**. It is believed that plants belonging to the Brassicaceae family possess diversified medicinal and therapeutic properties including inhibition of tumorigenesis (Lynn *et al*., 2006). *E. sativa* is a native of southern Europe and central Asia where it has been cultivated since centuries (Ugur *et al.,* 2010). Leaves of *E. sativa* contains proteins, carbohydrates, Mg, Ca and Na while seeds contain proteins, fats, P, Ca, Na, K and Mg (Bukhsh *et al*., 2007).

E. sativa aids in digestion (Jin *et al*., 2009), used as a carminative and to alleviate abdominal discomfort and improve digestion. It has been reported that the ethanolic extract of *E. sativa* seed possesses potent antioxidant and renal protective and diuretic activities (Sarwar *et al*., 2007; Jin *et al*., 2009; Sadiq *et al*., 2014). Previous studies reported medicinal and therapeutic properties of *E.sativa* include antihyperlipidemic, antihyperglycemic, hepatoprotective (Alqasoumi *et al*., 2009; Jin *et al*., 2009; Rafatullah *et al*., 2008), antiplatelet and antithrombotic activity (Fuentes *et al*., 2014). Furthermore, *E. sativa* possesses anti-secretory, anti-inflammatory, anti-cancer, cytoprotective and anti-ulcer activity against experimentally-induced gastric lesions which is possibly due to prostaglandin-mediated activity and/or through anti-secretory activity (Alqasoumi *et al*., 2009; Khan & Khan, 2014; Saleh *et al*., 2016). So far, it has been reported that *E. sativa* seed and leaves are potent antioxidants (Sadiq *et al*., 2014; Koubaa *et al*., 2015; Abdul-Jalil *et al*., 2016).

The *E. sativa* The seeds and tender leaves are known in Arabian countries to increase sexual desire and are considered to be an aphrodisiac (Yaniv *et al*., 1998; Sarwar *et al*., 2007). It was reported that the ethanolic extract of *E. sativa* has an androgenic activity or stimulate testicular steroid production which enhances the preputial gland as well as increase spermatogenesis in the testis of male mice (Nowfel & Al-Okaily, 2017).Treating mice with *E. sativa* leaves extract had a significant increase ($P \leq 0.05$) in testosterone level, sperm activity and a significant decrease ($P \leq 0.05$) in sperm mortality and abnormalities (Hussein, 2013).

In addition, *E.sativa* act as anti-cancer (Alqasoumi *et al*., 2009; Khan & Khan, 2014; Shaban *et al*., 2016). Lamy *et al*. (2008) reported the antigenotoxic effect of *E. sativa* against human hepatoma (HePG2) cells which are attributed to the presence of erucin and erysolin compounds in the plant extract. Also, the previous studies were evaluated their possible curative effects and considered *E. sativa* seed extract a promising natural product from cruciferous vegetables against cancer as breast cancer (Shaban *et al*., 2016). *In vitro* antitumor study of *E. sativa* 70% ethanolic extract (*ES*-EE) as well as its compounds kaempferol and glucopyranoside proved their cytotoxic activity in 4 different human tumor cell lines: HepG2 (liver carcinoma), MCF7 (breast carcinoma),

HCT116 (colon carcinoma), and Hep2 (larynx carcinoma). On the basis of these results, the *ES*-EE as well as its compounds, seem to have potential as a novel cancer preventive agent (Michael *et al*., 2011). Furthermore, *E. sativa* seed powder and seeds oil, crude water extract, aqueous extract as well as a methanolic extract of *E. Sativa* displayed highest antibacterial activity and showed variable degrees of antifungal inhibition (Rani *et al*., 2010; Gulfraz *et al*., 2011; Rizwana *et al*., 2016).

