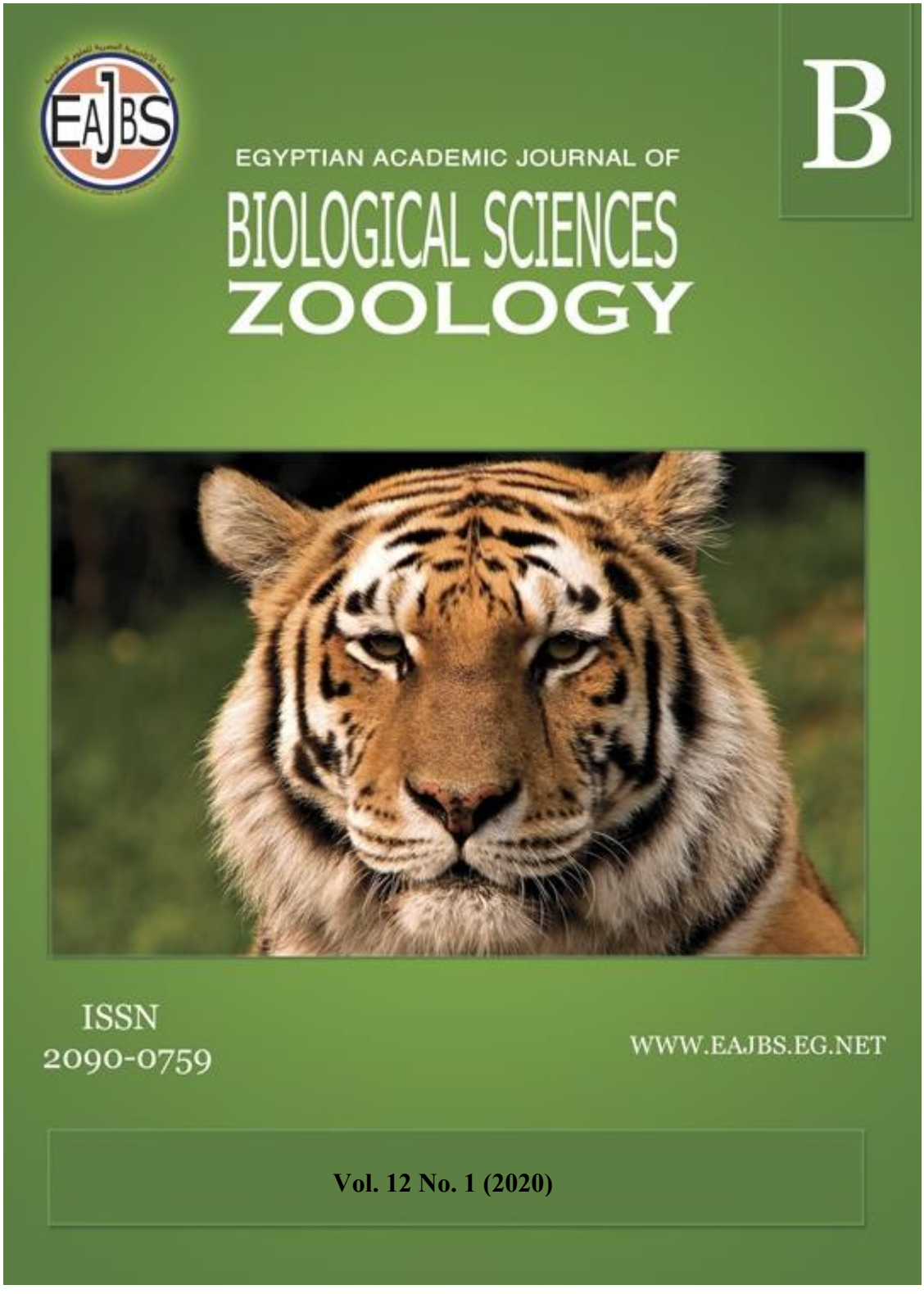

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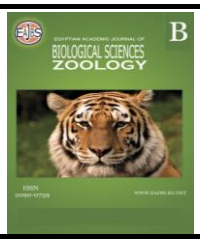

Comparative Hematological and Biochemical Studies on the Effect of Some Hepatoprotective Agents in Rats

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ABSTRACT

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

A total of 80 normal male albino rats, were divided into 8 groups, the first grou (group I): rats served as the control group and received commercial balanced diet all ov 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/k for the whole experimental period, group V: served as hepatotoxicated group, treated wi an intraperitoneal injection of 2mg /kg b. w. of carbon tetrachloride(CCl_4) dissolved in corn oil 1:1 at 11 and 12 days of an experiment to induce hepato-toxicity and groupV chamomile orally pretreated group (200mg/kg) for the whole experimental period with i injection of CCl_4 in days 11 and 12 of the experiment, group VII: grape seed oral pretreated group (100mg/kg) for the whole experimental period, with i/p injection of CC in days 11 and 12 of the experiment and group VIII: silymarin orally pretreated grou (100mg/kg) for the whole experimental period, with i/p injection of CCl_4 in days 11 and 1 of the experiment.

