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## Potential Protective Effects of Cauliflower Leaves and Prickly Pear Fruits Skin on Liver Disorders Induced by Carbon Tetrachloride in Rats

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### Abstract:

The present study aims to investigate the potential protective effects of food industries by-products, cauliflower leaves and prickly pear fruits skin, against liver disorders induced by carbon tetrachloride on experimental rats. Thirty rats were distributed into five equal groups as follow: Group 1, control (normal) group, group 2, control positive group i.e. rats infected with hepatotoxicity by  $CCl_4$ , group 3, infected rats treated with 5% prickly pear peels powder (PPP), group 4, infected rats treated with 5% cauliflower leaves powder (CLP), group 5, infected rats treated with mixture of 2.5% PPP plus 2.5% CLP.  $CCl_4$  induced a significant decrease ( $p \leq 0.05$ ) in BWG (-54.40), FI (-36.36) and FER (-23.33%) compared to normal controls. Supplementation of the diet with PPP, CLP and their mixture induced significant ( $p \leq 0.05$ ) increasing on body weight gain (BWG), feed intake (FI) and feed efficiency ratio (FER) by the ratio of 33.55, 40.39 and 57.56%; 23.35, 30.64 and 40.14%; and 6.76, 14.01 and 16.42% of the control positive group, respectively. Also, consumption the all-tested plant parts improved the biological parameters Expressing liver functions examination and biological antioxidant (glutathione, GSH). Reduced glutathione (GSH) as well as decreasing the formation of malonaldehyde (MDA) i.e. serum lipid peroxidation or oxidative stress. Furthermore, all the biochemical analyzes results were in agree with the results of the histological examination.

**Keywords:** *Weight gain, feed intake, feed efficiency ratio, glutathione, malonaldehyde*

### Introduction

Many physiological processes in our bodies are regulated by the liver, including metabolic, circulatory, immunological, storage, secretory, and excretory functions. It also plays a role in the detoxification of a wide range of medicines and xenobiotics. It also

plays an important part in the human body's glucose, protein, and fat metabolism<sup>(1)</sup>. The liver produces several vital protein components of blood plasma including albumins, fibrinogen, and prothrombin. Albumins are proteins that maintain colloidal osmotic pressure<sup>(2)</sup>. It plays a central role in lipid metabolism, serving as the center for lipoprotein uptake, formation and export to the circulation. Hepatic lipid metabolism changes might contribute to the development of chronic liver disease like nonalcoholic fatty liver disease (NAFLD) and accelerate the progression of other chronic liver diseases like hepatitis C. Furthermore, chronic liver disease can modify hepatic lipid metabolism, resulting in changes in circulating lipid levels, which can contribute to dyslipidemia<sup>(3)</sup>.

Plants have been used as remedies for thousands of years, according to historical sources. Several years ago, the plants have been utilized to treat ailments<sup>(4)</sup>. Traditional medicines are believed to be used by 80 percent of the world's population for primary healthcare<sup>(5)</sup>. This dependence is significantly due to the fact that plants are considered the only available, affordable and trusted medicine to bring about sustainable solutions to health problems. In spite of considerable progress in modern medicine, there are very few therapeutic agents that can protect the liver from damage and stimulate liver functions<sup>(6)</sup>. Due to their low cost, easy availability, and high content in polyphenolic compounds as well as other valuable active ingredients, some plant wastes and subsidiary products have been shown to be potential sources of antioxidants, which are effective in delaying or inhibiting the oxidation of lipids or other molecules<sup>(7-10)</sup>.

Patients with chronic liver illness are at risk of undergoing liver transplantation, which is not only expensive but also has long-term immunosuppressive side effects, including hyperlipidemia, hypertension, and renal failure<sup>(11)</sup>. As a result, many individuals with liver illness turn to herbal medicines to help them overcome their health issues<sup>(12)</sup>. Natural plant-based therapies are gaining popularity in South Africa as a therapy option. Traditional medicines are used by up to 80% of the South African people to satisfy their primary health care needs<sup>(13)</sup>.

Cauliflower (*Brassica oleraceae*) is the most common *Brassicaceae* crop and it is grown in large quantities all over the world. As a result of this concentrated manufacturing, tones of cauliflower ancillary goods are produced each year. These cauliflower by-products are primarily made up of leaves and stems, with cauliflower leaves accounting for almost half of the total<sup>(14)</sup>. As a result, recovering and using bioactive chemicals from cauliflower leaves is a significant difficulty. The antioxidant capacity of edible parts of cauliflowers is widely studied and has been associated with their flavonoids and phenolic acids contents<sup>(14,15)</sup>. Bioactive compounds derived from cauliflower by-products, on the other hand, have received little attention<sup>(16)</sup>. However, because several of the antioxidant phytochemicals found in cauliflower leaves have a limited bioavailability, they are unable to prevent meat oxidation with dietary supplementation<sup>(17,18)</sup>. Cauliflower is an excellent

source of vitamins B1, B2, B3, B5, B6, C, E and K, folic acid as well as dietary fiber, omega-3 fatty acids, proteins, potassium, phosphorus, magnesium manganese and iron<sup>(19)</sup>. Cauliflower also contains sulfur-containing glucosinolates, flavonoids, terpenes, S-methyl cysteine sulfoxide, coumarins, and other minor compounds, among which are sulfur-containing glucosinolates, flavonoids, terpenes, S-methyl cysteine sulfoxide, coumarins, and other minor compounds. These chemicals found in cauliflower and other Brassica vegetables have been shown to be useful in preventing and treating some types of cancer<sup>(14)</sup>. Glucosinolates from cauliflower and their breakdown products as well as polyphenols show also antioxidant, anti-inflammatory, antiallergic, anti-fungal, anti-virus, anti-mutagenic, and antibacterial properties<sup>(20)</sup>.

