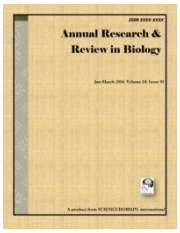

Annual Research & Review in Biology
4(20): 3119-3127, 2014

SCIENCEDOMAIN *international*
www.sciencedomain.org

The Effect of Water Extract of *Zataria multiflora* on Microvascular Permeability in Streptozocin Induced Diabetic Rats

Gholamreza Sepehri[1*] Masoomeh khazaee[2], Mohammad Khaksari[3] and Ahmad Gholamhoseinian Najar[4]

[1]Neuroscience Research Center, Institute of Neuropharmacoloy, Kerman University of Medical Sciences, Kerman, Iran.
[2]Department of Physiology, Medical School, Kerman University of Medical Sciences, Kerman, Iran.
[3]Physiology Research Center, Institute of Neuropharmacoloy, Kerman University of Medical Sciences, Kerman, Iran.
[4]Department of Biochemistry, Medical School, Kerman University of Medical Sciences, Kerman, Iran.

Authors' contributions

This work was carried out in collaboration between all authors. All authors have substantial contributions to conception and design, acquisition of data or analysis and interpretation of data. Author GS designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. All other authors took part in conducting the experiment and analyzing the results. All authors read and approved the final manuscript.

Short Communication

Received 11th February 2014
Accepted 10th April 2014
Published 31st May 2014

ABSTRACT

Objectives: The present investigation was aimed to study the effect of water extract of *Zataria multiflora* (*Z. multiflora*) on microvascular permeability in streptozocin (STZ)-induced diabetic rats.
Place and Duration of Study: Physiology Research Center, Kerman University of Medical Sciences, Kerman, Iran, between June, 2011 and June 2012.
Materials and Methods: diabetes was induced in rats by administration of STZ (55 mg/kg, i.p.). *Z. multiflora* (1000mg/kg/d) and glibenclamide (5mg/kg) were administered by intragastric gavage for 8 weeks after the induction of diabetes. Control, vehicle and

**Corresponding author: Email: gsepehri@yahoo.com;*

STZ_induced diabetes groups received the same volume of distilled water as Z. multiflora treated group. The fasting body weight, blood glucose level and glycated haemoglobin A1c (HbA1c) levels were estimated at the start of experiments and at the end of every 2 week for 8 weeks. Microvascular permeability was evaluated by the evans blue test and water content measurement, 8 weeks after the induction of diabetes.

Results: *Z. multiflora* caused a significant reduction in extravasated evans blue concentration (31.4±0.8µg/100mg) in STZ diabetics vs 12.3±0.7µg/100mg in *Z. multiflora* treated diabetic rats), (P< .001) as an indicator of microvascular permeability. However, *Z. multiflora* had no significant effect on water content percentage (80.2±0.1%) compared to STZ diabetic rats (81.4±0.5%). Also *Z. multiflora* administration caused a significant decrease in blood glucose level (P=.05), HbA1c level (P<.001) and body weight loss (P=.01) in STZ diabetic rats.

Conclusion: this study showed that *Z. multiflora* reduced microvascular permeability in STZ diabetic rats. The underlying mechanism(s) is not determined yet, but the H1-receptor inhibition, antioxidant and/anti-inflammatory effect of *Z. multiflora* may be involved in its benefit effects on STZ- induced diabetes.

Keywords: Zataria multiflora; diabetes; microvascular permeability; streptozocin (STZ).

1. INTRODUCTION

Diabetes mellitus (DM) and its complications are a significant cause of morbidity and mortality in both developed and developing countries [1]. For various reasons in recent years traditional plant (herbal) therapies as prescribed by indigenous systems of medicine with different mechanisms have commonly been used [2]. These natural compounds are used by traditional herbalists for the management of diabetes in several parts of Iran [2].

Acute hyperglycemia is associated with impaired vascular function through production of superoxide anion and endothelium-dependent vasodilation is impaired in patients with insulin-dependent and non-insulin-dependent diabetes mellitus [3].

