URTICA PILULIFERA UPREGULATES THE EXPRESSION OF HEAT SHOCK PROTEIN (HSP70) IN KIDNEYS OF DIABETIC RATS

Ahed J Alkhatib  
Jordan university of Science and Technology, Jordan

Fatima Laiche  
Al-albayt University, Jordan

Mosleh A Alkhatatbeh  
Jordan University of Science and Technology

Saleh A. Alrasheidi  
University of Arkansas, USA

Murtala Muhammad  
ALiyu Maje Bello

Ibrahim Ahmad Muhammad  
Ya'u Sabo Ajingi

Mustapha Garba Muhammad  
Aminu Faruk Kabara  
Jordan university of Science and Technology, Jordan

Abstract  
U. pilulifera has been used by people to treat various diseases including diabetes. There is a need to study cellular mechanisms associated with U. pilulifera. The purpose of the present study was to study the effects of the extract of U. pilulifera on the expression of HSP70 in the kidneys of diabetic rats. U. pilulifera was collected from various places in Jordan, air dried and extracted by Soxhlet cold extractor using absolute methanol as solvent and remained for three consecutive days. Extracted juice was kept in refrigerator at 4°C. Diabetes was induced through administration of alloxan 150 mg/kg body weight intraperitoneally. Study model included 4 groups: control group, diabetic group, diabetic group treated with 1.25 mg/kg body weight, and diabetic group treated with 1.88 mg/kg body weight. Study findings showed that diabetes lowered the expression of HSP70 significantly (P 0.000) compared with control group in kidneys. Treatment with either dose of U. pilulifera increased the expression of HSP70 significantly in
kidney tissue. Taken together, diabetic patients can benefit from U. pilulifera to cope with oxidative stress attributed to diabetes.

**Keywords:** diabetes, Urtica. Pilulifera, HSP70, kidney, oxidative stress.

**Introduction**

Urtica pilulifera L. belongs to the family of Urticaceae that has been extensively cultivated in the Mediterranean region (Irshaid and Mansi, 2009; Shuwayeb and Khatib, 2013). This plant is also known by other names such Nettle in Roman and as Qurraus in Jordan (Affif and Abu-Irmaileh, 2000; Ali-Shtayeh, Yaniv, Mahajna, 2000). The importance of U. pilulifera has been realized since a long time, and its extracts have been used to treat various diseases including Diabetes Mellitus (Kavalali et al., 2003; Lopatkin et al., 2005).

Diabetes is a disease characterized by having high glucose level in blood. Glucose is utilized within cells by insulin; a hormone that helps the glucose gets into cells to provide them with energy (Khatib, 2013). Diabetic symptoms include frequent urination, lethargy, excessive, thirst and hunger. Diabetes can be treated by changes in diet, medications, and in some cases, daily injections of insulin. Diabetes may occur as a result of either a lack of insulin or because of the present of factors that oppose the action in blood glucose, the result of insufficient action of insulin is an increase in blood glucose concentration (Tierney, McGhee, Papadakos, 2002).

Diabetes mellitus is not viewed as a single disease but it is rather a group of metabolic disorders include alterations in the carbohydrate, fat, and protein metabolism associated with absolute and/or relative deficiencies in insulin secretion (West, Ahuja, Bennett, 1983). High blood glucose levels were reported to be toxic, causing serious microvascular and macrovascular damages (Eizirik, 1995; Kelly et al., 2003).

Diabetes can cause diabetic nephropathy (DN) which is known as the main cause of end-stage renal failure in the Western world (Barutta et al., 2008). DN has various clinical features including elevated albumin excretion rate (AER) as well as increased deterioration in renal function (Estacio and Schrier, 2001; Molitch et al., 2004). In a study conducted by Cooper (2001), it has been found that hyperglycemia and glomerular capillary hypertension to be the main determinants in the onset and the progression of the complication of DN.

Heat shock proteins (HSP) are a family of proteins that are known by being ubiquitous, highly conserved intracellular proteins and categorized according to their molecular weight (Fink, 1999). It has been found that various sources of stress including thermal, oxidative, hemodynamic, osmotic, and hypoxic stresses have the ability to induce the expression of
various types heat shock protein members including HSP70 to offer cytoprotection (Kim, Morse, Choi, 2006; Kim, Hwang, Lee, 2007).

In his study Barutta et al (2008) found that diabetes and its related insults modify selectively the expression of some heat shock proteins including HSP27, HSP60, and HSP70 in the glomeruli and in the medulla which may explain the ability of renal cells to increase the effectiveness of cytoprotective response.

In the present study, we aimed to explore the effect of extract of U. pilulifera on the expression of HSP70 in rat's diabetic kidneys.

