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Effect Natural Products Isolated From Vitex Agnus-Castus On Periostin In Mice Induced With Behcet Disease

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Abstract:
Behçet's disease is a rare and intriguing vasculitic condition marked by the persistent presence of oral and genital ulcers, along with a spectrum of systemic symptoms, including uveitis. Periostin is a carboxylate matrix protein that founded in kidney lung and bone, among other extracellular contain a hydrophilic COOH terminal domain, internal homologous repeat region cysteine-rich domains , and a signal peptide sequence at the NH2 end make up periostin. The extracellular matrix protein periostin, also known as osteoblast-specific factor-2,(OSF-2). In the present study Periostin, MDA, TNF-alpha, IL-8, and GSH were measured by enzyme linked immune absorbent assay (ELISA). Biochemical parameters, creatinine that were measured by spectrophotometer technique in patients and healthy volunteers' groups. The study included the separation and diagnosis of various natural products from the Vitex agnus-castus, where the oily substance and its fatty acids were separated, which were identified HPLC) technology. Some flavonoids and alkaloids were also separated. The flavonoids were identified by applying high-performance liquid chromatography (HPLC) technology. The effect of these products on Periostin was studied and it was found that they had a positive effect in reducing the level of Periostin and some other measured variables. In addition, the study examined the effect of these natural products on some biochemical parameters related to Behcet disease in the blood serum of mice induced with the disease. Biochemical variables included: Malondialdehyde (MDA), Glutathione (GSH), Creatinine, Interlukin-8 (IL-8), and Tumor necrosis alpha (TNF-alpha). The results showed that the concentrations of Periostin, MDA, IL-8 , TNF-alpha, and creatinine increased significantly in infected mice, compared to healthy mice, and a significant decrease in the concentration of GSH was observed.

Keywords: Behcet Disease, Isolation, Mice, Natural products, Periostin.

Introduction:

Behçet syndrome is rare inflammation marked by ulcers in the genital and oral, diverse cutaneous lesions, and ocular abnormalities. Symptoms appear cavity in oral mucus inflammation (canker sores) and genital region (ulcers) that often appear and reappear currently. Ocular inflammation anterior, posterior, or pan uveitis) affect persons with Behcet condition. Different body including nervous joints, blood vessels system and tract digestive. The precise etiology of Behcet syndrome remains unclear. Behcet disease is globally prevalent. However, it is most prevalent in Turkey 420 cases, the Middle East 300 and the Far East 15 cases per in the United States 7 incidences per 100000 individuals. This condition may manifest at any age. However, symptoms often emerge 20 to 30 old [1].

Periostin is thought to play a part in the growth and remodeling of numerous tissues, including the thyroid, bone, heart, skin, kidney and lungs additionally to wound care[2]. Periostin participates in the pathogenic pathway during tissue remodeling and fibrosis, despite playing a crucial role as a structural mediator in tissue growth and repair. Periostin plays a role in the tumor genesis pathway of several types of solid tumors by being strongly expressed at the site of damage or inflammation following mechanical stress. Moreover, it has a role in a number of allergy conditions such atopic

dermatitis and rhino sinusitis. In response to damage, increased collagen deposition, tissue remodeling, and cell necrosis are all significantly correlated with periostin overexpression[3]

Material and Methods:

One of Mosul's city marketplaces provided the myrrh plant, which was then ground into powder using a machine. Oil extraction from the Vitex agnus-castus involves weighing 200 gm. of plant powder and then soaking it in petroleum ether solvent at a temperature of 60–80 ºC for a duration of one day. This process allows for the extraction of fatty acids and volatile oils. After that, a rotary evaporator was used to extract the solvent and the resultant oil product was refrigerated and kept in a sealed tube for future tests [4].

Fatty acids in plant-derived oils have been characterized using capillary gas chromatography separation column (SE- 30) with dimensions of 30 meters in length, 0.25 mm in diameter, and 0.25 mm in film thickness, together with a flame ionization detector (FID). The test took place in the Ministry of Science and Technology's laboratories in Baghdad, Department of Environment and Water. Nitrogen gas was used as a carrier gas. .

The extraction of flavonoids from the plant involved utilizing the leftover material from myrrh powder after oil extraction. We then dried this material to remove the petroleum ether. Subsequently, an extraction procedure was carried out for a duration of three days using 99% ethanol. The extracted product was stored in a sealed tube in a refrigerated environment for future investigations [5]. Flavonoids were diagnosed using high performance liquid chromatography (HPLC) After isolating both the oil and flavonoids, the residue was separated to obtain the alkaloids. The residue was then extracted by extraction using distilled water in extraction equipment for a period of three days, To remove all ethanol. After that, it was soaked in distilled water for one day, and the solution was placed in a freeze-drying condition to remove the water, obtaining a dry extract to be stored later in a sealed tube for subsequent tests [6].

Experimental Animals:

In this study, forty adult male Swiss albino mice, aged three months and weighing between (25 - 30g), were utilized. They were placed in a dedicated room with suitable conditions for feeding, lighting, temperature, and ventilation, located within the veterinary medicine college premises at the University of Mosul.

Induction of Behcet disease:

Behcet disease was induced using isolated viral (pSV40-Rluc-HSVTK-Fluc-ITGB6(human)-3UTR) provided by (men.solarbio company) induction of Behcet disease model by Scratch in the eye repeated once time every week for three week, and the induced condition was diagnosed through clinical examinations and histological analysis of the behcet disease[7]

Determination of Effective Dose of Vitex Angus-Castus Aqueous Extract:

Five groups of mice were taken, each group consisting of five mice, and then were intraperitoneally injected with doses of 100, 200, 300, and 400 mg/kg body weight of Vitex angus-castus plant extract.

Obtaining Blood and Tissue Samples:

Blood samples were collected from the orbital sinus by employing specialized capillary tubes following a temporary anaesthesia of the animals with ether for a few seconds[8] .The blood was obtained and placed in dry, clean, and sterilized tubes (specifically, plain tubes) without any substance to prevent blood clotting. Next, the serum was isolated from the blood to perform the necessary biochemical analysis. Additionally, the kidney tissue was extracted and placed in tubes containing 10% neutral buffered formalin solution for subsequent histological examination[9].

