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Comparing the effects of metformin with silymarin in biochemical parameters related to diabetes and pancreatic tissue in streptozotocin- induced diabetic rats

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ABSTRACT

Diabetes is a major public health problem worldwide and associated with serious side effects. Given the role of medicinal plant in controlling diabetes, this study aimed to compare the effects of silymarin with metformin on serum glucose, insulin, insulin resistance (HOMA.IR), pancreatic function (HOMA.B) and pancreatic tissue in diabetic rats. In this experimental study, frothy male Wistar rats weighing 180-240 g were randomly divided into 5 equal groups as follows: the healthy control (HC), the diabetic control (DC), the silymarin100 (S_{100}), the silymarin 200 (S_{200}) and the metformin 100 (M_{100}). Groups DC, S_{100}, S_{200} and M_{100} were injected with intraperitoneally of streptozotocin. Groups S_{100}, S_{200} and M_{100} received 100 mg/ kg of silymarin, 200 mg/ kg of silymarin and 100 mg / kg of metformin respectively. After 30 days of intervention, serum concentrations of glucose and insulin were determined by enzymatic and ELISA method respectively. Also the pancreatic tissue was studied by light microscopy. Serums concentrations of glucose, insulin and HOMA.IR significantly decreased, whereas HOMA.B increased in the S_{100}, S_{200} and M_{100} groups compared to the DC group. Glucose and insulin levels significantly decreased in the M_{100} group compared to the S_{100} and S_{200} groups ($p < 0.05$). Histological analysis demonstrated restoration effects of metformin and silymarin on pancreatic tissue. It seems that efficacy of metformin on diabetes is better than silymarin, however, more researches are needed to survey the effects of different timing (longer) and different concentrations of silymarin on diabetes.

Key words: Diabetes, Silymarin, Metformin, Insulin resistance

INTRODUCTION

Diabetes is one of the most important public health problems worldwide and is the most common metabolic disease [1]. The prevalence of this disease in the world is increasing rapidly, so that by 2025 the number of people with diabetes is expected to reach 300 million or more [2]. Diabetes imposes enormous costs on patients and their families and its prevalence between 7.8 to 15.5% have been reported in different ethnic groups in the world [1]. Many types of blood glucose lowering drugs have been produced; however, some of these drugs may have side effects such as severe hypoglycemia, lactic acidosis, liver damage, major neurological deficit, digestive disorders, dyslipidemia, headache, dizziness, and even death [3, 4]. Metformin as a hypoglycemic agent in the treatment of type 2 diabetes, decreased insulin resistance, reduced glucose absorption from the gastrointestinal tract and inhibited glucose production in the hepatic [5]. On the other hand, insulin injection in diabetic patients is a costly and time-consuming treatment until the end of the patients' life, along with the boring abundant side effects. In this regard, the study of medicinal plants offers natural solutions for the health problems of the diabetes. These medicinal plants

have been in attention due to their availability, low side effects, less toxicity and favorable price compared to the chemical medicine. Some of these plants such as *Otostegia persica* and *Stevia Rebaudiana* are effective in diabetes by decreasing serum glucose and insulin resistance [6, 7]. Milk thistle is one of the plants of the *Asteraceae* with scientific name of *Silybum marianum* is known as Milk thistle [8]. Silymarin as a known hepatoprotective drugs obtained from seeds of *Silybum marianum* [9], is a mixture of flavonoligans comprises of silybin, isosilybin, silydianin and silychristine [10]. With respect to pancreatic function in the etiology and incidence of complications associated with diabetes, it seems, the antioxidant property of silymarin is the cause of pancreatic tissue repair. This research survey the comparative effect of silymarin and metformin on the pancreatic tissue parallel with serum biochemical parameters related to diabetes.

MATERIALS AND METHODS

Animals and induction of diabetes

In this study, 40 male Wistar rats weighing 180 to 240 g were divided into 5 groups (8 rats per group) as follows: the healthy control (HC), the diabetic control (DC), the silymarin100 (S_{100}), the silymarin 200 (S_{200}) and the metformin 100 (M_{100}). Rats were maintained under standard condition at a temperature of 22 ± 3 °C, humidity 60-70% and 12h light/dark cycle and fed with standard pellet diet.

