Effect of Pumpkin (*Cucurbita pepo* L.) on Immune System and Liver Functions of Rats Induced with Liver Cirrhosis

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Abstract: Liver cirrhosis was estimated to be responsible for over 2% of all deaths worldwide therefore; this study was conducted to evaluate the effect of dried pumpkin supplementation for 8 weeks on immune system and liver functions in rats with induced liver cirrhosis. Chemical composition of Pumpkin recorded high content of vitamins, minerals and carbohydrates but low in fats. The methanolic extracts of fresh Pumpkin revealed the presence of total phenolic (6.82 mg GAE), flavonoid (9.53 mg CE) compound and has antioxidant activity (3.65 mg VE). Thirty five adult male albino rats were divided into five groups, the first group, negative control group (-ve) was fed on basal diet only, the other four groups (7 rats per each) were intraperitoneally injected with CCl_4 at a dose of 0.5ml/100g BW for the first time and followed by a dose of 0.3 ml/100g BW twice a week over a period of six weeks in order to induce liver cirrhosis. One group of them served as a positive control group and fed on basal diet only, groups (3,4 and 5) of rats were fed on basal diet supplemented with (7.5, 10 and 15%) dried Pumpkin respectively. The results indicated that, supplementation with dried Pumpkin at (7.5, 10 and 15%) to rats with induced liver cirrhosis significantly increased ($P<0.05$) the mean value of IgM and IgG compared to the positive control group. Hematological parameters were significantly increased ($P<0.05$) for the groups given Pumpkin at different levels. Moreover, rats with induced liver cirrhosis and treated with Pumpkin had significant improvement of liver functions, albumin level and lipid profile, compared to the positive control group. In addition to, the lowering effect of Pumpkin on lipid profiles in serum. The present study recommended the consumption of Pumpkin for patients with liver cirrhosis.

Key words: Pumpkin, chemical composition, immune functions, liver functions, Hematological parameters, rats.

Introduction

Liver organ is the most important organ, which plays a pivotal role in regulating various physiological processes in the body. It is the body organ in charge of many important life functions, including food digestion, glycogen storage space، control over metabolism, medicine detoxification and de las hormonas production **(Si-Tayeb *et al*., 2010)**. As liver is so essential to life, malfunction or failure of the body organ often results in high rates of morbidity and mortality. Various substances are recognized to cause liver destruction **(Gnanaprakash *et al*., 2010)**, and one of them is Carbon tetrachloride (CCl_4) has long been used as an oxidative chemical mixture for the induction of liver injury in animals through oxidation process and Kupffer cell account activation.Upon intra-peritoneal administration of CCl_4 in rats, oxidative injury is preferentially present in the liver with fatty changes (**Ahn *et al*., 2014**).

Cirrhosis is a late-stage of liver disease which occurs when scar tissue (fibrosis) replaces healthy tissue (**Byass, 2014)**. Globally, liver cirrhosis was estimated to be responsible for over one million deaths in 2010, which equates to approximately 2% of all deaths worldwide (**Mokdad *et al*., 2014)**. Hepatitis C virus (HCV) constitutes an epidemic in Egypt having the highest prevalence in the world of 14.7%. HCV is the main cause of liver cirrhosis and liver cancer in Egypt and, indeed, one of the top five leading causes of death. It kills an estimated 40,000 Egyptians a year (**Amer *et al*., 2015)**.

Considering that the liver injury advances, immune system neglects to combat prionflammatory cytokines and reactive oxygen types (ROS) which further cause oxidative liver organ injury. Simply by causing mitochondrial alterations, oxidative stress promotes hepatocyte necrosis, release of pro-fibrotic cytokines and activation of collagen gene expression in hepatic stellate cells contributing to intoxicating drinks induced sensitization of hepatocyte to pro-apoptotic action of tumor necrosis factor can progress to cirrhosis, hepatocellular carcinoma, and liver body organ inability **(Adhikari and Tirosh, 2012)**.

Phytochemicals are naturally occurring bioactive non-nutrient plant compounds in fruit, vegetables **(Gawlik-Dziki, 2012**), grains and other natural herb foods. These phytochemicals have various health benefits. A lot of studies show that natural substances from consumable and medicinal plant life showed strong antioxidant activity that could act against CCl_4-induced liver damage, because they contain lots of totally free radical scavenger such as phenolic acids, flavonoid compounds and therefore should be a signifi find it hard to part of our diet **(Wong *et al*., 2012 and Sachdeva *et al*., 2014)**.

Pumpkin is an important horticultural crop that belongs to family *Cucurbitaceae* that has experienced increased wish for recent times due to dietary and health basic safety effects **(Xanthopoulou *et al*., 2009)**. Pumpkins have become a part of healthy diet partly because of their high carotenoid contents (β-carotene) required for human health α-tocopherol, phenolics, flavonoids, vitamins (A, B1, B2,C and E), amino acids, carbohydrates, minerals **(Choi *et al*., 2012 and Valenzuela *et al*., 2014)**. Additionally, it also provides not only the important minerals including calcium, phosphorous, iron, magnesium, potassium, sodium but also the rare elements including zinc, selenium, copper, cobalt, nickel, chromium, and nicotinic acidity **(Hadad and Levy, 2012)**. Pumpkin is one of the very most popular vegetables used in the world، has been lately known a functional food **(Al-Jahani and Cheikhousman, 2017)**.