Objective: The present study was carried out to investigate the effect of or administration of ethanol extract of chamomile capitula extract, grape seed and silymar for 3 weeks on hepatotoxicity induced to rats by carbon tetrachloride (CCl_4). These effec were explored by measuring erythrogram and serum levels of liver enzymes; asparta 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 gra seed extract, and silymarin to hepato-injured rats for 21 days improved anemia ar significantly decreased the elevated serum levels of liver enzymes (AST, ALT, and ALP total bilirubin and increased serum total protein, albumin, when compared to th corresponding control, positive groups.

Conclusion: The results suggest that the chamomile extract, grape seed extract, ar silymarin induce potent hepato-protective effects in CCl_4 induced hepato-injury in rat This study recommends that intake of chamomile and grape seed extracts as an herbal ma 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 hepato-protective 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 (CCl_4) 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 0f 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 72hr, 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 200ml D.W, the suspension then filtered a 0.4*m*m 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 2ml /kg of CCl_4 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 CCl_4 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 CCl_4 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 CCl_4 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 CCl_4 in days 11 and 12 of the experiment.

Sampling:-

Blood 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 ($P \leq 0.05$).

RESULTS

Effect of CCl_4 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 compare to the normal control group, While there was a reduction in RBCs, Hb, PCV, MCV, MCH, and MCHC levels in CCl_4 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 CCl_4 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 Ccl_4 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 changed when compared to the normal control group. While i/P injection of CCl_4 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 CCl_4 hepatotoxicity as well as the protective effects of chamomile, grape seed and silymarin on erythrogram after 2 weeks.

Parameters / Groups	RBCs (10^6/µL)	Hb (g/dl)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)
Control	6.79±0.16^a	13.96±.37^a	42.03±1.07^a	61.89±0.43^a	20.55±0.22^a	33.22±0.11^a
Chamomile	6.70± 0.04^a	13.82 ±0.05^a	41.47±0.16^a	61.83±0.25^a	20.72±0.06^a	33.33±0.01^a
Grape seed	6.70± 0.07^a	13.87± 0.18^a	41.68±0.56^a	62.05 ±0.20^a	20.68±0.07^a	33.32±0.00^a
Silymarin	6.74± 0.07^a	13.70 ±0.15^a	41.10±0.46^a	60.93±0.97^a	20.30±0.32^a	33.23±0.10^a
CCl_4	4.73±0.65^c	8.32±0 .098^c	28.27±0.29^c	52.84±.032^b	17.09±0.24^b	29.43±0.33^b
Ch+CCl_4	5.90±0.16^b	12.08± 0.18^b	36.25±0.66^b	60.58±0.57^a	20.19±0.19^a	33.33±0.00^a
Gr+CCl_4	6.17± 0.17^b	12.45±0.18^b	37.37±0.55^b	60.54±0.48^a	20.15±0.17^a	33.33±0.00^a
Sily+CCl_4	6.04± 0.16^b	12.10±0. .21^b	36.30±0.65^b	60.08±0.68^a	20.03±0.23^a	33.30±0.02^a

$^{a-c}$ Means in the same column with different superscripts ±are significantly different ($p \leq 0.05$); values are presented as means ±SE.

CCl_4 (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.

Parameters / Groups	RBCs (10^6/µL)	Hb (g/dl)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)
control	6.82±0.09^a	14.21±0.17^a	42.63±0 .52^a	62.51±0.97^{ab}	20.82±0.32^{ab}	33.33±0.00^a
Chamomile	6.71±0.01^a	14.00±0.09^a	41.92±0.27^a	62.76±0.39^a	20.90±0.13^a	33.20±0.18^a
Grape seed	6.82±0.09^a	14.21±0.17^a	42.63±0.52^a	62.51±0.97^{ab}	20.82±0.32^{ab}	33.33±0.00^a
Silymarine	6.84±0.08^a	14.00±0.06^a	42.00±0.52^a	61.34±0.68^{ab}	20.44±0.22^{ab}	33.28±0.05^a
CCl_4	4.50±0.06^c	8.32±0.09^c	25.52±0.29^c	54.80±0.73^c	18.39±0.24^c	29.61±0.33^b
Ch+CCl_4	6.46±0.06^b	13.00±0.10^b	38.92±0.33^b	60.22±0.65^b	20.08±0.20^{ab}	33.33±0.00^a
Gr+CCl_4	6.77±0.11^a	13.85±0.14^a	41.88±0.74^a	61.83±0.27^a	20.78±0.19^{ab}	33.14±0.17^a
Sily+CCl_4	6.46±0.08^b	12.92±0.15^b	38.77±0.46^b	60.01±0.49^b	20.00±0.15^b	33.33±0.00^a

$^{a-d}$ Means in the same column with different superscripts are significantly different ($p \leq 0.05$); values are presented as means ±SE.

