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Amelioration of Hepatointoxication and Hyperglycemia by Diets Containing Herbs

Fatma El Zahra Amin , Mai Khafagy , Azza Ibraheem El-Sayed
Department of Nutrition and Food Sci., Faculty of Home Economics, Menoufia Univ.,Shebin El-Kom,Egypt

Abstract :

This study was conducted to investigate the effect of Elder berry (*Sambucus nigra*), Echinacea (*Echinacea purpurea*) , Astragalus (*Astragalus hispidulus*) and Curry leaves (*Murraya koenigii*) plants on impaired liver function of injected rats with Carbon Chloride Tetra (CCL4) and injected with Alloxan to induce hyper glycemia. Seventy eight mature albino rats weighting 150-160g B.Wt. each were used, and divided into 13 equal groups, one group was kept as a control –ve group , while the six groups were injected by (ccl4) once a day for a period of 4 days. The used plants in diets were : (Elderberry 5%, Echinacea 5% , Astragalus 5% , Curry leaves 5% , and as mixture of these plants 5%(1:1:1:1w/w) from the basal diet. And another six group were injected by Alloxan once a day .The used plants in diets were : (Elderberry 5%, Echinacea 5% , Astragalus 5% , Curry leaves 5% , and as mixture of this plants 5% (1:1:1:1w/w) from the basal diet .Serum liver function (ALT,AST,ALP) CAT,SOD and GPX, kidney function (urea , creatinine , uric acid),lipids profile(total cholesterol , triglycerides , lipoproteins "HDL , LDL , VLDL"),Gulcose and histopathological changes of liver & kidney were examined .The obtained results concluding that the feeding with the tested plant parts improved the kidney functions , liver functions and lipid profile specially when a mixture of these studied herbs used.

Key Words: Hyperglycemia, kidney functions, Histopathological changes

Introduction

Bodi *et al.*, (2014), EFSA (2017) and Mulder *et al*., (2018) reported that the liver performs many critical metabolic functions, including processing and distribution of nutrients. Liver diseases can be caused by infection, such as hepatitis B and C, or by genetic mutations. Other liver diseases can be triggered by autoimmune reactions or drug toxicity. The rise in obesity in the United States has led to a rise in nonalcoholic fatty liver disease. Many liver diseases place individuals at higher risk for developing liver cancer.

The only current treatment for end-stage liver disease is a liver transplant, and the number of livers available from deceased donors is limited. Thus NIDDK-supported liver research focuses on identifying liver disease early, preserving liver function in people with liver disease, and developing new treatment options, including transplants performed with liver tissue from living donors.

Subbiah, *etal*.,(2019) showed that diabetes mellitus is a complex metabolic disorder characterized by hyperglycemia, pancreatic beta (β) cells dysfunction, and abnormal lipid profile that result from metabolic deregulations, impaired insulin secretion and action, and inappropriate consumption of glucose . It is one of the most prevalent chronic diseases and leads towards severe complications such as increase in production of reactive oxygen species (ROS), impairment of antioxidant enzymes ,hyperglycemia ,dislipidemia ,alteration in insulin signaling pathway, and ROS-induced cellular damage .All these changes will result in diabetes-associated secondary complications like nephropathy, retinopathy, neuropathy, and cardiovascular morbidity.

Kahkeshani *etal*.,(2019) reported that experimental and clinical research studies have proved strong relation between oxidative stress induced by hyperglycemia and diabetes as ROS are produced in excessive amount through the oxidation of glucose Furthermore, pancreatic β cells are attacked by excessive ROS with the consequence of cellular damage due to weak intrinsic free radical scavenging potential .Several signaling pathways are also altered by oxidative stress resulting in the release of proinflammatory cytokines, formation of advanced glycation end products (AGEs), and cell death Therefore, interference in oxidative stress has been highlighted as an important strategy for treatment of diabetes .

Oral antihyperglycemic agents are being used for glycemic control, but they have severe adverse effects such as abdominal pain, obesity, hepatic disorders, and renal injury .According to latest research, **Ahmad *etal*., (2018)** indicated that plant-derived products have demonstrated wide range of valuable therapeutic activities without causing adverse effects ,

Plants rich in polyphenolics have gained much attention due to their wide spectrum of therapeutic benefits, as verified by both in vitro and in vivo studies .The polyphenols are reported to produce insulin-like effect in glucose consumption, lower ROS generation, and enhance free radical scavenging mechanism .These phytoconstituents protect cellular antioxidant defense mechanism from oxidative stress, stimulate insulin signaling pathway, and regulate transcription factors, hormones, peptides, and inflammatory pathways for the management of hyperglycemic condition and diabetes-associated complications .

Recent years have seen a surge in research concerning bioactive compounds in food. The plant world is a source of many substances affecting functionality of the human organism and its status **(Costa *etal.*, 2013).** One of the relatively little known sources of bioactive compounds is elderberry, the consumption of which provides prevention and therapy for a number of diseases.

Balciunaite *et al*,. (2015) declared that the huge importance of searching for alternative sources of antidiabetic agents and the limited number of studies dealing with elderberry extract supplementation to reduce diabetes complications, highlights the need to conduct more detailed studies on this topic. Previous studies revealed the potential of elderberry extracts in diabetes status management. The effects of acidified methanol elderberry extracts dietary supplementation 28–70 mg of extract/kg body weight (b.w.) of streptozotocin (STZ)-induced diabetic Wistar rats, during 12 to 16 weeks) were evaluated showing a reduction in serum glycemic and lipidic levels (cholesterol and triacylglycerol); reduction in the levels of oxidative markers (as superoxide dismutase and glutathione peroxidase activities) and inflammatory markers (as interleukin-6); and an increase in immunological parameters from T lymphocytes populations.