Zataria multiflora boiss. is a member of Laminaceae family that geographically grows in Iran, Pakistan, and Afghanistan and has been shown to posses the anesthetic, antiseptic, antioxidant, anti-inflammatory and antispasmodic property [4-5]. Previous studies reported that aqueous extract from *Z. multiflora* caused hypoglycemic effect through the inhibition of alpha-glucosidase in streptozocin (STZ) induced diabetic rats [6].

Since there is an increasing trend to use herbal medicine for the control of DM and *Z. multiflora* is commonly used as a folk medicine for control of DM in Iran, and there was no documented report on the effect of *Z. multiflora* on vascular permeability in diabetic conditions, so the aim of this study was to investigate the effect of aqueous extract from *Z. multiflora* on the microvascular permeability in STZ-induced diabetic rats [6].

2. MATERIALS AND METHODS

2.1 Animals

Wistar male rats weighing 250-300g were used for the study. Animals were housed 4 or 5 rat per cage under standard conditions of temperature (23±2ºC), 12h/12h light/dark cycle, and

had free access to a standard pellet diet (Pars Karadj, Karadj, Iran) and tap water. Sodium chloride (4.5g/L) was added to tap water to prevent dehydration in STZ diabetic rats. They were housed in standard polypropylene cages with wire mesh top. All studies were carried out using at least six rats in each group. Animal handling and experimental procedures were approved by the Ethics Committee of Kerman University of Medical Sciences (K/88/222).

2.2 Plant Material

Air-dried aerial parts of *Z. multiflora boiss* (locally called Avishan shirazi) were purchased from Kerman, Iran, on June, 2011. During spring season, the callus and flower-bearing tops of *Z. multiflora* were collected from herbal medicine stores, Kerman, Iran. The plant was identified and confirmed by the Pharmacognosy department of School of Pharmacy (Kerman, Iran) as Z. multiflora Boiss.(Laminaceae family) and were air-dried and powdered. Water extract of *Z. multiflora* was prepared by socking 100g of the powdered callus and flower-bearing tops of *Z. multiflora* in 1000mL of distilled water for 72h. The solution thereafter filtered and the filtrate was evaporated in an oven at 40ºC. The yield of the extract was 8.6% W/W with reference to powdered callus and flower-bearing tops. The extract was dissolved in distilled water to prepare a solution included 1000mg/ml concentration from dry weight of *Z. multiflora*.

2.3 Experimental Design

DM was induced by a single intraperitoneal injection of 55 mg/kg STZ (Sigma Chemical Co, St. Louis, Mo) dissolved immediately before administration in freshly prepared 50mmol/L citrate buffer (pH 4.5). To prevent the STZ-induced hypoglycemia, rats received 10% dextrose solution after 6h of STZ administration for next 24h [7]. Induction of diabetes was verified after 72h by measuring blood glucose level (300mg/mL) with strips using glucometer (Insopia , South Korea) [8].

The study comprised of 5 different groups as follows:

1- Control or untreated rats.
2- Vehicle group, which received a single i.p. injection of 0.1 ml of 50mmol/L citrate buffer (pH 4.5).
3- STZ_induced diabetic group which received a single i.p. injection of 55mg/kg STZ in citrate buffer (pH 4.5).
4- STZ_induced diabetic group, which received *Z. multiflora* (1000mg/kg/d) by intragastric gavage for 8 weeks after the induction of DM [6,9].
5- STZ_induced diabetic group, which received glibenclamide (5mg/kg) by intragastric gavage for 8 weeks after the induction of DM as positive control group for evaluation of antihyperglycemic protocol.

Control, vehicle and STZ_induced diabetic groups received the same volume of distilled water by intragastric gavage daily for 8 weeks as *Z. multiflora* treated group.

2.4 Sample Collection and Biochemical Assays

The fasting blood glucose level and glycated haemoglobin A1c (HbA1c) levels were estimated at the start of experiments and at the end of every 2 weeks for 8 weeks. The EDTA blood was used to estimate the HbA1c level [10]. HbA1c was assessed by

immunoturbidimetric determination by chromatography method (Glyco Hemoglobin kit, Germany). Fasting plasma glucose was measured by strips using glucometer (Insopia, South Korea). Also fasting body weight was measured by the same protocol.

