Protective Effect of Echinacea (Echinacea Angustifolia) , Rosemary (Rosmarinus Officinalis, L.) and Dandelion (Taraxacum Officinal) Powder in Alloxan-Diabetic Rats

By

Hala M.A. Wahba

Home Economics Department,

Faculty of Specific Education, Minufiya University

hala.ali77@yahoo.com

Research Journal Specific Education

Faculty of Specific Education

Mansoura University

ISSUE NO. 69, JULY , 2022

Protective Effect of Echinacea (Echinacea Angustifolia) , Rosemary (Rosmarinus Officinalis, L.) and Dandelion (Taraxacum Officinal) Powder in Alloxan-Diabetic Rats

*Hala M.A. Wahba**

Abstract:

This study was performed to assess the Protective effect of Echinacea (EP) , Rosemary (RP) and Dandelion(DP) powder on body weight, blood glucose (BG), insulin, immune status, total cholesterol (TC), triglycerides (TG) and kidney function parameters as well as on activities of renal tissue antioxidant enzymes in diabetic rats. sixty three mature Sprague Dawley male rats were randomized into 6 main groups (n= 7) they are divided into sub-groups. Group (1) was used as a negative control and fed on basal diet, while the remaining 5 groups were rendered diabetic by intraperitoneal injection of alloxan (120 mg/kg). After induction of diabetes, group (2) was kept positive control, while groups (3), (4) ,(5) and (6) were divided into group A, B were fed on diet supplemented with 5 % , 10% EP; RP ; DP and 5% from (EP+RP+DP) respectively for 10 weeks. Blood samples were collected for estimation of differential leucocytic count and activities of antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) in kidney tissues . Insulin, TC and TG levels, blood urea nitrogen (BUN), uric acid and creatinine (Cr) serum levels were also determined. The results showed that diet supplementation with EP,RP and DP significantly increased body weight and improved feed efficiency ratio of diabetic rats. Diet supplementation with EP,RP and DP decreased the number of lymphocytes, production of TNF-α and cytokines IL4 and IL8; normalized BG, insulin, TC and TG and BUN and Cr levels. There were enhanced the activity of SOD, GPx and CAT enzymes in renal tissues of diabetic rats. In conclusion, diet supplementation with EP,RP and DP has immunostimulant, antidiabetic, hypolipidemic, nephroprotective and

* Home Economics Department, Faculty of Specific Education, Minufiya University

antioxidant activities in diabetic rats. Therefore, intake of extract of EP,RP and DP may be beneficial for patients who suffer from diabetes mellitus.

Keywords: Echinacea, Dandelion, Rosemary, Immunostimulant, Antidiabetic, Antioxidant.

1. INTRODUCTION

The metabolic disorder of Diabetes mellitus (DM) is frequently diagnosed by hyperglycemia and lipid abnormalities leading to vascular disorders. Hyperglycemia occurs when the cells become unable to use glucose and/or the liver and skeletal muscles cannot store glycogen **(Luis-Rodríguez *et al.*, 2012)**. Activation of the innate immunity evident by increased secretion of inflammatory cytokines has been shown to be relevant to the pathogenesis of DM type 2 **(Jin *et al.*, 2013**; **Itariu and Stulnig , 2014)**. In addition, DM induced oxidative stress via generation of reactive oxygen species (ROS) that are causally linked to the development of cardiovascular morbidity and mortality **(Sturza *et al.*, 2015)**.

Rosmarinus bureinalis L. Extract (RE) from a rat normally caused by streptozotocin (STZ) diabetes **Rasoulian *et al.*,(2019) .** Echinacea purpurea extracts may enhance immunity is through modulating interferon-associated macrophage pathways and their effect may be stimulatory or suppressive **Senchina *et al.*, (2010) and (Torkan et al.2015),** Echinacea purpurea is an important medicinal plant with immunostimulatory and anti-inflammatory activities (**Khalaf *et al.*, 2019).**

Rosemary (Rosmarinus oficinalis L.) is one of the most important plants which has many benefits, with 1,8-cineole, camphor, α-pinene and α-terpineol as the principal volatile compounds . Rosemary is used in the food industry as flavoring agent ***(Gaya et al., 2013).*** In folk hygiene, R. Officeinalis has been used to treat headaches, loss of breathing, epilepsy, mild anti-inflammatory, analgesic conditions and essential Rosemary oils have strong antioxidant and antimicrobial characteristics and harm treatment activity **(Yu *et al.*, 2013 and Rašković *et al.*,2014**) ; Handling of the diabetic rats with RE developed hyperglycemia, hyperalgesia and motor

deficit, suppressed caspase-3 activation and reduced the Bax **(Rasoulian et al., 2019).**

Rosmarinus officinalis L. (Lamiaceae) is a herb which is widely used as an antiflammatory, diuretic, antimicrobial aroma, mainly because of its use in traditional medicine, as well as for the treatment and prevention of diseases(**Pérez-Mendoza et *al.*, (2019) and Rola *et al* . , 2019**).

Dandelion (Taraxacum officinale) dietary enhanced intestinal immunity through increasing goblet cells numbers and adaptable expression of immune-related genes and antioxidant abilities and intestinal barrier in golden pompano **(Xiaohong *et al.*, 2017**) ; Dietary Dandelium (Taraxacum officinale) presented significant rises in total plasma protein, four-complement content and alkaline phosphatase activities, lysozymes, reductase glutathione (GSR), but significant decreases compered to control group triglycerides, LDL cholesterol, malondialdehyde (MDA) **(Tan *et al.*, 2017).**

The present study was designed to evaluate the effect of fortification of diets with Echinacea *powder* , Rosemary *powder and* Dandelion *powder* on body weight, immune status; blood glucose, insulin, triglycerides and total cholesterol levels as well as function of kidney parameters and the activity of renal antioxidant enzymes in diabetic rats.

2. MATERIALS AND METHODS

2.1. Plant

Echinacea (Echinacea Angustifolia), Rosemary and Dandelion (Taraxacum Officinal) were bought from the local spice shops, Cairo , Egypt and were cleaned, sun- dried under vacuum at low temperature) and milled to fine powder. Both plant papers have been mixed and turned into a fine powder. The basic diets were supplemented with each powder at 5 % and 10% concentrations and combination of both powders at 5 % to formulate the experimental diets.

2.2. Rats

Sixty-three (63) Sprague — Dawley, 255-265 grams of male albino rats, have been used in this study. The rats were bought from Animal Colony Laboratory in Helwan, Egypt. The rats were fed basal and experimental diets during the study, and water was ad libitum.

2.3. Alloxan and biochemical kits

Alloxan was bought from El-Gomhoryia Company for Chemicals; Cairo, Egypt. Kits for biochemical determinations of blood glucose and insulin hormone Kits (enzyme-linked immunoassay) were obtained from Gamma Trade Company, Egypt

2-4 - Experimental diets :

The dietary supply of protein, fat, carbohydrates, vitamins and minerals was in accordance with the recommended dietary allowances for rats (American Institute of Nutrition, AIN) according to **Reeves *et al.* (1993)**.