CCl_4 (Carbon tetrachloride). Ch (chamomile). Gr(grape seed) . Sly (slymarin).

Table 3: The effects of CCl_4 hepatotoxicity as well as the protective effects of chamomile, grape seeds and silymarin on serum biochemical analysis after 2 weeks.

Parameters Groups	ALT (U/I)	AST (U/I)	ALP (U/I)	TP (g/dl)	ALB (g/dl)	Globulin (g/dl)	A/ B (Ratio)	Cholesterol (mg/dl)	T.BIL (mg/dl)	I.BIL (mg/dl)	D.BIL (mg/dl)	Glucose (mg/dl)
Normal control	32.8±1.9 c	116.6±2.9 c	239.0±23.0 c	7.63±.033 a	4.26±.032a	3.36±0.06 a	1.26±0.03a	106.60±.33 d	0.12±.006 c	0.08±0.00a	.043±.006 b	108.9±.88d
Chamomile	34.18±0.63 c	115.8±.85 c	238.3±0.59c	7.55±0.028 a	4.21±0.010 a	3.34±0.020a	1.25±0.00a	105.9±0.37 d	0.12±.00 c	0.08±0.00a	.040±.00b	110.1±3.03d
Grape seed	33±1.15 c	115.5±.35 c	238.1±1.09c	7.56±0.033a	4.18±0.00 a	3.38±0.030 a	1.23±0.00a	105.7±.68d	0.12±.00 c	0.08±0.00a	0.041±.00b	110±.98 d
silymarin	32.6±.56 c	116.5±2.40 c	241.1±.70c	7.53±0..033a	4.21±0.01 a	3.32±0.02a	1.26±0.00 a	106.6±.87 d	0.12±.00 c	0.08±0.00a	0.043±.06b	110±2.15d
CCl_4	82.66±5.0 a	221.33±9.4 a	451.6± 14.8 a	4.36±.08 c	2.30±0.57 c	2. ±0.05 c	1.15±0.03 b	182.10±1.18 a	0.30±0.01 a	0.08±0.00a	0.22±.011a	191.6±.97 a
Ch+CCl_4	67.50 ±6 b	178.3±6.7 b	292.6±10.4 b	6.13±0.06 b	3.33±0.83b	2.37±0.14 b	1.20±0.01a	123.07±0.52 b	0.18±.00 b	0..086±0.00a	0.093±0.00 b	127.8±1.5c
Gr+CCl_4	57.00±3.2 b	169.6±2.7 b	280.23 ±23.3 b	6.30±0.86 b	3.40±.08 b	2.90±0.23 b	1.21±0.07a	112.33±1.20 c	0.18±0.12 b	0.086±0.00a	0.093±.00b	131.5±.77 bc
Sily+CCl_4	62.66±5.3 b	164.3 ±7.8 b	326.7 ±2.4 b	6.0 ±0.114 b	3.30±.07 b	2.70±0.30b	1.22±0.06a	124.67±0.60 b	0.19±0.00 b	0.084±0.00a	0.10±.00 b	133.8±.31b

a-d Means in the same column with different superscripts are significantly different ($p \leq 0.05$); values are presented as means ±SE.

CCl_4 (Carbon tetrachloride). Ch (chamomile).Gr (grape seed) .Sly (slymarin).

Table 4: The effects of CCl_4 hepatotoxicity as well as the protective effects of chamomile, grape seeds and silymarin on serum biochemical analysis after 3 weeks**.**

