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The Effect of Hydroalcoholic Extract of Glaucium Flavum on the Activity of Anti Oxidative Enzymes in the Heart and Brain of Alloxan Induced Diabetic Rats

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ABSTRACT

BACKGROUND AND OBJECTIVE: Oxidative stress is the connection between diabetes and neuropathies and micro vascular disorders. This study was designed to investigate the effect of hydroalcoholic extract of Glaucium Flavum on the tissue activity of antioxidant enzymes in the heart and brain of Alloxan induced diabetic rats.

METHODS: In this experimental study, 32 male rats were randomly divided into four groups of eight including control, diabetic, diabetic rats treated by Glaucium Flavum with dose of 500mg/kg and diabetic rats treated with Glibenclamide 5µg/kg. Diabetes was induced by single injection of 120mg/kg of Alloxan. After one month, activity of SOD, CAT and GPX were measured in the heart and brain tissues and analyzed.

FINDINGS: Cardiac activity of all three enzymes in the diabetic + extract group were significantly higher than diabetic control ($p<0.001$). Activity of SOD in brain had a significant difference in comparison to diabetic + extract (8.79±1.4) and diabetic +drug groups (6.77±1.7) ($p=0.03$). As the same, CAT activity in diabetic+extract group (4.64±1.2) was significantly higher than diabetic + drug group (3.83±1.5) ($p<0.001$). Similar to the two previous state, GPX activity in diabetic+extract group (4.23±0.7) was significantly higher than diabetic+drug group (3.64±0.4) ($p=0.03$).

CONCLUSION: The present study declared that yellow Glaucium Flavum extract can promote the main protective enzymatic mechanisms against diabetic induced oxidative stress in heart and brain. In addition, the effect of the extract was more successful than the effect of Glibenclamide and this effect was more pronounced in brain tissue.

KEY WORDS: *Diabetes Mellitus, Glaucium flavum, Superoxide Dismutase, Catalase, Glutathione Peroxidase, Brain, Heart, Rat, Alloxan.*

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Introduction

Diabetes mellitus is a complex of metabolism-related disorders mediated by oxidative stress results in acute metabolic complications such as ketoacidosis, cardiovascular disorders and chronic complications such as neuropathy (1). Increase blood glucose in turn, induces beginning of a series of reactions cascade which ultimately leads to increased free radical production including oxygen free radicals in the blood and various tissues in the body. These compounds due to having power of chemical reactions cause damages to cells and tissues (2). One of the most important complications of diabetes is diabetic neuropathy, which occurs in peripheral nerves as well as the central nervous system. The main characteristics of nerve damage during diabetes are impaired cognitive function, memory loss and decreased learning. Recent findings in laboratory animals with type I diabetes have shown that hyperglycemia leads to impaired memory and learning (3). In addition, decreased pain threshold and the subsequent destruction of myelinated and non-myelinated nerve fibers are considered as other injuries due to diabetes which can reduce the quality of life of these people (4).

Studies have also shown that diabetes plays an important role in cardiomyocyte hypertrophy and induction of apoptosis, which occurs during the increase in oxidative stress mediated by inflammatory cytokines (5). Moreover, deposition of extracellular matrix proteins and formation of interstitial and perivascular fibrosis are other consequences (6). Cells are protected against free radicals especially reactive oxygen species (ROS) by several antioxidant compounds including glutathione, vitamin E, vitamin C and enzymes such as glutathione s-transferase (GST), superoxide dismutase (SOD), Glutathion Peroxidase (GPX) and Catalase (CAT). On the other hand, studies have shown a significant decrease in the amount of enzymatic and non-enzymatic antioxidants in the blood and cells of diabetic mice.