On 56th day , microvascular permeability was determined after intravenous injection of evans blue (20mg/kg) into jugular vein, and 30 min later, 2 samples were taken from duodenum through laparatomy from each group of rats. Microvascular permeability was evaluated by the evans blue test and water content measurement [11].

The 2nd duodenum sample was weighted and dried in an incubator at 65ºC for 96h and then tissue water content % was calculated by the following formula:

$$\text{Water Content} = (Wet\ Weight - Dry\ Weight) \times \frac{100}{Wet\ Weight}$$

2.5 Data Analysis

The data were presented as mean ± SEM of at least 6 rats in each group. Student pair T-test was used to compare the mean differences between two groups and one way ANOVA test (post hoc tukey) to compare the mean of effects among different groups. P values of less than .05 were considered as statistical significance.

3. RESULTS

3.1 Effect of *Z. multiflora* Administration on Extravasated Evans blue Concentration in Duodenum of Treated Groups

The amount of extravasated evans blue concentration in duodenum is shown in Table 1. One way ANOVA showed a significant difference in extravasated evans blue concentration in STZ diabetic rats (31.4±0.8µg/100mg) in comparison to control (16.9 ±0.7µg/100mg tissue) and vehicle group (15.9±0.8µg/100mg) (P<.001). *Z. multiflora* (1000mg/kg/d) administration for 8 weeks in STZ diabetic rats caused a significant reduction in extravasated evans blue concentration(12.31±0.70µg/100 mg) in duodenum as compared to STZ diabetic rats(31.42±0.8µg/100mg) (P<.001) (Table 1). Our results showed that there was no significant difference in extravasated evans blue concentration in *Z. multiflora* treated diabetic rats (12.31±0.70µg/100 mg)compared to control (16.9±0.7µg/100mg) and vehicle(15.9±0.8 µg/100 mg) groups.

3.2 Effect of *Z. multiflora* Administration on Tissue Water Content Percentage in Duodenum of Treated Groups

One way ANOVA showed that tissue water content percentage in duodenum of STZ diabetic rats(81.39±0.53%) was increased significantly in comparison to control(67.7±1.7%)) and vehicle group (58.7 2.8%)(P=.01) (Table 1)). Also water content percentage in duodenum of vehicle group was significantly lower than control rats (P=.05). However, there was no significant difference in tissue water content percentage of *Z. multiflora* treated STZ diabetic (80.2±0.1%) as compared to STZ diabetic rats (81.4±0.5%).

Table 1. The effect of *Z. multiflora* on variables of HbA1c, evans blue concentration, water content (%) and body weight (g) after 8 weeks of treatment

Variables	Control	Vehicle	STZ- DM	STZ-DM+Zataria
Evans blue concentration (µg/100mg)	16.9±0.7	15.9±0.8	31.4±0.8$^{ttt\ ###}$	12.3±0.7***
Water content (%)	67.7±1.7	58.7±2.8t	81.4±0.5**$^{###}$	80.2± 0.1***$^{##}$
Body weight (g)	210±6.5	208 ±8.8	131.8±2.3 $^{ttt\ ###\ ‡‡}$	160±9.3 $^{ttt\ #\ ‡‡}$
HbA1c concentration (%)	3.9±0.1	4.2±0.2	6.7±0.1 $^{ttt\ #}$	4.5±0.2***

*ttt = Significantly different from control (P<·001). ### = Significantly different from vehicle (P<·001). *** = Significantly different from STZ- diabetics (P<·001).** = Significantly different from control (P=. 01), ## = Significantly different from vehicle (P=.01), t = Significantly different from control (P=.05), ‡‡ = Significantly different from STZ Diabetic (P=.01)*

3.3 Effect of *Z. multiflora* administration on body weight in treated groups

Data showed that there was a significant decrease in BW in STZ diabetic rats in 2nd, 4th, 6th and 8th weeks compared to start of experiments and control group (P<.001). However, BW in control and vehicle groups increased significantly from 4th week post experiments respectively (P<.001) (Data not shown).

Our results showed a significant reduction in BW in both STZ diabetic and diabetic rats which received *Z. multiflora* in comparison to control and vehicle group ((*P*<·001) (Table 1). Also data showed a significant reduction in body weight loss at 8 weeks in diabetic rats which received *Z. multiflora* as compared to STZ diabetic rats **(**P=.05) (Table 1). Also there was no significant difference in body weight at 8 weeks of diabetic rats which received glibenclamide as compared to control and vehicle group (data not shown).