Groups of DC, S_{100}, S_{200} and M_{100} were injected with intraperitoneally of streptozotocin (60mg/kg). Fujimoto was found that streptozotocin causes inflammation and destruction of the pancreatic beta cells [11]. HC and DC groups received standard pellet diet. In addition to the pellet diet, groups of S_{100}, S_{200} and M_{100} as the treatment groups received 100 mg/ kg of silymarin, 200 mg/ kg of silymarin and 100 mg / kg of metformin respectively during 30 days (via gavages). In order to equalize the stress to the HC and the DC groups, 2 ml of distilled water for 30 days was applied (orally gavages). All experimental protocols were approved by the Animal Ethics Committee.

Collection of sample and experimental protocol

Following 30 days of the intervention, the animals after 12 hours of overnight fasting were anesthetized by isoflurane. Subsequently blood sample were given directly from the arterial of the neck and the concentrations of serum glucose were analyzed by enzymatic method with (Pars azmun Kit, Tehran, Iran) using a Selectra 2 auto analyzer (Vital Science, Spankeren, The Netherland S0), also insulin levels measured by using enzyme-linked immunosorbent assay (ELISA, MEDO kit).

Homeostasis model assessment insulin resistance (HOMA.IR) and homeostasis model assessment insulin-B cells (HOMA-B) were calculated by using the equations:

$$\text{HOMA. IR} = \frac{\text{Insulin}(\frac{\mu\text{IU}}{\text{ml}}) \times \text{FBS}(\frac{mg}{\text{dl}})}{405}$$

$$\text{HOMA. B} = \frac{20 \times \text{Insulin}\left(\frac{\mu\text{IU}}{\text{ml}}\right)}{\text{FBS}\left(\frac{\text{mmol}}{\text{ml}}\right) - 3.5}$$

Biopsy samples of pancreatic tissues were taken and then fixed in 10% formalin. The samples were dehydrated in alcohol, molded in paraffin, and 3µm microtome sections were prepared. Sections were stained with H&E Staining Protocol. After preparation of tissues, microscopic slides of each section were taken using light microscope equipped with digital camera (Moticam, model A-352, the Netherlands and China) at different magnifications.

Statistical Analysis

Statistical analysis of data was done by using SPSS statistical software version 17. Descriptive statistics for quantitative variables were presented as mean± SD. Data were compared by one-way ANOVA and $p<0.05$ was considered significant.

RESULTS

Results of this study indicated that injection of STZ increased serum levels of glucose in the DC group compared to the HC group (p: 0.000). Serum concentrations of glucose significantly reduced in the S_{100} and S_{200} groups compared to the DC group respectively (p: 0.000, p: 0.001, table 1). Metformin administration caused a significant decrease in serum glucose levels compared to the DC group (p: 0.000). Efficacy of M_{100} group in reducing of glucose is better than the S_{100} (p: 0.041) and S_{200} groups (p: 0.039).

As it can be seen in table 1, insulin levels in the DC group were significantly increased compared to the HC group (p: 0.000). Serum levels of insulin significantly decreased in the S_{100} and S_{200} groups compared to the DC group (p: 0.000). Subsequently, the amount of serum insulin in the M_{100} group was significantly decreased compared to the DC group (p: 0.000).The efficacy of metformin in reducing of serum insulin was more than the S_{100} and the S_{200} (p: 0.000).

HOMA.IR increased in the DC group compared to the HC group (p: 0.000), whereas this parameter as an insulin resistance was reduced in the M_{100}, S_{100} and S_{200} groups compared to the DC group (p: 0.000, table 1). There wasn't significant changes of HOMA.IR in the M_{100} group compared to the S_{100} and S_{200} groups (p>0.05).

Average of HOMA.B in the different groups is shown in table 1. HOMA.B in the DC group was significantly decreased compared to the HC group (p: 0.000), whereas this parameter as a pancreatic function was significantly increased in the M_{100}, S_{100} and S_{200} groups compared to the DC group (p: 0.000).There wasn't significant changes of pancreatic function in the M_{100} group compared to the S_{100} and S_{200} groups (p>0.05).