Echinacea purpurea has many beneficial features, especially activation of immune system by increasing the number of circulating white blood cells, stimulating phagocytosis, T-cell production, lymphocytic activity, cytokine production, cellular respiration, activity against tumor cell, inhibiting hyaluronidase enzyme secretion and trigger the alternate complement pathway. In previous studies, anti-inflammatory effects of this extract have been investigated in Arsenic induced hepatic toxicity **(Newman & Cragg 2012).**

Curry Leaf is a green leafy vegetable native to India. Curry leaves are natural flavoring agents with numerous health benefit They contain several medicinal properties that include it being anti-diabetic, antioxidant, antimicrobial, anti-inflammatory, anti-carcinogenic and with

hepato-protective properties .The antibacterial effects of curry leaves were also investigated **(Suman Singh *et al*, 2014).**

Materials & Methods

Matriels:

The Used Plants:

Elder berry (*Sambucus niara*), Curry leaves (*Murraya koenigii*) Echinacea (*Echinacea purpurea*) , were obtained from Harraz market in Cairo ,Egypt but Astragalus (*Astragalus membranaceus*) obtained from Emirates Arabic United

CARBON TETRACHLORIDE (CCL4):

Carbon tetrachloride was obtained from El-gomhoria Company for Med-Preparations, chemicals and Medical Equipments, Cairo-Egypt as 10%liquid solution. It was dispensed in white plastic bottles each containing one litre as atoxic chemical material for liver poisoning according to **(Passmore and Eastwood 1986)** in the same time it is mixed with 10% paraffin oil which obtained from the pharmacy for dilution during the induction.

Alloxan:

Alloxan obtained from El- gomhoriya Company, Cairo, Egypt and used as a dose of 150 mg/kg body weight.

Rats:

Seventy eight adult male albino rats, weighting 150-160g.from Medical Insects Research nstitute, Doki, Cairo, were used in this study.

Rats were housed in wire cages under the normal laboratory condition and were fed on standard diet for a week as an adaptation period. Diet was offered to rats in special food cups to avoid loser conditions of feed, water was provided to the rats by glass tubes supported to one side of the cage, feed and water provided ad-libitum and checked daily.

Methods:

Preparation Of Matriels

All herbs were grinded in to soft herb by using Electric grinder to give a powder and kept in dusky stoppered glass bottles in a cool and dry location till use according to **Russo (2001),** who reported that al herbs and plants are pest kept in a cool, dry, and dark location to reduce oxidation of their contents.

Experimental design:

The experimental study performed in the Faculty of Home Economics, Menuofia University, Shebin El-kom. Rats were housed in wire cages in a room temperature 25 c^{o} ,and kept under normal healthy conditions.

Rats were divided into the following groups:

Group 1: Negative Control Group – Normal Group(6 Rats)

In This Group Rats Were Kept On Basal Diet And Tap Water.

Group 2: Hepatopathy Induced Group (36 Rats)

In this group, rats were injected by 0.2 mg\kg body weight by Carbon Tetrachloride for two weeks to induce liver impaired. This main group was subdivided into 6 groups to feed on the experimental diets for (4) weeks according to the following:

Group (2): 6 rats: Positive control group (untreated group)
Group (3): 6 rats: Treated with 5% Elder berry.
Group (4): 6 rats: Treated with 5% Echinacea
Group (5): 6 rats: Treated with 5% Astraguls
Group (6): 6 rats: Treated with 5% Curry Leavs
Group (7):6 rats had basal diet supplemented with mixture of (Elder berry, Echinacea, Astraguls and Curry Leavs) 5% (1:1:1:1: w/w).

Preparations of Diabetic Rats: Diabetes was induced in normal healthy male albino rats via subcataneous injection of alloxan 150 mg /kg body weight according to the method described by **Desai and Bhide (1985).** Six hours after the injection of alloxan , fasting blood samples were obtained by retro-orbital method to estimate fasting serum glucose . Rats having fasting serum glucose more than 185 (mg/dl) were considered diabetics **(NDDG 1994).**

Group 3: Diabetes induced Group (36 Rats)

In this group, rats were induced by Alloxan for one day to induce hyperglycemia. This main group was subdivided into 6 groups to feed on the experimental diets for (4) weeks according to the following:

Group (8): 6 rats: Positive control group (untreated group)
Group (9): 6 rats: Treated with 5% Elder berry.
Group (10): 6 rats: Treated with 5% Echinacea
Group (11): 6 rats :Treated with 5% Astraguls
Group (12): 6 rats: Treated with 5% Curry Leavs
Group (13):6 rats had basal diet supplemented with mixture of (Elder berry, Echinacea, Astraguls and Curry Leavs) 5% (1:1:1:1: w/w).

Blood sampling and organs:

Blood samples were collected after 12 hours fasting at the end of the experiment using the abdominal aorta in which the rats were scarified under ether anetheized. Blood samples were received in to clean dry centerfuge tubes and left to clot at room temperature, then centerfuged for 10 minutes at 3000 rpm to separate the serum.

Serum was carefully aspirated, transferred in to clean cuvette tubes, and kept frozen at-20^{o}C for analysis. All serum samples were analyzed for determination the following parameters:

Serum glucose was measured using the modified kinetic method according to**(Kaplan ,1984),** Urea was determinated according to the enzymatic method of **(Patton and Crouch, 1977)** , Creatinine was determinated according to kinetic method of **(Henry 1974),** Uric acid was deter mined according to **(Schultz,1984),** Enzymatic calorimetric determination of alkaline phosphatases was carried out according to **(Belfield and Goldberg, 1971),** Determination of GPT was carried out according to the method of **Yound (1975),** Determination of GOT was carried out according to the method **of Henry (1974) and Yound (1975),** Enzymatic colorimetric determination of triglycerides was carried out according to **(Fossati and Prencipe, 1982),** The method used for total cholesterol determination was according to **(Allain, 1974),**HDL, according to **(Lopez, 1977),** VLDL (very low density lipoproteins) and LDL were carried out according to the method of **(Lee and Nieman, 1996).** The activity of antioxidant enzyme was estimated according to the method described by **(Oyanagui, 1984).** (SOD) Glutathione peroxidase (GPX) enzyme activity was determined according to the method of **Paglia and Valentine (1967).** The activity of antioxidant enzyme catalase (CAT) was determined according to the method of **Aebi (1984).**

Statistical Analysis:

The data were statistically analyzed using a computerized program by one way ANOVA .The results are presented as mean ± SD. Differences between treatments at $p \leq 0.05$ were considered significant.

Histopathological Examination:

At the end of the experiment which continued for 28 successive days, all rats were sacrificed, tissue samples including liver and kidney were taken for histopathological examination **(Carleton, 1978).**

Results and discussion:

Effect of some plants herbs of hepato intoxicated rats induced by ccl4:

a- Biological parametrs (BWG, FI and FER) :.

