Journal of Home Economics

Volume 25, Number (3), 2015

http://homeEcon.menofia.edu.eg

Journal of Home Economics

ISSN 1110-2578

Possible Employing Of Clover Berseem ,(*Trifolium Alexandrinum*) Plant Parts As Remedies For Hepatointoxication Of Male Albino Rats.

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Abstract

This study was conducted to investigate the effect of plant parts on impaired liver function by injecting rats with carbon tetrachloride (CCl_4). Thirty five adult male albino rats weighing 150 ±10g were divided into two main groups and five sub groups, each with five rats. One of main groups (not injected) was considered as negative control group which fed on standard diet and tap water, and the other main group was fed on standard diet and injected s/c by carbon tetrachloride (CCl_4) in olive oil 50% v/v (3ml/kg. B.wt.) twice a week for two weeks to induce hepatointoxication. This main group was divided into 6 groups to be fed on the experimental diets for (4) weeks according to the following :Group (2) :Positive control group (untreated group) , Group(3):Feeding with 5g/100g Flowers, Group (4): Feeding with 5g/100g Leaves, Group (5) :Feeding with 5g/100g Stems, Group (6) : Feeding with 5g/100g Roots, Group (7): Feeding with 5g/100g Seeds of berseem plant. At the end of experiment (4 weeks), the blood samples were collected after 12 hours fasting and serum was separated for determination of :Lipid profile:Total cholesterol (T.C),tri -glycerides (T.G), high density lipoprotein (HDL-C),low density lipo protein (LDL-C), very low density lipo protein (VLDL-C) and Atherogenic Index (AI) ,Serum glucose,Serum liver functions: Aspartate amino transaminase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), (AST/ALT) ratio,total protein, albumin, globulin, albumin/globulin ratio,total bilirubin, direct bilirubin, and indirect bilirubin, serum antioxidant enzymes: Glutathione peroxidase (GPX), Superoxide dismutase (SOD) and Catalase (CAT) and kidney functions (urea , creatinine ,uric acid).At the same time, the organs :Liver, Heart, Spleen ,

kidney and lungs were removed ,washed in saline solution, wiped by filter paper and weighted and histopathological changes of liver and kidney were examined.The obtained results revealed that treatment by 5g/100g berseem clover seeds led to significant increase in body weight gain, feed intake, feed efficiency ratio and significant decrease in urea and creatinine .Treatment by 5g/100g berseem clover flowers led to significant decrease in serum glucose. Treatment by 5g/100g berseem clover leaves led also to significant decrease in T.C, T. G, LDL- C, VLDL-C ,AI, AST, ALT, ALP, (AST/ALT) ratio, globulin, total bilirubin, direct bilirubin and indirect bilirubin, and significant increase of HDL-C, total protein, albumin, albumin/globulin ratio, Glutathione peroxidase (GPX), Superoxide dismutase (SOD) and Catalase (CAT).
Key words; Hepatointoxication, CCl_4, plant parts, Lipid profile, Liver and Kidney Functions.

Introduction

The liver is the body's largest gland . It is a vital organ that supports nearly every other organ in the body in some facet. It is located in the upper right portion of the abdomen cavity under the diaphragm and to the right of the stomach **(Abdel-Misih and Bloomston , 2010).**The liver can be considered a factory because it produces bile that is required in the digestion of food, in particular fats and stores it in a small pouch called the gallbladder which sits just under the liver, stores the extra glucose or sugar as glycogen, and then converting it back into glucose when the body needs it for energy , produces the blood clotting factors , produces amino acids (the building blocks for making proteins) including those used to help fight infection , process and storage of iron necessary for red blood cell production and manufactures of cholesterol and other chemicals required for fat transport. It is also a powerful detoxification center that handles many chemicals, alcohol, poisons and toxins as well as drugs and clears the blood (**Canadian Cancer Society, 2015**).

There are many types of liver disease that can be caused by a virus, damage from drugs or chemicals, obesity, diabetes or an attack from your own immune system. The major liver diseases that are responsible for the most morbidity and mortality are viral hepatitis (chronic hepatitis B and C), alcoholic liver disease, non-alcoholic fatty liver disease, cirrhosis and hepatocellular cancer **(Canadian Liver Foundation, 2013**) .

In past, several studies have been carried out to examine the effect of plants used traditionally by herbalists to support normal liver function and treat diseases of liver. So, various experimental evidences have confirmed the efficacy of plants such as *Silybum marrium* (milk

thistle),*Curcuma longa* (turmeric) **(Luper, 1999)**, *Nymphea stellata* **(Bhandarkar and Khan 2004)**.

In spite of significant advances in medicinal plant research and rapid strides in modern medicine, reachers still are in need for more precise, safe and effective treatment of liver disorders **(Oliveria *et al.*, 2005).**

Liver damage induced by Carbon Tetra Chloride (CCl_4) involves biotransformation of free radical derivatives, increased lipid peroxidation and excessive cell death in liver tissue (**Clawson, 1989 and Recknagel *et al*., 1989**). .

The principle causes of Carbon Tetra Chloride (CCl_4) is induced hepatic damage in lipid peroxidation and decreased activities of antioxidant enzymes and generation of free radicals **(Castro *et al*., 1974) and (Poli., 1993).**

Hepatotoxicity is one of very common aliment resulting into serious debilities ranging from severe metabolic disorders to even mortality(**Patel *et al.*, 2008)** .