2.5. Experimental design

Rats were randomized into to 6 groups of 7 animals each. Group (1) was used as negative control and fed on basal diet, while the other 5 groups were rendered diabetic. Diabetes mellitus was induced by single intraperitoneal injection of alloxan in a dose of 120 mg/kg/day as described by **Ashok *et al*., (2007)**. After diabetes, group (2) was maintained positive (diabetic) control and fed on basal diets, while groups (3a and 3b), (4a and 4b), (5a and 5b) and (6) had fed experimental diets with 5% EP supplements; 10% EP; 5% RP; 10% RP and 5% DP+10% DP respectively for a period of 10 weeks. the initial and final body weights of rats were recorded during the feeding period and changes were calculated in their body weight and feed efficiency. Blood samples have been collected for biochemical analysis at the end of the experiment.

2.6. Biochemical analyses

Blood samples were taken at the end of the experiment from all of the previously stated groups following a 12-hour fast. Blood was collected

into a dry clean centrifuge tube and allowed to coagulate in a water bath (37c) to separate the serum from the blood, it was centrifuged at 3000 rpm for 10 minutes enzymatic kit according to **Siest *et al*. (1981)** and Insulin was estimated using radioimmunoassay (RIA) assay **(Yallow and Bauman, 1983).** Serum levels of total cholesterol and triglycerides were chemically determined according to **Ratliff and Hall (1973)** and **Jacob and Van-Denmark (1963),** respectively.

2.7. Differential leucocytic count

Freshly collected blood samples of 20µl were spread on clean slides as a thin film. Each smear was left to air dried and fixed with methanol for 2–3min and then labelled. Blood smears were stained with 10% Giemsa's stain (Aldrich), examined under light microscopy and different types of blood leukocytes were counted **(Schalm *et al*., 1975)**.

2.8. Assay of tissue antioxidant enzymes

Kidney homogenates were centrifuged at 4000 rpm for 10 minutes at 4°C and the supernatants were used to assay the activity of antioxidant enzymes glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT) according to **Paglia and Valentaine (1979), Spitz and Oberley (1989), and Sinha (1972)**, respectively.

2.9. Kidney function

blood urea nitrogen (BUN) was determined using BioMérieux kits according to the method of **Patton and Crouch, (1977).** Serum uric acid (UA) was determined using the enzymatic colorimetric method as described by **Fossati *et al*., (1980)**. Serum creatinine (Cr) concentration was colorimetrically determined by the Jaffe reaction according to the method of **(Husdan and Rapoport, 1968).**

2.10- Histopathological study

kidneys specimens only were collected from rats of all experimental groups at the end of the experimental period, fixed in 10% neutral buffered formalin (pH=7.0), dehydrated in ethyl alcohol, then cleared in xylol and embedded in paraffin; 4-6 microns thickness sections prepared and stained

with heamtoxylin and eosin for examining the liver using light microscope at various magnification **(Carleton , 1976).**

2.11. Statistical analysis

Data were presented as means ± SD. Statistical analysis was performed using computerized Statistical Package of Social Sciences (SPSS) program with one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests according to **Snedecor and Cochran (1986).**

3. RESULTS AND DISCUSSION

The present study aimed to evaluate the effect of diet supplemented with EP, RP and DP and their combination on body weight, A number of herbal products are known to facilitate enhancement of the immune system. These include Echinacea, garlic, cat,s claw,

astragolus, licorice and other herbs. In comparison to negative (normal) control rats, the diabetic rats gained less weight and had lower feed efficiency. Diabetic rats fed substantially ($P < 0.05$) on diets supplemented with EP, RP, and DP, increasing body weight and enhancing feed effectiveness compared to the positive (diabetic) control (Table 1).

Results of the present study showed that diets fortified with EP, RP and DP and their mixture enhanced body weight improvement and enhanced feed efficiency ratio in diabetic rats. These result were in agreement with those reported by **Tan *et al* .,(2017)** they showed that supplement with dandelion powder could significantly increase final body weight (FBW), weight gain rate (WGR), specific growth rate (SGR), feed efficiency ratio , feed intake , protein efficiency ratio and protein deposit rate ($P < 0.05$).

Data in Table (2) and Fig(1)showed that Increased blood glucose and reduced insulin levels in animals, diabetes when compared to the normal control non diabetic group. Diets added with EP, RP and DP and their combination significantly decreased blood glucose and increased insulin levels as compared with the positive (diabetic) control group. This is in accordance with our data, where alloxan injection induced significant increase in blood glucose levels versus the control group. Blood glucose was significantly reduced after feeding diets added with EP, RP and DP and

their combination compared to the normal and diabetic groups. These results were agreement with those ***Richter et al.,*** **(2012)** and **Rasoulian *et al.,(*2019)** they reported that RP and DP reduce hyperglycemia

The results of ELISA test showed that diabetes mellitus in rats induced significant ($P < 0.05$) increases in serum tumor necrosis factor alpha (TNF-α) and inflammatory cytokines IL4 and IL8. Experimental diets supplemented with EP, RP and DP and their combination significantly ($P < 0.05$) decreased the elevated levels of serum TNF-α, IL4 and IL8 in diabetic rats when compared to the positive group (Table 3).

The high percentage of oxygenated monoterpenes in both important oils play an vital role in the antioxidant and healing potential observed herein, Rosemary important oils in chitosan-based preparations in suitable combination could efficiently promote different stages of wound healing **Labib *et al.*, (2019).**

Table 1: Effect of diets with Echinacea (EP *)* , Rosemary (RP),*and* Dandelion (DP) *on* body weight gain (BWG) and feed efficiency ratio (FER) in diabetic rats.

Groups	Parameters			
	Initial b.wt.	Final b.wt	BWG	FER
Control (-)	265.0±7.5[a]	301.0±7.3[a]	36	2.1±0.02[a]
Control (+)	255.0±7.3[a]	280.0±6.1[c]	25	1.38±0.03[c]
Group 3a (5% EP)	251.0±7.6[a]	285.0±4.2[b]	34	1.7±0.03[b]
Group 3b(10% EP)	253.5±6.9[a]	289.0±6.3[b]	35.5	1.6±0.04[b]
Group 4a (5% RP)	252.0±7.1[a]	270.0±5.9[b]	18	1.8±0.03[b]
Group 4b (10% RP)	254.5±6.9[a]	275.0±5.9[b]	21	1.2±0.02[b]
Group 5a (5% DP)	250.5±6.9[a]	280.0±5.9[b]	29.5	1.1±0.03[b]
Group 5b (10% DP)	259.5±6.9[a]	265.0±5.9[b]	5.5	0.55±0.02[a]

Means ± SD with different superscript letters in the same column are significant at $P < 0.05$ using one way ANOVA test. n=7 rats.

The present results correspond with these effects were previously reported for received Echinacea, DP and these extracts might have appreciable immunostimulatory activity of phagocytosis and IgM ($P < 0.05$)

(Isbaniah *et al.*, 2011 and **Torkan *et al.*, 2015)** ; **Rostami *et al.*,(2018**) suggest that dietary RP and VE additives can interact and modulate the humoral immunity of broilers.

