Hepatoprotective and antioxidant effects of *Ocimum basilicum* extract in CCL₄-induced hepatotoxic rats compared with Silymarin.

Sarhan, Hasnaa, Farid, Ayman and Mostafa, Khalid
Clinical Pathology Department, Faculty of Veterinary Medicine, Benha University

**ABSTRACT**

The aim of this study was to evaluate pulmonary function test, acute phase proteins, pro-inflammatory cytokines, electrolyte balance and electrocardiographic alterations in BRD affected calves compared to pen matched healthy control calves. A total of 30 calves (20 BRD affected calves and 10 apparently healthy calves) were used in this study. The BRD affected calves showed significant (*P*<0.05) reduction in blood pH, partial pressure of oxygen, oxygen saturation, calcium, phosphorus, magnesium, sodium and chloride, and significant (*P*<0.05) increase in partial pressure of carbon dioxide, total carbon dioxide and potassium. Acute phase proteins and Proinflammatory cytokines assessment revealed a significant (*P*<0.05) increase of serum amyloid A, haptoglobin, fibrinogen and interleukin-6 in diseased calves compared to healthy control calves. Electrocardiographic examination of BRD affected calves revealed a significant increase in heart rate with a significant (*P*<0.05) alteration in ECG wave trace parameters (P wave, QRS complex, T wave, PR interval, QT interval). In conclusion, measurement of pulmonary function test, APPs, cytokines and ECG could be used as valuable and early diagnostic tools for BRD diagnosis in feedlot cattle calves.

**Key words:** BRD, pulmonary function, acute phase proteins, cytokines, ECG.

1. **INTRODUCTION**

Bovine respiratory disease (BRD) is one of the most economically significant diseases in cattle industry especially in intensely raised, recently weaned and newly transported calves (Loneragan *et al.*, 2001). The biggest challenges in bovine medicine is early detection of clinical cases of diseases, especially important the subclinical form, which can be easily missed and cause important economic losses (Arslan and Ozcan, 2018). It has been reported that 37–68% of calves that never received treatment for BRD during the finishing period had lung lesions at the slaughter time (Thompson *et al.*, 2006). For this reason, early identifying of an accurate method to classify calves that are at greater risk of becoming sick is the key to optimal calves’ health (Abdallah *et al.*, 2016 and Zeineldin *et al.*, 2019). Recently, the possibility to use acute phase proteins (APPs), cytokines, pulmonary function test and electrocardiography (ECG) as diagnostic biomarkers of infection has expanded significantly in the context of respiratory medicine. The acute inflammatory process initiates the acute phase reaction that results in increase the concentration of APPs in diseased calves (Heller and Johns, 2015). APPs have been recently proposed as sensitive and rapid indicators of inflammatory processes in ruminants (Gonzales *et al.*, 2011). APPs play an important role in eliminating the infectious agents, and activate the repair process toward the normal function (Tothova *et al.*, 2015). Assessment of blood gases and related clinical parameters are also considered an important diagnostic indicator for BRD that help in early treatment decision (Constable *et al.*, 2017). ECG assessment is also a useful tool in accurate diagnosis and evaluation of cardiac diseases or cardiac dysfunctions secondary to other systemic disease (Ghanem, 1997). Therefore, our hypothesis was that assessment of pulmonary function test, APPs and ECG changes in BRD affected calves with other
clinical variables could be used to influence treatment decisions at the time of initial disease diagnosis. The objective of this study was to evaluate pulmonary function test, APPs, electrolyte balance and ECG alterations in BRD affected calves compared to pen matched healthy control calves.

2-MATERIAL AND METHODS

2.1. Animals and samples collection

This study was carried out in a farm in Gharbiya Governorate. A total of 30 calves (3-9 months old) were selected and used in this study. These calves were recently transported to the farm from sale barns before begin this study. All calves were classified based on clinical examination into the following groups: clinically healthy calves (Control, n=10), and calves suffered from bovine respiratory disease (BRD affected calves, n=20). Calves suspected to be suffering from BRD were visually examined for the presence of nasal or ocular discharge, respiratory distress, cough, depression and inappetance. When two or more of these clinical signs were observed, rectal temperature of calf was recorded. Using the clinical scoring system (McGuirk, 2008), a calf with a score of 5 or more was classified as morbid and included in the study. Calves were not included in the study if there was a presence of concurrent diseases.