Plants are natural resources of producing large number of bioactive chemical constituents in a most proficient way and with specific selectivity. Since the middle of the 19 century, different class of bioactive compounds have been isolated and characterized **(Rauf *et al*., 2012).** Many plant extracts and plant products have been shown to have significant antioxidant activity **(AL-Howiriny *et al* ., 2005 ; Hussein , 2009)** which may be an important property of medicinal plants associated with the treatment of several ill-fated diseases including liver toxicity. Thus, herbal plants are considered a useful means to prevent or ameliorate certain disorders, such as diabetes, atherosclerosis, hepatotoxicity, and other complications **(AL-Howiriny *et al* ., 2005 ; Hussein, 2008).**

Clovers are used mainly as a fodder and pasture crops but they also gain interest due to the content of secondary metabolites, in particular saponins and flavonoids. They are popular food additives or diet supplements and also find appliction in pharmaceutical or cosmetic industries **(Augustin *et al* ., 2011)**. The *Trifolium* (Leguminosae or Fabaceae) taxa is one of the most important genera of the Leguminosae family, both in terms of its agricultural value and the number of species (about 300) **(Zohary and Heler, 1984).** Only ten are agriculturally important as cultivated and pasture crops. *T.resupinatum* (Persian clover) and *T. Alexandrinum* (Egyptian clover or berseem) are commonly cultivated as winter annuals in tropical and subtropical areas such as Egypt, India, Pakistan, Turkey and the Mediterranean countries.
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The genus *Trifolium* is distributed in temperate and subtropical regions of both hemispheres **(Bisby *et al* ., 1994).** *T. pretense* (the best known of *Trifolium* species) and other species from the *Trifolium* genus are characterized by a wide range of therapeutic uses in folk medicine of different world regions. Some clovers are also listed as traditional wild feed **(Luczaj, 2012;Sansanelli and Tassoni , 2014).** It has been used by the Oriental and the European cultures, and more recently also by the Americans, as a medicinal herb for the treatment of eczema and psoriasis **(Klejdus *et al.*,2001,Figueiredo *et al.*, 2007).**

Native Americans traditionally valued red clover for the treatment of external skin problems and lung, nervous and reproductive system ailments. The isoflavone constituents in red clover have estrogenic properties.They are thought to have positive effects on menopausal disorders such as osteoporosis, cardiac risk factors or breast cancer **(Fugh-Berman and Kronenberg, 2001;Beck *et al.*, 2005)**. It is also used as expectorant, analgesic, antiseptic ,tonic **(Sabudak *et al.*, 2009),** sore throat, fever, pneumonia, and meningitis; skin problems and lung illnesses disorders of reproductive system **(Kołodziejczyk-Czepas 2012).**

Trifolium alexandrinu ,(L) (family: Fabaceae) (common name: Egyptian clover, berseem clover) is an annual plant cultivated in Egypt (**Muschler, 1970; Tackholm, 1974**). It is used in the treatment of various human dysfunctions. It is reported as to be an antibacterial and antidiabetic agent **(Khan *et al.*, 2012).** The phytochemistry of this plant has indicated the presence of terpenoind glycosides, amino acids and their derivatives , proteins , flavonoids and their glycosides , isoflavonoids , and fatty acids in different parts of the same plant **(Sharaf ,2008; Temine and Guler ,2009).**

It is a high-quality forage characterized by a high concentration of nutrients, primarily protein
(15-25% DM), minerals (11-19%) and carotene **(Sharma and Murdia 1974)**. Even as hay, the crude protein content of berseem can be higher than 20% DM **(Sharma and Murdia ,1974**).

Materials And Methods

1- Materials:

1.1- Plants:

The *Trifolium alexandrinum* medicinal plant was collected at flowering stage from local region during May 2015.Plant parts were separated and impurities were removed then cleaned. Collected plant samples were used after sun drying and ground mechanically into the fine powder.

1.2-Carbon Tetra Chloride (CCl_4):

Carbon Tetra Chloride (CCl_4) was obtained from EL-Gomhoryia Company for chemical Industries, Cairo, Egypt, as a toxic chemical for liver poisoning according to **Passmore and Eastwood (1986)** . In the same time, it is mixed with olive oil by equal volumes and used for induction.

1.3-Animals:

Thirty five (35) male albino rats (Spargue-Dawley strain) weighting (150±10g) were used in this study & obtained from Research Institute of Ophthalmology, Medical Analysis Department. Rats were housed in individual wire cages at room temperature of 25 C^o in the animal house of Home Economics, Menoufia University under the normal laboratory condition and fed on basal diet for 4 consecutive days 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. Feed and water checked daily. Rats were weighted at the begining of the experimental then weekly and at the end of the experimental.

1.4-Chemical Composition of Basal Diets:

Table (1):The composition of standard basal diet (Campbell, 1963).

Constituents	Percentage
Protein	12%
Corn oil	10%
Salt mixture	4%
Vitamins mixture	1%
Cellulose	5%
Choline Chloride	0.25%
Corn starch	up to 100%

The basal diet also contains salt mixture according to **(Hegested *et al.*, 1941)** and vitamins mixture according to**(Campbell, 1963).**

2- Methods:

2.1-Induction of Liver Intoxication in Rats:

Thirty (30) male albino rats, (Spargue - Dawley strain) weighting (150±10g) were injected by carbon tetrachloride in olive oil 50% v/v (3 ml/kg .B.wt) twice a week for 14 days to induce chronic damage of the liver according to the method described by **Jayasekhar *et al* ., (1997).**

2.2- Experimental Designs and Animal Groups:

Thirty five (35) male albino rats (Spargue - Dawley strain) were distributed into7groups each of 5 rats in which means of rats weight for

all groups were nearly equal. Each of the groups was kept in a single cage. All the groups of rats were fed on the experimental diet for 4 weeks according to the following groups:

Main group (1):Negative Control Group - Normal Group (5 Rats), which fed on basal diet.

