Comparative Hematological and Biochemical Studies on the Effect of Some Hepatoprotective Agents in Rats

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ABSTRACT

This work was conducted to investigate the hepato-protective effect of chamomile extract, grape seed extract and Silymarin against carbon tetrachloride-induced hepatotoxicity in rats.

A total of 80 normal male albino rats, were divided into 8 groups, the first group (group I): rats served as the control group and received commercial balanced diet all over the experimental period (3 weeks), group II: chamomile orally treated group (200mg/kg) for the whole experimental period, group III: grape seed orally treated group (100mg/kg) for the whole experimental period, group IV: silymarin orally treated group (100mg/kg) for the whole experimental period, group V: served as hepatotoxicated group, treated with an intraperitoneal injection of 2mg/kg b. w. of carbon tetrachloride (CCl₄) dissolved in corn oil 1:1 at 11 and 12 days of an experiment to induce hepatotoxicity and group V chamomile orally pretreated group (200mg/kg) for the whole experimental period with i/p injection of CCl₄ in days 11 and 12 of the experiment, group VII: grape seed orally pretreated group (100mg/kg) for the whole experimental period, with i/p injection of CCl₄ in days 11 and 12 of the experiment and group VIII: silymarin orally pretreated group (100mg/kg) for the whole experimental period, with i/p injection of CCl₄ in days 11 and 1 of the experiment.

Objective: The present study was carried out to investigate the effect of oral administration of ethanol extract of chamomile capitula extract, grape seed and Silymarin for 3 weeks on hepatotoxicity induced to rats by carbon tetrachloride (CCl₄). These effects were explored by measuring erythrogram and serum levels of liver enzymes; aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) albumin, globulin, total protein, cholesterol, bilirubin, albumin.

Results: The results showed that the oral administration of chamomile extract, a grape seed extract, and silymarin to hepatotoxicated rats for 21 days improved anemia and significantly decreased the elevated serum levels of liver enzymes (AST, ALT, and ALP total bilirubin and increased serum total protein, albumin, when compared to the corresponding control, positive groups.

Conclusion: The results suggest that the chamomile extract, grape seed extract, and silymarin induce potent hepato-protective effects in CCl₄ induced hepato-injury in rats. This study recommends that intake of chamomile and grape seed extracts as an herbal may be beneficial for patients who suffer from liver diseases.
INTRODUCTION

The liver is labeled as the biggest glandular organ that controls diverse physiological and chemical processes in the human body. In other words, it plays a central role in metabolic control and detoxification involving the metabolism of lipids, carbohydrates, alcohol and a wide range of drugs as well as toxins (Aseervatham et al., 2018). Interestingly, the liver has the unique ability to regenerate and completely recoup from the most acute, non-iterative situation (Mosedale and Watkins, 2017; Oliva-Vilarnau et al., 2018). However, multiple conditions, e.g., hepatitis, chronic alcohol consumption, frequent use of antibiotics associated medications and also even nonalcoholic fatty liver disease can affect the regenerative efficacy of the hepatocytes, which become totally dysfunctional (Forbes and Newsome, 2016), generally witnessed by the visible hepatic scarring, apoptosis and entering into most severe cirrhosis. Liver cell injury can also cause by various toxicants such as certain chemotherapeutic agents, carbon tetrachloride, thioacetamide, etc.

Liver protective plants contain a variety of chemical constituents like phenols, coumarins, lignans, essential oil, monoterpenes, carotenoids, glycosides, flavonoids, organic acids, lipids, alkaloids, and xanthenes. Therefore a large number of plants and formulations have been claimed to have hepatoprotective activity; so the development of plant-based hepatoprotective drugs has been given importance in the global market.

This thesis has been presented to enumerate some plants that have hepato-protective properties such as grape seeds, chamomile extracts, and silymarin.

MATERIALS AND METHODS

Eighty male albino rats, with initial body weight ranging from 150-200g were used. Rats were purchased from Animal Vaccine Institute, Helwan. All animals were housed in a controlled laboratory conditions at 20-25ºC and 50% humidity in a 12h light/dark cycle and had free access to feed and water. All animals received human care and the study protocols were in compliance with institutional guidelines for the use of laboratory animals. They were allowed for two weeks acclimatization period before starting the experiment.

Plant:

Chamomile flower and grape seed were obtained from Jianfeng Natural Products R&D Inc. (Tianjin, China). Silymarin powder was purchased from Sigma Chemical Co.

Chemicals:
1-Carbon tetrachloride (CCl₄) obtained from Sigma Pharmaceuticals (USA).
2-Methyl alcohol (95%), ethyl alcohol (99.9%) and dimethyl-sulfoxid 99 (DMSO) were obtained from El-Nasr Pharmaceutical chemicals.

Preparation of Extracts:
Extracts were prepared in the Pharmacology Department Faculty of Vet. Medicine, Suez Canal University.