All calves were subjected to complete clinical examination including body temperature, pulse rate, respiratory rate and thoracic auscultation (Radostits et al., 2000). Two blood samples were collected from each calf using jugular vein puncture. The first blood sample (2ml) of blood was collected in syringe containing heparin (50 I.U/ml) used for blood gases, plasma fibrinogen estimation (Fararh et al., 2017). The second blood sample was collected without anticoagulant, clotted at room temperature for 20 min, centrifuged at 3,000 rpm for 10 min, and then the clear non-hemolyzed serum samples were separated and stored at −20°C until subsequent biochemical analysis.

2.2. Blood gas analysis

Immediately after vein-puncture, the tip of the needle was sealed with a rubber stopper in order to prevent gas from moving in or out of it. The samples were placed in a bed of crushed ice, taken immediately to the laboratory for analyzed no more than one hour after collection. The blood samples were analyzed for pH, partial oxygen pressure (P\textsubscript{O}\textsubscript{2}), partial dioxide pressure (P\textsubscript{CO}\textsubscript{2}), bicarbonate (HCO\textsubscript{3}) concentrations, total carbon dioxide (tCO\textsubscript{2}) and oxygen saturation (SO\textsubscript{2}) using blood gas analyzer (Hussein and Aamer, 2013).

2.3. Acute phase proteins and proinflammatory cytokines measurement

Serum haptoglobin (Hp) concentration was determined by ELISA kit according to method described by Idoate et al. (2015). Serum amyloid A (SAA) concentration was measured with a commercially available ELISA kit according to method described by Alsemgeest et al. (1994). Serum fibrinogen (Fb) concentration was measured according to method described by Becker et al. (1984). Interleukin-6 (IL-6) level was determined from undiluted serum samples using commercially available ELISA kits according to method described by Kabu et al. (2016a).

2.4. Serum minerals and electrolytes measurement

Serum Ca, inorganic P, Cl, Na and K levels were determined using spectrophotometer according to the method described by Chessborough (1991). Serum magnesium (Mg) levels were determined by using atomic absorption spectrophotometer by as described by Devlin (1997).

2.5. Electrocardiographic examination

The ECG was recorded with base apex lead system II using limb lead. Calves were kept in standing position without any tranquilizers or sedative. When animals got calm (decreasing muscle tremors), the ECGs were recorded, using alligator-type electrodes attached to the skin after cleaning it with ethanol and applying ethyl alcohol to improve the contact. Base apex lead system II was applied as; the right forelimb electrode was placed on the right side of the neck along the jugular groove one third of the way up the neck. The left forelimb electrode was placed on the ventral midline under the apex of the heart. The ground cables were placed on the left and right stifle joints. Alligator clips moisten with alcohol were used
(Ghanem, 1997). The electrocardiogram (ECG) should reveal a distinct P wave (atrial depolarization), QRS complex (ventricular depolarization), and T wave (ventricular repolarization). All ECGs were obtained with a single channel electro-cardiographic machine (BTL-08 SD ECG, Industries Ltd.161 Cleveland Way, Steven age, SG1 6BU, UK) with paper speed of 25 mm/s and calibration of 10 mm equal to 1 mV. For measuring ECG parameters, the ECG was analyzed using a magnifying glass. In this method, precision of duration is 0.02 sec. and amplitude is 0.1 mV.

2.6. Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics version 20. Data was statistically analyzed using Independent sample t-test to compare control healthy with diseased animal as described by Bailey (2008). Values were represented as means ± standard error (SE). All differences were considered statistically significantly when P<0.05.

