Hepatoprotective Effect Of *Calendula Officinalis* Flowers On CCl₄ Induced Rats.

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**Abstract:** This study was carried out to evaluate the hepatoprotective Potential of *Calendula officinalis* flowers against aflatoxins induced liver damage. On impaired liver function of rats injected with Carbon Tetrachloride (CCl₄). Thirty-six male mature albino rats weighting 150-160g per each were used in this study and divided into 6 equal groups, the first group was kept as a control (–ve) group, the second group(+ve) which rats inflicted with hepatotoxicity by CCl₄ were fed on basal diet. The tested plant powder flower was given to the rats as a percent of *C.officinalis* 1, 2.5, 5, and 7.5% from the Basel diet for 28 days. At the end of the experimental the serum liver functions (GOT, GPT, ALP), kidney functions (Urea, Creatinine and Uric Acid), F.I, F.E.R, B.W.G%, High-density lipoprotein (HDL-c), Total Cholesterol (T.C) and Triglycerides (T.G) of rats and histopathological changes of liver were examined. The results of the obtained data indicated that tested plant significantly (P≤0.05) decreased serum, TG, TC, and increased HDL. Also, the tested plants improved liver and kidney functions.

**Keywords:** liver function, kidney function, HDL, TG, TC, *Calendula officinalis* and histopathological examination.

**Introduction**

The liver is responsible for the metabolism and detoxification of most of the components that enter the body (Nunez, 2006). Hepatotoxicity is the most widespread pathology worldwide, representing up to 83% of all cases. Hepatitis, viral infections, food additives, alcohol, toxic industrial chemicals, air, and water pollutants are the major risk factors of liver toxicity (Jemal et al., 2007). Carbon
Journal of Home Economics, Volume 29, Number (2,4), 2019

tetrachloride (CCl₄) is a potent environmental hepatotoxin (Guven et al., 2003), that in addition to hepatic problems, causes dysfunction of the kidneys, lungs, testis, brain, and blood by generating free radicals (Ozturk et al., 2003; Khan et al., 2009).

Carbon tetrachloride (CCl₄) is a highly toxic chemical agent that is used as an industrial solvent. CCl₄ is widely used to induce hepatic steatosis and to study the effects of protective agents, especially antioxidants. The toxic effects of CCl₄ on the liver have been extensively studied (Sheweita et al., 2001). Metabolic activation of CCl₄ by cytochrome P450 to the free radicals, namely trichloromethyl peroxy radicals, is reported to enhance lipid peroxidation and protein oxidation in the liver, resulting in widespread membrane damage and liver injury. Membrane damage also causes alterations in lipoprotein secretion and accumulation of lipoprotein and lipid droplets in hepatocytes (Junnila et al., 1998).

Synthetic drugs available in the market for liver treatment cause many complications (Sanjiv, 2002). The liver damages can be indicated by assessing the level of liver enzymes and proteins as well as assessing histopathological changes in liver tissues. Liver enzymes that are used in the detection of liver malfunction are; alanine aminotransferase (ALT) and aspartate aminotransferase (AST). The liver tissue damage can also be assessed through histological studies as increased permeability of liver cells is an important indicator of liver damage (Edet et al., 2011). The liver therapy can be achieved by traditional medicines from medicinal as they are safer, easily reachable, and economical and have fewer toxicities and side effects compared to synthetic medicines (Nair and Chanda, 2007). Therefore, there is a growing interest in herbal medicines (Hussain et al., 2009).

The medicinal activities of plants are attributed to their bioactive compounds that include phenolics, flavonoids, terpenoids, glycosides and alkaloids as they are proved to be efficient precursors for drug formation. These Phytochemicals act additively, individually or in a synergic way for the progress of human health (Schutz et al., 2006)

Nature has been a source of medicinal agents since the beginning of time. Herbal medicine is still the most common source for primary health care of about 65-80% of the world’s population, mainly in developing countries. Different parts of these plants including Leaves,
flowers, stems, roots, seeds, fruit, and bark can all be constituents of herbal medicines (Shibamoto et al., 2008). The medicinal values of these plants lie in their phytochemical components which produce definite physiological actions on the human body. The most important of these components are alkaloids, tannins, flavonoid and phenolic compounds (Shariff, 2001). Such components are extensively found at different levels in various medicinal plants and used in herbal medicine to treat diverse ailments such as cough, malaria, wounds, toothache and rheumatism diseases (Exarchou et al., 2002).