Parameters Groups	ALT (U/I)	AST (U/I)	ALP (U/I)	TP (g/dl)	ALB (g/dl)	Globulin (g/dl)	A/ B (Ratio)	Cholesterol (mg/dl)	T.BIL (mg/dl)	I.BIL (mg/dl)	D.BIL (mg/dl)	Glucose (mg/dl)
Normal control	31.16±1.16 d	118.6±2.4 d	240.6 ±22.8 c	7.61±0.19 a	4.21±0.044 a	3.4±0.23 a	1.24±0.09 a	101.50±01.2 e	0.13±0.00 c	0.09±0.01 a	.040±0.010 d	109.10±2.1 d
Chamomile	32.16±0.88 d	119.3±0.52 d	239.4.8±.63 c	7.58±0.04 a	4.11±0.07 a	3.4±0.07 a	1.22±0.03 a	103.20±1.12 e	0.13±0.01 c	0.09±0.01 a	.04±0.012 d	112.10±.88 d
Grape seed	32.30±1.20 d	119.4±.66 d	239.6±.21 c	7.60±0.03 a	4.12±0.06 a	3.33±0.06 a	1.3±0.02 a	102.10±1.41 e	0.13±0.00 c	0.09±0.00 a	.04±0.00d	110.00±1.23 d
Silymarin	34.60±.44 d	120.3±0.25 d	240.00±.56 c	7.56±0.03 a	4.12±0.04 a	3.28±0.04 a	1.30±0.02a	103.00±1.79 e	0.13±0.00c	0.09±0.00 a	.04±0.00 d	112.10±.88 d
CCl_4	80.10±5.6 a	200.3±6.7 a	442.00±4.1 a	4.66±0..03c	2.1±0.08 c	2.10±0.08 c	1.10±0.04 b	174.60±1.73 a	0.28±0.01 a	0.080±0.01a	..2±0.015 a	195.6±3.9 a
Ch+CCl_4	47.1.1±0.44 c	150±.57 c	248 ±1.28 c	6.50±0.17 b	3.6±0.05 b	2.90±0.24 b	1.24±0.05a	119.10±2.20c	0.156±0.00 b	0..083±0.1 a	.075±0.00 b	121.70±0.85 c
Gr+CCl_4	44.00±1.1 c	144±.57 c	239.50±8.3 c	6.53±0..14 b	3.60±0.07 b	2.93±0.26 b	1.22±0.66 a	110.40±.61 d	0.153±0.00 b	0..080±0.2 a	.06±0.01 c	122.00±2.8 c
Sily+CCl_4	54.50±.04 b	158±3.7 b	320.30±40 b	6.53±0.61 b	3.65±0.02 b	2.88±0.41 b	1.24±0.17 a	124.40±0.72 b	0.160±0.00 b	0..083±0.08a	.08±0.013 b	128.70±.28 b

a-dMeans in the same column with different superscripts are significantly different ($p \leq 0.05$); values are presented as means ±SE.

ccl_4 (Carbon tetrachloride). Ch (chamomile).Gr(grape seed) .Sly (slymarin).

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).

CCl_4 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 CCl_4-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 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 CCl_4 intoxicated rats than healthy control this may be attributed to the cytotoxic effect and suppression of the erythropoiesis caused by CCl_4. 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 CCl_4 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 CCl_4 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 CCl_4 has been known to produce hepatic damage by the generation of highly reactive trichloromethyl (CCl_3*) and trichloromethylperoxy (CCl_3OO*) radical when metabolized by cytochrome P_{450} (Britton and Bacon, 1994, Weber *et al.,* 2003).

These findings were in agreement with Zahran *et al.,* (2018) who revealed that CCl_4 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 CCl_4 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 CCl_4 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 haematologiacal 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 CCl_4 toxicity significantly reduced Hb level, RBCs count and PCV with respect to normal control. The intoxication of CCl_4 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 CCl_4-induced hepatic damage (Lu *et al.,* 2016 and Yang *et al.,* 2015) and were elevated in the CCl_4 group in our study. Therefore, results provided further evidence that all three extracts tested in this study exerted favorable hepatoprotective effects against CCl_4-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 CCl_4-induced serum AST, ALT elevations and ALP increase in these groups compared with the CCl_4 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 CCl_4. 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 CCl_4 (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 CCl_4 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 CCl_4. 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 CCl_4 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 CCl_4 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 CCl_4 intoxication. Also, Bouhrim *et al.* (2018) reported that serum glucose value was reduced in CCl_4-intoxicated rats. Studies have demonstrated decreased hepatic glycogen content after treatment with CCl_4, reflecting decreased gluconeogenesis by the liver.

The results of the present study have also established that the CCl_4 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 CCl_4 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 (CCl_4) 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 CCl_4 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 gibberllic 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 gibberllic. 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 gibberllic 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 indomethacin 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 CCl_4 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 CCl_4 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 CCl_4 caused to increase in glucose and to decrease in albumin, total cholesterol levels. 2.5% infusion of silymarin pre-teated improved these changes. All liver function tests were elevated by CCl_4 administration and then reduced, by silymarin pre-treatment. The study by Zhang *et al.,* (2013) evaluated that the protective effects of silymarin, as a marketed 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

REFERENCES

Abdalla E. El-Hadary & Mohamed F. Ramadan Hassanien (2016): Hepatoprotective effect of cold-pressed Syzygium aromaticum oil against carbon tetrachloride (CCl_4)-induced hepatotoxicity in rats. Pharma. Biol., 54:8, 1364-1372, DOI: 10.3109/13880209.2015.1078381.

Abdel-Salam f.EL-Sawyz, Zeynab Kh.El-Maddawy, Neveen rAshoura (2018): Role of Silymarin in Restoring the Deleterious Effect Induced by Dexamethasone in Male Rats. AJVS.Vol.59:125-135 2018.

Abid Ali WDh, Khudair ARN, AL-Masoudi EA (2015): Influence of silymarin extracted from Silybum marianum seeds compared to legalon against nickel chloride induced hematological and biochemical changes in male rabbits. Bas. J. Vet. Res. 2015; 14:293-305.

Aksoy L, Sözbilir NB (2012): Effects of Matricaria chamomilla L. on lipid peroxidation, antioxidant enzyme systems, and key liver enzymes in CCl_4-treated rats. Toxicol. Environ. Chem. 2012; 94:1780–1788.