Table 1- Changes serum concentrations of glucose, insulin, insulin resistance and pancreatic function index in the different groups

Groups	Glucose (mg/dl)	Insulin (µIu/ml)	HOMA.IR	HOMA.B
HC	113.83±8.20	1.07±0.11	0.30±0.05	7.71±0.88
DC	331.83±43.56*	2.34±0.26*	1.94±0.46*	3.17±0.28*
S_{100}	153.14±15.28**	1.62±0.09**	0.61±0.09**	6.57±0.74**
S_{200}	196.57±22.39**	1.95±0.21**	0.95±0.20**	5.30±0.45**
M_{100}	119.71±13.21**	1.12±0.12**	0.33±0.07**	7.32±1.00**

*HOMA. IR, Homeostasis model assessments for insulin resistance; HOMA.B, Homeostasis model assessment insulin-B cells; HC, the healthy control group; DC, the diabetic control group; S_{100}, dose 100 mg/kg of the silymarine group;S_{200}, dose 200 mg/kg of the silymarin group;M_{100}, dose 100 mg/kg of the metformin group; Data expressed as means±S.D,*Significant differences with the healthy control group; **Significant differences with the diabetic control group.*

Histopathological studies

Photomicrograph accepted from islands of langerhans has shown that Paranchymal cells and interstitial connective tissue in the HC group are uniform, regular and relatively normal and cells have containing cytoplasm staining and active eukaryotic nucleus and no specific histopathological changes was seen, but all tissues completely degenerate and degraded in the DC group. The Photomicrograph for islands of langerhans (white arrow) in the S_{100} and S_{200} comparing to the HC group and DC group (H & E Staining) × 400 was shown in Fig.(1).

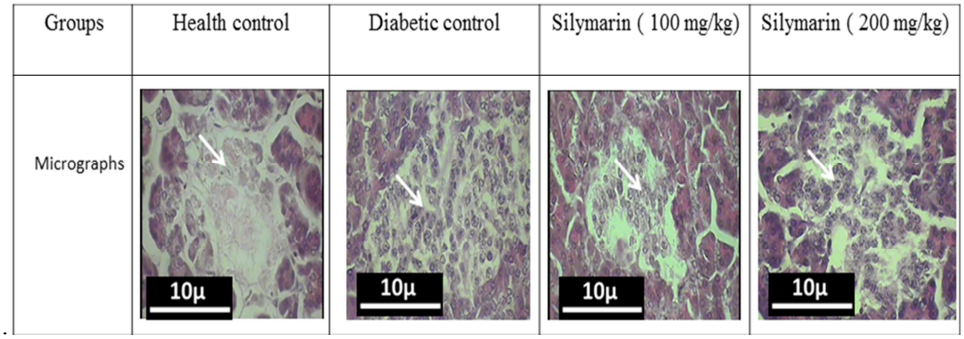

Fig. 1 Photomicrograph, islands of langerhans (white arrow) in the S_{100} and S_{200} comparing to the HC group and DC group (H & E Staining) × 400

The Photomicrograph for islands of langerhans (white arrow) in the M_{100} comparing to the HC group and DC group (H & E Staining) × 400 was shown in Fig (2).

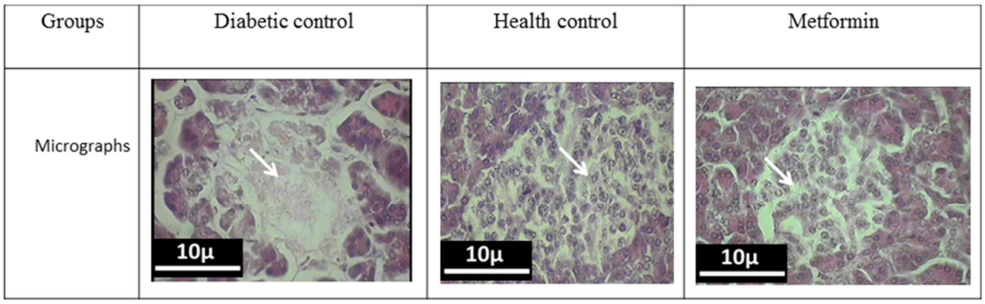

Fig. 2 Photomicrograph, islands of langerhans (white arrow) in the M_{100} comparing to the HC group and DC group (H & E Staining) × 400

All of tissues almost regenerated in the groups of S_{100}, $S_{200}$and M_{100} (Figs. 1 and 2).