Main group (2): Hepatointoxicated Group (30 Rats), which rats were injected by (CCl_4).

Group (2): Positive control group (un treated group, basal diet), **Group (3):**Feeding with 5g/100g Flowers, **Group (4)**:Feeding with 5g/100g Leaves,**Group (5)**:Feeding with 5g/100g Stems, **Group (6)** :Feeding with 5g/100g Roots, **Group (7)**: Feeding with 5g/100g Seeds of berseem plant.

2.3-Biological evaluation:

During the experimental period **(28days),** the diet consumed was recorded every day and body weight was recorded every week. The body weight gain **(BWG g),** feed efficiency ratio **(FER),** and organ/body weight % were determined according to **Chapman *et al*., (1959)** using the following equations:

Body Weight Gain = Final weight (g) – Initial weight (g).
Feed Efficiency Ratio (FER) = Gain in body weight (g) / Feed intake (g) .

2.4-Blood Samples and Organs Collection:

From all the previously mentioned groups, blood samples were collected after 12 hours fasting at the end of the experimental using the abdominal aorta in which the rats were scarified under ether anesthetized. Blood samples were received into dry clean centrifuge tubes and left to clot at room temprature for half an hours then centrifuged for 10 minutes at 3000 r.p.m toseparate the serum. Serum was carefully aspirated and transferred into clean quit fit plastic tubes and kept frozen at (-20 C^o) until the time of analysis **(Malhotra ,2003).** All serum samples were analyzed for determination the following parameters:Glucose according to the method of **Young (2001),** triglycerides according to **Fossati and Prencipe (1982)**, total cholesterol according to the method of **Allain (1974)**, HDL according to the method of **Lopez (1977),** VLDL and LDL according to the method of **Lee and Nieman (1996)**, (AST and ALT) according to the method of **Henry (1974) and Yound(1975)**, (ALP) according to the method of **Belfield and Goldberg (1971),** (Total Protein) according to the method of **Henry , (1974),** (albumin) according to the method of **Doumas *et al.*, (1971**),(SOD) according to the method of **Sun *et al.*,(1988)**, (GST)

according to the method of **John and Kathryn (1998),** (CAT) according to the method of
Diego, (2011). At the same time, the organs liver, kidney, lungs, heart and spleen were removed, washed in saline solution, wiped by filter paper & weighted according to the method described by **Drury and Wallington(1980),** and small specimens from liver and kidney were immersed in 10% neutral buffered formalin, dehydrated in ascending concentration of ethanol(70, 80 and 90%) , cleared in xylene and embedded in paraffin . Sections of (4 - 6) microns thickness were prepared and stained with hematoxylin and eosin according to **Bancroft *et al.*, (1996)**.

Results And Disscusion:

A- Bwg , Fi ,Fer :

Table (2) results illustrated the effect of feeding hepatointoxicated rats with plant parts on body weight gain (BWG),feed intake (FI) & feed efficiency ratio (FER). As shown in the table, the best (BWG, FI and FER) was recorded for group7 (hepatointoxicated rats fed on 5g/100g Seeds of berseem). These results agree with **Bakr (2009) ; Faramawy , Asmaa (2010) ; Abd EL- Halem, Eman (2012) ; Shehata , Rehab (2012) ; EL banna (2014) and Riad (2014)** they found that injecting rats by CCl_4 caused a decrease in BWG , FI , FER while treatment with tested plants reversed such a change.

B- Organs weight:

Table (3) results illustrated the effect of feeding hepatointoxicated rats with plant parts on organs weight. As shown in the table, the best **liver weight** was recorded for group3 (hepatointoxicated rats fed on 5g/100g Flowers of berseem), the best **heart weight** was recorded for group 4 (hepatointoxicated rats fed on 5g/100g Leaves of berseem), the best **kidney,spleen and lungs weight** recorded for group5 (hepatointoxicated rats fed on 5g/100g Stems of berseem).These results agree with **Bakr (2009) ; Faramawy , Asmaa (2010) ; Abd EL-Halem, Eman (2012) ; Shehata , Rehab (2012) ; EL banna (2014) and Riad (2014)** , they found that injecting rats by CCl_4 caused an increase in all organs weight , while treatment with tested plants reversed such a change.

C-Serum glucose:

Table (4) results show the effect of feeding hepatointoxicated rats with plant parts on serum glucose. As shown in the table, the best glucose level was recorded for group3 (hepatointoxicated rats fed on

5g/100g Flowers of berseem). This result is in agreement with **Amer *et al.*, (2004)** who found that daily intake of (water, hexane and ethanolic) extracts of *T. alexandrinum* in drinking water for 4 weeks immediately after diabetes induction caused significant decreases in glucose and glycated hemoglobin levels and increase in insulin level, **Al Rawi (2007**) who reported that the flower head of clover (CF) (*Trifolium alexandrinum*) was promising antidiabetic agent and **Aly *et al* ., (2015)** who concluded that ERS (Egyptian radish sprouts) and ECS (Egyptian clover sprouts) had hypoglycemic activities in diabetic rats, and had the potential to alleviate hyperglycemia in cases where diabetes is present and to serve in the primary prevention of diabetes mellitus.

D- Lipid profile: .

