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Hepatoprotective Effect Of *Calendula Officinalis* Flowers On Ccl_4 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 ($P \leq 0.05$) 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

tetrachloride (CCl_4) 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 (CCl_4) is a highly toxic chemical agent that is used as an industrial solvent. CCl_4 is widely used to induce hepatic steatosis and to study the effects of protective agents, especially antioxidants. The toxic effects of CCl_4 on the liver have been extensively studied **(Sheweita *et al*., 2001)**. Metabolic activation of CCl_4 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 accessed 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 CCl_4-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 (CCl_4, 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 CCl_4 (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 ^{0}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 CCl_4 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^{o}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 slain 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 steatorhea, 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.

Groups / Parameters	(G1) C^- Control Mean ±SD	(G2) C^+ Control Mean ±SD	(G3) *C. officinalis* (1%) Mean ± SD	(G4) *C. officinalis* (2.5%) Mean ± SD	(G5) *C. officinalis* (5%) Mean ± SD	(G6) *C. officinalis* (7.5%) Mean ± SD	LSD (P≤0.05)
BWG (%)	14.47[a] ± 0.89	7.34[de] ± 0.45	6.27[e] ± 0.95	9.16[c] ± 0.215	11.17[b] ± 0.58	8.28[cd] ± 0.28	1.219
%Change of positive control	97.13	-	-14.5	24.79	52.17	12.84	-
F.I (g/day/rat)	16.46[a] ± 0.91	12.26[b] ± 0.251	17.13[a] ± 1.205	16.23[a] ± 0.702	16.06[a] ± 0.802	16.033 ± 0.96	1.523
%Change of positive control	34.25	-	39.72	32.38	30.99	30.75	

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

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

1- Glotamic 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 adminis tered to CCl4-intoxicated livers in albino male Waster rats at a dose of 10 mL/kg, resulted in a reduction of hepatocytoly sis by 28.5% due to a reduction in glutamo-oxalate-trans-aminase (GOT) and glutamo pyruvate transaminase (GPT).

2- Glotamic 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 (u\l) 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

(U\L) 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 (hepatic 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 CCl_4 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.

Groups / Parameters	(G1) C^- Control Mean± SD	(G2) C^+ Control Mean± SD	(G3) *C.officinalis* (1%) Mean± SD	(G4) *C. officinalis* (2.5%) Mean± SD	(G5) *C. officinalis* (5%) Mean± SD	(G6) *C. officinalis* (7.5%) Mean± SD	LSD (P≤0.05)
GPT(U/L)	27.67^{d}±1.53	35.3^{a}±1	29.33^{cd}±0.57	31.11^{bc}±1.00	32.33^{b}±0.5	30.67^{bc}±1.53	1.97
%Change of positive control	-21.72	-	-17.11	-11.89	-8.41	-13.11	-
GOT(U/L)	136.00^{e}±1.00	228.00^{a}±1.52	181.66^{c}±1.53	172.66^{d}±1.53	196.33^{b}±1.53	169.67^{d}±1.53	3.195
%Change of positive control	-40	-	-20.28	-24.21	-13.71	-25.47	-
ALP(U/L)	99.66^{e}±1.53	142.33^{a}±1.53	131.33^{b}±2.52	132.66^{b}±1.53	121.33^{c}±1.53	111.33^{d}±1.53	3.08
%Change of positive control	-30.26	-	-7.84	-6.88	-14.84	-22.12	-

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

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 (3) Effect of *Calendula officinalis* flowers powder on kidney function (Creatinine, Uric Acid and Urea) of hepatic rats.

Groups / Parameters	(G1) C^- Control Mean ±SD	(G2) C^+ Control Mean ±SD	(G3) *C. Officinalis* (1%) Mean ±SD	(G4) *C. officinalis* (2.5%) Mean ±SD	(G5) *C. officinalis* (5%) Mean ±SD	(G6) *C. officinalis* (7.5%) Mean ±SD	LSD (P≤0.05)
Creatinine (mg/dl)	0.81^{b} ±0.03	1.033^{a} ±0.152	0.920^{ab} ±0.072	0.800^{b} ±0.100	0.77^{b} ±0.025	0.80^{b} ±0.020	0.1462
%Change of positive control	-20.88	_	-11.10	-20.52	-24.5	-21.83	_
Urea(mg/dl)	29.67^{d} ±1.53	43.00^{a} ±1.00	35.67^{b} ±1.52	33.67^{bc} ±0.577	36.33^{b} ±1.53	32.00^{c} ±1.00	2.218
% Change of positive control	-31	_	-17.04	-21.69	-15.51	-25.58	_
Uric Acid (mg/dl)	1.55^{d} ±0.05	4.66^{a} ±0.15	4.46^{a} ±0.1527	2.40^{b} ±0.100	2.46^{b} ±0.15	1.80^{c} ±0.10	0.22
%Change of positive control	-66.37	_	-4.28	-48.49	-48.62	-61.37	_

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

-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\dl.