**Calendula Officinalis** L., a member of the Asteraceae family, is an annual plant with yellow to orange flowers; it grows to about two feet tall with multiple branches. The flowers are the part used medicinally, it is also known as Gold-bloom, Marigold, Marybud, Pot Marigold (Gazim, et al., 2008). C.officinalis contains a high amount of carotenoids such as flavoxanthin, lutein, rubixanthin, β-carotene, g-carotene, and lycopene (Pintea, 2003).

Flavonoids are potent antioxidants and reported as having a wide range of biochemical functions (anti-allergic, anti-inflammatory, antimicrobial and anticancer) among these flavonoids quercetin and kaempferol are the most important and widely spread flavonols class (Asif and Khodadadi, 2013).

In the present study, we investigated proximate chemical composition, mineral and phytochemicals content and the hepatoprotective activity of *Calendula Officinalis* L against CCl₄-induced hepatotoxicity in male rats by assaying liver and kidney functions and histopathology of liver and kidney tissues.

### 2. Materials and Methods

**Materials:**

**Plant Materials:**

*Calendula Officinalis* were purchased from the local market of Shibin El- Kom, washed and dehydrated at 60°C for 6 hrs then ground to soft powder and kept in dusky Stoppard glass bottles.

**Rats:**

Male albino rats weighing 150-160 g per each were purchased from Medical Insects Research Institute, Cairo, Egypt.
Basal Diet:

The basal diet was prepared according to the following: protein (10%), corn oil (10%), vitamin mixture (1%), mineral mixture (4%), choline chloride (0.2%), methionine (0.3%), cellulose (5%), and the remained is corn starch (69.5%) according to Campbell, (1963). The vitamin mixture component was recommended by Hegested et al., (1941), while the salt mixture was formulated according to Drury and Wallington, (1980).

Chemicals:

All chemicals, solvents and buffers in analytical grade, carbon tetrachloride (CCl₄, 10% liquid solution), vitamin and salt mixtures components used for rats feeding were purchased from El- Gomhoria Company for Chemicals and Drug Trading, Cairo, Egypt. Casein was obtained from Morgan Chemical Co., Cairo, Egypt.

Methods:

Preparation of Liver Impaired Rats:

Liver impaired was induced in normal healthy male albino rats by subcutaneous injection of CCl₄ (0.2mg/kg body weight) for two weeks according to method described by Passmore and Eastwood, (1986).

Rats (n=36 rats) were housed individually in wire cages in a room maintained at 25 ± 2 °C and kept under normal healthy conditions the study protocol was approved by Ethical committee for laboratory animal feed and care. All rats (36 rats) were fed on basal diet for one-week before starting the experiment for acclimatization. After one week period, the rats were divided into two main groups, the first group (Group 6 rats) still fed on basal diet (control -) and the other main group (30 rats) was injected by CCl₄ for two weeks to induce liver impaired rats then classified into five sub groups as follow:

- Group(2): Hepatic rats fed on basal diet only as a positive control (control +)
- Group (3): Hepatic rats fed on basal diet containing 1% C. Officinalis flower powder.
- Group (4): Hepatic rats fed on basal diet containing 2.5% C. Officinalis flower powder.
- Group (5): Hepatic rats fed on basal diet containing 5% C.Officinalis flower powder.
Group (6): Hepatic rats fed on basal diet containing 7.5% *C. Officinalis* flower powder.

**Blood Sampling:**

At the end of experiment period, 28 days, blood samples were collected after 12 hours fasting using the abdominal aorta and rats were scarified under ether anesthetized. Blood samples were received into clean dry centrifuge tubes and left to clot at room temperature, then centrifuged for 10 minutes at 3000 rpm to separate the serum according to (Drury and Wallington, 1980). Serum was carefully aspirate, transferred into clean covet tubes and stored frozen at -20°C until analysis.

**Hematological Analysis**

Different tested parameters in serum were determination using specific methods as follow: alanine aminotransferase (ALT) according to (Yound 1975), aspartate aminotransferase (AST) according to (Tietz, 1976) and (Yound 1975), alkaline phsphatase (ALP), according to (Belfield and Goldberg, 1971), Urea was determined according to the method described by (Patton, and Crouch, 1977). Uric acid was determined according to the method described by (While et al., 1970). Creatinine was determined according to the method described by (Henry, 1974).

**Histopathological Examination**

Liver was removed, washed in saline solution, dried by filter paper, weighted, and stored frozen in formalin solution 10% for histopathological testing according to method mentioned by (Drury and Wallington, 1980).