Prickly pear fruit is a berry that weighs between 100 and 200 grams and has a thick fleshy skin or peel (30-40% of total fruit weight) and a high sugar content (10% of total rind weight)<sup>(21)</sup>. Prickly pears (*Opuntia spp.*, *Cactaceae*) are an important source of income in many arid places, and they are abundantly produced<sup>(21,22)</sup>. Polysaccharides (21%), cellulose (29.1%), hemicelluloses (8.5%), pectin (3%), protein (8.3%), and minerals (12.13%) were all found in significant amounts in prickly pear peel<sup>(23)</sup>. They went on to say that the prickly pear had a lot of colors and flavor chemicals, especially betalains and polyphenols. The peel fraction had a lipid level of 2.43 percent, according to the study (on dry weight basis). Prickly pear is a particularly rich plant in vitamins, minerals, amino acids, and carbohydrates, according to several studies. It has been utilized in meals, medical purposes, cosmetics, and the manufacturing of cochineal<sup>(24)</sup>. Elhassaneen<sup>(25)</sup> also discovered that PPP is a good source of dietary fiber as well as bioactive components including carotenoids and phenolics.

The best-characterized animal model of xenobiotic-induced, oxidative stress-mediated hepatotoxicity is carbon tetrachloride (CCl<sub>4</sub>), which is a frequently used industrial solvent<sup>(26)</sup>. CCl<sub>4</sub> causes liver injury by inducing the formation of a variety of reactive effects such as reactive metabolites, reactive oxygen species (ROS), inflammatory reactions, and an imbalance between cellular damage and defensive responses<sup>(27,28)</sup>.

This study aims to investigate the potential protective effects of food industries by-products against liver disorders induced by carbon tetrachloride on experimental rats.

## Materials and Methods

### Materials

**Plant parts:** Cauliflower leaves (*Brassica oleraceae*) and prickly pear (*Opuntia spp.*) peels were obtained from market in Shebin El-Kom, Menoufia Governorate, Egypt during 2020 harvesting period. The collected samples were transported to the laboratory and used immediately for their powder preparation.

**Basal diet:** Basal diet constituents were obtained from El-Gomhoryia Company for Trading Drug, Chemical and Medical Instruments, Cairo, Egypt.

**Rats:** thirty white male albino rats, weighting between (130-160g) were used in the study. The animals were obtained Research Institute of Ophthalmology, medical Analysis department, Doki, Giza, Egypt. Rats were housed in wire cages under the normal laboratory condition and fed on basal diet for one week as adaptation period. Diets were introduced to rats in a special non-scattering feeding cup to avoid loss of feed and contamination. Tap water was provided to rats by means of glass tubes projecting through wire cages from inverted bottles supported to one side of the cage.

**Kits:** For determination of liver functions, serum glucose, serum lipid profile, oxidative stress marker malondialdehyde (MDA) and Glutathione (GSH), which were obtained from Bio Diagnostic Co. Doki, Giza, Egypt.

**Chemicals:** All solvents used throughout the present work were of high analytical grade and obtained from different companies. ABTs, DPPH and substrates were purchased from (Sigma chemical Co, St. Louis, U S A). Carbon tetrachloride (CCl<sub>4</sub>) was obtained from El-Gomhoryia Company for Chemical Industries, Cairo, Egypt as 10% liquid solution used for liver poisoning according to Passmore and Eastwood, (1986). In the same time, it is mixed with olive oil which obtained from the pharmacy for dilution during the induction.

## **Methods**

### **Preparation of the dried powder of prickly pear peels (PPPs)**

Fresh mature prickly pear fruits were washed with tap water followed by distilled water, then they were carefully hand-peeled, and the peels (with a thickness of about 3-4mm) were cut into small pieces and dried in an oven at 55<sup>0</sup>C for 12h. The dried peels were ground in a blender (Moulinex) for 3min, passed through a 150 μm mesh sieve, packed in polyethylene bags and stored in a freezer (-25 <sup>0</sup>C) until further use <sup>(29)</sup>.

Thorns of fresh prickly pear fruits were removed manually by placing in the freezer for two hours before washed to remove thorns and hand-peeled by using stainless steel knife. The dried samples were sequentially extracted with 500ml ethanol solution 70% (30% distilled water). Each fraction was separately evaporated using a rotary vacuum evaporator at 45<sup>0</sup>C to obtain the powder. The obtained powder was kept in light-protected containers at 25<sup>0</sup>C until further use.

### **Preparation of the dried powder of cauliflower leaves (CLP)**

Weighed amount of fresh leaves were roasted on taws, initially for 5 minutes at low flame, so that the leaves become crisp and brittle to touch. Then the roasted leaves were finely grinded into fine powder to make cauliflower leaf powder (CLP). Source of heat was gas oven. The freshly prepared C L P was weighed and stored at room temperature and kept in dusky stoppered glass bottles in a cool and dry location till use.

### **Rats diet**

The standard diet (Table A) was formulated according to AIN<sup>(30)</sup>, and salt mixture (Table B) and vitamins (Table C) mixture such as mentioned by Hegsted et al<sup>(31)</sup> and Campbell,<sup>(32)</sup> respectively.

### **Induction of liver Intoxication in rats**

Twenty-four (24) male albino rats were administrated by intraperitoneal injection of carbon tetrachloride (CCl<sub>4</sub>) in olive oil 50% V/V (2ml / kg B.wt.) twice a week for two weeks to induce chronic damage of the liver according to the method described by<sup>(33)</sup>.

### **Experimental Designs and Animal Groups**

The experimental was done in the Faculty of Home Economics, Menoufia University, Shebin El-Kom. Rats were housed in wire cages in a room temperature 25<sup>0</sup>C and kept under normal healthy conditions. All mice were fed standard diet for seven consecutive days as adaptation period. Then, rats were distributed into five groups each of six rats in which means of rats' weight for all groups were nearly equal. All the groups of the experimental were according to the following:

Group 1: Control negative group (-ve), in which normal rats were fed on basal diet and tap water.

Group 2: control positive group (+ve), in which rats infected with hepatotoxicity by CCl<sub>4</sub> were fed on basal diet and tap water.