3-RESULTS

Clinical examination of apparently healthy calves (control group) revealed good healthy condition with no apparent diseases. The healthy control calves showed normal behavior during handling and examination, moist muzzle, good rumination, shiny hair, healthy eyes, with no ocular and nasal discharge. The body temperature, pulse and respiration of clinically healthy calves were within the normal range. On other hand calves suffering from BRD are diagnosed based on McGuirk’s clinical scoring system. The most common recognizable clinical signs of those calves that develop BRD were varying degree of depression, loss of appetite, in-appetence, loss of body weight, nasal discharge, sever dyspnea with mouth breathing in some cases, congestion of ocular mucous membrane with ocular discharge and some BRD cases suffered from painful cough. Auscultation of the lung in BRD affected calves showed abnormal lung sounds including loud wheezing, crackling sound and moist rales. Frictional sound and exaggerated vesicular sounds were also heard. BRD affected calves also showed significant (P<0.05) increase in body temperature (40.62±0.09), respiratory rate (54.25±2.75) and pulse rate (154.05±2.66) compared to clinically healthy calves body temperature (38.94±0.11), respiratory rate (29.1±1.15) and pulse rate (93.3±1.71).

3.1. Blood gas analysis

The BRD affected calves showed significant (P<0.05) decrease in pH, PO2 and SO2, and significant (P<0.05) increase in PCO2, HCO3, and tCO2, when compared to healthy control calves (Table, 1).

3.2. Acute phase proteins and proinflammatory cytokines

Assessment of APPs and cytokines in BRD affected calves showed a significant (p<0.05) increase in Hp, SAA, Fb, IL-6 levels when compared to healthy control calves (Table, 2).

3.3. Serum minerals and electrolytes

Serum minerals and electrolyte assessment in BRD affected calves showed a significant decrease of Ca, P, Mg, Na and Cl when compared to healthy calves. On other hand diseased calves revealed a significant increase in K level when compared to healthy calves (Table, 3).

Table (1): Biochemical parameters of rats in different groups at 1st check point on 16th day of experiment

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameters</th>
<th>AST (U/L)</th>
<th>ALT (U/L)</th>
<th>GGT (U/L)</th>
<th>ALP (U/L)</th>
<th>T.B (mg/dl)</th>
<th>D.B (mg/dl)</th>
<th>Ind.B (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>96.23±3.9a</td>
<td>53.84±3.60b</td>
<td>9.68±0.80b</td>
<td>117.98±5.39b</td>
<td>0.81±0.05b</td>
<td>0.2±0.02b</td>
<td>0.58±0.04b</td>
</tr>
<tr>
<td>Silymarin</td>
<td></td>
<td>95.92±4.24a</td>
<td>54.12±4.62b</td>
<td>9.48±0.92b</td>
<td>123.66±6.66b</td>
<td>0.81±0.06b</td>
<td>0.24±0.03b</td>
<td>0.58±0.05b</td>
</tr>
<tr>
<td>Ocimum</td>
<td></td>
<td>98.85±4.28b</td>
<td>54.67±4.64b</td>
<td>9.58±0.93b</td>
<td>124.88±5.71b</td>
<td>0.82±0.06b</td>
<td>0.24±0.03b</td>
<td>0.58±0.06b</td>
</tr>
<tr>
<td>CCL4</td>
<td></td>
<td>155.56±4.83a</td>
<td>98.14±6.18b</td>
<td>15.49±1.28a</td>
<td>188.57±8.74a</td>
<td>1.40±0.15a</td>
<td>0.39±0.04a</td>
<td>1.01±0.16a</td>
</tr>
<tr>
<td>Silymarin</td>
<td></td>
<td>113.55±4.60b</td>
<td>63.53±4.24b</td>
<td>11.42±0.94b</td>
<td>139.22±6.35b</td>
<td>0.96±0.06b</td>
<td>0.27±0.02b</td>
<td>0.69±0.05b</td>
</tr>
<tr>
<td>Ocimum</td>
<td>CCL4</td>
<td>104.38±4.62b</td>
<td>58.89±5.03b</td>
<td>10.31±1.00b</td>
<td>134.56±6.16b</td>
<td>0.88±0.06bc</td>
<td>0.26±0.03b</td>
<td>0.63±0.06b</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± S.E.M.
Superscript letters (a, b and c) indicate significant differences (P ≤ 0.05) between any two means, within the same column
Table (2): Biochemical parameters of rats in different groups at 2nd check point on 22nd day of experiment