**Statistical Analyses**

Results were analyzed statistically using a complete randomized design (CRD), and tested significantly with least significant differences (L.S.D) at level (P< 0.05) to indicate the significant of results (Al-Rawy and Kalafallh, 2000).

### 3. Results and Discussion

**Effect of *Calendula officinalis* flowers powder on BWG (%) and FI of hepatic rats.**

1. **Body weight gain (BWG %).**

Table (1) Revealed the mean value of BWG% of hepatic rats fed on *Calendula officinalis*. It could be noticed that the mean value of
BWG% of control (+) group was lower than control (-) group, being 7.34± 0.445 and 14.47 ± 0.896 respectively, showing significant difference. The percent of increase was 97.13 % for control (-) as compared to control (+) group. All hepatic rats fed on *C.officinalis* indicated significant increases in mean values as compared to control (+) group except group (3) showed decrease, but this reduction didn’t show any significantly differences. The values were 6.27± 0.947, 9.16 ± 0.215, 11.17 ± 0.585, 8.28 ± 0.28 respectively for *C.officinalis* 1, 2.5, 5 and 7.5% respectively. The percent of decrease and increases were –14.5, 24.79, 52.17 and 12.48 for groups 3, 4, 5 and 6 respectively. The better BWG% was consider for group 5 (hepatic rats fed on *C.officinalis* 5%). This may explain the decrease in body weight in hepatic rats. Ingestion on various digestive enzymatic activities that give rise to a malabsorption syndrome, characterized by steatorrhea, hypocarotenoidemy, and to lowering of bile, pancreatic lipase, trypsin, and amylase (*Osborne et al.,* 1982).

2. Feed Intake (F.I) (g/day/rat)

Table (2) revealed the mean value of F.I (g/day/rat) of hepatic rats fed on *Calendula officinalis*. It could be noticed that the mean value of F.I of control (+) group was lower than control (-) group, being 12.26+.251 and 16.46+.907 (g/day/rat) respectively, showing significant difference. The percent of increase was 34.25% for control (-) as compared to control (+) group. All hepatic rats fed on *C.officinalis* indicated significant increases in mean values as compared to control (+) group. The values were 17.13±1.205, 16.23±0.702, 16.06±0.802, and 16.033 ± 0.96 (g/ day/rat) respectively for *C.officinalis* 1, 2.5, 5 and 7.5% respectively. The percent of increases were 39.72, 32.38, 30.99 and 30.75 for groups 3, 4, 5 and 6 respectively. This result is in the same line with *Hamzawy et al.,* (2013), they reported that hepatic rats are significant reduction of the body weight and food intake.
**Table (1) Effect of *Calendula officinalis* flowers powder on BWG% and F.I of hepatic rats.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>(G1) C- Control Mean ±SD</th>
<th>(G2) C+ Control Mean ±SD</th>
<th>(G3) <em>C. officinalis</em> (1%) Mean ± SD</th>
<th>(G4) <em>C. officinalis</em> (2.5%) Mean ± SD</th>
<th>(G5) <em>C. officinalis</em> (5%) Mean ± SD</th>
<th>(G6) <em>C. officinalis</em> (7.5%) Mean ± SD</th>
<th>LSD (P&lt;0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BWG (%)</td>
<td>14.47 ± 0.89</td>
<td>7.34 ± 0.45</td>
<td>6.27 ± 0.95</td>
<td>9.16 ± 0.215</td>
<td>11.17 ± 0.58</td>
<td>8.28 ± 0.28</td>
<td>1.219</td>
</tr>
<tr>
<td></td>
<td>% Change of positive control</td>
<td>97.13</td>
<td>-</td>
<td>-14.5</td>
<td>24.79</td>
<td>52.17</td>
<td>12.84</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>F.I (g/day/rat)</td>
<td>16.46 ± 0.91</td>
<td>12.26 ± 0.251</td>
<td>17.13 ± 1.205</td>
<td>16.23 ± 0.702</td>
<td>16.06 ± 0.802</td>
<td>16.033 ± 0.96</td>
<td>1.523</td>
</tr>
<tr>
<td></td>
<td>% Change of positive control</td>
<td>34.25</td>
<td>-</td>
<td>39.72</td>
<td>32.38</td>
<td>30.99</td>
<td>30.75</td>
<td></td>
</tr>
</tbody>
</table>

Means in the same row with different litters are significantly different. Significant (p ≤ 0.05).