Table (5) results show the effect of feeding hepatointoxicated rats with plant parts on serum lipid profile. As shown in the table, the best (T.C, T.G, HDL, LDL, VLDL and AI) was recorded for group 4 (hepatointoxicated rats fed on 5g/100g Leaves of berseem). These results agree with **Ammar *et al* ., (2016),** they found that Luteolin-7-O-glycoside (LG) which considered one of the major constituents of the aqueous methanol extract of *Trifolium alexandrinum* induced a significant improvement in plasma lipid profile.

E- Liver enzymes activities:

Table (6) results show the effect of feeding hepatointoxicated rats with plant parts on liver enzymes activities. As shown in the table, the best (AST, ALT, ALP, AST/ALT,Albumin , Albumin/Globulin ratio, total bilirubin, direct bilirubin and indirect bilirubin) recorded for group 4 (hepatointoxicated rats fed on 5g/100g Leaves of berseem), the best serum total protein was recorded for groups 4 (hepatointoxicated rats fed on 5g/100g Leaves of berseem), 5 (hepatointoxicated rats fed on 5g/100g Stems of berseem) and 7(hepatointoxicated rats fed on 5g/100g Seeds of berseem), the best serum globulin was recorded for groups 3(hepatointoxicated rats fed on 5g/100g Flowers of berseem) and 4 (hepatointoxicated rats fed on 5g/100g Leaves of berseem).These results agree with that of **El-Gendy (2012)** who reported that the *Trifolium alexandrinum* extracts showed a remarkable hepatoprotective against Paracetamol effect and **Sakeran *et al.*, (2014)**, they reported that TAR extract has beneficial properties and can reduce the liver damage and toxicity induced by APAP.

F-Serum antioxidant enzymes:

Table (7) results illustrate the effect of feeding hepatointoxicated rats with plant parts on Serum antioxidant enzymes . As shown in the table, the best (GPX, SOD and CAT) was recorded for group 4 (hepatointoxicated rats fed on 5g/100g Leaves of berseem).

G-Serum kidney function:

Table (8) results illustrated the effect of feeding hepatointoxicated rats with plant parts on serum kidney function. As shown in the table, the best serum Creatinine was recorded for groups 5 (hepatointoxicated rats fed on 5g/100g Stems of berseem) & 7(hepatointoxicated rats fed on 5g/100g Seeds of berseem), the best serum Urea was recorded for group 7 (hepatointoxicated rats fed on 5g/100g Seeds of berseem) and the best serum Uric acid was recorded for group 3 (hepatointoxicated rats fed on 5g/100g Flowers of berseem). These results agree with **Bakr (2009) ; Faramawy , Asmaa (2010) ; Abd EL- Halem, Eman (2012) ; Shehata , Rehab (2012) ; EL banna (2014) and Riad (2014),** they found that hepatointoxicationion raised Creatinine, Urea Uric acid in serum, while treatment with tested plants reversed such a change.

Table (2) : Effect of Berseem (clover) plant parts on BWG , FI , FER

Parameters / Groups	BWG		FI (g/rat		FER	
	Mean ±SD	Change of (+ve) group %	Mean ±SD	Change of (+ve) group %	Mean ±SD	Change of (+ve) group %
(G1) Control (-)	0.52^{a}±	+ 27.14	20.15^{a}	+ 63.0	0.026	+ 136.36
(G2) Control (+)	0.14^{e} ± 0.01	-	12.36^{g} ±0.005	-	0.011^{c} ±0.002	-
(G3) 5% Berseem (clover) flowers	0.36^{d} ± 0.03	+ 15.71	17.83^{e} ±0.005	+44.26	0.020^{b} ±0.005	+ 81.82
(G4) 5% Berseem (clover) leaves	0.38^{cd} ±0.05	+ 17.14	17.47^{f} ±0.001	+ 41.34	0.022^{ab} ±0.005	+ 100.00
(G5) 5% Berseem (clover) stems	0.48^{ab} ±0.02	+ 24.29	19.50^{c} ±0.104	+ 57.77	0.025^{ab} ±0.001	+ 127.27
(G6) 5% Berseem (clover) roots	0.43^{bc} ±0.02	+ 20.17	18.25^{d} ±0.005	+ 47.65	0.024^{ab} ±0.001	+ 118.18
(G7) 5% Berseem (clover) seeds	0.50^{a} ± 0.05	+ 25.71	19.65^{b} ±0.001	+ 58.98	0.026^{a} ±0.003	+ 136.36
LSD: p ≤ 0.05	0.055		0.069		0.005	

Means in the same column with different litters are significantly different (p≤0.05).

Table (3) : Effect of Berseem (clover) plant parts on Liver , Heart , Kidney, Spleen and Lungs weight :

