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Effect of Artemisia fruit extract on TNF- α and IL-6 levels in streptozotocin-induced diabetic mice

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Objective This study was conducted to investigate the effects of Artemisia extract on some immunological parameters in streptozotocin-induced diabetic mice.

Methods After preparation of Artemisia extract, many chemical tests were used to identify the type of element and compounds presented in this plant using many chemical techniques. Thirty five streptozocin (STZ)-induced diabetes mice were divided into five groups; the first group provided only with water, the other four groups were consumed orally ingested the plant extract in four different concentration (2000, 1000, 500, 250) mg/kg of body weight. Another 10 mice did not injected with STZ were divided into two groups; one consumed Artemisia (Art group), and the second consumed only normal saline (Cont. group). After 14 days of diabetes induction and Artemisia extract treatment, the mice were sacrificed. Blood and tissue (brain, spleen and kidney) were collected. Fasting blood sugar and insulin levels were determined in the serum. Furthermore tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) levels were determined in the serum and aliquots of homogenize tissues.

Results Results declared that the extract of Artemisia fruit contains high levels of active compounds especially antioxidant compounds. IL-6 and TNF-α levels were decreased while insulin and glucose levels were increased in the STZ-induced mice group. Artemisia extract effects differently on glucose and insulin levels depending on its concentration. Interestingly, IL-6 and TNF-α levels increase in serum, brain, and spleen of the STZ-induced mice group consumed different concentration of Artemisia but it normalized in the STZ-induced mice group consumed 250 mg/kg Artemisia, as well as insulin and glucose levels for the same group, while there was no difference in kidney.

Conclusion Artemisia can control diabetes in 1000 and 500 mg/kg through controlling insulin level, and in the other hand, using the plant extract in 250 mg/kg, acts as an immune modulator for anti-inflammatory agents like IL-6 and TNF-α.

Keywords Artemisia, tumor necrosis factor-alpha, interleukin-6

Introduction

Diabetes mellitus (DM) is the most common metabolic and endocrine disorder worldwide, which is related to metabolism disorder of carbohydrate, fat, and protein.[1] Diabetes mellitus becomes globally important because of its escalate unabated occurrences. Hundred millions of people are suffering from diabetes around the world, and this number increase every year which will reach about 400–500 millions in 2030. Diabetes produces many complications which may cause the death.[2] In 2011, WHO reported that more than 80% of diabetes dies in low- and middle-income countries every year.[3]

One of these complications is the inflammation, which occurs more often in diabetic patients than those without. In this patient, the inflammation course is very complex. Immunological disorders are considered as one of the possible causes of the elevated rate of inflammations accompanying to diabetes mellitus.[4] The chronic insulitis, inflammation of pancreatic islets caused by destruction pancreatic cells mediated by auto-reactive T cells, play an important role in the diabetes mellitus type-1 development[5] while the triggering alteration in the diabetes mellitus type-2 is primary insulin resistance. Insulin resistance disrupts the balance of fat, cytokines and the production of adipocins, leading to increased systemic inflammation. Thus, inflammatory markers levels increase in the diabetes such as C-reactive protein, interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α).[6–10] Many studies explain the role of T-cells and TFN-α producing cells in the glucose homeostasis disorder of both type-1 and 2 DM.[11–14]

Recently, modern medicine include traditional medicine practices[15] such as vegetables, culinary herbs, and medicinal plants which are used in the management of diabetes.[15,16] WHO documented that the herbal medicine was used by 80% of world population.[17]

Artemisia is classified as follows: kingdom Plant, subkingdom Tracheobointa, superdivision Spermatophyta, Division Magnoliophyta, class Magnoliopsida, subclass Asteridae, order Asterales, Family Asteraceae, subfamily Asteroideae, genus *Artemisia* L., which is an important species used as medicinal plant species which is thoroughly studied.[18] This plant grows in the limestone Wadis of the desert, Red Sea regions and Sinai Oases.[19] Artemisinin is the major active constituent of Artemisia. The other compounds include arteether, artemether, artemotil, artenimol, artesunate, dihydroartemisinin, deoxyartemisinin, artemisinic acid, arteannuin-B, stigmasterol, friedelin, friedelan-3 beta-ol, artemetin, and quercetagetin-tetramethyl ether.[17,18,20,21] It is widely used to treat drug-resistant and non-drug resistant malaria, for gastrointestinal disorders, and urinary tract disorder as antispasmotic.[22] Artemisia has many effects such as anti-inflammatory,[23] antioxidant,[24] antihypertensive, and anti-hyperlipidemia,[19] and antitumor.[25] It was found that there is no acute or subchronic toxicity of ethanolic extracts of Artemisia in mice[26] therefore, can open the aspects to use this herb in controlling diabetes. Artemisia extract have been used to treat diabetic states in Middle East since a long time ago.[27]

The aim of this work is to study the effect of Artemisia extract on the physiological and immunological parameters in streptozotocin-induced diabetic BALB/c mice type.

