Potential Therapeutic Effects of some Egyptian Plant Parts on Hepatic Toxicity Induced by Carbon Tetrachloride in Rats

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Abstract: This study was conduced to investigate the effect of some Egyptian herbs on impaired liver function of rats injected with Carbon Tetrachloride (CCl_4). Seventy mature albino rats, weighting 150-160g per each, were used and divided into 12 equal groups, one was kept as a control-ve group, while the other groups were injected subcutaneous (s/c) by CCl_4 in paraffin oil 50% v/v (2 ml/kg B.W.), twice a week for tow weeks. The tested herbs were given as a percent of 5% and10% from the Basel diet. Serum liver function (GOT, GPT, ALK) total protein, albumin, globulin, total bilirubin, indirect bilirubin, direct bilirubin, and histopathological changes of liver were examined. The results indicated that rats treated with CCl_4 recorded significantly increasing in the activity levels of all biomarker liver enzymes (SGOT, SGPT and SALP) and decreasing in serum total protein, albumin, globulin and bilirubin with significant values when compared with the control group after the experiment period, 2 weeks. Addition of tested plant parts such Henada *(Jasonia Montana)*, lemon balm leaves (*Melissa officinalis*), hawthorn leaves *(Crataegus azorolus),* rose of jericho *(Anastatica hierochuntica)* and corn cob silk *(zea mayz)* by 5 and 10% of the diet intake in the presence of CCl_4 induced significant improvements in all liver functions. Also, CCl_4 treated group had histopathological changes on liver through degeneration hyperemia, inflammatory reaction. The post-treatment of tested plant parts with CCl_4 leads to prevent some of the previous histopathological changes. It could be concluded that the tested plant parts were effective in protecting against CCl_4 -induced adverse liver functions and its histopathological changes. These results supported our hypothesis that the tested plant parts contain several compounds that are able to prevent or inhibit CCl_4 toxicity. Therefore, we recommended those tested plant parts by a moderate amount to be included in our daily diets and drinks.

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1. Introduction

Liver is a vital organ present in all vertebrates. It has a wide range of functions, including detoxification, protein synthesis, and production of biochemical necessary for digestion (Voet and Voet,1990). This organ plays a major role in metabolism and has a number of functions in the body, including glycogen storage, decomposition of red blood cells, plasma protein synthesis, hormone production, and detoxification. It lies below the diaphragm in the abdominal-pelvic region of the abdomen. It produces bile, an alkaline compound which aids in digestion via the emulsification of lipids. The liver's highly specialized tissues regulate a wide variety of high-volume biochemical reactions, including the synthesis and breakdown of small and complex molecules, many of which are necessary for normal vital functions (Maton *et al.,* 1993).The liver is necessary for survival; there is currently no way to compensate for the absence of liver function long term, although liver dialysis can be used short time.

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, because of better cultural acceptability, and compatibility with the human body as well as fewer side effects. 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 cough, malaria, wounds, toothache and rheumatism diseases (Exarchou *et al., 2002*).

Few decades ago, there has been renewed attention and interest in the use of traditional medicine globally (WHO, 2002). According to estimate of the world health organization, 80% of the world population is primarily reliant on traditional

methods of healing which use empirical knowledge based o the use of medicinal plants (Mueller and Mechler, 2005). Therefore, the present study aims to evaluate the therapeutic effect of some Egyptian plant parts on impaired liver function of rats injected with carbon tetrachloride (CCl_4). Also, histopathological effects of the these plant parts on liver will be in the scope of this investigation.

2. Materials and Methods

2.1 Materials:

Plants: Henada *(Jasonia Montana)* were obtained from Sant Catherine, Sinai while lemon balm leaves (*Melissa officinalis*), hawthorn leaves *(Crataegus azorolus),* rose of jericho *(Anastatica hierochuntica)* and corn cob silk *(zea mayz)* were obtained from local markets, Shebin El-kom City, Egypt. All plants parts were grinded using Electric grinder (Moulinex, France) to give a powder and kept in dusky Stoppard glass bottles until use.

Rats: Sixty adult male albino rats, weighing 150-160g per each were obtained from Medical Insects Research Institute, Dokki, Cairo.