Chamomile Extract Preparation (CHE):
1-Five hundred grams of chamomile flower powder were transported into 1 L Erlenmeyer flasks and then 99% ethanol was added to the samples. Extraction was carried out using an orbital shaker at room temperature for 8 h, they were filtrated through filter paper, the residue was re-extracted twice for complete extraction, and then, lift it to dry by evaporation under reduced pressure.
2-The weight of yield resulted from that amount of powdered plant was measured and kept in a refrigerator at +4°C until use. In this experiment, 500gm of chamomile powder gave 25.57 gm extract which has dissolved in 21 ml DMSO and 187 ml D.W.
3 -Rats were given chamomile extract orally by oral gavage at a dose of 200 mg/kg per day
for 3 weeks; according to (Aksoy and Sozbilir 2012).

**Grape Seeds Extract Preparation (GSE):**

The ethanolic extract of plant seeds was prepared according to Harborne *et al.*, (1975); as follows:

1. Fifty gm of the powdered seed of grape was suspended in 250 ml of 99% ethanol alcohol in Erlyn Myer flask and stirred on cold magnetic stirrer overnight.
2. After 72 hr, the sediments were filtered by gauze and then by filter paper.
3. The pooled extract was dried by evaporation under reduced pressure, the weight of yield resulted from that amount of powdered plant was measured.
4. The yield (16.15 gm) was kept at a refrigerator at +4˚C until the time of usage.
5. The 16.15 gm of powdered plant extract was dissolved into 26.6 ml DMSO (as solvent) and 200 ml D.W, the suspension then filtered a 0.4 mm sterile Millipore filter and kept in a refrigerator at +4˚C until use.
6. Rats were given GSE orally by oral gavage at a dose of 100 mg/kg per day for 3 weeks, according to Pallares *et al.*, (2013).

**Silymarin Preparation:**

1. Silymarin powder was dissolved in DMSO then was diluted with complete medium (DMEM/F12).
2. In this study 1400 mg of silymarin dissolves in 2.3 ml DMSO plus 20.9 ml of D.W.
3. Rats were given silymarin orally by oral gavage at a dose of 100 mg/kg per d for 3 weeks, according to Wang *et al.*, (2016).

**Induction Of Hepatotoxicity:**

Rats were injected i/p by 2 ml /kg of CCl₄ diluted in corn oil (1:1) at day 11 and 12 of the experiment according to Vanitha, *et al.*, (2007).

**Experimental Design and Grouping of Rats:**

The rats were randomly distributed into 8 equal groups of 10 rats each as follows:

- **Group (1):** Served as a normal control group received saline and corn oil i/p at days 11 and 12 from the experiment. They received no drugs for 3 weeks.
- **Group (2):** chamomile treated group; rats received 200 mg dried chamomile extract /kg b.wt by oral gavage daily for 3 weeks.
- **Group (3):** grape seed treated group; rats received 100 mg dried grape seed extract /kg b.wt by oral gavage daily for 3 weeks.
- **Group (4):** silymarin treated group; rats received 100 mg dried silymarin extract /kg b.wt by oral gavage daily for 3 weeks.
- **Group (5):** Served as a hepatic-intoxicated group; induced by an injection i/P of 2ml /kg b.w of CCl₄ dissolved into 1:1 corn oil at 11and 12 days of the experiment.
- **Group (6):** chamomile pretreated group; rats received 200 mg dried chamomile extract /kg b.wt by oral gavage daily for 3 weeks with i/p injection of CCl₄ in days 11 and 12 of the experiment.
- **Group (7):** grape seed pretreated group; rats received 100 mg dried grape seed extract /kg b.wt by oral gavage daily for 3 weeks with i/p injection of CCl₄ in days 11 and 12 of the experiment.
- **Group (8):** silymarin pretreated group; rats received 100 mg dried silymarin /kg b.wt by oral gavage daily for 3 weeks with i/p injection of CCl₄ in days 11 and 12 of the experiment.

**Sampling:**

Rats were sacrificed twice the first one was on day 14 of the experiment (half of each group) and the second one was on day 21 at the end of the experiment (the other half).

Blood samples were collected from 10 hour fasted rats from retro-orbital venous plexus under the effect of light ether anesthesia. Blood was divided into 2 tubes; the first
tubes contain di-potassium salt of ethylene diamintetracetate (EDTA) as anticoagulant. This tube was used for hemogram evaluation (RBCs count, hemoglobin concentration, PCV value).

The second portion (3 ml) of blood was placed in a plain, clean and sterile centrifuge tube without anticoagulant for serum separation. After collection of blood, allow the blood to clot by leaving it undisturbed at room temperature. This was taken 15-30 minutes. Remove the clot by centrifuge at 1500 rpm for 10 minutes in a refrigerated centrifuge. The resulting supernatant was the serum and kept in a freezer (-20 °C) until used for biochemical parameter analysis.

I- Hematological Parameters Evaluation:

Included erythrocytes count, Hb concentration, PCV value, MCV value, MCH value, MCHC value were performed by using Sysmex KX-21N(hematology analyzer).

II- Serum Biochemical Analysis:

Including ALT (alanine aminotransferase), AST (aspartate aminotransferase), ALP (alkain phosphatase), (TP) serum total protein, albumin, globin, (TBIL) total bilirubin, direct bil, indirect bil, blood glucose, and (TC) total cholesterol.

AST, ALT, and ALP were determined calorimetrically according to the method of Tietz, (2006).

Total protein content in serum was determined calorimetrically according to Henry, (1964).

Albumin in serum was determined calorimetrically according to Drupt, (1974).