Materials and Methods

Chemical Part

The plant tree is collected, cut and dried in the shade at room temperature for 1 week. Then, the fruit are taken and grind by an electric mill and kept in glass cans away from light, heat and humidity until use.

Three grams of Artemisia powder was placed in a glass flask. About 8 ml of nitric acid and 2 ml of 60% hydrochloric acid were added and left until the next day after covering with a watch bottle. It was placed in a sandy bath of 80°C for approximately 6 h. The volume is adjusted to 50 ml with distilled ion-free water. Finally, the elements (Zn, Ni, Cu, Cd, Cl, Pb) were estimated by atomic absorption device without flame, according to the method referred to in Kotani et al.[28]

About 10 ml of organic solvents mixture composed of hexane and ethyl acetate were added to 0.1 g of the Artemisia powder in 1:1 ration. The solution was filtered. The resulting filtrate was used to measure the FTIR spectra using FTIR spectrum analyzer from Shimadzu FTIR-8400S and gas chromatography-mass (GC-mass) using Gas chromatography–mass spectrometry from Shimadzu GCMS-QP2010 Ultra.[29]

About 50 g of powdered Artemisia fruit were dissolved in each of two volumetric flasks containing 1 L and 500 ml distilled water. The first flask was left in cold water solution. The second was placed in the Shaker incubator at 50°C for 48 h as hot solution. Both extracts are filtered by the filter paper (Whatman 1). The filtrate was centrifuged for 6 min (4000 cycles/min) to completely separate the solution from the remaining minutes of the previous filtration process and take the last solution to make the required measurements.

About 1.037 mg of fruit extract and 1.025 mg of the standard material for calibration, using the European[30] instrument of the Italian company (EuroVector S.p.A.) from EA 3000 have been used to analyze the presence of carbon, hydrogen, nitrogen, and sulfur elements in the fruit extract.

Biology Part

Forty five BALB/c mice were used in this study, from Prevention Research Center, Baghdad, Iraq. These mice aged about 12 week and weighted about 25 g. Thirty five mice were injected inter peritoneum (IP) with 50 mg/kg streptozocin (STZ) to induce diabetic disease. After 5 days, the fasting blood sugar (FBS) levels were determined. The STZ-induced mice with high blood sugar level (<150 mg/dl) were selected as diabetic models. The STZ-induced mice were divided into five groups (five mice in each group); the first group was provided only with water and served as control (STZ group). The other groups consumed Artemisia in different concentrations: 250, 500, 1000, 2000 g Artemisia/kg of mouse body weight. Another 10 mice did not inject with STZ divided into two control groups (five mice in each group); (Art group) which consumed 2000 mg/kg Artemisia, and (Cont. group) which consumed only normal saline.

The FBS was measured, using ACCU-CHEK Active from Roche, through the next 5 days after STZ-injection to select diabetic mice, and to determine FBS in zero time, in 7 and 14 days after treatment with Artemisia.

The mice were scarified by cervical distraction after 14 days. Spleen, kidney, and brain were homogenized with D.W. by Crucible. Homogenized samples were centrifuged at 20 000 rpm at 4°C for 10 min, and supernatants were collected and used to determine the IL-6 and TNF-α concentrations in tissues.

The blood was also collected and centrifuged for 10 min at 10,000 rpm. The serum was collected and was used to determine the insulin, IL-6, and TNF-α.

Serum samples were used to determine insulin level by Insulin-Products-Diesse from Diagnostica Senese, Spain. The serum samples and the eloquent of homogenized tissue were used to determine IL-6 by Mouse IL-6 ELISA Kit and TNF by Mouse TNF-a ELISA Kit from Koma Biotech Inc.

Statistical Analysis

Results were expressed as mean ± standard error. Data were analyzed by one-way analysis of variance followed by Fisher's test for multiple comparisons, using Statview version 5.0. Differences were considered significant when $p < 0.05$.

Results

Chemical Part

The results in Table 1 shows that the extract of the fruit of the Artemisia contains the elements of carbon, hydrogen, and nitrogen in different percentages. Carbon% was the highest element while the lowest element was nitrogen%. The sulfur and oxygen component was not mentioned in Table 1 because it was very low.

Many heavy materials were found in the extract of Artemisia fruit in different concentration. The highest was Cu while Ni was the lowest (Table 2).