**Methodology**

**Plant collection and preparation of extraction**:

We followed the procedures published by Irshaid and Mansi (2009). U. pilulifera leaves were collected from various areas at Jordan, air-dried in shad well-ventilated area and then ground into fine powder. About 350 g of powder was put in a Soxhlet cold extractor using absolute methanol as solvent and remained for three consecutive days (Sadki et al., 2001). The extract was concentrated to dryness in rotary evaporator under reduced pressure and controlled temperature (45°C) to yield an 11.4% viscous greenish-colored extract. The extract was kept at 4°C in a glass container until use. Wister rats were used in this study, in which their average weight was 170 g. The conditions in animal house were to place rats in stainless steel cages under 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. After determination of lethal dose (LD50), two doses were selected 1.25 g/kg and 1.88 g/kg of body weight. Doses were prepared through dissolving required amount of the viscous extract in 10 mL Tween-20: 0.9% NaCl (1:9, V/V).

**Diabetic model**

Diabetes was induced depending on alloxan so that rats were injected by alloxan monohydrate "B.O.H chemical LTD England" intraperitoneally at a dose of 150 ml/kg body weight (dissolved in fresh normal saline) to 18 hr fasted rat. Rats were monitored for blood glucose and rats with blood glucose level over 200 mg/ml, were considered diabetic and employed in the study.

Animals were assigned into the following groups:

Group I: control group; Group II: diabetic group; Group III: diabetic treated with 1.25 mg/kg of body weight; Group IV: diabetic treated with 1.88 mg/kg of body weight.
Immunohistochemistry

Immunohistochemical detection of HSP70 was performed using commercially available mouse monoclonal antibodies. Immunohistochemical detections of HSP70 was demonstrated by using labeled streptavidin biotin LSAB kit, which consists of secondary biotinylated goat anti-mouse antibody and conjugated streptavidin. Horse raddish peroxidase was followed by 3',3'-Diaminobenzidine (DAB) chromogen. Sections were processed for immunohistochemistry using conventional techniques (Khatib, 2013).

Immunohistochemical Assessment of Stained Sections

Slides were assessed using adopy photoshop software. Photos for sections were taken and divided into pixels. The total number of pixels was computed and represented both colours (blue and brown), then the brown colour (the colour of the marker under study) was computed and divided by the total number of pixels (Khatib, 2013).

Statistical Analysis

The expression of HSP70 was compared between groups using T test. P value $\leq 0.05$ was considered statistically significant.

Study results

The expression of HSP70 in control group was about 38%, whereas in diabetic group, it decreased to about 23%, this variation in the expression of HSP70 is statistically significant (P 0.000). Treating diabetic groups with 1.25 mg/kg of body weight increased the expression of HSP70 in kidney to about 81% and compared with diabetic group, this was statistically significant (P 0.000). The data of the present study also revealed significant expression of HSP70 (about 64%, P 0.000) in diabetic group treated 1.88 mg/kg of body weight compared with diabetic group (figure 1).

![Figure 1: The expression of HSP70 among study groups](image-url)
Discussion

The present study was conducted to investigate the effects of the extract of U. pilulifera on expressing patterns of HSP70 in kidney tissues of diabetic rats using immunohistochemical techniques. After the end of experiment, the data of the present study showed that the expression of HSP70 was significantly reduced in diabetic group compared with control group (P 0.000). The findings of our study confirm previous studies in which diabetes has adverse effects on kidneys and can lead to DN (Barutta et al., 2008). Other studies also showed that DN is able to increase deterioration in renal function (Estacio and Schrier, 2001; Molitch et al., 2004).

The data of the present study revealed significant expression of HSP70 attributed to treating diabetic groups with either dose of U. pilulifera. Both doses, 1.25 mg/kg of body weight and 1.88 mg/kg of body weight increased the expression of HSP70 significantly in kidneys (P 0.000; P 0.000 respectively). These findings confirm the previous trends in research in which U. pilulifera has been significantly used to treat various diseases including Diabetes Mellitus (Kavalali et al., 2003; Lopatkin et al., 2005). Other studies have indicated that various sources of stress including thermal, oxidative, hemodynamic, osmotic, and hypoxic stresses have the ability to induce the expression of various types heat shock protein members including HSP70 to offer cytoprotection (Kim, Morse, Choi, 2006; Kim, Hwang, Lee, 2007). Diabetes has oxidative properties and U. pilulifera has been shown through various studies to exert antioxidative properties (Abo-elmatty et al., 2013; Ghaima et al., 2013).

Conclusion

The present study demonstrated less expression of HSP70 in diabetic group compared with control group. The extract of U.pilulifera increased significantly the expression of HSP70 in diabetic groups. Taken together, diabetic patients can benefit from U. pilulifera to cope with oxidative stress attributed to diabetes.

References: