Evaluation the Impact of Colocynth and Teucrium Diets of Diabetic Albino Rats

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Abstract:

This investigation aimed to evaluate the effect of whole colocynth fruit, teucrium whole plant and mixture of both on male diabetic rats to raise immunity. Thirty (30) adult male Sprague Dawley rats were divided into five groups. Group (1): Normal rats fed on basal diet as control negative (C-), Group (2): Control positive (C+) (untreated group). Group (3): Diabetic rats fed on basal diet and colocynth (5%).Group (4): Diabetic rats fed on basal diet and teucrium (5%). Group (5): Diabetic rats fed on basal diet and mixture of both dried plants (5%). At the end of experiment, after 28 days of feeding, all serum samples were analyzed for biochemical parameters. Injection with alloxan caused a significant decrease in the level of Hb, Ht, RBC, WBC, Neutrophils, Lymphocytes, Monocytes, Eosinphils and Basophils while a significant increase was recorded in organs weight and Plt while changes of histopathological structure of liver and kidney occamed .Diabetic rats treated with various diets, the results showed the improvement in all previous parameters. Teurium diet strengthened immune than coloynth diet and the Mix diet was the best.

Key words: Diabetes, Colocynth, Teucrium and mixture of both.

Introduction:

Type 2 diabetes (T2D) is a major worldwide health problem. This metabolic disease is indicated by high blood glucose levels due to insufficient insulin production by the pancreas. Inflammatory response occurs as a result of immune response to high blood glucose levels as well as the presence of inflammatory mediators produced by adipocytes and macrophages in fat tissue. This low and chronic inflammation leads to damage of the pancreatic beta cells and insufficient insulin production, which results in hyperglycemia. Hyperglycemia in diabetes is thought to cause dysfunction of the immune

response, which results in failure to control the spread of invading pathogens in diabetic subjects. Therefore, diabetic subjects are known to more susceptible to infections. The increased prevalence of T2D will increase the incidence of infectious diseases and related comorbidities **(Berbudi *et al*., 2019)**.

Citrullus colocynthis (L.) Schrad. is a Cucurbitaceae family plant **(Riaz *et al*., 2015)**. The plant is generally accessible in the Sahara and Arabian deserts, Sudan and a Southern piece of Asia including Pakistan, India and Southern Islands. The fruit is intense and globular with a smooth surface. It is hard and has a skin around it and contains 200–300 seeds/gourd. Seeds are small (6mm in length), ovoid, compressed, smooth and brownish when ripe. Seed constitute about 75% of the weight of fruit of *Citrullus colocynthis* **(Hussain *et al*., 2014)**.

A few dynamic synthetic constituents of *C. colocynthis* plant were surveyed. They are grouped as saponins, carbohydrates, tannins, glycosides, alkaloids, flavonoids and essential oils. Plant-based characteristic constituents can be obtained from any part of the plant like leaves, roots, flowers, stems, fruits, and seeds. Various plant secondary metabolites including flavonoids and cucurbitacins have already been accounted for from *C. colocynthis* **(Salama, 2012)**.

Citrullus colocynthis (L.) Schrad has a wide range of therapeutic and nutritional uses. It possessed antioxidant, antidiabetic, antimicrobial, anticancer, anti-inflamatory, analgesic, gastrointestinal, reproductive, protective and many other pharmacological effects **(Al-Snafi, 2016)**. Traditionally this plant is used in the treatment of diseases like cancer, leucoderma, ulcers, asthma, bronchitis, urinary discharge, enlargement of spleen, tuberculosis glands of the neck, dyspepsia, constipation, anemia and throat diseases **(Dhakad *et al*., 2017)**.

Teucrium polium is perennial, herbaceous, with almost woody plants, to a height of 30 cm and has a white appearance and cotton. Flowers can be seen in white, yellow and white to yellow. This variability is seen not only in color, but also in flower stems that are branched or lying **(Ricci *et al*., 2005)**.

Some compounds of *T. polium* have been intro-duced in different investigations including tannin, terpenoid, saponin, flavonoid, sterol, β-caryophyllene, diterpenoids, caryophyllene oxide, asparagine, ditryne and resinous sub-stances **(Niazmand *et al*., 2008)**.

The effects of *T. polium* on the liver, kidney, stomach, brainhas investigated and antidiabetic, antioxidant, antimicrobial, and anticancer effects of this agent, have introduced. Several studies revealed that *T. polium* has a hypoglycemic effect and can help to control blood sugar. In addition, due to the

undeniable effects of this plant againstcancer cells, it can be considered as a natural resource, for the treatment of cancer **(Khazaei *et al*., 2018)**.

Materials and Methods

Materials:

Colocynth whole fruit and teucrium whole plant were obtained dry from herb shop in Cairo, Egypt.

Chemicals:

Alloxan obtained from El-Gomhoria Company, Cairo .Egypt.

Animals:

Thirty (30) adult male Sprague Dawley rats, average body weight (150± 10 g) were used in this study. Rats were obtained from Research Institute of Ophthalmology, Medical Analysis Department, Giza, Egypt.

Methods:

Basal diet composition of tested rats:

The basal diet in the experiment consisted of casein (12%), corn oil (10%), mineral mixture (4%), vitamin mixture (1%), cellulose (5%), chorine chloride (0.2%), methionine (0.3%) and the remained is corn starch (67.5%) according to **AIN (1993)**.

Preparation of materials:

All materials were milled to soft powder by using electric grinder and kept in dusky stoppered glass bottles in a cool and dry location till use according to **Russo (2001)**.