Through the analysis of spectroscopy using the technique of gas chromatography–mass spectrometry, there are more than 21 compounds in the organic extract of the Artemisia fruit. It was found that it contains antioxidant groups and other compounds. Lilac aldehyde A was the highest occurrence in the extract while 2-(triethoxsilyl)propylamine was the lowest occurrence in this extract (Table 3 and Fig. 1).

Table 1. **CHNS analysis results of Artemisia fruit extract**

Type	Name	N%	C%	H%	S%	O%	Weight (mg)
Bypass	Bypass						
Blank	Blank						
Stander	Acetanilide	10.363	71.089	6.711			1.025
Sample	Artemisia	1.921	48.015	6.989			1.037

Table 2. **The elements in the extract of Artemisia fruit powder**

Mineral elements	Amount of concentration (mg/L)
Pb	0.3
Cl	0.35
Cu	0.82
Ni	0.1
Cd	0.6
Zn	0.4

Table 3. **GC-mass results of fruit extract of Artemisia**

Name	A/H	Height (%)	Height	Area (%)	Area	R. time	Peak#
Ethoxycarbonyl isothiocyanate	3.04	5.08	46287	4.06	140905	3.705	1
Acetic acid	4.15	16.32	148674	17.75	616411	5.886	2
Dimethyl sulfoxide	4.15	1.85	16868	2.01	69925	8.130	3
5,5-Dimethyl-2(5*H*)-furanone	3.63	5.73	52220	5.46	189582	8.877	4
Lilac aldehyde B	2.73	2.84	25889	1.76	61241	9.768	5
2(3*H*)-Furanone, 5-ethenyldihydro-5-methyl	3.45	5.83	53089	5.28	183279	9.968	6
Spiro(tetrahydrofuryl)2.1′(decalin)5′,5′,8′*a*-trimethyl	3.00	6.68	60903	5.26	182532	10.84	7
2-(Triethoxsilyl)propylamine	1.69	0.53	4795	0.23	8087	11.975	8
7-Oxabicyclo[4.1.0]heptane, 2-methyl	2.21	1.01	9218	0.59	20355	12.233	9
2(1*H*)-Naphthlenone, octahydro-4*a*-methyl-, cis-	1.60	0.68	6198	029	9909	13.086	10
Butylated hydroxytoluene	2.73	2.81	25651	1.75	60786	13.686	11
2-Cyclopenten-1-one, 3-methyl-2-(2-pentenyl)-, (*Z*)-	2.79	2.10	19094	1.54	53331	14.050	12
2-(4-Hydroxybutyl)cyclohexanol	2.73	0.87	7914	0.62	21594	14.849	13
Nonadecane, 1-chloro	1.85	1.16	10556	0.56	19534	15.003	14
Lilac alcohol B	2.91	3.73	34011	2.85	98804	15.233	15
Heneicosane	2.37	2.12	19351	1.32	45789	16.265	16
Docosane	3.13	5.21	47486	4.29	148838	17.703	17
Spiro[2.4]heptane-5-methanol, 5-hydroxy-	3.33	2.74	24966	2.39	83046	18.434	18
Tricosane	3.70	8.05	73467	7.82	271365	19.445	19
Lilac aldehyde A	5.37	17.17	156481	24.19	839843	20.922	20
Triacontane	5.08	7.49	68252	9.99	347046	21.657	21
Total		100.00	911270	100.00	3472211		

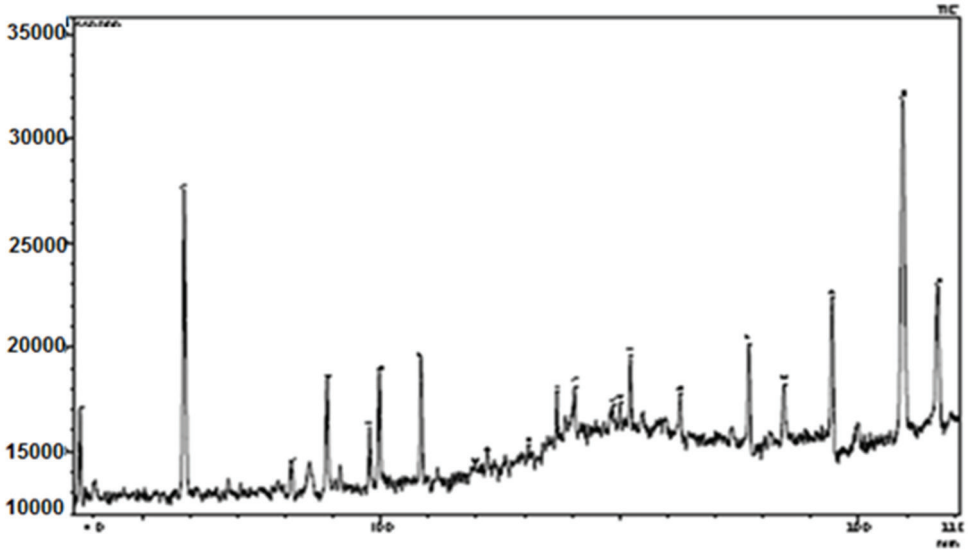

Fig. 1 **GC-mass results of fruit extract of Artemisia.**

FTIR spectrum analysis was used to detect the nature of the compounds and the active groups of these compounds present in the extract of Artemisia. As a result of vibration of electrons between the bonds, and by the active groups appear peaks in different places within the areas of measurement of the spectrum (Fig. 2).