Al-Jubouri HHF, Al-Jalil BH, Farid I, Jasim F, Wehbi S. (1990): The effect of chamomile on hyperlipidemias in rats. J. Fac. Med. Baghdad 32: 5 –11.

Amany Ahmed Mohamed Abd-Allah, Nasr Abdelwahab Mohamed Nasr El-Deen, Wafaa Abdou Mohamed, Fatma Mohamed Naguib (2018): Mast cells and pro-inflammatory cytokines roles in assessment of grape seeds extract anti-inflammatory activity in rat model of carrageenan-induced paw edema .Basic. Med. Sci. 2018:21:97-107.

Amany Ahmed Mohamed Abd-Allah, Nasr Abdelwahab Mohamed Nasr El-Deen, Wafaa Abdou Mohamed Mohamed, Fatma Mohamed Naguib (2017): Protective effect of grape seeds proanthocyanidins against naphthalene-induced hepatotoxicity in rats, Department of Clinical Pathology, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt MVSc of Clinical Pathology, Faculty of Veterinary Medicine, Zagazig University, Sep 28, 2017.

Ammar A. Attar, Amr A. Rezq, Aml F. Elgazar, Baz M. Sameh and Mohamed H. Mukhtar (2018): Studying Some of Biological Effects of Grape Seeds on Carbon Tetrachloride (CCl_4)-Induced Liver Damage in Rats. Middle-East J. of Sci. Res. 26 (2): 217-226, 2018 ISSN 1990-9233.

Aseervatham, G.S.B., Ananth, D.A., and Sivasudha, T. (2018): "The Liver: Oxidative Stress and Dietary Antioxidants," in The Liver. Elsevier), 239-246.

Bagchi, D.; Bagchi, M.; Stohs, S.J.; Das, D.K.; Ray, S.D.; Kuszynski, C.A.; Joshi, S.S. and Pruess, H.G. (2000): Free radicals and grape seed proanthocyanidin extract: Importance in human health and disease prevention. Toxicol., 148(2–3): 187−197.

Bentivegna, S.S. and Whitney, K.M. (2002): Subchronic 3-month oral toxicity study of grape seed and grape skin extracts. Food and Chem. Toxicol., 40:1731–1.

Bouhrim M, Ouassou H, Choukri M, Mekhfi H, Ziyyat A, Legssyer A, Aziz M, Bnouham M (2018): Hepatoprotective effect of *Opuntia dillenii* seed oil on CCl_4 induced acute liver damage in rat. Asian Pac. J. Trop. Biomed. 2018;8: 254-60.

Britton, R. S., Bacon, B. R. (1994): Role of free radicals in liver diseases and hepatic fibrosis.Hepatogastro-enterol. 41: 343 - 348.

Burtis, C. A., Ashwood, E. R., Bruns, D.E. and Tietz, N.W. (2006): Textbook of clinical chemistry and molecular biology, St. Louis: Elsevier Saunders.

Dalal A.A. Al-Baroudi1, Reham A. Arafat and Thana A. El-kholy (2014): Hepatoprotective Effect of Chamomile Capitula Extract against 2, 4-Dichlorophenoxyacetic Acid-Induced Hepatotoxicity in Rats. Life Sci. J. 2014; 11.

Droge, W., (2002): Free radicals in the physiological control of cells. Physiol. Rev. 82: 47-95.

Drupt F. (1974): Colorimetric method for determination of albumen. Pharm. Bio. 9: 777-779.

Duncan D. B. (1955): Multiple range and multiple F tests. Biometrics11: 1–42.

El-Adawi, H.; Abdel Mohsen, M.; Youssef, D. and El-Sewedy, S. (2006): Study on the Effect of Grape Seed Extract on Hypercholesterolemia: Prevention and Treatment. Int. J. Pharm., 2: 593- 600.

Elshater A.A, Salman M.M.A, Moahmed S. (2013): The hepato-ameliorating effect of *Solanum nigrum* against CCL_4 induced liver toxicity in Albino rats. Egypt. Acad. J. Biolog. Sci.(C,Physiology and Molecular Biology) Vol. 5(1): 59-66.

Forbes, S.J., and Newsome, P.N. (2016): Liver regeneration—mechanisms and models to clin. Appl. Nature Rev. Gastroentero. & Hepatol. 13, 473.

Gabr SA, Zahran, Mohammed Faten, Hassanin WF, Sharoud MN , and Mesalam (2018): Therapeutic Effect of Camel Milk Against Hepatotoxicity Induced by CCl_4 in Rats. Res. J. of Pharm., Biol. and Chem. Sci. Page No. 601.

Gihan, M. Hammoud, Nivin, S. Nail, Khaled, M. ELSawy, Adel, S. Abd El-Rahman, Mervat, S.H. Youssef and Mohamed, S. Masoud (2014): Protective Effect Of Grape Seed Extract Against Sodium Nitrate –Iduced Toxicity And Oxidative Stess In Az. J. Pharm. Sci. Vol. 49, March, 2014.