Induced diabetic for rats:

Rats were injected by Alloxan at150 mg /kg body weight than times in these consecutive days to induce male diabetic for rats. Injected rats were fed on basel diet, then blood sugar determined. Serum glucose of about 200mg\dl indicates hypoglycemia **(NDDG, 1994)**.

Experimental design and animal groups:

Rats were housed in wire cages under the normal laboratory condition, and were fed on basal diet for a week as an adaptation period. The rats were divided into 5 groups each of 6 rats. All groups of rats were housed in wire cages at room temperature 25 C^0, and kept under normal healthy condition. Rats were divided into the following groups:

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

Group (2): Control positive group (+), in which diabetic rats were fed on basal diet.

Group (3): Diabetic rats fed on colocynth 5% diet.
Group (4): Diabetic rats fed on teucrium 5% diet.
Group (5): Diabetic rats fed on mixture of both 5% diet.

Determination of Biochemical Blood Parameters:

Blood samples were collected after 12 hours fasting at the end of experiment using the abdominal aorta. The rats were scarified under ether anaesthesia. Blood samples were received into in clean dry centrifuge tubes, in which blood was left to clot at room temperature, and then centrifuged for 10 minutes at 3000 r.p.m to separate the serum. Serum was carefully aspirated and transferred into clean cuvette tubes and stored frozen at-20^{o}C for biochemical analysis as described by **Schermer, (1967)**. CBC carried out according to **Fischbah (1966)**. Histopathological investigation of liver and kidney carried out according to **Canleton (1979)**.

Organs weight:

Heart, lungs, liver, spleen and kidneys of the sacrificed rats were carefully removed, washed in saline solution dried with filter paper and weighted independently. Percent of organs weight of final body weight calculated.

Statistical Analysis:

The data were statistically analyzed using a computerized Costat Program by one way ANOVA using a Completely Randomized Factorial Design **(SAS, 1988)** when a significant mean effect was detected, the means were separated with the Duncan's Multiple Range Test. Differences between treatments at $P \leq 0.05$ were considered significant. The results are presented as mean ± SD.

Results and Discussion:

Data presented in table (1) show the effect of colocynth, teucrium and mixture of both on organs weight of diabetic rats. It could be observed that the mean value of liver of control (+) group was higher than control (-) group, being 8.6±0.03 and 6.9±0.04 g respectively. The best liver weight showed for groups 5 (rats fed on basal diet containing 5% mixture of both plants) when compared to control (+) group.

It could be observed that the mean value of heart weight of control (+) group was higher than control (-) group, being 1.50± 0.004 and 0.62±0.002 g respectively. The best heart weight showed for group 5 (rats fed on basal diet + 5% mixture of both plant diets) when compared to control (+) group.

The same table indicated that the mean value of lungs weight of control (+) group was higher than control (-) group, being 1.55±0.001 and 0.80±0.004 g

respectively. The best lungs weight showed for group 4 (rats fed on basal diet + 5% teucruim) when compared to control (+) group.

Also, data of table (1) noticed that the mean value of spleen weight of control (+) group was higher than control (-) group, being 0.91±0.002 and 0.60±0.007 g respectively. The best spleen weight showed for group 5 (rats fed on basal diet + 5% mixture of both plant diets) when compared to control (+) group.

It could be noticed that the mean value of kidneys weight of control (+) group was higher than control (-) group, being 2.00± 0.03 and 1.50±0.05 g respectively. The best kidneys weight showed for group 4 (rats fed on basal diet + 5% teucruim) when compared to control (+) group, provided that non-significant differences between G4 and G5.

Rasekh *et al*., (2005) found that subchronic toxicity of *Teucrium polium* total extract at 100mg/kg in rats reduced liver and kidney weight at 21, 44 days of study.

Benariba *et al*., (2012) reported that *Citrullus colocynthis L*. seed extracts lower liver and kidneys weight in streptozotocin-induced diabetic rats.

Table (1): Effect of colocynth, teucrium and mixture of both on organs weight (g) of diabetic rats

Parameters / Groups	Liver (g) Mean ±SD	Heart (g) Mean ±SD	Lungs (g) Mean ±SD	Spleen (g) Mean ±SD	Kidneys (g) Mean ± SD
G1: Control –ve	6.9^{c}± 0.04	0.62^{e}± 0.002	0.80^{c}± 0.004	0.60^{d}± 0.007	1.50^{c}± 0.05
G2: Control+ve	8.6^{a}± 0.03	1.50^{a}± 0.004	1.55^{a}± 0.001	0.91^{a}± 0.002	2.00^{a}± 0.03
G3: Colocynth (5%)	8.0^{b}± 0.5	0.77^{b}± 0.005	0.91^{b}± 0.002	0.67^{b}± 0.006	1.73^{b}± 0.05
G4: Teucrium (5%)	7.8^{b}± 0.05	0.72^{c}± 0.006	0.40^{d}± 0.068	0.65^{c}± 0.009	1.65^{b}± 0.04
G5: Mixture of both (5%)	7.6^{b}± 0.07	0.69^{d}± 0.003	0.89^{b}± 0.003	0.64^{c}± 0.005	1.66^{b}± 0.08
LSD	0.41	0.0077	0.055	0.011	0.096

Values of same letters in the same column indicate non-significant differences at $p \leq 0.05$.

Data presented in table (2) show the effect of colocynth, teucruim and mixture of both on Hb, Ht, RBC, PLt and WBC of diabetic rats.

It could be observed that the mean value of (Hb) of control (-) group was higher than control (+) group, being 18.9±1.2 and 10.1±0.4 g/dl respectively. The best Hb was shown for group 5 (rats fed on basal diet + 5% mixture of both plant diets) when compared to control (+) group.