**Effect of *Calendula officinalis* on liver function of hepatic rats.**

1- Glutamic Pyrofic Transaminase (G.P.T) (U/L):

Table (2) revealed the mean value of GPT (U/L) of hepatic rats fed on *Calendula officinalis*. It could be noticed that the mean value of GPT (U/L) of control (+) group was significantly higher than control (-) group, being 35.3 ± 1 and 27.63 ± 1.52 (u/l) respectively, with percent of decrease -21.72% % as compared to positive control group. All hepatic rats fed on *C. officinalis* indicated no significant decreases in mean values as compared to control (+) group. The values were 29.33 ± 0.577, 31.11 ± 1, 32.33 ± 0.5, and 30.67 ±1.52 U/L, for *C. officinalis* 1, 2.5, 5 and 7.5% respectively. The percent of decreases were -17.11, -11.89, -8.41 and -13.11 % for groups 3, 4, 5 and 6 respectively. The best serum GPT was consider for group 3 (hepatic rats treated with *C. officinalis* 1%) as compared with control (-) group.

This results are in agreement with (Abdel-Wahhab et al., 2002) they found that significant increase in ALT, AST, ALP, urea and creatinine. The significant increase of ALT, AST and ALP in aflatoxins treated animals indicate changes in the hepatic tissues and biliary system. Lin et al. (2002) showed that the hot water extract of C. officinalis flowers exhibited anti-hepATOMA activity against human liver
cancer cells with an inhibitory effect of 25-26%. These results are supported by the results of Hepatoprotective for *C. officinalis* by Khalid and Silva, (2012) who observed that the hydroalcoholic extract of the flowers, when administered to CCl4-intoxicated livers in albino male Waster rats at a dose of 10 mL/kg, resulted in a reduction of hepatocytolysis by 28.5% due to a reduction in glutamoxaloacetate transaminase (GOT) and glutamopyruvate transaminase (GPT).

2- Glutamic Oxaloacetic Transaminase G.O.T (AST) (U/L):

Table (2) indicated the mean value of GOT (U/L) of hepatic rats fed on *C. officinalis*. It could be noticed that the mean value of GOT (U/L) of control (+) group was significantly higher than control (-) group, being 228 ±1.5 and 136 ± 1 (uL) respectively, with percent of decrease - 40% % as compared to control (+) group, the significant increase in AST ,ALT, ALP , as a result of cellular damage and structural damaging of liver integrity, because these enzymes are cytoplasmic in location and released into plasma (El-Agamy, 2010 and El-Nekeety et al., 2011). All hepatic rats fed on *C. officinalis* indicated no significant decreases in mean values as compared to control (+) group. The values were 181.66 ±1.5, 172.66 ± 1.52, 196 ± 1.5, and 169.67 ± 1.527 U/L, for *C. officinalis* 1, 2.5, 5 and 7.5% respectively. The percent of decreases were -20.28, -24.21, -13.71 and -25.47 % for groups 3, 4, 5 and 6 respectively. The better serum GOT was consider for group 6 (hepatic rats treated with *C. officinalis* 7.5%) as compared with control (-) group. These results are agree with Hamzawy et al., (2013) who showed that the Calendula extract succeeded to improve the biochemical parameters, inflammatory cytokines and decreased the oxidative stress. The Calendula extract has potential hepatoprotective effects against aflatoxins due to its antioxidant properties and radical scavenging activity.

Also Preethi et al., (2006) found that the extract of *C. officinalis* had significant increase in glutathione levels in blood and liver. Glutathione reductase was found to be increased, whereas glutathione peroxidase was found to be decreased after administration of Calendula extract.

3- Alkaline Phosphatase (ALP) enzyme U/L.

Table (2) showed the mean value of ALP (U/L) of hepatic rats fed on *Calendula officinalis*. It could be noticed that the mean value of ALP
(UL) of control (+) group was significantly higher than control (-) group, being 142.33 ±1.52 and 99 ± 1.52 (u\l) respectively, with percent of decrease – 30.26% % as compared to positive control group. All hepatic rats fed on *C.officinalis* indicated no significant decreases in mean values as compared to control (+) group. The values were 131.33 ± 2.51, 132.63 ±1.52, 121 ±1.52 and111±1.527 U/L, for *C.officinalis* 1, 2.5, 5 and 7.5% respectively. The percent of decreases were -7.84, -6.88, - 14.84 and -22.12 % for groups 3, 4, 5 and 6 respectively. The better serum ALP was consider for group 6 (hepa tic rats treated with *C.officinalis* 7.5%) as compared with control (-) group.