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameters</th>
<th>AST (U/L)</th>
<th>ALT (U/L)</th>
<th>GGT (U/L)</th>
<th>ALP (U/L)</th>
<th>T.B (mg/dl)</th>
<th>D.B (mg/dl)</th>
<th>Ind.B (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>106.46±4.52b</td>
<td>65.79±3.67b</td>
<td>9.42±0.49b</td>
<td>115.51±9.39b</td>
<td>1.13±0.02b</td>
<td>0.39±0.02b</td>
<td>0.74±0.02b</td>
</tr>
<tr>
<td>Silymarin</td>
<td></td>
<td>110.70±4.71b</td>
<td>68.49±3.87b</td>
<td>9.79±0.51b</td>
<td>120.13±9.76b</td>
<td>1.17±0.03b</td>
<td>0.41±0.02b</td>
<td>0.76±0.02b</td>
</tr>
<tr>
<td>Ocimum</td>
<td>CCL4</td>
<td>113.49±5.72b</td>
<td>70.45±4.76b</td>
<td>10.24±0.49b</td>
<td>115.39±10.19b</td>
<td>1.18±0.03b</td>
<td>0.40±0.02b</td>
<td>0.79±0.03b</td>
</tr>
<tr>
<td>Silymarin</td>
<td>CCL4</td>
<td>199.60±8.32a</td>
<td>128.54±5.12a</td>
<td>16.88±0.85a</td>
<td>214.96±22.04a</td>
<td>2.77±0.13a</td>
<td>0.72±0.04a</td>
<td>2.04±0.11a</td>
</tr>
<tr>
<td>Ocimum</td>
<td>CCL4 +</td>
<td>122.41±5.21b</td>
<td>75.73±4.28b</td>
<td>10.83±0.56b</td>
<td>132.84±10.80b</td>
<td>1.30±0.03b</td>
<td>0.45±0.02b</td>
<td>0.85±0.02b</td>
</tr>
<tr>
<td>Ocimum</td>
<td>CCL4</td>
<td>117.81±5.94b</td>
<td>73.14±4.94b</td>
<td>10.63±0.50b</td>
<td>119.78±10.58b</td>
<td>1.23±0.04b</td>
<td>0.41±0.02b</td>
<td>0.81±0.03b</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± S.E.M.
Superscript letters (a, b and c) indicate significant differences (P ≤ 0.05) between any two means, within the same column.

Table (3): Antioxidant parameters of rats in different groups at 1st check point on 16th day of experiment

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameters</th>
<th>MDA (nmol/mg)</th>
<th>SOD (U/L)</th>
<th>GPX (ng/mg)</th>
<th>CAT (ng/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>0.11±0.01d</td>
<td>0.27±0.02ab</td>
<td>0.34±0.03a</td>
<td>0.27±0.01ab</td>
</tr>
<tr>
<td>Silymarin</td>
<td></td>
<td>0.12±0.01cd</td>
<td>0.32±0.02a</td>
<td>0.29±0.02ab</td>
<td>0.30±0.02a</td>
</tr>
<tr>
<td>Ocimum</td>
<td>CCL4</td>
<td>0.25±0.02b</td>
<td>0.18±0.01c</td>
<td>0.26±0.02b</td>
<td>0.27±0.02b</td>
</tr>
<tr>
<td>Silymarin</td>
<td>CCL4</td>
<td>0.31±0.02c</td>
<td>0.11±0.01d</td>
<td>0.09±0.02c</td>
<td>0.14±0.02c</td>
</tr>
<tr>
<td>Ocimum</td>
<td>CCL4</td>
<td>0.17±0.02c</td>
<td>0.31±0.01a</td>
<td>0.26±0.02b</td>
<td>0.25±0.04b</td>
</tr>
<tr>
<td>Ocimum</td>
<td>CCL4</td>
<td>0.23±0.01b</td>
<td>0.22±0.02bc</td>
<td>0.20±0.02b</td>
<td>0.19±0.03bc</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± S.E.M.
Superscript letters (a, b and c) indicate significant differences (P ≤ 0.05) between any two means, within the same column.