Pumpkin is also known as a rich source of fiber **(Ratnayake *et al*., 2003)**. Pumpkins have antioxidant β-carotene that really help to enhance the immune function and can decrease the chance of diseases like heart disease and cancer. Also it consists of proteins and polysaccharides that have shown anticancer activity against melanoma **(Muntean *et al*., 2013)**. Pumpkin is also popular in traditional medication for several ailments (antidiabetic, antihypertensive, antitumor, immunomodulation، antibacterial, anti-hypercholesterolemia, intestinal antiparasitia, antiinflammation, antalgic) **(Sharma *et al*., 2013; Elella and Mourad, 2015 and Muruganantham *et al*., 2016)**.

Pumpkin is one such vegetable which has been frequently used as functional food or medication **(Caili *et al*., 2006)**.The occurrence of many biologically active components including high levels of α- and β-carotene، β-cryptoxanthin, lutein and zeaxanthin, polysaccharides, phytosterols, unsaturated fatty acids, protein and peptides makes pumpkin extremely attractive for the phytochemical manufacturing industry **(Boiteux *et al*., 2007and**

Rodriguez-Amaya *et al*., 2008). Pumpkin exhibiting important physiological properties as wound healing, tumour growth inhibition, hypoglycaemic effects and immune modulating **(Chaturvedi, 2012)**. The present study was conducted to evaluate the effect of dried Pumpkin fruit at three different levels on hematology, immune system and liver functions in rats with induced liver cirrhosis.

Materials and methods

Materials

Plant: Fresh Pumpkin (***Cucurbita pepo L.***) fruits were purchased from a local market in Egypt. The peel was separated, the seeds were removed manually and raw fruits were cut with a slicer and dried in an air-circulated oven at 40˚C till complete dryness, they were powdered in a domestic mixer. The powdered samples were packed in (Metallocene polyethylene (MPE) pouches until the time of analysis.

Rats: Thirty five adult male albino rats of Sprague Dawley strain, weighing (210±5g.) were purchased from Helwan Farm for Experimental Animals, Cairo, Egypt.

Chemicals: Kits for biochemical analysis were purchased from Biodiagnostic Company for Pharmaceutical and chemicals, Dokki, Egypt. Carbon tetra-chloride (CCl_4), Casein, vitamins, minerals, cellulose and choline were obtained from Morgan Chemical Factory, Cairo, Egypt.

Methods

Chemical composition of pumpkin fruit: protein, carbohydrate, ash, fiber, fat and moisture contents of dried pumpkin were determined according to **AOAC, (2010)**. The content of potassium (K), calcium (Ca), sodium (Na), phosphorus (P), magnesium (Mg), iron (Fe) and zinc (Zn) in pumpkin pulp according to the method previously reported by **Subramanian *et al*., (2012)**. β-carotene and L-ascorbic acid were determined using HPLC method as described previously by **Hymavathi and Khader, (2005)** and **Liu *et al*., (2013)** respectively and are expressed as milligram per 100 g of fresh weight (FW).

Determination of total phenols and total flavonoid: The concentration of total phenolic and total flavonoid content were expressed as mg of gallic acid equivalent (GAE) and catechin equivalent (CE) per100 g FW of sample, respectively and determined according to the procedure of **Zilic *et al*., (2012)**.

Determination of antioxidant activity by DPPH radical-scavenging assay: The effect of pumpkin extracts on DPPH radical was estimated according to the method reported previously by **Blois, (1958)**.

Experimental Design

Thirty five adult male albino rats Sprague Dawely Strain, weighting (210 ± 5 g), were housed in well aerated cages under hygienic condition and fed on basal diet for one week for adaptation. After this week the rats were divided into five groups as follows: the first group (7 rats) was kept as negative control group (-ve) and fed on basal diet only was preparing according to **Reeves *et al*., (1993)**. The other four groups (7 rats each) were intraperitoneally injected with CCl_4 at a dose of 0.5ml/100 g. body weight for the first time and followed by a dose of 0.3 ml/100 g b.w. twice a week over a period of eight weeks in order to induce liver cirrhosis (**Zhao *et al*., 2014)**. Four rats from the injected rats were chosen randomly and killed for pathological examination to determine liver cirrhosis. One group of them served as a positive control group, the other three groups were fed on basal diet and supplemented with 7.5,10 and 15% of dried pumpkin respectively. At the end of the experiment (8 weeks) the rats were fasted for 12 hour, and then sacrificed under ether anesthesia. Two Blood samples were collected from medial canthus of the eyes of rats by means of fine capillary glass tubes. The first sample was collected into a tube containing disodium salt of Ethylene Diamine Tetra Acetic Acid (EDTA) as anticoagulant and used for assessment of erythrocytes indices. These parameters were quantified by standard hematological assay analyzer. The second blood sample was collected into a centrifuge tube without any anticoagulant and centrifuged for 15 minutes at 3000 r.p.m. to obtain serum which was stored at- 20°C until used for subsequent analysis.