Chemicals: All chemicals, solvents and buffers in analytical grade, carbon tetrachloride (CCl_4, 10% liquid solution) and vitamin and salt mixtures components used for rats feeding were purchased from Elgomhoria Company for Chemicals and Drug Trading, Cairo, Egypt. Casein was obtained from Morgan Chemical Co., Cairo, Egypt. Paraffin oil (10%) obtained from Eltampashawy Pharmacy, Shebin El-Kom City, Egypt.

2.2 Biological Experiments:

2.2.1 Basal Diet:

The basic diet prepared according to the following formula as mentioned by (AIN, 1993) as follow: 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%). The used vitamin mixture component was that recommended by (Campbell, 1963) while the salt mixture used was formulated according to (Hegsted, 1941).

2.2.2 Preparation of liver impaired rats:

Liver impaired was induced in normal healthy male albino rats by subcutaneous injection of CCl_4 (0.2 mg/kg body weight) for two weeks according to method described by Passmore and Eastwood, (1986).

2.2.3 Experimental design

All biological experiments were done at the biology lab, Nutrition and Food Science Dept., Faculty of Home Economics, Minoufiya University, Shebin El-Kom, Egypt. Rats (n=60 rats) were housed individually in wire cages in a room maintained at 25 ± 2 ^{0}C and kept under normal healthy conditions. All rats (60 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 1, 5 rats) still fed on basal diet and the other main group (55 rats) was injected by CCl_4 for two weeks to induce liver impaired rats then classified into eleven sub groups as follow:

– Group (2): fed on standard diet only as a positive control
– Group (3): fed on standard diet containing 5% henada powder.
– Group (4): fed on standard diet containing 10% henada powder.
– Group (5): fed on standard diet containing 5% lemon palm powder.
– Group (6): fed on standard diet containing 10% lemon palm powder.
– Group (7): fed on standard diet containing 5% Jericho rose powder.
– Group (8): fed on standard diet containing 10% Jericho rose powder.
– Group (9): fed on standard diet containing 5% hawthorn powder.
– Group (10): fed on standard diet containing 10% hawthorn rose powder.
– Group (11): fed on standard diet containing 5% corn cob silk powder.
– Group (12): fed on standard diet containing 10% corn cob silk powder.

2.2.4 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.

2.2.5 Hematological analysis

Different tested parameters in serum were determination using specific methods as follow: glotamic oxaloacetic transaminas (GOT), glotamic pyruvic transaminas (GPT), alkaline phsphatase (ALP), total Bilirubin, direct Bilirubin and indirect bilirubin, albumin, globulin and total protein according to Yound, (1975), Tietz, (1976), Belfield and Goldberg, (1971), Doumas *et al.,* (1973), Chary and Sharma, (2004), Spencer and Price, (1977), Chary and Sharma, (2004) and Weissman *et al.,* (1950), respectively.

2.2.6 Histopathological examination

After rats were scarified under ether anesthetized, 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).

2.3. Statistical analyses

Statistical analyses were made by using SPSS computer program (1998).

3. Results and Discussion

3.1 Serum GOT, GPT And ALP Activities (U\L) Of Rats Injected Ccl_4 And Consumed Some Plant Parts

Table (1) illustrate the mean value of serum (GOT) of rats' livers fed on various diets. It could be noticed that GOT of control (-) group was lower than control (+) group by the ratio of -40.8 %. Rats' livers fed on different tested diets revealed significant decreases in GOT activities compared with the rats injected with CCl_4. The percent of decrease as compared to control (+) group were - 42.14, - 46.17, - 41.18, - 43.48, and - 45.21; and -43.87, - 44.06, - 37.55, - 40.8 and - 45.21 % for *henada, lemon palm, Jericho rose, hawthorn and Corn cob silk by 5 and* 10%, respectively. Non significant differences were observed amongst rats groups fed on henada 10%, lemon palm 5 and 10%, hawthorn 5% and corn cob silk 5 and 10%. Considering (GOT) activity, *lemon palm 5%* group recorded the best treatment was observed for when compared to control (-) group. According to amelioration degree of GOT activity, the sequence of potency of that plant parts were lemon palm 5%> corn cob silk 5 > corn cob silk 10 > lemon palm 10%> Henada 10%> hawthorn 5%> henada 5%> Jericho rose 5%> hawthorn 10%> Jericho rose 10%. The same behaviour was observed for the mean value of serum (GPT) and (ALP) except the sequence of plant parts potency which were Jericho rose 5%> lemon palm 5%> hawthorn 5%> Henada 10%> lemon palm 10%> henada 5%> hawthorn 10%>> corn cob silk 10> Jericho rose 10% >corn cob silk 5 for GPT and hawthorn 10%> Jericho rose 5%> lemon palm 5%> Henada 5%> hawthorn 5%> henada 10%> Jericho rose 10% > lemon palm 10> corn cob silk 10> corn cob silk 5 for ALP. In similar study Shaban *et al.,* (2012) reported that the methanolic extract of Jericho rose (*A. hierochuntica*) was significantly decreased the activity levels of all some biomarker liver damage enzymes including GOT, GPT and ALP and restored them to normal values in all treated group of alloxan diabetic rats.