Although medicinal and therapeutic benefit of *E. sativa* but some side effects of were mentioned by Bajilan & Al-naqeeb (2011) who assessed the effect of the hot aqueous extract of *E. sativa* leaves (250 and 500 mg/kg body weight) on the histological structure of kidney, liver and spleen in male albino mice which treated orally for 30 days. It was found that the weight of the liver for the two treated groups had a significant increase as compared with control and only the 500 mg/kg group shows a significant increase in weight of the kidney. Also, the histological study for the treated groups shows hypertrophy of the hepatic cells with the accumulation of glycoprotein granules. Sections of the spleen revealed the expansion of the white pulp and red pulp areas in addition to the presence of megakaryocytes and haemosiderosis in some areas. While sections of the kidney did not show remarkable changes. Reactive oxygen species (ROS) that includes hydrogen peroxide, hypochlorous acid, superoxide anion, singlet oxygen, lipid peroxides, hypochlorite and hydroxyl radical are involved in growth, differentiation, progression, and death of the cell. They can react with membrane lipids, nucleic acids, proteins, enzymes and other small molecules. Low concentrations of ROS has an indispensable role in intracellular signaling and defense against pathogens, while, higher amounts of ROS play a role in a number of human diseases (Rajendran *et al*., 2014). which could attribute to increased ROS formation and oxidative stress and impair the redox balance. We cannot avoid endogenous and exogenous free radical formation due to normal metabolism and exposure to environmental oxidants (Poljsak *et al*., 2013). The most important endogenous sources of oxidizing agents contributing to aging are mitochondrial: electron transport chain and nitric oxide synthase reaction. Nonmitochondrial sources of free radicals are Fenton's reaction, microsomal cytochrome P450 enzymes, peroxisomal beta-oxidation, and respiratory burst of phagocytic cells (Gilca *et al*., 2007).

A biological antioxidant has been defined as any substance that is significantly delayed or prevents the oxidation of that substrate (Pooja, 2016). An ideal antioxidant should be readily absorbed by the body and should prevent or quench free radical formation or chelate redox metals at physiologically relevant levels. It should work in aqueous and/or membrane domains and affect gene expression in a positive way (Rahman, 2007). Cellular redox homeostasis is carefully maintained by an elaborate endogenous antioxidant defense system, which includes endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), glutathione (GSH), proteins, and low molecular-weight scavengers, like uric acid, coenzyme Q, and lipoic acid (Halliwell, 2011).

Acetylcholinesterase (AChE) enzyme is essential in maintaining the normal function of the nervous system since it rapidly terminates the action of ACh released into the synapse (Kwon *et al*., 2009). One of the treatment strategies to enhance cholinergic functions is the use of AChE inhibitors that increase the availability of AChE in central cholinergic synapses (Mount *et al*., 2006; Kwon *et al*., 2009).

Both metallothionein (MT) and cytochrome (CYP1A1) (Cytochrome P450, family 1, subfamily A, polypeptide 1) are considered as general stress proteins, and their transcription has been shown to be affected by oxidative stress (Morel and Barouki, 1999; Andrews, 2000). MT is small, cysteine-rich and heavy metal-binding proteins, which participate in an array of protective stress responses (Ruttkay-Nedecky *et al*., 2013) and is an efficient scavenger of the hydroxyl radicals (OH–).Yeast and mammalian MTs can functionally substitute for SOD in protecting yeast from oxidative stress (Andrews, 2000).

Cytochrome P450 (CYP) is a superfamily of hemoproteins, with monooxygenase activity, which are biological catalysts that metabolize endogenous compounds such as hormones, bile acids, cholesterol, and xenobiotics like environmental pollutants and drugs (Santes-Palacios *et al*., 2016). CYP450 enzymes are essential for the production of cholesterol, steroids, prostacyclins, and thromboxane A2. They also are necessary for the detoxification of foreign chemicals and the metabolism of drugs. CYP450 enzymes are so named because they are bound to membranes within a cell (cyto) and contain a heme pigment (chrome and P). There are more than 50 CYP450 enzymes that absorb light at a wavelength of 450 nm (Wilkinson, 2005). These enzymes are predominantly expressed in the liver, but they also occur in the small intestine (reducing drug bioavailability), lungs, placenta, and kidneys (Slaughter & Edwards,1995).

The studies are limited in the literature about effects of *E. sativa* leaves extract alone on ROS, TAC, MT, AChE, cytochrome P450 (CYP1A1) concentrations. Therefore, this study designed to find out the effect of two high doses 250&500 mg/kg of the 20% methanolic extract of *E. sativa* leaves in ROS, TAC, MT, AChE, cytochrome P450 (CYP1A1) concentrations and their correlations with a hematological parameter in male albino rats.

2-MATERIALS AND METHODS:

2.1. Plant collection and identification

Fresh leaves of *E. Sativa* were obtained from the local markets, Hilla city, Iraq and identified by Taxonomist in the herbarium of the Biology Department, University of Babylon, Iraq.