Biochemical analysis

Immunoglobulin M (IgM) and immunoglobulin G (IgG) were measured according to **Ziva and Pannall, (1984)**. Total leucocytic count (TLC), red blood cell (RBC) count, haemoglobin concentrations were estimated, and packed cell volume (PCV) was determined using standard haematological technique as described by **Ochei and Kolharktar (2008)**. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined according to method of **Reitman and Frankel, (1957)** and alkaline phosphatase (ALP) according to **Roy, (1970),** while**,** albumin was estimated according to **Weissman *et al*., (1950)**. Total cholesterol and triglycerides were carried out according to the method of **Richmond**, **(1973)** and **Fossati and Praneipe, (1982)** respectively. Determination of HDL-c level was carried out according to the method of **Richmond, (1973)**. VLDL-c and LDL-c were calculated according to the equation of **Friedewald *et al*., (1972)**.

Statistical analysis:

The results were expressed as mean ± standard error (SE). The statistical analysis was carried out by using SPSS, PC statistical software (Verion 18.0 SPSS Inc., Chieago) using the Dunk 'test multiple range post-hoc test. Data were analyzed by one way analysis variance (ANOVA). The values were considered significantly different at $P < 0.05$ **(Snedecor and Cochran, 1980)**.

Results

The concerned chemical composition of Pumpkin in Table (1) indicated that, Pumpkin is rich in moisture (90.50%), total carbohydrates (74.23%), and proteins (22.6%). Crude fiber was 1.64% but low in fats (0.17%). Regarding the minerals content, it contains a high level of Ca, P, Fe, Zn, K, Mg and Na were 27.98, 31.33, 0.34, 0.26, 183.01, 18.29 and 1.97mg/100g, respectively.

Table (1): The chemical composition of dried Pumpkin

Nutrients		Values
	Moisture	90.05
Nutrients (g/100 g dry matter)	Proteins	22.6
	Fats	0.17
	Carb.	74.23
	Fiber	1.64
	Ash	1.36
Minerals (mg/100 g dry matter)	Ca	27.98
	P	31.33
	Iron	0.34
	Zn	0.26
	K	183.01
	Mg	18.29
	Na	1.97

Table (2): Antioxidant activities and related compounds β- carotene, L- ascorbic acid, total phenols and total flavonoids content in fresh pulp pumpkin.

Sample / **Parameters**	Pumpkin pulp/100g
β-carotene	0.17 (mg/100g FW)
L-ascorbic acid	14.49 (mg/100g FW)
Total phenols	6.82 (mg GAE/100g FW)
Total flavonoids	9.53 (mg CE/100g FW)
DPPH	3.65 (mg VE/100g FW)

FW: fresh weight, GAE: Gallic acid equivalent, CE: Catchin equivalent.

The antioxidant activity and related antioxidant compounds such as β-carotene, L-ascorbic acid, phenols and flavonoids were analyzed in fresh pulp pumpkin were recorded in Table (2). It contains have β-carotene, L-ascorbic acid, total phenols, total flavonoids and antioxidant activity in the following concentrations (0.17 mg, 14.49 mg, 6.82mg GAE, 9.53 mg CE and 3.65 mg VE/100g sample, respectively.

Results recorded in table (3) show the effect of pumpkin at different levels on serum immunoglobulins (IgG and IgM) of rats with induced liver cirrhosis. The injection with CCl_4 to induce liver cirrhosis in rats caused significant decrease ($P<0.05$) in the mean value of IgG and IgM compared to control the control negative group. The addition of different levels of Pumpkin at (7.5, 10, 15%) significantly increased ($P<0.05$) the mean level of IgG and IgM compared to the control positive group. Pumpkin supplementation at (7.5, 10, 15%) significantly increased ($P<0.05$) the mean level of IgG and IgM compared to the control positive group. There was a significant difference ($P<0.05$) in the level of IgM and IgG between the three groups that fed on the different levels of Pumpkin. The higher supplementation with Pumpkin, the higher immunoglobulins IgG and IgM level.

Table (3): Effect of pumpkin supplementation on immunoglobulins of rats with induced liver cirrhosis

Groups \ Parameters	IgG (mg/ml)	IgM (mg/ml)
Control (-ve)	4.85±0.25 [a]	3.05±0.05 [a]
Control (+ve)	1.52±0.07 [e]	0.82±0.01 [e]
Pumpkin (7.5%)	2.35±0.05 [d]	1.59±0.05 [d]
Pumpkin (10%)	2.89±0.01 [c]	2.22±0.12 [c]
Pumpkin (15%)	3.67±0.12 [b]	2.61±0.07 [b]

Values are expressed as means ± SE. Values at the same column with different letters are significantly different at P<0.05.