Groups / Parameters		(G1) Control(-)	(G2) Control (+)	(G3) 5%Berseem (clover) flowers	(G4) 5%Berseem (clover) leaves	(G5) 5% Berseem (clover) stems	(G6) 5% Berseem (clover) roots	(G7)5% Berseem (clover) seeds	LSD: p ≤ 0.05
Liver	**g**	5.53^{d}± 0.40	7.67^{a}± 0.60	5.58^{d}± 0.40	7.12^{b}± 0.70	6.58^{c}± 0.50	6.54^{c}± 0.30	6.58^{c}± 0.20	0.005
	Change of (+ve) group	-27.90	-	-27.25	-7.17	-14.21	-15.91	-14.21	
Heart	**g**	0.68^{f}± 0.036	1.98^{a}± 0.004	1.56^{b}± 0.006	0.80^{e}± 0.012	0.88^{d}± 0.0031	0.90^{d}± 0.022	1.25^{c}± 0.031	0.039
	Change of (+ve) group	-65.66	-	-21.21	-59.60	-55.56	-54.55	-36.87	
kidney	**g**	1.34^{g}± 0.003	2.36^{a}± 0.001	1.60^{c}± 0.01	1.45^{d}± 0.01	1.42^{f} ± 0.001	1.43^{e}± 0.007	1.65^{b}± 0.001	0.008
	Change of (+ve)	- 43.22	-	-32.20	-38.56	-39.83	-39.41	-30.08	
Spleen	**g**	0.78^{f}± 0.003	1.90^{a}± 0.03	1.50^{b}± 0.004	1.00^{d}± 0.04	0.78^{f} ± 0.003	0.94^{e}± 0.004	1.35^{c}± 0.003	0.037
	Change of (+ve) group	-58.95	-	-21.05	-47.37	-58.95	-50.53	-28.97	
Lungs	**g**	1.33^{d}± 0.30	2.3^{a} ± 0.70	1.72^{b}± 0.50	1.46^{c}± 0.30	1.34^{d}± 0.60	1.46^{c}± 0.40	1.73^{b}± 0.90	0.011
	Change of (+ve) group	-42.17	-	-25.22	-36.52	-41.74	-36.52	-24.78	

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

Table(4): Effect of Berseem (clover) plant parts on serum glucose mg\dl

Variable	(G1) Control (-)	(G2) Control (+)	(G3) 5%Berseem (clover) flowers flowers	(G4) 5% Berseem (clover) leaves	(G5) 5% Berseem (clover) stems	(G6) 5% Berseem (clover) roots	(G7) 5% Berseem (clover) seeds	LSD: p ≤ 0.05
Glucose (mg\dl)	125.00^{f} ±0.133	168.00^{a} ±0.004	129.00 ±0.221	140.00^{c} ±0.102	132.00^{d} ± 0.111	145.00^{b} ± 0.101	140.00^{c} ± 0.103	0.113
Change of (+ve) group%	25.60-		23.21-	16.67-	21.43-	13.70-	16.67-	

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

Table(5): Effect of Berseem (clover) plant parts on serum lipid profile (mg\dI)

Parameter \ Groups		(G1) Control(-)	(G2) Control(+)	(G3) 5%Berseem (clover) flowers	(G4) 5% Berseem (clover) leaves	(G5) 5% Berseem (clover) stems	(G6) 5% Berseem (clover) roots	(G7) 5% Berseem (clover) seeds	LSD: p ≤ 0.05
TC	**(mg\dl)**	80.00^{g}± 0.111	136.00^{a}± 0.02	98.00^{c}± 0.103	82.00^{f}± 0.004	84.00^{e}± 0.031	97.00^{d}± 0.201	99.00^{b}± 0.101	0.122
	Change of (+ve)	- 41.18	-	-27.94	- 39.71	-38.24	-28.68	-27.21	
TG	**(mg\dl)**	34.00^{g}± 0.009	65.00^{a} ± 0.021	40.00^{c}± 0.186	35.00^{f}± 0.122	37.00^{e}± 0.231	38.00^{d}± 0.195	43.00^{b}± 0.214	0.163
	Change of (+ve) group %	- 47.69	-	-38.46	- 46.15	- 43.08	- 41.54	-33.85	
HDL	**(mg\dl)**	60.00^{a}± 0.216	34.00^{f} ± 0.111	53.00^{e}± 0.006	59.00^{b}± 0.133	57.00^{c}± 0.004	55.00^{d}± 0.046	53.00^{e}± 0.231	0.166
	Change of (+ve) group %	+76.47	-	55.88+	+73.53	76.65+	61.76+	55.88+	
LDL	**(mg\dl)**	13.20^{f}± 0.02	89.00^{a} ± 0.111	37.00^{b}± 0.01	16.00^{e}± 0.003	20.00^{d}± 0.152	29.00^{c}± 0.011	37.00^{b}± 0.012	0.115
	Change of (+ve) group %	-85.17	-	-58.43	-82.02	-77.53	67.42-	-58.43	
VLDL	**(mg\dl)**	6.80^{g} ± 0.85	13.00^{a} ± 1.00	8.00^{c} ± 0.94	7.00^{f} ± 0.99	7.40^{e} ± 0.78	7.60^{d} ± 0.97	8.60^{b} ± 0.98	0.148
	Change of (+ve) group%	- 47.69	-	-38.46	- 46.15	- 43.08	- 41.54	-33.85	
AI	**(mg\dl)**	0.33^{e}± 0.009	3.00^{a} ± 0.10	0.85^{b} ± 0.001	0.39^{e} ± 0.003	0.48^{d} ± 0.001	0.67^{c} ± 0.001	0.86^{b} ± 0.001	0.066
	Change of (+ve) group%	-89.00	-	-71.67	-87.00	-84.00	-77.67	-71.33	

Means in the same column with different litters are significantly different (p≤0.05)