2.2. Plant Extract Preparation

Leaves were dried at room temperature and then ground into fine powder form by the electrical grinder. Powdered samples stored in clean bags and preserved at 4ºC for further analysis (Sadiq *et al*., 2014). Organic extract of leaves was prepared using a mixture of two different

solvents with increasing polarity (methanol and distilled water). Dried leaves powder was weighed accurately and subjected to extraction in a ratio of 1gm leaves powder: 3ml solvent (20% methanol:80%.distelled water V/V) then homogenized by electrical blender for half hour at room temperature then filter by using gauze and dried at 40-45ºC (Sato *et al*.,1990). The extracted powder preserved in plastic bags at 4ºC until use to prepare the required doses, further analysis, and experiments.

2.3. Experimental animals

Male albino rats were used at 8-12 weeks (of) old, were allowed to adapt for 2-3 weeks. The animals caged in a cage at 60×50×60 cm. The rats randomly divided into three experimental groups each one has five rats.

2.4. Methods of intubation

Oral intubation of *E. sativa* 20 % hydro-methanolic extract (250 & 500 mg/kg) groups for 40 days were used as well as distilled water treated rats as placebo-treated control group. During the experiments, the animals were fed by a pellet and drinking water *ad libidum*.

2.5. Biochemical analyzes

Quantitative Sandwich ELISA technique was used for measuring serum ROS, metallothionein (MT1), acetylcholinesterase (AChE), cytochrome CYP1A1 (Cytochrome P450, family 1, subfamily A, polypeptide 1) concentrations according to manufacturing company (Elabscience Biotechnology Co., Ltd. China.).Whereas, Apak *et al.* (2008) method used to measure total antioxidant capacity (TAC).

2.6. Hematological profile

Complete blood picture was done by using full automated Hematological analyzer, Methic 18 Vet/Orphee/France.

2.7. Statistical Analysis

Values in tables and figures are given as mean ± S.E. Data were analyzed using SPSS version 22. Differences between groups were analyzed by a one-way analysis of variance (ANOVA). *p*-value ≤0.05 were considered significant. Also, canonical correspondence analysis (CCA) used in present study.

3- RESULTS:

3.1. Effect of E. sativa in ROS & TAC Concentration

The result that presented in Table (1) and Figure (1A) no statistically significant differences were observed in ROS concentrations between treated with both doses 250 and 500 mg/ kg of 20 % methanolic extract of E. sativa while both doses showed significant decreasing in TAC concentration as compared with and control group.

Table 1 Effect of methanolic extract of *E. sativa* in ROS and TAC compared in treated male rats (Mean ± S.E).

Group	ROS (pg/ml)	Total Antioxidant capacity (pg/ml)
Control	1644.67 ± 44.2 a	830.73 ± 12.00 a
E .sativa 250 mg/kg	1772.50 ± 28.44 a	738.79 ± 0.63 b
E. sativa 500 mg/kg	1564 ± 26.91 a	706.32 ± 22.15 b

Different letters refer to significance differences (P ≤ 0.05) between groups

Table 2 Effect of methanolic extract of *E. sativa* in MT, CYP1A1 and AChE level in treated male rats (Mean ± S.E).

Group	MTs (ng/ml)	CYP1A1 (ng/ml)	AChE (ng/ml)
Control	1525.83 ± 417.23 a	8.55 ± 2.90 a	7.87 ± 3.32 a
E.sativa 250 mg /kg	4338.67 ± 380.17 b	9.24 ± 3.19 a	8.18 ± 4.02 a
E.sativa 500 mg /kg	4029.83 ± 754.38 b	22.63 ± 13.53 a	37.13 ± 15.23 b