Table (1): Effect of feeding hepatic rats with elderberry,echinacea , astragalus and curry leaves diets on BWG, FI and FER

Parameters / Groups	BWG (g/ day)	FI (g/day)	FER(g/28day)
	Mean±SD	Mean±SD	Mean±SD
Group 1 control – ve	0.75±0.01[a]	15.75±0.2[a]	0.048±0.04[a]
Group 2 control + ve	0.11±0.002[f]	11.5±0.1[e]	0.010±0.001[d]
Group 3 5%Elder berry	0.59±0.01[a]	13.5±0.05 [d]	0.044±0.04[b]
Group 4 5% Echinacea	0.54±0.02[b]	14.75±0. 1[b]	0.037±0.04[d]
Group 5 5% Astragalus	0.59±0.01[a]	14±0.2[c]	0.042±0.04[c]
Group 6 5% Curry leaves	0.33±0.02[c]	13.3±0.2[d]	0.011±0.02[d]
Group 7 5% Mixture	0.65±0.01[a]	15.5±0.03[a]	0.042±0.04[c]
LSD	0.197	0.264	0.002

Means in the same column with different letters are significantly different.* Significance ($p < 0.05$).

- The rats fed on Echinacea diets showed significantly lower body weights and energy intakes than rats fed on casein diets.
- In human research, an *S. nigra* (elderberry)-containing diet decreased mean body weight **(Chrubasik *et al.*, 2008; Hasani-Ranjbar *et al.*, 2009).**
- These results also agree with those of **(Suman Singh *et al,* 2014).** He reported that *Curry leaves* administered rats showed a progressive decrease in body weight.
- These results agree with those of **(Agyemang *et al.*, 2013**) indicating that Astraglus administered rats showed a progressive decrease in body weight.

- **Table (2):** Effect of feeding hepatic rats with elderberry, echinacea , astragalus and Curry leaves diets on relative organs weight (g/100 g. B.Wt.)

Relative organs weight (g/100 g. B.Wt.)					
Groups	Liver	Spleen	Lungs	Heart	Kidneys
Group 1 control – ve	3.7±0.01^{g}	0.4±0.01^{e}	0.7±0.01^{b}	0.4±0.02^{f}	0.7±0.03^{f}
Group 2 control + ve	4.3±0.002^{a}	0.6±0.02^{a}	0.8±0.02^{a}	0.7±0.01^{a}	1.3±0.05^{a}
Group 3 5% Elder berry	4. 1±0.01^{c}	0. 4±0.02^{e}	0.65±0.1^{c}	0. 5±0.02^{d}	1±0.01^{c}
Group 4 5% Echinacea	4.2±0.01^{b}	0.55±0. 01^{b}	0.65±0. 2^{c}	0.45±0.01^{e}	1.2±0.02^{b}
Group 5 5% Astragalus	3.9±0.02^{e}	0.5±0.01^{c}	0.8±0.01^{a}	0.6±0.03^{b}	0.9±0.02^{d}
Group 6 5% Curry leaves	4±0.01^{d}	0. 4±0.02^{e}	0. 6±0.01^{d}	0.55±0.01^{c}	0.99±0.01^{c}
Group 7 5%Mixture	3.8±0.03^{f}	0.45±0.02^{d}	0.69±0.02^{c}	0.5±0.02^{d}	0.85±0.01^{e}
LSD	0.062	0.009	0.009	0.034	0.034

- Means in the same column with different letters are significantly different.* Significance ($p < 0.05$).
- From results of table (2) it is evidence that group (7) was the best.
- This result was agreed with those of **(ZakayRones *et al*., 2004) ,** she reported that the Histopathological observations in liver rat's tissues revealed that methanolic Sambucus was non-toxic.
- The results are the agreement with that of **(Anita Joshi *et al,* 2009).** considering feeding of hepatic rats on certain plants.

b- Biochemical parametrs:

Table (3): (A) Effect of the hepatic rats with parts of elderberry, echinacea , astragalus and curry leaves diets on serum levels of TC, TG and HDLc

Groups	TC (mg/dL)	TG (mg/dL)	HDLc. (mg/dL)
Group 1 control – ve	145±0.3[f]	97.4±0.1[g]	51.8±0.2[a]
Group 2 control + ve	278±0.4[a]	. 80.6±0.4[a]	25.3±0.2[f]
Group 3 5% Elderberry	146.3±0.2[e]	115.5±0.25[e]	45.6±0.2[b]
Group 4 5% Echinacea	150.2±0.3[c]	120.2±0.2 [c]	42±0.1[d]
Group 5 5% Asrraglus	149.5±0.5[d]	117.1±0.1[d]	44±0.5 [c]
Group 6 5% Curry leaves	152.3±0.2[b]	122.3±0.2[b]	39.8±0.2 [e]
Group 7 5% Mixture	149±0.5[d]	110.8±0.2[f]	46.3±0.4 [b]
LSD	0.689	0.370	0.889

Table (3): (B) Effect of the hepatic rats with parts of elderberry, echinacea , astragalus and curry leaves diets on serum levels of LDLc, VLDLc. and AI

Groups	LDLc. (mg/dL)	VLDLc. (mg/dL)	AI (mg/dL)
Group 1 control – ve	74±0.1[f]	19.5±0.5 [f]	1.8±0.01 [g]
Group 2 control + ve	261.6±0. [a]	36.1±0.1 [a]	9.99±0.01 [a]
Group 3 5% Elderberry	77.6±0.4 [e]	23.1±0.2[d]	2.21±0.01 [e]
Group 4 5% Echinacea	84.2±0.3 [c]	24±0. 2 [c]	2.58±0.01[c]
Group 5 5% Asrraglus	82.1±0.5 [d]	23.4±0.1 [d]	2.40±0.01[d]
Group 6 5% Curry leaves	88±0.4 [b]	24.5±0.2 [b]	2.83±0.02[b]
Group 7 5% Mixture	74.4±0.2 [f]	22.2±0.1 [e]	2.06±0.05[f]
LSD	0.469	459	0.045

Means in the same column with different letters are significantly different.

* Significance ($p < 0.05$).

Best treatment of table (3) was that of group 7.

- In human research, ***Sambucus*** reduced postprandial lipid levels **(Vlachojannis *et al.*, 2010).** In human research, elderberry juice decreased cholesterol concentrations and had minor effect on other serum lipids **(Mulleder *et al*., 2002).**
- Result of table (3) agree with **Das and Biswas (2012),** they reported that oral administration of the aerial parts of ethanolic extract of the aerial parts of **Curry leaves** at a concentration of 150 mg/kg b.w. daily to rats for 15 days showed a significant protection against-induced decrease in serum holesterol.
- Also **Sparreboom *et al.*, (2004)** reported that when **Echinacea** added at 5% on the diet of hypercholesterolemic rat serum TG decreased
- Moreovers **Fang *et al.*, (2009)** who reported that **Astraglus** has been shown to lower cholesterol, LDL (bad cholesterol) levels.

