

The Effects of Methanol Extract Derived from *Urtica pilulifera* Leaves on Some Hematological and Biochemical Parameters of Diabetic Rats

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Abstract: This research was carried out to examine the effects of methanol leaf extract of *U. pilulifera* on some hematological and biochemical parameters in diabetic rats. Forty rats weighting 150-185 g were divided into four groups: group I treated with a vehicle (control); group II including diabetic rats treated with a vehicle; groups III and VI including diabetic rats treated with 1.0 and 2.0 g kg⁻¹ of the extract for 3 weeks, respectively. Rats were made diabetic by injection with a single dose of streptozotocin (70 mg kg⁻¹, i.p). At the end of the experimental period, rats were killed and blood samples were taken for biological analysis. The blood glucose level, Erythrocyte Sedimentation Rate (ESR), Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), urea and creatinine levels significantly increased in diabetic rats as compared to control. By contrast, significant reduction of the erythrocyte and leukocytes counts and Pack Cell Volume (PCV), Thyroxine (T₄) and Triiodothyronine (T₃) and Thyroid Stimulating Hormone (TSH) levels in diabetic group were also noticed in diabetic rats as compared to control. Treatment of diabetic rats with this extract caused a significant decrease in blood glucose levels, ESR, urea and creatinine, whereas ALT and AST slightly lowered. In addition, in diabetic treated groups, the erythrocyte and leukocyte counts and PCV, T₃, T₄ and TSH values significantly increased, whereas the leukocyte count slightly increased. We can conclude that *U. pilulifera* extract improves the diabetic status in terms of blood sugar and alleviates the diabetes-induced disturbances of some hematological and biochemical parameters of rats.

Key words: Antioxidants, blood, thyroid hormones, hyperglycemia, rats, streptozotocin, *Urtica*

INTRODUCTION

Diabetes Mellitus (DM) is mostly due to low level of insulin production or an inability of the body to use insulin properly which, in turn, leads to hyperglycemia. Hyperglycemia plays a central role in progression and complications of DM. The complications of DM are many and include poor metabolic control, nephropathy, hepatopathy, depression of the thyroid axis and even death (Narayan *et al.*, 2003). Once, the individuals have been diagnosed with DM, the aim of treatment is to keep blood glucose close to normal physiological level and to reduce the risk of complications from hyperglycemia. Blood is the vital fluid that plays a key role in control the delivery of nutrients, endocrine hormones, metabolic excretion and immunological processes, as well as homeostatic responses (Clark and Wallis, 2003). Therefore, for the purpose of disease diagnosis, prevention and treatment, blood parameters are still the most highly accurate, sensitive and reliable parameters that investigators highly used and depend on.

Drugs from plant products have long been used in the treatment of diabetic patients via improving the diabetic status in terms of blood sugar (Marles and Fansworth, 1995; Sabu and Kuttan, 2002). In addition, drugs from plant extracts are considered to be safer and induce fewer side effects than synthetic drugs. On other hand, undesirable effects such as hypoglycemic episodes and others appear during intake of oral hypoglycemic synthetic drugs. Thus, the use of medicinal plants to control DM as alternative treatment to synthetic treatment has been highly recommended by the World Health Organization (WHO) especially, in countries, where access to the synthetic treatment of diabetes is inadequate (WHO, 1998).

For long period of time, several species of medicinal plants are being used in the treatment of DM in traditional systems of medicine throughout the world (Marles and Fansworth, 1995; Sabu and Kuttan, 2002; Afif and Abu-Irmaileh, 2000). One of these, *Urtica pilulifera* L. (*U. pilulifera*) is an annual herb of family Urticaceae that is widely distributed in the Mediterranean region,