Different letters refer to significance differences (P ≤ 0.05) between groups

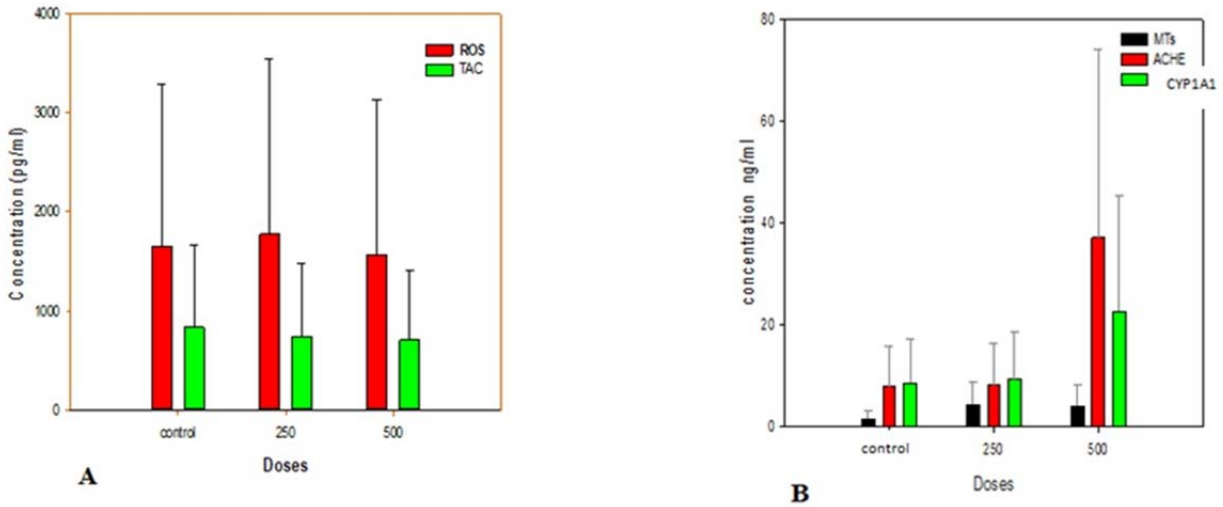

Figure 1. Effect of 20% methanolic extract of *E. sativa* 250 and 500 mg/ kg in the means of studied parameters. A- Effect of treatment in ROS & TAC. B- Effect of treatment in MTs, CYP1A, and AChE.

Table 3 Effect of 20 % methanolic extract of *E. sativa* in total and differential WBC count in treated male rats(Mean±S.E)

Group	Total WBC count (10^{*3}/ µl)	Monocytes (10^{*3}/ µl)	Lymphocytes (10^{*3}/ µl)	Granulocytes (10^{*3}/ µl)	Lymphocytes %	Monocytes %	Granulocytes %
Control	14.55 ± 1.05 a	١.3 ± 0.228 a	11.35 ± 0.35 a	2.60 ± 0.54 a	49.96 ± 1.07 a	25.65 ± 0.13 a	24.38 ± 1.14 a
E.sativa 250 mg/kg	8.73 ± 1.52 b	1.80 ± 0.52 a	5.38 ± 0.82 b	1.55 ± 0.19 a	63.15 ± 1.76 b	18.18 ± 2.66 b	18.68 ± 18.68 a
E.sativa 500 mg/kg	13.55 ± 1.53 a	2.30 ± 0.31 a	8.35 ± 0.68 c	2.85 ± 0.65 a	62.85 ± 3.10 b	17.15 ± 0.96 b	20.00 ± 2.90 a

Different letters refer to significance differences (P ≤ 0.05) between groups

Table 4
Effect of 20% methanolic extract of *E. sativa* in RBC count and indices in treated male rats (Mean ±S.E).

Group	Total RBC count (10^{*6}/ µl)	Hemoglobin Concentration (g/dL)	HCT %	MCH (pg)	MCHC (g/dL)	MCV (µm*3)	Platelates count 10^{*3}/µl	MPV µm ^{*3}	RDW %
control	8.6 ± 0.18 a	14.83 ± 0.19 a	43.75 ± 0.70 a	17.25 ± 0.20 a	33.90 ± 0.31 a	50.90 ± 0.24 a	803.25 ± 62.91 a	6.70 ± 0.06 a	18.18 ± 0.04 a
E.sativa 250 mg/kg	9.1 ± 0.52 a	15.93 ± 1.04 a	45.35 ± 2.46 a	17.20 ± 0.22 a	34.53 ± 0.39 a	49.90 ± 0.51 a	474.50 ±125.23 a	7.05 ± 0.36 a	18.93 ± 0.41 a
E.sativa 500 mg/kg	8.7 ± 0.62 a	15.1 ± 1.27 a	44.00 ± 3.04 a	17.38 ± 0.46 a	34.05 ± 0.52 a	50.98 ± 0.96 a	774.50 ±146.07 a	6.50 ± 0.19 a	18.40 ± 0.46 a