Statistical Analysis:

The Statistical Packages of Social Sciences-SPSS (2019) program was used to detect the effect of difference groups in study parameters. test (ANOVA) was used to significant compare between means. Estimate of correlation coefficient between difference parameters in this study. LSD-Least significant difference and T-test test was used to significant compare between means. Chi-Square test

was used to significant compare between percentage. Estimate of Correlation coefficient between difference variables in patient groups in this study[10].

Results:

The presence of active components in the oil, flavonoids, and alkaloids of the Vitex agnus-castus plant was determined using colorimetric assays or physical techniques. Their biological efficacy was further assessed by studying their therapeutic effects on newly induced Bechet disease in mice. Additionally, the fatty acid composition in the plant's oil was identified. Compounds such as, were also detected.

Used the HPLC technique to analyze the fatty acids in isolated oils from the Vitex agnus-castus, resulting in multiple peaks (Fig.1), (Table1.) previous study[11] confirms that oil, comprising palmitic, stearic, oleic, linoleic, and linolenic acids, is present in Vitex agnus-castus.

The results of the analysis of the standard sample were purified using the high-performance liquid chromatography technique (Fig. 2), (Table1) and there were eight peaks of phenolic compounds that were identified. The Vitex agnus-castus contains Vanillic acid, Syringeic acid, Caffeic acid, Ferulic acid, Catechine, Qurcetine, Rutin, and gallic acid.the result is agreement with previous study were detected phenolic compounds from Vitex agnus-castus [12].

TABLE 1. Fatty Acids Compounds Separated from Vitex agnus-castus Plant Using HPLC Equipment

No	Retention Time (Standard Substance)	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	4.05	4.07	6125.65	620.80	20.00	20.00	0.08	Stearic
2	5.33	5.36	8114.90	800.14	25.00	25.00	0.10	Lenolic
3	6.00	6.01	6325.90	613.65	20.00	20.00	0.08	Palmatic
4	7.90	7.96	7221.45	677.41	20.00	20.00	0.08	Oleic
5	8.80	8.83	5325.90	562.65	15.00	15.00	0.05	Lenolinic
Total		33113.30	3271.90	100.00	100.00			

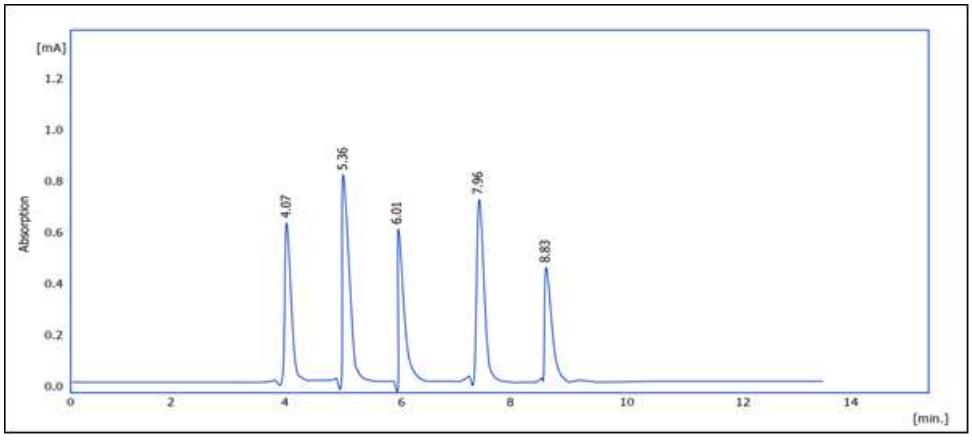

Fig. 1. HPLC Chromatogram of Separated Fatty Acids Extract from Vitex agnus-castus Plant

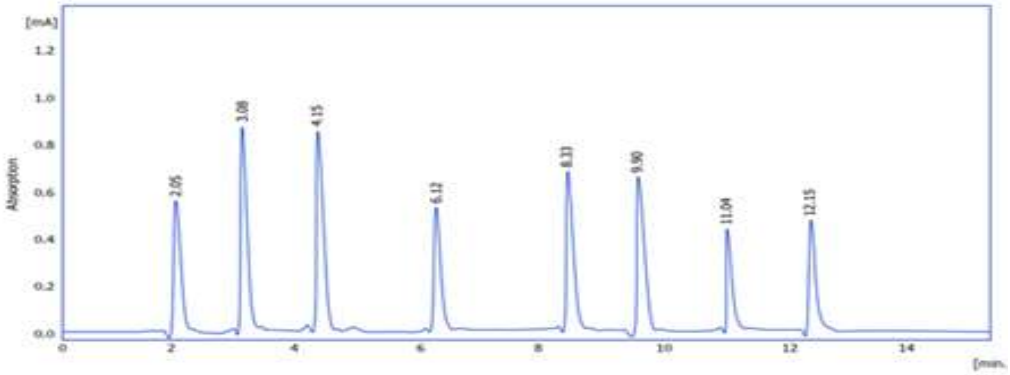

Fig. 2. HPLC Chromatogram of Separated Flavonoids Extract from Vitex agnus-castus Plant

TABLE 2. Flavonoids Compounds Separated from Vitex agnus-castus Plant Using HPLC Equipment

No	Retention Time (Standard Substance)	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	2.05	2.05	4521.98	594.28	11.00	11.00	0.03	Ferulic acid
2	3.0	3.08	9852.11	889.98	16.00	16.00	0.08	gallic acid
3	4.11	4.15	9745.14	887.44	16.00	16.00	0.08	Rutin
4	6.15	6.12	4011.25	596.65	11.00	11.00	0.03	caffeic acid
5	8.33	8.33	5236.98	641.14	14.00	14.00	0.05	Qurcetine
6	9.9	9.90	5412.66	640.11	14.00	14.00	0.05	catechine
7	11.8	11.04	3211.41	496.65	9.00	9.00	0.03	Vanillic acid
8	12.12	12.15	3126.98	513.05	9.00	9.00	0.03	Syringeic acid
Total		54118.98	5259.30	100.00	100.			