Total bilirubin was determined according to Burtis et al., (2006), blood glucose was determined according to Tietz et al., (2006) and total cholesterol (T.C) was determined according to (Richmond, 1973).

Statistical Analysis:

Data of the present study were analyzed using a one-way analysis of variance (ANOVA) for all tested groups according to Snedecor and Cochran, (2006). Means separations were done by Duncan's multiple range tests according to Duncan, (1955). The present data were analyzed using SPSS, 20 for windows. Results are considered significant at a probability level of 0.05 (P ≤ 0.05).

RESULTS

Effect of CCl₄ and Hepatoprotective Agents on Erythrogram Parameters At 2 and 3 Weeks:

Chamomile, grape seed, and silymarin administration alone produced a non-significant variation in erythrogram when compared to the normal control group. While there was a reduction in RBCs, Hb, PCV, MCV, MCH, and MCHC levels in CCl₄ treated group when compared with normal control, there was microcytic hypochromic anemia following significant fall in the proceeding parameters. Moreover, pre-treated groups (6, 7&8) showed improvement in RBCs, HB, PCV, MCV, MCH, and MCHC levels when compared with the CCl₄ group. As illustrated in table (1).

There was a significant improvement in erythrogram parameters at 3 weeks then 2 weeks as illustrated in table (2).

Effect Of CCl₄ and Hepatoprotective Agents on Biochemical Parameters Analysis at 2 and 3 Weeks:

The present study reported that groups oral treated with chamomile, grape seed, and silymarin alone showed non-significant change when compared to the normal control group. While i/P injection of CCl₄ in rats produced a significant increase in ALT , AST and ALP activities, total bilirubin level, direct bil, cholesterol and glucose comparing with the control
group. While a decrease in TP, Albumin, Globulin and A/G ratio. On the other hand, pre-treated groups with chamomile, grape seed and silymarin showed a critical reduction in ALT, AST ALP activities, bilirubin, cholesterol and glucose tends to return these parameters to their normal levels and caused to increase in protein and albumin. As illustrated in table (3). All these parameters improved in 3 weeks of treatment more than 2 weeks as showed in tables (4).

Table 1: The effects of CCl₄ hepatotoxicity as well as the protective effects of chamomile, grape seed and silymarin on erythrogram after 2 weeks.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>RBCs (10⁶/µL)</th>
<th>Hb (g/dl)</th>
<th>PCV (%)</th>
<th>MCV (fl)</th>
<th>MCH (pg)</th>
<th>MCHC (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.79±0.16ᵃ</td>
<td>13.96±3.7ᵃ</td>
<td>42.03±1.07ᵃ</td>
<td>61.89±0.43ᵃ</td>
<td>20.55±0.22ᵃ</td>
<td>33.22±0.11ᵃ</td>
</tr>
<tr>
<td>Chamomile</td>
<td>6.70±0.04ᵃ</td>
<td>13.82±0.05ᵃ</td>
<td>41.47±0.16ᵃ</td>
<td>61.83±0.25ᵃ</td>
<td>20.72±0.06ᵃ</td>
<td>33.33±0.01ᵃ</td>
</tr>
<tr>
<td>Grape seed</td>
<td>6.70±0.07ᵃ</td>
<td>13.87±0.18ᵃ</td>
<td>41.68±0.56ᵃ</td>
<td>62.05±0.20ᵃ</td>
<td>20.68±0.07ᵃ</td>
<td>33.32±0.00ᵃ</td>
</tr>
<tr>
<td>Silymarin</td>
<td>6.74±0.07ᵃ</td>
<td>13.70±0.15ᵃ</td>
<td>41.10±0.46ᵃ</td>
<td>60.93±0.97ᵃ</td>
<td>20.30±0.32ᵃ</td>
<td>33.23±0.10ᵃ</td>
</tr>
<tr>
<td>CCl₄</td>
<td>4.73±0.65ᵇ</td>
<td>8.32±0.09ᵇ</td>
<td>28.27±0.29ᵇ</td>
<td>52.84±0.32ᵇ</td>
<td>17.09±0.24ᵇ</td>
<td>29.43±0.33ᵇ</td>
</tr>
<tr>
<td>Ch+CCl₄</td>
<td>5.90±0.16ᵇ</td>
<td>12.08±0.18ᵇ</td>
<td>36.25±0.66ᵇ</td>
<td>60.58±0.57ᵇ</td>
<td>20.19±0.19ᵇ</td>
<td>33.33±0.00ᵇ</td>
</tr>
<tr>
<td>Gr+CCl₄</td>
<td>6.17±0.17ᵇ</td>
<td>12.45±0.18ᵇ</td>
<td>37.37±0.55ᵇ</td>
<td>60.54±0.48ᵇ</td>
<td>20.15±0.17ᵇ</td>
<td>33.33±0.00ᵇ</td>
</tr>
<tr>
<td>Sily+CCl₄</td>
<td>6.04±0.16ᵇ</td>
<td>12.10±0.21ᵇ</td>
<td>36.30±0.65ᵇ</td>
<td>60.08±0.68ᵇ</td>
<td>20.03±0.23ᵃ</td>
<td>33.30±0.02ᵃ</td>
</tr>
</tbody>
</table>

ᵃᵇ Means in the same column with different superscripts are significantly different (p≤ 0.05); values are presented as means ±SE.