Table(4): Effect of feeding hepatic rats with parts of elderberry (*Sambucus nigra*), echinacea (*Echinacea purpurea*) , astragalus (*Astragalus hispidulus*) and curry leaves (*Murraya koenigii*) diets on urea and uric acid in serum

Parameters / Groups	**Urea (U/ L)**	**Uric acid (U/ L)**	**Creatinine(U/L)**
	Mean±SD	Mean±SD	Mean±SD
Group 1 control – ve	22.6±0.1^{f}	3.1±0.1^{e}	0.76±0.01^{b}
Group 2 control + ve	48.1±0.15^{a}	7.9±0.1^{a}	1.4±0.05^{a}
Group 3 5% Elder berry	28.5±0.12^{e}	4.1±0. 03 ^{d}	0.86±0.01^{b}
Group 4 5% Echinacea	30.7±0. 3^{d}	4.5±0.05^{c}	0.94±0.005^{b}
Group 5 5% Astragalus	35.2±0.2^{c}	4.6±0.03^{bc}	0.94±0.005^{b}
Group 6 5% Curry leaves	40.1±0.1^{b}	4.7±0.01^{b}	0.78±0.21^{b}
Group 7 5% Mixture	27.8±0.2^{e}	4.1±0.1^{d}	0.85±0.1^{b}
LSD	0.872	0.120	0.390

Means in the same column with different letters are significantly different.
* Significance ($p < 0.05$).

- Mixture 5 % diet proved to be the best group.
- This indicated the possible synergestic action when the three tested plants combined.

- In human research, **Sambucus** (anthocyanins) lacked significant effects on urea and creatinine **(Curtis *et al.*, 2009).**
- **Rajendran *et al.*, (2014)** mentioned that **Curry leaves** prevented glomerular hyper filtration.
- **Cech *et al.*,(2006)** concluded that **Echinacea** in diuretic rat at a dose of 500mg/kg body weight **.**Ameliorated Nemopath without affecting proximal tubular function, Na+, or uric acid excretion.

Table (5): Effect of feeding hepatic rats with parts of elderberry, echinacea , astragalus and curry leaves diets on serum levels of AST, ALT and ALP enzyme

Parameters / Groups	**AST (U/ L)**	**ALT (U/ L)**	**ALP (U / L)**	**AST\ALT (U/L)**
	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Group 1 control – ve	48±0.2^{f}	35±0.1^{f}	111±1^{f}	1.23±0.2^{e}
Group 2 control + ve	155±1^{a}	75±1^{a}	150±2^{a}	2.07±0.02^{a}
Group 3 5% Elder berry	59±0.39^{e}	45.02±0. 3 ^{e}	129±0.05^{d}	1.31±0.05^{d}
Group 4 5% Echinacea	67.9±0.23^{c}	47±0.5^{d}	134.9±0.39^{c}	1.45±0.01^{c}
Group 5 5% Astragalus	63±0.5^{d}	50.06±0.9^{c}	126±0.7^{e}	1.26±0.02^{e}
Group 6 5% Curry leaves	74±0.6^{b}	51.07±0.8^{b}	138±0.1^{b}	1.45±0.05^{cb}
Group 7 5% Mixture	62.08±0.1^{d}	42.05±0.4^{e}	127±0.22^{e}	1.52±0.02^{b}
LSD	0.531	0.567	0.055	0.040

Means in the same column with different letters are significantly different.

* Significance ($p < 0.05$).

From results of table (3) best group was that of the 5 % mixture diet (Group7).

- Astudy **(Harokopakis *et al.*, 2006)** was carried out to determine efficiency of ethanolic extract of **Sambucus** as antioxidant and anticancer agents for liver.
- **(Mathur A, *et al.*, 2010)**, reported that oral administration of **Curry leaves** at a concentration of150 mg/kg b.w. daily to rats treated with iron for 31 prior to iron overloaded-administration significantly prevented the increase in liver, kidney and serum iron, serum ferritin, serum transferrin levels, γ- GT, α-GST and γ-GT activities as well as serum NO and TNF- α level and hepatic MDA level as compare to iron-overloaded treated rats.

- **Ali (2008)** reported that **Echinacea** has protective effects on the liver against CCl4**.**
- **Astragalus** can reduce CCl4-induced liver fibrosis in rats as well as platelet-derived growth factor-stimulated hepatic stellate cell migration **(İlçim and Behçet, 2016).**

Table(6): Effect of feeding nephritic rats with parts of elderberry (Sambucus nigra), echinacea (Echinacea purpurea) , astragalus (Astragalus hispidulus) and curry leaves (Murraya koenigii) diets on serum antioxidation enzyme activities

Parameters / Groups	**SOD (U/ mL)**	**GPX (U/ mL)**	**CAT(U/mL)**
	Mean±SD	Mean±SD	Mean±SD
Group 1 control – ve	40.6±0.4^{a}	44.6±0.4^{a}	82.1±0.4^{d}
Group 2 control + ve	8.3±0.0.3^{f}	17.2±0.3^{g}	27.5±0.3^{g}
Group 3 5% Elder berry	35±0.15^{b}	35.2±0. 15 ^{d}	75.4±0.15^{c}
Group 4 5% Echinacea	33±0. 2^{d}	34.1±0.2^{e}	72±0.2^{d}
Group 5 5% Astragalus	33.5±0.1^{c}	38±0.1^{c}	70.6±0.1^{e}
Group 6 5% Curry leaves	32.5±0.2^{e}	33±0.2^{f}	69.7±0.2^{f}
Group 7 5% Mixture	35.2±0.3^{b}	39.5±0.3^{b}	79.4±0.3^{b}
LSD	0.455	0.436	0.451

- Means in the same column with different litters are significantly different.
  * Significance ($p < 0.05$).
- Group (7) proved to be the best treatment to then diets groups.
- Astudy **Harokopakis *et al.*, (2006)** efficiency of ethanolic extract of **Sambucus** as antioxidant and anticancer agents for liver.
- **Mathur A, *et al.*, (2010)**, reported that oral administration of **Curry leaves** at a concentration of150 mg/kg b.w. daily to rats treated with iron for 31 prior to iron overloaded-administration significantly prevented the increase in liver, kidney and serum iron, serum ferritin, serum transferrin levels, γ- GT, α-GST and γ-GT activities as well as serum NO and TNF- α level and hepatic MDA level as compare to iron-overloaded treated rats.