especially, in Jordan, where is known as Qurrais and Nettle in Romman (Afif and Abu-Irmaileh, 2000). For decades, this plant is very valuable herbal medicine because, it has been used in a number of areas of the world as traditional medicine for the treatment of various diseases including stemming internal bleeding, anemia, excessive menstruation, hemorrhoids, arthritis, rheumatism, hay fever, kidney problems and pain and skin complaints especially, eczema. In addition, this plant has demonstrated a wide range of biological activities such as antiasthmatic, antitumor, astringent, diuretic, antidandruff, galactagogue, deputative and anti-hyperglycemic (Tahri *et al.*, 2000; Chrubasik *et al.*, 2007; Mahmoud *et al.*, 2006; Kavalali *et al.*, 2003). For instance, anti-hyperglycemic chronic effects of crude extract of *U. pilulifera* have been demonstrated in rat model, in which diabetes mellitus has been induced by administration of Streptozotocin (STZ) (Kavalali *et al.*, 2003). Furthermore, in rural areas of the United Kingdom and Canada, *U. pilulifera* makes an excellent spinach substitute and can also be added to soups and stews. In addition, a tea made from the leaves of this plant has also been traditionally used as a stimulating tonic and blood purifier as well as hemostatic (Chrubasik *et al.*, 2007). However, the efficacy and safety of this herb have not been reported yet in details in animals or humans despite widespread use of this plant as a vegetable and as a folk medicine in treatment of DM. It is also important to note that STZ-induced diabetes exhibit a number of defects in rat organs that resemble those seen in diabetic humans. Thus, it would be worthwhile to study the efficacy and safety of this herb to blood, liver, kidney and thyroid gland in diabetic rat. Therefore, the present study was undertaken to explore the effects of three weeks dosing of methanol extract of *U. pilulifera* on some hematological and biochemical parameters of STZ-induced diabetic rats.

MATERIALS AND METHODS

Plant collecting and processing: Leaves of *U. pilulifera* were collected during the month of May from Sail Husban, Nauor, Jordan. The plant was identified by Professor Jamil Lahham, taxonomist, at the herbarium of the Department of Biological Sciences, Faculty of Sciences, Yarmouk University, Irbid, Jordan. The voucher specimen (No.AHE-3-007) was deposited in the Department of Biological Sciences, Faculty of Sciences, Al al-Bayt University, Al-Mafraq, Jordan. The leaves of *U. pilulifera* were dried in the shade. Then, the leaves were crushed and finely powdered. The powder (500 g) was placed on a Soxhlet cold extractor using absolute methanol as solvent and remained for three consecutive days. The

extract was concentrated to dryness in rotary evaporator under reduced pressure at 45°C to yield a viscous greenish-colored extract (57 g). The extract was stored in a refrigerator at 4°C in a glass container until use. No sign of toxicity was noticed on rats when exposed to *U. pilulifera* methanol leaf extract up to 20 g kg⁻¹ of body weight. Two doses of *U. pilulifera* methanol extract (1.0 and 2.0 g kg⁻¹ of body weight per day) were selected based on the LD₅₀ and used in this study. For preparation of the 2 tested doses, appropriate amount of this viscous extract was dissolved in 1 mL Tween 20. This was followed by adding 9 mL of 0.9% NaCl to each mixture. The vehicle was obtained by dissolving 1 mL of Tween 20 in 9 mL of 0.9% NaCl.

Animals: Forty Wister rats were obtained from the animal house of the Jordan University of Science and technology, Irbid, Jordan. The rats weighting 150-185 g were used for this study. The rats were harbored in stainless steel cages under standard laboratory condition of 12 h light/dark cycle throughout the experimental periods. They had access to food (Top Fed, Sapele) and water *ad libitum*. The animals were carefully checked and monitored every day for any changes. Daily body weight measurement for each rat was recorded. Food and water consumption of the rats were also daily recorded.

Experimental design: To induce diabetes, rats were first anesthetized with inhalation of gaseous nitrous. STZ was purchased from Sigma Company (St Louis, MO, USA) and was prepared in freshly citrate buffer (0.1 M, pH 4.5). The STZ solution was injected intraperitoneally at a concentration of 70 mg kg⁻¹ of body weight in a volume of 1 mL per rat (Suthagar *et al.*, 2009). After the STZ injection, the rats were given a 5% glucose solution for 48 h and then were subjected to overnight fasting. After measuring fasting blood sugar, diabetic status was determined. Rats with blood glucose of 250 mg dL⁻¹ or more were classified as diabetic rats and were used for the subsequent experiments.

Forty rats (30 diabetic surviving rats, 10 control rats) were assigned to four experimental groups of 10 rats each. Group I consisted of nondiabetic rats that received only the vehicle (0.5 mL kg⁻¹ body weight) and served as a control group; group II consisted of diabetic rats that received only vehicles (0.5 mL kg⁻¹ body weight); groups III and IV consisted of diabetic rats that received methanol extract of *Urtica pilulifera* orally, 3 days after the STZ injection, at daily doses of 1.0 and 2.0 g kg⁻¹, respectively. Treatments were orally administrated every day by gavage, using feeding needle. Rats were maintained in these treatment regimens for 3 weeks with

free access to food and water *ad libitum*. These experiments complied with the guide-lines of the animal ethics committee, which was established in accordance with the internationally accepted principles for laboratory animal use and care.