Aminotransferases are normally intracellular enzymes. Thus, the presence of elevated levels of aminotransferase in the plasma indicates damage to cells rich in these enzymes. For example, physical trauma or a disease process can cause cell lysis, resulting release of intracellular enzymes into the blood. Two aminotransferases were found in plasma are of particular diagnostic value GOT and GPT. These enzymes are elevated in nearly all liver diseases, but are particularly high in conditions that the causes extensive cell necrosis, such as severe viral hepatitis and prolongated circulatory collapse. Serial enzyme measurements are often useful in determining the course of liver damage. Also, aminotransferases may be elevated in nonhepatic disease, such as myocardial infraction and muscle disorders; however, these disorders can usually be distinguished clinically from liver disease (Champe and Harvey, 1994). Alkaline phosphatase (ALP) is an enzyme which catalyzes the hydrolysis of phosphate esters at an alkaline pH to give pi and the corresponding alcohol, phenol or sugar.

Table (1). Serum GOT, GPT and ALP activities (u\l) of rats injected CCl_4 and consumed some plant parts

Groups		GOT (U\L)		GPT (U\L)		ALP (U\L)	
		Mean±SD	% of change	Mean±SD	% of change	Mean±SD	% of change
Control (-)		$103^{bc} \pm 5$	- 40.80	$33.6^{de} \pm 1.4$	- 68.53	$165^{bcde} \pm 5$	- 21.55
Control (+)		$174^{a} \pm 5.29$	------	$106.8^{a} \pm 21.5$	------	$210.33^{a} \pm 1.52$	------
Henada	5%	100.66 ± 4.04	- 42.14	$47.2^{bc} \pm 1.3$	- 55.80	$160.66^{cde} \pm 6.02$	- 23.61
	10%	$97.66^{cd} \pm 2.51$	- 43.87	$43.2^{bcde} \pm 1.9$	- 59.55	$161.66^{bcde} \pm 2.08$	-23.13
Lemon palm	5%	$93.66^{cd} \pm 2.51$	- 46.17	$33.6^{de} \pm 1.4$	- 68.53	$160^{de} \pm 4.58$	-23.92
	10%	$97.33^{cd} \pm 3.05$	- 44.06	$45.2^{bcd} \pm 1.3$	- 57.67	$164^{bcde} \pm 4.58$	-22.02
Jericho rose	5%	$102.33^{bc} \pm 3.78$	- 41.18	$31.8^{e} \pm 1.6$	- 70.22	$159.66^{de} \pm 1.52$	-24.09
	10%	$108.66^{b} \pm 3.21$	- 37.55	$49.4^{bc} \pm 2.6$	- 53.74	$162^{bcde} \pm 3.60$	-22.97
Hawthorn	5%	$98.33^{cd} \pm 1.52$	- 43.48	$39^{cde} \pm 2.5$	- 63.48	$161.33^{bcde} \pm 2.51$	-23.29
	10%	$103.^{bc} \pm 4.35$	- 40.80	$48^{bc} \pm 3.8$	- 55.05	$157.33^{e} \pm 2.51$	-25.19
Corn cob silk	5%	$95.33^{cd} \pm 2.51$	- 45.21	$53.4^{b} \pm 2.1$	- 50.00	$170^{bc} \pm 2.00$	-19.17
	10%	$95.66^{cd} \pm 4.04$	- 45.02	$48.4^{bc} \pm 6.1$	- 54.68	$168^{bcd} \pm 2.00$	-20.12
Sig.		*		*		*	
L.S.D ($p \leq 0.05$)		6.153		8.02		5.732	