Different letters refer to significance differences (P ≤ 0.05) between groups

3.3. Effect of E. sativa on hematological parameters

3.3.1. Effect of E. sativa in Total and Differential WBC Count

There was significant ($p<0.05$) decrease in total WBC count in group treated with 250 mg/kg of *E. sativa* as well as significant ($p<0.05$) decrease in lymphocyte count and percentage of monocytes in both treated groups (250 & 500 mg/kg) of *E. sativa* while percentage of lymphocyte was significantly increased ($p<0.05$) in both treated groups as compared to normal control group. Also, there was no significant difference in the percentage of monocytes, granulocytes, and percentage of granulocytes (Table 3 and Figure 2).

3.3.2. Effect of E. sativa in RBC Count and Indices

The hematological parameters (RBC, HGB, HCT, MCH, MCHC, MCV, Platelets, MPV, RDW) did not record any significant alterations in any of *E. sativa* administered groups (Table 4).

In control group, the sum of all canonical eigen values was 0.256. ROS were closely related with granulocytes and MCH while AChE correlated with lymphocytes. TAC correlated with monocytes and WBC while CYP correlated with granulocytes, MT had no correlation with other parameters except RDW and MCHC (figure -3-A). In the group treated with *E.sativa* leaves extract 250 mg/kg, the sum of all canonical eigenvalues was 0.005 where there was no correlation between parameters except MT with granulocytes and ROS and TAC were closely related with RBC (figure -3-B). In the group treated with *E.sativa* leaves extract 500 mg/kg, the sum of all canonical eigenvalues was 0.089 where there was no correlation between parameters except CYP with monocytes and AChE was closely related with RBC (Figure -3-C).

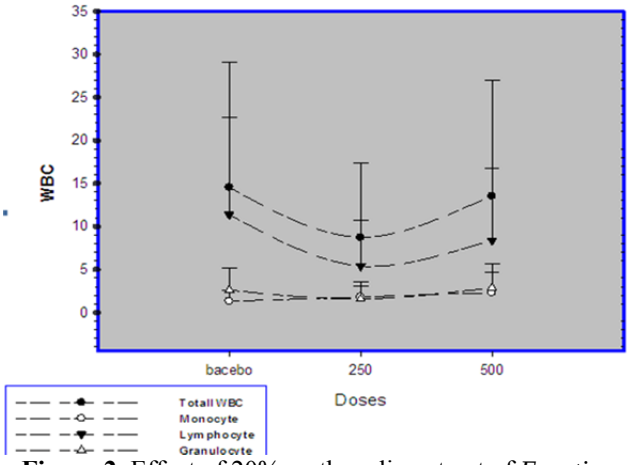

Figure 2. Effect of 20% methanolic extract of *E. sativa* 250 and 500 mg/ kg in the total WBC, monocytes, lymphocytes, and granulocytes count.