Table(6): Effect of Berseem (clover) plant parts on Liver function

Parameter / Groups		(G1) Control (-)	(G2) Control (+)	(G3) 5%Berseem (clover) flowers	(G4) 5%Berseem (clover) leaves	(G5) 5%Berseem (clover) stems	(G6) 5%Berseem (clover) roots	(G7) 5%Berseem (clover) seeds	LSD: $p \leq 0.05$
AST	**(U/L)**	$46.00^{f} \pm 0.103$	110.00^{a} 0.02	$66.00^{b} \pm 0.111$	$53.00^{e} \pm 0.123$	$58.00^{d} \pm 0.101$	$63.00^{c} \pm 0.116$	$63.00^{c} \pm 0.003$	0.088
	Change of (+ve) group %	-58.18	-	- 40.00	-51.82	-47.27	-42.73	-42.73	
ALT	**(U/L)**	$22.00^{f} \pm 0.012$	40.00^{a} ±	$29.00^{b} \pm 0.003$	$25.00^{e} \pm 0.105$	$27.00^{d} \pm 0.009$	$28.00^{c} \pm 0.001$	$29.00^{b} \pm 0.001$	0.068
	Change of (+ve) group %	-45.00	-	-27.50	-37.50	-32.50	-30.00	-27.50	
ALP	**(U/L)**	109.00± 0.03	270.00^{a} ± 0.05	$130.00^{c} \pm 0.04$	$117.00^{f} \pm 0.015$	$119.00^{e} \pm 0.01$	$197.00^{b} \pm 0.003$	125.00^{d} ± 0.022	0.052
	Change of (+ve) group %	-59.63	-	-51.85	-56.67	-55.93	-27.04	-53.70	
AST/ ALT	**(U/L)**	$2.09^{g} \pm 0.002$	$2.75^{a} \pm 0.003$	$2.28^{b} \pm 0.001$	$2.12^{f} \pm 0.004$	$2.15^{e} \pm 0.006$	$2.25^{c} \pm 0.005$	$2.17^{d} \pm 0.004$	0.008
	Change of (+ve) group %	-24.00	-	-17.09	-22.91	-21.82	-18.18	-21.09	
T. P	**(g/dl)**	$6.50^{a} \pm 0.65$	$5.40^{d} \pm 0.41$	$5.70^{c} \pm 0.43$	$6.40^{ab} \pm 0.54$	$6.40^{ab} \pm 0.50$	$6.30^{b} \pm 0.49$	$6.40^{ab} \pm 0.52$	0.140
	Change of (+ve) group %	20.37+	-	+5.56	+18.52	18.52+	16.67+	+18.52	
ALB	**(g/dl)**	$3.60^{a} \pm 0.002$	$2.00^{f} \pm 0.003$	$2.80^{e} \pm 0.004$	$3.50^{b} \pm 0.01$	$3.40^{c} \pm 0.003$	$3.28^{d} \pm 0.001$	$3.40^{c} \pm 0.002$	0.033
	Change of (+ve) group %	+80.00	-	+40.00	+75.00	+70.00	+64.00	+70.00	
GLOB	**(mg//dl)**	$2.90^{c} \pm 0.21$	$3.40^{a} \pm 0.29$	$2.90^{c} \pm 0.15$	$2.90^{c} \pm 0.18$	$3.00^{bc} \pm 0.32$	$3.10^{b} \pm 0.27$	$3.00^{bc} \pm 0.09$	0.145
	Change of (+ve) group %	-14.71	-	-14.71	-14.71	-11.76	-8.82	-11.76	
ALB/ GLOB	**(mg/dl)**	$1.24^{a} \pm 0.01$	$0.59^{c} \pm 0.044$	$0.97^{b} \pm 0.09$	$1.21^{a} \pm 0.11$	$1.13^{ab} \pm 0.105$	$1.03^{b} \pm 0.02$	$1.13^{ab} \pm 0.111$	0.149

	Change of (+ve) group %	+110.17	-	+ 64.41	+105.08	+ 91.52	+ 74.58	+ 91.52	
T.Bili	**(mg/dl)**	0.75^{d} ± 0.026	1.20^{a} ± 0.11	0.95^{b} ± 0.103	0.81^{cd} ± 0.023	0.87^{bc} ± 0.011	0.92^{b} ± 0.0119	0.90b ± 0.061	0.075
	Change of (+ve) group %	-37.50	-	-20.83	-32.50	-27.50	-23.33	-25.00	
D.Bili	**(mg/dl)**	0.58^{e} ± 0.044	0.93^{a} ± 0.037	0.70^{b} ± 0.041	0.62^{d} ± 0.058	0.66^{c} ± 0.017	0.69^{b} ± 0.049	0.68^{bc}± 0.036	0.023
	Change of (+ve) group %	-37.63	-	-24.73	-33.33	-29.03	-25.81	-26.88	
IN D.Bili	**(mg/dl)**	0.17^{f} ± 0.03	0.27^{a} ± 0.045	0.25^{b} ± 0.032	0.19^{e} ± 0.039	0.21^{d} ± 0.046	0.23^{c} ± 0.019	0.22^{cd} ± 0.048	0.018
	Change of (+ve) group %	-37.04	-	-7.41	-29.63	-22.22	-14.81	-18.52	

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

Table(7): Effect of Berseem (clover) plant parts on Serum antioxidant enzymes

Groups / Parameters		**(G1) Control (-)**	**(G2) Control (+)**	**(G3) 5%Berseem (clover)flowers**	**(G4) 5%Berseem (clover) leaves**	**(G5) 5% Berseem (clover) stems**	**(G6) 5% Berseem (clover) roots**	**(G7)5% Berseem (clover) seeds**	**LSD: p ≤ 0.05**
GPX	**(ng/ml)**	40.32^{a}± 0.002	22.30^{g}± 0.01	35.81^{e}± 0.003	38.65^{b} ± 0.004	38.40^{c}± 0.01	36.90^{d}± 0.01	30.43^{f}± 0.001	0.012
	Change of (+ve) group%	+ 80.81	-	+ 60.58	+ 73.32	+ 72.20	+ 65.47	+ 36.46	
SOD	**(U/L)**	38.94^{a}± 0.92	10.32^{g}± 0.81	30.16^{e}± 1.00	36.15^{b}± 0.69	34.20^{c}± 0.85	31.72^{d}± 0.79	29.14^{f} ± 0.96	0.192
	Change of (+ve) group%	+277.33	-	+192.25	+250.29	+231.40	+207.36	+182.36	
CAT	**(nmol/L)**	77.60^{a}± 0.04	39.11^{g}± 0.032	65.67^{e}± 0.008	74.11^{b} ± 0.003	71.05^{c} ± 0.01	68.34^{d}± 0.008	65.40^{f} ± 0.018	0.037
	Change of (+ve) group%	+ 98.41	-	+ 67.91	+ 89.49	+ 81.67	+74.74	+ 67.22	