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

The greatest concentration of ALP is found in bone, liver, intestine and the placenta. However, practically every body tissue contains at least a small amount of ALP. Because of this wide distribution limited information can be obtain from a total AP assay. Elevated serum and leukocytic ALP leaves in patients with Hodgkin`s and non-Hodgkin`s lymphoma were reported by several investigators. Also, Aiba *et al*., (1980), found that the elevated leukocyte AP in patients who have hairy cell leukemia was inversely correlated to absolute number of neutrophils in the peripheral blood, *i.e*. the patients who had high leukocyte ALP scores has low or normal peripheral blood neutrophil counts. Abnormal leukocyte ALP scores are characteristic of certain myloprolifrative and lymphoproliferative disorders. Gobbi *et al.,* (1982) found that among liver function tests that have been investigated in Hodgkin's disease, serum AP activity was elevated in 20 out of 133 patients while it was elevated in 10 out of 20 patients with initial bone disease.

Such as reviewed in Beattic *et al.,* (2005) plant parts including berries arc a rich source of such phytochemicals, in particular anthocyanins and flavonols. phenolic phytochemicals were extracted from blueberries, blackberries, strawberries, raspberries, cranberries, and Noble muscadine grapes by Wang *et al.,* (2000). Many studies reported that the effect of many plant parts like raspberry on decreasing the serum liver function enzymes activity could be attributed to their high level content of that phytochemicals. For examples, El-Nashar, (2007) found that different doses of cinnamon extract showed slight-decreased in serum GOT, GPT, ALP, total protein and globulin after 12 week of feeding when compared with control group while showed increased in serum total protein, globulin and total protein in liver homogenates with a significant on serum total protein and serum globulin after 12 week when compared with the control group. Dawson, (1998) reported that flavonoid is known to block the hepatocellular uptake of bile acids. It is reviewed in Beattic *et al.,* (2005) that flavonoids (found in berries) pretreatment improved the antioxidant capacity of the liver, diminished the bilirubin concentration compared with the groups without treatment. Also, flavonol glycosides reduced the elevated levels of the following serum enzymes, GOT, GPT and ALP. Finally, it reviewed in El-Nashar, (2007) that pre-treatment with flavonoids were not only able to suppress the elevation of GOT and GPT but also reduce the damage of hepatocytes *in vitro*. Also, they found that flavonoids have exhibited strong antioxidant activity against reactive oxygen species (ROS) *in vitro*. The hepatoprotective activity of flavonoids was possibly due to its antioxidant properties, acting as scavengers of reactive oxygen species (ROS).According to the present study, we strongly suggested that supplementation of the diet with berries has some hepatoprotective properties and therapeutic effects against hyperglycemia. In similar study, Mohammed, (2008) reported that oral administration of both the aerial parts extracts of Henada *(J. Montana)* at a concentration of 150 mg/kg b.w daily for 30 days leads to effectively normalize the impaired antioxidant status in Streptozotocin induced diabetes than the glibenclamide-treated groups. The extract exerted rapid protective effects against lipid peroxidation by scavenging of free radicals by reducing the risk of diabetic complications. The effect was more pronounced in ethanolic extract as compared to aqueous extract.

3.2 Serum Albumin, Globulin And Total Protein (G\Dl) Of Rats Injected Ccl_4 and Consumed Some Plant Parts

Data in Table (2) illustrate the mean value of serum albumin (ALB) of rats' livers fed on various diets. It could be noticed that ALB of control (-) group was lower than control (+) group by the ratio of -34.90 %. Rats' livers fed on different tested diets revealed significant increases in ALB activities compared with the rats injected with CCl4. The percent of increase as compared to control (+) group were 56.00, 56.00, 52.00, 39.20 and 58.00; and 50.00, 34.00, 43.20, 51.20 and 54.80 % for henada, lemon palm, Jericho rose, hawthorn and Corn cob silk by 5 and 10%, respectively. Non significant differences were observed amongst rats groups fed on henada 5%, henada 1%, lemon palm 5%, Jericho rose5%, hawthorn 10% and corn cob silk 5 and 10%. Considering (ALB) level, con cob silk 5% group recorded the best treatment was observed for when compared to control (-) group. According to enhancement on ALB level, the sequence of potency of that plant parts were corn cob silk 5 > henada 5%> lemon palm 5%> corn cob silk 10 > Jericho rose 5%> hawthorn 10%> Henada 10%> Jericho rose 10% >hawthorn 5%> lemon palm 10%. The same behaviour was observed for the mean value of serum globulin and total protein except the sequence of plant parts potency which were Jericho rose 10% > henada 5%> lemon palm 5%> Henada 10%> lemon palm 10%> hawthorn 10%> Jericho rose 5%> hawthorn 5%>> corn cob silk 5 > corn cob silk 10 for globulin and corn cob silk 5> corn cob silk 10> hawthorn 5%> Jericho rose 5%> hawthorn 10%> Henada 5%> lemon palm 5%> henada 10%> Jericho rose 10% > lemon palm 10 for total protein.