Biology Parts

Fasting blood sugar of induced diabetic disease and controls groups was determined in zero time and after 7 and 14 days from treatment. In all groups of induced diabetes, the FBS reduced after Artemisia treatment compared with the controls groups, significant differences were detected between 14 days and zero time in mice consumed Artemisia in 250, 500, and 1000 g/kg concentrations and between 7 days and zero time in mice consumed Artemisia in 250 and 500 g/kg concentrations (Table 4).

In Fig. 3, significant decrease in the insulin levels in the induced diabetic disease group consumed only water (STZ) compared with other control groups while there was no

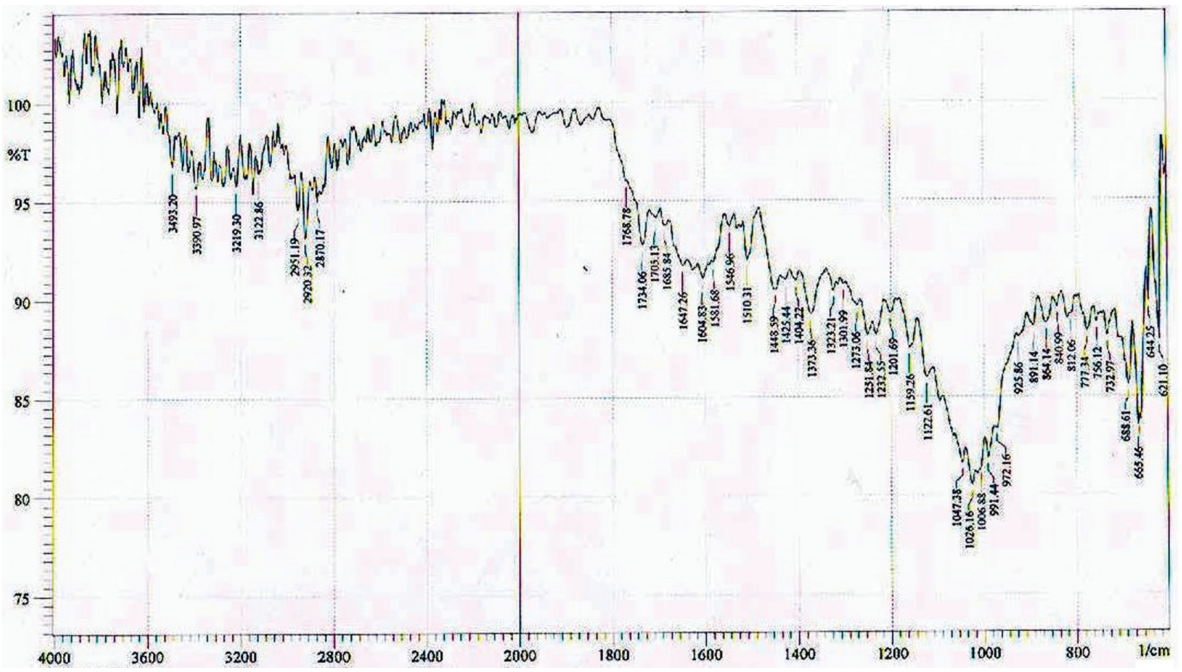

Fig. 2 **FTIR spectroscopy results in the fruit extract of Artemisia.**

Table 4. **The FBS level in induced diabetic disease and controls groups in zero time and after 7 and 14 days from treatment**

Groups	FBS (mg/dl)		
	Zero time	7 days	14 days
Cont	111.7 ± 3.7	112.0 ± 6.4	107.7 ± 1.5
Art	108.0 ± 6.6	112.3 ± 3.3	117.0 ± 0.6
STZ	166.4 ± 18.4	152.4 ± 13.2	167.5 ± 15.5
250	157.2 ± 5.0	139.5 ± 7.2*	138.5 ± 3.2§
500	179.8 ± 17.4	119.3 ± 15.7*	102.0 ± 21.0§
1000	162.0 ± 6.6	148.4 ± 6.8	130.3 ± 6.8§
2000	150.9 ± 8.9	150.3 ± 5.7	137.0 ± 4.5

*Significant differences in zero time vs. 7 days.
§Significant differences in zero time vs. 14 days.