The effect of natural products isolated from myrrh plants on certain biochemical parameters: as shown in **Table 3**

Parameters	**Control**	**mice induced Behcet disease (Untreated)**	**mice induced Behcet disease (Treated with (Oil)**	**mice induced Behcet disease (Treated with (Flavonoids)**	**mice induced Behcet disease (Treated with Alkaloids)**
POSTN (pg/ml)	a 258±8.88	d 547.8±58.0	c 352±7.59	b 303±10.23	c 328±6.32

MDA (ng/ml)	a 35.47±2.71	d 96.4±8.59	c 60.45±1.64	b 44.12±3.37	c 54.56±2.83
GSH (µg/ml)	e 4.41±0.31	a 2.15±0.12	b 2.48±0.126	c 3.22±0.19	d 2.88±0.09
CREAT. (mg/dL)	a 0.63±0.11	c 1.16±0.05	b 0.96±0.02	b 0.93±0.03	b 0.94±0.02
TNF-α (pg/mL)	a 20.36±2.41	d 66.3±2.39	c 40.2±1.68	b 29.2±1.89	c 38.06±0.99
IL8 (pg/mL)	a 27.58±5.80	d 94.6±4.44	c 82.2±3.87	b 61.1±13.0	c 68.8±3.39

Histological changes in experimental animal organs:

control sample

The histological analysis of the animals in the control sample showed that the kideny a typical and healthy shape. Figure (2) (3) illustrates the natural histological features of the kidney, including the glomeruli (G), distal tubules (DT), and proximal tubules (PT). Hematoxylin and eosin stain. Magnification 100X, scale-bar = 100 µm.

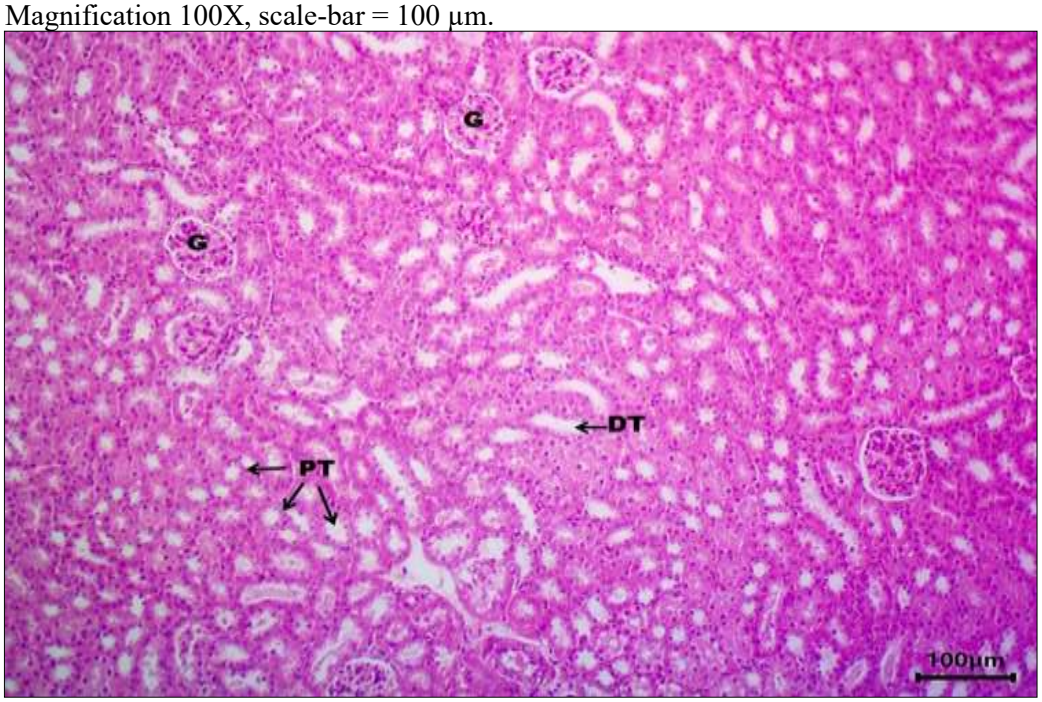

Figure (2): Histological section of a rat kidney from the control (healthy) group. showing normal histological features of the glomeruli (G), distal tubules (DT), and proximal tubules (PT). Hematoxylin and eosin stain. Magnification 100X, scale-bar = 100 µm.

