U. PILULIFERA MIMICS THE ACTION OF METFORMIN IN LOWERING LIVER ENZYMES IN ALLOXAN DIABETIC MODEL

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Abstract

Liver plays a significant role in glucose homeostasis and acts to retain normal glucose levels during fasting and in the postprandial period. The association between liver function tests and incidence of diabetes is not well established. The present study was conducted to investigate the association between liver enzymes and diabetes, and to investigate the effects of the extract of *U. pilulifera* and metformin on liver enzymes. Study methodology included collection of *U. pilulifera*, preparation of its extract, induction of diabetic model through alloxan injection. Study findings showed a positive relationship between studied liver enzymes ALT, AST, and ALP and diabetes. Treating diabetic groups using either the extract of *U. pilulifera* or metformin showed protection potential for liver through restoring liver enzymes to levels approximate to control groups. Taken together, the extract of *U. pilulifera* mimics the action of metformin in treating diabetes through restoring liver function tests to levels approximate to control group.

Keywords: Liver, diabetes, liver function tests, AST, ALT, ALP, alloxan, metformin, *U. pilulifera*

Introduction

Diabetes mellitus is a very wide prevalent disease in both developed and developing countries, and its world prevalence has been estimated as 25% of the world population (Kavishankar et al., 2011). Diabetes mellitus results from disturbed carbohydrate metabolism, and this is associated with either insufficient blood insulin or insulin insensitivity (Maiti et al., 2004).

Irrespective to witnessing progress in treating diabetes using oral synthetic agents, there still a need to find out new medications because of the limitations of available medications. On the other hand, there is a need to formulate herbal plants with potential use as antidiabetic therapy (Wadkar et al., 2008).

Diabetes is not a single disease, but rather, a group of metabolic diseases. It leads to diabetic complications. Antidiabetic herbs act through increasing insulin secretion, enhance glucose uptake by adipose or muscle tissues and inhibit glucose absorption from intestine and glucose production from liver (Hongxiang et al., 2009).

Metformin is an oral antidiabetic drug in the biguanide class. It is the first-line drug of choice for the treatment of type 2 diabetes, in particular, in overweight and obese people and those with normal kidney function. It is effective only in the presence of insulin. It does not directly stimulate insulin secretion. Its major effect is to increase insulin action. One important effect appears to be suppression of glucose output from the liver (Kavishankar et al., 2011).

Liver has a significant role in glucose homeostasis and acts to retain normal glucose levels during fasting and in the postprandial period (Duckworth, Hamel, Peavy, 1988). The role of liver in developing of type 2 diabetes has attracted much interest. Furthermore, it is thought that abnormal function of liver attributed to insulin-resistance syndrome may lead to development of type 2 diabetes (Marchesini et al., 2001). Liver function test is assessed through using liver enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) (Hall and Cash, 2012). Both AST and ALT are considered markers of hepatocellular health. ALT is considered the most specific biomarker of liver pathology and is found mainly in liver (Lee et al., 2004). Because AST and ALP can be found in other tissues, they are thought to be less specific biomarkers of liver function (Lee et al., 2003). The relationship between concentrations of liver enzymes and the incidence of type 2 diabetes has been investigated through several studies. Some studies showed a significant relationship between serum AST and diabetes (Hanley et al., 2004; Nannipieri et al., 2005). Other studies have investigated the relationship between AST and ALT and risk of type 2 diabetes, with varied results (Doi et al., 2007; Goessling et al., 2008; Jiamjarasrangsi et al., 2008; Monami et al., 2008; Sato, Hayashi, Nadamura,

2008). Another study by Tibi et al (1988) showed that liver ALP was significantly higher in the diabetics compared with the control group.

Among well-known herbal plants of potential therapy for diabetes is *Urtica pilulifera* (Kavalali et al., 2003; Lopatkin et al., 2005; Kavishankar et al., 2011; Irshaid and Mansi, 2009; Kavishankar et al., 2011; Shuwayeb and Khatib, 2013). *Urtica pilulifera L*. is one species of the family *Urticaceae* that has been extensively cultivated in the Mediterranean region (Irshaid and Mansi, 2009; Shuwayeb and Khatib, 2013).

Study objectives

The main objectives of the present study are to investigate the impacts of induced diabetes on liver function tests in liver of diabetic rats and to evaluate the effects of the extract of *U. pilulifera* and metformin on liver function test.

Methodology

Methodology followed in this study included the following steps: collection of *U. pilulifera* and preparation of its extracts, induction of diabetic model and biochemical evaluation of liver function tests.

Collection of *U. pilulifera* and preparation of its extraction

U. pilulifera leaves were collected from appropriate areas at 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 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 employing 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.

Evaluation of liver function tests

Liver function tests were evaluated using a commercial biochemistry analyzer (Roche

P800 MODULAR; Roche Diagnostics, Indianapolis, IN, USA).

Statistical analysis

Data analysis was carried out using SPSS 20. Data were presented as mean and standard deviation. T test was used to investigate the difference between liver enzymes in study groups. Significance level was considered at alpha level ≤ 0.05.

Study findings

Concentrations of AST among study groups

As shown in table 1, concentration of AST in control group was 36.8 $\pm$ 3.35 U/L, and this was significantly increased in diabetic group to 98.78 $\pm$ 12.82 ($P < 0.05$). Treatment with the extract of *U. pilulifera* (1.25 mg/kg body weight) decreased the concentration of AST (43.46 $\pm$ 3.48 U/L) significantly ($P < 0.05$). Treatment with the extract of *U. pilulifera* (1.88 mg/kg body weight) decreased the concentration of AST (51.53 $\pm$ 4.62 U/L) significantly ($P < 0.05$). Treatment with metformin (14.2 mg/kg body weight) decreased the concentration of AST (37.94 $\pm$ 2.34 U/L) significantly ($P < 0.05$).

Concentrations of ALT among study groups

As shown in table 1, concentration of ALT in control group was 46.93 $\pm$ 7.32 U/L, and this was significantly increased in diabetic group to 84.72 $\pm$ 11.68 ($P < 0.05$). Treatment with the extract of *U. pilulifera* (1.25 mg/kg body weight) decreased the concentration of ALT (58.42 $\pm$ 2.24 U/L) significantly ($P < 0.05$). Treatment with the extract of *U. pilulifera* (1.88 mg/kg body weight) decreased the concentration of ALT (51.62 $\pm$ 3.25 U/L) significantly ($P < 0.05$). Treatment with metformin (14.2 mg/kg body weight) decreased the concentration of ALT (47.82 $\pm$ 2.17 U/L) significantly ($P < 0.05$).

Concentrations of ALP among study groups

As shown in table 1, concentration of ALP in control group was 41.64 $\pm$ 6.64 U/L, and this was significantly decreased in diabetic group to

35.73 ± 2.13 (P <0.05). Treatment with the extract of *U. pilulifera* (1.25 mg/kg body weight) increased the concentration of ALP (46.8 ± 1.91 U/L) significantly (P <0.05). Treatment with the extract of *U. pilulifera* (1.88 mg/kg body weight) increased the concentration of ALP (4.9.74 ± 1.56 U/L) significantly (P <0.05). Treatment with metformin (14.2 mg/kg body weight) increased the concentration of ALP (46.72 ± 4.59 U/L) significantly (P <0.05).

Table 1: Concentration of liver enzymes among study groups

Group	Treatment	AST ± SD (U/L)	ALT ± SD (U/L)	ALP ± SD (U/L)
I	Control	36.8 ± 3.35	46.93 ± 7.32	41.64 ± 6.64
II	Diabetic	98.78 ± 12.82 *	84.72 ± 11.68 *	35.73 ± 2.13 *
III	Diabetic treated with 1.25 mg/kg	43.46 ± 3.48 **	58.42± 2.24 **	46.8 ± 1.91 **
IV	Diabetic treated with 1.88 mg/kg	51.53± 4.62 **	51.62 ± 3.52 **	49.74 ± 1.56 **
V	Diabetic treated with metformin 14.2 mg/kg	37.94 ± 2.34 **	47.82 ± 2.17 **	46.72 ± 4.59 **

* Compares diabetic group with control group

** Compares treated groups with diabetic group

Discussion

The present study was conducted to investigate the impacts of diabetes on liver function tests in diabetic experimental model and to compare the effects of the extract of *U. pilulifera* and metformin on liver function tests.

Liver function tests including AST, ALT and ALP were investigated among study groups. The data of the present study showed significant variations in liver function tests between control and diabetic groups. The findings of our study agree and confirm previous studies in which liver has a significant role in glucose homeostasis and acts to retain normal glucose levels (Duckworth, Hamel, Peavy, 1988). We agree with other studies that showed a role of liver in developing type 2 diabetes. Such studies proposed that abnormal function of liver attributed to insulin-resistance syndrome may lead to development of type 2 diabetes (Marchesini et al., 2001). However, liver function test is assessed through using liver enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) (Hall and Cash, 2012). Our data are consistent with other studies in which AST and ALT were considered markers of hepatocellular health (Lee et al., 2004). Our data also confirmed other previous studies that proved a significant relationship between concentrations of liver enzymes and the incidence of type 2 diabetes (Hanley et al., 2004; Nannipieri et al., 2005). It is worth to mention that the relationship between AST and ALT and

risk of type 2 diabetes is not well established and our data did not agree with such studies (Doi et al., 2007; Goessling et al., 2008; Jiamjarasrangsi et al., 2008; Monami et al., 2008; Sato, Hayashi, Nadamura, 2008). Our results showed that ALP was significantly decreased in diabetic group compared with control group ($P < 0.05$), and this is not consistent with findings reported by Tibi et al (1988) who showed that liver ALP was significantly higher in the diabetics compared with the control group.

The data of this study revealed a significant role of the extract of *U. pilulifera* and metformin in restoring the liver function tests to levels approximates to control group. Within this context, it is plausible to share other researchers that there is a need to formulate herbal plants with potential use as antidiabetic therapy (Wadkar et al., 2008). It is also possible that the extract of *U. pilulifera* acts through increasing insulin secretion, enhance glucose uptake by adipose or muscle tissues and inhibit glucose absorption from intestine and glucose production from liver (Hongxiang et al., 2009). Our data showed that metformin has more profound effects in restoring liver function tests compared with the extract of *U. pilulifera*. This finding can be explained by taking into consideration the dose level since metformin concentration was higher than that of *U. pilulifera.*

Conclusion

The extract of *U. pilulifera* mimics the action of metformin in treating diabetes through restoring liver function tests to levels approximate to control group.

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