Table (4): Antioxidant parameters of rats in different groups at 2nd check point on 22nd day of experiment

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameters</th>
<th>MDA (nmol/mg)</th>
<th>SOD (U/L)</th>
<th>GPX (ng/mg)</th>
<th>CAT (ng/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>0.12±0.00c</td>
<td>0.16±0.01b</td>
<td>0.27±0.03ab</td>
<td>0.22±0.01c</td>
</tr>
<tr>
<td>Silymarin</td>
<td></td>
<td>0.13±0.02c</td>
<td>0.38±0.05a</td>
<td>0.29±0.05a</td>
<td>0.35±0.03b</td>
</tr>
<tr>
<td>Ocimum</td>
<td>CCL4</td>
<td>0.23±0.02b</td>
<td>0.18±0.01b</td>
<td>0.28±0.02ab</td>
<td>0.23±0.01c</td>
</tr>
<tr>
<td>Silymarin</td>
<td>CCL4</td>
<td>0.31±0.02a</td>
<td>0.15±0.03b</td>
<td>0.15±0.01c</td>
<td>0.19±0.01c</td>
</tr>
<tr>
<td>Ocimum</td>
<td>CCL4</td>
<td>0.16±0.04c</td>
<td>0.31±0.04a</td>
<td>0.33±0.06a</td>
<td>0.41±0.03a</td>
</tr>
<tr>
<td>Ocimum</td>
<td>CCL4</td>
<td>0.30±0.02ab</td>
<td>0.18±0.03b</td>
<td>0.22±0.03ab</td>
<td>0.2±0.02c</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± S.E.M.
Superscript letters (a, b and c) indicate significant differences (P ≤ 0.05) between any two means, within the same column.

Table (5). Relative expression of TNFa, IL4 and NFkB/B gene in liver of different groups

<table>
<thead>
<tr>
<th>Group</th>
<th>TNF alpha</th>
<th>IL4</th>
<th>NFkB/B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control (G1)</td>
<td>1.00 ± 0.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Silymarin group (G2)</td>
<td>0.96 ± 0.05</td>
<td>1.15</td>
<td>1.04</td>
</tr>
<tr>
<td>Ocimum group (G3)</td>
<td>1.05 ± 0.06</td>
<td>1.26</td>
<td>1.16</td>
</tr>
<tr>
<td>CCL4 group (G4)</td>
<td>5.54 ± 0.31</td>
<td>10.06</td>
<td>6.45</td>
</tr>
<tr>
<td>Silymarin +CCL4 (G5)</td>
<td>2.69 ± 0.10</td>
<td>3.01</td>
<td>3.16</td>
</tr>
<tr>
<td>Ocimum +CCL4(G6)</td>
<td>3.92 ± 0.18</td>
<td>5.03</td>
<td>4.14</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± S.E.M.
Superscript letters (a, b and c) indicate significant differences (P ≤ 0.05) between any two means, within the same column.
Fig.1. A: Liver of rat (control) showing normal hepatocytes arranged in cords around the central vein (arrow), H&E, bar= 100 µm.; B: Liver of rat administered with silymarin showing normal hepatocytes arranged in cords around the central vein (arrow), H&E, bar= 100 µm.; C: Liver of rat administered with *O. basilicum* showing normal hepatocytes arranged in cords around the central vein (arrow), H&E, bar= 100 µm.; D: Liver of rat intoxicated with CCl₄ showing marked centrilobular hepatic vacuolation (arrow) associated with mononuclear cells infiltration (arrowhead), H&E, bar= 100 µm.; E: Liver of rat intoxicated with CCl₄ and protected with silymarin showing mild to moderate degree of fatty change (arrow), H&E, bar= 100 µm.; F: Liver of rat intoxicated with CCl₄ and protected with *O. basilicum* showing marked decrease of the hepatic necrosis and cellular apoptosis (arrow), H&E, bar= 100 µm.