Graw A, Cowan R.A, O'Reilly D.S.J, Stevant M.J and Stephard, J (1999): Clinical biochemistry- an illustrated color text. Is ted. New York: Churchill Livingstone. 1999; 51-53.

Gupta AK, Misra N (2006): Hepatoprotective Activity of Aqueous Ethanolic Extract of Chamomile capitula in Paracetamol Intoxicated Albino Rats. Am. J. Pharmacol. Toxicol. 2006; 1:17–20.

Hanaa HA, Samiha MA, Fatehya MM, Nermeen MF(2015): Biological significance of grape seed extract against lung injury induced by formaldehyde inhalation in rats. Der. Pharma. Chem. 2015; 7:64-78.

Harborne, JB.; Marbay, TJ. and Mabray, H. (1975) : Physiology and function of flavonoids.Academic Press, New York,Pp: 970.

Havel RJ (1986): Functional activities of hepatic lipoproteins receptors. Ann. Rev. Physiol. 48:119–134.

Hebat Allah M. Mahmoud, M.Sc.; Fatma A. Mahmoud, M.D.; Ghada Hashem, M.D.; Hanan H. Ahmed, M.D. and Hesham M. Mahmoud, M.D (2018): The Beneficial Effects of Silymarin in Treatment of Experimentally Induced Non-Alcoholic Fatty Liver Disease in Rats Med. J. Cairo Univ., Vol. 86, No. 2, March: 1055-1064, 2018.

Henry R.J. (1964): Colorimetric determination of total protein In: Clinical Chemistry. Harper and Row Publ.; New York, USA, 181.

Kumar, S., S. Sharma and Suman (2010): Anti-diabetic potential of alcoholic extract of Vitis vinifera roots in streptozotocin nicotinamide induced diabetic rats. Inventi Rapid: Ethnopharmacology2010.Inventi:ep/61/10http://www.inventi.in/Article/ep/61/10.aspx.

Lu Y., Hu D., Ma S., Zhao X., Wang S., Wei G., … Wang J. (2016): Protective effect of wedelolactone against CCl_4-induced acute liver injury in mice. Int. Immun., 34, 44–52. 10.1016/j.intimp.2016.02.003

Luke Obuyanwe Nwoye (2013): Protective and therapeutic effects of Chamomilla Recutita extract on subacute ethanol intoxication in white albino rats.Afr. J. of Biotech..Vol 12(18), pp.2378-2385.

M. Ozturk1, M. Akdogan, I. Keskin, A.N. Kisioglu1, S. Oztas4 and K. Yildiz (2012): Effect of *Silybum marianum* on acute hepatic damage caused by carbon tetrachloride in rats. Biom. Res. 2012; 23 (2): 268-274.

Magbolah Salem Helal Alzahrani (2018): The effect of different levels of Matricaria chamomilla on functional status of liver in rats injected with carbon tetrachloride (CCl_4). Researcher 2018; 10(6):92-100]. ISSN 1553-9865 (print); ISSN 2163- 8950.

Makni M, Yassine C, Hamadi F, El Mouldi G, Mohamed B, Chama M, Choumous K, Najiba Z (2012): Erythrocyte oxidative damage in rat treated with CCl_4 protective role of vanillin. Saga J. 28: 908-916.

Mandal A, Karmakar R, Bandyopadhyay S, Chatterjee M (1998): antihepatotoxic potential of *Trianthema portulacastrum* in carbon tetrachloride-induced chronic hepatocellular injury biochemical characteristics. Arch. of Pharm. Res. 21: 223-230.

Mansour Amraei, Ehsan Shirzadpour, Mahmoud Mohamadpour, Seyedeh Fatemeh Mousavi, Mojtba Mohamadpour, Ayub Ghorbani (2018): Surveying Antioxidant Activity of Hydroalcoholic Extract of Matricaria chamomilla L. and Comparing it to Lovastatin in Rat. Asian J. of Pharm. • Oct-Dec 2018 (Suppl) • 12 (4) | S1527.

Mohamed-Amine Jabri Mamane Sani, Kais Rtibi, Lamjed Marzouki, Jamel El-Benna, Mohsen Sakly, and Hichem Sebai (2016): Chamomile decoction extract inhibits human neutrophils ROS production and attenuates alcohol-induced haematological parameters changes and erythrocytes oxidative stress in ra.t Published online 2016 Mar 31. doi: 10.1186/s12944-016-0233-4.

Mona, N.M., Olfat A.H., Fatma, H.S., M.E., M., S., S.A (2014): Transplantation of Modified and Fresh Hepatocyte Reduces Hepatotoxicity Induced by Carbon Tetrachloride. Life Sci. J. 11.