3.4 Effect of *Z. multiflora* Administration on HbA1c Concentration in Treated Groups

The results of this study showed that the HbA1c values at time 0 in STZ diabetic rats (3.8±0.2%) showed no significant difference with control rats(3.9±0.1%). However, data showed that HbA1c concentration in STZ diabetic rats after 8 weeks of treatment (6.7±0.1%) was significantly higher than control (3.9±0.1%) and vehicle group (4.2±0.2%) (P<.001). *Z. multiflora* administration significantly decreased HbA1c level (4.5±0.2%) in comparison to STZ diabetic rats (6.7±0.1%), (P<.001), (Table 1). However, the values are significantly higher than control and vehicle groups ((*P*<·001).

3.5 Effect of *Z. multiflora* Administration on Blood Glucose Level in Treated Groups

Blood glucose level of 4 groups of rats is shown in Table 2. Statistical analysis showed that blood glucose in STZ_induced diabetic group was significantly higher than control and vehicle groups at start of experiment (341±10mg/dl), 2nd week (325.5±19.4mg/dl), 4th weeks (387.0±31.5mg/dl), 6th week (406.5±40.7mg/dl), and 8th week (411±37.6mg/dl)(P<.001).

Z. multiflora administration caused a significant decrease in blood glucose level in comparison to STZ diabetic rats at 6th week (243.2±29.9mg/dl) and 8th week (246.0±30.5 mg/dl) (P=.01). However, blood glucose level in STZ diabetic rats which received *Z. multiflora* for 8 weeks was significantly higher than control and vehicle group (P<.001). Our results showed that glibenclamide (5mg/kg) reduced significantly blood glucose level as compared to STZ diabetic rats during 8 weeks of drug treatment (P<.001).

Table 2. Effect of *Z. multiflora* administration on blood glucose level (mg/dl) at baseline and during the intervention period

Time	Control	Vehicle	STZ DM	STZ DM+ Zataria	STZ DM+ Glibenclamide
(Day 0)	67±5.7	70.0±4.6	341.0±10.0***	343.5±20.1***	336±28.4***
Week 2	70.7±2.7	69.0±4.0	325.5±19.4***	295.5±19.2***	93.4±17.8 ###
Week 4	74.3±1.8	74.2±3.3	387.2±31.5***	282.3±8.4***	94.6±13.9 ###
Week 6	80± 2.8	78.3±4.2	406.5±40.7***	243.2±29.9***##	98.7±15.8 ###
Week 8	79.2±3.5	77.9±4.5	411.0±37.6***	246.0±30.5***##	99.2 ±14.6 ###

*Diabetes was induced by 55 mg/kg/i.p. STZ in citrate buffer (pH 4.5). Rats received Z. multiflora (1000mg/kg/d) and glibenclamide (5mg/kg) by intragastric gavage for 8 weeks after the induction of diabetes. All data are expressed as mean ± SEM (n=6). *** = STZ diabetic compared to control and vehicle (P< .001), # # = Z. multiflora compared to STZ diabetic (P = .01), # # # = STZ DM + Glibenclamide compared to STZ diabetic (P< .001)*

4. DISCUSSION

In this study, *Z. multiflora* showed a good potential to decrease capillary permeability in rats as indicated by significant decrease in extravasated evans blue concentration, in duodenum of STZ diabetic rats, which is an established indicators of vascular permeability [11]. However, *Z. multiflora* treatment had no significant effect on water content percentage. Also *Z. multiflora* caused a significant reduction in blood glucose level and HbA1c concentration as compared to STZ diabetic rats.

Advanced glycation end products (AGEs) have been implicated as important factors in the pathogenesis of diabetic vascular complication [12], Endothelial dysfunction will cause alterations in blood flow, pressure and permeability which lead to microangiopathy, pathogenesis of various diabetic complications, however, the underlying mechanism(s) is not determined yet [3]. Recent studies have shown that hyperglycaemia can alter the glycocalyx structure, and parallel findings have shown that the apparent increase in permeability demonstrated in hyperglycaemia may be due to an increase in the permeability of the vessels to water, and not an increase in protein permeability, an effect attributable to altered glycocalyx [3].