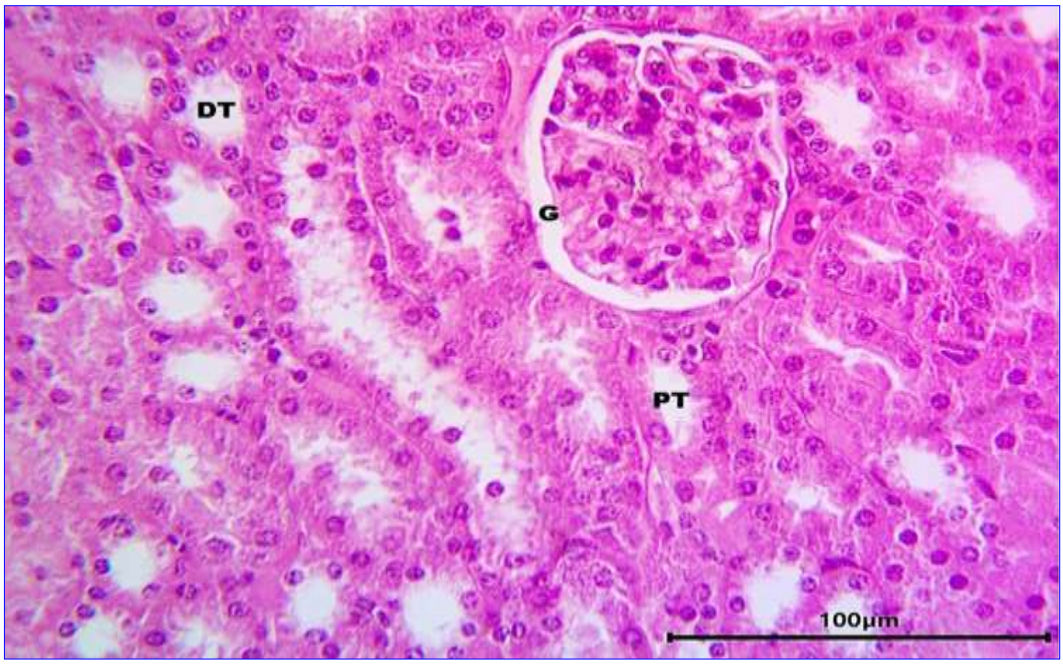

Figure (3):Histological section of a rat kidney from the control (healthy) group showing normal histological features of the glomeruli (G), distal tubules (DT), and proximal tubules (PT). Hematoxylin and eosin stain. Magnification 400X, scale-bar = 100 µm.

The Group Afflicted with Experimentally Induced Behcet Disease Model:

The histological examination of the group of animals affected with experimentally induced behcet disease model by using isolated viral (pSV40-Rluc-HSVTK-Fluc-ITGB6(human)-3UTR) provided by (men. solarbio company)induction of Behcet disease model by Scratch in the eye repeated once time every week for three week, and the induced condition was diagnosed through clinical examinations and histological analysis of the behcet disease compared to the control group[13]. Figure (3) and(4) showed severe nephritis characterized by infiltration of multiple inflammatory cells and mononuclear cells (iC), necrosis (N), cellular swelling of the epithelial cells lining the renal tubules (CS), deposition of proteinaceous material (PD), edema (E), hyperemia (HP), and hemorrhage (H).

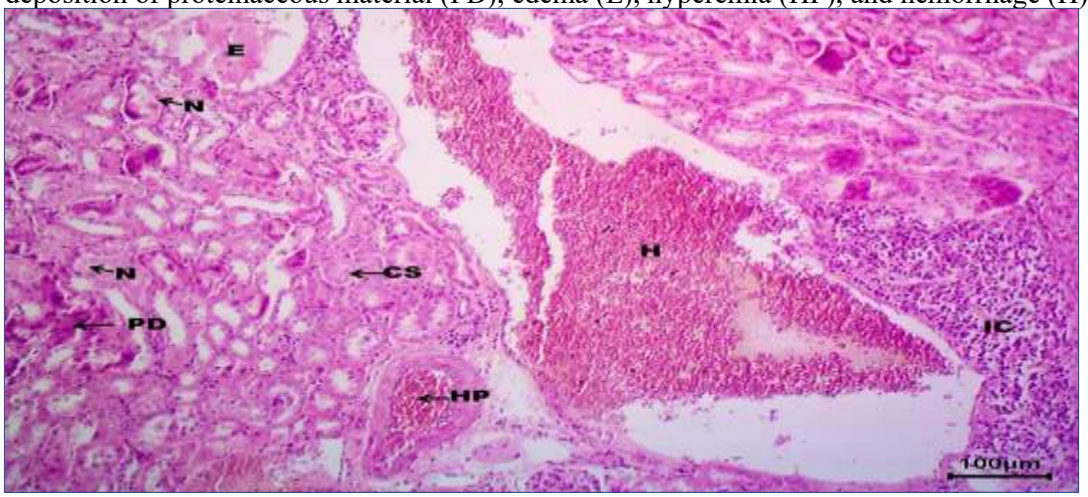

Figure (3):Histological section of a rat kidney from the control (infected) group showing severe nephritis characterized by infiltration of multiple inflammatory cells and mononuclear cells (iC), necrosis (N), cellular swelling of the epithelial cells lining the renal tubules (CS), deposition of proteinaceous material (PD), edema (E), hyperemia (HP), and hemorrhage (H). Hematoxylin and eosin stain. Magnification 100X, scale-bar=100µm.

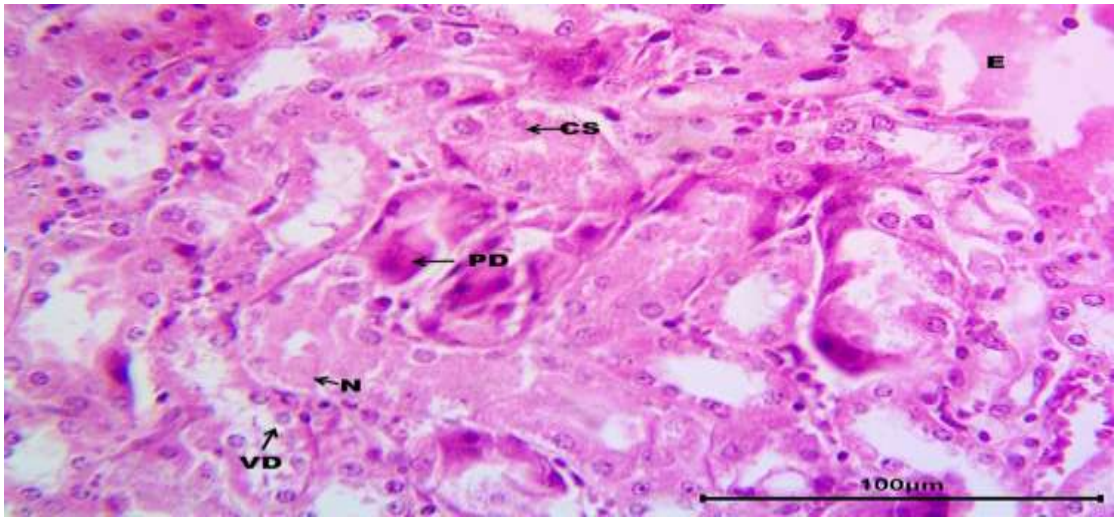

Figure (4):Histological section of a rat kidney from the control (infected) group showing severe lesions represented by necrosis (N), vacuolar degeneration (VD), cellular swelling of the epithelial cells lining the renal tubules (CS), protein deposition (PD), and edema (E). Hematoxylin and eosin stain. Magnification 400X, scale-bar = 100 µm.