Mosedale, M., and Watkins, P.B. (2017): Drug-induced liver injury: advances in mechanistic understanding that will inform risk management. Clinical Pharmacology & Therapeutics 101, 469-480.

Mritunjay Kumar, DB Mondal, Ajay Kumar and Monalisha Sahu (2018): Clinico-patho-physiological changes associated with carbon tetrachloride (CCl_4) induced subacute hepatotoxicity in rats. Int.J. of Chem. Stud. 2018; 6(3): 2670-2676.

Mukherjee PK (2003): Plant products with hypocholesterolemic patientials, Adv. Food Nutr. Res., 47: 277–338.

Naglaa Kamal Madkourand Amal Attia El-Morsy Ibrahim (2016): The prophylactic effect of silymarin on hepatic damage and IL-1β, IL-6 and TNF-α expression in rats with hepatic fibrosis. Int. J. Adv. Res. 4(9), 2188-2196.

Nouf Al-Rasheed, Laila Faddah1, Iman A Sharaf, , Azza M Mohamed, Nawal Al-Rasheed1 and Nayira Abdelbaky (2015): Assessment of the Potential Role of Silymarin Alone or in Combination with Vitamin E and/ or Curcumin on the Carbon Tetrachloride Induced Liver Injury in Rat Braz. Arch. Biol. Technol. v.58 n.6: pp. 833-842, Nov/Dec 2015.

Oliva-Vilarnau, N., Hankeova, S., Vorrink, S., Mkrtchian, S., Andersson, E., and Lauschke, V.M. (2018): Calcium signaling in liver injury and regeneration. Frontiers in medicine 5, 192.

Pallarès V, Fernández-Iglesias A, Cedó L, Castell-Auví A, Pinent M, (2013): Grape seed procyanidin extract reduces the endotoxic effects induced by lipopolysaccharide in rats. Free Radic. Biol. Med. 60: 107-114.

Richmond W. (1973): "Preparation and properties of a cholesterol oxidase from Nocardia sp. and its application to the enzymatic assay of total cholesterol in serum. Clin. Chem. 19.12: 1350-1356.

Roychowdhury, S.; Wolf, G.; Keilhoff, G.; Bagchi, D. and Horn, T. (2001): Grape seed proanthocyanidin extract (GSPE) protects astroglia against nitrosative/oxidative stress. Nitric Oxide, 5: 137−149.

Rui AC, John GJ, Chris MG, Dean AS, Craig RM (2002): Hepatic gluconeogenesis and krebs cycle fluxes in a CCl_4 model of acute liver faliure. NMR Biomed. 15: 45–51.

Saba AB, Oyagbemi AA and Azeez OI (2010): Amelioration of carbon tetrachloride-induced hepatotoxicity and haemotoxicity by aqueous leaf extract of Cnidoscolus aconitifolius in rats Nig. J. Physiol. Sci. 25(December 2010) 139 – 147.

Salamon I (1992): Chamomile, a medicinal plant. Herb Spice Med. Plant Digest.10:1-4.

Sally A. El-Awdan, Gehad A. Abdel Jaleel, Dalia O. Saleh (2013): Grape seed extract attenuates hyperglycaemia-inducedin rats by streptozotocin. Bulletin of Faculty of Pharmacy, Cairo University51, 203-209.

Samira Bouhalit and Zine Kechrid (2019): Protective Effect of Silymarin Extracted from *Silybum marianum* Seeds upon Nickel-Induced Hepatotoxicity in Albino Wistar Rats. Ann. Microb.Immunol. 2019; 1(1): 1005.

Sedlak TW, Snyder SH (2004): Bilirubin benefits: cellular protection by a biliverdin reductase antioxidant cycle. Pediatrics 2004; 113: 1776_82.

Sethupathy, C. Elanchezhiyan, K. Vasudevan, G. Rajagopal(2002): Antiatherogenic effect of taurine in high fat diet fed rats. Ind.J. Exp. Biol., 40 (10) (2002), pp. 1169-1172.

Shahin Nargesi , Ardeshir Moayeri , Ayub Ghorbani , Yaser Seifinejad , Ehsan Shirzadpour Mansour Amrae (2018):The effects of Matricaria chamomilla L. hydroalcoholic extract on atherosclerotic plaques, antioxidant activity, lipid profile and inflammatory indicators in rats. Biom. Res. and Therapy, 5(10):2752- 2761,2018.

Shaymaa Ismaiel Salem, 1Abeer Ahmed Abd El-Baky and Faten Fathy Mohammed (2017): Cytoprotective Effect of Silymarin on Cisplatin Induced Hepatotoxicity and Bone Marrow Toxicity in Rats. Asian J. of Animal Sci. 11.140.152.

Snedecor George, Cochran W. and William G. (2006): Statistical Methods, Eighth Edition, Iowa State University Press.SPSS (20.0): Command Syntax.

Sodikoff CH (1995): Laboratory profiles of small animal diseases. A guide to laboratory diagnosis. St. Louise: Mosby. 1995 ; 1-36.