Preethi and Kuttan, (2009) they observed that Protective effect of *C.officinalis* flower against CCl₄ induced acute hepatotoxicity. Extract has been found to contain several carotenoids of which lutein, zeaxanthin and lycopene predominates. Possible mechanism of action of the flower extract may be due to its antioxidant activity and reduction of oxygen radicals. Also, (Maysa et al., 2015) reported that *C.officinalis* extract afford a protection against CCl₄ induced toxicity and showed an improvement in liver function due to significant antioxidant activity and free radical scavenging activity of bioactive metabolites including flavonoids and terpenoids present in Calendula. These bioactive metabolites have potent activities for scavenging the hydroxyl radicals (OH.) and superoxide radicals (O.2) resulted from CCl₄ metabolites.

### Table (2) Effect of *C.officinalis* on (GPT (ALT), GOT (AST) and ALP) Enzymes of hepatic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>(G1) C Control Mean± SD</th>
<th>(G2) C Control Mean± SD</th>
<th>(G3) <em>C.officinalis</em> (1%) Mean± SD</th>
<th>(G4) <em>C.officinalis</em> (2.5%) Mean± SD</th>
<th>(G5) <em>C.officinalis</em> (5%) Mean± SD</th>
<th>(G6) <em>C.officinalis</em> (7.5%) Mean± SD</th>
<th>LSD (P&lt;0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPT(UL)</td>
<td>27.67±1.53</td>
<td>35.3±1</td>
<td>29.33±0.57</td>
<td>31.11±1.00</td>
<td>32.33±0.5</td>
<td>30.67±1.53</td>
<td>1.97</td>
</tr>
<tr>
<td>% Change of positive control</td>
<td>-21.72</td>
<td>-17.11</td>
<td>-11.89</td>
<td>-8.41</td>
<td>-13.11</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>GOT(UL)</td>
<td>136.00±1.00</td>
<td>228.00±1.52</td>
<td>181.66±1.53</td>
<td>172.66±1.53</td>
<td>196.33±1.53</td>
<td>169.67±1.53</td>
<td>3.195</td>
</tr>
<tr>
<td>% Change of positive control</td>
<td>-40</td>
<td>-20.28</td>
<td>-24.21</td>
<td>-13.71</td>
<td>-25.47</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>ALP(UL)</td>
<td>99.66±1.53</td>
<td>142.33±1.53</td>
<td>131.33±2.52</td>
<td>132.66±1.53</td>
<td>121.33±1.53</td>
<td>111.33±1.53</td>
<td>3.08</td>
</tr>
<tr>
<td>% Change of positive control</td>
<td>-30.26</td>
<td>-7.84</td>
<td>-6.88</td>
<td>-14.84</td>
<td>-22.12</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Means in the same row with different litters are significantly different. Significant (p ≤ 0.05).
Effect of *Calendula officinalis* on kidney function for hepatic rats

1- Creatinine (mg/dl).

Data of table (3) indicated the mean value of serum creatinin (mg/dl) of hepatic rats fed on various diets. It could be observed that the mean value of creatinin of control (+) group was higher than control (-) group, being 1.02 ±0.15and 0.8 ±0.0305 respectively, showing significant difference with percent of decrease -20.88% of control (-) group when compared to control (+) group. All hepatic rats fed on different diets revealed significant decreases in mean values as compared to control (+) group. The values were 0.920±0.072, 0.810±0.1, 0.77±0.025, and 0.797±0.02 mg/dl for *C.officinalis* 1, 2.5, 5 and 7.5% respectively. The percent of decreases were -11.10, -20.52, - 24.5 and -21.83 % for groups 3, 4, 5 and 6 respectively. The better serum creatinin was consider for group 6 (hepatic rats treated with *C.officinalis* 7.5%) as compared with control (-) group. This results are in agreement with (Abdel-Wahhab et al., 2002) who found that significant increase in urea and creatinine. Also Preethi et al., (2009) observed that *C. officinalis* flower extract inhibit the cisplatin induced oxidative stress and reduces the kidney damage, The renal accumulation of platinum leads to nephrotoxicity, The Calendula extract reduces the kidney damage due to its anti-oxidant activity, and The increased activity of SOD, CAT and increased level of GSH in extract treated group leads to the protection against cisplatin induced renal damage.

2- Urea (mg/dl).