Group 3: Rats with impaired liver, treated with 5% prickly pear peels powder diet.

Group 4: Rats with impaired liver, treated with 5% cauliflower leaves powder diet.

Group 5: Rats with impaired liver, treated with mixture of 2.5% prickly pear peels powder plus 2.5% cauliflower leaves powder diet.

Each of the above groups was kept in a single cage for 28 days. Rats were weighted at the beginning of experimental then weekly and at the end of the experimental period.

### **Blood sampling and Organs**

From all the previously mentioned groups, blood samples were collected after 12 hour fasting at the end of the experiment. Using the retro-orbital method, by means of a micro capillary glass, blood was collected into a dry clean centrifuge tube, and left to clot at room temperature for half hour. The blood was centrifuged for 10 minutes at 300 r.p.m. to separate the serum. Serum was carefully aspirated and transferred into clean quit fit plastic tubes and kept frozen at (-20c) until the time of analysis. The organs (liver, kidney, heart, lungs and spleen) were removed and washed in saline solution, weighted and stored in (10%) formalin solution according to methods described by Drury and Wallington,<sup>(34)</sup>.

### **Biological evaluation**

During the experimental period (28 days), the diet consumed was recorded every day and body weight was recorded every week. The body weight gain (B.W.G.%). Food efficiency ratio (F.E.R), and organ/ body weight% were determined according to

Chapman et al., (1959). Using the following equations: BWG (%) = (Final weight – Initial weight)/ Initial weight  $\times$ 100 and ER = Grams gain in body weight (g/28 day)/Grams feed intake (g/28 day)

### **Biochemical Analysis**

#### **Determination of Liver enzymes**

##### **Assay of alanine aminotransferase (ALT) Activity**

Alanine aminotransferase (ALT) catalyzes the transamination of alanine to alpha ketoglutarate to form glutamate and pyruvic acid, which then reacts with 2, 4-dinitrophenylhydrazine to form hydrazine derivative of pyruvate. Alanine aminotransferase were determined according to Reitman and Frankel, <sup>(35)</sup>.

##### **Assay of aspartate aminotransferase (AST) activity**

Aspartate aminotransferase (AST) catalyzes the transamination of aspartate to alphaetoglutarate to form glutamate and oxaloacetate, which then reacts with 2,4-dinitrophenylhydrazine to form hydrazine derivative of oxaloacetate, Aspartate aminotransferase were determined according to Reitman and Frankel <sup>(35)</sup>.

##### **Assay of Alkaline Phosphatase (ALP) Activity**

Serum alkaline phosphatase was determined as IFCC, <sup>(36)</sup>.

##### **Determination of Bilirubin Content**

This was determined calorimetrically according to the method described by Jendrassik and Grof <sup>(37)</sup>.

##### **Glucose tolerance tests (GTT)**

Glucose tolerance tests (GTT) were done on day 28 of the animal treatment. After an overnight fast for 8 hours, rats in all groups were intraperitoneally dosed with a D-glucose solution (3.0 g/kg BW) in 0.9% normal saline. This solution was prepared by dissolving 45 g of D-glucose anhydrous 36 in 60 ml distilled water (0.75 g/ml). Blood glucose concentrations were measured by tail pricking at 0, 30, 60, 90, and 120 minutes, using glucometer (OneTouch select®; Lifescan Inc., Milpitas, California, USA). The Area under the Curve (AUC) was calculated from blood glucose-time curves and presented as AUC units (mg/dl/min) <sup>(38)</sup>.

##### **Determination of Antioxidant Enzymes**

Glutathione was measured according to the method of Ellman, <sup>(39)</sup> and Raja et al <sup>(40)</sup>. Serum glutathione peroxidase, glutathione reduced, and superoxide dismutase enzymes were determined by autoanalyzer (Roche-Hitachi, Japan) using commercial kits according to the methods described in Hissin and Hiff <sup>(41)</sup>, Kakkar et al <sup>(42)</sup>, and Sinha <sup>(43)</sup>, respectively.

##### **Determination of malondialdehyde (MDA) content**

MDA content was estimated calorimetrically using the thiobarbituric acid reactive substance (TBARS) assay kit <sup>(44)</sup>.

**Histopathological examination:**

Specimens of the internal organs (liver) were taken immediately after sacrificing rats and immersed in 10% neutral buffered formalin. The fixed specimens were then trimmed and dehydrated in ascending grades of alcohol, cleared in xylene, embedded in paraffin, sectioned (4-6 micrometer thickness), stained with hematoxylin and eosin and examined microscopically <sup>(45)</sup>.

**Statistical Analysis**

All the obtained data were statistically analyzed using a computerized costat program by one way ANOVA. The results are presented as mean  $\pm$  SD. Differences between treatments at  $P \leq 0.05$  were considered significant <sup>(46)</sup>.

**Results and Discussion**

Effect of food industries by-products on BWG, FI and FER of hepatic disorders induced by  $CCl_4$  in rats were shown in Table (1). From such data it could be noticed that  $CCl_4$  induced a significant decrease ( $p \leq 0.05$ ) in BWG (-54.40), FI (-36.36) and FER (-23.33%) compared to normal controls. Supplementation of the diet with PPP, CLP and their mixture induced significant ( $p \leq 0.05$ ) increasing on BWG, FI and FER by the ratio of 33.55, 40.39 and 57.56%; 23.35, 30.64 and 40.14%; and 6.76, 14.01 and 16.42% of the control positive group, respectively. The higher effects in manipulation of the BWG, FI and FER disorders induced by  $CCl_4$  in mice were recorded for the by-product mixtures followed by CLP and PPP, respectively. These results are in agreement with that observed by other studies <sup>(47,48,49)</sup>. Also, such results are in the same line with Hamzawy et al <sup>(50)</sup> and Abd El-Rahman <sup>(51)</sup> who reported that hepatic rats reveal significant reduction of the body weight and feed intake. Furthermore, El-Shatanovi et al <sup>(52)</sup> found that after 10 weeks of feeding 5% of cauliflower juice body weights were significantly higher in rats group fed on atherogenic diet (animal fat diet) compared to other groups. They also discovered that the type of dietary fat in the diet had an effect on BWG. The lipids accumulated in the bodies of rats fed an atherogenic diet without any vegetable juice cauliflower leaves. Related to FI, Morresion and Hark, <sup>(53)</sup> showed that liver disease can lead to malnutrition and the major causes of malnutrition in patients with liver disease are poor dietary/feed intake, maldigestion, malabsorption and abnormalities in the metabolism and storage of macro and micronutrients. On the other side, Dickerson and Lee, <sup>(54)</sup> reported that many patients with acute or chronic liver disease are ill and commonly lose weight. Additionally, Elbanna <sup>(55)</sup>, Abd El-Khareem <sup>(56)</sup>, Mansour <sup>(57)</sup>, Tahoon <sup>(58)</sup>, and Elhassaneen et al <sup>(59)</sup> found that injected rats by  $CCl_4$  caused decrease in both FER and BWG and improved by consumption plant parts.