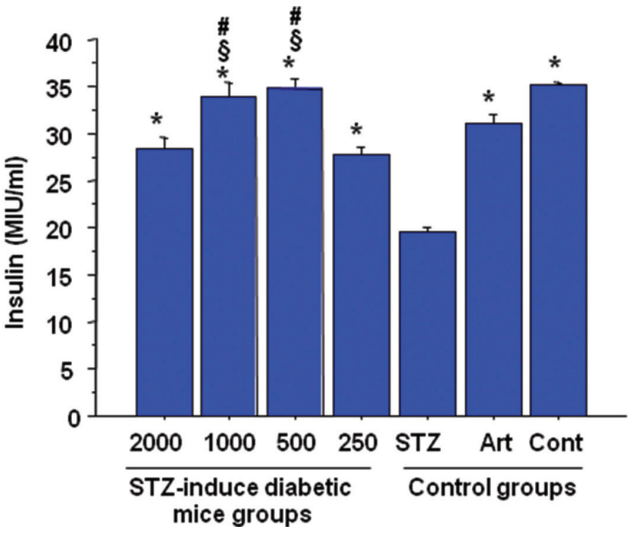

Fig. 3 **Insulin level in STZ-induced diabetic mice and controls groups after 14 days from treatment with Artemisia. *Significant difference in STZ vs. other groups, §significant difference in 2000 vs. other groups, and #significant difference in 250 vs. other groups**

significant difference between control groups consumed water (cont) and control groups consumed Artemisia (Art). Insulin level in all STZ-induced diabetic mice groups treated with Artemisia (250, 500, 1000 and 2000) was significantly high compared with STZ control group. It can be observed that insulin level was significantly higher in the STZ-induced diabetic mice groups consumed 500 and 1000 g/kg than those consumed 250 and 2000 g/kg Artemisia (Fig. 3).

The differences in serum TNF-α and IL-6 levels after 14 days from treatment among all studied groups are shown in Fig. 4. In the controls groups, while there was no significant difference between (cont) group and (Art) group, significant lower TNF-α and IL-6 levels in STZ group compared with other controls groups were detected. TNF-α and IL-6 levels in all STZ-induced diabetic mice groups treated with Artemisia (250, 500, 1000 and 2000 g/kg) was significantly elevated as compared with STZ group. An extraordinary increasing concentration of TNF-α and IL-6 levels were shown in mice group consumed 2000 mg/kg Artemisia and significantly lowest in the STZ-induced diabetic mice groups consumed 250 mg/kg Artemisia extract.

The differences in TNF-α and IL-6 levels after 14 days from treatment with Artemisia were determined in tissues, brain as central nervous system (CNS), spleen as lymphoid tissue, and kidney as non-lymphoid tissue. The results are shown in Figs. 5 and 6.

In kidney, there were no differences in TNF-α level seen, but significant differences were shown in brain and spleen as compared with STZ-control group as follows. In the control groups, significant decrease in TNF-α level in the induced diabetic disease group consumed only water (STZ) compared with other control groups while there was no significant difference between control groups consumed water (cont) and control groups consumed Artemisia (Art). TNF-α level in all STZ-induced diabetic mice groups treated with Artemisia (250, 500, 1000 and 2000 g/kg) was significantly higher compared with STZ control. Diabetic animals treated with 1000 mg/kg of Artemisia showed the highest level of TNF-α in the examined tissue (Fig. 5).

