PharmacologyOnLine

Archives • 2021 • vol.3 • 38-44

ANTI-INFLAMMATORY EFFECT OF ANTIDIABETIC MIXTURE ON A MODEL OF CARRAGEENAN EDEMA

Savych, Alona*

[1]Department of Pharmacognosy with Medical Botany, I. Horbachevsky Ternopil National Medical University, Ukraine

*alonasavych@gmail.com

Abstract

The association between hyperglycemia, inflammation and vascular complications in DM is now well established. Antidiabetic mixture of different medicinal plants can relieve inflammation due to the various biologically active substances present in it.

The aim of our study was to investigate an anti-inflammatory activity of antidiabetic mixture, which consists of *Equiseti arvensis herba, Sambuci flores, Inulae rhizomata et radices, Hyperici herba, Tiliae flores, Polygoni avicularis herba, Myrtilli folium, Urticae folia*.

Anti-inflammatory properties were studied on male albino rats weighing between 180 g and 200 g by carrageenan-induced pleurisy. The inflammation was inducted in rats by a subplantar injection of 0.1 mL of carrageenan suspension (100 μg/paw). The rats were orally treated with diclofenac (8 mg/kg) and aqueous extracts of antidiabetic mixture at doses of 9 mL/kg, 12 mL/kg and 25 mL/kg .

An aqueous extracts of antidiabetic mixture at a dose of 9 mL/kg did not show significant anti-edema effect during the experiment.

After 6 hours, an aqueous extracts of antidiabetic mixture in doses of 12 mL/kg and 25 mL/kg showed significant ($p<0.05$) an anti-inflammatory effect, it was 43.1% and 41.6%, respectively. The highest activity was observed in diclofenac, as standard nonsteroidal anti-inflammatory drug, and it was 69.6%.

Keywords: *antidiabetic herbal mixture, anti-inflammatory, edema, carrageenan, diabetes mellitus*

Introduction

The prevalence of diabetes mellitus (DM) is on the rise, with 415 million people affected worldwide according to recent data from the International Diabetes Federation [1, 2]. This number is predicted to increase further, with 642 million people expected to develop DM by 2040 [2, 3, 4]. While many factors are known to contribute to the development of DM and its complications, the involvement of the immune system in the pathogenesis of metabolic diseases has been gaining interest [5, 6, 7]. It has long been appreciated that inflammation is central to the pathology of the pancreatic islet in DM type 1 [8, 9, 10, 11]. However, growing evidence suggests that inflammation also plays a key role in the pathogenesis of DM type 2, including obesity-related insulin resistance, impaired insulin secretion, and diabetes-related vascular complications [12, 13, 14, 15]. Existing research suggest that anti-inflammatory treatments may improve glycemia, β-cell function, and/or insulin resistance in patients with DM type 2 [16, 17, 18, 19, 20]. In DM, hyperglycemia and elevated free fatty acids may promote inflammation by stimulating glucose utilization along with alterations in oxidative phosphorylation [21, 22, 23, 24]. Glucotoxicity and lipotoxicity might also exert oxidative and endoplasmic reticulum stress, which in turn elicits an inflammatory response [25, 26, 27, 28, 29]. Therefore, the optimization of pharmacotherapy, searching and study of new drugs with anti-inflammatory activity for the prevention and treatment of DM type 2 and its dangerous complications is a top issue of pharmacy and medicine [30, 31, 32, 33, 34, 35].

One of these areas is phytotherapy, as it has several advantages over traditional therapy with using oral synthetic agents, namely, it is low-toxic, has a mild pharmacological effect and possibility to be used for long time without significant side effects [36, 37, 38, 39, 40], is well combined with other drugs, has a complex activity through a numerous of biologically active compounds [41, 42, 43, 44, 45, 46]. The combination of different medicinal plants deserves special attention because such herbal mixtures are expected to have more biologically active substances that influence on all links of the pathogenetic mechanism of DM type 2 and its complications development [47, 48, 49, 50, 51, 52, 53]. One of such combinations is an antidiabetic herbal mixture (*Equiseti arvensis herba, Sambuci flores, Inulae rhizomata et radices, Hyperici herba, Tiliae flores, Polygoni avicularis herba, Myrtilli folium, Urticae folia*) with previously studied antidiabetic activity *in vivo* [54].

Plant biocompounds have a wide range of pharmacological action and a variety influencing mechanisms on DM type 2 development and its angiopathies [55, 56, 57, 58].