Data of table (3) illustrate the mean value of serum urea (mg/dl) of hepatic rats fed on various diets. It could be noticed that the mean value of urea of control (+) group was higher than control (-) group, being 43.00 ±1.00 and 29.67 ± 1.528 mg/dl respectively, indicating significant difference with percent of decrease -31 % of control (-) group when compared to control (+) group. All hepatic rats fed on different diets revealed significant decreases in mean values as compared to control (+) group. The values were 35.67±1.528, 33.67±.577, 36.33±1.528, and 32 ±1.0 mg/dl for *C.officinalis* 1, 2.5, 5 and 7.5% respectively. The percent of decreases were -17.04, -21.69, - 15.51 and -25.58 % for groups 3, 4, 5 and 6 respectively. The better serum UA was Consider for group 6 (hepatic rats treated with *C.officinalis* 7.5%) as compared with control (-) group. These findings are in agreement with study conducted by Verma et al., (2016) who observed that treatments with ethanolic floral extract of *C. officinalis* protect plasma and renal tissue in cisplatin
induced nephrotoxicity by restoring antioxidant system of the renal tissue.

3- **Uric Acid (mg/dl).**

Results of table (3) indicated the mean value of serum (U. A) (mg/dl) of hepatic rats fed on various diets. It could be observed that the mean value of UA of control (+) group was higher than control (-) group, being 4.66±0.15 and 1.55 ± 0.05 mg/dl respectively, indicating significant difference with percent of decrease -66.73% of control (-) group when compared to control (+) group. All hepatic rats fed on different diets revealed significant decreases in mean values as compared to control (+) group. The values were 4.466±0.152, 2.40 ± 0.10, 2.40 ± 0.152 and 1.80 ± 0.10 mg/dl for *C.officinalis* 1, 2.5, 5 and 7.5% respectively. The percent of decreases were -4.28, -48.49, -48.62 and -61.37 % for groups 3, 4, 5 and 6 respectively. The better serum UA was consider for group 6 (hepatic rats treated with *C.officinalis* 7.5%) as compared with control (-) group.

The protective role of the flower extract of *C. officinalis* against cisplatin induced nephrotoxicity, extract has been found to contain several carotenoids of which lutein, zeaxanthin and lycopene predominates. Possible mechanism of action of the flower extract may be due to its antioxidant activity and reduction of oxygen radicals **Preethi and Kuttan, (2009).**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>(G1) C Control Mean ±SD</th>
<th>(G2) C’ Control Mean ±SD</th>
<th>(G3) <em>C. officinalis</em> (1%) Mean ±SD</th>
<th>(G4) <em>C. officinalis</em> (2.5%) Mean ±SD</th>
<th>(G5) <em>C. officinalis</em> (5%) Mean ±SD</th>
<th>(G6) <em>C. officinalis</em> (7.5%) Mean ±SD</th>
<th>LSD (P&lt;0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine (mg/dl)</td>
<td></td>
<td>0.81&lt;sup&gt;b&lt;/sup&gt; +0.03</td>
<td>1.033&lt;sup&gt;b&lt;/sup&gt; +0.152</td>
<td>0.920&lt;sup&gt;b&lt;/sup&gt; +0.072</td>
<td>0.800&lt;sup&gt;b&lt;/sup&gt; +0.100</td>
<td>0.77&lt;sup&gt;b&lt;/sup&gt; +0.025</td>
<td>0.80&lt;sup&gt;b&lt;/sup&gt; +0.020</td>
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<td>% Change of positive control</td>
<td></td>
<td>-20.88</td>
<td>-11.10</td>
<td>-20.52</td>
<td>-24.5</td>
<td>-21.83</td>
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<td>_</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td></td>
<td>29.67&lt;sup&gt;a&lt;/sup&gt; +1.53</td>
<td>43.00&lt;sup&gt;d&lt;/sup&gt; +1.00</td>
<td>35.67&lt;sup&gt;b&lt;/sup&gt; +1.52</td>
<td>33.67&lt;sup&gt;d&lt;/sup&gt; +0.577</td>
<td>36.33&lt;sup&gt;b&lt;/sup&gt; +1.53</td>
<td>32.00&lt;sup&gt;a&lt;/sup&gt; +1.00</td>
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<tr>
<td>Uric Acid (mg/dl)</td>
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<td>4.66&lt;sup&gt;b&lt;/sup&gt; +0.15</td>
<td>4.46&lt;sup&gt;c&lt;/sup&gt; +0.1527</td>
<td>2.40&lt;sup&gt;d&lt;/sup&gt; +0.100</td>
<td>2.46&lt;sup&gt;c&lt;/sup&gt; +0.15</td>
<td>1.80&lt;sup&gt;d&lt;/sup&gt; +0.10</td>
<td>0.22</td>
</tr>
</tbody>
</table>

Means in the same row with different litters are significantly different. Significant (p ≤ 0.05).
-Effect of Calendula officinalis on total cholesterol (T.C.), triglycerides (T.G) and high density lipoprotein cholesterol (H.D.L.c) of hepatic rats.