Table 2: Effect of diets with Echinacea (EP) , Rosemary (RP),and Dandelion (DP) on blood glucose levels (BG) and insulin hormone in diabetic rats.

Parameter	Groups	
	BG (mg/dL)	Insulin (µU/ml)
Control (-)	112.7 ± 1.9^{d}	45.5 ± 0.1^{a}
Control (+)	320.0 ± 3.1^{a}	16.3 ± 0.1^{d}
Group 3a (5% EP)	222.0 ± 1.5^{b}	28.1 ± 0.1^{c}
Group 3b(10% EP)	210.5 ± 2.3^{b}	27.2 ± 0.2^{c}
Group 4a (5% RP)	215.0 ± 2.1^{b}	25.8 ± 0.2^{b}
Group 4b(10% RP)	210.6 ± 3.1^{b}	29.8 ± 0.1^{c}
Group 5a (5% DP)	260.6 ± 5.1^{b}	34.8 ± 0.1^{b}
Group 5b (10% DP)	255.6 ± 3.1^{b}	30.8 ± 0.1^{b}
Group 6 (5% EP+5% RP + DP5%)	195.0 ± 1.3^{c}	43.3 ± 0.1^{a}

Means ± SD with different superscript letters in the same column are significant at $P < 0.05$ using one way ANOVA test. n=7 rats.

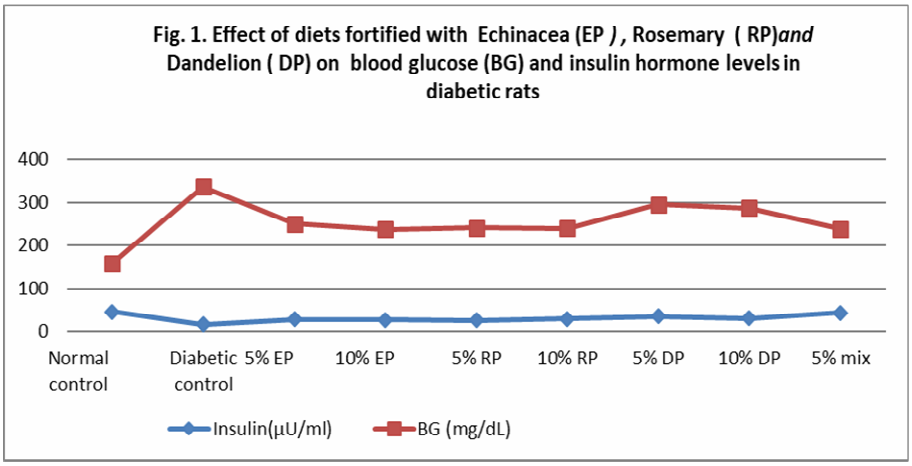


Table 3: Effect of diets with Echinacea (EP) , Rosemary (RP),and Dandelion (DP) on serum tumor necrosis factor alpha (TNF-α) interleukins IL-4 and IL-8 in diabetic rats.

Groups	Serum concentration of		
	TNF-α (ng/ ml)	IL4 (pg/ml)	IL8 (pg/ml)
Control (-)	1.99 ± 0.02^{e}	856.8±3.5^{c}	255.4±5.3^{d}
Control (+)	4.92 ± 0.07^{a}	945.4±5.3^{a}	645.1±4.3^{a}
Group 3a (5% EP)	2.66 ± 0.02^{c}	810.8±5.2^{d}	332.2±5.3^{c}
Group 3b (10% EP)	2.69 ± 0.03^{c}	813.2±4.2^{d}	335.3±5.4^{c}
Group 4a (5% RP)	3.06 ± 0.02^{b}	844.5±2.5^{b}	425.1±5.3^{b}
Group 4b (10% RP)	3.34 ± 0.01^{b}	845.2±5.2^{b}	427.1±3.2^{b}
Group 5a (5% DP)	2.35 ± 0.01^{b}	830.2±5.2^{b}	440.1±4.2^{b}
Group 5b (10% DP)	2.34 ± 0.01^{b}	845.2±5.2^{b}	428.1±3.2^{b}
Group 6 (5% EP+5% RP + DP5%)	2.00 ± 0.02^{d}	821±3.2^{c}	242.2±5.3^{d}

Means ± SD with different superscript letters in the same column are significant at $P < 0.05$ using one way ANOVA test. n=7 rats.

Table 4: Effect of diets with Echinacea (EP *) , Rosemary (RP),and* Dandelion (DP) on differential leukocyte count in diabetic rats.

Groups	Parameters			
	Monocytes (%)	Neutrophils(%)	Eosinophils (%)	Lymphocytes(%)
Control (-)	58.4±3.2^{b}	6.6±0.5 ^{b}	25.6±0.5^{a}	8.4±0.8^{b}
Control (+)	62.2±2.4^{a}	6.5±0.3 ^{a}	20.4±0.6^{b}	11.8±0.2^{a}
Group 3a (5% EP)	60.8±4.2^{b}	6.3±0.2 ^{a}	19.8±0.6^{b}	10.60±0.2^{a}
Group 3b(10% EP)	59.2±4.4^{b}	6.2±0.2 ^{b}	19.9±0.5^{b}	10.30±0.2^{a}
Group 4a (5% RP)	60.7± 2.4^{b}	5.9± 0.2 ^{a}	21.9±0.9^{b}	8.70±0.3^{a}
Group 4b(10% RP)	59.5±2.6^{b}	6.8± 0.3 ^{a}	19.5±0.6^{b}	10.80±0.2^{a}
Group 5a (5% DP)	61.8±2.4^{b}	5.2± 0.2 ^{b}	20.9±0.9^{b}	8.80±0.3^{a}
Group 5b (10% DP)	60.9±2.5^{b}	6.9± 0.2 ^{b}	18.9±0.9^{b}	9.70±0.3^{a}
Group 6 (5% EP+5% RP + DP5%)	58.6.±3.6^{b}	6.2 ±0.4 ^{b}	20,3 ±0.8^{b}	10.50±0.4^{a}

Means ± SD with different superscript letters in the same column are significant at $P < 0.05$ using one way ANOVA test. n=7 rats.

Feeding experimental diets supplemented with EP, RP and DP and their combination increased neutrophils and Eosinophils percentages, but decreased lymphocytes and monocytes percentage when compared with the normal control group (Table, 4).

Table (5) Fig. (2) and show effect of EP, RP and DP and their combination on diabetes rats (total proteins, albumin (Alb) and globulin (Glb)) . It was revealed that (TP, Alb and GLb (g/dl) for control (+) were significantly less than that of control (-). Also it is clear that groups of EP, RP and DP and their combination treatments showed significantly differences ($P<0.005$) higher levels compared with control (+)respectively for (total proteins, albumin (Alb) and globulin (Glb).

