URTICA PILULIFERA AMELIORATES DIABETIC IMPACTS THROUGH UPREGULATION THE EXPRESSION OF HSP70 IN LIVER OF DIABETIC RATS

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Abstract

Diabetes has impacts in liver through downregulation of heat shock protein response. People used to use herbal remedies to treat diabetes among which is Urtica pilulifera (U. pilulifera). We conducted the present study to investigate the impacts of diabetes on liver through exploring the diabetic effects on expression of HSP70 in liver tissue and to explore the effects of the extract of U. pilulifera on liver of diabetic groups treated with U. pilulifera, and to investigate its effects on the expression of HSP70 in liver tissue. 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 downregulated the expression of HSP70 significantly in liver (P 0.000). There was a significant upregulation of HSP70 in diabetic groups treated with either dose of U. pilulifer (1.25 mg/kg of body weight, 1.88 mg/kg of body weight; P 0.000). Taken together, the present study

demonstrated significant roles of using U. pilulifera in upregulating the expression of HSP70 in liver of diabetic rats. The induction of HSP response through U. pilulifera is unique, exciting, and inexpensive.

Keywords: Diabetes, heat shock protein, liver, downregulation, upregulation, U. pilulifera.

Introduction

The present study investigates two basic aspects related to diabetes; the first of which is the impact of diabetes on liver and how it interferes with hindering the antioxidative system through impairment of the expression of heat shock protein 70 (HSP70). The second aspect is to explore the therapeutic potential of Urtica pilulifera on improvement of diabetic status.

HSPs regulate chaperone proteins in their response to stress on cellular level through repairing of damaged proteins and retaining physiological functions of the cell (Kylie et al., 2011). There is a significant role for heat shock factor 1 (HSF1) which acts through binding to heat shock elements in the promoter region of the HSP genes leading transcription of HSP70 mRNA (Akerfelt, Morimoto, Sistonen, 2010).

It has been shown that HSPs once induced offer protection of proteins that have already been translated by the endoplasmic reticulum and also protect oxidized proteins and increase intracellular antioxidant mechanisms which result in minimizing the chronic inflammatory conditions associated with insulin resistance (Okada et al., 2004; Hotamisligil, 2006). Several studies have demonstrated that both patients who are insulin-resistant and hyperglycemic have lowered HSP70 protein and gene expression (Kurucz et al., 2002; Bruce et al., 2003; Chung et al., 2008).

The study of Ozcan et al (2006) pointed to the possibility of restoring insulin sensitivity in animal models of obesity through lowering cellular stress. Furthermore, Kars et al (2010) pointed to the possibility of improving insulin signaling in obese people. These findings agree with the study of Chung et al (2008) who reported that elevated levels of HSPs using several methods including heat, pharmacological induction, and muscle overexpression inhibited obesity-associated insulin resistance in rodents.

Hooper (1999) showed that heat therapy was able to significantly reduce glycosylation of hemoglobin A1c (A1c%). In another study by Literati-Nagy et al (2009), it has been shown that introducing HSP co-inducer for one month in people improved glucose uptake by tissues. Another study showed that there was a significant reduction in expression of HSF1/HSPs in liver and reduced plasma HSP70 levels from nonhuman primates with type 2 DM (Kavanagh, Zhang, Wagner, 2009).

Urtica pilulifera L. is a member of the family of Urticaceae (Irshaid and Mansi, 2009; Shuwayeb and Khatib, 2013). U. pilulifera is one of herbal remedies that has used to treat various diseases including Diabetes Mellitus (Kavalali et al., 2003; Lopatkin et al., 2005).

Study objectives:

The main objectives of the present study are to investigate the impacts of diabetes on liver through exploring the diabetic effects on expression of HSP70 in liver tissue. The second objective is to explore the effects of the extract of U. pilulifera on liver of diabetic groups treated with U. pilulifera, and to investigate its effects on the expression of HSP70 in liver tissue.

Methodology

Methodology section covers plant collection and processing, inducing diabetic model and immunohistochemistry.

Plant collection and processing

U. pilulifera leaves were collected from several parts in Jordan, air-dried in shade 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.

Results

The expression percentage of HSP70 in rat liver of control group was about 0.89 under physiological conditions. Due to diabetic impacts on liver tissue, the expression of HSP70 was decreased significantly in diabetic group (0.371, P 0.000).

The results of the present study revealed significant increased expression of HSP 70 in diabetic group treated with 1.25 mg/kg of body weight (0.744, P 0.000). Further increased expression of HSP70 (0.76) was observed in diabetic group treated with 1.88 mg/kg of body weight. The variation between diabetic group and diabetic group treated with 1.88 mg/kg of body weight was statistically significant (P 0.000) (figure 1).

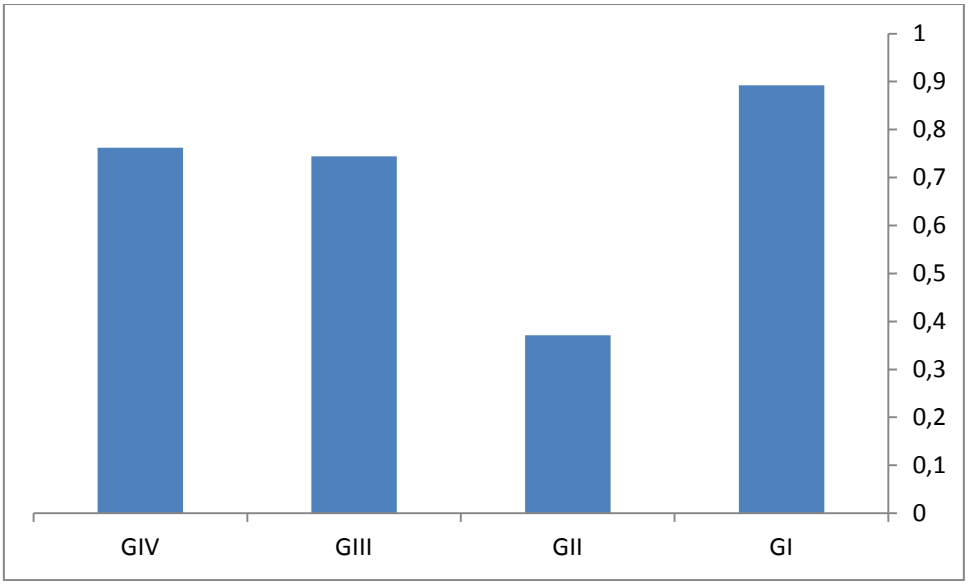

Figure 1: The expression of HSP70 of liver among study groups.

Discussion

The present study was conducted to explore the therapeutic potential of the extract of U. pilulifera to treat diabetes. We depended on the expression of HSP70 in liver under physiological conditions, diabetic models and in groups treated with U. pilulifera.

Our data showed that diabetes downregulated the expression of HSP70 in liver tissue of diabetic rats significantly compared with control group. This finding is consistent with other studies in literature in which it has been shown that there was a significant reduction in expression of HSF1/HSPs in liver and reduced plasma HSP70 levels from nonhuman primates with type 2 DM (Kavanagh, Zhang, Wagner, 2009).

The data of the present study demonstrated upregulation of the expression of HSP70 attributed to the use of the extract of U. pilulifera for about one month. This upregulation of HSP70 in treated group compared with diabetic group was statistically significant (P 0.000). These finding confirm other reported studies in literature in which using U. pilulifera showed beneficial remedies against Diabetes Mellitus (Kavalali et al., 2003; Lopatkin et al., 2005). The importance of the present study comes from two basic considerations. The first consideration implies that the upregulation of the expression of HSP70 by the use of U. pilulifera is unique, exciting, practical and inexpensive. The second consideration implies that various ways to combat diabetes are mediated through upregulation of HSP70 in diabetic liver. Several studies confirmed these considerations. Studies by Kylie et al (2011) suggested that HSPs respond to stress on cellular level through repairing of damaged proteins and retaining physiological functions

of the cell. Other studies put focus on the role of induced HSP70 to minimize the chronic inflammatory conditions associated with insulin resistance (Okada et al., 2004; Hotamisligil, 2006). Other studies confirm the consideration that induced HSPs play a role in reducing glucose level including the study of Hooper (1999) who induced HSPs through heat therapy. It is of great interest the results of Literati-Nagy et al (2009) who showed that introducing HSP co-inducer for one month in people improved glucose uptake by tissues.

Conclusion

The present study demonstrated significant roles of using U. pilulifera in upregulating the expression of HSP70 in liver of diabetic rats. The induction of HSP response through U. pilulifera is unique, exciting, and inexpensive.

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