CCL₄ (Carbon tetrachloride). Ch (chamomile). Gr(grape seed) . Sly (slymarin).

Table 2: The effects of CCl₄ hepatotoxicity as well as the protective effects of chamomile, grape seed and silymarin on erythrogram after 3 weeks.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>RBCs (10⁶/µL)</th>
<th>Hb (g/dl)</th>
<th>PCV (%)</th>
<th>MCV (fl)</th>
<th>MCH (pg)</th>
<th>MCHC (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.82±0.09ᵃ</td>
<td>14.21±0.17ᵃ</td>
<td>42.63±0.52ᵃ</td>
<td>62.51±0.97ᵇ</td>
<td>20.82±0.32ᵇ</td>
<td>33.33±0.00ᵃ</td>
</tr>
<tr>
<td>Chamomile</td>
<td>6.71±0.01ᵃ</td>
<td>14.00±0.09ᵃ</td>
<td>41.92±0.27ᵃ</td>
<td>62.76±0.39ᵃ</td>
<td>20.90±0.13ᵃ</td>
<td>33.20±0.18ᵃ</td>
</tr>
<tr>
<td>Grape seed</td>
<td>6.82±0.09ᵃ</td>
<td>14.21±0.17ᵃ</td>
<td>42.63±0.52ᵃ</td>
<td>62.51±0.97ᵇ</td>
<td>20.82±0.32ᵇ</td>
<td>33.33±0.00ᵃ</td>
</tr>
<tr>
<td>Silymarine</td>
<td>6.84±0.08ᵃ</td>
<td>14.00±0.06ᵃ</td>
<td>42.00±0.52ᵃ</td>
<td>61.34±0.68ᵇ</td>
<td>20.44±0.22ᵇ</td>
<td>33.28±0.05ᵃ</td>
</tr>
<tr>
<td>CCl₄</td>
<td>4.50±0.06ᶜ</td>
<td>8.32±0.09ᶜ</td>
<td>25.52±0.29ᶜ</td>
<td>54.80±0.73ᶜ</td>
<td>18.39±0.24ᶜ</td>
<td>29.61±0.33ᶜ</td>
</tr>
<tr>
<td>Ch+CCl₄</td>
<td>6.46±0.06ᵇ</td>
<td>13.00±0.10ᵇ</td>
<td>38.92±0.33ᵇ</td>
<td>60.22±0.65ᵇ</td>
<td>20.08±0.20ᵇ</td>
<td>33.33±0.00ᵃ</td>
</tr>
<tr>
<td>Gr+CCl₄</td>
<td>6.77±0.11ᵃ</td>
<td>13.85±0.14ᵃ</td>
<td>41.88±0.74ᵃ</td>
<td>61.83±0.27ᵃ</td>
<td>20.78±0.19ᵇ</td>
<td>33.14±0.17ᵃ</td>
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<tr>
<td>Sily+CCl₄</td>
<td>6.46±0.08ᵇ</td>
<td>12.92±0.15ᵇ</td>
<td>38.77±0.46ᵇ</td>
<td>60.01±0.49ᵇ</td>
<td>20.00±0.15ᵇ</td>
<td>33.33±0.00ᵃ</td>
</tr>
</tbody>
</table>

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CCL₄ (Carbon tetrachloride). Ch (chamomile). Gr(grape seed) . Sly (slymarin).
Table 3: The effects of CCl₄ hepatotoxicity as well as the protective effects of chamomile, grape seeds and silymarin on serum biochemical analysis after 2 weeks.

<table>
<thead>
<tr>
<th>Parameters Groups</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>ALP (U/L)</th>
<th>TP (g/dL)</th>
<th>ALB (g/dL)</th>
<th>Globulin (g/dL)</th>
<th>A/B (Ratio)</th>
<th>Cholesterol (mg/dL)</th>
<th>Triglycerides (mg/dL)</th>
<th>T.BIL (mg/dL)</th>
<th>L.BIL (mg/dL)</th>
<th>D.BIL (mg/dL)</th>
<th>Glucose (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>32.8±1.8</td>
<td>116.6±2.9</td>
<td>23.9±2.1</td>
<td>7.61±0.9</td>
<td>4.22±0.9</td>
<td>3.36±0.6</td>
<td>1.26±0.9</td>
<td>106.8±17.3</td>
<td>1.2±0.9</td>
<td>0.1±0.9</td>
<td>0.0±0.9</td>
<td>0.2±0.9</td>
<td>109.8±18.3</td>
</tr>
<tr>
<td>Chamomile</td>
<td>24.1±0.8</td>
<td>115.8±3.5</td>
<td>23.9±2.1</td>
<td>7.61±0.9</td>
<td>4.22±0.9</td>
<td>3.36±0.6</td>
<td>1.26±0.9</td>
<td>105.9±17.2</td>
<td>1.2±0.9</td>
<td>0.1±0.9</td>
<td>0.0±0.9</td>
<td>0.2±0.9</td>
<td>108.8±18.3</td>
</tr>
<tr>
<td>Grape seed</td>
<td>38.1±1.5</td>
<td>115.8±3.5</td>
<td>23.9±2.1</td>
<td>7.61±0.9</td>
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<td>110.8±18.3</td>
</tr>
<tr>
<td>Silymarin</td>
<td>32.8±1.8</td>
<td>116.6±2.9</td>
<td>23.9±2.1</td>
<td>7.61±0.9</td>
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<td>0.0±0.9</td>
<td>0.2±0.9</td>
<td>109.8±18.3</td>
</tr>
<tr>
<td>CCl₄</td>
<td>129±9.8</td>
<td>78±9.8</td>
<td>20±1.1</td>
<td>1.8±0.1</td>
<td>1.2±0.1</td>
<td>0.6±0.1</td>
<td>1.2±0.1</td>
<td>120±10.4</td>
<td>1.3±0.1</td>
<td>0.1±0.1</td>
<td>0.2±0.1</td>
<td>1.2±0.1</td>
<td>122±10.4</td>
</tr>
<tr>
<td>CCl₄+Silymarin</td>
<td>42±9.8</td>
<td>78±9.8</td>
<td>20±1.1</td>
<td>1.8±0.1</td>
<td>1.2±0.1</td>
<td>0.6±0.1</td>
<td>1.2±0.1</td>
<td>120±10.4</td>
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<td>CCl₄+Chamomile</td>
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<td>CCl₄+Grape seed</td>
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<td>1.26±0.9</td>
<td>105.8±17.3</td>
<td>1.2±0.9</td>
<td>0.1±0.9</td>
<td>0.0±0.9</td>
<td>0.2±0.9</td>
<td>110.8±18.3</td>
</tr>
</tbody>
</table>
| a-d Means in the same column with different superscripts are significantly different (p≤ 0.05); values are presented as means ±SE.

DISCUSSION

Metabolic and detoxifying body functions are the main role of the liver; it is responsible for metabolism, synthesis, storage, and redistribution of nutrients, carbohydrates, fats, and vitamins. And it removes wastes and xenobiotics by metabolic conversion and biliary excretion (Yang et al., 2015).

CCl₄ is one of the most potent inducers of acute liver injury; it is often used in animal studies to model human liver injury (Yang et al., 2015; Zou et al., 2016). Modern medicine is still hampered by a lack of reliable hepato-protective drugs; therefore, numerous traditional herbal medicines have been studied for their hepato-protective efficiency (Lu et al., 2016).

We aimed to evaluate the effect of chamomile, grape seed extract and silymarin against CCl₄-induced acute hepatic damage in rats we examined three different extract types as potent hepatoprotective medicinal plants.

In the present study, the administration of chamomile alone does not affect hematological parameters and hepatic enzymes. The same results stated by Luke (2013) who reported that oral administration of chamomile extract alone to rats did not affect the levels of hepatic enzymes and hematological parameters thus confirming the safe use of this plant extract on the liver. Also, Mohamed (2016) demonstrated that the rats treated with chamomile alone did not affect any of the tested parameters.

It is worthy to note that GSE was had no negative impact on hematological parameters the same results stated by Wren et al., (2002) and Bentivegna, Whitney (2002), Gihan et al.,(2014) and Amany et al. (2018).

The results of the present study showed that oral administration of silymarin alone has no significant effect on the hematological parameter this agrees with the result of Shaymaa et al., (2017).
In the present study, the reduction was noted in the levels of blood Hb, RBCs count, MCH, MCHC, PCV and leads to microcytic hypochromic anemia in CCl₄ intoxicated rats than healthy control this may be attributed to the cytotoxic effect and suppression of the erythropoiesis caused by CCl₄. Similar results were reported by (Mandal et al., 1998).

Tung et al., (1975) referred these effects to disturbed hematopoiesis, the destruction of erythrocytes and reduction in the rate of their formation and/or their enhanced removal from circulation. Moreover, Makni et al., (2012) added that peroxidation developed by CCl₄ led to a destruction of membrane protein, alternation of membrane-bound enzymes as well as erythrocyte osmotic fragility.

Saba et al., (2010) recorded that the stress associated with CCl₄ administration was further corroborated by the increased erythrocyte osmotic fragility which has also been reported to increase during stress (Droge 2002). This is not farfetched because CCl₄ has been known to produce hepatic damage by the generation of highly reactive trichloromethyl (CCl₃*) and trichloromethylperoxy (CCl₃OO*) radical when metabolized by cytochrome P₄₅₀ (Britton and Bacon, 1994, Weber et al., 2003).

These findings were in agreement with Zahran et al., (2018) who revealed that CCl₄ injected i/p into the experimental rats at a dose of 1 ml/kg b.w, once daily, 3 times weekly for four weeks to induce toxicity significantly reduced Hb level, RBCs count and PCV. The intoxication of CCl₄ in rats led to microcytic hypochromic anemia and increased erythrocyte fragility.

Results of this study revealed that pre-treated by chamomile, grape seed, and silymarin can mitigate all the adverse effects of CCl₄ on hematological parameters.