In rats, Diabetes in serum triglycerides (TG) and total cholesterol (TC) have increased significantly. Diets supplementation with EP, RP and DP and their combination significantly decreased the elevated serum levels of TC and TG as compared with the positive (diabetic) control group (Table 6) and Fig (3). Feeding diet supplemented with EP, RP and DP and their combination produced hypocholesterolemic and hypolipidemic activities. These results were agree with those reported by **Xue *et al.*, (2008)** for DP and their combination and by **Richter *et al.*, (2012)** for RP ; anti-inflammatory , antioxidant and lipid decreasing properties in rosmary and its principal components ; Rosemary in the prevention and management of dyslipidemia and other cardio-metabolic diseases **Farkhondeh *et al.*, (2019).**

The present results explain that diet supplemented with EP, RP and DP and their combination significantly improved kidney function as Elevated blood urea nitrogen (BUN) uric acid and creatinine (Cr.) as recorded in Table (7).

These effects were previously reported by **(Erboga *et al.*, 2015 and Park *et al.*, 2019**) DP and their combination (EP, RP and DP) feeding to diabetic rats produced nephroprotective and renal tissue antioxidant effects.

The results showed that feeding diet supplemented with EP, RP and DP and their combination to diabetic rats significantly increased the activity of renal tissue antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) as recorded in Table (8). The present results showed that DP and their combination feeding to diabetic rats produced nephroprotective and renal tissue antioxidant effects. These effects were previously reported for DP and their combination **(Louay *et al*., 2014)**

Table (5): Effect of diets with Echinacea (EP *)* , Rosemary (RP),*and* Dandelion (DP) on serum levels of total proteins (TP), albumin (Alb) and globulin (Glb) in diabetic rats.

Groups	Parameters		
	TP(g/dL)	Alb (g/dL)	Glb (g/dL)
Control (-)	8.30±0.03^{a}	4.40±0.14^{a}	3.40±0.10^{a}
Control (+)	3.53±0.02^{e}	3.15±0.12^{d}	1.95±0.02^{d}
Group 3a (5% EP)	7.85±0.05^{b}	3.60±0.11^{b}	3.10±0.02^{a}
Group 3b(10% EP)	8.35±0.04^{a}	4.10±0.21^{a}	3.20±0.01^{a}
Group 4a (5% RP)	5.56±0.07^{d}	3.21±0.13^{b}	2.61±0.04^{c}
Group 4b(10% RP)	6.45±0.03^{c}	3.78±0.16^{b}	2.66± 0.01^{c}
Group 5a (5% DP)	6.35±0.04^{c}	3.10±0.21^{d}	2.20±0.01^{b}
Group 5b (10% DP)	7.55±0.07^{b}	3.20±0.13^{b}	2.90±0.04^{ab}
Group 6 (5% EP+5% RP + DP5%)	8.45±0.04^{a}	4.20±0.21^{a}	3.50±0.01^{a}

Means ± SD with different superscript letters in the same column are significant at $P < 0.05$ using one way ANOVA test. n=7 rats.

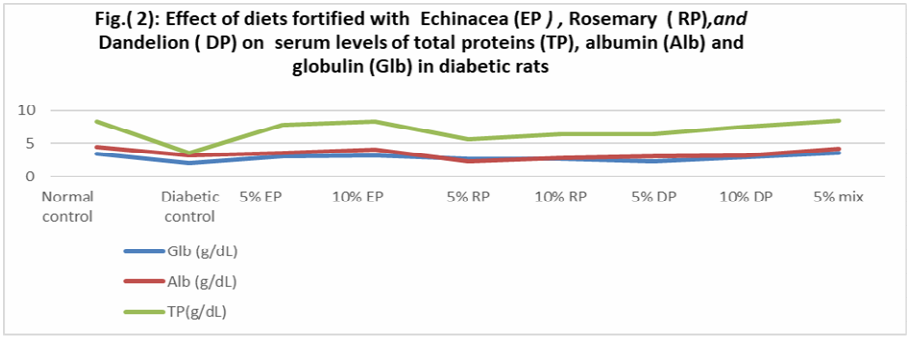


Table 6: Effect of diets with Echinacea (EP) , Rosemary (RP),*and* Dandelion (DP) on serum total cholesterol (TC) and triglycerides (TG) levels in diabetic rats.

Groups	Parameter	
	TC (mg/dL)	TG (mg/dL)
Control (-)	99.50 ± 3.1^{c}	79.00 ± 3.4^{d}
Control (+)	200.70 ± 4.1^{a}	108.00 ± 5.2^{a}
Group 3a (5% EP)	182.80 ± 4.8^{b}	101.00 ± 3.1^{b}
Group 3b(10% EP)	175.60 ± 3.7^{b}	99.50 ± 4.5^{b}
Group 4a (5% RP)	150.50 ± 3.6^{bc}	95.50 ± 4.3^{b}
Group 4b(10% RP)	149.70 ± 3.6^{bc}	81.50 ± 3.6^{c}
Group 5a (5% DP)	189.70 ± 4.6^{b}	102.50 ± 4.6^{c}
Group5b(10% DP)	188.50 ± 3.6^{b}	101.51± 3.5 ^{b}
Group 6 (5% EP+5% RP + DP5%)	102.81 ± 3.8^{c}	79.55 ± 3.1^{d}

Means ± SD with different superscript letters in the same column are significant at $P < 0.05$ using one way ANOVA test. n=7 rats.

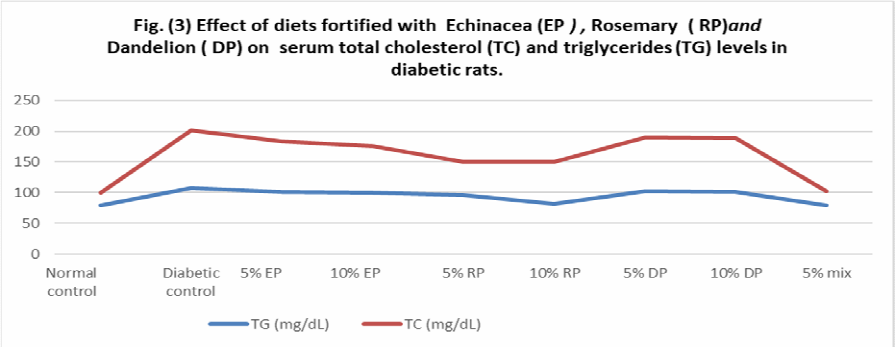


Table 7: Effect of diets with Echinacea (EP) , Rosemary (RP),*and* Dandelion (DP) on Blood urea nitrogen (BUN), uric acid (UA) and creatinine (Cr.) in diabetic rats.