Sample collection: Blood sample was collected from each animal by cardiac puncture and rats were sacrificed by cervical dislocation under light ether anesthesia. Part of the blood sample was put in EDTA bottles and used for determining the some hematological parameters described below. The remaining blood sample was put into test tubes and allowed to clot for 30 min before centrifuging using a bench top centrifuge for determining the biochemical parameters described. Serums, for biochemical and hormonal determinations were stored at -20°C until the day of measurement.

Hematological analysis: The CBC is commonly performed on an automated hematology analyzer using whole well mixed blood to which, EDTA is added to prevent clotting. ESR was determined by Wintergreen's method. Differential leukocyte count was performed on Geimsa stained blood smears.

Biochemical analysis: Glucose, creatinine, urea, ALT and AST were determined by using commercial analytical kits from Sigma (Lab-Kit, Spain). Serum T₃, T₄ and TSH levels were measured by radioimmunoassay (Gupta *et al.*, 1997).

Statistical analysis: The results were expressed as mean±SD. Differences between groups were analyzed with student's t-test. Differences between groups were considered at 95% confidence limit and probability level of 0.05. The results were taken as significant if p<0.05.

RESULTS

Effect on blood glucose level: The blood glucose level in diabetic group was significantly higher (p<0.05) than those of the control group (Table 1). On the other hand, administration of methanol leaf extract of *U. pilulifera* for 21 days was found to lower blood glucose significantly in a dose dependent manner in treated diabetic groups (p<0.05) when compared with those of the diabetic group. In addition, significant (p<0.05) weight loss was observed in diabetic group II when, compared with those of the treated diabetic groups III and IV and control group I.

Effect on some hematological parameters: As can be seen (Table 2), the erythrocyte count and PCV value in diabetic group reduced significantly (p<0.05) compared to control group at the end of the experimental period (Table 2). By

Table 1: Effect of treatment with methanol extract of *U. pilulifera* for 3 weeks on serum glucose concentration (mg dL⁻¹) in STZ-induced diabetic rats

Groups	Days		
	7	14	21
I	83.8±6.2	87.8±11.6	79.4±8.9
II	349.7±28.5*	332.7±32.7*	337.9±37.2*
III	280.6±21.4**	273.3±24.1**	255.8±31.6**
IV	274.6±27.4**	262.5±33.4**	246.3±27.3**

Table 2: Effect of treatment with methanol extract of *U. pilulifera* for 3 weeks on some hematological parameters (erythrocyte, leukocyte, PCV and ESR) in STZ-induced diabetic rats

Groups	Erythrocyte (10 ⁶ µL ⁻¹)	Leukocyte (10 ³ µL ⁻¹)	PCV (%)	ESR (%)
I	6.7±0.4	13.8±2.3	39.2±4.5	11.8±2.9
II	4.3 ±0.6*	11.1±1.6	34.9±3.8*	16.9±4.3*
III	5.4±0.4**	11.5±2.2	46.3±5.2**	12.7±2.6**
IV	5.9±0.5**	11.9±1.5	44.7±5.1**	12.1±3.4**

Values are expressed as the mean±SD of 10 rats. *Statistically significant when compared to control group (I) at p<0.05, **Statistically significant when compared to untreated diabetic group (II) at p<0.05

contrast, significant increase in the erythrocyte counts and PCV values were also recorded in treated diabetic groups compared to untreated diabetic group (p<0.05). The data also indicated that ESR value of the diabetic group increased significantly compared to control group (p<0.05), whereas a significant decreased in ESR values were observed in treated diabetic groups compared to diabetic group (p<0.05). Furthermore, leukocyte count significantly lowered in diabetic group as compared to control group (p<0.05). By contrast, treated diabetic groups showed slight increase in leukocyte counts after 21 days of treatment with *U. pilulifera* leaf extract compared to diabetic group and this increase was not statistically significant.