It could be showed that the mean value of (Ht) of control (-) group was higher than control (+) group, being 53.2±0.5 and 28.4±1.3 % respectively. The best Ht was shown for group 5 (rats fed on basal diet containing 5% mixture of both plant diets) when compared to control (+) group.

The same table indicated that the mean value of (RBC) of control (-) group was higher than control (+) group, being 5±0.5 and 3±0.3 (10^6/µL) respectively. The best RBC was shown for group 3 (rats fed on basal diet +5% colocynth) when compared to control (+) group.

Also, data of table (2) observed that the mean value of (Plt) of control (+) group was higher than control (-) group, being 700±7 and 406±3 (10^3/µL) respectively. The best AI was shown for group 5 (rats fed on basal diet + 5% mixture of both plant diets) when compared to control (+) group.

It could be noticed that the mean value of (WBC) of control (-) group was higher than control (+) group, being 10.8±0.05 and 7.1±0.81 (10^3/µL) respectively. Group 5 (rats fed on basal diet +5% mixture of both plant diets) recorded the best result as compared to control (+) group.

Rahmouni *et al*., (2017) found that *Teucrium polium* (TP) aqueous extract and vitamin C (Vit C) against carbon tetrachloride (CCl4) increased white blood cell (WBC) and red blood cell count (RBC) induced toxicity in rats.

Elalfy *et al*., (2019) reported that *Citrullus colocynthis* at dose of 25 mg/kg increased significantly level of hemoglobin (Hb), hematocrit (Ht), total leukocyte count (WBCs) but non-significantly increase of red blood cells (RBCs) while *Citrullus colocynthis* at dose of 12.5 reduced significantly platelets in Sprague Dawley rat short term of toxicity bioassay.

Table (2): Effect of colocynth, teucrium and mixture of both on Hb, Ht, RBC, PLt and WBC of diabetic rats

Parameters / Groups	Hb (g/dl) Mean ±SD	Ht (%) Mean ±SD	RBC (10^6/µL) Mean ±SD	PLt (10^3/µL) Mean ±SD	WBC (10^3/µL) Mean ±SD
G1: Control –ve	18.9^{a}±1.2	53.2^{a}±0.5	5.0^{a}±0.5	406^{d}±3	10.8^{a}±0.05
G2: Control+ve	10.1^{c}±0.4	28.4^{c}±1.3	3.0^{b}±0.3	700^{a}±7	7.1^{d}±0.81
G3: Colocynth (5%)	16.0^{b}±1.1	46.2^{b}±0.8	4.3^{ab}±0.4	440^{b}±2	7.9^{c}±0.08
G4: Teucrium (5%)	16.9^{ab}±0.6	47.8^{b}±0.7	4.1^{ab}±0.3	430^{c}±3	8.2^{c}±0.03
G5: Mixture of both (5%)	17.2^{ab}±1.0	48.4^{b}±1.4	4.0^{ab}±1	400^{d}±4	9.5^{b}±0.04
LSD	1.66	1.82	1.026	7.589	0.66

Values of same letters in the same column indicate non-significant differences at $p \leq 0.05$.

Data presented in table (3) illustrate the effect of colocynth, teucruim and mixture of both on Neutrophils, Lymphocytes, Monocytes, Eosinophils and Basophils (10^9/L) of diabetic rats.

It could be noticed that the mean value of (Neutrophils) of control (-) group was higher than control (+) group, being 4.55±0.004 and 2.99±0.009 respectively. The best Neutrophils was shown for group 5 (rats fed on basal diet + 5% mixture of both plant diets) when compared to control (+) group.

It could be observed that the mean value of (Lymphocytes) of control (-) group was higher than control (+) group, being 5.01±0.001 and 3.29±0.004 respectively. The best Lymphocytes was shown for group 5 (rats fed on basal diet containing 5% mixture of both plant diets) when compared to control (+) group.

The same table revealed that the mean value of (Monocytes) of control (-) group was higher than control (+) group, being 1.03±0.05 and 0.68±0.05 respectively. The best Monocytes was shown for group 5 (rats fed on basal diet +5% mixture of both plant diets) when compared to control (+) group.

Also, data of table (3) observed that the mean value of (Eosinophils) of control (-) group was higher than control (+) group, being 0.12±0.001 and 0.01±0.007 respectively. The best Eosinophils was shown for group 5 (rats fed on basal diet + 5% mixture of both plant diets) when compared to control (+) group.

It could be noticed that the mean value of (Basophils) of control (+) group

was higher than control (-) group, being 0.07±0.004 and 0.04±0.007 respectively. Group 5 (rats fed on basal diet +5% mixture of both plant diets) recorded the best result as compared to control (+) group.

Khleifat *et al*., (2002) found that chronic treatment with small sublethal doses (20 mg/kg and 50 mg/kg) of *Teucrium polium* ethanolic extract increased Lymphocytes and Monocytes in rats.

Elgerwi *et al*., (2013) indicted that extract of *Citrullus colocynthis* increased monocytes, lymphocytes, neutrophils, eosinophiles and basophiles in treated groups compared with those in the control group in rats.