- **Ali (2008)** reported that **Echinacea** has protective effects on the liver against ccl4**.**
- **Astragalus** can reduce CCl4-induced liver fibrosis in rats as well as platelet-derived growth factor-stimulated hepatic stellate cell migration **(İlçim and Behçet, 2016)**

Table (7): Fasting serum glucose mmol\dl for negative control (1), positive control (2), and all treated groups as affected by some plant herbs

Variables	*(1)* **Negative control**	**(2) Positive control**	*(3)* **Elderberry** *5%*	*(4)* **Echinacea** *5%*	*(5)* **Astragalus** *5%*	*(6)* **Curry leaves** *5%*	*(7)* **Mixture of plants** *5 %*	***Sig***	***L.S.D*** **(p ≤ 0.05)**
	Mean SD±	**Mean SD±**	**Mean SD±**	**Mean SD±**	**Mean SD±**	**Mean SD±**	**Mean SD±**		
Glucose mmol\dl	88.5^{g} ± 0.20	201.6^{a} ± 0.50	140^{d} ± 0.44	161^{c} ± 1	174^{b} ± 0. 88	129^{e} ± 0.7	125^{f} ± 0. 57	±	0.947

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

The best treatment was that of group "7" (5% mixture of herbs).

- In vitro research **(Gray *et al.*, 2000)** refuted earlier research reported **Sambucus** plant showed stimulation of glucose metabolism and promotion of insulin secretion from beta cells.
- In human research **(Disegha *et al.*, 2014). ,** they repoted that **Curry leaves** improved blood sugar levels
- Study of (**J.C., 2017).** showed that **Echinacea** in your bloodstream can help keep your blood sugar from spiking if you're diabetic or prediabetic
- **Lee *et al.*, (2010)** ,they repoted that **Astraglus** can help against insulin resistance and treat diabetes. Saponins,polysaccharides and flavonoids all has the capability to treat both Type 1 and Type 2 Diabetes Mellitus.

Effect of Some Plant herbs on hperglycemic rats induced by Alloxan.

(A) Biological parametrs

Table (8): Effect of feeding hyperglycemic rats with elder berry **(***Sambucus nigra*), echinacea (*Echinacea purpurea*) , astragalus (*Astragalus hispidulus*) and curry leaves (*Murraya koenigii*) diets on BWG, FI and FER

Parameters / Groups	BWG (g/ day)	FI (g/day)	FER(g/28day)
	Mean±SD	Mean±SD	Mean±SD
Group 1 control – ve	0.75±0.01[a]	15.75±0.2[b]	0.047±0.04[a]
Group 2 control + ve	0.13±0.02[e]	12.5±0.2[g]	0.010±0.04[g]
Group 3 5% Elder berry	0.63±0.01[b]	13.8±0.19 [f]	0.045±0.04[c]
Group 4 5% Echinacea	0.55±0.02[c]	15±0. 3[c]	0.036±0.04[e]
Group 5 5% Astragalus	0.61±0.01[b]	14.2±0.3[e]	0.043±0.04[d]
Group 6 5% Curry leaves	0.25±0.01[d]	14.6±0.1[d]	0.013±0.04[f]
Group 7 5% Mixture	0.75±0.01[a]	16.4±0.2[a]	0.046±0.04[b]
LSD	0.026	0.305	0.001

Means in the same column with different letters are significantly different.
* Significance ($p < 0.05$)

Data of table(7) indicated that the best treatment was that of group "7" diet (5% mixture of all herbs.

-In new study of **Farrel, *et al*., (2015)** the results concurred **(Sambucus and Curry leavs)** play a critical role in reduce BWG of mice due to resisting with diseases such as type 2 diabetes

-Results disagree with those of **Agyemang *et al*., (2013)** he reported that **Astraglus** administered rats showed a progressive increase in body weight.

Table (9): Effect of feeding hyperglycemic rats with elder berry (*Sambucus nigra*), echinacea (*Echinacea purpurea*) , astragalus (*Astragalus hispidulus*) and curry leaves (*Murraya koenigii*) diets on relative organs weight (g/100 g. B.Wt.)

ns weight (g/100 g. B.Wt.)					
Groups	Liver	Spleen	Lungs	Heart	Kidneys
Group 1 control – ve	3.7±0.01^{g}	0.4±0.01^{a}	0.7±0.01^{b}	0.4±0.02^{a}	0.7±0.03^{f}
Group 2 control + ve	4.3±0.002^{a}	0.6±0.02^{a}	0.8±0.02^{a}	0.65±0.03^{a}	1.3±0.05^{a}
Group 3 5% Elder berry	4. 1±0.01^{c}	0. 4±0.02^{a}	0.65±0.1^{c}	0. 6±0.01^{b}	1±0.01^{c}
Group 4 5% Echinacea	4.2±0.01^{b}	0.55±0. 01^{b}	0.65±0. 2^{c}	0. 5±0.02^{c}	1.2±0.02^{b}
Group 5 5% Astragalus	3.9±0.02^{e}	0.5±0.01^{c}	0.8±0.01^{a}	0.5±0.02^{c}	0.9±0.02^{d}
Group 6 5% Curry leaves	4±0.01^{d}	0. 4±0.02^{a}	0. 6 ±0.01^{d}	0.4±0.01^{e}	0.99±0.01^{c}
Group 7 5%Mixture	3.8±0.03^{f}	0.40±0.02^{d}	0.8±0.02^{a}	0.438±0.03^{d}	0.8±0.01^{e}
LSD	0.062	0.009	0.009	0.016	0.034

Means in the same column with different letters are significantly different.* Significance ($p < 0.05$).

- Feeding an experimental herb diets improved the weight of different internal organs, specially group 7.

B- Biochemical parametrs:

Table (10): (A) Effect of the hyperglycemic rats with parts of elderberry (*Sambucus nigra*), echinacea (*Echinacea purpurea*) , astragalus (*Astragalus hispidulus*) and curry leaves (*Murraya koenigii*) diets on serum levels of TC, TG and HDLc.

	TC (mg/dL)	**TG (mg/dL)**	**HDLc. (mg/dL)**
Group 1 control – ve	145±0.3^{c}	97.4±0.1^{g}	51.8±0.2^{a}
Group 2 control + ve	240.7±0.3^{a}	. 109.2±0.3^{e}	38.5±0.5^{g}
Group 3 5% Elderberry	143±0.1^{e}	110.6±0.4^{d}	48.4±0.4^{c}
Group 4 5% Echinacea	49±0.3^{b}	118.3±0.2 ^{b}	44.1±0.1^{c}
Group 5 5% Asrraglus	145.6±0.4^{c}	114.4±0.4^{c}	46.2±0.3 ^{d}
Group 6 5% Curry leaves	144.7±0.3^{d}	119.6±0.3^{a}	40±0.3 ^{f}
Group 7 5% Mixture	140.2±0.2^{f}	99.4±0.1^{a}	50.4±0.4 ^{b}
LSD	0.335	0.432	0.625

Means in the same column with different letters are significantly differents.* Significance ($p < 0.05$).