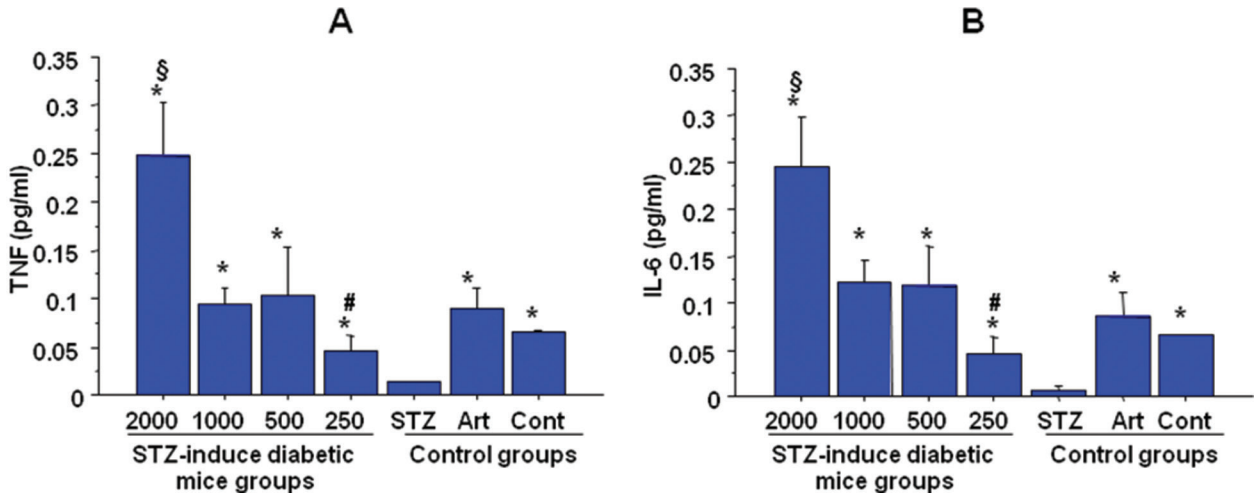

Fig. 4 **TNF-α (A) and IL-6 (B) levels in STZ-induced diabetic mice and controls groups after 14 days from treatment with Artemisia. *Significant difference in STZ vs. other groups, §significant difference in 2000 vs. other groups, and #significant difference in 250 vs. other groups.**

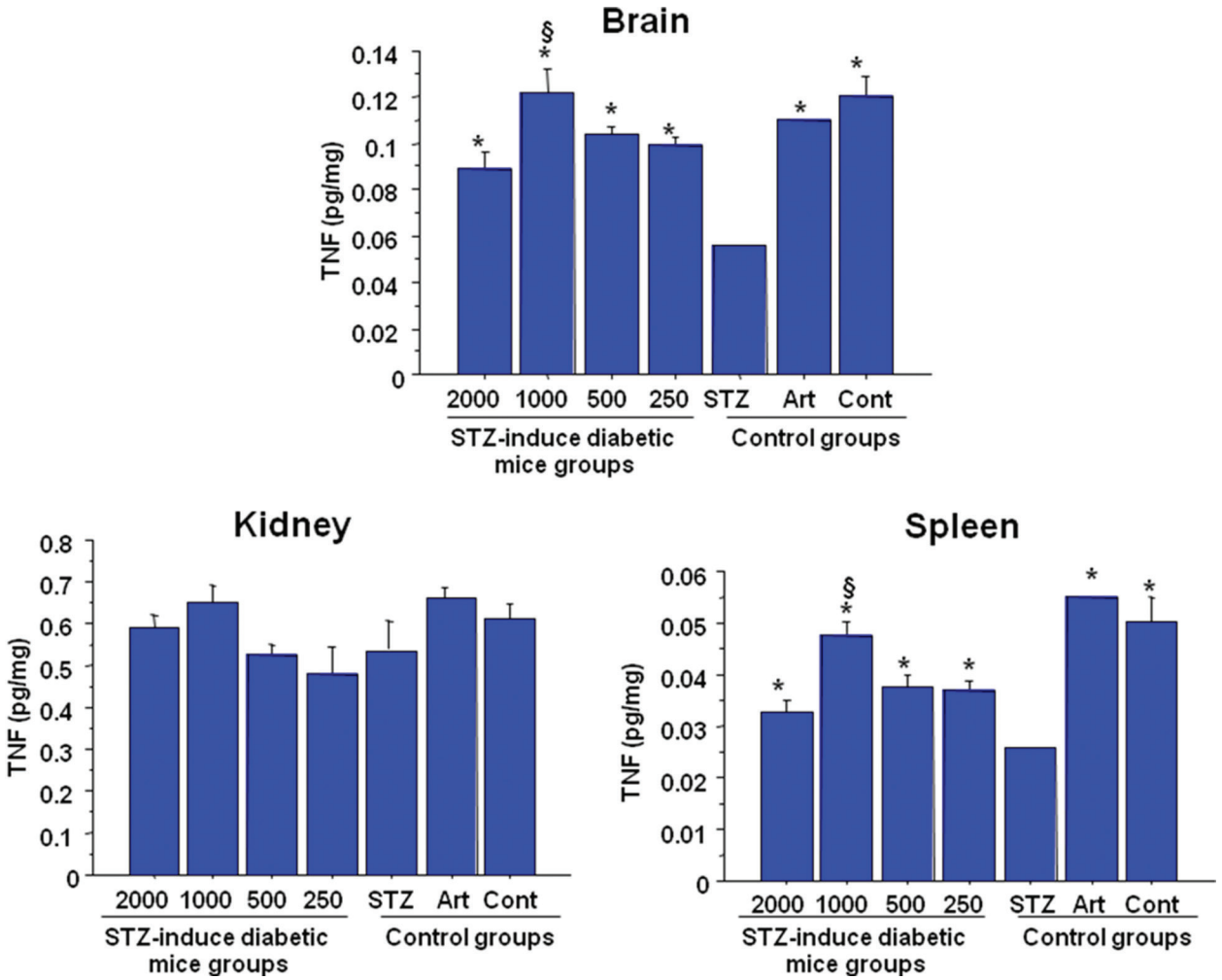

Fig. 5 **The TNF-α level into brain, spleen and kidney of STZ-induced diabetic mice and controls groups after 14 days from treatment with Artemisia. *Significant difference in STZ vs. other groups, §significant difference in 1000 vs. other groups.**