**Table 1. Effect of food industries by-products on BWG, FI and FER of hepatic disorders induced by  $CCl_4$  in rats\***

Parameters	Control (-) Std diet	Control (+) CCl <sub>4</sub>	Food industries by-products conc. (5 %, w/w)			LSD (P≤0.05)
			PPP	CLP	PPP+CLP	
<b>BWG (%)</b>						
Mean ±SD	39.74 <sup>a</sup> ±	18.12 <sup>c</sup> ±	24.2 <sup>d</sup> ±	25.44 <sup>b</sup> ±	28.55 <sup>c</sup> ±	0.31
% of Change	1.22 ----	1.11 -54.40	2.01 33.55	1.12 40.39	0.89 57.56	-
<b>FI (g/day/rat)</b>						
Mean±SD	19.85 <sup>a</sup> ±	12.63 <sup>c</sup> ±	15.58 <sup>d</sup> ±	16.5 <sup>c</sup> ±	17.7 <sup>b</sup> ±	0.31
% of Change	1.16 ----	2.13 -36.36	1.11 23.35	0.89 30.64	0.74 40.14	-
<b>FER</b>						
Mean±SD	2.70 <sup>b</sup> ±	2.07 <sup>d</sup> ±	2.21 <sup>d</sup> ±	2.36 <sup>a</sup> ±	2.41 <sup>c</sup> ±	0.13
% of Change	0.13 ----	0.16 - 23.33	0.13 6.76	0.19 14.01	0.18 16.42	-

\*PPP, prickly pear powder, CLP, culiflower leaves powder; Means in the same row with different letters are significantly different at  $P \leq 0.05$

The effect of food industries by-products on liver functions of hepatic disorders induced by CCl<sub>4</sub> in rats were shown in Table (2). From such data it could be noticed that CCl<sub>4</sub> induced a significant increased ( $p \leq 0.05$ ) in AST (42.31%), ALT (21.52%) and ALP (61.17%) compared to normal controls. Supplementation of the diet with PPP, CLP and their mixture induced significant decreasing on liver AST, ALT and ALP activities by the ratio of -1.63, -2.43 and -8.07; -5.67, -9.56 and -12.0; and -20.81, -23.16 and -31.69 of the control positive group, respectively. The higher effects in manipulation of the liver enzymes disorders induced by CCl<sub>4</sub> in rats were recorded for the by-product mixtures followed by CLP and PPP, respectively. Such data are in accordance with that reported by Sayed Ahmed <sup>(60)</sup> who tested the breads blended with different agricultural processing by-products including potato, onion and cauliflower peels powder in obese rats.

**Table 2. Effect of Food industries by-products on liver functions of hepatic disorders induced by CCl<sub>4</sub> in rats\***

Parameters	Control (-) Std diet	Control (+) CCl <sub>4</sub>	Food industries by-products conc. (5 %, w/w)			LSD (P≤0.05)
			PPP	CLP	PPP+CLP	



Serum aspartate aminotransferase (AST,U/L)						
<b>Mean±SD</b>	129.29 <sup>d</sup> ± 10.28	184 <sup>a</sup> ± 11.00	181 <sup>b</sup> ± 13.78	179.52 <sup>b</sup> ± 4.71	169.15 <sup>c</sup> ± 6.78	1.56
<b>% of Change</b>	----	42.31	-1.63	-2.43	-8.07	
Serum alanine aminotransferase (ALT,U/L)						
<b>Mean±SD</b>	36.66 <sup>c</sup> ± 3.21	44.55 <sup>a</sup> ± 5.51	42.02 <sup>b</sup> ± 3.46	40.29 <sup>c</sup> ± 2.33	39.2 <sup>d</sup> ± 3.72	0.90
<b>% of Change</b>	----	21.52	-5.67	-9.56	-12.0	
Serum alkaline phosphatase (ALP,U/L)						
<b>Mean±SD</b>	184 <sup>c</sup> ± 11.56	296.55 <sup>a</sup> ± 18.51	234.83 <sup>b</sup> ± 9.76	227.86 <sup>c</sup> ± 15.02	202.55 <sup>d</sup> ± 10.51	1.56
<b>% of Change</b>	----	61.17	-20.81	-23.16	-31.69	

\*PPP, prickly pear powder; CLP, cauliflower leaves powder; Means in the same row with different letters are significantly different at  $P \leq 0.05$

Such data are in accordance with that previously reported<sup>(25,48,59,61,62, 63,64)</sup>. Also, Ding et al.,<sup>(65)</sup> who induced liver fibrosis by injecting CCL<sub>4</sub> IP in rats for eight weeks and observed that disruption of tissue architecture, large fibrous septa formation, pseudo lobe separation and collagen accumulation. Electron microscopic examination indicated distorted cell organoids. These changes were accompanied by increase of the levels of ALT and AST, while albumin was decreased significantly. CCl<sub>4</sub> also increased serum liver enzymes ALT, AST, and ALP, lactate dehydrogenase, nitric oxide, tumor necrosis factor alpha (TNF), and liver malondialdehyde content, collagen fiber percent, and decreased liver reduced glutathione content as an endogenous antioxidant, according to Ahmed et al.<sup>(66)</sup>. Furthermore, Mohamed et al.,<sup>(67)</sup> observed that phenolic chemicals present in onions, garlic, cabbage, and cauliflower inhibited the increase in AST and ALT levels in the blood. Also, Giakoustidis et al.,<sup>(68)</sup> noticed that pretreatment with epigallocatechin-3- gallate (found in cauliflower) decreased serum levels of AST and ALT. Finally, the aqueous extract from cladodes (2 ml/kg) decreased the AST and ALT levels in the CCl<sub>4</sub>-induced hepatotoxic Wistar male rats<sup>(69)</sup>.