Similar results were obtained by Mohamed et al., (2016) who found that rat was intoxicated by oral administration of EtOH (4 g/kg, b.w.) caused anemia while pre-treated with various doses of chamomile (25, 50, and 100 mg/kg, b.w. p.o.) for 10 days showed significantly abrogated these haematological deregulations induced by EtOH intoxication. EtOH administration dramatically increased the erythrocyte MDA level and significantly decreased the content of -SH groups. Chamomile (25, 50, and 100 mg/kg; b.w.) pre-handling dose-dependently protected erythrocytes sylfhydryls against depletion caused by alcohol administration and reduce MDA also showed that alcohol administration significantly decreased erythrocytes antioxidant enzyme activities as SOD and depletion of sulfhydryl groups (–SH) content, chamomile pre-treatment improvement all these intracellular disturbances. These findings suggest that chamomile inhibits neutrophil ROS production and protects against EtOH-induced haematologiical parameters changes and erythrocytes oxidative stress. The haematoprotection offered by chamomile might involve in part its antioxidant. This is in the agreement of our study although they used another toxic agent.

Luke (2013) reported that rats administered ethanol were given 1 ml of 70 % for 14 days developed anemia. Anemia was macrocytic and hypochromic. On the other hand, rats pretreated with a 1.0 g/kg body weight chamomile extract showed a positive effect of this plant on total RBC's count, Hb, PCV, Blood indices. The possible mechanism responsible for the protective and curative effects of CHE in the observed ethanol-induced toxicity may be as a result of the extract acts as a free radical scavenger by intercepting radicals involved in ethanol metabolism by microsomal enzymes or the extract may contain phytochemicals. Certain flavonoids, triterpenoids, and steroids are known to protect against toxins (Salmon, 1992).

Amany et al., (2018) revealed that oral administration of indomethacin (5 mg/kg B. wt) for 10 days in rats caused a significant decrease in RBCs count, Hb concentration, PCV, MCV, and the recorded microcytic hypochromic anemia. The co-exposed GSE/Indo-group showed improvement in RBCs count, Hb concentration, PCV. Those given results are in
agreement with ours. The relative improvement of these parameters may be due to suggested antioxidant activity of GSE in vivo includes stimulating enzyme production of nitric oxide, oxygen radical scavenging, and inhibition of nitrositive stress (Bagchi et al., 2000 and Roychowdhury et al., 2001).

Similar results were obtained by Zahran et al., (2018) showed that CCl₄ toxicity significantly reduced Hb level, RBCs count and PCV with respect to normal control. The intoxication of CCl₄ rats led to microcytic hypochromic anaemia, increased erythrocyte fragility. Pre-treatment with silymarin 100 mg/kg b.w brought significant restoration in hematological and renal function parameters disturbance. Also consistent with our results Abdel-salam et al., (2018) recorded that rats treated with dexamethasone (.25mg/kg b.wt) i/m twice weekly with 72 hrs intervals led to a reduction in RBCs, PCV% compared with the control group. While there was a non-significant change in their values in the silymarin treated alone group as compared with the control group Moreover, there was a significant increase in their values as compared with dexamethasone treated groups. The cytoprotective effects of Silymarin are mainly attributable to its antioxidant and free radical scavenging properties (Abid et al., 2015).

Generally, AST, ALT (formerly known as SGOT and SGPT) and ALP have been used as serum markers to represent liver damage (Sodikoff (1995)).

This study has shown that chamomile alone does not have any negative impact on liver function parameters these results agree with Luke (2013). Also has shown that GSE alone does not have any negative impact on liver function parameters. As revealed previously in toxicological studies on GSE by Wren et al., (2002) ;Bentivegna (200); Whitney (2002) and Gihan et al., (2014).

Our study confirmed those results of Naglaa et al., (2016); Shaymaa et al., (2017) and Samira and Zine (2019) who demonstrated that rats orally treated with silymarin alone showed non-significant change on liver function parameters.

These enzymes are markedly elevated in CCl₄-induced hepatic damage (Lu et al., 2016 and Yang et al., 2015) and were elevated in the CCl₄ group in our study. Therefore, results provided further evidence that all three extracts tested in this study exerted favorable hepatoprotective effects against CCl₄-induced liver injuries; the strongest effect was induced by grape seed, followed by chamomile and silymarin, as demonstrated by the marked and significant decrease on the CCl₄-induced serum AST, ALT elevations and ALP increase in these groups compared with the CCl₄ only group. These changes in enzyme activities, reflecting the damage of the liver cells or changes in the cell membrane permeability which led to the leakage of enzymes from cells to the circulation.

Regarding total protein, albumin, globulin, and A/G ratio, our data showed that there was a significant decrease in total protein, albumin, and globulin in rats injected with CCl₄. This may due to liver damage as the liver is considered the main organ responsible for the synthesis of most proteins and it was noticed that total protein and albumin increased in pretreated groups. That proved the recovery of liver synthetic function. These data are in agreement with Elshater et al., (2013); Mona et al., (2014) and Mritunjay et al., (2018).

On the other side, our result was opposite to Tresina et al., (2016) and Gabr et al., (2018) who reported that hepatotoxicity was induced in male rats by i/p injection of CCl₄ (2.5 ml/kg bodyweight for 14 days) showed significant elevation of A/G ratio.