Effect on some biochemical parameters: The present study also indicates that ALT and AST increased significantly (p<0.05) in diabetic group as compared to control group as shown in Table 3. However, administration of *U. pilulifera* leaf extract for 21 days was capable to slightly lower ALT and AST levels in diabetic groups. Along the same line, serum urea and creatinine levels significantly (p<0.05) increased in diabetic group when compared with those of the control group. On other hand, after treatment of the diabetic groups with this extract, the serum urea and creatinine levels (p<0.05) reduced significantly when compared to those of the diabetic group (Table 4). Furthermore, the present study also indicates that serum T₃, T₄ and TSH levels significantly decreased (p<0.05) in diabetic rats when compared with those of the control group (Table 5). By contrast, after treatment the lowered serum T₃, T₄ and TSH levels significantly increased (p<0.05), when compared to diabetic group. In addition, serum TSH levels for treated diabetic groups were slightly higher than that

Table 3: Effect of treatment with methanol extract of *U. pilulifera* for 3 weeks on aspartate Aminotransferase (ALT) and alanine Aminotransferase (AST) in STZ-induced diabetic rats

Groups	ALT (U L ⁻¹)	AST (U L ⁻¹)
I	3.4±1.5	9.0±2.5
II	5.8±0.9*	12.6±3.1*
III	5.2±1.2	11.8±1.2
IV	4.9±0.7	10.9±3.5

Table 4: Effect of treatment with methanol extract of *U. pilulifera* for 3 weeks on urea and creatinine in STZ-induced diabetic rats

Groups	Urea (mg dL ⁻¹)	Creatinine (mg dL ⁻¹)
I	0.78±0.18	38.5±3.8
II	0.89±0.16*	47.6±4.2*
III	0.80±0.10**	39.3±3.4**
IV	0.76±0.13**	38.2±3.0**

Table 5: Effect of treatment with methanol extract of *U. pilulifera* for 3 weeks on T₃, T₄ and TSH in STZ-induced diabetic rats

Groups	T ₃ (pg mL ⁻¹)	T ₄ (ng dL ⁻¹)	TSH (iU mL ⁻¹)
I	12.8±2.2	38.2±4.5	11.8±3.9
II	8.1±1.6*	27.9±3.8*	8.5±2.3*
III	12.2±2.2**	44.8±4.2**	12.4±2.6**
IV	12.4±1.5**	46.5±5.1**	12.5±2.4**

Values are expressed as the mean±SD of 10 rats. *Statistically significant when compared to control group (I) at p<0.05, **Statistically significant when compared to untreated diabetic group (II) at p<0.05

from the control group. *U. pilulifera* treatment also significantly increased the serum T₄ when, compared with those of the control group (p<0.05).

DISCUSSION

Insulin is known as an anabolic hormone that plays a central role in maintenance of body growth and regulation of overall body metabolism (Clark and Wallis, 2003; Takac *et al.*, 1992). By contrast, insulin deficiency causes hyperglycemia which, in turn, leads to secondary complications affecting overall body growth and metabolism (Kennedy and Baynes, 1984). Similarly, the present study reports that STZ induces hyperglycemia as indicated by high blood glucose level in rats. In addition, STZ induces anemia as indicating by low levels of erythrocyte and leukocyte numbers as well as PCV and ESR values. This is in agreement with earlier studies, which indicated that STZ induces diabetes and its complications by stimulating the formation of free radicals in experimental animals (Szudelski, 2001). It has also been reported that occurrence of anemia in DM is more common and is mostly due to an increase in non-enzymatic glycosylation of erythrocyte membrane glycoproteins, which correlates with hyperglycemia (Ceriello, 2003; Kennedy and Baynes, 1984). These increases in oxidation of glycoproteins induce an increase in the production of lipid peroxides causing a hemolysis of erythrocyte. However, the question regarding the relationship between anemia and diabetes is still unclear.

Interestingly, the data clearly indicate that these negative effects are mainly due to the cytotoxic action of STZ in rats. It has also been noted that the cytotoxic action of STZ is mediated by the formation of free radical species which, in turn, causes rapid increase in glycosylation and oxidation of membrane glycoproteins (Szudelski, 2001). This is followed by rapid destruction of the insulin secreting β -cells of pancreas leading to insulin deficiency and hyperglycemia (Golalipour and Khorri, 2007). The changes observed in the STZ-diabetic group of rats support further the above notion and also reinforce the importance of insulin in maintaining body growth and metabolism.

The study also reveals that administration of *U. pilulifera* extract lower blood glucose level significantly in diabetic rats. The results are in agreement with earlier study of Kavalali *et al.* (2003) and Golalipour and Khorri (2007). In addition, the current study also reveals for the first time that *U. pilulifera* extract ameliorates some disturbed hematological parameters of STZ-diabetic rats such as RBC count and PCV and ESR values. This coincides with the traditional use of *U. pilulifera* in folk medicine as anti-hyperglycemic agent, a stimulating tonic, blood purifier and haemostatic and for the enhancement of hemoglobin concentration (Chrubasik *et al.*, 2007; Kavalali *et al.*, 2003).