The result of Pumpkin at different levels on red blood cell parameters of rats with induced liver organ cirrhosis in Table (4). The positive control group had significant decrease (P<0.05) in the mean value of Hemoglobin, RBC, and PCV compared with the control healthy group, however the level of TLC significantly increased (P<0.05), in the positive control group. On the other hand, the supplementation with (7.5, 10, 15%) significantly increased (P<0.05) the mean level of Hemoglobin, RBC, and PCV but significantly decreased (P<0.05) the level of TLC compared to the positive control group. There were significant differences in serum level of Hemoglobin, RBC, PCV, and TLC between the low and the high levels of Pumpkin, but there were no significant differences in serum level of Hemoglobin and RBC, between the low and the medium levels of Pumpkin. Furthermore, there are no significant changes (P<0.05) in serum level of PCV between medium and the high levels of Pumpkin. It was also observed that, the higher level of Pumpkin significantly increased (P<0.05) the degree of TLC compared to the negative control group. The greatest increase in the relative red blood cell parameters concentrations was obtained by fed on supplemented diet with 15% of Pumpkin.

Table (4): Effect of Pumpkin supplementation at different levels on RBC parameters and TLC of rats with induced liver cirrhosis.

Groups \ Parameters	Hemoglobin (%)	RBCs (million/c.mm)	PCV (%)	TLC (Th.c.mm)
Control (-ve)	13.55±0.15 [a]	4.85±0.15 [a]	48.65±1.05 [a]	4.90±0.10 [d]
Control (+ve)	8.70±0.20 [d]	2.95±0.05 [c]	31.55±1.55 [d]	13.80±0.30 [a]
Pumpkin (7.5%)	10.20±0.30 [c]	3.85±0.35 [b]	37.45±0.85 [c]	10.15±0.65 [b]
Pumpkin (10%)	10.75±0.35 [c]	3.95±0.05 [b]	41.75±1.05 [b]	8.40±0.30 [c]
Pumpkin (15%)	12.15±0.35 [b]	4.65±0.06 [a]	43.10±0.90 [b]	6.15±0.15 [d]

Values are expressed as means ± SE. Values at the same column with different letters are significantly different at P<0.05

Results illustrated in Table (5) revealed the effect of Pumpkin at different levels on liver functions of rats with induced liver cirrhosis. Rats injected with CCl_4 (positive control rats) had significant increase (P<0.05) in the mean value of serum ALT, AST, and ALP compared to the healthy control group. It could be observed that, these rats had significant reduction (P<0.05) in the mean value of serum albumin compared with the negative control group. On the other hand, the supplementation with different levels of Pumpkin significantly decreased (P<0.05) the mean level of serum liver enzymes compared with the control positive group, while had a significant increase (p<0.05) in serum level of serum albumin compared to the same group. There were significant distinctions in serum level of AST, ALT, ALP and albumin between the low and the high levels of Pumpkin, but there were no significant distinctions in serum level of AST, ALP and albumin between low and the medium levels of Pumpkin. Moreover, there may be significant difference in serum ALT variables between the three groups fed the several levels of Pumpkin. The level of serum albumin parameters significantly increased (P<0.05) with the high level of Pumpkin supplementation compared with the low level. Moreover, supplementation with 15% of Pumpkin to rats with induced liver cirrhosis caused no significant difference compared to the negative control group. The best results of liver functions were recorded for group fed on supplemented diet with 15% of Pumpkin.

Table (5): Effect of Pumpkin at different levels on liver functions of rats with induced liver cirrhosis

Parameters / Groups	AST	ALT	ALP	Albumin
	(µ/L)			(g/dl)
Control (-ve)	86.20±3.80 [d]	25.75±0.65 [d]	112.40±2.40 [d]	5.10±0.10 [a]
Control (+ve)	140.00±4.00 [a]	46.10±1.50 [a]	193.00±3.00 [a]	2.55±0.55 [d]
Pumpkin (7.5%)	126.25±1.75 [b]	37.60±2.20 [b]	150.50±3.50 [b]	3.65±0.05 [c]
Pumpkin (10%)	117.65±3.65 [b]	32.60±0.80 [c]	142.10±2.10 [b]	3.95±0.15 [bc]
Pumpkin (15%)	101.95±2.95 [c]	27.55±0.55 [d]	127.15±1.85 [c]	4.85±0.05 [ab]

Values are expressed as means ± SE. Values at the same column with different letters are significantly different at P<0.05.

Results illustrated in Table (6) revealed the effect of Pumpkin at different levels on lipid profile in rats. The positive control group had significant increase (P<0.05) in serum amounts of TC, TG, VLDL-c, and LDL-c induced significant reduction in serum HDL-c, as compared to the healthy control group. On the other hand, the supplementation with different levels of Pumpkin fruit (7.5, 10, 15%) had significant decreased (P<0.05) in serum levels of TC, TG, VLDL-c, and LDL-c as compared to the positive control group, While had a significant increase (p<0.05) in serum level of serum HDL-c, compared to the same group. In addition there are a significant changes (P<0.05) in the levels of TC,TG, HDL-c, VLDL-c and LDL-c between low and the high levels of Pumpkin, however there were no significant changes in the levels of TC,TG, HDL-c, VLDL-c and LDL-c between the low and the medium levels of Pumpkin. The greatest decrease of lipid profile are recorded for group fed on supplemented diet with 15% of Pumpkin.