In general, aminotransferases are normally intracellular enzymes. Thus, the presence of elevated levels of aminotransferase plus ALP 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<sup>(2)</sup>. These enzymes are raised in

almost all liver illnesses, but they're especially high in circumstances that induce significant cell necrosis, like acute viral hepatitis and prolonged circulatory collapse. The use of serial enzyme tests to track the progression of liver damage is common <sup>(25)</sup>. Non-hepatic illness, such as myocardial infarction, muscular problems, and obesity, can also cause an increase in aminotransferases <sup>(60,70)</sup>. Moreover, many authors found that the liver inflammation and functions-improving effects were evaluated according to ALT, AST (serum biochemical indicators for liver inflammation), albumin, total protein (liver cell regeneration indicators) <sup>(25, 63)</sup>.

Such as reviewed in several studies plant parts including cauliflower leaves and prickly pear skin are a rich source of different classes of phytochemicals such flavanols, phenolic acids, anthocyanins, alkaloids, carotenoids, phytosterols and organosulfur compounds <sup>(25,60,71,72,73,74)</sup>. The influence of numerous plant parts as by-products in food processing on serum liver function enzyme activity could be related to their high level content of such phytochemicals, according to the current study and others. For example, Mahran et al. <sup>(75)</sup> showed that after eight weeks of ingesting food processing by-product extracts (high in bioactive compounds like those found in teste by-products), rats treated with benzo(a)pyrene had significantly lower serum AST, GLT, and AP. Rats injected with nitrosamine and treated with apricot kernel extracts had the same results <sup>(76)</sup>. Active ingredients in sweet violet (*Viola odorata* L.) blossom powder prevented partially the rise of mean serum ALT, AST and AP activities induced by CCl<sub>4</sub> injection <sup>(77)</sup>. The possible mode of action of liver serum enzymes-lowering activity of the tested by-products including PPP and CLP, as individually or mixture, could be explained by one or more of the following process: 1) bioactive compounds found in all the tested by-products are known to block the hepatocellular uptake of bile acids <sup>(78)</sup>, 2) bioactive compounds improved the antioxidant capacity of the liver, diminished the bilirubin concentration <sup>(73)</sup>. 3) bioactive compounds have exhibited strong antioxidant activity against reactive oxygen species (ROS) <sup>(4,75,79)</sup> phytochemicals were able to reduce the damage of liver i.e. suppresses the elevation of AST, ALT and ALP through the improvement of antioxidant defense system in red blood cells <sup>(25,67)</sup>. Take in our consideration all of these mechanism of actions, the higher improvement in liver function parameters recorded in rats feeding selected food industries by-products in particular the mixture could be attributed to the antagonism effects induced by their content of different categories of bioactive compounds.

The effect of food industries by-products on serum bilirubin content, biomarkers of liver functions stress, of hepatic disorders induced by CCl<sub>4</sub> in mice were shown in Table (3). From such data it could be noticed that CCl<sub>4</sub> induced a significant ( $p \leq 0.05$ ) increased in both total (65.51%) and direct bilirubin (87.5%) compared to normal controls. Supplementation of the diet with PPP, CLP and their mixture induced significant ( $P \leq 0.05$ ) decreasing on liver total and direct bilirubin levels by the ratio of -4.16, -22.91 and -37.50%; and -12.5, -62.5 and -87.5% of the control positive group, respectively. The higher effects in manipulation of the serum bilirubin disorders induced by CCl<sub>4</sub> in rats were recorded for the by-product mixtures followed by CLP and PPP, respectively.

**Table 3. Effect of Food industries by-products on serum bilirubin content in hepatic disorders induced by CCl<sub>4</sub> in rats\***

Parameters	Control (-) Std diet	Control (+) CCl <sub>4</sub>	Food industries by-products			LSD (P≤0.05)
			PPP	conc. (5 %, w/w) CLP	PPP+CL P	
<b>T. Bilirubin (mg/dl)</b>						
Mean±SD	0.29 <sup>c</sup> ± 0.091	0.48 <sup>a</sup> ± 0.015	0.46 <sup>b</sup> ± 0.014	0.37 <sup>c</sup> ± 0.013	0.30 <sup>a</sup> ± 0.016	1.56
% of Change	----	65.51	-4.16	-22.91	-37.5	
<b>D. Bilirubin (mg/dl)</b>						
Mean±SD	0.01 <sup>d</sup> ± 0.04	0.08 <sup>a</sup> ± 0.001	0.07 <sup>b</sup> ± 0.011	0.03 <sup>c</sup> ± 0.006	0.01 <sup>d</sup> ± 0.004	1.56
% of Change	---	87.5	-12.5	-62.5	-87.5	

\*PPP, prickly pear powder, CLP, culiflower leaves powder; Means in the same row with different letters are significantly different at P≤0.05