Animals treated with natural products of the Vitex agnus-castus plant.

The treatment of animals with induced behcet disease using natural extracts from the myrrh plant for three weeks resulted in tissue recovery and restoration of the kidney structure in the afflicted animals, approaching normalcy. Particularly, animals treated with oil, flavonoids and alkaloids, when compared to tissues from untreated afflicted animals, displayed significant improvements. displayed significant improvements. In the third group (Group 3) Figure (5) and figure (6). Treated with the oily extract, a notable improvement in tissue showed slight infiltration of inflammatory cells (iC), slight necrosis (N), slight cellular swelling of the epithelial cells lining the renal tubules (CS), proteinaceous hyaline casts (HC), and hyperemia (HP). Result agreement with previous studies showed Histopathologic section of kidney of mice treated orally with clove oil extract contain fatty acid showed normal renal tubules and glomeruli in cortex area with mild vacuolar degeneration of glomerulus surround by normal tubules[14]

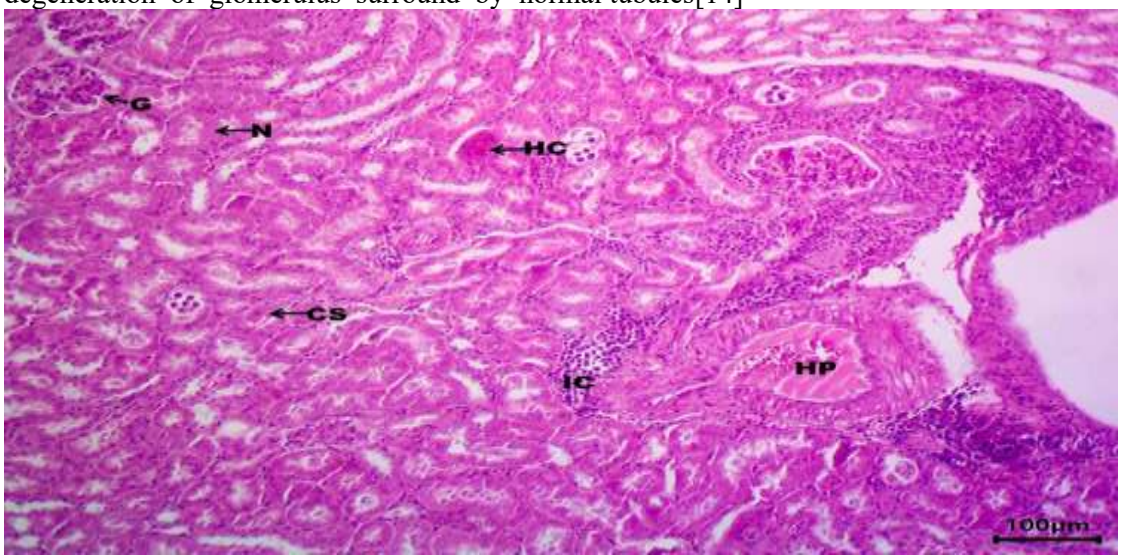

Figure (5):Histological section of a rat kidney from group A (injured + oil) showing slight infiltration of inflammatory cells (iC), slight necrosis (N), slight cellular swelling of the epithelial cells lining the renal tubules (CS), proteinaceous hyaline casts (HC), and hyperemia (HP). H&E stain. Magnification 100X, scale-bar=100µm.

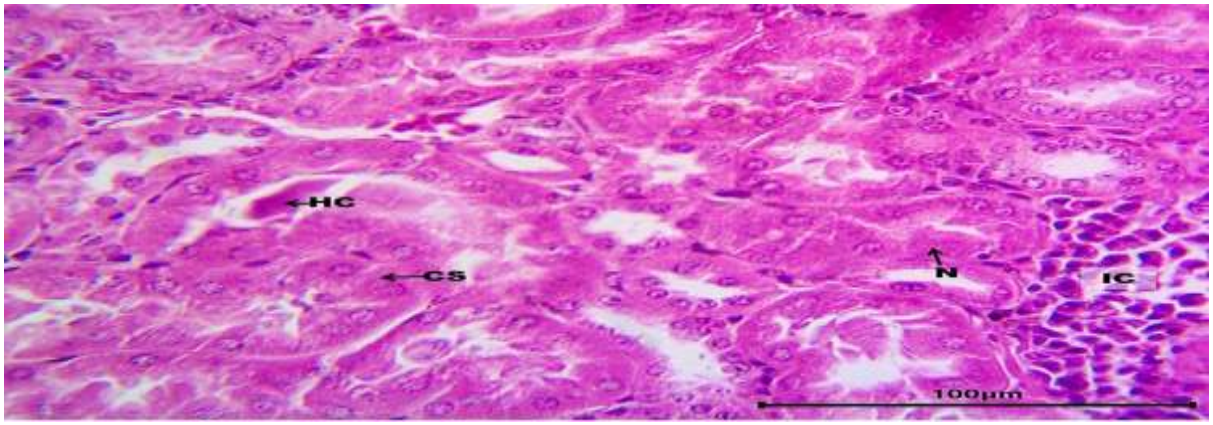

Figure (6):Histological section of a rat kidney from group A (injured + oil) showing slight infiltration of inflammatory cells (iC), slight necrosis (N), and slight cellular swelling of the epithelial cells lining the renal tubules (CS) and proteinaceous hyaline casts (HC). Hematoxylin and eosin stain. Magnification 400X, scale-bar=100µm.