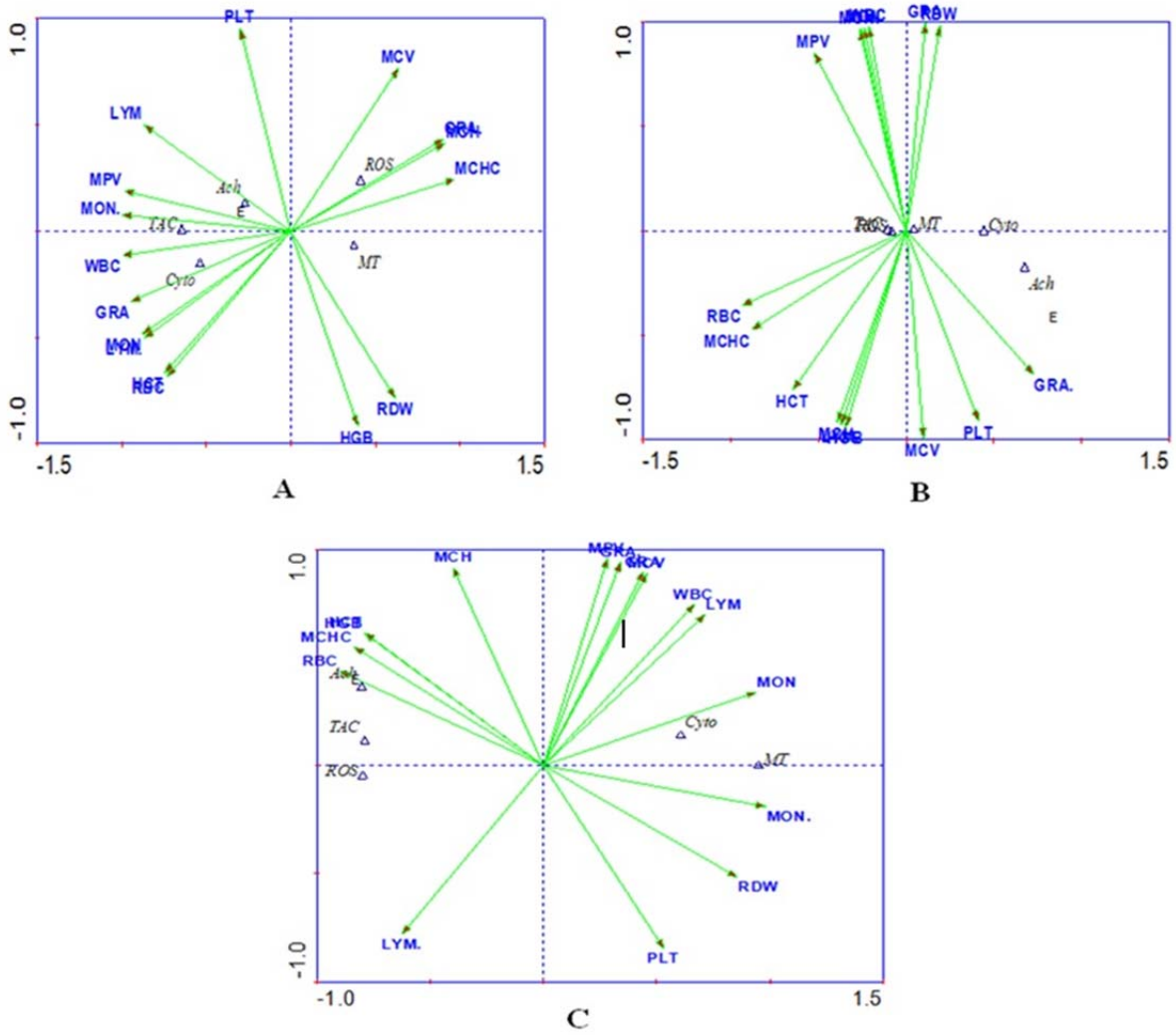

Figure 3. Canonical correspondence analysis (CCA) plot of the relations of variables to hematological parameters. The variables shown are related significantly to one or more of the CCA axes. A-Control group. B- Group treated with *E.sativa* leaves extract 250 mg/kg of. C- Group treated with *E.sativa* leaves extract 500 mg/kg.TAC=Total Antioxidant Capacity, ROS= Reactive Oxygen Species, MT= Metallothionein, CYP=Cytochrome P450 (CYP1A1), AChE=Acetylcholinesterase, WBC=White Blood Cells, MON= Monocytes, GRA= Granulocytes, LYM= Lymphocytes, RBC= Red Blood Cells, HCT= Hematocrit, MCH= Mean Corpuscular Hemoglobin, MCHC= Mean Corpuscular Hemoglobin Concentration, MCV= Mean Corpuscular Volume, HGB= Hemoglobin, RDW=Red Cell Distribution Width, PLT=Platelets, MPV= Mean Platelets Volume.

3.4. Relationships between Oxidative Markers and Hematological Parameters

In recent years, *E.sativa* has gained greater importance as a vegetable and spice around the world, it is also considered to be an important chemoprotective plant and used by in many countries for several medical purposes. For our knowledge, there are no studies concerning the effect of this plant alone on ROS, TAC, MT, AChE, cytochrome P450 (CYP1A1) and their correlations with hematological parameters in normal male rats. Therefore, the present study designed to assessment the effects of supplementation with 20% methanolic extract of *E.sativa* leaves on some oxidative biomarkers as TAC, ROS and stress proteins as MT and CYP1A1 in addition to AChE and their correlation with hematological parameters in male rats.