DISCUSSION

The results of this study showed that administration of silymarin at doses of 100mg / kg and 200 mg/ kg significantly decreased the blood glucose level compared to the DC group .Guigas demonstrated that silymarin at dose of 100mg/ kg reduces blood glucose levels in diabetic rats by affecting the kinetics of glucose-6-phosphatase and inhibition of gluconeogenesis within 28 days [12].

Data have been obtained from studies on animals, was shown that silymarin may be effective on reducing blood glucose levels via possible mechanisms by protection of pancreas from damage, lowering insulin resistance, inhibition of aldose reductase and so on [13-15]. In this study was found, repaired and improved of pancreatic tissue and reduces insulin resistance in the group treated with silymarin.

In addition flavonoids such as silymarin may be effective on reducing blood glucose levels by modulate the activity of liver enzymes responsible for the metabolism of carbohydrates such as reduce enzyme liver phosphorylase activity, increase glycogen synthase and glucokinase activity [16]. Bailey was made claims that silymarin reduces blood sugar and insulin levels, that this combined effect is beneficial in treatment of type 2 diabetes [17]. Impaired free fatty acid metabolisms in patients susceptible to diabetes induce the production of oxygen free radicals and oxidative stress. This metabolic disorder cause insulin resistance, beta-cell dysfunction and impaired insulin production [18, 19]. Silymarin-containing compounds such as flavonoids and phytosterols with antioxidant properties is effective in increase cellular glutathione levels and stabilize cell membranes that these function may be led to inhibition of metabolic disorder in susceptible individuals diabetic and diabetic patients [20- 22].

At the end of intervention, silymarin at doses of 100 and 200mg / kg, reduced insulin levels compared to the diabetic control group. According to previous studies, silymarin has no role in the stimulation and increased insulin secretion, in this study administration of silymarin at both doses, with reducing in insulin resistance due to decrease in insulin levels, a finding that was consistent with previous studies [23]. Soto suggests that silymarin induces pancreatic function recovery by expression of insulin and glucagon [24]. Also, Wang showed that the silybine exist in silymarin, may cause pancreatic beta-cell regeneration and thereby improve the hyperglycemia [25]. Referring to figure (1) has shown that both groups of silymarin repaired the damaged tissues of the pancreas.

Metformin as an oral medication of type 2 diabetes, reduce blood sugar via prevention of hepatic glucose production, decreased insulin resistance and reduced glucose absorption from the gastrointestinal tract [5]. It is thought that this drug increases quantity or strength of insulin binding to cell membrane receptors, since is effective in the presence of androgen insulin and healthy a portion of their pancreas cells. The effects of metformin on pancreatic beta cells are not entirely clear, but it causes the survival and preservation of beta cells and increases of insulin receptors [26].

Metformin with prevention of hepatic glucose production and decrease insulin resistance cause decrease serum glucose and serum insulin that these findings are consistent with previous studies. According to the results of this study, it was found that the effect of metformin on reducing levels of serum glucose and serum insulin more meaningful than both doses of silymarin.

CONCLUSION

Our results suggest that silymarin at doses of 100 and 200 mg/kg and metformin at a dose of 100 mg/kg for 30 days decreased serum concentrations of glucose and insulin, also reduced insulin resistance and improved pancreatic

tissue in diabetic rats. But the effect of metformin on reducing levels of glucose and insulin is greater than both doses of silymarin. In general, documentary evidence suggests that silymarin in animal models of diabetes, prevent deterioration of pancreatic beta cells, but its effectiveness is less than metformin It seems that chemical drugs function specific and faster than herbal drug. In this regard, further studies are needed to determine the different concentrations of silymarin during different timing (longer) in diabetic patients.

Authors' contributions

Conceived and designed the experiments: AS; Performed the experiments: BH, RR, FP, MF, BA, DA and PK; Contributed reagents/materials/analysis tools: BH, RR, FP, MF, BA, DA, PK, and GM; Wrote the paper: AS; and GM.

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