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Effect of Hydroethanolic Extract of *Rubus fruticosus* on Neuropathic Pain in Wistar Diabetic Rats

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ABSTRACT

Background: Diabetes mellitus is the most common metabolic disorder with many complications such as neuropathic pain which relief from it is a dilemma.
Objectives: To investigate the effect of hydroethanolic extract of *Rubus fruticosus* on neuropathic pain in diabetic rats.
Materials and Methods: In this experimental case –control study, forty eight male Wistar rats (250±20 g) were divided randomly to non-diabetic and diabetic groups. Streptozotocin (60 mg/kg, intra-peritoneal) was used to induce experimental diabetes. Each group was divided into three groups: receiving orally normal saline, 100 and 200 mg/kg doses of *Rubus fruticosus* extract for 30 days. At the end; the rats were subjected to Tail-Flick test. The data were analyzed by one-way and two-way ANOVA followed by Tukey's test in SPSS 21.
Results: In a paired comparison, all diabetic groups had shorter latency of tail-flicking time ($p < 0.01$) and lower pain tolerance threshold.
Also among diabetic rats, significant differences existed between saline group and group receiving the extract at a dose of 100 mg/Kg ($p < 0.01$), between saline group and the group receiving the extract at a dose of 200 mg/Kg ($p < 0.001$) and also between the groups receiving extract at two different doses ($p < 0.001$). But, only diabetic group receiving the extract at a dose of 200 mg/Kg showed positive significant difference with non-diabetic control group ($p < 0.01$).
Conclusions: The hydroethanolic extract of *Rubus fruticosus* can heighten the pain tolerance threshold and reduce the neuropathic pain induced by diabetes mellitus.

Keywords: *Rubus fruticosus*; Diabetes Mellitus; Diabetic Neuropathies

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Introduction

Diabetes mellitus is one of the common metabolic disorders associated with many complications in various organs (1). The worldwide prevalence of diabetes in 2000 was 2.8%, i.e. 171 million people, which is expected to reach 4.4%, i.e. 366 million in 2030 (2). It is epidemiologically and economically important in both developing and developed countries (3). Nowadays it is known as a disabling disorder owing to a wide variety of irreversible complications such as renal

failure, retinopathy, non-traumatic amputation, neuropathy and increasing risk of coronary heart disease, stroke and vascular diseases (4, 5, 6).

More than half of diabetic patients will suffer from neuropathic problems after 1-2 decades (7). In streptozotocin (STZ) induced diabetic rats, this time is reduced to two to four weeks after diabetes induction (8). Diabetic pain is one of the most common symptoms of diabetic neuropathy and is characterized by spontaneous pain and hyperalgesia (9, 10). Its under-lied mechanism is complicated (11). It is one of the major complaints of diabetic patients affecting their quality of life. Thus, relieving pain is of significant importance (12). Some studies have shown that oxidative stress due to hyperglycemia in diabetic patients can cause changes such as hypersensitivity of spinothalamic neurons and primary afferent neurons responsible for pain, and induce spontaneous impulses in them (3, 13). It is also suggested that biochemical changes in glial cells and peripheral nerves, failure of fatty acid production and decreased stimulation threshold and nerve conduction velocity are important in creating neuropathic pain in diabetic patients (14, 15).

Despite of developing of pharmaceutics and many chemical medicines to relieve pain; in recent decades scientists and researchers have focused on curative effects of herbal medicine thanks to their fewer complications, easier access and less costs (16).

Rubus fruticosus is belonging to the Rosaceae family. The plant is found in many parts of the world and has many biologically beneficial effects including antidiabetic, antibacterial, anti-inflammatory, antioxidant, antidiarrheal, anti-tumor, anti-fertilization, neuroprotective and also wound healing properties (17, 18). Several studies show that its phenolic compounds and anthocyanins have neuroprotective and analgesic and sedative properties, and can improve the age related memory impairment and learning disability (19). Its antidiabetic and neuroprotective effects are supposed to be due to some phenolic compounds such as ellagic acid and also anthocyanins, magnesium and manganese components (17, 18, 20).