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

Table(8): Effect of Berseem (clover) plant parts on kidney function

Groups / Parameters		(G1) Control (-)	(G2) Control (+)	(G3) 5%Berseem (clover) flowers	(G4) 5%Berseem (clover) leaves	(G5) 5% Berseem (clover) stems	(G6) 5% Berseem (clover) roots	(G7) 5% Berseem (clover) seeds	LSD: p ≤ 0.05
Creatinine	**(mg/dl)**	0.70^{f} ± 0.02	2.10^{a} ± 0.04	1.10^{b}± 0.05	1.00^{c} ± 0.023	0.80^{e} ± 0.028	0.90^{d} ± 0.041	0.80^{e} ± 0.023	0.046
	Change of (+ve) group	- 66.67	-	-47.62	- 52.38	- 61.90	- 57.14	- 61.90	
Urea	**(mg/dl)**	9.00^{g} ± 0.46	40.00^{a}± 0.222	20.00^{b}± 0.345	18.00^{c}± 0.395	14.00^{e}± 0.41	16.00^{d}± 0.33	10.00^{f}± 0.19	0.176
	Change of (+ve) group	-77.50	-	-50.00	-55.00	- 65.00	- 60.00	- 75.00	
Uric acid	**(mg/dl)**	0.80^{d}± 0.029	01.90^{a}± 0.012	0.80^{d}± 0.029	0.90^{c} ± 0.031	0.90 ± 0.011	1.10^{b} ± 0.06	0.90^{c} ± 0.02	0.059
	Change of (+ve) group	- 57.89	-	-57.89	- 52.63	- 52.63	- 42.11	- 52.63	

Means in the same column with different litters are significantly different (p≤0.05).

Histopathological Results

1- Liver:

A	B	C	D
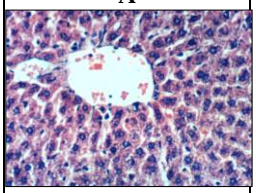	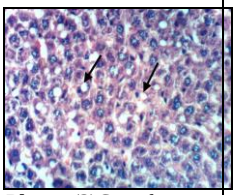	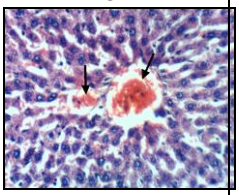	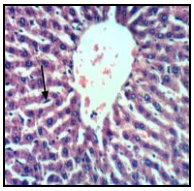
Photo: (1) normal histological structure of hepatic lobule (Control "-").	**Photo: (2)**Cytoplasmic vacuolation of hepatocytes (Control "+",basal diet).	**Photo:(3)**congestion of central veins (clover flowers diet).	**Photo: (4)**slight Kupffer cells activation (clover leaves diet).

E

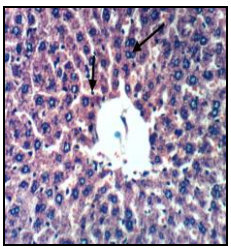

Photo: (5)slight Kupffer cells activation and binucleation of hepatocytes (clover stems diet).

F

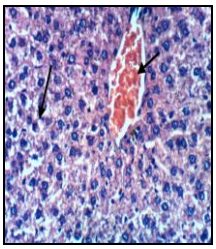

Photo: (6)congestion of central vein and slight hydropic degeneration of hepatocytes (clover roots diet).

G

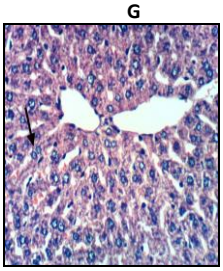

Photo: (7)binucleation of hepatocytes (clover seeds diet).

2-Kidney:

E

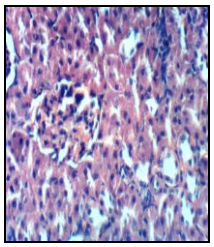

Photo: (8) normal histological structure of renal parenchyma (Control "-").

F

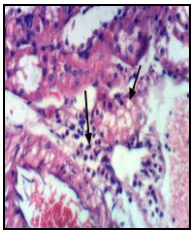

Photo :(9)vacuolation of epithelial lining renal tubules and perivascular inflammatory cells infiltration (Control "+",basal diet)

G

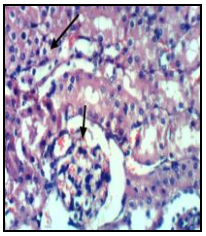

Photo: (10) vacuolation of epithelial lining renal tubules and congestion of glomerular tuft (clover flowers diet)

H

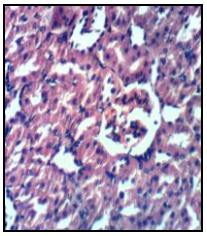

Photo: (11)no histopathological changes (clover leaves diet).