Groups	Parameters		
	BUN(mg/dL)	UA(mg/dL)	Cr(mg/dL)
Control (-)	20.0 ± 1.4^{d}	2.50 ± 0.01^{b}	0.75 ± 0.01^{d}
Control (+)	46.0 ± 2.4 ^{a}	4.49 ± 0.06^{a}	1.64 ± 0.04^{a}
Group 3a (5% EP)	22.1 ± 2.6 ^{c}	1.48 ± 0.02^{a}	1.03 ± 0.02^{b}
Group 3b(10% EP)	25.8 ± 2.3 ^{bc}	2.49 ± 0.04^{b}	1.22 ± 0.02^{b}
Group 4a (5% RP)	31.5 ± 2.1^{b}	2.50 ± 0.01^{a}	1.0 ± 0.03^{b}
Group 4b(10% RP)	29.4 ± 2.7^{b}	2.47 ± 0.05^{b}	0.94 ± 0.02^{c}
Group 5a (5% DP)	30.4 ± 2.5^{b}	2.98 ± 0.05^{b}	1.04 ± 0.02^{b}
Group 5b (10% DP)	28.4 ± 2.6^{b}	2.97± 0.05^{b}	.99 ± 0.02^{c}
Group 6 (5% EP+5% RP + DP5%)	20.6 ± 1.9^{d}	2.48 ± 0.01^{b}	0.92 ± 0.01^{c}

Means ± SD with different superscript letters in the same column are significant at $P < 0.05$ using one way ANOVA test. n=7 rats.

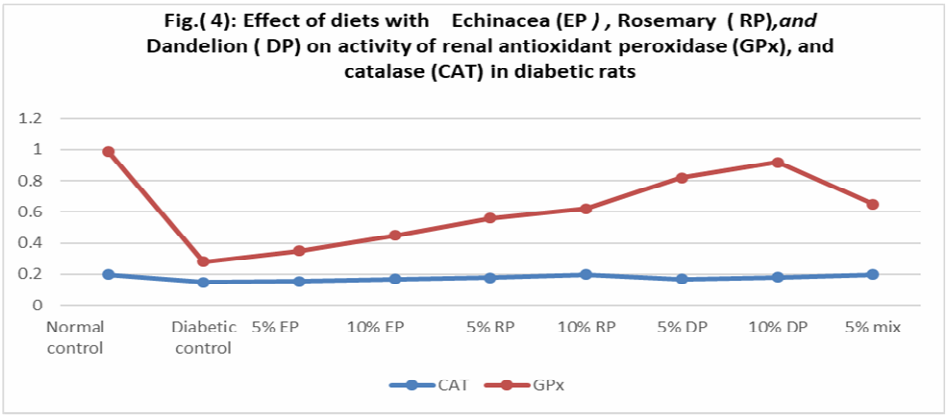
Table 8: Effect of diets with Echinacea (EP *)* , Rosemary (RP),*and* Dandelion (DP) on activity of renal antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) in diabetic rats.

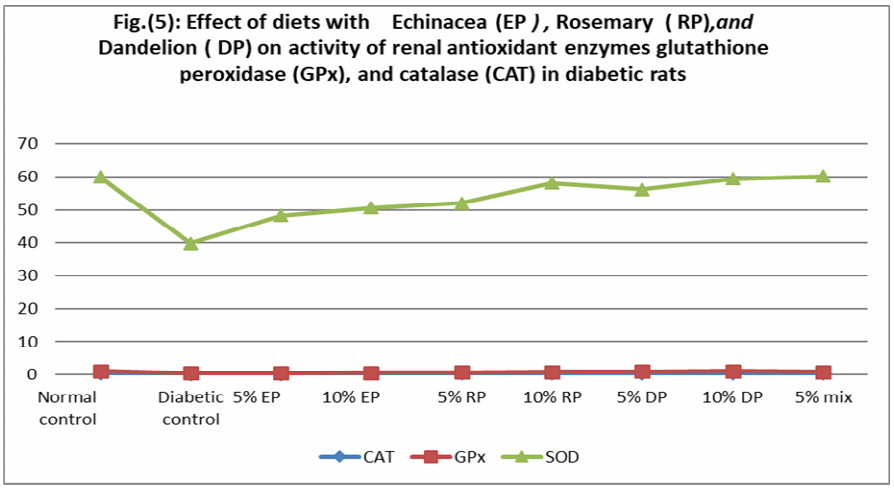
Groups	Parameters		
	SOD (U/mg protein)	GPx (nmol/min/mg protein)	CAT (nmol/min/mg protein)
Control (-)	58.70 ± 2.22^{a}	0.99 ± 0.01^{a}	0.198 ± 0.01^{a}
Control (+)	39.50 ± 2.68^{d}	0.28 ± 0.04^{d}	0.148 ± 0.02^{d}
Group 3a (5% EP)	47.74 ± 3.26^{c}	0.35 ± 0.03^{c}	0.155 ± 0.01^{c}
Group 3b(10% EP)	49.95 ± 2.38^{c}	0.45 ± 0.01^{c}	0.169 ± 0.01^{b}
Group 4a (5% RP)	51.25 ± 2.83^{b}	$0.56 \pm 0.01b^{c}$	0.178 ± 0.02^{b}
Group 4b(10% RP)	57.24 ± 3.23^{b}	$0.62 \pm 0.01b^{c}$	0.199 ± 0.02^{a}
Group 5a (5% DP)	55.24 ± 3.24^{b}	0.82 ± 0.01^{b}	0.169 ± 0.02^{b}
Group 5b (10% DP)	58.24 ± 4.24^{b}	0.92 ± 0.01^{c}	0.179 ± 0.02^{b}
Group 6 (5% EP+5% RP + DP5%)	59.30 ± 4.53^{b}	0.65 ± 0.01^{c}	0.199 ± 0.01^{a}

Means ± SD with different superscript letters in the same column are significant at $P < 0.05$ using one way ANOVA test. n=7 rats.

Unit of GPx = nmol of GSH utilized/min/mg protein.

Unit of CAT = nmol of H_2O_2 utilized/min/mg protein





Histopathological examination of Microscopically kidneys of rat the best effect of plants from group 1 (control -) showed the normal histology of renal parenclyme (Photo 1). On the other hand, kidneys of rat from group 2 (control +) revealed congestion of renal blood vessels and presence of easinophilic proteinaceous casts in the lumen of renal tubules (Photo 2). However kidneys sections from rats in groups 3a, (Echinacea (EP) revealed apparent normal renal parenclyme with no histopathological changes (Fig. 3). Meanwhile, kidneys of rat from group 4 a (Rosemary (RP) showed presence of proteinaceous cast in the lumen of some renal tubules (**Photo**. 4). Kidney of rat from group 5 a Dandelion (DP) revealed atrophy of some glomeruler tufts (**Photo** 6). while, kidneys of rat from group 6 (5% mix of herbs)) showed no changes except granularity of the cytoplasm of epithelial lining renal tubules (Photo 6).