Considering the importance of diabetes and its neuropathic complications finding out the new analgesic medication with minimal complications is essential. This study was conducted to assess the analgesic effects of hydroethanolic extract of *Rubus fruticosus* on neuropathic pain of diabetes.

Materials and Methods

The present study was performed in department of Biology, Bu-Ali Sina University of Hamadan on 48 adult male wistar rats purchased from Razi Institute. The weight of rats was in the range of 250±20 grams. The rats were transferred into Animal Science Research Laboratory of the Science Faculty and were housed at 22±2°C and 12-12 hour light-dark cycle with free access to food and water. In order to adapt to the laboratory environment, the rats were settled in this place one week before the experiment. All ethical issues were carefully regarded in this project according to international rules of animal research and it was confirmed by ethic committee of animal research of Hamadan Bu-Ali Sina University. In late summer, trimmings of blackberry bush were obtained from Damavand. After identification by a botanist (with herbarium number of 944-15) those were transferred to the laboratory in Hamadan research center of agriculture and natural resources and were air-dried in the dark. Then 500 grams of grinded herb were mixed with ethanol 80% at a ratio of 1 to 4 in a beaker and kept in the laboratory

environment for two weeks. Beaker contents were stirred twice per day by a glass stirrer. Thereafter the contents of the beaker were filtered through a filter paper and the prepared solution was placed in a rotary (Heidoilph WB2000) at the temperature of 80°C with a medium speed. After condensation of the extract and removal of the solvent, the resulting extract was placed under the laminar flow hood for one day until complete drying. The prepared extract with the approximate weight of 56 grams transferred to the freezer.

At first the animals were divided into two groups of streptozotocin (STZ), and saline for induction of diabetes. To induce diabetes, streptozotocin (STZ), (Sigma, Germany), was used intraperitoneally (ip) at a single dose of 60 mg/Kg. Three days after injection, the rats' glucose levels were measured and which blood glucose levels were greater than 250 mg/dl were considered as diabetic group. They showed symptoms of diabetes such as polydipsia, polyuria and weight loss. The others were in non-diabetic group who received 0.5 milliliters of normal saline.

Each diabetic and non-diabetic group was randomly divided into three groups which would receive (1) 0.5cc normal saline, (2) 100 mg/kg and (3) 200 mg/kg doses of *Rubus fruticosus* extract per orally.

According to the papers, neuropathy in rats occurs 2-4 weeks after diabetes induction (8), so the rats were attended for 30 days.

A sufficient amount of extract was dissolved in saline and the target dose was administered by gavage on a daily basis for 30 days to the groups which had been determined to receive the extract. The others received 0.5cc saline every day. On the last day, a half hour after gavage, the rats were tested and the results were recorded. To determine the heat pain threshold in rats, the Tail-Flick (TF) instrument (Borj Sanat Iran Company) was used. The test was performed according to the model proposed by Lee and McCarty (21). The test was conducted as follows: first, the rat was kept horizontally for about 45 minutes inside the container for adaptation. Then its tail was exposed to the light emitted from a heat source and the tail-flick latency was recorded by a digital chronometer. TF test was performed 3 times, once every 5 minutes, for regulating the thermal flux to set the tail-flick latency as 5-6 seconds in the control rats. The cut of time of light radiance to the tail was considered as 10 seconds. The mean of the Tail-Flick latency time was calculated in all groups.

The findings of the experimental groups were analyzed in SPSS21 calculating Mean ± SEM (Standard Error of Mean). One way and two-way ANOVA were used to compare the results and Turkey's test was used for pairwise comparison of groups. Data differences with $p < 0.05$ were considered significant.

Results

The effect of diabetes and extract of Rubus fruticosus on blood glucose level and body weight:

At first; there wasn't any difference between studied groups in terms of blood glucose level and body weight. In the end of study, non-diabetic groups have shown body weight increment, whereas diabetic groups demonstrated decrement (table 1).

STZ induced diabetes in the considered groups, but which received extract of *Rubus fruticosus* at a dose of 100 and 200 mg/Kg significantly showed reduction of serum level of blood glucose in comparison with diabetic control group ($p < 0.01$ and $p < 0.001$ respectively) (table 1).