4. DISCUSSION

4.1. Effect of E. sativa in oxidative response (ROS, TAC, MT and CYP concentrations)

The altered activities of oxidative response were observed between groups treated with both concentration 250 and 500 mg/kg of 20% methanolic extract of *E. sativa* leaves include marked reduction of TAC although non-significant differences in ROS concentrations, significant increasing in MT1 concentration while there were non-significant increasing in CYP1A1 between groups. These findings showed that the serum TAC of treated rats was considerably lower than the control group. It has been claimed that serum antioxidants can be decreased compared to established normal values may be as a consequence of reducing some antioxidant level and concentration when protecting against disease (Rajendran *et al*., 2014). Antioxidant capacity of serum is the primary

measure and marker to evaluate the status and potential of oxidative stress in the body (Tiwari *et al*., 2013). In fact, the capacity of known and unknown antioxidants and their synergistic interaction is therefore assessed, thus giving an insight into the delicate balance in vivo between oxidants and antioxidants (Serafini & Rio, 2004). The antioxidant enzymes, SOD, GSH-Px, and catalase work together to eliminate active oxygen species and prohibit the harmful effects of oxidant molecules on tissues and cells. Small deviations in physiological concentrations of these enzymes may result in a defect of body defense system and vulnerability of biomolecules to oxidative damages (Goel *et al*., 2005).

Significant increasing in MT1 concentration may refer that *E. sativa* functions not only as a nutrient but also as a potent inducer of metallothionein. In this study, the attention is paid to metallothioneins (MTs) as small, cysteine-rich and heavy metal-binding proteins, which participate in an array of protective stress responses. MT protects cells from exposure to oxidants and electrophiles. Moreover, MT plays a key role in the regulation of zinc levels and distribution in the intracellular space. The connections between zinc, MT and cancer are highlighted (Ruttkay-Nedecky *et al*., 2013). Previous studies were evaluated their possible curative effects and considered *E. sativa* seed extract a promising natural product from cruciferous vegetables against cancer (Michael *et al*., 2011; Shaban *et al*., 2016).

Several reports are found in the literature about normalization antioxidant activity of alcoholic extract of *E. sativa* and decreased levels of lipid peroxidation (LPO) and nitric oxide (NO) (El-Gayar *et al*., 2014). Other results confirm the protective role of *E. sativa* leaves extract against oxidative stress induced by H2O2 in rats and showed significant decrease in the level of MDA and play an important role in decreasing the harmful effect of the free radicals in the animals studied (Abdalrahman *et al*., 2010, AL-Okaily and Nowfel, 2015). *E. sativa* extracts may exert their prophylactic and treatment role against oxidative stress produced by CCl4 by increasing/maintaining the levels of antioxidant molecules and antioxidant enzymes (Ahmed *et al*., 2013). However, supplementing the diet of roosters subjected to oxidative stress induced by hydrogen peroxide with rocket salad seeds powder resulted in significant improvement concerning histological traits involved in this experiment (Al-Daraji & Razuki, 2014).

These effects are attributed to a range of phytochemicals including flavonoids and glucosinolates, both of which are found in high levels in Brassicaceous crops (Jin *et al*., 2009). *E. sativa* used for its antioxidant constituents including glucosinolates, flavonoids, carotenoids, etc. (Barillari *et al*., 2005). It is well established that *E. sativa* seed extract (ES-SE) contains high yields of total phenolics, flavonoids, alkaloids, triterpenoids, antioxidant capacity, anti-lipid peroxidation, reducing power and DPPH radical scavenging effect (Abdel-Rahman *et al*., 2015). Phytochemical investigations of the aqueous extract of *E. sativa* fresh leaves afforded the presence of nine natural flavonoid compounds. On the basis of these results, the ES-EE as well as its compound kaempferol seem to have potential as a novel cancer preventive agent (Michael *et al*., 2011).

Although flavonoids are widely described as antioxidants and this activity is generally related to beneficial effects on human health Therefore, despite they expected scavenger action over free radicals an oxidants, kaempferol, quercetin, and isoquercitrin extracted from *E.sativa* could be very lesive to living organisms by acting over erythrocytes and may be other cellular types (Vellosa *et al*., 2011).