Table (3): Effect of colocynth, teucruim and mixture of them on Neutrophils, Lymphocytes, Monocytes, Eosinophils and Basophils (10^9/L) of diabetic rats

Parameters / Groups	Neutrophils Mean ± SD	Lymphocytes Mean ± SD	Monocytes Mean ± SD	Eosinophils Mean ± SD	Basophils Mean ± SD
G1: Control –ve	4.55^{a}± 0.004	5.01^{a}± 0.001	1.03^{a}± 0.05	0.12^{ab}± 0.001	0.07^{a}± 0.004
G2: Control+ve	2.99^{c}± 0.009	3.29^{e}± 0.004	0.68^{c}± 0.05	0.01^{c}± 0.007	0.04^{c}± 0.007
G3: Colocynth (5%)	3.33^{c}± 0.008	3.67^{d}± 0.005	0.75^{c}± 0.09	0.11^{b}± 0.005	0.05^{bc}± 0.008
G4: Teucrium (5%)	3.43^{c}± 0.005	3.81^{c}± 0.006	0.78^{c}± 0.04	0.12^{ab}± 0.003	0.05^{bc}± 0.006
G5: Mixture of all (5%)	4.00^{b}± 0.6	4.41^{b}± 0.007	0.90^{b}± 0.03	0.13^{a}± 0.008	0.06^{ab}± 0.001
LSD	0.49	0.0092	0.10	0.0099	0.010

Values of same letters in the same column indicate non-significant differences at $p \leq 0.05$.

Histopathololgical changes:

Microscopically section of liver from healthy (control -) group 1revealed the normal histopathological structure of hepatic lobule (Photo 1). Sections of liver from diabetic group 2 (control +) showed congestion of central vein and hepatic sinusoids (Photo 2) and hydropic degeneration of hepatocytes as well as congestion of hepatic sinusoids (Photo 3). Rats of liver sections of group 3 (citrullus diets) showed slight activation of kupffer cell and congestion of hepatic sinusoids (Photo 4& 5).Nevertheless liver sections of teucrium diet

(group 4) indicated no histopathological alterations (Photo 6). While liver sections of mix diet groups (Photo 7) showed only slight activation of kupffers cells.

Kidney sections of healthy rat (control -) group 1 showed the normal histopathological structure of renal parenchyma (Photo 8). Diabetic rats sections (control +) group 2 showed cytoplasmic vacuolization of epithelial lining some renal tubules and congestion of renal blood vessel (Photo 9) as well as congestion of glomerular tuft (Photo 10).Citrullus diets of group 3 showed cytoplasmic vacuolization of epithelial lining some renal tubules and congestion of glomerular tuft (Photo 11&12). Kidney sections from teucrium diet group 4 showed no histopathological alternations (Photo 13&14).Kidney sections of mix diet group 5 showed no histopathololgical alternations (Photo 15).

It may conclude that citrullus, teucrium and specially the mix of both restored the original structure of liver and kidne, which was paralled to the record changes to the biochemical parameters.

Khleifat *et al*., (2002) revealed that chronic treatment with 50 mg/kg *T. polium* induced marked cytoplasmic vacuolation of liver. The ultrastructural appearance also revealed marked cytoplasmic vacuolation of liver cells in rats.

Baradaran *et al*., (2013) found that nephrototoxicity of hydroalcoholic *Teucrium polium* may be associated with kidney tubular injury and this herbal medicine should be used with caution in rats.

Ebrahimi *et al*., (2016) indicted that hydro-alcoholic leaf extract of *C. colocynthis* on histopathological changes in streptozotocin-induced diabetic rats showed improvement in their liver histological structure. The hepatocytes of kidney showed some degree of histological regeneration and less sinusoid dilatation in the absence of inflammatory cells. , improved in the diabetic rats treated with the extract, which demonstrateda normal glomeruli structure, normal urinary space, and areduction in tubular damage.

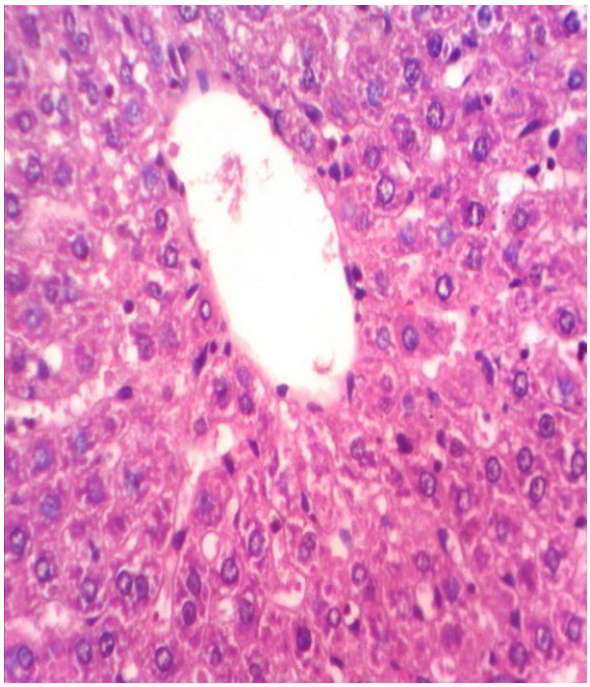

Photo (1): Liver of rat from group 1(control -) showing the normal histological structure of hepatic lobule (H & E X 400).

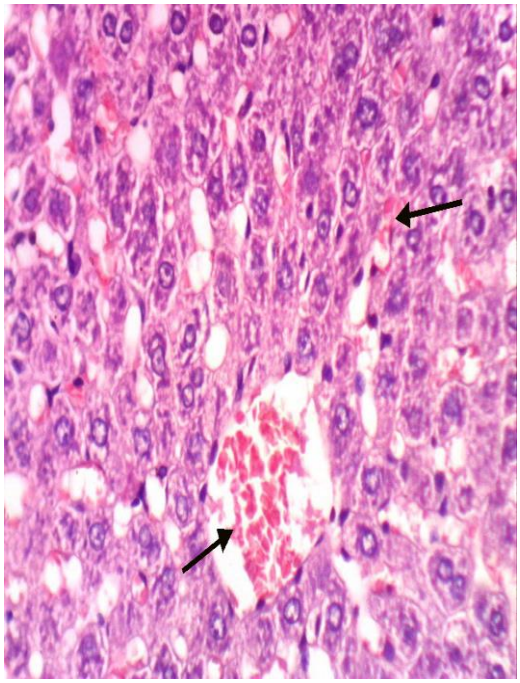

Photo (2): Liver of rat from group2 (control +) showing congestion of central vein and hepatic sinusoids (H & E X 400).