Therefore, the aim of our study was to investigate an anti-inflammatory activity of antidiabetic mixture, which consists of *Equiseti arvensis herba, Sambuci flores, Inulae rhizomata et radices, Hyperici herba, Tiliae flores, Polygoni avicularis herba, Myrtilli folium, Urticae folia*.

Methods

Plant materials: The herbal raw materials harvested in June to August 2017 in Ternopil region and Charpathians (*Vaccinium myrtillus* leaf) (Ukraine) were used. After harvesting, the raw materials were dried, crushed and stored according to the general GACP requirements [59]. The plants were identified by Prof. S. M. Marchyshyn. The voucher specimens of herbal raw materials have been deposited in Herbarium of Pharmacognosy with Medical Botany Department, I. Horbachevsky Ternopil National Medical University, Ternopil, Ukraine.

Extraction procedure: The sample of the herbal raw material was grinded into a powder by laboratory mill. Then 10 g of powdered herbal mixture was put into a 100 mL conical flask and 120 mL of distilled water was added. The aqueous extract was obtained by heating in the boiling water bath for 30 min. The extract was filtered using Whatmann filter paper No. 1. Then the filtrate was evaporated by rotary evaporator and was lyophilized to dryness. The lyophilized powder of herbal mixture was stored at 4 °C for further use.

Experimental Protocol: The study was performed on male albino rats weighing between 180 g and 200 g, which were bred at the animal house of the Central Research Laboratory of I. Horbachevsky Ternopil National Medical University, where they

were kept under appropriate conditions (at a constant room temperature of 22 ± 1 °C, 40-70 % humidity conditions and a 12-hour light/dark cycle). Throughout the experimental period, the animals received standard rat diet and water *ad libitum.* The animals were treated in accordance with the internationally accepted standard ethical guidelines for laboratory animal use and care as described in the European Community Guidelines [60]. All protocols for animals' experiment were approved by the animal ethical committee of I. Horbachevsky Ternopil National Medical University.

Carrageenan-induced pleurisy in rats: According to the described method of Winter et al. [61], 30 rats equally were divided into five groups of six; they were fasted for 6 hours for experimentation. The initial volume (V_0) of the right hind paw was measured for each rat.

The rats were orally treated with diclofenac as standard anti-inflammatory drug (8 mg/kg) (reference group), water (control group) or aqueous extracts of antidiabetic mixture at doses of 9 mL/kg, 12 mL/kg and 25 mL/kg (experimental groups). After 1 hour of treatment, the inflammation was inducted in rats by a subplantar injection of 0.1 mL of carrageenan suspension (100 µg/paw). The paw volumes were determined hourly using a plethysmometer for 6 hours. The paw edema rate and inhibition rate of each group were calculated as follows:

Paw edema rate (%) = $V_t - V_0/V_0$

Inhibition rate (%) = $E_c - E_t/Ec$,

where Vt is the paw volume of the rat after carrageenan injection and V0 is the paw volume of the rat before carrageenin injection; Ec is the edema rate of the control group and Et is the edema rate of the treated group.

Statistical analysis: Statistical evaluation was carried out with StatView. Data were expressed as mean ± SEM. Statistical differences were evaluated by One-way ANOVA and the Student's *t*-test. *P* values <0.05 were considered statistically significant.

Results and Discussion

The presence of DM type 2 causes the development of several metabolic disorders in the body, primarily carbohydrate metabolism, as well as protein and lipid with subsequent changes in immune status. This forms the basis for frequent exacerbations of inflammatory processes [62, 63]. Therefore, it is necessary to study an anti-inflammatory activity of the presented herbal antidiabetic mixture.

Given that the initial stage of any inflammatory process is exudation, it was advisable to study the anti-inflammatory properties of herbal antidiabetic mixture in the model of acute exudative inflammation.

In the pathogenesis of acute inflammation caused by carrageenan, in the first 30-90 minutes involved biogenic amines - serotonin and histamine. In the interval between 1.5-2.5 hours - the system of kinins (bradykinin, fragments of complement), and between 2.5-5.5 hours - leukotrienes and prostaglandins. The role of free radicals in the development of acute inflammation caused by carrageenan has also been proven [64].

The anti-inflammatory activity of the studied objects was determined by their ability to reduce the development of foot edema in rats and was displayed as a percentage compared to the control group.