Therefore, the use of antioxidant compounds, especially natural antioxidants, to prevent oxidative damage in diabetic patients with high oxidative stress conditions can be beneficial (7). Nowadays, considering the side effects of synthetic drugs, researchers have focused on the use of herbs and herbal medicinal compounds (8). Glaucium flavum is a species of Papaveraceae that grows in spring and it is of great interest because of its medicinal and economic importance. Among the therapeutic uses of this plant treatment of dental abscesses, angina, asthma, bronchitis, pertussis and insomnia can be mentioned (9,10). The most well-known components of this plant are alkaloids, including aporphine, protopine, and protoberberine. Meanwhile, Glaucine in the aporphine family is considered to be the most important alkaloid compound (11). The alkaloid compounds of this plant are widely used in the pharmaceutical industry as analgesic, decongestant and anti-tussive (10). Emphasis on the antioxidant properties of this plant has been strengthened by the emergence of its antiviral and anticancer properties (12,13). The above mentioned herb is used in the southern regions of Iran as "Kelatin" and is used to reduce the complications of diabetes. The blood sugar-lowering ability of this drug in healthy rabbits has also been scientifically validated and studied (14). Alloxan is one of the toxic analogs of glucose that is transduced into pancreatic beta cells by transcription by the glucose transporter 2 (GLUT2); and in the presence of thiols, it forms reactive oxygen species (ROS). These groups cause cell death by producing superoxide and hydrogen peroxide radicals. Recent evidence from review studies has shown an increase in oxidative stress while reducing the levels of vitamins and suppressive enzymes of these deleterious agents in both type 1 and type 2 diabetes (2). Since no study has been performed to investigate the possible effects of Glaucium flavum extract on heart and brain antioxidant enzymes, this study was performed to determine the antioxidant properties of aqueous-ethanolic extract of Glaucium flavum in the brain and heart of adult male rats with alloxan induced diabetes compared with commercial and conventional drug glibenclamide.

Methods

This experimental study was approved by the Ethics Committee of the Islamic Azad University of Kazerun Branch under the Ethics Code of IR.IAU.KAU.REC.049.1398.32 adult male rats weighting 200-250 g were purchased from the Laboratory Animal Care Center of the Islamic Azad University of Kazerun Branch and studied in the animal laboratory. Animals were randomly divided into 4 groups of 8 healthy control, diabetic control, diabetic group treated with extract 500 mg/kg (diabetic+extract) and diabetic treated with 5 µg/kg glibenclamide daily (15,16). Diabetes was induced in groups 2 to 4 by injection of alloxan.

Plant collection: Glaucium Flavum plant samples were collected from pastures around Kazeroon in early spring. The shoots and flowers of the plants were dried

after exposure to light in the electric mills and transferred to the laboratory for extraction. The resulting powder was soaked in 50/50 ratio of 96% ethanol with water and alcohol for 72 hours and then smoothed and then placed in a final oven at 40 °C to evaporate the water and alcohol. The extract was combined with saline for oral administration and the suspension was fed daily to animals by gavage (17). Glibenclamide commercial pills (Najou, Iran) were also powdered with mortar and dissolved in titrated physiological serum with about pH=6 to reach the animal's oral intake (18).

Experimental diabetes induction method: In order to increase the effect of alloxan, animals were starved for eight hours before drug injection. Diabetes was induced by single-dose intraperitoneal injection of 120 mg/kg alloxan monohydrate (Sigma Alderich, USA) (19). Blood was drawn from the tail end to ensure that they were diabetic. Diabetes was confirmed by blood glucose testing using Easygluco (South Korea) glucometer. Cultivars above 250 mg/dl were considered diabetic (17).

Biochemical sampling and testing: At the end of the experiment the animals were anesthetized with ether and their heart and brain samples were removed. Then, while the blood in the target tissues was removed, equal weights of each were separated, homogenized on ice with the help of a homogenizer (Edmund Buhler, Germany), followed by refrigerated centrifugation (Sigma, USA). The supernatant was extracted at G18000 for 10 min. The Bradford method was used to measure protein. Standard protein curves were obtained using bovine serum albumin (BSA) (20). Catalase activity was measured by the Aebi method by following hydrogen peroxide decomposition at 240 nm using a spectrophotometer (cecil series 2040, England) (21). Tissue extract activity of GPX and SOD enzymes was determined using Randox kit (Randox kit, UK) based on kit instructions and spectrophotometry device at wavelengths of 340 nm and 560 nm, respectively in units of mg protein. Data were analyzed using one-way ANOVA and tukey post-hoc tests for enzymes and multivariate analysis of variance (MANOVA) for blood glucose in SPSS 20 software. P<0.05 was considered significant.

Results

Blood glucose: In the diabetic group treated with the extract, there was no significant difference between the means of the first days (273.6±22.04) and the fifteenth day (259±35.07). However, there was a significant decrease (217.25±11.98) between the first and thirty days (p=0.02); and a significant decrease in the 15th and 30th days (p=0.01). In diabetic group treated with glibenclamide, comparison of first day 270.4±19.7 and fifteenth day 197±19.11 indicated significant decrease in blood glucose level (p<0.001). In addition, in the comparison of the first and thirty days 198.87±15.22 this difference was maintained at the same level (p<0.001), but the difference was not significant (p<0.001) (Fig 1).