Additionally, ROS levels were not changed significantly may be because several free radicals cannot cross cell membranes due to their charge, or they are so short-lived that their diffusion is negligible. As such they cannot enter the blood from an affected region or organ. As there is no direct correlation between the oxidative stress markers in blood and their levels within the cells (Poljsak *et al*., 2013).

There were non-significant increasing in CYP1A1 in groups treated with *E. sativa* leaves extract and this result in consistence with Hanlon *et al*., 2008 who revealed that purified erucin, the dietary secondary metabolite contained in a rocket (*E. sativa* Mill) failed to influence cytochrome P450 activity in either human or rat liver.

4.2. Effect of E. sativa in AChE concentration

The results indicate that the crude 20% methanolic extract of *E. sativa* at a dose 500 mg/kg body weight exhibits AChE stimulatory activity which due to the phytochemical compounds of extract. The stimulation of AChE by the 20% methanolic extract of *E. sativa* is, to our knowledge, reported in this study for the first time. The need emerges of further studies aimed at understanding the effects of 20% methanolic extract of *E. sativa* on the regulation of acetylcholine release and the effects on the functioning of acetylcholine receptors.

4.3. Effect in Hematology

The hematopoietic system is one of the most sensitive targets for toxic compounds and hence it is mandatory to record any possible alterations resulting from a test substance. On the other hand, change in hematological parameters has a higher predictive value, when the data of drug toxicity in animal studies are translated for clinical usage (Olson *et al*., 2000). A normal RBC count and indices in groups treated with *E. sativa* extract but there was significant decrease ($P \leq 0.05$) in total WBC count in group treated with 250 mg/kg of *E. sativa* as well as significant decrease ($P \leq 0.05$) in lymphocyte count and percentage of monocytes in both treated groups (250 & 500 mg/kg) of *E. sativa* while percentage of lymphocyte was significantly increased ($P \leq 0.05$) in both treated groups as compared to normal control group.

In light of these findings, we may conclude that *E. sativa* leaves extract had some side effects in blood parameters. Many medicinal herbs and pharmaceutical drugs are therapeutic at one dose and toxic at another (Saad *et al*., 2006). This result in consistent with the previous study that revealed the treatment of *E. sativa* leaves extract reduces oxidative stress induced by H2O2 and showed a significant

decrease in lymphocytes number and level of blood glucose, total cholesterol TG, LDL-C, VLDL-C and atherogenic index, blood urea, and MDA. Also, the same study showed a significant increase of the eosinophils, monocytes, basophils, and HDL-C. However, *E. sativa* leaves extract treatment showed no significant difference in the levels of Hb, PCV, total count of leukocytes and albumin. Significant elevated in monocytes count and a significant reduction in lymphocytes count treated with 250 mg/kg of *E. sativa* leaves extract i/p for 21 days (Abdalrahman *et al*., 2010).

The altered activities of stress proteins, lymphocytes, and monocytes as well as raised levels of lymphocytes percentage and marked reduction of total antioxidant capacity were observed in rats receiving 20**%** hydro-methanolic extract of *E. sativa*. These effects are attributed to a range of phytochemicals including flavonoids and glucosinolates, both of which are found in high levels in Brassicaceous crops (Jin *et al*., 2009). *E. sativa* used for its antioxidant constituents including glucosinolates, flavonoids, carotenoids, etc. (Barillari *et al*., 2005). It is well established that *E. sativa* seed extract (SE) contains high yields of total phenolics, flavonoids, alkaloids, triterpenoids, antioxidant capacity, anti-lipid peroxidation, reducing power and DPPH radical scavenging effect (Abdel-Rahman *et al*., 2015). This result agrees with previous studies which showed some negative effects of a hot aqueous extract of *E. sativa* leaves represented by cellular hypertrophy and vacuolation of the cytoplasm of hepatocytes while kidneys showed mild degenerative changes in the renal tubules that mean this extract has a slight adverse effect on kidney (Bajilan & Al-naqeeb, 2011).

5. CONCLUSIONS

It was possible to observe that 20 % methanolic extract of *E. sativa* leaves acted through the phytochemical compound, stand out direct and indirect antioxidant actions. It is important to notice that besides their effect on antioxidant capacity, *E. sativa* promoted variable effects in white blood cells. Finally, it is interesting to note that *E. sativa* leaves extract gave AChE stimulatory activity have, in fact, properties that may suggest new applications.

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