Table (2). Serum albumin (ALB), globulin (GLB) and total Protein (TP) (g\dl) of rats injected CCl_4 and consumed some plant parts

Groups		Albumin (g\dl)		Globulin (g\dl)		Total Protein (g\dl)	
		Mean±SD	% of change	Mean±SD	% of change	Mean±SD	% of change
Control (-)		3.84 ^{ab} ± 0.093	53.60	2.28^{f} ± 0.27	25.00	6.12 ^{d} ± 0.07	10.46
Control (+)		2.5 ^{e} ± 0.158	-------	3.04^{a} ± 0.04	-----	5.54 ^{e} ± 0.15	------
Henada	5%	3.90 ^{a} ± 0.74	56.00	2.39^{ef} ± 0.16	- 21.38	6.29 ^{d} ± 0.15	13.53
	10%	3.75 ^{abc} ± 0.106	50.00	2.47^{def} ± 0.16	- 18.75	6.22 ^{d} ± 0.12	12.27
Lemon palm	5%	3.90 ^{a} ± 0.061	56.00	2.37^{ef} ± 0. 1	- 22.03	6.27 ^{cd} ± 0.02	13.17
	10%	3.35 ^{d} ± 0.120	34.00	2.53^{def} ± 0.16	- 16.77	5.7 ^{e} ± 0.15	2.88
Jericho rose	5%	3.80 ^{abc} ± 0.081	52.00	2.72^{cd} ±0. 1	- 10.52	6.52 ^{bc} ± 0.15	17.68
	10%	3.58 ^{cd} ± 0.137	43.20	2.34^{ef} ±0.16	- 23.02	5.92 ^{e} ± 0.19	6.58
Hawthorn	5%	3.48 ^{d} ± 0.076	39.20	2.75^{bcd}±0.085	- 9.53	6.53 ^{bc} ± 0.15	17.87
	10%	3.78 ^{abc} ± 0.072	51.20	2.71^{cd} ± 0.12	- 10.85	6.49 ^{bc} ± 0.13	17.14
Corn cob silk	5%	3.95 ^{a} ± 0.058	58.00	2.98^{b} ± 0.094	- 4.93	6.84 ^{ab} ± 0.15	23.46
	10%	3.87 ^{ab} ± 0.186	54.80	2.91^{bc} ± 0.15	- 4.26	6.78 ^{a} ± 0.08	22.38
Sig.		*		*		*	
L.S.D (p≤0.05)		0.176		0.187		0.171	

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

Albumin and globulin constitute most of the protein within the body and are measured in the total protein. Albumin is a protein that is formed within the liver. This makes up approximately 60% of the total protein. The major effect of albumin within the blood is to maintain colloidal osmotic pressure. Furthermore, albumin transports important blood constitutes such as drugs, hormones, and enzymes. Globulins are the key building block of antibodies (Champe and Harvey, 1994). Their role in maintaining osmotic pressure is far less than that of albumin. Globulins, to a lesser degree, also act as transport vehicles. Albumin is synthesized within the liver and is therefore a measure of hepatocyte function. When disease and their factors (CCl_4 in the present study) affect the liver cell, the hepatocyte loses its ability to synthesize albumin. The serum albumin level is greatly decreased (Pagana and Pagana, 1997). In some diseases, albumin is selectively diminished and globulins are normal or increased to maintain a normal total protein level. Another group of diseases similarly associated with low albumin, high globulin, and normal total protein levels is chronic liver diseases. In these diseases, the liver cannot produce albumin but globulin is adequately made in the reticuloendothelial system. Using of the all tested plant parts in the present study leads to repair the impaired hepatocyte induced by CCl_4 by different ratios which could be probably attributed to their content of some active ingredients i.e. phytochemicals. In similar study mentioned by Mohammed and Abdel-Gawad, (2009) showed that oral administration of ethanolic extract of the aerial parts of henada *(J. Montana)* at a concentration of 150 mg/kg b.w. daily to rats for 15 days leads to significant increase in hepatic total protein.