Another significant difference in IL-6 level into brain and spleen were shown, while no differences in the kidney were detected, in addition there was no significant difference between control groups consumed water (cont) and control groups consumed Artemisia (Art) group, but significant low IL-6 level in STZ control group as compared with other controls groups were detected. IL-6 level in all STZ-induced diabetic mice groups treated with Artemisia (250, 500, 1000 and

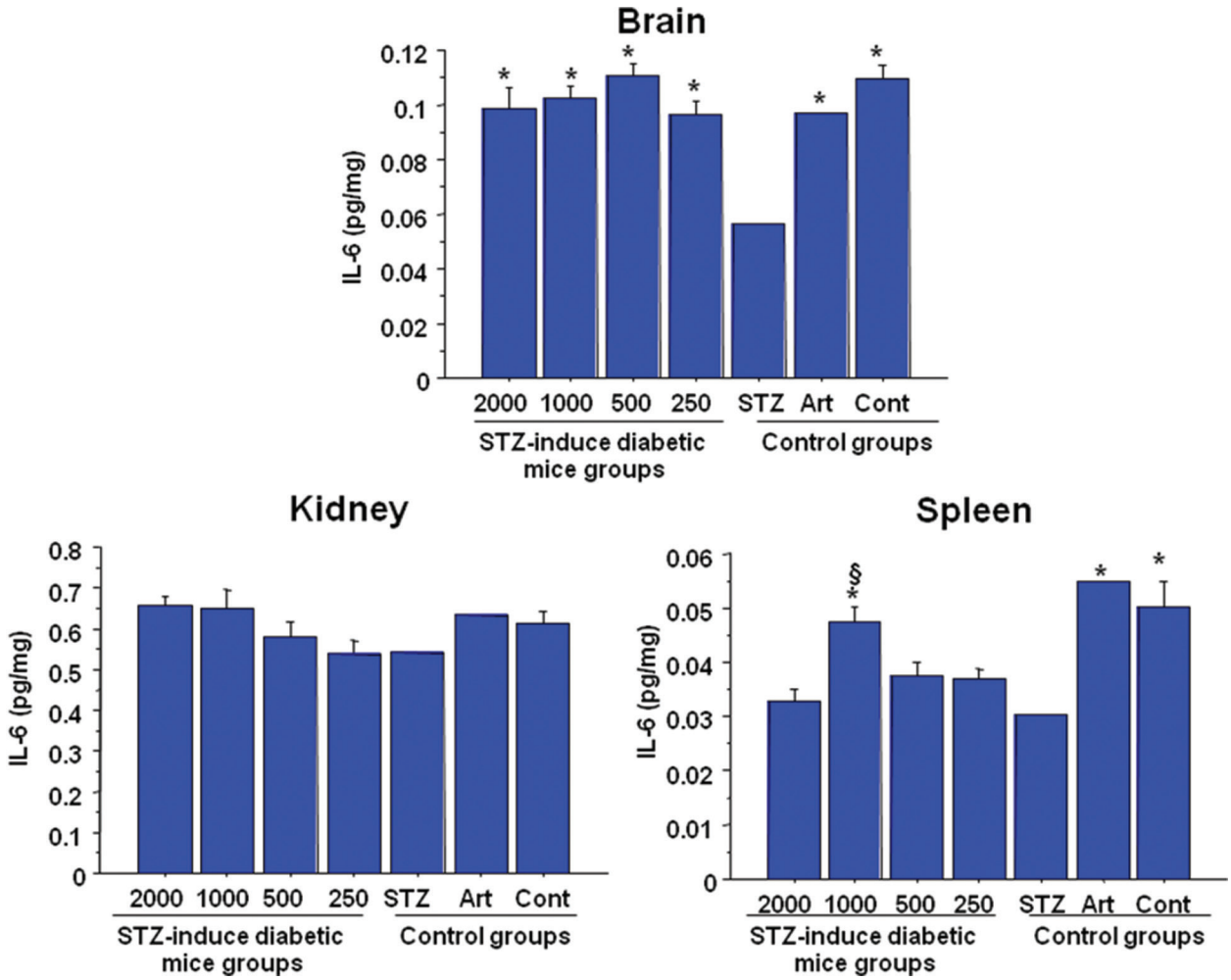

Fig. 6 **The IL-6 levels brain, spleen, and kidney of STZ-induced diabetic mice and controls groups after 14 days from treatment with Artemisia. *Significant difference in STZ vs. other groups, §significant difference in 1000 vs. other groups.**

2000 g/kg) was significantly higher compared with STZ into brain, but into spleen only the STZ-induced diabetic mice group consumed 1000 g/kg Artemisia was significantly higher compared with STZ group and other STZ-induced diabetic mice groups consumed Artemisia (Fig. 6).