As shown by the results of the experiment (Table 1), during the 1st hour of the experiment, the most pronounced anti-inflammatory activity was found by diclofenac (60.7%). An aqueous extracts of antidiabetic mixture at doses of 12 mL/kg, 25 mL/kg showed only a tendency to anti-inflammatory action. At the 2nd hour, an aqueous extracts of antidiabetic mixture at doses of 12 mL/kg and 25 mL/kg and diclofenac contributed to a significant (p<0.05) reduction in paw edema in experimental animals. The highest activity was observed in diclofenac (69.6%).

An aqueous extracts of antidiabetic mixture at a dose of 9 mL/kg did not show significant anti-edema effect for 2-3 hours of the experiment. At the end of the 3rd hour, the studied extract of antidiabetic mixture at a dose of 25 mL/kg (25.1%) showed a significant (p<0.05) anti-edematous effect. After 6 hours, an aqueous extracts of antidiabetic mixture at doses of 12 mL/kg and 25 mL/kg showed high an anti-inflammatory effect (43.1% and 41.6%, respectively).

Thus, the maximum anti-inflammatory activity of aqueous extracts of antidiabetic mixture was shown

at a dose of 12 mL/kg and 25 mL/kg, at which it was inferior to diclofenac.

Conclusions

It was conducted the study of anti-inflammatory activity of the herbal antidiabetic mixture, which consists of *Equiseti arvensis herba, Sambuci flores, Inulae rhizomata et radices, Hyperici herba, Tiliae flores, Polygoni avicularis herba, Myrtilli folium, Urticae folia*, on male albino rats by a subplantar injection of 0.1 mL of carrageenan suspension (100 µg/paw).

Research has shown that an aqueous extracts of antidiabetic mixture at a dose of 9 mL/kg did not show significant anti-edema effect, but an aqueous extracts of antidiabetic mixture at doses of 12 mL/kg and 25 mL/kg showed significant ($p<0.05$) an anti-inflammatory effect, it was 43.1% and 41.6%, respectively. The highest activity was observed in diclofenac, as standard nonsteroidal anti-inflammatory drug, and it was 69.6%.

References

1. American Diabetes Association (2020). Standards of Medical Care in Diabetes. *Diabetes care*, *43*, 1212.
2. International Diabetes Federation (2019). *IDF Diabetes Atlas, 9th ed.* Brussels, Available at: https://www.diabetesatlas.org
3. Savych, A. O., & Marchyshyn, S. M. Phytochemical composition of the herbal mixtures, which causes their effectiveness in diabetes mellitus. *Challenges and achievements of medical science and education: Collective monograph*. Riga, Latvia: "Baltija Publishing", 276-290.
4. Savych, A., Marchyshyn, M., Basaraba, R., & Lukanyuk, M. (2020). Antihyperglycemic, hypolipidemic and antioxidant properties of the herbal mixtures in dexamethasone-induced insulin resistant rats. *PharmacologyOnLine*, 2, 73-82.
5. Savych, A., Marchyshyn, M., & Naconechna, S. (2021). Influence of some herbal mixtures on insulin resistance and glucose tolerance in rats. *PharmacologyOnLine, 1*, 356-364.
6. Ndjaboue, R., Farhat, I., Ferlatte, C. A., Ngueta, G., Guay, D., Delorme, S., Ivers, N., Shah, B. R., Straus, S., Yu, C., & Witteman, H. O. (2020). Predictive models of diabetes complications: protocol for a scoping review. *Systematic reviews*, *9*(1), 137.
7. Savych, A., & Mazur, O. (2021). Antioxidant activity *in vitro* of antidiabetic herbal mixtures. *PharmacologyOnLine,* 2, 17-24.
8. Feshchenko, H., Marchyshyn, S., Budniak, L., Slobodianiuk, L., & Basaraba, R. (2021). Study of antibacterial and antifungal properties of the lyophilized extract of fireweed (*Chamaenerion angustifolium* L.) herb. *Pharmacologyonline,* 2, 1464-1472.
9. Budniak, L., Slobodianiuk, L., Marchyshyn, S., & Parashchuk, E. (2021) Determination of carbohydrates in burnet saxifrage (*Pimpinella saxifraga* L.). *Pharmacologyonline,* 2, 1374-1382.
10. Dukhnich, N.Yu., Mishchenko, O.Ya., Larianovska, Yu.B. & Kalko, K.O. (2021). Effect of complex pharmaceutical composition at the histostructure of the pancreas under the conditions of experimental metabolic syndrome in rats. *PharmacologyOnLine*, 2, 1192-1202.
11. Savych, A., Marchyshyn, M., & Basaraba, R. (2020). Screening study of hypoglycemic activity of the herbal mixtures (Message 1). *ScienceRise: Pharmaceutical Science,* 4(26), 40-46.
12. Savych, A., Basaraba, R., & Gerush, O. (2021). Comparative analysis of hypoglycemic activity of herbal mixtures by glucose tolerance tests (message 2). *PharmacologyOnLine*, 2, 1118-1127.
13. Zotsenko, L., Kyslychenko, V., Kalko, K. & Drogovoz, S. (2021). The study of phenolic composition and acute toxicity, anti-inflammatory and analgesic effects of dry extracts of some elsholtzia genus (lamiaceae) species. *PharmacologyOnLine*, 2, 637-649.
14. Savych, A., Gerush, O., & Basaraba, R. (2021). Determinationof hypoglycemic activity of the herbal mixtures by means of glucose loading tests (message 3). *PharmacologyOnLine*, 2021, 2, 1128-1137.
15. Savych, A., & Sinichenko, A. (2021). Screening study of hypoglycemic activity of the herbal mixtures used in folk medicine (message 4). *PharmacologyOnLine*, 2, 1254-1262.
16. Budniak, L., Slobodianiuk, L., Marchyshyn, S., Basaraba, R., & Banadyga, A. (2021). The antibacterial and antifungal activities of the extract of *Gentiana cruciata* L. herb. *PharmacologyOnLine,* 2, 188-197.