The mixture diet (group7) proved to be the best.

Table (10): (B) Effect of the hyperglycemic rats with parts of elderberry (*Sambucus niara*), echinacea (*Echinacea purpurea*) , astragalus (*Astragalus hispidulus*) and curry leaves (*Murraya koenigii*) diets on serum levels of LDLc, VLDLc. and AI

Groups	LDLc. (mg/dL)	VLDLc. (mg/dL)	AI (mg/dL)
Group 1 control – ve	74±[d]	19.5±0.1 [f]	1.8±0.01 [f]
Group 2 control + ve	190.4±0.6 [a]	21.8±0.2 [d]	7.45±0.02 [a]
Group 3 5% Elderberry	72±0.5 [e]	22.1±0.2[c]	1.96±0.02 [e]
Group 4 5% Echinacea	81.2±0.3 [b]	23.7±0. 3 [a]	2.38±0.03[c]
Group 5 5% Asrraglus	76.5±0.2 [c]	22.9±0.1 [b]	2.15±0.04[d]
Group 6 5% Curry leaves	80.8±0.2 [b]	23.9±0.1 [a]	2.62±0.02[b]
Group 7 5% Mixture	69.9±0.1 [f]	19.9±0.2 [e]	1.78±0.01[f]
LSD	0.551	0.226	0.045

Means in the same column with different letters are significantly different.
* Significance ($p < 0.05$).

The mixture diet (group7) proved to be the best.

- Results showed that the LPO contents in **Sambucus** , very low density lipoprotein (VLDL), LDL and high density lipoprotein (HDL) decreased significantly in the 2 h post-dose phase and thia agree with **Zakay-Rones *et al*., (2004).**
- Feeding rats on diets containing **Astraglus and Echinacea** , induced a significant decrease in plasma (LDL+VLDL)-cholesterol but not in HDL-cholesterol. Hepatic cholesterol and triacylglycerol were also reduced **(Barak *et al*., 2001)**
- The **Curry leavs** reduceD serum total cholesterol and LDL-cholesterol, as well as the atherogenic index, in hypercholesterolemic rats **(Nemeth and Bernath ., 2001).**

Table(11): Effect of feeding hyperglycemic rats with parts of elderberry (*Sambucus nigra*), echinacea (*Echinacea purpurea*) , astragalus (*Astragalus hispidulus*) and curry leaves (*Murraya koenigii*) diets on urea and uric acid in serum

Parameters / Groups	**Urea (U/ L)**	**Creatinine(U/L)**	**Uric acid (U/ L)**
	Mean±SD	Mean±SD	Mean±SD
Group 1 control – ve	22.6±0.1^{g}	0.76±0.01^{b}	3.1±0.1^{cd}
Group 2 control + ve	37.2±0.3^{b}	1.3±0.02^{a}	7±0.3^{a}
Group 3 5% Elder berry	25±0.2^{e}	0.65±0. 02 ^{d}	3.2±0.2^{cd}
Group 4 5% Echinacea	29.4±0. 1^{d}	0.68±0.01^{cd}	3.5±0.1^{bc}
Group 5 5% Astragalus	33.6±0.2^{c}	0.7±0.03^{c}	3.8±0.2^{b}
Group 6 5% Curry leaves	38.2±0.3^{a}	0.71±0.01^{c}	3.9±0.1^{b}
Group 7 5% Mixture	24.3±0.2^{f}	0.64±0.1^{d}	3±0.2^{d}
LSD	0.173	0.034	0.338

Means in the same column with different letters are significantly different.

* Significance ($p < 0.05$).

Data of table (11) revealed that the best treatment was that of group "7" (mixture of herbs diet)

- **Jung *et al.*, (2008**) showed that Treatment with **Astraglus** prevents deposits of calcium oxalate crystals formation and of micro calcifications in the kidney, and reduces the risk of fibrosis sub capsular.
- **Sambucus** produced a significant dose-dependant decrease in serum urea and creatinine levels, this results agree with **(Steffek, 2002**)
- **Ali (2008)** results agreed with that of table , he said **Echinacea** is rich in potassium may help to reduce kidney stones.

Table (12): Effect of feeding hyperglycemic rats with parts of elderberry (*Sambucus nigra*), echinacea (*Echinacea purpurea*) , astragalus (*Astragalus hispidulus*) and curry leaves (*Murraya koenigii*) diets on serum levels of AST, ALT and ALP enzyme

Parameters / Groups	**AST (U/ L)**	**ALT (U/ L)**	**ALP (U / L)**	**AST/ALT (U/L)**
	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Group 1 control – ve	48±0.2^{g}	35±0.1^{g}	111±0.5^{ab}	1.37±0.02^{C}
Group 2 control + ve	120±1^{a}	68±0.4^{a}	112±1^{a}	2.07±0.02^{a}
Group 3 5% Elder berry	52±0.2^{e}	41±0. 3 ^{e}	106±0.2^{c}	1.31±0..01^{d}
Group 4 5% Echinacea	57±0. 3^{c}	43±0.2^{d}	105±0.3^{c}	1.45±0.01^{b}
Group 5 5% Astragalus	55±0.4^{d}	46±0.5^{c}	99±0.5^{d}	1.26±0.02^{e}
Group 6 5% Curry leaves	65.9±0.3^{b}	47±0.2^{b}	110±1^{b}	1.45±0.01^{b}
Group 7 5% Mixture	49±0.4^{f}	39±0.3^{f}	98.01±0.29^{d}	1.33±0.02^{cd}
LSD	0.465	0.239	1.091	0.040

Means in the same column with different litters are significantly different.

* Significance ($p < 0.05$).

- Treatment of group 7 was the best.
- In human research for (**USDA-ARS, 2003**), , **Sambucus** (anthocyanins) lacked significant effects on levels of alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), and albumin; however, a significant change in plasma bilirubin was measured, but the clinical impact was minimal, as the bilirubin levels were still within normal physiological levels.
- Significant increase in plasmatic ALT and AST activities were observed in untreated I/R rats compared with sham-operated animals, whereas treatment with **(Curtis *et al.*, 2009) , Astraglus** rutin or l-arginine in I/R rats reduced hepatic damage.