Bilirubin is an endogenous molecule that can be hazardous in some circumstances, yet modest unconjugated hyperbilirubinemia may protect against cardiovascular disease (CVD) and tumor formation. Under a variety of clinical situations, serum bilirubin levels are frequently elevated. CCl<sub>4</sub> caused hepatotoxins in the experimental study. Many studies indicated that the selected food industries by-products contains different classes of bioactive compounds including saponins<sup>(60,64)</sup>. The reduction of plasma cholesterol and bile salt concentration by the selected by-product probably attributed to the presence of certain saponins in the diet. However, some saponins can form insoluble complexes with minerals, such as zinc and iron, which make the minerals unavailable for absorption in the gut<sup>(85)</sup>. Data of the present study are in agreement with several studies<sup>(80-82)</sup> which attributed this reduction in serum bilirubin to the bioactive compounds found in the tested functional foods (such found in PPP and CLP) which stimulates the clearance of bile from the liver, preventing congestion in the liver and diminishing the liver damage. Also, Fati,<sup>(83)</sup> reported that the rate of improvement in serum total bilirubin was increased with the mixture treatment including different bioactive categories gave maximum reduction yield of serum total bilirubin when compared with the tested function foods separated. It could be mean that a combination of different bioactive categories may be more efficient for reducing serum total bilirubin, the biomarkers of liver functions stress, because the

interactive effects occurred by different categories of bioactive compounds content of functional foods such PPP and CLP.

Data of table (4) illustrate the serum (GSH) ( $\mu\text{mol/L}$ ) of hepatic rats fed on different diets. It could be observed that the mean value of (GSH) of control (+) group was lower than control (-) group, being  $5.44 \pm 1.87$  and  $8.98 \pm 1.33$  respectively, showing significant difference with percent of decrease -39.42 % 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  $6.43 \pm 1.45$ ,  $6.59 \pm 2.01$  and  $7.22 \pm 1.98$   $\mu\text{mol/L}$ . for Prickly pear peels powder 5%, Cauliflower leaves powder 5% and mixture of them 5% (1:1) respectively. The percent of increases were 18.20, 21.14 and 32.72 % for groups 3, 4 and 5 respectively. Rats fed on group 3 and 4 revealed nonsignificant differences between them. Also rats fed on all groups showed significant differences. As compared to control (+) group. Numerically the better serum (GSH) was showed for group 5 (Mix PPP+CLP 5% (1:1)) when compared to control (-) group. Such data are in accordance with that reported by different studies <sup>(25,61,62,63,84,85)</sup>. Glutathione is a major, non-protein thiol in living organisms which performs a key role in coordinating innate antioxidant defense mechanisms <sup>(86)</sup>. Reduced glutathione (GSH) plays a key role in the detoxification of the reactive toxic metabolites as reported by Recknagel et al., <sup>(87)</sup>. The antioxidant functions of GSH includes its role in the activities of the antioxidant enzymes system (GSH-Px and GSH-Rd). In addition, GSH can apparently serve as a nonenzymatic scavenger of oxyradicals/reactive oxygen species <sup>(88 Elmaadawy et al., 2016)</sup>.

**Table 4. Effect of Food industries by-products on serum biological antioxidants (serum glutathione, GSH,  $\mu\text{mol/L}$ ) in hepatic disorders induced by  $\text{CCl}_4$  in rats\***

Parameters	Control (-) Std diet	Control (+) $\text{CCl}_4$	Food industries by-products conc. (5 %, w/w)		
			PPP	CLP	PPP+CLP
<b>Range</b>	7.20 – 10.11	3.02 – 8.17	5.03 – 8.52	4.10 – 8.45	4.89 – 9.65
<b>Mean</b>	8.98 <sup>a</sup>	5.44 <sup>d</sup>	6.43 <sup>c</sup>	6.59 <sup>c</sup>	7.22 <sup>b</sup>
<b>SD</b>	1.33	1.87	1.45	2.01	1.98
<b>% of Change</b>	0.00	-39.42	18.20	21.14	32.72

\*PPP, prickly pear powder; CLP, cauliflower leaves powder; Means in the same row with different letters are significantly different at  $P \leq 0.05$

Several studies have shown that MLP has a strong antioxidant activity, reducing lipid peroxidation and oxidative stress in a variety of organs and tissues, including the liver <sup>(89)</sup>. These findings, along with those of others, suggested that intracellular structural breakdown and an increase in lipid peroxide content could limit GSH secretion from the

liver to the bloodstream <sup>(90)</sup>. MLP was defined by its increased level of antioxidative phytochemicals such as carotenoids, quercetin, flavonoids, and phenolics as well as anticarcinogenic properties <sup>(91)</sup>.

The effect of food industries by-products on serum MDA content, biomarker of lipid peroxidation and oxidative stress, of hepatic disorders induced by CCl<sub>4</sub> in mice was shown in Table (5). From such data it could be noticed that CCl<sub>4</sub> induced a significant ( $p \leq 0.05$ ) increased in MDA level (49.64%) compared to normal controls. Supplementation of the diet with PPP, CLP and their mixture induced significant ( $p \leq 0.05$ ) decreasing on serum MDA level by the ratio of -19.38, -20.08 and -26.39 of the control positive group, respectively. The higher effect in manipulation of the serum MDA disorders induced by CCl<sub>4</sub> in rats was recorded for the by-product mixtures followed by CLP and PPP, respectively. The present data are in accordance with that reported by different studies <sup>(25,59,61,84,92,93)</sup>. Also, Bohm et al., <sup>(94)</sup> found that a combination of different bioactive compounds ( $\alpha$ -tocopherol and  $\beta$ -carotene) i.e. such as found in CLP and PPP interact synergistically to inhibit lipid peroxidation subsequently decreased MDA in some model systems. Also, Abd El-Razek et al., <sup>(95)</sup> found that the potential value and regular ingestion of concentrated prickly pear fruit juice especially at level of 3 ml/day for 8 weeks reduced oxidative damage/peroxidation of lipids by elevated levels of MDA compared to control due to partially the greatly restored the activity of antioxidant enzymes. So, our present data with the others interpreted the effect of CLP and PPP on reducing the serum lipid peroxidation which could be attributed to several effects such as scavenging free radicals, inhibiting oxidation, and reducing atherogenic risk <sup>(59,62,93)</sup>.