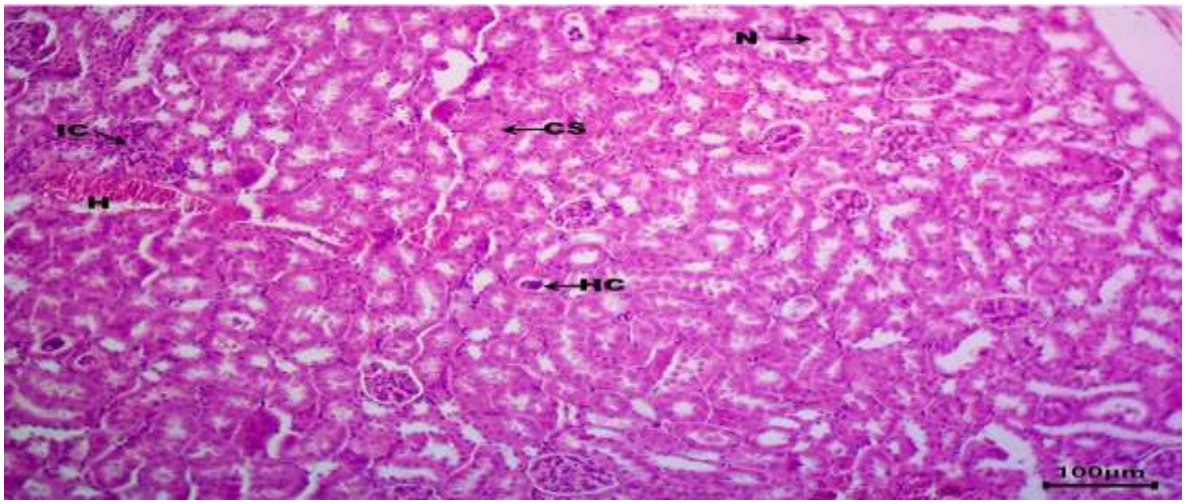

Figure (7):Histological section of a rat kidney from group B (infected + flavonoid) showing minimal infiltration of inflammatory cells (iC), slight necrosis (N), slight cellular swelling of the epithelial cells lining the renal tubules (CS), proteinaceous hyaline casts (HC), and hemorrhage (HP). H&E stain. Magnification 100X, scale-bar=100µm.

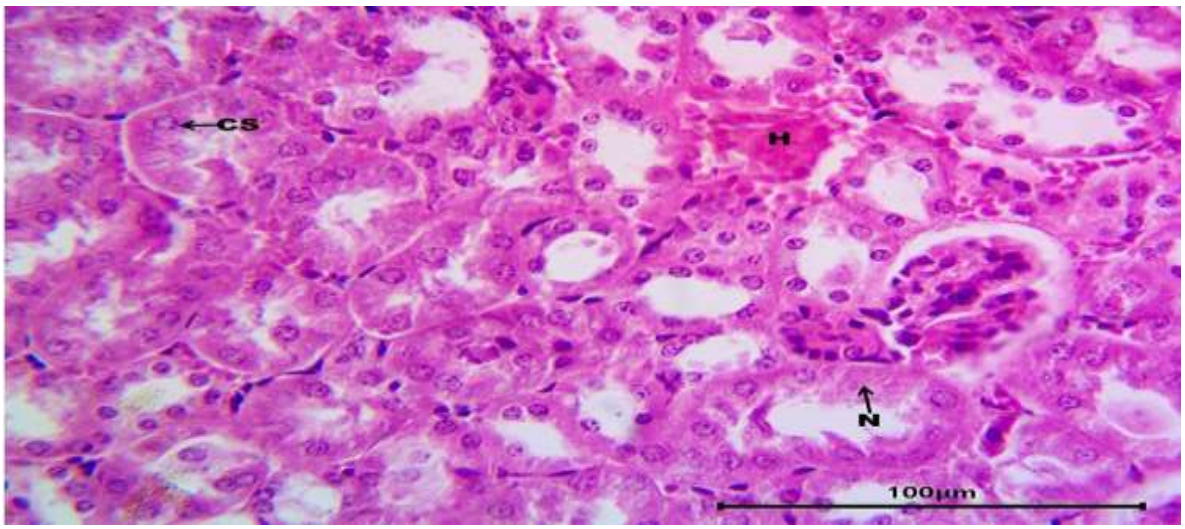

Figure (8):Histological section of a rat kidney from group B (infected + flavonoid) showing slight necrosis (N), slight cellular swelling of the epithelial cells lining the renal tubules (CS), and hemorrhage (HP). Hematoxylin and eosin stain. Magnification 400X, scale-bar = 100 µm.