Table 1. Comparison of body weight (g) and blood sugar (mg/dl) in the beginning and ending of the trial in all studied groups

Experimental Groups	Body Weight (g)		Blood Glucose Level (mg/dl)	
	Beginning of the trial	Ending of the trial	Beginning of the trial	Ending of the trial
Non-diabetic control	251.87 ± 6.61	300.12 ± 5.27	107.75 ± 3.01	120.12 ± 6.51
Diabetic Control	251.9 ± 5.24	[c] 191.62 ± 3.63	104.87 ± 5.98	[c] 574.37 ± 23.54
Non-diabetic 100 mg/kg Extract	254.25 ± 6.41	[c] 297.75 ± 5.3	98 ± 3.52	101.12 ± 6.59
Non-diabetic 200 mg/kg Extract	255.12 ± 6.07	[c] 293 ± 5.88	103.62 ± 1.97	102.25 ± 4.93
Diabetic 100 mg/kg Extract	254.87 ± 6.85	[b,e] 222.75 ± 13.41	100.12 ± 4.34	[c,d] 495.62 ± 22.09
Diabetic 200 mg/kg Extract	250.37 ± 3.6	[a,e] 238 ± 2.54	98.87 ± 4.14	[c,e] 339.37 ± 18.11

Values represent the mean±SEM of the (n = 8) male wistar male rats.
[a] $p < 0.05$ Compared to beginning of the trial in each group
[b] $p < 0.01$ Compared to beginning of the trial in each group
[c] $p < 0.001$ Compared to beginning of the trial in each group
[d] $p < 0.01$ The difference between the diabetic groups received extract and saline
[e] $p < 0.001$ The difference between the diabetic groups received extract and saline

The effect of diabetes on Tail-Flick latency time and pain tolerance threshold:

Non-diabetic and diabetic control groups were significantly different in terms of Tail-Flick latency time ($p < 0.001$). This difference was also found out between non-diabetic and diabetic groups receiving the extract at a dose of 100 mg/Kg ($p < 0.05$) and also between non-diabetic and diabetic groups receiving the extract at a dose of 200 mg/Kg ($p < 0.01$). In all of them diabetic groups had shorter latency of tail-flicking time which demonstrates the reduction of pain tolerance threshold among diabetic cases, four weeks after induction of diabetes compared with non-diabetic rats (Diagram 1).

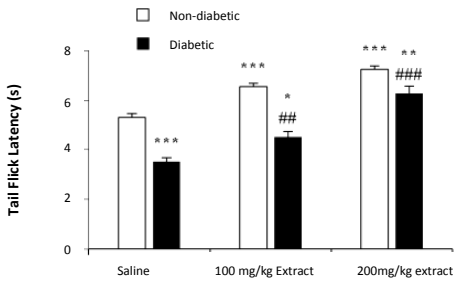

Diagram 1: Comparison of Tail Flick Latency in the experimental groups
Values represent the mean±SEM of the (n = 8) male Wistar male rats.
Represent difference of groups to non-diabetic control group (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$) *
Represent difference of groups to diabetic control group (##$p < 0.01$, ###$p < 0.001$)

The effect of extract of Rubus fruticosus on Tail-Flick latency time and pain tolerance threshold in diabetic rats:

As shown in diagram 1, a significant difference exists between diabetic control group and diabetic group receiving the extract at a dose of 100 mg/Kg in terms of Tail-Flick latency time ($p < 0.01$). Also there is a quite significant difference between diabetic control group and diabetic group receiving the extract at a dose of 200 mg/Kg ($p < 0.001$) and also between both diabetic groups receiving the extract at a dose of 100 and 200 mg/Kg ($p < 0.001$).

Considerably only diabetic group receiving the extract at a dose of 200 mg/Kg showed positive significant difference with non-diabetic control group ($p < 0.01$).

Discussion

In this study, adult male wistar rats were used in order to be confident about development of nervous system. STZ was used to induce diabetes through destruction of islets of Langerhans. As previously mentioned, neuropathy, as one of the common complications of diabetes in rats, occurs 2-4 weeks after induction of diabetes. Severe pain, hyposthesia or anesthesia, diabetic foot and

amputation are common neuropathic complications (22).