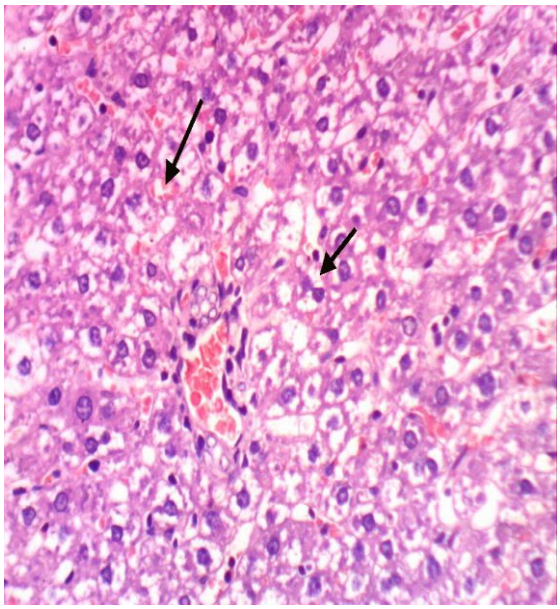

Photo (3): Liver of rat from group 2 (control +) showing hydropic degeneration of hepatocytes and congestion of hepatic sinusoids (H & E X 400).

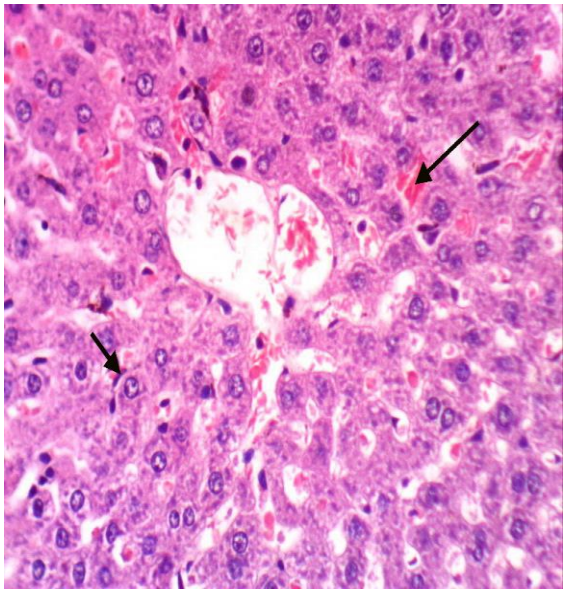

Photo (4): Liver of rat citrullus from group 3 showing slight activation of Kuppfer cells and congestion of hepatic sinusoids (H & E X 400).

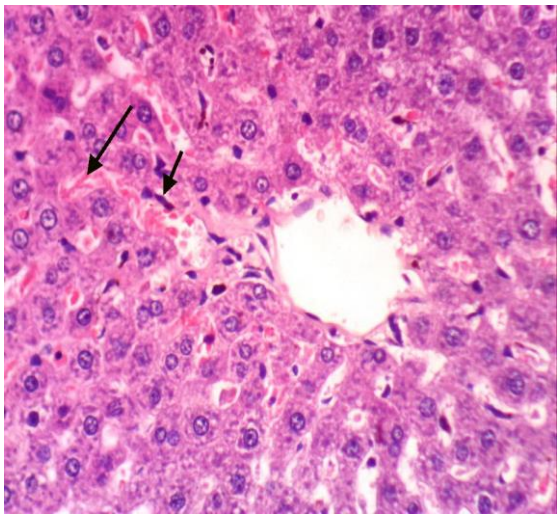

Photo (5): Liver of rat from citullus group 3 showing slight activation of Kupffer cells and congestion of hepatic sinusoids (H& E X 400).

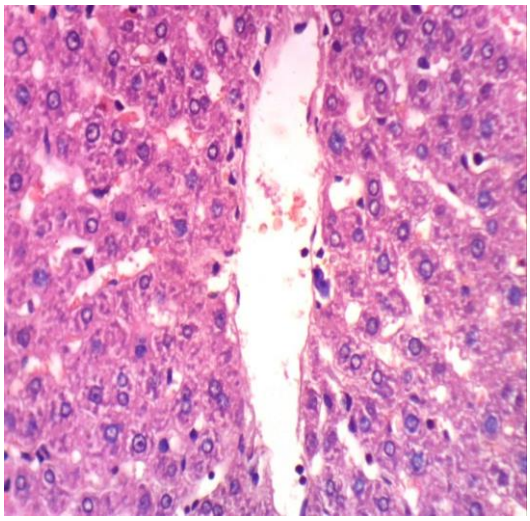

Photo (6): Liver of rat teucrium group 4 showing no histopatological alterations (H & E X 400).