Suwannaphet, A. Meeprom, S. Yibchok-Anun, S. Adisakwattana (2010): Preventive effect of grape seed extract against high-fructose diet-induced insulin resistance and oxidative stress in rats. Food Chem. Toxicol., 48 (7) (2010), pp. 1853-1857.

Thnaian Althnaian1, Ibrahim Albokhadaim2 and Sabry M El-Bahr (2013): Biochemical and histopathological study in rats intoxicated with carbontetrachloride and treated with camel milk lthnaian et al. SpringerPlus 2013, 2:57 http://www.springerplus.com/ content/ 2/1/57.

Tietz, N. W. (2006): Textbook of clinical chemistry and molecular diagnostics, Edited by: C.A. Burtis, E.R. Ashwood and D.E. Bruns, CA: Elsevier Saunders.

Tresina PS, Sornalakshmi V1, Paulpriya K1, Mohan VR, Arumugasamy K (2016): Hepatoprotective Effece Of Hedyotis Leschenaultiana DC, Ethanol Extract In CCL_4 Induced Hepatotoxicity In Rars. Asian J. Pharm. Clin. Res., Vol 9, Suppl. 1, 2016, 164-167.

Tung HT, Cook FW, Wyatt RD, Hamilton PB (1975): The anemia caused by aflatoxin. Poult. Sci. 54: 1962-1969.

Vanitha A, Murthy KN, Kumar V, Sakthivelu G, Veigas JM, Saibaba P and Ravishankar GA (2007): Effect of the carotenoid producing alga, *Dunaliella bardawil*, on CCl_4-induced toxicity in rats. Int. J. Toxicol., 26: 159-167.

Vargas-Mendoza N, Madrigal-Santillán E, Morales-González A, Esquivel-Soto J, Esquivel-Chirino C, García-Luna Y, González-Rubio M (2014):Hepatoprotective effect of silymarin. World J. of Hepatol.6:144–149.

Wang, R., Feng, X., Zhu, K., Zhao, X., & Suo, H. (2016): Preventive activity of banana peel polyphenols on CCl_4-induced experimental hepatic injury in Kunming mice. Exper. and Therap.Med., 11, 1947‑1954. https://doi.org/ 10.3892/etm.2016.3155.

Weber, L. W. D., Meinrad Boll, and Andreas Stampfl, (2003): Hepatotoxicity and Mechanism of Action of Haloalkanes: Carbon Tetrachloride as a Toxicological Model. Critical Rev.in Toxicol., 33 (2): 105–136.

Weidner C Wowro SJ, Rousseau M, Freiwald A, Kodelja V, Abdel-Aziz H, Kelber O, Sauer S. (2013): Antidiabetic effects of chamomile flowers extract in obese mice through

transcriptional stimulation of nutrient sensors of the peroxisome proliferator-activated receptor (PPAR) family. PLoS One. 2013 Nov 12; 8(11):e80335. doi: 10.1371/ journal.pone.0080335. Collection 2013.

Wren, A. F.; Cleary, M.; Frantz, C.; Melton, S. and Norris, L. (2002): 90-Day Oral Toxicity Study of a Grape Seed Extract (IH636) in Rats. J. Agric. Food Chem., 50(7):2180-2192.

Yang B. Y., Zhang X. Y., Guan S. W., & Hua Z. C. (2015): Protective effect of procyanidin B2 against CCl_4-induced acute liver injury in mice. Molecules, 20, 12250–12265. 10.3390/molecules200712250.

Yousef MI, Mahdy MAFH, Abdou HM (2019): The potential protective role of grape seed proanthocyanidin extract against the mixture of carboplatin and thalidomide -induced hepatotoxicity and cardiotoxicity in male rats. 2: DOI: 10.15761/PMCH.1000136.

Yousef, M. I.; Saad, A. A. and El- Shennawy, L. K. (2009): Protective effect of grape seed proanthocyanidin extract against oxidative stress induced by cisplatin in rats. Food and Chem. Toxicol., 47(6):1176- 1183.

Zahran F, Gabr SA, Abd El-Moneim AE, Sharoud MN, Hassanin WF, Mesalam NM2 (2018): Prophylactic Effect Of Camel Milk On Physioliogical And Biochemical Changes In CCL_4-Intoxicated Rats,DOI:http://dx.doi.org/10.18006/2018.6(1). 211. 219.

Zhang, B., Xu, D., She, L., Wang, Z., Yang, N., Sun, R., … Xie, Y. (2018): Silybin inhibits NLRP3 inflammasome assembly through the NAD(+)/SIRT2 pathway in mice with nonalcoholic fatty liver disease. The FASEB Journal, 32, 757– 767.

Zou J., Qi F., Ye L., & Yao S. (2016): Protective Role of grape seed proanthocyanidins against CCl_4 induced acute liver injury in mice. Med. Sci. Monitor, 22, 880–889. 10.12659/MSM.895552