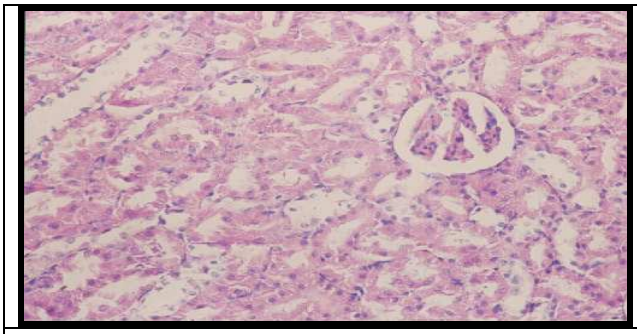


Photo (1): Kidney of rat from group1(control-) showing the normal histology of renal parenchyma (H and e x 200)

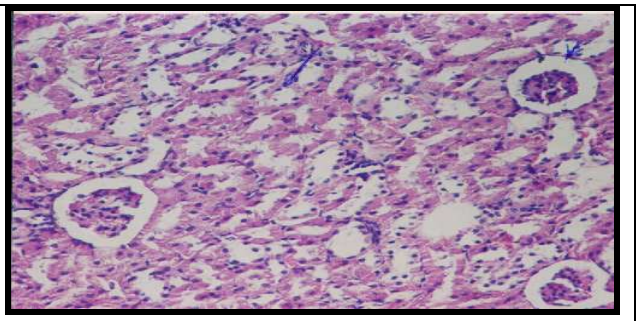


Photo (2): Kidney of rat from group2 (control+) showing eosinophilic proteinaceous casts in the lumen of renal tubules (H and E X 200

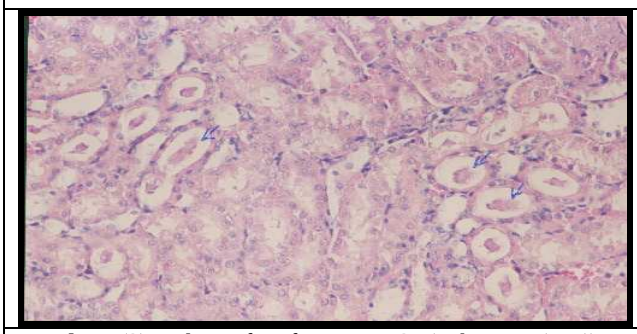


Photo (3): Kidney of rat from group3a (Echinacea (EP)) showing apparent normal renal parenchyma (H and E X 200)

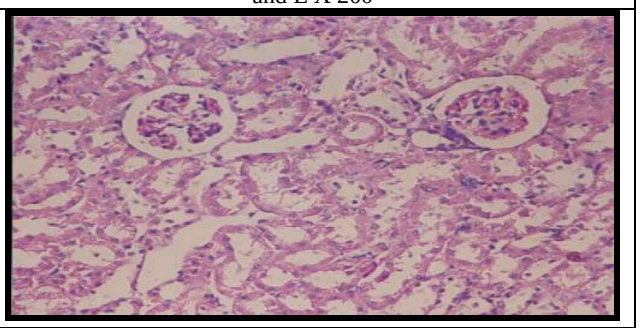
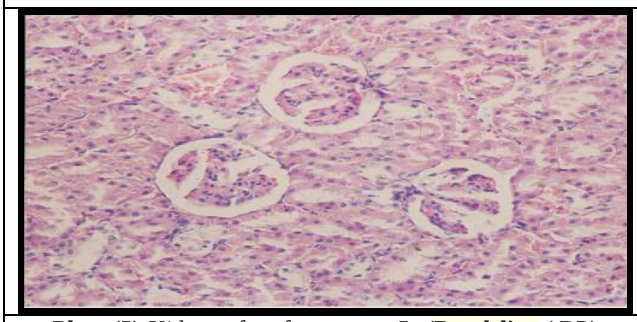


Photo (4): Kidney of rat from group 4a (Rosemary (RP),) showing eosinophilic proteinaceous cast in the lumen of some renal tubules (H and E X 200)



Photo(5): Kidney of rat from group 5a (**Dandelion** (DP) showing no histopathological changes (H and E X 200)

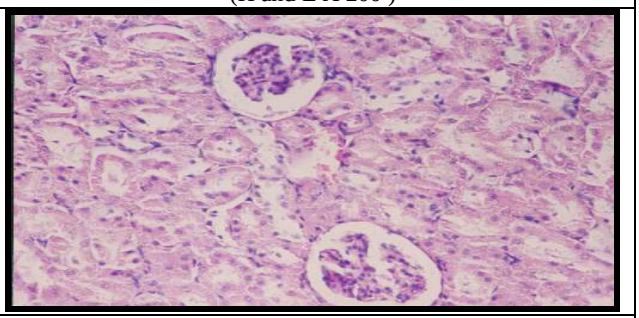


Photo (6): Kidney of rat from group 6 (5% mix of herbs) showing apparent normal renal parenchyma (H and E X 200)

Conclusion

the results denote that diet supplementation with EP, RP and DP and their combination exhibits good immunostimulant, hypoglycemic. The study recommends that intake of baked foods and sweets supplemented with both EP, RP and DP that may be beneficial for patient with type 2 diabetes mellitus. In addition, isolation of bioactive constituents from with EP, RP and DP and their combination is necessary to find safe and bioactive natural substances for diabetes mellitus treatment

REFERENCES

- **Abu-Al-Basal, M.A.(2010):**.Healing potential of *Rosmarinus officinalis* L. on full-thickness excision cutaneous wounds in alloxan-induced-diabetic BALB/c mice. J Ethnopharmacol.;131(2): 443–50.
- **Ashok, D.C.; Shrimant, N.P.; Panadeep, M.G. and Akalpita, U.A.(2007):** Optimization of alloxan dose is essential to induce stable diabetes mellitus for long period. Asian J. Biochem.; 2(6):402-408.
- **Carleton, H. (1976):** Carleton's Histological technique. 4th Edition, London, Oxford University press New York, Toronto.
- **Erboga, M.; Kanter, M.; Aktas, C.; Sener, U.; Erboga, Z.; Bozdemir D.Y. and Gurel,** A. (2015): Thymoquinone Ameliorates Cadmium-Induced Nephrotoxicity, Apoptosis, and Oxidative Stress in Rats is Based on its Anti-Apoptotic and Anti-Oxidant Properties. Biol. Trace Elem. Res.; 1: 1-8.
- Farkhondeh ,T. **;** Samarghandian , S.and Pourbagher-Shahri, A .M.(2019): Hypolipidemic **effects of Rosmarinus officinalis L.** J Cell Physiol. 29. doi: 10.1002
- **Fossati, P.; Prencipe, L. and Berti, G. (1980):** Use of 3, 5 dichloro-2- hydroxyl benzene sulfonic acid /4-amylphenazone chromogenic system in direct enzymatic assay of uric acid in serum and urine. Clin. Chem.; 26: 227-231.
- **Gaya, V. ; Repetto, J. ; Toneatto, C. ; Anesini, G. ; Piwien-Pilipuk, and Moreno, S. (2013):** "Antiadipogenic effect of carnosic acid, a natural compound present in Rosmarinus officinalis, is exerted through the C/EBPs and PPARγ pathways at the onset of the differentiation program," Biochimica et Biophysica Acta (BBA)-General Subjects, vol. 1830, no. 6, pp. 3796–3806.