Furthermore, there is a growing evidence that antioxidant compounds from plant species were found to exhibit anti-hyperglycemia activity by improving secretion of insulin and/or increasing glucose uptake by the peripheral tissues. Thus, an increase intake of food rich with antioxidants can prevent the development of diabetes and its complications (Ceriello, 2003; Shetty *et al.*, 2004). In addition, recent study reported that methanol extract of all parts of *U. pilulifera* extracts were found to exhibit the best antioxidant activity against various oxidative systems *in vivo* and this activity has been attributed to the reduction of lipid peroxidation and elevation of antioxidant enzyme activities (Mahmoud *et al.*, 2006). Therefore, the presence of antioxidant compounds such as vitamins, flavonoids and minerals in this plant provides further evidence for the beneficial chronic effects of *U. pilulifera* leaf extract on the STZ-induced diabetic rat model. Taken together, it is possible to suggest that this extract might play a vital role in improving the diabetic status in terms of blood sugar by restoring the structural and functional properties of β -cells of pancreas, a primary target organ for STZ.

The present data also shows that leukocyte number slightly improves, when rats were given *U. pilulifera*. In accordance with the present study, an earlier study reported that flavonoid glycosides from *U. dioica* L. have

in vitro immunomodulatory activities, indicating that these extracts were useful for patients suffering from neutrophil deficiency (Akabay *et al.*, 2003). Thus, it is possible to suggest that *U. pilulifera* treatment might increase the defense mechanism of the rats against infections by slightly increased the lowered neutrophil count of leukocyte in diabetic rats. The present study also, indicates that a single dose of STZ is effective in inducing thyroid dysfunction as shown by disturbance of the serum T₃ and T₄ levels. In consistent with these results, different laboratories have reported that insulin, T₃, T₄ and TSH levels in serum are significantly reduced in animals during STZ-induced DM (Vondra *et al.*, 2005; Ghubb *et al.*, 2005). On other hand, The data indicate that oral administration of methanol extract of *U. pilulifera* restores serum T₃ and T₄ back to their normal levels, thus, effectively protected thyroid cell functions and structure.

Furthermore, the present results also show that injection of STZ induces a hepatocellular damage, which is another characteristic change in diabetes as evidenced by high serum levels of AST and ALT in untreated diabetic groups. These increases may be due to the leakage of these enzymes from the liver cytosol into the blood stream and/or change in the permeability of liver cell membranes takes place. On other hand, oral administration of *U. pilulifera* extract slightly lowered the ALT and AST levels. Therefore, it is possible to suggest that this extract is safe and might confer protection against STZ induced hepatocellular damage as evidenced by normal serum levels of AST and ALT in treated diabetic groups. In addition, the results also go along with previous report, which indicated that *U. dioica*, a closely related species, has hepatoprotective activity and the main component is polyphytychol (Dashinamzhilov *et al.*, 1997). Therefore, the hepatoprotective activity of *U. pilulifera* might be due to the presence of such compound. We also measured the serum levels of urea and creatinine as a waste product formed during the digestion of proteins. An increase in serum urea and creatinine levels in STZ-diabetic rats may indicate diminished ability of the kidneys to filter these waste products from the blood and excrete them in the urine. This is also another characteristic change in diabetes (Harvey, 2003). On other hand, the results indicate that treatment of diabetic groups with *U. pilulifera* extract significantly reduced serum urea and creatinine levels. Based on these findings, the extract of this plant may enhanced the ability of the kidneys to remove these waste products from the blood as indicated by the reduction in serum urea and creatinine levels and thus, confer a protective effect on the kidney of diabetic rats. Taken

together, it is also possible to suggest that this extract might directly improve the structural and functional integrities of cells of the blood, liver, kidney and thyroid gland.

CONCLUSION

It is reasonable to conclude that injection of single dose of STZ induces diabetes and disturbances of some hematological and biochemical parameters as markers injury of blood, kidney, liver, thyroid gland cells. The data also, provide evidence for the first time that *U. pilulifera* alleviates some of the hematological and biochemical parameters of STZ-induced diabetic rats. Therefore, oral administration of *U. pilulifera* is of interest because, it is reverse the negative effects of STZ compounds, nontoxic and safe for rats. Based on these findings, it is highly likely that this extract could be used to unlock and/or improve some of the complications of DM on humans including thyroid dysfunction, nephropathy and hepatopathy. Therefore, the mechanisms and mode of *Urtica pilulifera* action in diabetic rats and humans need to be addressed and investigated.

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