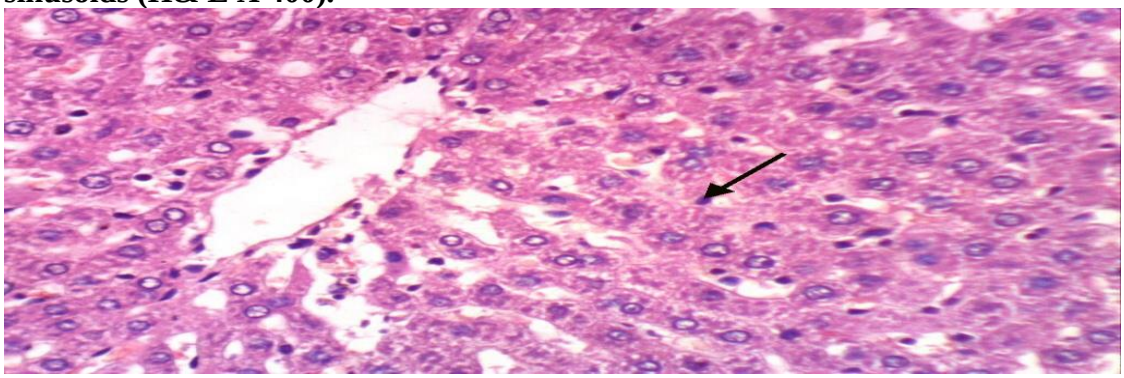

Photo (7): Liver of rat from mix diet group 5 showing slight activation of Kupffer cells (H & E X 400).

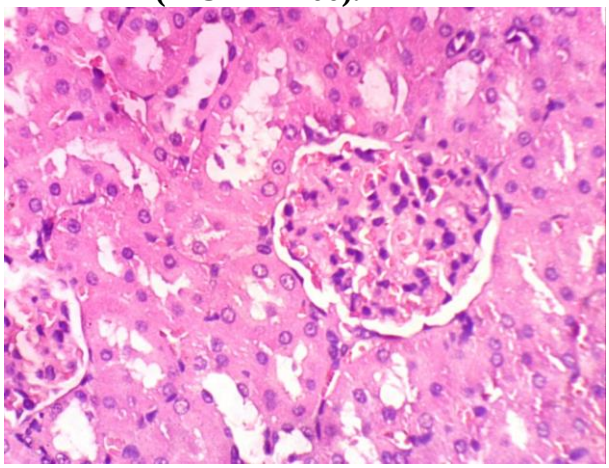

Photo (8): Photomicrograph of kidney of rat from group 1 (control -) showing the normal histological structure of renal parenchyma (H & E X 400).

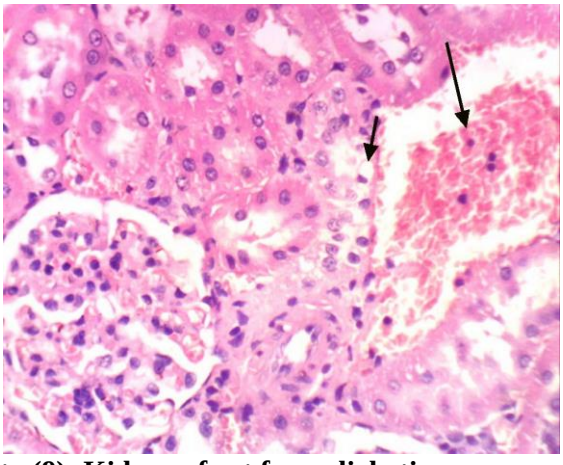

Photo (9): Kidney of rat from diabetic group 2 (control +) showing vacuolization of epithelial lining some renal tubules and congestion of renal blood vessel. (H & E X 400).

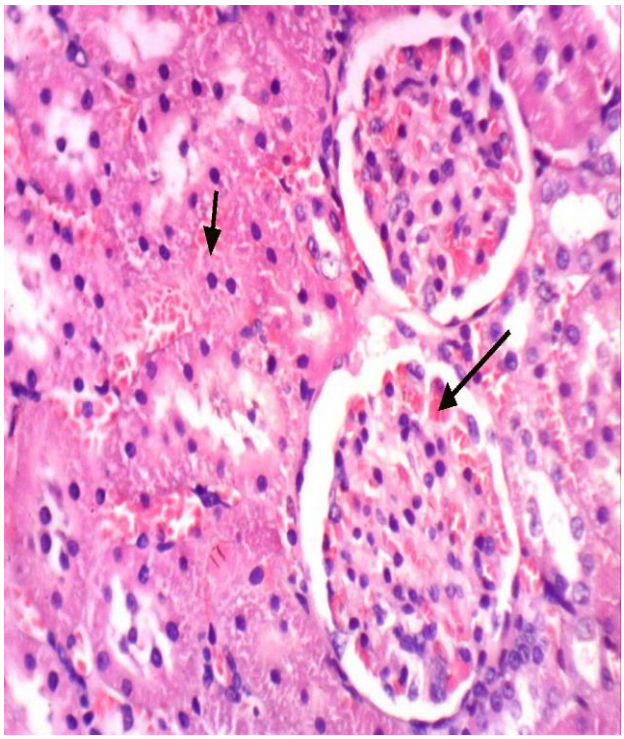

Photo (10): Kidney of rat from diabetic group 2 (control +) showing granular degeneration of epithelial lining some renal tubules and congestion of glomerular tuft (H & E X 400).