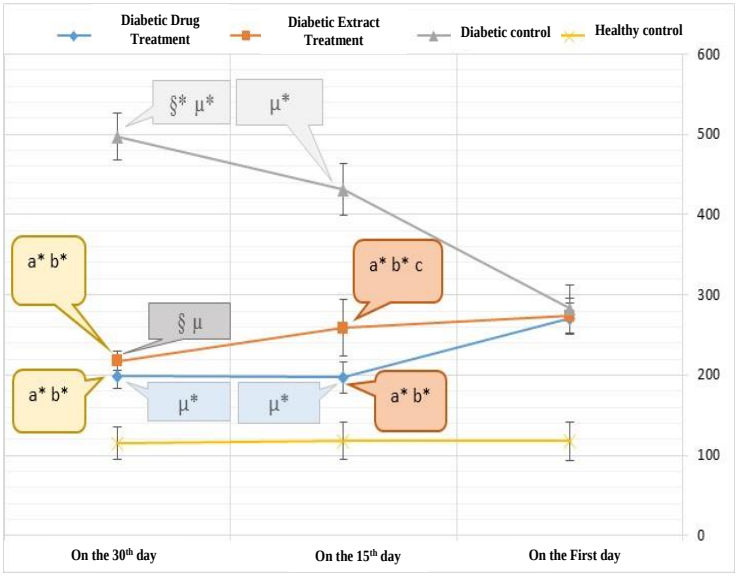

Figure 1. Comparison of mean blood glucose concentration in mg/dl in three days of measurement in 4 groups (n=8). µ: Significant difference with the first day at the level (p<0.05); §: Significant difference with the 15th day at the level (p<0.05), also the Latin letters indicate comparison between the experimental groups and the control groups on separate days: a: Significant difference with healthy control group, b: Significant difference with diabetic control group, c: Significant difference between experimental group. *: Significant difference in level (p<0.001)

In comparison between groups on the first day, mean of healthy control group was significantly lower than all diabetic groups (p<0.001). On the 15th day, glucose concentration in the diabetic control group was significantly higher than that of the diabetic group treated with the extract and drug treatment (p<0.001). Moreover, in comparison of the two experimental groups a significant decrease was found in treatment group with the extract than drug treated group (p=0.003). Finally, on the 30th day, glucose concentration was not significantly different between the diabetic and extract-treated diabetic groups.

Superoxide dismutase (SOD): Cardiac tissue SOD levels were significantly increased in the diabetic + extract group (17.05±0.9) than diabetic control group (12.86±2.5) ($p<0.001$). But there was no significant difference between the diabetic + extract and diabetic + drug groups (18.22±1.2) (Table 1). There was a significant increase in brain tissue in diabetic control group (3.44±1.47) and diabetic+extract (8.79±1.4) ($p<0.001$). Finally, the diabetic+extract experimental group had significantly more activity than the diabetic+drug group (6.77±1.7) ($p=0.03$) (Table 1).

Catalase (CAT): In the heart tissue, the activity of this enzyme had a significant difference in the diabetic control group (11.82±1.92) with both diabetic+extract (16.16±1) and diabetic + drug (16.57±1.5) ($p=0.001$). But there was no significant difference between the diabetic+extract and diabetic + drug groups. In brain tissue, the activity of catalase enzyme significantly increased in the treated diabetic groups as diabetic+extract (4.64±1.2) and diabetic + drug (3.83±1.5) compared with diabetic control group (0.88±0.19) ($p<0.001$). Finally, the observed increase for the diabetic + extract group was significantly higher than the diabetic + drug group ($p=0.001$) (Table 1).

Glutathione peroxidase (GPX): Cardiac activity of this enzyme was not significant in healthy controls (5.64±1.1) compared with diabetic + extract (4.25±0.9) and diabetic + drug (4.05±0.6). But in the diabetic control group (1.29±0.68) compared with the diabetic + extract and diabetic+drug groups there was a significant difference ($p<0.001$). There were no significant differences between the two experimental groups, diabetic + extract and diabetic + drug. In brain tissue, comparison of experimental groups including diabetic + extract (4.23±0.7) and diabetic + drug (3.69±0.4) also showed that the mean of the extract treated group was significant compared with the drug treated group ($p=0.03$)(Table 1).