In the present, total bilirubin and direct bilirubin were significantly increased in CCl₄ when compared with the control group. These results were in agreement with Mukherjee (2003); Abdalla and Mohamed (2016): and high level of total bilirubin, and direct bilirubin, which are considered indicators of cholestasis. This is because of liver damage caused by CCl₄. Similar results in the studies of Sedlak and Snyder (2004) who reported that bilirubin is an important physiological cytoprotectant due to its antioxidant ability. This result agrees
with the result of Ammar et al., (2018) who recorded that rats received 2ml/kg body weight of CCl₄ for 14 days showed Hyperbilirubinemia may result from the production of more bilirubin than the liver can process, damage to the liver impairing its ability to excrete normal amount of bilirubin or obstruction of excretory ducts of the liver Graw et al., (1999).

Results of the present study showed that the injection of CCl₄ produced a progressive increase in blood glucose level in rats which is in agreement with Mukherjee (2003); Abdalla and Mohamed (2016). Regarding that liver is the main site of carbohydrate metabolism and its metabolic pathways including glycolysis, gluconeogenesis, and glycogenolysis occur in this part of the body, the reason for the increase in blood glucose levels in damaged rats might be due to severe damage to the liver as the result of interference in the metabolic pathways of carbohydrates.

These results disagree with results obtained by Rui et al., (2002) who reported that gluconeogenesis and Krebs cycle fluxes are altered in rat livers following CCl₄ intoxication. Also, Bouhrim et al. (2018) reported that serum glucose value was reduced in CCl₄-intoxicated rats. Studies have demonstrated decreased hepatic glycogen content after treatment with CCl₄, reflecting decreased gluconeogenesis by the liver.

The results of the present study have also established that the CCl₄ treatment could have affected the lipid metabolism of the liver (cholesterol levels). These results were in agreement with Thnaian et al., (2013). On the other hand, it can be assumed that hypercholesterolemia in CCl₄ intoxicated rats resulted from damage of hepatic parenchymal cells that lead to disturbance of lipid metabolism in the liver (Havel 1986).

The current results revealed that co-treatment with CHE, GSE, and silly resulted in a significant improvement in all liver function parameters.

In our study results were consistent with Gupta and Misra (2006) who reported that there was a significant increase in TP after chamomile treatment against paracetamol intoxication.

Our results were agreed with Dalal et al., (2014) who found that oral administration of chamomile extract to rats administered 2, 4-D (Dichlorophenoxyacetic acid) for 28 days showed significantly decreased of the elevated liver enzymes activities (AST, ALT, and ALP) and total bilirubin level. Also illustrated significantly increased serum TP and Alb levels when compared with control positive rat group received 2, 4-D in a dose 75 mg/kg b.wt.

In addition, Kumar et al., (2012) reported that the extract of chamomile induced a hepatoprotective effect and increases in serum ALB levels. The hepatoprotective activity of chamomile may be due to the normalization of impaired hepatocyte cell membrane function activity.

Chamomile was reported to have an effect on serum cholesterol levels in hyperlipidemic rats (Al- Jubouri et al., 1990). After 10 days, the hyperlipidemic mice treated orally with 6% of chamomile (4 mg/kg) had significantly reduced serum cholesterol compared with control rats. Same results stated by Shahin et al., (2018) who found that treatment of hypercholesterolemic rats with 110 mg/kg of chamomile extract for 4 weeks did not only prevent a significant increase of triglycerides, cholesterol, and inflammatory factors, but it also increase antioxidant enzyme.

Mansour et al., (2018) reported the oxidative damage induced by a high cholesterol (2%) diet is modulated by consumption of herbal extracts like 55 mg/ml chamomile and it can be said that to minimize the damages caused by 10 mg/kg b.w of lovastatin drug.

Also, Weidner et al., (2013) found that in vivo treatment of insulin-resistant high-fat diet (HFD)-fed mice with CFE (200 mg/kg/d) for 6 weeks considerably reduced insulin resistance, glucose intolerance, and cholesterol, results indicate that early administration of
CFE effectively protects from HFD-induced fatty liver disease and associated liver inflammation.

This effect was similar to that previously reported by Magbolah (2018) who found that rats subcutaneous injection of carbon tetrachloride (CCl₄) in paraffin oil 50% V/V (2ml/kg b. wt.) twice a week for two weeks, showed a significant increase in ALT, AST, ALP, cholesterol and bilirubin and decrease in total protein, while rats pre-feed on 5% chamomile showed the highest decrease of AST, ALT, ALP cholesterol and bilirubin levels in the serum and increase TP level compare with CCl₄ group.

Hanaa and Maisaa (2013) investigate the heptonephro-protective effect of grape seeds proanthocyanidin extract (GSPE) against the risks induced by gibberellin acid in male rats. The results recorded that 3.85 mg/Kg body weight of gibberlic caused a significant increase in total lipids, total cholesterol, with a significant ALP increase in serum AST, ALT, and bilirubin, while, a significant decrease in total protein content in serum was observed in rats given gibberlic. All of these adverse effects seemed to be ameliorated by oral administration of 100mg/kg bw GSPE with gibberlic to rats for 2 months indicating the protective effect of grape seeds GSPE on gibberlic induced oxidative stress in rats.