Serum albumin represents one of the most important metal binding proteins. It is a large protein secreted by the liver. Albumin transports a number of primarily hydrophobic compounds in the circulation, including free fatty acids and some drugs. The free (unestrified) fatty acids move through the cell membrane of the adipocyte and immediately bind to albumin in the plasma, which carries them to the tissues where the fatty acids diffuse into the cells and are oxidized for energy (Champe and Harvey, 1994). Serum albumin is a sacrificial antioxidant that can bind copper tightly and iron weakly to its surface serving as a target for their related free radical reactions. Thus it inhibits copper ion dependent lipid peroxidation (Gutteridge and Wilkins, 1983). The data of the present study are in agree with that observed by Mohammed and Abdel-Gawad, (2009) who showed that oral administration of ethanolic extract of the aerial parts of henada *(J. Montana)* at a concentration of 150 mg/kg b.w. daily to rats for 15 days leads to significant increase in hepatic total protein.

3.3 Serum Total Bilirubin, Direct Bilirubin and Indirect Bilirubin of Rats Injected Ccl_4 and Consumed Some Plant Parts

Data of the Bilirubin of rats injected CCl_4 and consumed some plant parts were tabulated in table (3). It could be noticed that total Bilirubin of control (-) group was lower than control (+) group by the ratio of -28.57 %. Rats' livers fed on different tested

diets revealed significant decreases in total Bilirubin levels compared with the rats injected with CCl_4. The percent of decrease as compared to control (+) group were -8.92, -16.07, -7.14, -17.85 and -16.07; and -12.5, -12.5, -16.07, -16.07 and -12.50 % for henada, lemon palm, Jericho rose, hawthorn and Corn cob silk by 5 and 10%, respectively. Non significant differences were observed amongst rats groups fed on Jericho rose10%, hawthorn 5 % and corn cob silk 10%. Considering total Bilirubin level, hawthorn 5 % group recorded the best treatment was observed for when compared to control (-) group. According to enhancement on total bilirubin level, the sequence of potency of that plant parts were hawthorn 5%> lemon palm 5%> Jericho rose 10% > hawthorn 10%> corn cob silk 5> Henada 10%> corn cob silk 10 > lemon plam 10%> henada 5%> Jericho rose 5%. The same behaviour was observed for the mean value of direct Bilirubin and indirect Bilirubin except the sequence of plant parts potency which were lemon palm 5%> Jericho rose 10% > corn cob silk 10> hawthorn 5%> henada 5%> hawthorn 10%> Henada 10%> lemon plam 10%> Jericho rose 5%> corn cob silk 5 for direct Bilirubin and Henada 5%> Jericho rose 5%> henada 10%> Jericho rose 10% > lemon plam 10> corn cob silk 10> hawthorn 5%> hawthorn 10%> lemon palm 5%> corn cob silk 5 for indirect Bilirubin.

Table (3). Serum total bilirubin, direct bilirubin and indirect bilirubin of rats injected CCl_4 and consumed some plant parts

Groups		Total Bilirubin (mg\dl)		Direct Bilirubin (mg\dl)		Indirect Bilirubin (mg\dl)	
		Mean±SD	% of change	Mean±SD	% of change	Mean±SD	% of change
Control (-)		0.40 ^{g}±0. 15	28.57-	0.12^{c} ±0.015	- 57.14	0.38^{bc}±0.015	35.71
Control (+)		0.56 ^{a}±0.02	-----	0.28^{a} ±0.015	-----	0.28^{e} ±0.02	-----
Henada	5%	0.51 ^{bc}±0. 15	- 8.92	0.12^{c} ±0.015	- 57.14	0.39^{b} ±0.015	39.28
	10%	0.49 ^{bcd}± 0. 15	- 12.50	0.13^{bc} ±0.015	- 53.57	0.36^{bcd}±0.015	28.50
Lemon palm	5%	0.47 ^{def}± 0.15	- 16.07	0.11^{c} ±0.017	- 60.71	0.33^{d} ±0.015	17.85
	10%	0.49 ^{cde}± 0. 15	- 12.50	0.14^{bc} ±0.011	- 50.00	0.35^{cd} ±0.015	25.00
Jericho rose	5%	0.52 ^{b} ± 0.15	- 7.14	0.14^{bc} ±0.032	- 50.00	0.38^{bc} ±0.015	35.71
	10%	0.47 f ±0.13	- 16.07	0.11^{c} ±0.013	- 60.71	0.36^{bcd}±0.011	28.50
Hawthorn	5%	0.46 ^{f}±0.15	- 17.85	0.12^{bc} ±0.011	- 57.14	0.34^{a} ±0.015	21.43
	10%	0.47^{def}±0.015	- 16.07	0.13^{bc} ±0.011	- 53.57	0.34^{cd} ±0.022	21.42
Corn cob silk	5%	0.47 ^{def}±0.15	- 16.07	0.14^{bc} ±0.013	- 50	0.33^{d} ±0.02	17.85
	10%	0.46 ^{f} ±0.15	- 12.50	0.11^{c} ± 0.01	60.71	0.35^{bcd}±0.023	25.00
Sig.		*		*		*	
L.S.D (p≤0.05)		0.022		0.020		0.020	