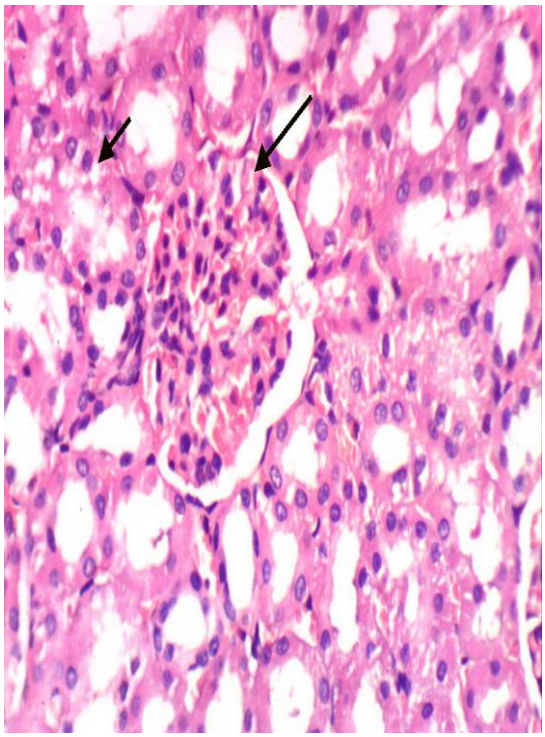

Photo (11): Kidney of from citrullus group 3 showing cytoplasmic vacuolization of epithelial lining some renal tubules and congestion of glomercular tuft (H & E X 400).

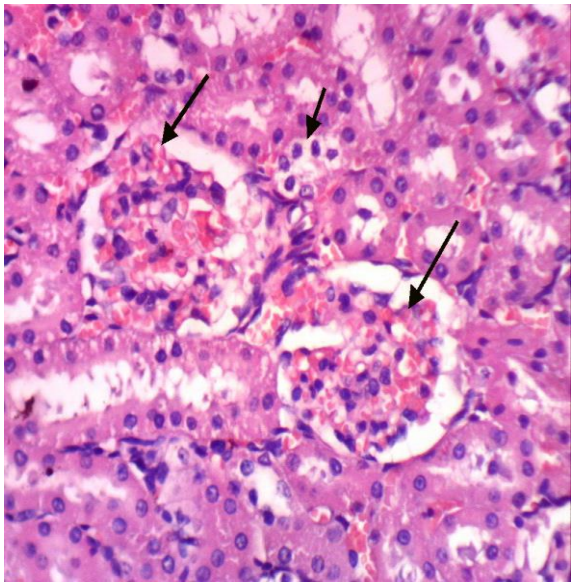

Photo (12): Kidney of rat from citrullus diet from group 3 showing cytoplasmic vacuolization of epithelial lining some renal tubules and congestion of glomerular tuft (H & E X 400).

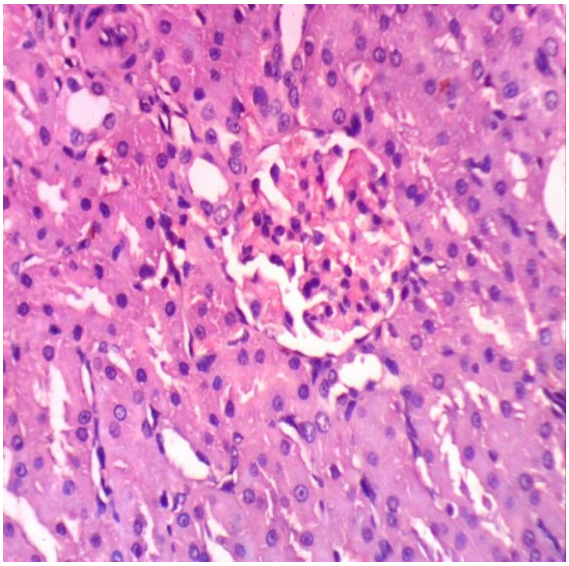

Photo (13): Kidney of rat from teurcrium 4 showing no histopatological alternations (H & E X 400).

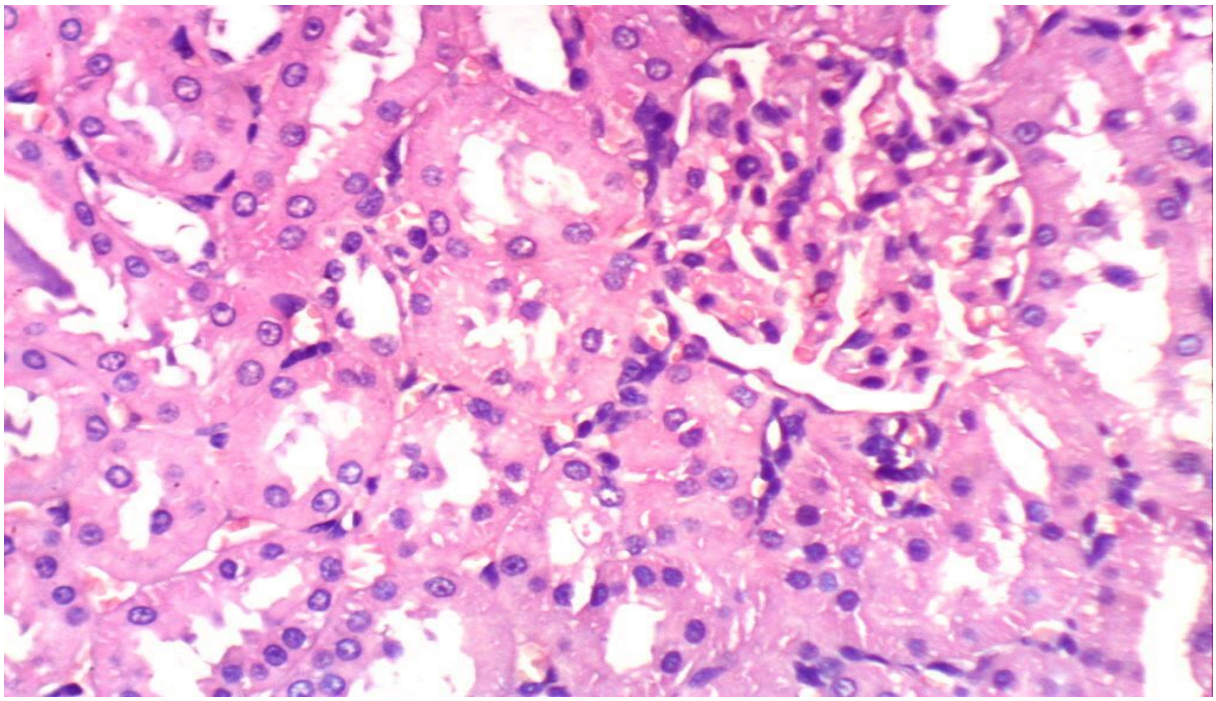

Photo (14): Kidney of rat from teucrium group 4 showing no histopathological alternations (H & E X 400).