Our results showed that *Z. multiflora* caused significant reduction in vascular permeability in STZ diabetic rats. Also data confirmed the previous report showing the hypoglycemic effect of *Z. multiflora* through its inhibitory effect on the alpha glucosidase in STZ diabetic rats [6]. The underlying mechanism(s) by which *Z. multiflora* affects vascular permeability is not known and needs further investigation. *Z. multiflora* showed significant antioxidant activity in rats, so benefit effects of *Z. multiflora* in STZ diabetic rats could be mediated through its antioxidant capacity [9].

The Benefit effects of *Zataria multiflora* Boiss in experimental model of mouse inflammatory bowel disease have been reported by previous studies and since subclinical inflammatory reaction has a role in the pathogenesis of type 2 diabetes, so, the anti-inflammatory effect of *Z. multiflora* may be involved in its benefit effects on STZ- induced diabetes [13-14].

Flavonoids(such as thymol , λ-terpinene , ρ-cymene, carvacrol , Rosmarinic acid (RA) and linalol) as the main constituent of *Z. multiflora* extract are a class of plant phenolics with significant antioxidant properties [15]. Their positive effects come from their ability to inhibit lipid peroxidation, chelate redox-active metals and attenuating other procedure involving reactive oxygen species [16]. In addition, the anti-inflammatory effects of flavonoids (mainly carvacrol constituent of *Z. multiflora* extract) seem to be related to a decrease in vascular permeability and extravasated evans blue concentration [4-5]. *Z. multiflora* and its constituent carvacrol showed inhibitory effect on histamine H (1) receptors which result in decrease in vascular permeability [17]

Thus, we conclude that part of the *Z. multiflora* extract on vascular permeability is due to its flavonoid component such as rosmarinic acid and carvacrol via two independent mechanisms: scavenging of reactive oxygen radicals and inhibition of the inflammatory response [4,18]. Also *Z. multiflora* may reduce vascular permeability through H1-receptor inhibition [17].

This study demonstrated that *Z. multiflora* extract partly reversed the increased blood glucose and HbA1c levels and weight loss in STZ diabetic rats which confirms the previous reports of its hypoglycemic effect which results in reduction in vascular permeability [19-20]. Although *Z. multiflora* decreased the HbA1c and blood glucose level in STZ diabetic rats , however, the values are were significantly higher than control and vehicle groups which indicates that biochemical values and microvascular permeability were partly reversed by extract, mainly through the inhibition of alpha glucosidase activity [6].

CONCLUSION

In summary this study showed that *Z. multiflora* reduced capillary permeability in STZ diabetic rats which was characterized by a significant decrease in extravasated evans blue concentration in duodenum of STZ diabetic rats. The underlying mechanism(s) is not determined yet, but the H1-receptor inhibition, antioxidant and/anti-inflammatory effect of *Z. multiflora* may be involved in its benefit effects on STZ- induced diabetes.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

Animal handling and experimental procedures were approved by the Ethics Committee of Kerman University of Medical Sciences (K/88/222).