Data of table (4) illustrate the mean value of serum (T.C.) (mg\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\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\dl.

Table (4) show the mean value of serum (T.G.) (mg\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\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

and hydroxyl radicals generated by Fenton reaction and inhibited in vitro lipid peroxidation.

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 13.83 ±1.04, 14.33 ±1.52, 15±2, &15.33 ±1.527 mg\dl for *C.officinalis* 1, 2.5, 5 and 7.5% respectively. The percent of increases 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)**.

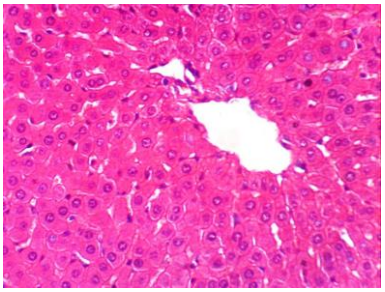
Table (4) Effect of *Calendula officinalis* flowers powder on T.C, T.G and H.D.L of hepatitis rats.

Groups / Parameters	(G1) C^- Control Mean ±SD	(G2) C^+ Control Mean ±SD	(G3) *C.officinalis* (1%) Mean ±SD	(G4) *C.officinalis* (2.5%) Mean ±SD	(G5) *C.officinalis* (5%) Mean ±SD	(G6) *C.officinalis* (7.5%) Mean ±SD	LSD (P≤0.05)
T.C (mg/dl)	69.67^{d} ±1.528	86^{a} ±1.000	81^{b} ±1.000	80.33^{b} ±1.528	77.33^{c} ±1.528	71^{d} ±1.000	2.29
%Change of positive control	-18.98	-	-5.81	-6.59	-10.08	-17.44	
T.G (mg/dl)	97.67^{e} ±1.528	160.6^{a} ±3.782	112.00^{b} ±1.00	107.33^{c} ±1.528	104.33^{cd} ±1.528	101.67^{d} ±1.528	3.811
%Change of positive control	-38.05	-	-28.9	-31.92	-33.83	-35.51	-
HDL (mg/dl)	16.667^{a} ± 0.577	13.800^{a} ± 1.587	13.833^{a} ± 1.040	14.333^{a} ± 1.527	15^{a} ± 2.0	15.333^{a} ± 1.527	2.57
%Change of positive control	20.77	-	0.239	3.86	8.69	11.08	-

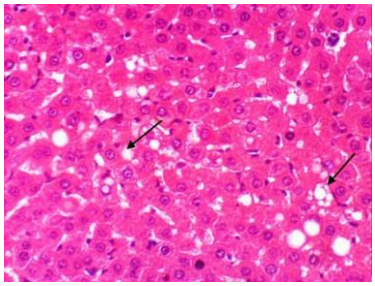
Means in the same row with different litters are significantly different. Significant ($p \leq 0.05$).

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).



Photo(1)



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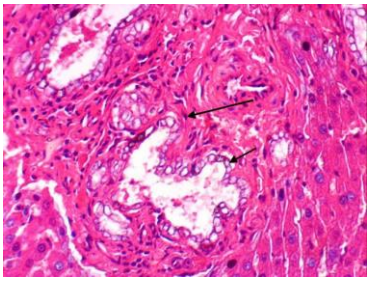
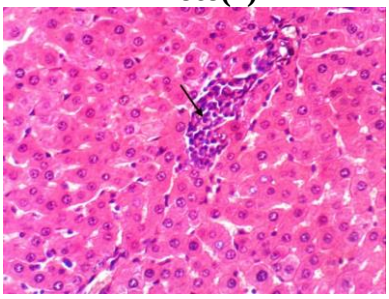
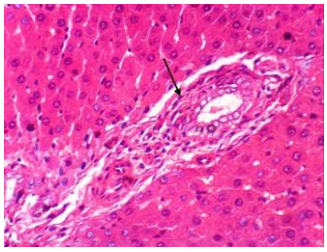