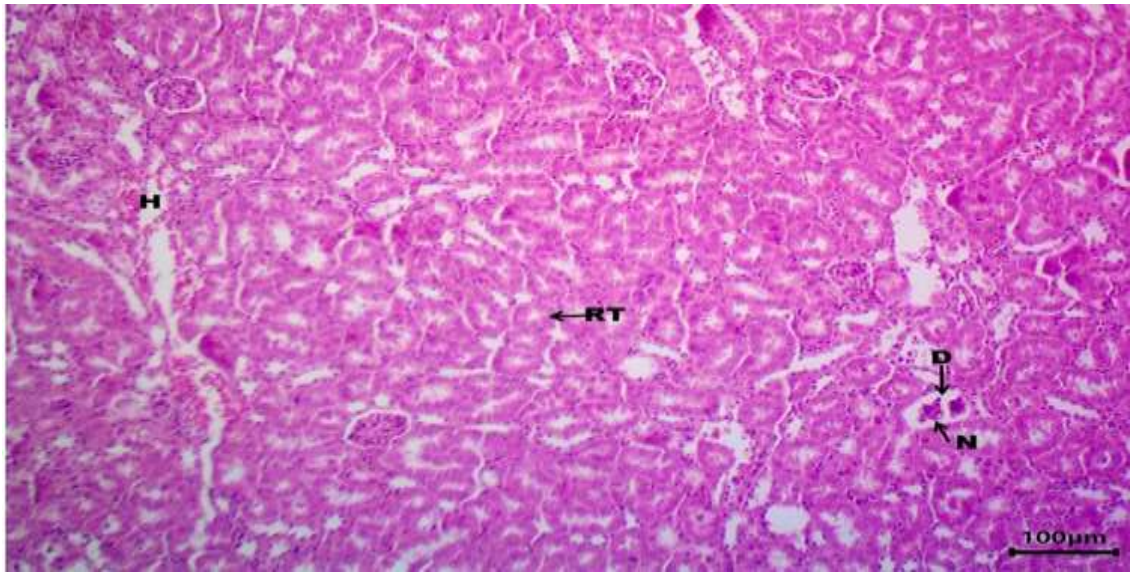

Figure (9):Histological section of a rat kidney from group C (injured + alkaloid) showing mild glomerular atrophy (A), slight dilatation of Bowman's capsule (D), hemorrhage (HP), and normal morphology of renal tubules (RT). Hematoxylin and eosin stain. Magnification 100X, scale-bar = 100 µm.

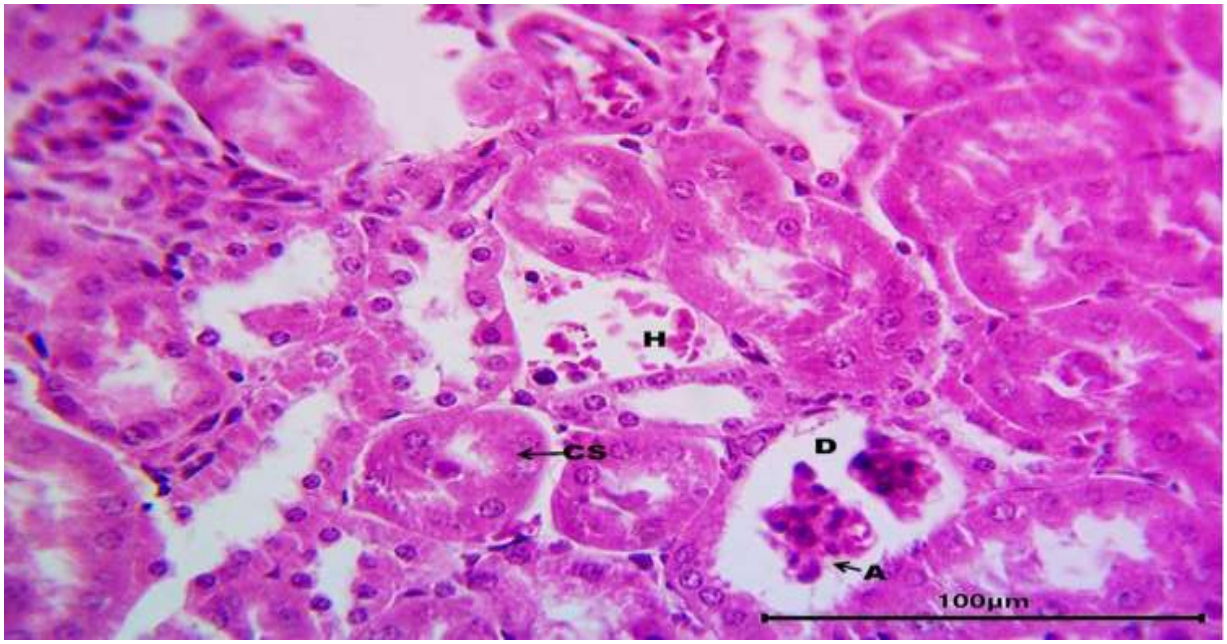

Figure (10):Histological section of a rat kidney from group C (injured + alkaloid) showing mild glomerular atrophy (A), slight dilatation of Bowman's capsule (D), hemorrhage (HP), and slight cellular swelling of the epithelial cells lining the renal tubules (CS). H&E stain. Magnification 400X, scale-bar=100µm.

In the forth group (Group 4) Figure (7) and figure (8), Treated with the flavonoid extract, a notable improvement in tissue showed minimal infiltration of inflammatory cells (iC), slight necrosis (N), slight cellular swelling of the epithelial cells lining the renal tubules (CS), proteinaceous hyaline casts (HC), and hemorrhage (HP),Flavonoid kaempferol significantly reduced renal inflammation, fibrosis,

and kidney dysfunction in diabetic mice by regulating tumor necrosis factor receptor, It also ameliorated renal injury and fibrosis[15].

In the fifth group (Group 5) Figure (9) and figure (10)**,** Treated with the alkaloid extract, a notable improvement in tissue showed mild glomerular atrophy (A), slight dilatation of Bowman's capsule (D), hemorrhage (HP), slight cellular swelling of the epithelial cells lining the renal tubules (CS) and normal morphology of renal tubules (RT). Result agreement with previous studies showed Histopathologic section of kidney analysis revealed that diabetic kidneys showed damaged nephric duct and glomeruli which are recovered after consecutive plant treatments of alkaloids ribulus terrestris (L) and Curcuma amada (R)[18]

Discussion:

Periostin in Mice:
Table (3):shown significantly increase level of POSTN in mice induced Behcet disease (547.8±58.0) compare with control (258±8.88). The effective dose of the oil(352±7.59), flavonoids(303±10.23), and alkaloids (328±6.32) (0.73, 1.26, and 0.2 mg/kg of body weight, respectively), POSTN significantly decreased suggested a positive impact of these compounds in reducing injury and slowing down disease progression, Vitex agnus-castus contain a proportion of omega-3 fatty acids, POSTN significantly decreased level than in mice induced behcet disease because of fatty acids Play an essential role in reducing the impact of vascular disorders, Including thrombosis and atherosclerosis. Studies have shown that omega-3 is effective in lowering the risk of vascular thrombosis, fatty acid composition, antioxidant properties, and enzyme composition. dietary supplementation with PUFAs is commonly used for their potential vessel protective effects, including their antiplatelet effects[16]. In mice treatment with flavonoids from the Vitex Agnus-Castus, POSTN significantly decreased level than in mice induced behcet disease , previous studies show that flavonoids affect gene expression related to inflammatory autoimmune diseases. A key anti-inflammatory mechanism is the inhibition of NF-κB. That important regulating immune responses and inflammation. By modulating NF-κB activity, flavonoids may help alleviate autoimmune disease symptoms and serve as potential treatment strategies[17]. In mice treatment alkaloids from the Vitex Agnus-Castus, POSTN significantly decreased level than in mice induced behcet disease, certain alkaloids exhibit potent anti-inflammatory properties and are crucial in managing various conditions, including BD and other autoimmune diseases[19]

