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-Shehabz 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 (Bukhash 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 anti-hyperlipidemic, anti-hyperglycemic, hepatoprotective (Alqasoumi et al., 2009; Jin et al., 2009; Rafatullah et al., 2008), antiplatelet and anti-thrombotic 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 ≤ 0.05) in testosterone level, sperm activity and a significant decrease (P ≤ 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; Gullfraz et al., 2011; Rizwan 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 hyper trophy 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., 2004). CYP450 enzymes are so named because they are bound to membranes within a cell (cyto) and contain a heme pigment (chrome and P). The studies are limited in the literature about effects of *E. sativa* leaves extract alone on ROS, TAC, MT, AChE, cytochrome P450 (CYP1A1) concentrations and their correlations with a 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 (Ruttikay-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 4°C 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%.distilled water V/V) then homogenized by electrical blender for half hour at room temperature then filter by using gauze and dried at 40-45°C (Sato et al.,1990). The extracted powder preserved in plastic bags at 4°C 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.

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).

<table>
<thead>
<tr>
<th>Group</th>
<th>ROS (pg/ml)</th>
<th>Total Antioxidant capacity (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1644.6 ± 44.2</td>
<td>836.73 ± 12.00</td>
</tr>
<tr>
<td>E. sativa 250 mg/kg</td>
<td>1722.50 ± 28.64</td>
<td>738.79 ± 0.63</td>
</tr>
<tr>
<td>E. sativa 500 mg/kg</td>
<td>1564.0 ± 26.91</td>
<td>763.32 ± 22.15</td>
</tr>
</tbody>
</table>

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).

<table>
<thead>
<tr>
<th>Group</th>
<th>MTs (ng/ml)</th>
<th>CYP1A1 (ng/ml)</th>
<th>AChE (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1525.8 ± 417.23</td>
<td>8.55 ± 2.90</td>
<td>7.87 ± 3.32</td>
</tr>
<tr>
<td>E. sativa 250 mg/kg</td>
<td>4338.67 ± 380.17</td>
<td>9.24 ± 3.19</td>
<td>8.18 ± 4.02</td>
</tr>
<tr>
<td>E. sativa 500 mg/kg</td>
<td>4029.83 ± 754.38</td>
<td>22.63 ± 13.53</td>
<td>37.13 ± 15.23</td>
</tr>
</tbody>
</table>

Different letters refer to significance differences (P ≤ 0.05) between groups.
Table 3 Effect of 20 % methanolic extract of *E. sativa* in total and differential WBC count in treated male rats (Mean±S.E)

<table>
<thead>
<tr>
<th>Group</th>
<th>Total WBC count (10^3/µl)</th>
<th>Monocytes (10^3/µl)</th>
<th>Lymphocyte s (10^3/µl)</th>
<th>Granulocyte s (10^3/µl)</th>
<th>Lymphocyte %</th>
<th>Monocytes %</th>
<th>Granulocyte %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14.55 ± 1.05</td>
<td>1.3 ± 0.228</td>
<td>11.35 ± 0.35</td>
<td>2.60 ± 0.54</td>
<td>49.96 ± 1.07</td>
<td>25.65 ± 0.13</td>
<td>24.38 ± 1.14</td>
</tr>
<tr>
<td><em>E. sativa</em> 250 mg/kg</td>
<td>8.73 ± 1.52</td>
<td>1.80 ± 0.52</td>
<td>5.38 ± 0.82</td>
<td>1.55 ± 0.19</td>
<td>63.15 ± 1.76</td>
<td>18.18 ± 2.66</td>
<td>18.68 ± 1.68</td>
</tr>
<tr>
<td><em>E. sativa</em> 500 mg/kg</td>
<td>13.55 ± 1.53</td>
<td>2.30 ± 0.31</td>
<td>8.35 ± 0.68</td>
<td>2.85 ± 0.65</td>
<td>62.85 ± 3.10</td>
<td>17.15 ± 0.96</td>
<td>20.00 ± 2.90</td>
</tr>
</tbody>
</table>

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).

<table>
<thead>
<tr>
<th>Group</th>
<th>Total RBC count (10^6/µl)</th>
<th>Hemoglobin Concentration (g/DL)</th>
<th>HCT %</th>
<th>MCH (pg)</th>
<th>MCHC (g/DL)</th>
<th>MCV (µm³)</th>
<th>Platelets count (10^3/µl)</th>
<th>MPV µm³</th>
<th>RDW %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8.5 ± 0.18</td>
<td>14.83 ± 0.19</td>
<td>43.75 ± 0.70</td>
<td>17.25 ± 0.20</td>
<td>33.90 ± 0.31</td>
<td>50.90 ± 0.24</td>
<td>803.25 ± 62.91</td>
<td>6.70 ± 0.06</td>
<td>18.18 ± 0.04</td>
</tr>
<tr>
<td><em>E. sativa</em> 250 mg/kg</td>
<td>9.1 ± 0.52</td>
<td>15.93 ± 1.04</td>
<td>45.15 ± 2.46</td>
<td>17.20 ± 0.12</td>
<td>34.53 ± 0.39</td>
<td>49.90 ± 0.51</td>
<td>747.50 ± 125.23</td>
<td>7.05 ± 0.36</td>
<td>18.93 ± 0.41</td>
</tr>
<tr>
<td><em>E. sativa</em> 500 mg/kg</td>
<td>8.7 ± 0.62</td>
<td>15.1 ± 1.27</td>
<td>44.80 ± 3.04</td>
<td>17.38 ± 0.46</td>
<td>34.05 ± 0.52</td>
<td>50.98 ± 0.96</td>
<td>774.50 ± 146.07</td>
<td>6.50 ± 0.19</td>
<td>18.40 ± 0.46</td>
</tr>
</tbody>
</table>

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 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.

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 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

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 (Ruttkey-Nedecy 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-Gayyar 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 CC14 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 (Velloso 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≤0.05) in total WBC count in group treated with 250 mg/kg of *E. sativa* as well as significant decrease (P≤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≤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 escoinphils, monocytes, basophils, and HDL-C. However, *E. sativa* leaves extract treatment showed no significant difference in the levels of Hb, PCV, total count of leucocytes 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 Brassicaceae 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 male reproductive system.

REFERENCES