I

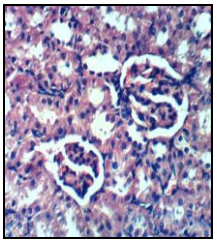

Photo :(12) no histopathologic al changes

J

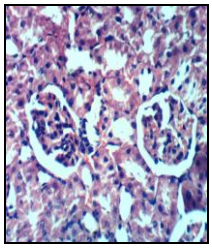

Photo :(13) no histopathologic al changes

K

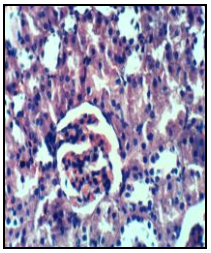

Photo :(14) no histopathologi

Hepatoprotective & Renoprotective Effect Of Trifolium Alexandrinum Plant Parts On Ccl_4- Induced Hepatointoxication (H & E X 400) Was Evident As Indicated By The Photos (**A- K),** While Was Parallel To The Biochemical Results. (**A&E)** Normal Rats ,Control "-" ,Basal Diet,(**B&F)** Hepatointoxicated ,Control "+",Basal Diet, **(C& G**) Hepatointoxicated ,5% Flowers Berseem, **(D& H)** Hepatointoxicated ,5% Leaves Berseem , **(E&I)** Hepatointoxicated ,5% Stems Berseem ,(**F&J**) Hepatointoxicated ,5% Roots Berseem , (**G&K**) Hepatointoxicated ,5% Seeds Berseem.

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Journal of Home Economics, Volume 25, Number (3), 2015

إمكانية توظيف اجزاء نبات البرسيم المصرى لعلاج التسمم الكبدى فى الفئران البيضاء.

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المستخلص العربى

تم اجراء الدراسة الحالية لمعرفة التاثيرات المحتملة لاجزاء نبات البرسيم المصرى على الخلل الفسيولوجى المحدث فى كبد الفئران المصابة برابع كلوريد الكربون . تم استخدام ٣٥ فار ابيض بالغ يتراوح وزن كل منهم ١٥٠±١٠جم وتم تقسيمهم الى ٧ مجموعات متساوية تركت احداها كمجموعة ضابطة سالبة اما المجموعة الست الاخرى فتم احداث تسمم للكبد باستخدام رابع كلوريد الكربون المخلوط مع زيت زيتون ٥٠ % بالحجم بنسبة ٣ مل/كجم من وزن الجسم مرتين اسبوعيا لمدة اسبوعين وتمت تغذية المجموعات كالاتى :الثانية :مجموعة ضابطة موجبة (غير معالجة) ,الثالثة: تم تغذيتها على٥ جم/١٠٠جم ازهارالبرسيم, الرابعة: تم تغذيتها على٥ جم/١٠٠جم اوراق البرسيم ,الخامسة: تم تغذيتها على٥ جم/١٠٠ جم سيقان البرسيم ,السادسة: تم تغذيتها على٥ جم/١٠٠جم جذورالبرسيم والسابعة: تم تغذيتها على٥ جم/ ١٠٠جم بذورالبرسيم وفى نهاية التجربة (٢٨يوم) تم ذبح الفئران بعد صيام ١٢ساعة وتجميع عينات الدم لتقديرالاتى: الكوليسترول الكلى والجليسريدات الثلاثية واللليبوبروتينات العالية , المنخفضة , المنخفضة جدا فى الكثافة ومعامل تصلب الشرايين ومستوى الجلوكوز بالدم وانزيمات الكبد (AST, ALT, ALP),البروتين الكلى, الالبيومين, الجلوبيولين, نسبة الالبيومين/الجلوبيولين, البيليروبين الكلى,البيليروبين المباشروالبيليروبين غيرالمباشر ومضادات الاكسدة الانزيمية (GPX , SOD, CAT) ووظائف الكلى (الكرياتينين- اليوريا- حمض اليوريك) وفى نفس الوقت تم فصل الاعضاء الداخلية (الكبد, القلب , الكلى, الطحال والرئتين) ووزنها وحفظ الكبد والكلى فى فورمالين ١٠% لاجراء الفحص الهستوباثولوجى. وقد اظهرت نتائج هذه الدراسة ان المجموعة السابعة (تغذت على٥ جم/ ١٠٠جم بذورالبرسيم) سجلت ارتفاعا معنويا عند ($p \leq ٠.٠٥$) فى وزن الجسم المكتسب, متوسط الماخوذ اليومى من الغذاء ومعدل كفاءة الغذاء وسجلت انخفاضا معنويا عند ($p \leq ٠.٠٥$) فى حمض اليوريك والكرياتينين.المجموعة الثالثة (تغذت على٥ جم/ ١٠٠جم ازهار البرسيم) سجلت انخفاضا معنويا عند ($p \leq ٠.٠٥$) فى مستوى الجلوكوز بالدم. المجموعة الرابعة (تغذت على٥ جم/١٠٠جم اوراق البرسيم) سجلت انخفاضا معنويا عند ($p \leq ٠.٠٥$) فى الكوليسترول الكلى والجليسريدات الثلاثية واللليبوبروتينات المنخفضة , المنخفضة جدا فى الكثافة ومعامل تصلب الشرايين وانزيمات الكبد(AST, ALT, ALP), الجلوبيولين, البيليروبين الكلى,البيليروبين المباشر, البيليروبين غير المباشر وسجلت ارتفاعا معنويا عند ($p \leq ٠.٠٥$) فى اللليبوبروتينات العالية الكثافة, البروتين الكلى, الالبيومين, معدل الالبيومين /الجلوبيولين ومضادات الاكسدة الانزيمية (GPX , SOD, CAT).

الكلمات المفتاحية: تسمم الكبد - رابع كلوريد الكربون - اجزاء النبات – انزيمات الكبد- وظائف الكلى.