- **Suman Singh *et al.*, (2014)** , they agreed **that Curry leaf** has tannins and carbazole alkaloids exhibited good hepato-protective properties.
- Liver enzymes activities were decreased to the normal levels found in control young rats with non statistical significance after administration of **(Echinacea**) and water**,** it could be found that serum total protein, albumin, globulin, AST and ALT levels were improved by administering Echinacea **(Mishima *et al.*, 2004**)

Table(13): Effect of feeding hyperglycemic rats with parts of elderberry (*Sambucus nigra*), echinacea (*Echinacea purpurea*) , astragalus (*Astragalus hispidulus*) and curry leaves (*Murraya koenigii*)diets on serum antioxidation enzyme activities

Parameters / Groups	**SOD (U/ mL)**	**GPX (U/ mL)**	**CAT(U/mL)**
	Mean±SD	Mean±SD	Mean±SD
Group 1 control – ve	40.6±0.4^{a}	44.6±0.4^{a}	82.1±0.1^{a}
Group 2 control + ve	16.7±0.0.3^{f}	20.5±0.2^{f}	80.5±0.1^{b}
Group 3 5% Elder berry	36±0.2^{c}	37.4±0. 1^{c}	80.2±0.2^{b}
Group 4 5% *Echinacea*	35.4±0. 2^{d}	35.6±0.2^{d}	77.5±0.3^{c}
Group 5 5% Astragalus	34±0.3^{e}	34.1±0.2^{e}	76.8±0.2^{d}
Group 6 5% Curry leaves	33.6±0.3^{e}	33.9±0.2^{e}	74.5±0.5^{e}
Group 7 5% Mixture	37.6±0.1^{b}	42.7±0.3^{b}	80.4±0.3^{b}
LSD	0.407	0.336	0.331

Means in the same column with different letters are significantly different.* Significance ($p < 0.05$).

- In table (13) the best treatment was that of mixture diet.
- A study of **Harokopakis *et al.*, (2006)** was carried supported the efficiency of ethanolic extract of **Sambucus** as antioxidant and anticancer agents for liver.

- **Mathur A, *et al.*,(2010)**, reported that oral administration of **Curry leaves** at a concentration of150 mg/kg b.w. daily to rats treated with iron for 31 prior to iron overloaded-administration significantly prevented the increases in liver, kidney and serum of iron, serum ferritin, serum transferrin levels, γ- GT, α-GST and γ-GT activities as well as serum NO and TNF- α level and hepatic MDA level as compared to iron-overloaded treated rats.
- **Ali (2008)** reported that **Echinacea** has protective effects on the liver against CCl4**.**
- **Astragalus** can reduce CCl4-induced liver fibrosis in rats as well as platelet-derived growth factor-stimulated hepatic stellate cell migration. **İlçim and Behçet, 2016)**

Table (14): Fasting serum glucose mmol\dl for negative control (1), positive control (2), and all treated groups as affected by some plant herbs

Variables	*(1)* **Negative control**	**(2) Positive control**	*(3)* **Elderberry** *5%*	*(4)* **Echinacea** *5%*	*(5)* **Astragalus** *5%*	*(6)* **Curry leaves** *5%*	*(7)* **Mixture of** ***plants*** *5 %*	***Sig***	***L.S.D*** **(p ≤ 0.05)**
	Mean SD±	**Mean SD±**	**Mean SD±**	**Mean SD±**	**Mean SD±**	**Mean SD±**	**Mean SD±**		
Glucose mmol\dl	88.5^{g} ± 0.3	280^{a} ± 0.1	158^{d} ± 0.5	169^{c} ± 0.2	180^{b} ± 1	151^{e} ± 0.4	149^{f} ± 0. 3	±	0.596

- Means in the same column with different litters are significantly different. Significant ($p \leq 0.05$).
- Data of table (14) showed that the best treatment was that of 5 % mixture diet (group7) .

-Histopathological results of hepatopatic rats fed on some plants diets :

a- Histopathological examination of Liver:

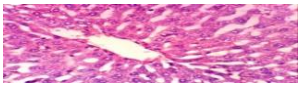

Photo. (1): Liver of rat from group 1(control "-") showing the normal histological structure of hepatic lobule .

Photo (2): Liver of rat hepatopathic from group 2 (control "+") showing cytoplasmic vacuolization of hepatocytes and multiple focal hepatic necrosis associated with inflammatory cells infiltration.

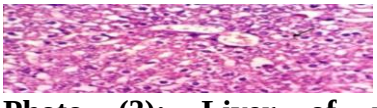

Photo (3): Liver of rat hepatopathic from group 3 (Sambucus diet) showing hydropic degeneration of hepatocytes .

(Echinacea diet) showing slight hydropic degeneration of some hepatocytes .

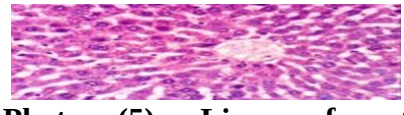

Photo (5): Liver of rat hepatopathic from group 5 (Astraglus diet) showing no histopathological alterations

Photo (6): Liver of rat hepatopathic from group 6 (Curry leaves diet) showing slight Kupffer cells activation..

Photo (7): Liver of rat hepatopathic from group 7 (Mixture diet) showing slight focal cytoplasmic vaculization of hepatocytes .

b- Histopathological examination of kidneys:

Photo (8): Kidney of rat from group 1 (control "-") showing the normal histological structure of renal parenchyma..

Photo (9) :Kidney of rat hepatopathic from group 2 (control "+") showing cytoplasmic vacuolization of epithelial lining renal tubules (short arrow) and congestion of renal blood vessels (long arrow)

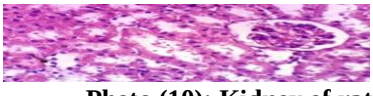

Photo (10): Kidney of rat hepatopathic from group 3 (elderberry diet) showing slight cytoplasmic vacuolization of epithelial lining some renal tubules (arrow)

Photo (11): Kidney of rat hepatopathic from group 4 (Echinacea diet) showing necrobiosis of epithelial lining some renal tubules (arrow) .

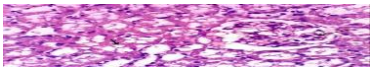

Photo (12): Kidney of rat hepatopathic from group 5 (Astraglus diet) showing cytoplasmic vacuolization of epithelial lining renal tubules (short arrow) and endothelial lining glomerular tuft (long arrow) .