4. DISCUSSION

Regarding to liver enzymes, rats injected with CCl₄ showed significant increase in the ALT and AST activities in 1st and 2nd check points. These results agree with Shah et al., (2015) who attributed that CCl₄ causes injury of the membrane and so leakage of the cytosomal enzymes. That is may be due to the reactive intermediate free radicals which are produced by CCl₄ bioactivation by cytochrome P450 (Weber et al., 2003). Also, CCl₄ treatment causes an increase in the level of serum GGT, ALP and total, direct and indirect bilirubin. These results agree with Li et al., (2015). The increase may be attributed to the increase synthesis of GGT and ALP in case of the biliary pressure increase (Muriel et al., 1992). Silymarin protected group significantly reduced the elevated liver enzymes and bilirubin (total, direct and indirect) after CCl₄ administration in 1st and 2nd check points. These findings agree with Saller et al., (2007).

Silymarin contain natural products as tocopherol and ascorbic acid 6 hexadecanoate, sterols, sterylglycosides and squalene, which could diminish the membrane peroxidation as well as the leakage of enzymes (Habib-ur-Rehman et al., 2009).

In 1st and 2nd check points, the administration of OBE significantly decreased the serum ALT and AST, ALP, GGT, total, direct and indirect bilirubin close to normal control rats. Results from this study conform with Atangwho et al., (2014) and Meera et al., (2009). These results could be explained that OBE protects the hepatocytes from injuries and improves the function of liver (Chiu et al., 2012).