- **Husdan, H. and Rapoport, A. (1968):** Estimation of creatinine by Jaffe reaction method. Clin. Chem.; 14: 222-228.
- Isbaniah, F. ; Wiyono, W.H; Yunus ,F. ; Setiawati, A. ; Totzke, U.and Verbruggen, M.A.(2011): **Echinacea purpurea along with zinc, selenium and vitamin C to alleviate** exacerbations **of chronic obstructive pulmonary disease: results from a randomized controlled trial.** J Clin Pharm Ther. **;36(5):568-76.**
- **Itariu, B.K. and Stulnig, T.M. (2014):** Autoimmune aspects of type 2 diabetes.
- **Jacob, N.J. and Van-Denmark, P.J. (1963):** A chemical method for the determination of triglycerides. Arch. Biochem. Biophys., 88: 250-255.
- **Jin, C.; Henao-Mejia, J. and Flavell, R.A. (2013):** Innate immune receptors: key regulators of metabolic disease progression. Cell Metab.; 17(6): 873-882.
- Khalaf, A.A., Hussein, S., Tohamy, A., F., Marouf, S ., Yassa, H.D., Zaki, A. R. and Bishayee, A .(2019): **Protective effect of Echinacea purpurea (Immulant)** against **cisplatin-induced immunotoxicity in rats.** Daru.**;27(1):233-241.**
- Labib, R .M. ; Ayoub ,I.M; Michel ,H.E . ; Mehanny, M .Kamil, V; Hany , M. ; Magdy, M . ; Moataz, A . ; Maged ,B .and Mohamed, A .(2019): Appraisal on **the wound healing potential of Melaleuca alternifolia and** Rosmarinus **officinalis L. essential oil-loaded chitosan topical preparations.** PLoS One. **16;14(9):e0219561.**
- **-Louay, L.; Usama, E. ; Yasse,.r and Mahmoud, I. (2014):** The Effects of Rosemary (Rosmarinus officinalis) Leaves Powder on Glucose Level, Lipid Profile and Lipid Perodoxation. International Journal of Clinical Medicine, 5, 297-304.

  http://www.scirp.org/journal/ijcm

  http://dx.doi.org/10.4236/ijcm.2014.5604
- **Luis-Rodríguez, D.; Martínez-Castelao, A.; Gorriz, J.L.; De-Alvaro, F. and Navarro- Gonzalez, J.F. (2012):** Pathophysiological role and therapeutic implications of inflammation in diabetic nephropathy. World J. Diabetes; 15:7-18.

- **Nobuyuki, T . ; Takayuki, A. ; Khiro,K. ; Yoshitaka, N.and Takashi , Y.(1990):** Alloxan-induced DNA Strand Breaksin pan creatic islets. The Journal of Biological Chemistry. Vol.266.no.4 pp 2112-2114. U.S.A.
- **Paglia, D.E, and Valentine, W.N. (1979):** Determination of glutathione peroxidase in tissue by UV method. J. Lab. Clin. Med.; 70:158-169.
- Park , J. **;** Rho, S.J**.and** Kim, Y.R **.(2019**): Enhancing antioxidant and antimicrobial activity of carnosic acid in rosemary (Rosmarinus officinalis L.) extract by complexation with cyclic glucans. Food Chem. 2019 Nov 30;299:125119.
- Richter, C. ; Dirks, M.E. ; Gronover, C.S. ; Prüfer. D. and Moerschbacher, B.M**.(2012):** Silencing and heterologous expression of ppo-2 indicate a specific function of a single polyphenol oxidase isoform in resistance of dandelion (Taraxacum officinale) against Pseudomonas syringae pv. tomato. Mol Plant Microbe Interact. 2012 Feb;25(2):200-10. doi:
- **Patton, C. J. and Crouch S.R. (1977):** Enzymatic colorimetric method for determination of urea in serum. Anal. Chem.; 49: 464-465.
- Pérez-Mendoza , M.B. ; Llorens,-E. ; Vanegas-E. ; P. ; Cifuentes, A. ; Ibáñez ,E. and Villar, D.(2019): **Chemical characterization of leaves and calli extracts of Rosmarinus officinalis by UHPLC-MS. Electrophoresis10.**1002/**elps.201900152.**
- Rasoulian , B.**;** Hajializadeh, Z.**;** Esmaeili-Mahani , S. **;** Rashidipour, M. **;** Fatem, I .and Kaeidi, A (2019): **Neuroprotective and antinociceptive effects of rosemary (Rosmarinus officinalis L.) extract in rats with painful diabetic** neuropathy**.** J Physiol Sci. **2019 Jan;69(1):57-64. doi:**
- Rašković ,A. ; Milanović, I. ; Pavlović, N. ; Ćebović,. T. ; Vukmirović, S. and Mikov, M. (2014) **: Antioxidant activity of rosemary (*Rosmarinus officinalis* L.) essential oil and its hepatoprotective potential. BMC Complementary and** Alternative **Medicine.; 14(1): 225.**
- **Ratliff, C.R. and Hall, F. (1973):** A new method for direct colorimetric determination of serum cholesterol. Cited in Laboratory Manual of Clinical Biochemistry, Scoot and White Memorial Hospital publication, Texas, USA.

- Rola ,M. ; Labib, Iriny, M. ; Ayoub, H. E. ; Michel, M .a; Mehanny, . K, ;Meryl, H; Mirette, M. ; Aya , M. ; Boula , A . and Ahmed ,M.(2019) : **Appraisal on the wound healing potential of *Melaleuca alternifolia* and *Rosmarinus officinalis* L. essential oil-loaded chitosan topical preparations.** PMC's policy on retraction ; ; 14(9): e0219561.
- **Reinhold, D.; Bank, U.; Buhling, F.; Lendeckel, U.; Ulmer, A.J. and Flad, H.D. (1994):** Transforming growth factor h1 (TGFh1) inhibits DNA synthesis of PWM-stimulated PBMC via suppression of Il-2, IL-6 production. Cytokine. 6:382-388.
- **Reeves, P.G.; Nielson, F.H. and Fahmy, G.C. (1993):** Reports of the American Institute of Nutrition, ADHOC willing Committee on reformulation of the AIN 93, rodent diet. J. Nutri.; 123: 1939-1951.
- Rostami , H. ; Seidavi, A . ; Dadashbeiki, M. ; Asadpour, Y. ; Simões, J. ; Shah, A.A. ; Laudadio, V. ; Losacco, C. ; Perillo , A.**and** Tufarelli ,V.**(2018):** Supplementing dietary rosemary (Rosmarinus officinalis L.) powder and **vitamin** E in broiler chickens: evaluation of humoral immune response, lymphoid organs, and blood proteins. Environ Sci Pollut Res Int. ;25(9):8836-8842.
- **Schalm, O.W.; Jain, N.C. and Carroll, E.J. (1975)**: Veterinary Hematology. The 3 rd Edition, Lea and Febiger, Philadelphia, U.S.A., Page 42-54.
- **Sinha, KA. (1972):** Colorimetric assay of catalase enzyme. Anal Biochem; 47: 389- **394**.
- **Siest, G., Henny, F. and Schiele, F. (1981):** Enzymatic determination of glucose. **Interpret**, Exam. Lab.; 2: 206-213.
- **Snedecor, G.W. and Cochran, W.G. (1986):** Statistical Methods, 7th Edition, **Iowa** State University Press, Ames, USA, Page 90.
- **Spitz, D.R. and Oberley, L.W. (1989):** An assay for superoxide dismutase activity in mammalian tissue homogenates. Anal. Biochem.; 179: 8-18.
- **Sturza, A.1.; Duicu, O.M.; Vaduva, A.; Dănilă, M.D., Noveanu, L., Varró, A., Muntean, D.M. (2015):** Monoamine oxidases are novel sources of **cardiovascular** oxidative stress **in** experimental diabetes. Can. J. Physiol. Pharmacol.; 12:1-

- **Senchina , D.S. ; Martin, A.E. ; Buss, J.E.and Kohut, M.L.(2010):** Effects of Echinacea extracts on macrophage antiviral activities. Phytother Res. 2010 Jun;24(6):810-6. Doi.
- Tan, X. ; Sun ,Z . ; Chen, S. ; Chen, S. ; Huang, Z. ; Zhou, C. ; Zou ,C. ; Liu, Q ; Ye, H. ; Lin, H . ; Ye , C . and Wang , A .(2017): **Effects of dietary dandelion extracts on growth performance, body composition, plasma biochemical parameters, immune responses and disease resistance of juvenile golden pompano Trachinotus ovatus.** Immunol. **Jul;66:198-206 .**
- **Takak, I. ; Bersani-Amado, L.E. ; Vendruscolo, A. ; Sartoretto, S.M. ; Diniz S.P. and Bersani-Amado ,C.A. (2008):** Anti-inflammatory and **antinociceptive** effects of *Rosmarinus officinalis* L. essential oil in experimental animal models. J Med Food; 11(4): 741–6. 10.1089.
- **Torkan, S. ; Khamesipour, F. and Katsande, S .(2015**): Evaluating the effect of oral administration of Echinacea hydroethanolic extract on the **immune** system in dog. utacoid Pharmacol. ;35(1-g2):9-13.
- Xiaohong,T. ;Zhen, C.;. PengweiXun, Q. ;ianqian,.H. ; HeizhaoL,I.and Chaoxia. ,Y.((2017): **Effects of dietary dandelion extract on intestinal morphology, antioxidant status, immune function and physical barrier function of juvenile golden pompano Trachinotus ovatus.** https://doi.org/10.1016/j.fsi.12.020**et**
- Xue, J. ;Xu ,Y. ; Jin ,L. ; Liu, G. ; Sun, Y. ; Li ,S. and Zhang J.(2008): **Effects of traditional Chinese medicine on immune responses in abalone, Haliotis discus hannai Ino.** Fish Shellfish Immunol. **;24(6):752-8.**
- **Yallow, R. and Bauman, W.A. (1983**): Plasma insulin in health and disease. In: Diabetes Mellitus: Theory and Practice. Edrs: Ellenberg M and Rifkin H. Excerpta Medica; 15:119- 120.
- **Yu, M.H. ; Choi, J.H .; Chae, I.G. ; Im, H,G. ; Yang, S,A. ; More, K. ; Lee, I.S. and Lee, J. (2013):**Suppression of LPS-induced inflammatory activities by *Rosmarinus officinalis* L. Food Chemistry**.;** 136(2): 1047–1054. 10.1016 / j.foodchem.2012.08.085 .

التأثير الواقي لمسحوق الاشناسيا وإكليل الجبل والهندباء في الفئران المصابة بمرض السكرى

هالة محمد على وهبه*

الملخص العربي:

أجريت هذه الدراسة لتقييم التأثير الواقي لمسحوق الاشناسيا وإكليل الجبل والهندباء على وزن الجسم ، تركيز سكر الدم وهرمون الانسولين، الحالة المناعية، كوليستيرول الدم والدهون الثلاثية وكذا نشاط الانزيمات المضادة للأكسدة بنسيج الكليتين في الفئران المصابة بمرض السكرى. فتم توزيع عدد ٦٣ من فئران التجارب عشوائيا إلى ٦ مجموعات رئيسيه كل منها ٧ فئران وتقسم الى مجاميع فرعيه منها ليصبح عددها ٩ مجموعات . استخدمت المجموعة الاولى كمجموعة ضابطة سالبة تم تغذيتها على الوجبة الأساسية . و تم إحداث مرض السكرى فى بقية المجموعات وظلت المجموعة الثانية مصابة بالمرض وتغذت على الوجبة الأساسية. اما المجموعات الثالثة والرابعة الخامسة والسادسة فتم تغذيتها على وجبات غذائية مدعمة بما يلى: ٥٪ و١٠ ٪ الاشناسيا ، ٥٪ و١٠ ٪ إكليل الجبل و ٥٪١٠٪ الهندباء و ٥٪ من كلا من (الاشناسيا + كليل الجبل +الهندباء) على التوالي لمدة ١٠ اسابيع. وفى بداية ونهاية فترة التغذية تم وزن الفئران وحساب معدل التغير فى وزن الجسم ، وفى نهاية فترة التجربة تم سحب عينات دم لقياس مستويات دلالات الحالة المناعية، سكر الدم وهرمون الانسولين، كوليستيرول الدم والدهون الثلاثية وتم قياس نشاط الانزيمات المضادة للأكسدة فى نسيج الكليتين . وأظهرت النتائج أن تغذية الفئران المصابة بمرض السكرى على وجبات غذائية مدعمة بمسحوق الاشناسيا وإكليل الجبل والهندباء أدت إلى زيادة وزن الجسم المكتسب وتحسن معدل الاستفادة للطعام. وكان هناك تحسن فى دلالات الحالة المناعة وعودة تركيزات سكر الدم وهرمون الانسولين، كوليستيرول الدم والدهون الثلاثية الى مستويات متقاربة من المستوى الطبيعي . وكذا زيادة نشاط الانزيمات المضادة للأكسدة فى نسيج الكليتين فى الفئران المصابة بداء السكرى. ودلت النتائج على أن تغذية الفئران المصابة بمرض السكرى على وجبات غذائية مدعمة بمسحوق الاشناسيا وإكليل الجبل والهندباء أدت الى زيادة وزن الجسم ومعدل مدى كفائه الاستفادة من الغذاء وتحسن الحالة المناعية وخفض سكر الدم والكوليسيرول والدهون الثلاثية و زيادة نشاط الانزيمات المضادة للأكسدة بنسيج الكليتين فى الفئران المصابة بمرض السكرى . وتوصي الدراسة بأن تناول مستخلص الاشناسيا وإكليل الجبل والهندباء قد يكون مفيدا للمرضى الذين يعانون من مرض السكرى.

الكلمات المفتاحية: الاشناسيا- والدهون الثلاثية- الهندباء – المناعة

* قسم الاقتصاد المنزلي – كلية التربية النوعية – جامعة المنوفية