Table (6): Effect of Pumpkin supplementation at different levels on lipid profile in rats

Parameters / Groups	TC	TG	HDL	VLDL-C	LDL-C
	(mg/dl)				
Control (-ve)	69.65±0.35[c]	59.50±2.50[d]	49.55±0.45[a]	11.90±0.50[d]	8.20±0.30[c]
Control (+ve)	107.75±2.25[a]	94.35±0.65[a]	34.20±1.20[c]	18.87±0.13[a]	54.68±3.58[a]
Pumpkin (7.5%)	96.30±4.30[b]	82.50±4.50[b]	40.00±1.00[b]	16.50±0.90[b]	39.80±2.40[b]
Pumpkin (10%)	89.35±1.35[b]	75.70±1.30[bc]	41.45±0.55[b]	15.14±0.26[bc]	32.76±1.64[b]
Pumpkin (15%)	77.10±2.10[c]	68.40±1.60[cd]	48.85±0.15[a]	13.68±0.32[cd]	14.57±2.57[c]

Values are expressed as means ± SE. Values at the same column with different letters are significantly different at P<0.05.

Discussion

The liver, the key organ involved in numerous metabolic functions and detoxification of hazardous substances, plasma protein synthesis, and glycogen storage. Oxidative stress is considered as the imbalance between reactive oxygen species (ROS) production and antioxidant protective mechanism. It is principal cause of the development of hepatic disorders **(Galicia-Moreno and Guti´errez-Reyes, 2014)**. Complications of cirrhosis include jaundice, tumors, metabolic and degenerative lesions and liver cell necrosis etc **(Raj *et al*., 2013)**.Cirrhosis is characterized by architectural disruption, aberrant hepatocyte regeneration, nodule formation and vascular changes. Cirrhosis is also associated with an increased risk of liver failure, portal hypertension high blood pressure levels and liver cancer (**Hernandez-Gea and Friedman, 2010)**. The stage of liver fibrosis is critical for decision of treatment and prediction of outcomes, as life threatening complications highly develop in cirrhotic patients (**Mobarak, 2016)**.

Despite developments in medical sciences, treatment of diseases relating to liver and kidney is very expensive, relatively unavailable with high incidence of adverse effects and failure **(Mistry *et al*., 2013)**. The management of liver disorders is still a challenge to the modern medicine (**Bouasla *et al*., 2014)**. This kind of spurs the need for a more efficient, cost effective and easily available regime to combat liver organ and kidney pathologies especially in the developing countries. Natural products with the second metabolites may be effective and have got antioxidant possibilities that scavenge free radicals and reduce oxidative stress **(Wong *et al*., 2012)**.

There exists a growing request for diet supplements that are now made using different fruits and veggies and also herbs in order to provide natural curative as well as preventive methods for combating diseases and poor health **(Abbas *et al*., 2015)**. Fruits and fresh vegetables have been consumed by humans for centuries. They have low energy content and are an important method to obtain sugars, minerals, amino acid, fiber and vitamins **(Bressy *et al*., 2013). Oyebode *et al*., (2014)** proved that an increased consumption of fruits and vegetables is known to reduce cases of cancers, inflammatory, immunomodulatory effects and cardiovascular mortality. They may contain phytochemicals such polyphenols, coumarins, carotenoids, alkaloids, vitamins and minerals. Antioxidant play an natural part in inhibition of hydrolytic and oxidative digestive enzymes, anti-inflammatory action and various other natural or medicinal activities along with free radical scavenging of dangerous effective oxygen species **(Ignat *et al*., 2011 and Sachdeva *et al*., 2014)**.

Although our bodies can synthesis antioxidant enzymes, we also need additional intake of dietary antioxidants to enhance our immunity and protect us from the harmful effects of free radicals and oxidative stress. Pumpkin has a high nutritive value and antioxidant substances, however there is no documented evidence of any investigation of its immunomodulatory actions. In this attention, **Robinson and Decker-Walters, (1997)** mentioned that different parts of the pumpkin plant have been used in the form of various food regimens throughout its distribution area in America. The unripe fruit is eaten as a boiled vegetable, while the flesh of the ripe fruit is used to prepare sweets and soft or slightly alcoholic drinks.

The current results revealed that chemical composition of Pumpkin in (Table 1) indicated that, Pumpkin is rich in moisture, proteins, carbohydrates and fiber, but low in fats. Regarding the minerals content, it is cleared that, contains a high level of Ca, P, Fe, Zn, K, Mg and Na. Huge amounts of Ca, Mg and P made pumpkin a good source of minerals. These results were in accordance with **Mukesh *et al*., (2010)** who mentioned that Pumpkin has a good β- carotene content and has a moderate content of carbohydrates, vitamins and minerals. These results were also in accordance with **Zhao *et al*., (2015)** who mentioned that all pumpkin cultivars exhibited high levels of K, Ca, Mg and P content but low Na content. The mineral contents obtained in this study were generally higher when compared with pumpkin in research by **Mahabir and Verma (2012)**.