Photo (13): Kidney of rat hepatopathic from group 6 (Curry leaves diet) showing cytoplasmic vacuolization of epithelial lining renal tubules (arrow) .

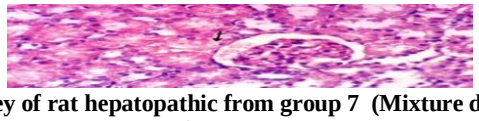

Photo (14): Kidney of rat hepatopathic from group 7 (Mixture diet) showing very slight cytoplasmic vacuolization of epithelial lining some renal tubules (arrow)

D-Diabetic rats

A-Histopathological examination of Liver:

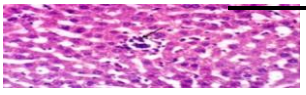

Photo (15): Liver of rat diabetic from group 2 (control"+") showing focal hepatocellular necrosis associated with inflammatory cells infiltration.

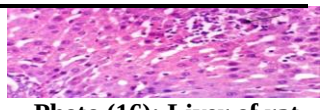

Photo (16): Liver of rat diabetic from group 3 (Sambucus diet) showing focal hepatocellular necrosis associated with inflammatory cells infiltration .

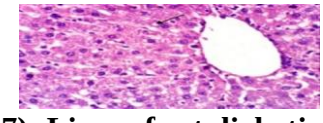

Photo (17): Liver of rat diabetic from group 4 (Echinacea diet) showing slight cytoplasmic vacuolization of hepatocytes.

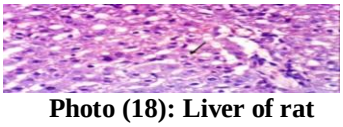

Photo (18): Liver of rat diabetic from group 5 (Astraglus diet) showing cytoplasmic vacuolization of hepatocytes

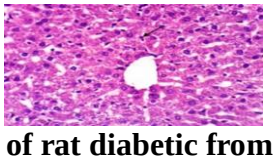

Photo (19): Liver of rat diabetic from group 6 (Curry leaves) showing Kupffer cells activation

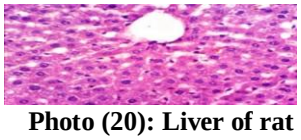

Photo (20): Liver of rat diabetic from group 5 (Astraglus diet) showing no histopathological alterations .

b-Histopathological examination of kidneys:

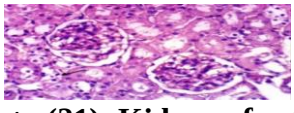

Photo (21): Kidney of rat diabetic from group 2 (control "+") showing slight cytoplasmic vacuolization of epithelial

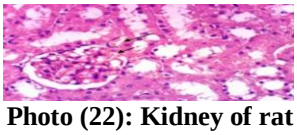

Photo (22): Kidney of rat diabetic from group 3 (Sambucus diet) showing slight congestion of glomerular tuft (short arrow) and intertubular blood capillaries (long arrow) .

Photo (23): Kidney of rat diabetic from group 4 (Echinacea diet) showing no histopathological changes

Photo (24): Kidney of rat diabetic from group 5 (Astraglus diet) showing no histopathological changes

Photo (25): Kidney of rat diabetic from group 5 (curry leavs diet) showing slight congestion of

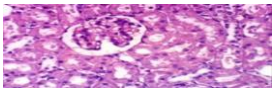

Photo (26): Kidney of rat diabetic from group 6 (mixture diet) showing no histopathological changes

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تخفيف التسمم الكبدى وارتفاع جلوكوز السيرم بواسطة اغذية تحتوى على اعشاب

فاطمة الزهراء أمين الشريف, مى الحسينى خفاجى , عزة إبراهيم السيد
قسم التغذية وعلوم الأطعمة –كلية الإقتصاد المنزلي – جامعة المنوفية- مصر

المخلص العربي

تم إجراء الدراسة الحالية لمعرفة التأثيرات المحتملة لنباتات الخمان الأسود والقنفذية الأجوانية والقتاد الأشعث واوراق الكارى علي مرضى الكبد والسكر في بعض الفئران المصابة برابع كلوريد الكربون والالوكسان. تم استخدام 78 فأر أبيض بالغ يتراوح وزن الفأر (150-160) جرام، تم تغذيتها على الوجبة الأساسية لمدة أسبوع ثم قسمت بعد ذلك إلى مجموعتين متساويتين ، المجموعة الاولى تم إصابتها تجريبيا باستخدام 2مجم/كجم من وزن الجسم رابع كلوريد الكربون مره يوميا لمدة 4 ايام والمجموعة الثانية , ثم حقنها بالالوكسان لمدة يومين بهدف اصابة الفئران بارتفاع جلوكوز الدم . وأضيفت المواد النباتية المستخدمة كل على حدة بنسبة 5% من الوجبة وكذلك خليط منهما بنسبة 5%. استمرت التجربة لمدة 28 يوم وفى نهاية التجربة تم وزن الفئران ثم ذبحهم وتجميع عينات الدم بعد صيام 12 ساعة ثم تقدير وظائف الكبد (الجلوتاميك أوكسالو أستيك ترانس أمينيز، الجلوتاميك بيرفيك ترانس أمينيزوالألكالين فوسفاتيز) ، ووظائف الكلى (اليوريا وحمض اليوريك والكرياتينين) و الكولسترول الكلى و الجلسريدات الثلاثية,(HDL-c LDL-c and VLDL-c) ومؤشر (AI) وسكر الدم ثم تم فصل الأعضاء الداخلية (الكبد والكلى والطحال والرئتين والقلب)ووزنها وتم حفظ الكبد والكلي في فورمالين 10% متعادل لإجراء الفحوص الهستوباثولوجية وأيضا تم تقدير وزن الجسم المكتسب، والمأخوذ من العلف ونسبة الاستفادة من الغذاء. وقد أظهرت الدراسة أن تناول هذه النباتات وخليطها قد نتج عنه زيادة معنوية فى كل من وزن الفئران ونسبة الاستفادة من الغذاء وكذلك زيادة نسبة البروتين الدهني عالي الكثافة و و كذلك تحسن في وظائف الكبد و الكلى وجلوكوز السيرم سواء للمرضي أو للأصحاء ولذلك يمكن استخدام هذه المواد النباتية لتحسين وظائف الكلى والكبد ودهون الدم .

الكلمات المفتاحية: مرض الكلى، انزيمات الكلى ،إنزيمات الكبد ، الفحص الهستوباثولوجى