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

Bilirubin is a breakdown product of hem (a part of haemoglobin in red blood cells). The liver is responsible for clearing the blood of bilirubin. It does this by the following mechanism: Bilirubin is taken up into hepatocytes, conjugated (modified to make it water-soluble), and secreted into the bile, which is excreted into the intestine (Nyblom *et al.,* 2004). The total serum Bilirubin level is the sum of the conjugated (direct) and unconjugated (indirect) bilirubin. Normally, the unconjugated bilirubin makes up 70% to 85% of the total bilirubin. If direct bilirubin is elevated, then the liver is conjugating bilirubin normally, but is not able to excrete it. Bile duct obstruction by gallstones, hepatitis or cancer should be suspected (Nyblom *et al .,* 2004). Active ingredients such phytochemicals content of the all tested plant parts in the present study may cause decreased levels of the total bilirubin induced by CCl_4. In similar study mentioned by Mohammed and Abdel-Gawad, (2009) showed that oral administration of ethanolic extract of the aerial parts of henada *(J. Montana)* at a concentration of 150 mg/kg b.w. daily to rats for 15 days showed a significant protection against-induced decrease in serum bilirubin levels.

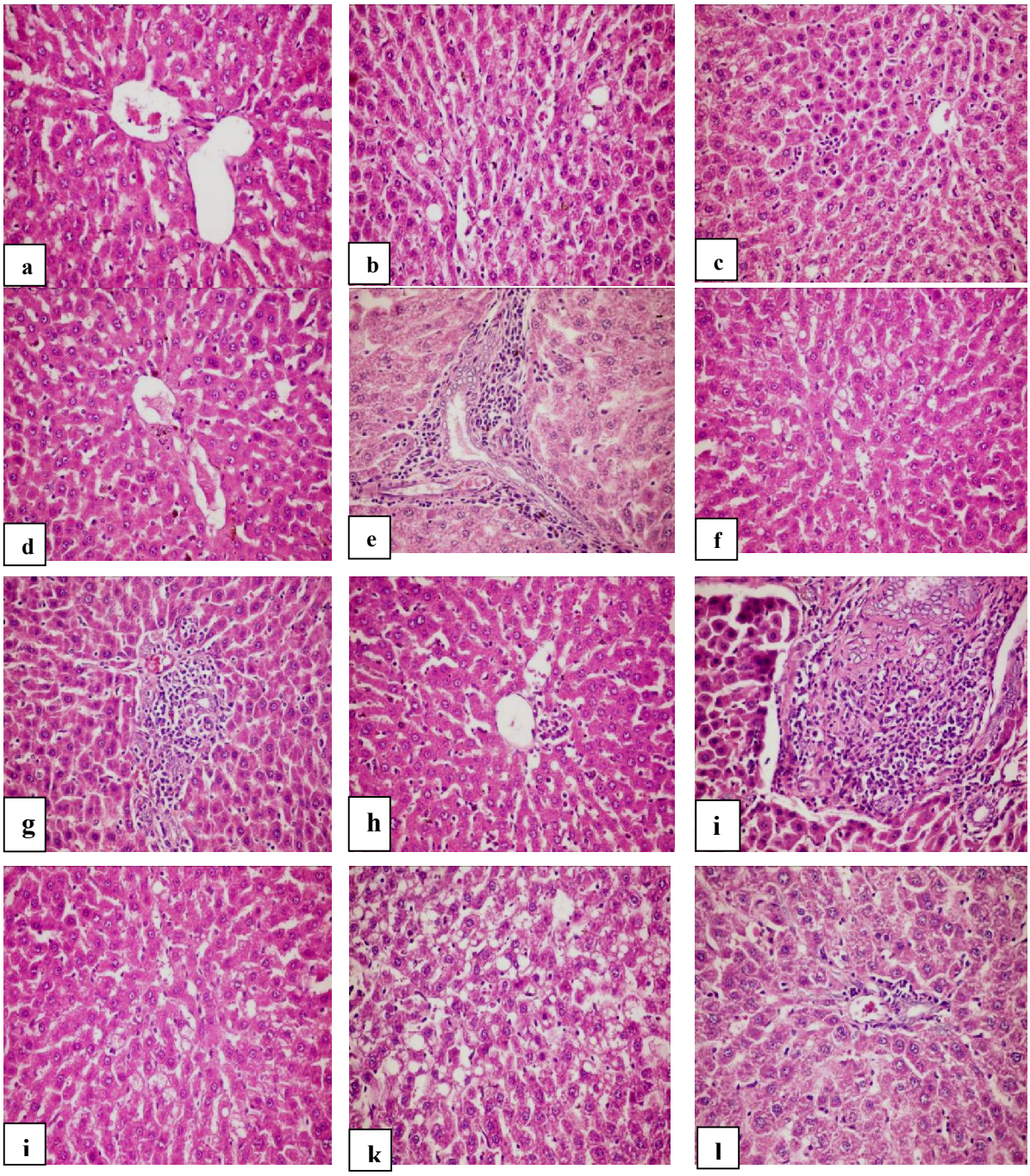

Fig. 1: The effect of tested plant parts on liver histopathological changes induced by CCl4 in rats. a, normal (control diet); b, c, fed the control diet and administrated CCl4 for 2 weeks; d, e, fed diet containing jasonia for 4 weeks after treated with CCl4; f,g,h, fed diet containing melissa for 4 weeks after treated with CCl4; i, j, fed diet containing crataegus azorolus for 4 weeks after treated with CCl4; and k, l, fed diet containing corn cob silk for 4 weeks after treated with CCl4. (H&E, X 40)

3.4 Histopathological Examination

Changes in histopathological parameters as the result of CCl_4 subjection has been studied by many authors. Several universities and academic centers have paid attention towards the methods could be used successively in reducing like of these