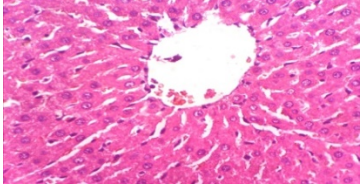
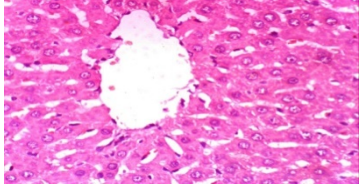
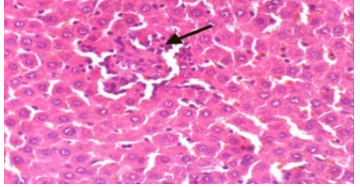
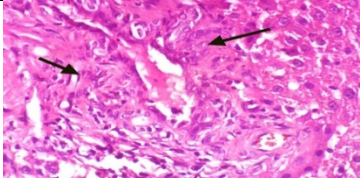
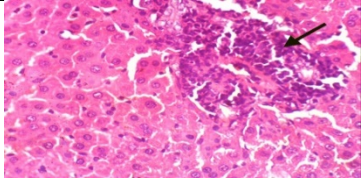
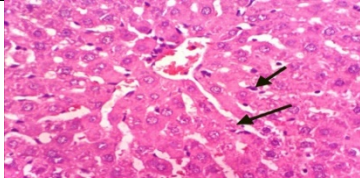
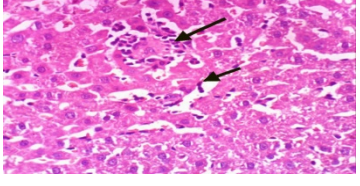
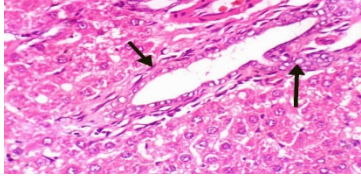
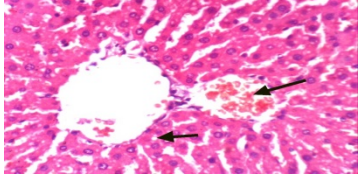
**Table 5. Effect of PPP and CLP on serum biological oxidants (malonaldehyde concentration, MDA, nmol /mL) in hepatic disorders induced by CCl<sub>4</sub> in rats\***

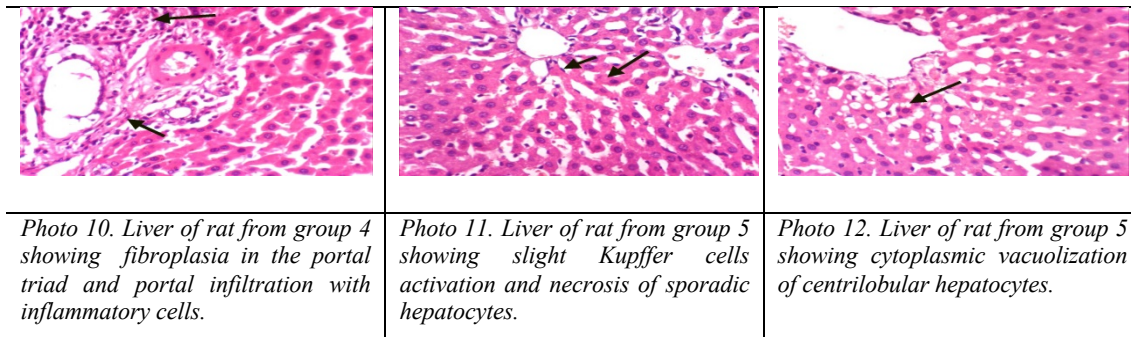
Parameters	Control (-)	Control	Food industries by-products conc.			LSD ( $P \leq 0.05$ )
	Std diet	(+) CCl <sub>4</sub>	PPP	(5 %, w/w) CLP	PPP+CLP	
Mean $\pm$ SD	2.76 $\pm$ 0.45 <sup>c</sup>	4.13 $\pm$ 0.78 <sup>a</sup>	3.33 $\pm$ 0.67 <sup>b</sup>	3.30 $\pm$ 1.01 <sup>b</sup>	3.04 $\pm$ 0.55 <sup>b</sup>	1.58
% of Change	0.00	49.64	-19.38	-20.08	-26.39	----

\*PPP, prickly pear powder; CLP, culiflower leaves powder; Means in the same row with different letters are significantly different at  $P \leq 0.05$

The effect of selected food industries by-products on liver histopathological disorders induced by CCl<sub>4</sub> in rats are shown in Figure (1). From such microscopically examination it could be noticed that liver of rats from group (1) revealed the normal histological structure of hepatic lobules (Photos 1 and 2). On contrary, liver of rats from group (2) showed focal hepatocellular necrosis associated with inflammatory cells infiltration (Photo 3), marked fibroplasia in the portal triad, appearance of newly formed bile ductules (Photo 4) and portal infiltration with inflammatory cells (Photo 5). However,

liver of rats from group (3) showed slight Kupffer cells activation, necrosis of sporadic hepatocytes (Photo 6), focal hepatocellular necrosis associated with inflammatory cells infiltration (Photo 7), slight fibroplasia in the portal triad and appearance of newly formed bile ductules (Photo 8). Meanwhile, liver of rats from group (4) revealed vacuolar degeneration of hepatocytes, congestion of central vein (Photo 9), fibroplasia in the portal triad and portal infiltration with inflammatory cells (Photo 10). On the other hand, liver of rats from group (5) exhibited slight Kupffer cells activation, necrosis of sporadic hepatocytes (Photo 11) and cytoplasmic vacuolization of centrilobular hepatocytes (Photo 12).