It was reported that about 29 g of protein is essential by adults daily **(AVRDC,1992)**. It means that pumpkin leafy vegetable can certainly satisfy the protein required by the per day. The plant protein produced by pumpkin leafy vegetable undoubtedly will contribute significantly to the mitigation of protein deficiency in the nutrition of people in developing countries. Though, some essential amino acids are not present in high concentration in plant protein (e.g. Methionine, Lysine, Thiamine), the % crude protein from pumpkin leafy vegetable is still comparable to that of some animal products. For example, animal protein per 100 g in Chicken is 20.6 g, Beef is 17.9 g, Egg is 12.1 g while fish (sardine) gives 19.6 g **(FAO, 2010)**. **Prerona *et al*., (2011)** reported that some phytochemicals found in pumpkin fruit such as flavonoids, polyphenolics, saponins, proteins and carbohydrates.

As of the most important antioxidant plant components, β-carotene, L-ascorbic acid, phenols and flavonoids in fresh pulp pumpkin were also analyzed in (Table 2). **Kubola and Siriamornpun, (2008)** who reported that the presence of various phytochemicals including flavonoids and phenolic acids in gourd vegetables. Additionally, **Wattermen, (1992) and Chonoko and Rufai, (2011)** established the presence of various phytochemicals including flavonoids, alkaloids, tannin, saponin etc. in raw form of Pumpkin (*Cucurbita pepo*). Flavonoids are considered as most popular natural antioxidants and effective secondary metabolic products **(Gyamfi and Aniya, 2002)**. These results were in accordance with **Mala and Kurian, (2016)** who reported that the pulp was found to be a rich source of β-carotene, polyphenols and antioxidants. Also, **Kim *et al*., (2012)** reported that pumpkin contained a significant amount of antioxidants, tocopherols, and carotenoids. Fat-soluble antioxidants (tocopherols and carotenoids),vitamin C is a strong water-soluble antioxidant that protects cells and cellular components from free radicals by donating electrons, and regenerating other antioxidants, such as vitamin E (tocopherols) **(Keith *et al*., 2006)**.Therefore, high pumpkin intake has various benefits to improve overall health.

Injection with CCl_4 caused a significant reduction in immunoglobulins and red blood cell parameters, these results agreed with the finding of **Bystry *et al*., (2011)** who reported that CCl_4 is a chemoattractant for natural killer cells, monocytes and a variety of other immune cells. The addition of Pumpkin at different levels improved the concentrations of IgM and IgG in the rats with induced liver cirrhosis. This enhancement of immune functions may be related to the chemical composition of Pumpkin that contains a high levels of antioxidant compounds such as phenolics, flavonoids, α-tocopherol, vitamins (ascorbic acid, Carotene), amino acids, minerals. These results agreed with **Prerona *et al.*, (2011) and Muntean *et al.*, (2013)** who indicated that Pumpkins have antioxidant β-carotene, that really help to increase the immune function and can reduce likelihood of diseases like heart disease and cancers. Also these results were in accordance with **Koryachkina *et al.*, (2016)** who indicated that addition of pumpkin powder contributed to the activation of blood immune system. **Gropper *et al.*, (2005)** reported that tocopherols and carotenoids have recently been suggested to be fat-soluble antioxidants. Antioxidants play an important role in reducing DNA damage, reducing lipid peroxidation, maintaining immune system function, and inhibiting cancerous modification in vitro, that are thought to prevent some diseases. **Jafaryan *et al.*, (2012)** observed all various extracts of winter squash fruit significantly improved edema of rat's legs and probably are regulator for immune system.

Polyphenols are classified according to the chemical structures into phenolic acids and bio-flavonoids (**Tsao, 2010)**. Polyphenols of several sources have shown a modulator effect on epigenetic mechanisms as genetics methylation, histone modifications and posttranscriptional regulation by microRNAs, and these mechanisms in change, can modulate immune system influencing both activation and differentiation of multiple cellular types included in the immune system response (**Cuevas *et al.*, 2013)**.

Marbun *et al.*, (2018) reported that pumpkin pulp and seeds revealed the occurrence of many phytochemical constituents such as flavonoids, tannis, steroids, terpenoids, and saponins. Therefore, pumpkin consumption has various benefits to boost immune system. **Aghaei *et al.*, (2014)** described that Phenolic compounds such as polyphenols flavonoids as well as vitamins and zinc in medicinal plant life such pumpkin are credited factors for antioxidant activity.

Vitamin C is an essential water-soluble nutrient that generally exerts its effect on host immunity process and induces the immune system (**Pavlovic and Sarac, 2010)**. Consumption of diet containing vitamins E and C could help to ameliorate the oxidation effect caused by gasoline (as a free radical) increasing the haemoglobin concentration and decreasing white cell count **(Ibitoroko *et al.*, 2011)**. Ascorbic acid enhances antioxidant defenses of T-cells (**Pavlovic *et al.*, 2009)** and also increase T-cell responsiveness to antigens, suggesting a role in regulating immune system (**Wu *et al.*, 2000**). Supplement with vitamin C was shown to improve individual immune response, such as antimicrobicidal, natural monster cell activities, lymphocyte expansion and chemotaxis (**Pavlovic *et al.*, 2005**), suggesting the most beneficial role of this supplement in regulating the immune system response.