17. Budniak, L., Slobodianiuk, L., Marchyshyn, S., & Klepach, P. (2021). Investigation of the influence of the thick extract of common centaury (*Centaurium erythraea* Rafn.) herb on the secretory function of the stomach. *Pharmacologyonline*, 2, 352-360.
18. Derymedvid, L.V., Horopashna, D.O., Kalko, K.O., Mishchenko, O.Ya., Okipniak, I.V., Komissarenko, A.M., Komisarenko, M.A., & Sevastianova, T.V. (2021). Anti-inflammatory properties of raspberry shoot extract. *PharmacologyOnLine*, 2, 657-662.
19. Savych, A., Basaraba, R., Muzyka, N., & Ilashchuk, P. (2021). Analysis of fatty acid composition content in the plant components of antidiabetic herbal mixture by GC-MS. *Pharmacia*, *68*(2), 433-439.
20. Budniak, L., Vasenda, M., & Slobodianiuk, L. (2021). Determination of flavonoids and hydroxycinnamic acids in tablets with thick extract of *Primula denticulata* SMITH. *PharmacologyOnLine*, 2, 1244-1253.
21. Budniak, L., Slobodianiuk, L., Darzuli, N., & Honcharuk, Ya. (2021). The antibacterial activity of the tablets with dry extract of round-leaved wintergreen leaves. *PharmacologyOnLine*, 2, 672-679.
22. Savych, A., Marchyshyn, S., & Basaraba, R. (2020). Determination of fatty acid composition content in the herbal antidiabetic collections. *Pharmacia*, *67*(3), 153–159.
23. Kalko, K.O., Derimedvid, L.V., Korang, L.A., Toziuk, O.Yu., Okipniak, I.V., Domar, N.A., & Zhurenko, D.S. (2021). Anti-inflammatory, membraneprotective and capillary strengthening properties of sweet flag leaf extracts. *PharmacologyOnLine*, 2, 145-157.
24. Drogovoz, S., Kalko, K., Borysiuk, I., Barus, M., Horoshko, V., Svyshch, O., & Liulchak, S. (2021). Potential risks and pharmacological safety features of hypoglycemic drugs. *PharmacologyOnLine*, 2, 1164-1171.
25. Savych, A., Marchyshyn, S., & Milian, I. (2021). Inhibition of pancreatic α-amylase by water extracts of some herbal mixtures. *PharmacologyOnLine*, 2, 1443-1449.
26. Savych, A., & Marchyshyn, S. (2021). Inhibition of pancreatic α- glucosidase by water extracts of some herbal mixtures. *PharmacologyOnLine*, 2, 1450-1456.
27. Savych, A., & Marchyshyn, S. (2021). Inhibition of pancreatic lipase by water extracts of some herbal mixtures. *PharmacologyOnLine*, 2, 1457-1463.
28. Oguntibeju O. O. (2019). Type 2 diabetes mellitus, oxidative stress and inflammation: examining the links. *International journal of physiology, pathophysiology and pharmacology*, 11(3), 45–63.
29. Savych, A., & Nakonechna, S. (2021). Determination of amino acids content in two herbal mixtures with antidiabetic activity by GC-MS. *Pharmakeftiki*, *33*(2), 116-123.
30. Savych, A., Duchenko, M., Shepeta, Y., Davidenko, A., & Polonets, O. (2021). Analysis of carbohydrates content in the plant components of antidiabetic herbal mixture by GC-MS. *Pharmacia*, *68*(4), 721–730.
31. Marchyshyn, S., Slobodianiuk, L., Budniak, L., & Ivasiuk, I. (2021). Hypoglycemic effect of *Cyperus esculentus* L. tubers extract. *Pharmacologyonline*, 2, 1383-1392.
32. Slobodianiuk, L., Budniak, L., Marchyshyn, S., Kostyshyn, L., & Ezhned, M. (2021). Determination of amino acids content of the *Tagetes lucida* Cav. by GC/MS. *Pharmacia*, *68*(4), 859-867.
33. Savych, A., & Basaraba, R. (2021). Ascorbic acid content in the herbal mixture with antidiabetic activity. *PharmacologyOnLine*, 2, 76-83.
34. Slobodianiuk, L., Budniak, L., Marchyshyn, S., & Demydiak, O. (2021). Investigation of the anti-inflammatory effect of the dry extract from the herb of *Stachys sieboldii* Miq. *Pharmacologyonline*, 2, 590-597.
35. Slobodianiuk, L., Budniak, L., Marchyshyn, S., Berdey, I., & Slobodianiuk, O. (2021). Study of the hypoglycemic effect of the extract from the tubers of *Stachys sieboldii* Miq. *Pharmacologyonline*, 2, 167-178.
36. Savych, A., Marchyshyn, S., Kyryliv, M., & Bekus, I. (2021). Cinnamic acid and its derivatives in the herbal mixtures and their antidiabetic activity. *Farmacia*, 69(3), 595-601.
37. Governa, P., Baini, G., Borgonetti, V., Cettolin, G., Giachetti, D., Magnano, A. R., et al. (2018). Phytotherapy in the management of diabetes: a review. *Molecules*, 23(1), 105.