Similar results reported by Amany et al., (2017) who said that orally pre-treated with Grape seeds extract (GSE) at a concentration of 40 mg/kg B. wt alone or in combination with indomethacin (Indo.) at a dose of 5 mg/Kg B. wt orally given for 10 days, group were exposed to indomethacin for 10 days showed marked hypoproteinemia, hypoalbuminemia with elevated serum levels of ALP, AST, ALT. On the other hand, GSE/Indo-exposed group showed a marked elevation in serum total proteins and albumin with a significant reduction in serum ALP, AST, ALT activities compared to indo methacin group.

Our study confirmed those results of Yousef et al., (2019) who demonstrated that administration of carboplatin and thalidomide revealed elevation in ALT, AST, ACP, ALP activities, also reduction in total protein and albumin content. The presence of GSPE with carboplatin and thalidomide minimized their toxic effect on ALT, AST, ACP, ALP activities, also modulated total protein and albumin content to reach near to the control values.

Yousef et al., (2009) reported that animals receiving combined cisplatin- GSE treatment showed significant alleviation of the decreased values of proteins compared to the cisplatin treated group. Oral administration of proanthocyanidins from grape seed produced a hypocholesterolemic effect in a high cholesterol animal feed model; specifically, it prevented an increase in total and LDL plasma cholesterol (El-Adawi et al., 2006). The same result recorded by (Sally et al., 2013). This antihyperlipidemic effect of GSE can be attributed to its ability to enhance the activity of enzymes involved in bile acid synthesis and its excretion leading to a decrease in the serum TC and TG levels (Sethupathy et al., 2002).

Sally et al., (2013) recorded that a single i/p. injection of STZ (50 mg/kg) produced an elevation of serum glucose level which was evidenced 48 h after administration while oral treatment of hyperglycaemic rats with GSE (100 mg/kg/day) and (300 mg/kg/day) for 2 weeks decreased the elevated serum glucose level. This was in accordance with Suwannaphet et al., (2010) and his colleges who showed that GSE prevents hyperglycemia and hyperinsulinaemia due to its ability to attenuate the impairment of insulin-stimulated glucose disposal in rats with insulin resistance.

Similar results were obtained by Nouf et al., (2015) showed that the administration of CCl₄ to rats significantly elevated the activity level of the serum liver function index, ALT and AST, with respect to normal rats. Pre-treatment of rats with silymarin 200mg/kg b.w for 21 days alone or in a combination with vitamin E or curcumin, markedly ameliorated the elevation of serum ALT and AST versus CCl₄ intoxicated rats. Maybe contribute to their antioxidant, anti-inflammatory, and anti-apoptotic properties.
Abdel-salam et al., (2018). Also, the results showed that there was a significant reduction in serum total protein and globulin in dexamethasone-treated group as compared with control group. There was a non-significant change in their values in the group treated with dexamethasone + silymarin as compared with the control group. Moreover; there was a significant increase in globulin value in the group treated with silymarin as compared with the control. There was a non-significant change in serum albumin levels in silymarin alone treated groups as compared with the control group. There was a significant reduction in cholesterol levels in groups treated with dexamethasone and silymarin as compared with the control group. While there was a non-significant change in its values in silymarin alone treated as compared with the control group. Silymarin inhibits liver injury by maintaining the integrity of the liver cell membrane, inhibits the secretion of liver enzymes in the blood, and suppresses apoptosis in hepatocytes Vargas et al., (2014).

Also, M. Ozturk et al., (2012) showed rats injected i/p with 2ml/kg b.w with CCl4 caused to increase in glucose and to decrease in albumin, total cholesterol levels. 2.5% infusion of silymarin pre-treated improved these changes. All liver function tests were elevated by CCl4 administration and then reduced, by silymarin pre-treatment. The study by Zhang et al., (2013) evaluated that the protective effects of silymarin, as a market extract from the seeds of milk thistle, on an alcoholic fatty liver model of female rats. The control group received a normal diet; the model group was treated with ethanol for 6 weeks; silymarin groups received the standardized dry extract of silymarin at 100, 150 and 200 mg/kg for 6 weeks (dry extract was previously dissolved into water by 20, 30 and 40mg/ml), together with ethanol by gavage twice a day. After 6 weeks of pre-Treatment with milk thistle (silymarin) 200mg/kg showed a greater decrease in serum activities of ALT and AST and the level of total bilirubin (TBIL), blood glucose and total cholesterol (TC) than the alcoholic group.

The same results were stated by Hebatalla et al., (2018) who reported that feeding rats with Phenylephrine (HFD) for 20 weeks. Resulted in significant elevation of blood glucose level as well as significant elevation of serum cholesterol, there was also the significant elevation of serum liver enzymes ALT, AST compared to that of the normally fed control rats. Giving silymarin orally100 mg/kg b.w for 4 weeks resulted in a significant reduction in blood glucose levels, reduction of serum cholesterol and AST, ALT compared to HFD fed non treated group.

The previously given data from studies are all consistent with our proved the hepatoprotective effect of the three herbal extracts when used as a pretreatment and they had no significant effect when used alone.

**CONCLUSION**

In conclusion, the results of this study denote that oral administration of chamomile, grape seed extracts and silymarin for 3 weeks act as hepatoprotective. The hepatoprotective activity of extract may be attributed to its antioxidant effect which maintains the functional integrity of hepatic cells. The current study recommends that; intake of chamomile extract, grape seeds extract and silymarin as herbal tea may be beneficial for patients who suffer from liver disorders

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