Zinc deficiency can cause a spectrum of clinical indications, such as poor of appetite, loss of body hair, altered taste and smell, testicular atrophy, objective and immune dysfunction, and diminished drug elimination capacity. These are common symptoms in patients with serious liver diseases, especially liver organ cirrhosis. The liver is the key organ in charge of the zinc metabolism that can be damaged by liver diseases. However, zinc deficiency may change hepatocyte functions and also immune responses in other liver diseases (**Grüngreiff *et al*., 2016)**.

Pumpkin in the current study caused a significant increase (P<0.05) in haematological indices such as PCV, RBC and Haemoglobin concentration of the treatment plan rats. This can be attributed to the free radical scavenging activities of flavonoids and other plant phenolics or to the metal-binding activity and immune system stimulating properties of polyphenolics (**Middleton and Kandaswami, 1992)**.

The rise during these variables suggests a greater production of vast majority of the cells included in the immunity process which are produced in the stem skin cells of the bone marrow. This can be thought to have a stimulatory impact on the immune reactions since increased production of the immune system cells may imply a greater immune system function (**Sainis *et al*., 1997**). Like a dietary component, flavonoids are thought to have health-promoting properties because of the high antioxidant capacity in vivo and in vitro systems (**Cook and Samman, 1996)**.

Many studies proved that Pumpkin as a rich resource of iron, phytostrol, omega3, omega6. Some scientists have proposed the use of pumpkin in treatment of anemia, following studies which reported that extracts of pumpkin helps to maintain blood level in subjects given its extracts **(Fiona and Latunde-Dada, 2011)**. These results are accordance with those reported by **Xia and Wang, (2006)** reported that oral functions of pumpkin extract (300 and 600 mg /kg body wt. per day) for 30 days lead in a substantial decrease in blood vessels glucose, glycosylated haemoglobin, and an increase in the plasma insulin and total haemoglobin.

The obtained results revealed that, supplementation with different levels of Pumpkin decreased the elevated level of liver functions. Pumpkin extract significantly increased superoxide dismutase and glutathione peroxidase activities in mouse liver **(Chang *et al*., 2004)**. **Asgary *et al*., (2010)** mentioned that treatments with pumpkin powder in diabetic rats caused a significant decrease in levels of enzymes compared with the diabetic groups and consumption of pumpkin had significant effects on reducing the score of liver inflammation in diabetic groups .These results were matching with the present study. Also these results agreed with **Koryachkina *et al*., (2016)** who reported that addition of Pumpkin powder contributed to the activation of protein metabolism increase (raise of total protein and urea, reduction in bilirubin level, AST activity).

The protective actions of hepatoprotective medicinal plants are mediated by their flavonoids or alkaloids components or by their combination via antioxidant and free radicals scavenging activities **(Adeneye *et al*., 2008)**. Phenolic compounds can act as antioxidants by many potential pathways such as free radical-scavenging, oxygen radical absorbance, and chelat-ing of metal ions **(Halliwell *et al*., 1995 and Reddy *et al*., 2005)**.

Moreover, Vitamin C was reported to normalized levels of serum ALT, AST, gamma glutamine, ALP, lactate dehydrogenase and MDA and serum bilirubin in intoxicated animals. It potentiates the activities of free radical scavengers, superoxide dimutase, and catalase glutathione peroxidase thereby protecting against microsomal lipid peroxidation, liver fibrosis, liver organ necrosis and hepatic infection. Hepatoprotective property of supplement C is related to it antioxidant property (**Adikwu and Deo, 2013)**. Treatment with supplement Vitamin c previous exposure to thioacetamide-activated liver cirrhosis is sufficient to avoid hepatic cirrhosis in rats **(Al-Attar, 2011)**.

The obtained results agreed with the finding of **(Hussein *et al*., 1997)** who mentioned that zinc may have some beneficial effect in the treatment of liver cirrhosis by significant decrease in plasma levels of AST, ALT, gamma-glutamyl aminotransferase, and total bilirubin. In addition most of the hepatocytes appeared normal in zinc-treated as compared with untreated cirrhotic animals. Flavonoids of vegetables have been reported to prevent liver damage which occurs in rats fed cholesterol containing diets **(Nakayama *et al*., 2007; Aoyama *et al*., 2008 and Roldan-Marin *et al*., 2009)**.

The obtained results exposed that, supplementation with different levels of Pumpkin decreased the increased of lipid profile in rats with induced liver cirrhosis, while had significant embrace serum level of HDL-c. This effect is probably due to its phytochemical compounds. These results were in accordance with **Zhao *et al*., (2014a)** who mentioned that administration of Pumpkin could significantly decrease the levels of plasma TG, TC, and LDL-c and HDL-c. Also **Song and Sun, (2017)** reported that Pumpkin natural powder diet reduced body weight gain, the levels of the plasma insulin, lipid profile and liver organ glycogen. **Takada *et al*., (1994) and Romero *et al*., (2002)** described that fibers reduce the plasma LDL level by suppressing the absorbtion of cholesterol and bile acids and increasing the experience of LDL receptors, therefore it could be concluded that pumpkin reduces the effect of triglycerides through its fibers. Dietary fiber can also reduce triglyceride levels by suppressin lipogenesis in the liver.