38. Savych, A., Marchyshyn, S., Harnyk, M., Kudria, V., & Ocheretniuk, A. (2021). Determination of amino acids content in two samples of the plant mixtures by GC-MS. *Pharmacia*, *68*(1), 283-289.
39. Marchyshyn, S., Polonets, O., Savych, A., & Nakonechna, S. (2020). Determination of carbohydrates of *Chrysanthemum morifolium L.* leaves and flowers by GC-MS. *Pharmakeftiki*, *32*(4), 202-212.
40. Savych, A., & Milian, I. (2021). Total flavonoid content in the herbal mixture with antidiabetic activity. *PharmacologyOnLine*, 2, 68-75.
41. Budniak, L., Slobodianiuk, L., Marchyshyn, S., Klepach, P., & Honcharuk, Ya. (2021). Determination of carbohydrates content in *Gentiana cruciata* L. by GC/MS method. *International Journal of Applied Pharmaceutics*, *13*(1), 124–128.
42. Slobodianiuk, L., Budniak, L., Marchyshyn, S., & Basaraba, R. (2020). Investigation of the hepatoprotective effect of the common cat's foot herb dry extract. *Pharmacologyonline, 3,* 310–318.
43. Savych, A., Bilyk, O., Vaschuk, V., & Humeniuk, I. (2021). Analysis of inulin and fructans in *Taraxacum officinale* L. roots as the main inulin-containing component of antidiabetic herbal mixture. *Pharmacia*, *68*(3), 527-532.
44. Slobodianiuk, L., Budniak, L., Marchyshyn, S., Sinichenko, A., & Demydiak, O. (2021). Determination of amino acids of cultivated species of the genus *Primula* L. *Biointerface Research in Applied Chemistry, 11*, 8969–8977.
45. Darzuli, N., Budniak, L., & Slobodianiuk, L. (2021). Investigation of the antibacterial and antifungal activity of the *Pyrola rotundifolia* L. leaves dry extract. *PharmacologyOnLine, 1*, 395–403.
46. Savych, A., Marchyshyn, S., Kozyr, H., & Yarema, N. (2021). Determination of inulin in the herbal mixtures by GC-MS method. *Pharmacia*, *68*(1), 181-187.
47. Savych, A., Marchyshyn, S., & Milian, I. (2021). Determination of carbohydrates in the herbal antidiabetic mixtures by GC-MC. *Acta Pharmaceutica*, *71*(3), 429-443.
48. Savych, A., Marchyshyn, S., Basaraba, R., & Kryskiw, L. (2021). Determination of carboxylic acids content in the herbal mixtures by HPLC. *ScienceRise: Pharmaceutical Science*, 2(30), 33-39.
49. Marchyshyn, S., Budniak, L., Slobodianiuk, L., & Ivasiuk, I. (2021). Determination of carbohydrates and fructans content in *Cyperus esculentus* L. *Pharmacia, 68*(1), 211–216.
50. Budniak, L., Slobodianiuk, L., Marchyshyn, S., & Demydiak, O. (2020). Determination of *Arnica foliosa* Nutt. fatty acids content by gc/ms method. *ScienceRise: Pharmaceutical Science, 6*(28), 14–18.