According to studies about pain pathways, involvement of central pain system, direct stimulation of pain receptors mainly by means of chronic inflammatory processes which has been associated with the activation of glial cells can lead to hyperalgesia (15, 23-25). Various evidences have indicated that glial cells, particularly microglia, are activated in uncontrolled hyperglycaemic conditions in the spinal cord (26, 27). After some phenotypic changes, they release various proinflammatory cytokines, including interleukin 1β (IL-1β) and tumour necrosis factor α (TNF-α), which have been implicated directly in the induction of neuropathic pain (26). In confirmation of these theories, some proinflammatory cytokines are expressed in the dorsal horn of the spinal cord which is thought to contribute to the pathogenesis of diabetic neuropathic pain (11).

This study showed that hydroethanolic extract of *Rubus fruticosus* has analgesic effect in diabetic rats which was especially more with higher dose.

Considering by thermal tests such as Tail-Flick which used for pain assessment in this trial, central pathways of pain are mainly assessed (28). There is a possibility that *Rubus fruticosus* can have analgesic effects by affecting the central system.

It is also reported that neuropathy occurs in diabetes due to diabetes-induced oxidative stress and inflammatory processes associated with prostaglandins PG-I_2 and PG-E which have destructive effects on neurons and eventually lead to chronic pain in these patients. The pathophysiology of diabetic neuropathy involves a complex cascade of several interrelated mechanisms, but not all components have been known yet in detail (29).

In summary, hyperglycemia induces spontaneous oxidation of glucose through a variety of enzymatic and non-enzymatic activities, and increases the advanced glycation end products (AGEs), protein kinase C pathway activity, polyol pathway activity (aldose reductase), poly ADP-ribose polymerase (PARP) pathway activity, hexosamine flow, and decreases growth factors, all of which are the key components of the mentioned complex cascade. This pathway finally leads to oxidative stress of nerve cells through stimulating the production of active oxygen and active nitrogen species (30, 31). Oxidative stress will eventually cause a lot of neuropathic lesions by activation of many degenerative pathways such as reduction of intracellular antioxidant enzymes activity, vascular damage, increased synthesis of free radicals in the mitochondria, decreased nitric oxide, induced endoneurial hypoxia, apoptosis, degradation of cellular components and increased expression of inflammatory factors (32).

Previous studies show that the plants of genus *Rubus* contain the compounds with antioxidant and anti-inflammatory properties including a variety of antioxidants (such as Neoxanthin, Violaxanthin, β-carotene, Lutein) and anthocyanins (such as Cyanidin-3-glucoside, cyanidin-3-xyloside, cyanidin-3-rutinoside, cyanidin-3-malonylglucoside and dioxalylglucoside-cyanidin-3), vitamin C, flavonoid and phenolic compounds (such as ellagic acid, catechin, epicatechin, rutin, quercetin, caffeic acid, p-coumaric acid) and minerals (such as calcium, iron, magnesium, phosphorus, potassium, sodium, zinc, manganese and selenium) (17, 33-35).

So by abovementioned description, it can be assumed that compounds in hydroethanolic extract of aerial parts of *Rubus fruticosus* suppress inflammation caused by diabetes by

affecting on inflammatory mechanisms and exerting anti-inflammatory effects and inhibiting enzymes involved in this pathway such as lipoxygenase and cyclooxygenase. Similarly it leads to reduction of oxidative stress in diabetic rats because of their antioxidant compounds. Also it may possibly act by affecting some receptors in the nervous system and neuronal changes. Through these ways it can reduce neuropathic complications of diabetes and increased pain tolerance threshold in studied rats.

Further studies on *Rubus fruticosus* and its compounds will better show whether the plant can be used to treat diabetes and improve neurological complications.

Conclusion

The hydroethanolic extract of aerial parts of *Rubus fruticosus* can reduce neuropathic complications of diabetes, heighten the pain tolerance threshold and so reduce the neuropathic pain induced by diabetes mellitus.

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Conflict of Interest

No conflict of interest.

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