Concerning to antioxidants parameters, the increase of MDA in CCl₄ administered rats in 1st and 2nd check points compared to normal rats may be attributed to the trichloromethyl radicals that resulted from CCl₄ metabolism. Those radicals stimulate the process of lipid peroxidation with the formation of by-
products such as MDA. (Madubuike et al., 2015)
Also, in the present study, the hepatic antioxidant enzymes SOD and CAT (in 1st check point) and GPx (in 1st and 2nd check points) were significantly decreased activities in CCl₄-intoxicated rats compared with control rats. These results partially match with Tsai et al., (2009). Decrease in enzyme activity may be attributed to the deactivation of their isoenzymes by oxidation of a cysteine residue near the active centre (Tamai et al., 1990).
Rats protected with Silymarin showed a significant decrease in MDA levels. On the contrary, SOD, catalase and GPx activities were significantly elevated by administration of silymarin to CCl₄-intoxicated rats, that reflects its ability to restore these enzymes activities in the liver damaged by CCl₄. These result is in agreement with Trappoliere et al., (2009). Silymarin contain linoleic acid that could be converted into certain isomers of conjugated linoleic acids (CLA) in the gastrointestinal tract of rats. CLA inhibit lipoperoxidation and as a result, MDA decreased (Arab et al., 2006). Also due to its content of tocopherol (Sudheesh et al., 2013), CLA (Belury, 2002), sterols (Conforti et al., 2008), ascorbic acid 6-hexadecanonoate (Klein and Weber., 2001) and sterylglucosides (Mirmiran et al., 2014), it recovers the activities of CAT and SOD, as well as of GPx as these substances have antioxidant effects.
Rats administered OBE only compared with normal rats showed significant increase in liver MDA in 1st and 2nd check points and significant decreases in liver SOD and GPx levels in 2nd check point. These results partially agree with Karaali et al., (2018) who found that pretreatment with basils extract caused increase in serum ALT and AST and MDA in liver.
OBE protected group demonstrated significant increases in GPx and SOD compared with CCl₄ injected rats, while a significant decrease in MDA was observed in the 1st check point. Dasgupta et al., (2007) found that Ocimum basilicum increased the activity of xenobiotic metabolizing phase 1 and phase 11 enzymes, elevating antioxidant-enzyme response.
Regarding to proinflammatory and anti-inflammatory cytokine expression, CCl₄ cause a significant increase in TNF-α and NFκB expression. The free radicals and ROS that generated from CCl₄ metabolism by cytochrome P450 2E1 (CYP2E1), stimulate the upregulation of TNF-α (Jeong, 2008).
Chávez et al., (2008) mentioned that CCl₄ increase NFkB, which regulates the several genes transcription including cytokines in rat. Liver injury caused by CCl₄ leads to rapid recruitment of eosinophils, which secrete IL-4 to stimulate the proliferation of quiescent hepatocytes. IL-4 is able to stimulate cell cycle progression via IL-4Rα in liver cells (Goh et al., 2013).
Silymarin protected group showed a lower expression level of TNF-α, IL-4 and NFκB compared to CCl₄ injected rats. This trend agrees with Schümann et al., (2003). That result reflects the ability of silymarin to ameliorate inflammatory markers (Altaei, 2012). Silibinin considered to be an immuneresponse modifier in vivo, suppressing the NF-κB activation which suppresses the subsequent synthesis of TNF and stimulating synthesis of IL-10 within the liver. In addition, while the synthesis of IL-10 was stimulated, the intrahepatic production of IL-4 was inhibited (Abenavoli et al., 2008).
OBE causes a significant decrease in the expression of genes (TNFα, IL4 and NFκB) compared with CCl₄ injected rats. These results partially agree with Athari et al., (2018). Rosmarinic acid which present in large amounts in OBE (Makino et al., 1998), suppress the induction of TNFα by inhibiting the proliferation of mesangial cell which secrets TNFα as autocrine factors. The antiproliferative effects exhibited by rosmarinic acid are due to its suppressive effects on PDGF (platelet derived growth factor) and c-myc m-RNA expression which are essential in mesangial cell proliferation (Makino et al., 2000). Ocimum labiatum extract treatment causes down-regulation of the IL-4 cytokine by reconditioning the function of Th2 effector cells (Taylor et al., 2012).
Concerning to histopathological changes, CCl₄ causes centrilobular hepatic degeneration and coagulative necrosis with marked fatty changes. The present results are in accordance with those of Sreelatha et al.,( 2009). CCl₄ is metabolized by cytochrome P-450 enzymes that leads to liberation of free radicles as trichloromethyl and trichloro-methylperoxyl radicals. These radicals bind to hepatocyte components and also affect unsaturated fatty acids that results in phospholipid destruction.
(Cheeseman et al., 1985), which results in accumulation of fat (fatty liver) and damage of the liver (Chamulitrat et al., 1995). The current results revealed that silymarin treatment was shown to decrease fatty change, hepatic necrosis and inflammation. These findings agree with those of Abdel-Salam et al., (2007). The hepatoprotective role of silymarin acted by antioxidative, anti-fibrotic, anti-lipid peroxidative, membrane stabilizing and liver regenerating mechanisms (Chlopčíková et al., 2004; Pradhan and Girish, 2006). The anti-inflammatory effect of silymarin is mediated through inhibition of neutrophil migration (De La Puerta et al., 1996). The present study showed that OBE decrease hepatic necrosis and vacuolation. These results agree with Ogaly et al., (2015). OBE has anti-inflammatory and hepatotonic effects acting by stimulating p450 detoxification enzymes help in neutralization of free radicals and overtly aggressive oxygen species. Moreover, ocimum basilicum contains methyl eugenol which has antioxidant and anti-inflammatory properties (Duke, 2003). In addition, ocimum basilicum can decrease the conversion of hepatic stellate cells into myofibroblasts (Salmah et al., 2005).

5. CONCLUSION

The results of present study demonstrate that OBE has hepatoprotective ability against CCl4 hepatotoxicity in rats comparing with silymarin. The results also imply that the hepatoprotective effects of OBE may be due to the presence of flavonoids compounds with strong antioxidant activities. However, silymarin has more potent hepatoprotective and antioxidant effect than ocimum.

6. REFERENCES


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