Malondialdehyde in Mice:
Table(3):shown increase significantly level of MDA in mice induced Behcet disease (96.4±8.59) compare with control (35.47±2.71), Result in table (3) agreement with previous studies showed MDA is a final product of lipid oxidation that reflects the degree of free radical attacks, and its overexpression exacerbates in vessel and cell membrane damage[20,21]. Table (3):shown the result with effective dose of the oil(60.45±1.64), flavonoids(44.12±3.37), and alkaloids (54.56±2.83), Mice treatment with Oil from the Vitex Agnus-Castus showed significant decreased in MDA level than in mice induced Behcet disease, Previous studies showed oil contained PUFAs was found to significantly boost GPx and serum total antioxidant capacity, while reducing MDA levels[22]. Mice treatment with Flavonoids from the Vitex Agnus-Castus showed significant decreased in MDA level than in mice induced Behcet disease, Previous studies showed Flavonoids may reduce pro-inflammatory cytokine production and enhance antioxidant responses and decreased MDA level[23]. Mice treatment with alkaloid from the Vitex Agnus-Castus showed significant decreased in MDA level than in mice induced Behcet disease, Previous studies showed significantly lower value of testicular Malondialdehyde (MDA) was observed in the treated groups with the total alkaloid extract of P. harmala compared to the control group[24]

Concentration of Glutathione in Mice:
Table (3):shown decrease significantly level of GSH in mice induced Behcet disease (2.15±0.12) compare with control (4.41±0.31) in serum GSH levels in mice induced with induced Behcet disease compared to the control group. This decrease in GSH levels may be attributed to increased levels of

lipid peroxides observed in mice exposed induced Behcet disease, GSH, a non-enzymatic antioxidant, helps eliminate free radicals in the body. In its inactive form, GSSG interacts with proteins to form a compound called protein-glutathione disulfide, The formation of this compound stimulates the thiol transferase enzyme, reducing the amount of active GSH available [25]. Natural products from the Vitex Agnus-Castus significantly increased. GSH concentration in the blood serum of mice with Behcet disease, As presented in Tables Table (3). GSH levels increased due to activation of the gamma-glutamyl transpeptidase enzyme or improved glutathione reductase activity. The enzyme converts oxidized glutathione to its reduced form, assisted by the NADPH enzyme[26].

Concentration of Creatinine in Mice:

Table (3):showed increase significantly level of Creatinine in mice induced Behcet disease (1.16±0.05) compare with control (0.63±0.11) Oxidative stress is caused by the increase in reactive oxygen species (ROS) and reactive nitrogen species (RNS) inside cells, Many pathophysiological mechanisms related to kidney disease[27], inflammation increase oxidative stress leading to direct damage to kidney cells, including mesangial cells, podocytes, tubular epithelial cell and reduction in antioxidant mechanisms can lead to the progression of CKD, Accordingly, the kidneys of animals with CKD induced by nephrectomy had a high expression of NADPH-oxidase[28]. Natural products from the Vitex Agnus-Castus significantly decreased Creatinine concentration in the blood serum of mice with Behcet disease, As presented in Tables Table (3) Polyphenols primarily demonstrate their antioxidant capacity by directly scavenging reactive oxygen species and inhibiting their formation[29]. Oil contain fatty acid could have a beneficial reduce oxidative and renal stress in rat[30]. Alkaloid treatment regulates immune cell infiltration, inhibits oxidative stress, and suppresses apoptosis in the kidney in mice [31].

Concentration of Tumor Necrosis Factor-Alpha (TNF-α) in Mice

Table (3):showed increase significantly level of TNF-α in mice induced Behcet disease (66.3±2.39) compare with control (20.36±2.41) Previous studies indicated inflamation increases (TNF-α) production in mice [32]. However TNF-alpha concentrations significantly decreased after administration of Vitex Agnus-Castus extracts. This corresponds with prior research indicating that flavonoids and alkaloids reduce TNF-alpha concentrations. Flavonoids were associated with anti-inflammatory effects, while alkaloids demonstrated pharmaceutical effects. A previous study showed that lipophilic alkaloids can cross the blood-brain barrier and inhibit neuroinflammatory responses, which may involve the modification of TNF-alpha levels[33,34,35].

Concentration of Interleukin-8 in Mice:

Table (3):showed increase significantly level of IL-8 in mice induced Behcet disease (94.6±4.44) compare with control (27.58±5.80) IL-8 indicate the activation of immune system in BD, Serum IL-8 and TNF-alpha seem to be related to disease activity, Increased lipid peroxidation suggests oxidative stress in BD and therefore tissue damage in such patients[36,37]. However IL-8 concentrations significantly decreased after administration of Vitex Agnus-Castus extracts. This corresponds with prior research indicating that flavonoids and alkaloids reduce TNF-alpha concentrations. Flavonoids were associated with anti-inflammatory effects, while alkaloids demonstrated pharmaceutical effects. A previous study showed that lipophilic alkaloids can cross the blood-brain barrier and inhibit neuroinflammatory responses, which may involve the modification of IL-8 levels[38].

Conclusions

The study showed there was a correlation between increasing the concentration of the Periostin and the Behcet disease

This study found the natural products isolated from Vitex Agnus-Castus plant carrying plant extracts have a positive effect on the Behcet disease

through their therapeutic effect, as they led to the reduction of inflammatory factors and other oxidation factors.

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Conflicts of interest
There are no conflicts of interest.

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