Silveira and Alvarez Leite, (1996) mentioned that pumpkin pectin and hydrolyzing pumpkin have remarkable effect on lowering cholesterin in blood plasma and improving metabolization of cholesterin of rats. **Kumar and Sudheesh, (1997**) mentioned that diet containing 5% pectin from pumpkin for 45 days significantly decreased serum cholesterol levels. Triacylglycerols were notably reduced in liver, and fatty acids were obviously decreased in blood. Pectin increases the activity of lipoprotein lipase in fat cells and cardiovascular, resulting in higher consumption of triglyceride rich lipoproteins (VLDL and chylomicron) in tissues other than liver organ in promoting their breakdown and therefore lowering triglyceride levels **(Sedigheh *et al*., 2011)**. These results were matching with the present study.

Chung, (2004) reported that saponins and flavonoids cause reduction of triglycerides and total cholesterol by formation of large micelles excreted in bile. These types of bioactive compounds are decrease consumption of cholesterol in the intestines and serum levels of LDL-c. These results were matching with the current study. It was reported by **Orsavova *et al*., (2015)** that MUFA may reduce LDL-c, while it may increase HDL-c. Phytosterols can lower both total serum cholesterol and LDL-cholesterol in humans by inhibiting the absorption of dietary cholesterol **(Piironen *et al*., 2000)**. **Uemura *et al*., (2011)** also reported that presence of phytosterols reduces the serum levels of TG by inhibiting accumulation of TG and expression of the lipogenic genes. These results were matching with the present study. Moreover, flavonoids are reported to inhibit triglycerides accumulation by inhibiting pancreatic lipase activity **(Kang *et al*., 2013 and Patra *et al*., 2015)**.

In conclusion, Pumpkin supplementation generally showed immune stimulatory effect in rats with induced liver cirrhosis due to the presence of phytochemicals like flavonoids and total phenols. Furthermore, clinical trials on humans can be conducted to test in patients with hepatic cirrhosis.

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تأثير القرع العسلى (اليقطين) علي الجهاز المناعي ووظائف الكبد في الفئران المصابة بتليف الكبد

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قسم الإقتصاد المنزلى – كلية التربية النوعية - جامعة بورسعيد- بور سعيد- مصر

الملخص

لقد أوضحت الإحصائيات أن تليف الكبد قد أصبح مسئولا عن أكثر من 2٪ من مجموع الوفيات في جميع أنحاء العالم. لذلك أجريت الدراسة الحالية لتقييم تأثير اعطاء القرع العسلى لمدة ثمانية أسابيع على وظائف الدم ، الجهاز المناعي ووظائف الكبد في الفئران المصابة بتليف الكبد. تشير نتائج التحليل الكيميائي للقرع العسلى ارتفاع محتواها من الفيتامينات، الاملاح المعدنية والكربوهيدرات وانخفاض الدهون بها. ويحتوي المستخلص الميثانولي من القرع العسلى علي مركبات الفينول (6.82ملجم GAE)، الفلافونيد (9.53ملجم CE) وقدرتها المضادة للاكسدة (3.65ملجم VE). تم تقسيم عدد 35 من ذكور الفئران البالغة من سلالة الالبينو الي خمس مجموعات، المجموعة الاولي وهي المجموعة الضابطة السالبة وتتغذي علي الغذاء الاساسي فقط . بينما الثلاث مجموعات الاخري (7 فئران في كل مجموعة) تم حقنهم بمادة رابع كلوريد الكربون بجرعة 0.5مللي/كجم من وزن الجسم في المرة الاولي ثم بجرعة 0.3مللي/كجم من وزن الجسم مرتين بالاسبوع ولمدة ستة اسابيع لاحداث تليف بالكبد للفئران. وتم تغذية احد هذه المجموعات علي الغذاء الاساسي فقط وهي تمثل المجموعة الضابطة الموجبة ، تم خلط القرع العسلى واعطائها الي الثلاث مجموعات الاخرى (7.5، 10، 15) علي التوالى. تشير النتائج الي أن اعطاء القرع العسلى عند مستوي (7.5، 10، 15) الي الفئران المصابة بتليف الكبد أدي الي حدوث ارتفاع معنوى P<0.05 في قيم المناعة IgG, IgM علي التوالي مقارنة بالمجموعة الضابطة الموجبة. مقاييس الدم قد ارتفعت ارتفاع معنوى P<0.05 ايضا. علاوة علي ذلك ، فان الفئران المصابة بتليف الكبد قد انخفض بدرجة ذات دلالة احصائية P<0.05 انزيمات الكبد المرتفعة عندها وارتفاع معنوى في قيم الالبيومين مقارنة بالمجموعة الضابطة الموجبة. بالإضافة إلى ذلك ، فإن تأثير خفض القرع العسلى على صورة دهون الدم . وتوصى الدراسة الحالية باستخدام القرع العسلى لمرضى التليف الكبدى .

الكلمات المفتاحية: القرع العسلى، التركيب الكيميائى، الوظائف المناعية، وظائف الكبد، المقاييس الهيماتولوجية، الفئران.