Table 1. Comparison of mean activity of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) enzymes in cardiac and brain tissues of rats in four groups (n = 8)

Tissue	Group / enzyme u/mg pro	Healthy control Mean±SD	Diabetic control Mean±SD	Diabetic treated with extract (diabetic+extract) Mean±SD	Diabetic treated with drug (diabetic+extract) Mean±SD
Heart	SOD	18.67±1.4	12.86±2.5 a*	17.05±0.9 ab*	18.22±1.2 b*
	CAT	19.01±0.9	11.82±1.92 a*	16.16±1 ab*	16.57±1.5 ab*
	GPX	5.64±1.1	1.29±0.68 a*	4.25±0.9 b*	4.05±0.6 b*
Brain	SOD	10.45±1.3	3.44±1.47 a*	8.79±1.4 ab*	6.77±1.7 abc
	CAT	6.11±1.1	0.88±0.19 a*	4.64±1.2 ab*	3.83±1.5 a*b*c
	GPX	5.14±0.7	1.27±0.55 a*	4.23±0.7 ab*	3.69±0.4 a*b*c

One-way analysis of variance test, Latin letters indicate significant differences at the level ($p<0.05$); a: significant difference with the healthy control group, b: significant difference with the control diabetic group, c: significant difference with group treated with the extract and * indicates significant difference in level ($p<0.001$)

Discussion

In this study, increased activity of antioxidant enzymes SOD, CAT and GPX was observed in the extract-treated group than in the diabetic control group, which was generally more pronounced in the brain and more significantly for the GPX enzyme. Also, comparison of mean blood glucose concentration in diabetic group treated with Glaucium Flavum with diabetic control and diabetic group treated with drug confirms hypoglycemic effect of this plant, but this effect is less effective than glibenclamide. These results are consistent with the study by Cabo et al (14). To explain this observation, it can be noted to study of Gyurkovska et al. that attributed suppressing of JAK / STAT pathway and IL-1, IL-6, IL-7, IL-12 and M-CSF cytokines and thus suppressing the inflammation to glaucine extracted from Glaucium Flavum (22). Gurzov et al. also demonstrated that impairment of the JAK /

STAT pathway in the pancreas, liver, muscle, and adipose tissue is an important factor in the development of obesity and diabetes (23); Yoshida et al. also observed higher concentrations of IL-12 and M-. CFS in diabetic patients attributed these factors to macrophage-mediated vascular wall fibrosis in these patients (24). A study on streptozotocin-induced diabetic rats showed that Malondialdehyde (MDA) as an index of diabetes-induced oxidative stress was increased and the administration of Terrestrial fruit extract significantly decreased the MDA levels and increased SOD activity in brain tissue of treated rats (25). On the other hand, the triggering oxidative stress and lipid peroxidation lead to complications in metabolic diseases such as diabetes, and ultimately lead to neuropathy and neurodegeneration. In the present study, this effect was well seen in the brains of diabetic controls. But this effect was modulated by treatment with the Glaucium Flavum extract. Considering the presence of alkaloids and antioxidant compounds of Glaucium Flavum, it is possible to justify the possibility of increasing GPX, SOD and CAT enzymes in groups receiving 500 extracts (12,13). In one study, administration of vitamin E as an antioxidant was able to modulate this effect in brain tissue (27). In the present study, in the heart tissue of diabetic rats, the activity of all three enzymes was significantly reduced. This finding is supported by similar results by Tanoorsaz et al., Doustar et al., and Kandhare et al. (28-30). It has been shown that the antioxidant defense of heart tissue during diabetes is strongly affected and suppressed (31). Researchers see this phenomenon as a precursor to cardiac cell apoptosis as a product of an inflammatory process with the presence of cytokines such as IL-1, TNF-α and IFN-γ (32). But comparing the results of the groups treated with the extract, it was found that the protective effect of the Glaucium Flavum extract was similar to that of glibenclamide, which is consistent with a study by Shirpoor et al. reported protective and antioxidant effect of vitamin E against diabetes-induced apoptosis in rat hearts (33). On the other hand, Mohamadifard et al., In a comparison between ash, barberry and silymarin plants, reported that silymarin was superior to the rest of the flavonoids in antioxidant properties (34). From the present study it can be concluded that administration of the Glaucium Flavum extract at a dose of 500 mg / kg definitely decreases blood glucose concentration and reduce oxidative stress and enhance brain and heart antioxidant enzymes activity possibly by suppressing inflammation.

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