		
<i>Photo 1. Liver of rat from group 1 showing the normal histological structure of hepatic lobule.</i>	<i>Photo 2. Liver of rat from group 1 showing the normal histological structure of hepatic lobule.</i>	<i>Photo 3. Liver of rat from group 2 showing focal hepatocellular necrosis associated with inflammatory cells infiltration.</i>
		
<i>Photo 4. Liver of rat from group 2 showing marked fibroplasia in the portal triad and appearance of newly formed bile ductules .</i>	<i>Photo 5. Liver of rat from group 2 showing portal infiltration with inflammatory cells.</i>	<i>Photo 6. Liver of rat from group 3 showing slight Kupffer cells activation and necrosis of sporadic hepatocytes.</i>
		
<i>Photo 7. Liver of rat from group 3 showing slight Kupffer cells activation and focal hepatocellular necrosis associated with inflammatory cells infiltration.</i>	<i>Photo 8. Liver of rat from group 3 showing slight fibroplasia in the portal triad and appearance of newly formed bile ductules.</i>	<i>Photo 9. Liver of rat from group 4 showing vacuolar degeneration of hepatocytes and congestion of central vein.</i>



**Figure 1. The effect of selected food industries by-products on liver histopathological disorders induced by CCl<sub>4</sub> in rats (H and E X 400)**

Data of the present study are going well with that showed in similar studies. For example, Ding et al.,<sup>(65)</sup> observed disruption of tissue architecture, large fibrous septa formation, pseudolobe separation, and collagen accumulation after inducing liver fibrosis in rats with CCl<sub>4</sub> IP for eight weeks. These changes were accompanied by an increase in ALT and AST levels, while albumin levels decreased significantly. CCl<sub>4</sub> caused histopathological changes such as regenerative nodules, deteriorated parenchyma, and lobules that were infiltrated with fat and structurally altered, according to Ahmed et al.<sup>(66)</sup>. Increased serum liver enzymes ALT, AST, and ALP, lactate dehydrogenase, nitric oxide, tumor necrosis factor alpha (TNF), and liver malondialdehyde content, collagen fiber percent, and decreased liver reduced glutathione content as endogenous antioxidant accompanied these changes. Furthermore, Elhassaneen<sup>(25)</sup> and Shehata and Rdwan<sup>(96)</sup> reported that rats treated with CCl<sub>4</sub> showed congestion of central vein and cytoplasm vacuolization of hepatocytes. Feeding on diet containing Cape Gooseberry fruits and Mulberry leaves powder (containing the same bioactive compounds with the parts used in the present study) showed Kupffer cells activation and slight hydropic degeneration of some hepatocytes. Such histopathological changes were confirmed by the biochemical results. In conclusion, selected plant parts (PPP and CLP) were effective in protecting against CCl<sub>4</sub>-induced liver disorders. These results supported our hypothesis that such plant parts contains several classes of phytochemicals that are able to prevent or inhibit CCl<sub>4</sub> hepatotoxicity through one or more of the following mechanisms: 1) Inhibition of excessive enzymatic activity expressed in liver functions, 2) improving the state of serum antioxidant defense system, and 3) reducing the degree of oxidative stress in serum i.e. formation of oxidants. Therefore, we recommended like of that plant parts powder by a concentrations up to 5% (w/w), amount to be included in our daily diets, drinks and food supplementation.

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**التأثيرات الوقائية المحتملة لأوراق القرنبيط وجلد ثمار التين الشوكي على اضطرابات الكبد التي يسببها رابع كلوريد الكربون في الفئران**  
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**الملخص العربي**

تهدف الدراسة الحالية إلى التحقق من التأثيرات الوقائية المحتملة للمنتجات الثانوية للصناعات الغذائية والتي تشمل أوراق القرنبيط وجلد ثمار التين الشوكي ضد اضطرابات الكبد التي يسببها رابع كلوريد الكربون في فئران التجارب. لذلك قسمت ثلاثون فأراً على خمس مجموعات متساوية على النحو التالي: المجموعة الاولى ، المجموعة الضابطة (العادية) ، المجموعة الثانية (المجموعة الضابطة الموجبة) أي الفئران المصابة بالسمية الكبدية بواسطة CCl4 ، المجموعة 3 ، الفئران المصابة المعاملة بمسحوق جلد ثمار التين الشوكي بنسبة 5% ، المجموعة 4 ، الفئران المصابة المعاملة بمسحوق أوراق القرنبيط 5% ، المجموعة الخامسة ، الفئران المصابة المعاملة بمخلوط 2.5% بمسحوق قشر ثمار التين الشوكي بالإضافة إلى 2.5% مسحوق أوراق القرنبيط. تسبب رابع كلوريد الكربون في انخفاض معنوي ( $P \leq 0.05$ ) في معدل الزيادة في وزن جسم الفئران بنسبة 54.40% ، المستهلك من الغذاء -36,36%) ومعدل كفاءة التغذية (-23,33%) مقارنة بالضوابط العادية. أدى تكميل النظام الغذائي باستخدام مسحوق قشر ثمار التين الشوكي ، مسحوق أوراق القرنبيط وخليطهما إلى زيادة معنوية ( $P \leq 0.05$ ) في زيادة وزن الجسم ، وتناول العلف ، ونسبة كفاءة التغذية بنسبة 33.55 ، 40.39 ، 57.56% ، 23.35 ، 30.64 ، 40.14% ؛ 6.76 ، 14.01 ، 16.42% من المجموعة الضابطة الموجبة على التوالي. أيضًا ، أدى استهلاك جميع أجزاء النبات المختبرة إلى تحسين المعايير البيولوجية المعبرة عن وظائف الكبد ومضادات الأكسدة البيولوجية (الجلوتاثيون) وكذلك تقليل تكوين مركب المالونالدهيد المعبر عن تكوين بيروكسيدات في دهون الدم أو الإجهاد التأكسدي. علاوة على ذلك ، كانت جميع نتائج التحاليل البيوكيميائية متوافقة مع نتائج الفحص النسيجي .

الكلمات المفتاحية: زيادة وزن الجسم، المدخول الغذائي، نسبة كفاءة التغذية، الجلوتاثيون، المالونالدهيد