changes. One of the most effective methods commonly tested in the last decade is the using of origin-plant parts. Some of these parts exhibited significant roles in reducing the adverse effects of CCl_4 including the histopathological changes. In the present study the effect of phytochemical containing diet on rats treated with CCl_4 have been investigated. Liver of rats fed the normal diet showed the normal histological structure of the central veins and surrounding hepatocytes (Figure 1-a). Rats treated with CCl_4 for 2 weeks showed kupffer cells activation, cytoplasmic vacuolization of hepatocytes (Figure 1-b) and sinusoidal leucocytosis (Figure 1-c). Feeding on diet containing the tested plant parts for 4 weeks after treated with CCl_4 induced some improvement /amelioration on the histological structure of rat livers as follow: jasonia showed Kupffer cells activation (Figure 1-d) and portal infiltration with leucocytes (Figure 1-e), melissa showed kupffer cells activation (Figure 1-f), hierochuntica showed portal infiltration with leucocytes (Figure 1-g) and few leucocytes in hepatic sinusoids (Figure 1-h), *crataegus azorolus* showed portal infiltration with leucocytes and appearance of newly formed bile ductuoles (Figure 1-i) and cytoplasmic vacuolization of some hepatocytes (Figure 1-j), and corn silk showed cytoplasmic vacuolization of hepatocytes (Figure 1-k) and kupffer cells activation and apoptosis of hepatocytes (Figure 1-l). From such data it cold be reported that some of the tested plant parts act as a protector against CCl_4 toxicity mainly on liver. This finding agreed with that noticed by Wu *et al.,* (2006) and Elhassaneen *et al.,* (2009) who found that all histopathological changes in liver such as fatty infiltration, variation in mitotic figures and focal necrosis, which induced by dibutylnitrosamine were reversed by the administration of phytochemicals extract of cinnamon plant parts.

In conclusion, the tested plan parts in the present study were effective in protecting against CCl_4-induced histopathological changes. These results supported our hypothesis that tested plan parts contain several free and conjugated compounds that are able to prevent or inhibit CCl_4 toxicity. Therefore, we recommended the tested plant parts by a moderate amount to be included in our daily diets and drinks.

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