1- Total Cholesterol (T.C) mg\(\text{dl}\).

Data of table (4) illustrate the mean value of serum (T.C.) (mg\(\text{dl}\)) of hepatic rats fed on *Calendula officinalis*. It could be observed that the mean value of (T.C.) of control (+) group was higher than control (-) group, being 86 ±1 and 69.67 ±1.51 respectively. All hepatic rats fed on *Calendula officinalis* revealed significant decreases in mean values as compared to control (+) group. The values were 81±1, 80.33±1.528, 77.33±1.528, and 71.00±1mg\(\text{dl}\) for *C.officinalis* 1, 2.5, 5 and 7.5% respectively. The percent of decreases were -5.81, -6.59, -10.08 and -17.44 % for groups 3, 4, 5 and 6 respectively. The better serum T.C was consider for group 6 (hepatic rats treated with *C.officinalis* 7.5%) as compared with control (-) group.

These results are supported by the results published by Cordova et al., (2002). Who observed that the study of *Calendula officinalis* L. (marigold) against lipid peroxidation of rat liver microsomes and action as free radical scavenger. Suggest that the butanolic fraction of C. officinalis possesses a significant free radical scavenging and antioxidant activity and that the proposed therapeutic efficacy of this plant could be due, in part, to these properties.

2- Triglycerides (T.G) mg\(\text{dl}\).

Table (4) show the mean value of serum (T.G.) (mg\(\text{dl}\)) of hepatic rats fed on different diets. It could be noticed that the mean value of (T.G.) of control (+) group was higher than control (-) group, being 160.6 ±3.78and97.67±1.52 respectively. All hepatic rats fed on different diets revealed significant decreases in mean values as compared to control (+) group. The values were 112±1, 107.33±1.52, 104.33±1.528, &101 ±1.528 mg\(\text{dl}\) for *C.officinalis*1, 2.5, 5 and 7.5% respectively. The percent of decreases were -28.9, -31.92, -33.83 and -35.51% for groups 3, 4, 5 and 6 respectively. The better serum T.G was consider for group 6 (hepatic rats treated with *C.officinalis* 7.5%) as compared with control (-) group. Preethi et al., (2006) they found that the alcoholic extract of *Calendula officinalis* Linn. (Compositae) was evaluated for its antioxidant potential in vitro. Calendula officinalis extract was found to scavenge superoxide radicals generated by photoreduction of riboflavin
3. High Density Lipoprotein (HDL) mg/dl.

Table (4) indicate the mean value of serum HDL (mg/dl) of hepatic rats fed on different diets. It could be observed that the mean value of (HDL) of control (+) group was lower than control (-) group, being 13.8 ± 1.58 and 16.66±.577 respectively, showing significant difference with percent of increase +20.77% of control (-) group as compared to control (+) group. All hepatic rats fed on different diets revealed significant increases in mean values as compared to control (+) group. The values were 0.239, 3.86, 8.69 and 11.08% for groups 3, 4, 5 and 6 respectively. The better serum HDL was consider for group 6 (hepatic rats treated with C.officinalis 7.5%) as compared with control (-) group.

Phenolics and hydroxyl group containing flavonoids found in Calendula are antioxidants with radical radicalization activities, and plays a very important role in protecting the body from the types of reactive oxygen resulting from oxidative stress Jan and John, (2017).


<table>
<thead>
<tr>
<th>Parameters</th>
<th>(G1) C Control Mean ±SD</th>
<th>(G2) C Control Mean ±SD</th>
<th>(G3) C.officinalis (1%) Mean ±SD</th>
<th>(G4) C.officinalis (2.5%) Mean ±SD</th>
<th>(G5) C.officinalis (5%) Mean ±SD</th>
<th>(G6) C.officinalis (7.5%) Mean ±SD</th>
<th>LSD (p ≤ 0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T.C (mg/dl)</td>
<td>69.67±1.528</td>
<td>86±1.000</td>
<td>81±1.000</td>
<td>80.33±1.528</td>
<td>77.33±1.528</td>
<td>71±1.000</td>
<td>2.29</td>
</tr>
<tr>
<td>% Change of positive control</td>
<td>-18.98</td>
<td>-</td>
<td>-5.81</td>
<td>-6.59</td>
<td>-10.08</td>
<td>-17.44</td>
<td></td>
</tr>
<tr>
<td>T.G (mg/dl)</td>
<td>97.67±1.528</td>
<td>160.6±3.782</td>
<td>112.00±1.00</td>
<td>107.33±1.528</td>
<td>104.33±1.528</td>
<td>101.67±1.528</td>
<td>3.811</td>
</tr>
<tr>
<td>% Change of positive control</td>
<td>-38.05</td>
<td>-</td>
<td>-28.9</td>
<td>-31.92</td>
<td>-33.83</td>
<td>-35.51</td>
<td>-</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>16.66±0.577</td>
<td>13.800±1.587</td>
<td>13.833±1.040</td>
<td>14.333±1.527</td>
<td>15±2.0</td>
<td>15.333±1.527</td>
<td>2.57</td>
</tr>
<tr>
<td>% Change of positive control</td>
<td>20.77</td>
<td>-</td>
<td>0.239</td>
<td>3.86</td>
<td>8.69</td>
<td>11.08</td>
<td>-</td>
</tr>
</tbody>
</table>