Discussion

Results obtained in this research, using GC-mass technique to elucidate the nature of phytochemical compounds in terms of molecular weight and partial formula, show that the extract of Artemisia fruit contains high levels of active compounds especially antioxidant compounds group representing the bulk of the fruit and its ability to link with other active groups. These results agree with many other researches.[31,32] As well as Artemisia extract found to have certain mineral elements that trigger the activation of enzymes in the body[28,33] and the presence of flavonoids, which capture free radicals,[34,35] and leprosy. They activate the enzymes and transporter proteins in the membrane of the cell.[28] Since Artemisia has better phytochemical compounds[36] which may have role in much of activities.[36,37] Moreover, Swamy et al. in 2016 reported that Artemisia enter into various fields such as medical, pharmaceutical, fragrance, and flavor manufacturing. Therefore, it became one of the medical plants which form a large part of natural plants.[38]

The usage of STZ to induce diabetes, in congenitally albino mice, was reported in many sources to induce diabetes because STZ, a glucosamine-nitrosuria which derived from *Streptomyces achromogenes*, which is used as a chemotherapeutic agent in carcinoma of pancreatic β cell. These cells are damaged by STZ leading to hypoinsulinemia and hyperglycemia and induce adiabatic state. STZ has chemical structure similar to glucose which allow it to link with the receptor of GLUT2 glucose transporter by which allow STZ to enter and accumulate in β cells.[39–41] Thus, STZ induce immune-mediated insulin deficient diabetes and depression of immune reactivity.[42] These evidences can explain the decrease of IL-6 and TNF-α, and the increase of insulin and glucose levels in the mice group treated with STZ compared with control.

It is well established that Artemisia extract is very useful in diabetes. Antidiabetic ability of various extracts of Artemisia due to the presence of any secondary metabolites (flavonoids, alkaloids, phenols, glycosides and terpenes) at various concentrations in Artemisia. It has been reported that the secondary metabolites have differently antidiabetic potential, which is the proposed cause of variation in the activities of these extracts.[43,44] These evidences can explain the different effects of Artemisia on glucose and insulin levels, depending on its concentration, as shown in this study which comes in agreements with Chang et al.[45] and Yagi et al.[46]

It seems that Artemisia might boost the innate immunity[47] which led to use it as antifungal agent in many fungal infections.[48] Since IL-6 and TNF-α soluble mediators are secreted by different innate immune cells,[49] decreased by STZ effect; Artemisia try to boost it.[50] In our results, the levels of both IL-6 and TNF-α were increased in the STZ-induced mice group treated with different concentrations of Artemisia but it is normalized in the STZ-induced mice group treated with 250 mg/kg Artemisia, in which the insulin and glucose levels also normalized.

It is at present well established that immune cells produce soluble mediator that can influence the CNS and modify its activity in different ways.[51,52] On the other hand, CNS exerts numerous immunomodulatory functions in lymphoid organs.[53] The immunomodulatory effect of CNS mediators is mainly exerted by stimulation of β_2-adrenoreceptors, which are expressed by B lymphocytes, $CD4^+$, and $CD8^+$ T cells, innate immunity cells, and Th1 helper cells.[54] In our results, the brain, CNS, spleen, and lymphoid organ, were affected by STZ and Artemisia, in which the IL-6 and TNF-α were decreased in STZ group compared with control these levels increased in the STZ-induced mice group treated with Artemisia and normalized in the STZ-induced mice group treated with 250 mg Artemisia. Interestingly, these results were different in the kidney, non-lymphoid organs, in which, there was no differences in IL-6 and TNF-α levels among all groups.

Conclusion

From the results in this work, it can be conclude that the STZ caused diabetic by increase insulin and glucose levels in the blood resulting from damage in the pancreatic β cell; and depression of immune activity. Artemisia can be a good treatment for this case because of the effectiveness of its chemical components by correcting the imbalance in the β cells and raising the insulin and glucose levels in the blood. On the other hand, it boost the innate immunity which secreted IL-6 and TNF-α and normalized the levels of IL-6 and TNF-α in the blood, CNS, and lymphoid organs which connected in different ways, while kidney were not affected by STZ or Artemisia.

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Conflict of Interest

None.

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