ACKNOWLEDGEMENTS

This study was carried out in Physiology Research Center of Kerman University of Medical Sciences. The funding of this study was provided by the Vice Chancellor of Research, Kerman University of Medical Sciences, Kerman, Iran (Grant No: 88/10/61/21818).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Mohan V. Age-and sex-specific prevalence of diabetes and impaired glucose regulation in 11 Asian cohorts. Diabetes care. 2003;26(6):1770-80.
2. Hasani-Ranjbar S, Larijani B, Abdollahi M. A systematic review of Iranian medicinal plants useful in diabetes mellitus. Arch Med Sci. 2008;4(3):285-92.
3. Perrin RM, Harper SJ, Bates DO. A role for the endothelial glycocalyx in regulating microvascular permeability in diabetes mellitus. Cell Biochem Biophys. 2007;49(2):65-72.
4. Boskabady MH, Mehrjardi SS, Rezaee A, Rafatpanah H, Jalali S. The impact of *Zataria multiflora* Boiss extract on *In vitro* and *In vivo* Th1/Th2 cytokine (IFN-gamma/IL4) balance. J Ethnopharmacol. 2013;150(3):1024-31.
5. Hosseinzadeh H, Ramezani M, Salmani G. Antinociceptive, anti-inflammatory and acute toxicity effects of *Zataria multiflora* Boiss extracts in mice and rats. J Ethnopharmacol. 2000;73(3):379-85.
6. Gholamhosseinian A, Falah H, Mirtajadini SM. The inhibitory effect of some Iranian plants extracts on the alpha glucosidase. Iranian Journal of Basic Medical Sciences. 2008;11(1):1-9.
7. Tanko Y, Yerima M, Mahdi M, Yaro A, Musa K, Mohammed A. Hypoglycemic Activity of Methanolic Stem Bark of Adansonnia digitata Extract on Blood Glucose Levels of Streptozocin-Induced Diabetic Wistar Rats. International Journal of Applied Research in Natural Products. 2008;1(2):32-6.
8. Sabitha V, Ramachandran S, Naveen KR, Panneerselvam K. Antidiabetic and antihyperlipidemic potential of Abelmoschus esculentus (L.) Moench. in streptozotocin-induced diabetic rats. J Pharm Bioallied Sci. 2011;3(3):397-402.
9. Babaie M, Yasa N, Mohammadirad A, Khorasani R, Abdollahi M. On the anti oxidative stress potential of *Zataria multiflora* Boiss (Avishan shirazi) in Rats. International Journal of Pharmacology. 2007;3(6):510-4.
10. Jeppsson JO, Kobold U, Barr J, Finke A, Hoelzel W, Hoshino T, et al. Approved IFCC reference method for the measurement of HbA1c in human blood. Clin Chem Lab Med. 2002;40(1):78-89.
11. Udaka K, Takeuchi Y, Movat HZ, editors. Simple method for quantitation of enhanced vascular permeability, Royal Society of Medicine; 1970.
12. Rojas A, Morales MA. Advanced glycation and endothelial functions: a link towards vascular complications in diabetes. Life sciences. 2004;76(7):715-30.
13. Spranger J, Kroke A, Möhlig M, Hoffmann K, Bergmann MM, Ristow M, et al. Inflammatory cytokines and the risk to develop type 2 diabetes. Diabetes. 2003;52(3):812-7.

14. Nakhai LA, Mohammadirad A, Yasa N, Minaie B, Nikfar S, Ghazanfari G, et al. Benefits of *Zataria multiflora* Boiss in experimental model of mouse inflammatory bowel disease. Evidence-Based Complementary and Alternative Medicine. 2007;4:1.
15. Ebrahimzadeh H, Yamini Y, Sefidkon F, Chaloosi M, Pourmortazavi SM. Chemical composition of the essential oil and supercritical CO2 extracts of *Zataria multiflora* Boiss. Food chemistry. 2003;83(3):357-61.
16. Gabrieli CN, Kefalas PG, Kokkalou EL. Antioxidant activity of flavonoids from Sideritis raeseri. J Ethnopharmacol. 2005;96(3):423-8.
17. Boskabady MH, Tabanfar H, Gholamnezhad Z, Sadeghnia HR. Inhibitory effect of *Zataria multiflora* Boiss and carvacrol on histamine (H(1)) receptors of guinea-pig tracheal chains. Fundam Clin Pharmacol. 2012;26(5):609-20.
18. Kordsardouei H, Barzegar M, Sahari MA. Application of *Zataria multiflora* Boiss. and Cinnamon zeylanicum essential oils as two natural preservatives in cake. Avicenna Journal of Phytomedicine. 2013;3(3):238-47.
19. Ding H, Triggle CR. Endothelial dysfunction in diabetes: multiple targets for treatment. Pflügers Archiv European Journal of Physiology. 2010;459(6):977-94.
20. Niiya Y, Abumiya T, Yamagishi S, Takino J, Takeuchi M. Advanced glycation end products increase permeability of brain microvascular endothelial cells through reactive oxygen species-induced vascular endothelial growth factor expression. J Stroke Cerebrovasc Dis. 2012;21(4):293-8.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=543&id=32&aid=4773