Photo(3)



Photo(4)



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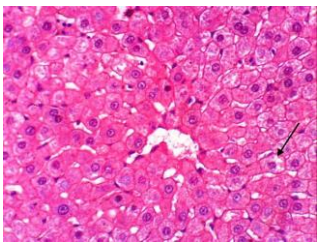

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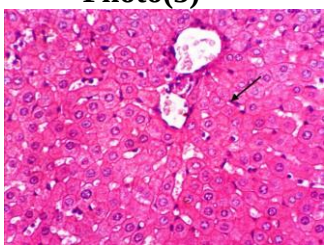

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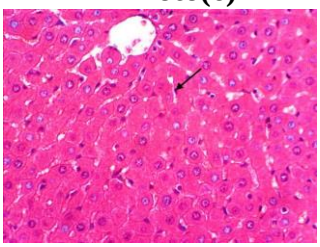
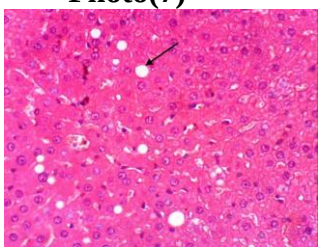


Photo(8)



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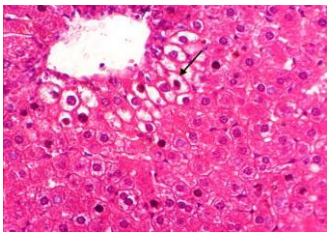

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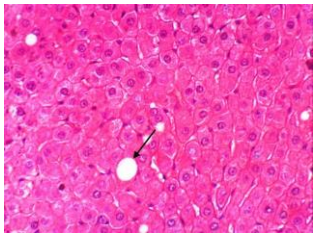

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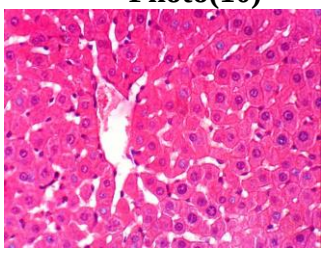

Photo(12)

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.

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التأثير الوقائي لزهور الأذريون علي كبد الفئران المحقونه برابع كلوريد الكربون

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الملخص العربي

أجريت هذه الدراسة لمعرفة التأثيرات الوقائيه المحتمله لزهور الآذريون علي وظائف الكبد في الفئران المصابة والمحقونه برابع كلوريد الكربون (CCl4). تم إستخدام 36 فأر بالغ من الذكور يتراوح وزنهم من 150-160 جرام وتم تقسيمها إلى 6 مجموعات متساوية ، وتم الاحتفاظ بالمجموعة الأولى كمجموعة قياسية سالبه (ve–) والمجموعة الثانية (ve +) التي تم حقن الفئران برابع كلوريد الكربون على نظام غذائي أساسي. أعطيت زهرة الاذريون كمسحوق للفئران المجموعات المتبقيه كنسبة مئوية من 1 و 2.5 و 5 و 7.5 ومضافه علي الغذاء الاساسي لمده 28 يومًا. في نهاية التجربة تم اخذ عينات الدم للحصول علي السيرم وعمل تحليل لوظائف الكب (GOT، GPT، ALP) ، وظائف الكلى (اليوريا ، الكرياتينين وحمض اليوريك) ، ، البروتين الدهني عالي الكثافة (HDL-c) ، إجمالي الكوليسترول (TC) وفحص الدهون الثلاثية(TG) ،و تقدير المأخوذ من الطعام ونسبة الزياده في الوزن (FI، FER، BWG٪). ولقد أشارت نتائج البيانات التي تم الحصول عليها إلى أن الاذريون أدي إلي انخفاض معنوي ($P\leq0.05$)انخفضت في TG، TC، وزيادة HDL كما ادي الاذريون ايضا إلي تحسن في وظائف الكبد والكلى وقد ظهر هذا في تشريح الكبد وفحص الانسجه الذي اوضح ان هناك تاثير ملحوظ.

الكلمات المفتاحية: وظائف الكبد ، وظائف الكلى ، HDL، TG، TC، الاذريون وفحص الأنسجة.