Means in the same row with different litters are significantly different. Significant (p ≤ 0.05).
Histopathological examination of Liver:

Microscopically, liver of rats from group 1 revealed the normal histological structure of hepatic lobule (photo 1). On the other hand, liver of rats from group 2 revealed steatosis of hepatocytes (photo 2), hyperplasia of epithelial lining bile duct and fibroplasia in the portal triad (photo 3). However, liver of rats from group 3 showed small focal hepatic necrosis associated with inflammatory cells infiltration (photo 4) and slight fibroplasia in the portal triad (photo 5). Meanwhile, liver from group 4 revealed no changes except slight hydropic degeneration of some hepatocytes (photo 6) and slight activation of Kupffer cells (photo 7). Examined sections from group 5 showed slight activation of Kupffer cells (photo 8) and steatosis of focal hepatocytes (photo 9). However, some sections from group 6 showed hydropic degeneration of focal hepatocytes (photo 10) and steatosis of sporadic hepatocytes (photo 11), whereas, other sections revealed no histopathological alterations (photo 12).
Photos (1:12): Liver rats of control (-ve), control (+ve), and all treated groups as a percent of *C. officinalis* 1, 2.5, 5, and 7.5% from the Basel diet for 28 days.

**Conclusion**

From this results it could be concluded that feeding on Calendula officinalis had protective effect on liver against CCl4 and had improvement effect on liver and kidney functions, it may be due to its antioxidant, anti-inflammatory properties, and free radical scavenging activities.
4- References


التأثير الوقائي لزهور الأذريون علي كبد الفئران المحقوه برابع كلوريد الكربون
حمديه أحمد هلال، عبير نزية عبد الرحمن، سارة حمدي يونس

الملخص العربي
أجريت هذه الدراسة لمعرفة التأثيرات الوقائية المحتملة لزهور الأذريون على وظائف الكبد في الفئران المحوسد والمحقوه برابع كلوريد الكربون (CCl4). تم استخدام 36 فأر بالغ من الذكور يتراوح وزنهم من 150-160 جرام وتم تقسيمها إلى 6 مجموعات متساوية، وتم الاحتفاظ بالمجموعة الأولى كمجموعة قياسية سالبة (C+)، والمجموعة الثانية (C−) التي تم حقن الفئران برابع كلوريد الكربون على نظام غذائي أساسي. أعطتهم زهرة الأذريون كمحمول للفران المجموعات المتبقية كنسبة من 1 و 2.5 و 5 و 7.5 ومضافة علي الغذاء الأساسي لمدة 28 يوماً. في نهاية التجربة تم اخذ عينات الدم للحصول على السيرم وعمل تحليل لوظائف الكبد (ALP، GPT، GOT، وظائف الكلي (البوريا، الكريتبيني وحمض الوربيك)، البروتين الدهني عالي الكثافة (HDL)، إجمالي الكوليسترول (TC) وفحص الدهون الثلاثي (TG)، وتقييم المأخوذ من الطعم ونسبة الزيداء في الوزن (BWG). وفقاً لأشارت نتائج البيانات التي تم الحصول عليها إلى أن الأذريون أدى إلى انخفاض معنوي (P<0.05) في انخفاض في TC، TG، HDL، وزيادةkinson في TC، TG، HDL، كفاءة الأذريون أيضاً إلى تحسن في وظائف الكبد بالكلي وقد ظهر هذا في تشريح الكبد وفحص الأنسجة الذي أوضح أن هناك تأثير ملحوظ.

الكلمات المفتاحية: وظائف الكبد، وظائف الكلي، TC، TG، HDL، الأذريون وفحص الأنسجة.