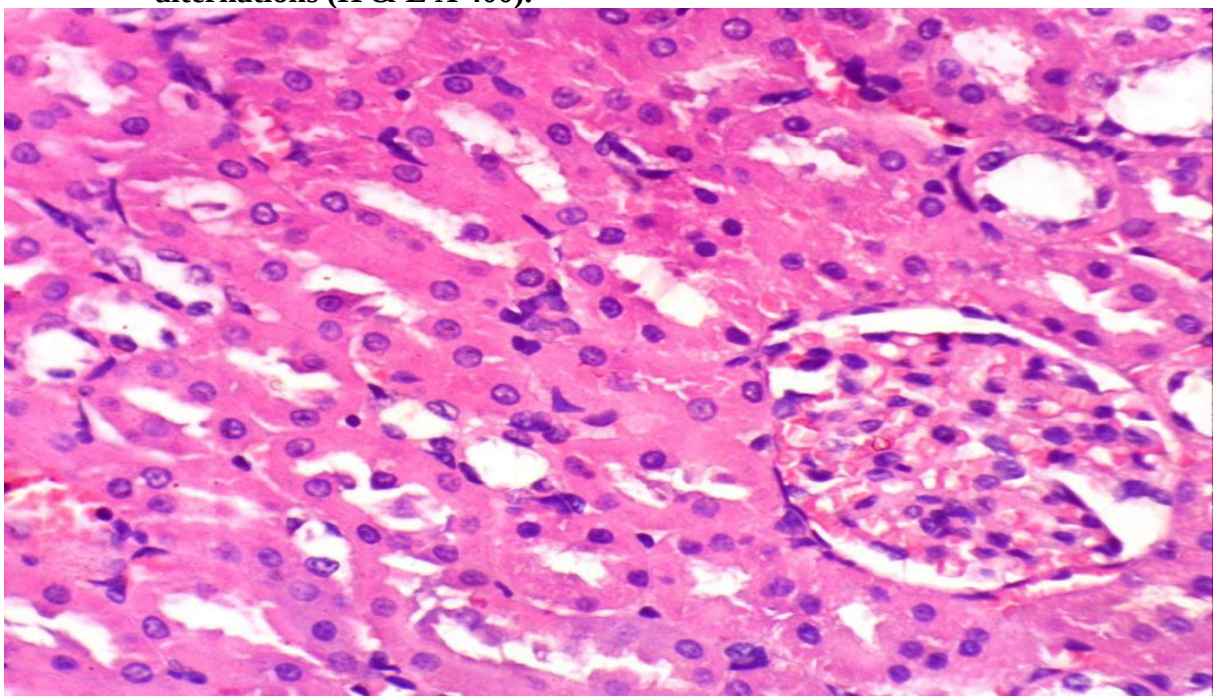

Photo (15): Kidney of rat from mix diet group 5 (mix diets) showing no histopathological alternations (H & E X 400).

In conclusion, best results obtained with the Mix diet, indicating synergistic action. Moreover results were better for *T. polium* than coloynthis diets. This indicated that restoration of the immune activity was better for *T. polium* diet, considering the diabetic rats.

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تقييم تأثير أغذية بها الحنظل والجعدة لرفع المناعة في الفئران المصابة بمرض السكري

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الملخص العربى

يهدف هذا البحث إلى تقييم تأثيرثمار الحنظل كاملة ونبات الجعدة الكامل والخليط منهما علي ذكور الفئران المصابة بالسكرلرفع المناعة. تم تقسيم ثلاثون فأر من الذكور البالغين سبراغ داولي إلى خمس مجموعات. مجموعة (1): وهي المجموعة الضابطة السالبة (-) تغذت على الوجبة الأساسية ، المجموعة (2): وهي المجموعة الضابطة الموجبة (+) وهي الفئران المصابة بالسكري وتغذت على الوجبة الأساسية. المجموعة (3): الفئران المصابة بالسكري التي تغذت علي نبات الحنظل بنسبة 5%. المجموعة (4): الفئران المصابة بالسكري التي تغذت على نبات الجعدة بنسبة 5 %. المجموعة (5): الفئران المصابة بالسكر التي تغذت على الاثنين معا بتركيز 5%.. في نهاية التجربة ، بعد 28 يومًا من التغذية ، تم تقديرتأثير المعاملات. الحقن بالألوكسان سبب ارتفاع في وزن الأعضاء والصفائح الدموية وانخفاض مستويات الهيموجلوبين والهيماتوكريت وكرات الدم الحمراء والبيضاء والخلايا المناعية وخلل في البناء الهستوباثولوجي للكبد والكلي في الفئران المصابة بالسكر وتحسنت النتائج باستخدام الأغذية المعالجة. وقد لوحظ أن تقوية المناعة كانت أكثر في حالة أغذية الجعده في حين أن الخليط كان الأفضل.

الكلمات المفتاحية : مرض السكر, أغذية الحنظل, أغذية الجعدة والخليط من الاثنين معا.