51. Budniak, L., Slobodianiuk, L., Marchyshyn, S., Kostyshyn, L., & Horoshko, O. (2021). Determination of composition of fatty acids in *Saponaria officinalis* L. *ScienceRise: Pharmaceutical Science, 1*(29), 25-30.
52. Marchyshyn, S., Slobodianiuk, L., Budniak, L., & Skrynchuk, O. (2021). Analysis of carboxylic acids of *Crambe cordifolia* Steven. *Pharmacia, 68*(1), 15–21.
53. Savych, A., Marchyshyn, S., Mosula, L., Bilyk, O., Humeniuk, I., & Davidenko, A. (2021). Analysis of amino acids content in the plant components of the antidiabetic herbal mixture by GC-MS. *Pharmacia* [In press].
54. Savych, A., & Polonets, O. (2021). Study of hypoglycemic activity of antidiabetic herbal mixture on streptozotocin-nicotinamide-induced rat model of type 2 diabetes. *PharmacologyOnLine*, 2, 62-67.
55. Budniak, L., Slobodianiuk, L., Marchyshyn, S., Ilashchuk, P. (2021). Determination of polysaccharides in *Gentiana cruciata* L. herb. *Pharmacologyonline, 2,* 1473-1479.
56. Slobodianiuk, L., Budniak, L., Marchyshyn, S., Parashchuk, E., & Levytska, L. (2021). Experimental studies on expectorant effect of extract from *Pimpinella saxifraga* L. *PharmacologyOnLine, 1*, 404–410.
57. Wang, H., Jiang, M., Li, X., Zhao, Y., Shao, J., Liu, Z., Lin, L., Xu, Q., Wang, L., Lu, X., Zhang, H., Chen, Y., & Zhang, R. (2021). Anti-inflammatory Therapies for Coronary Heart Disease: A Systematic Review and Meta-Analysis. *Frontiers in cardiovascular medicine*, *8*, 726341.
58. Nunes, C., Barreto Arantes, M., Menezes de Faria Pereira, S., Leandro da Cruz, L., de Souza Passos, M., Pereira de Moraes, L., Vieira, I., & Barros de Oliveira, D. (2020). Plants as Sources of Anti-Inflammatory Agents. *Molecules (Basel, Switzerland)*, 25(16), 3726.
59. WHO Guidelines on good agricultural and mixture practices (GACP) for medicinal plants (2003). *World Health Organization*, Geneva, Switzerland, 72.

60. EEC. "Council directive 2010/63/EU, of the 22nd September 2010 on the approximation of laws, regulations and administrative provisions of the member states regarding the protection of animals used for experimental and other scientific purposes". Offical Journal of the European Communities, 2010:1–29.
61. Winter, C. A., Risley, E. A., Nuss, G. W. (1962). Carrageenin-induced edema in hind paw of the rat as an assay for antiiflammatory drugs. *Proc Soc Exp Biol Med*, *111*, 544-547.
62. Ebtehaj, S., Gruppen, E. G., Parvizi, M., Tietge, U., & Dullaart, R. (2017). The anti-inflammatory function of HDL is impaired in type 2 diabetes: role of hyperglycemia, paraoxonase-1 and low grade inflammation. *Cardiovascular diabetology*, *16*(1), 132.
63. Kuryłowicz, A., & Koźniewski, K. (2020). Anti-Inflammatory Strategies Targeting Metaflammation in Type 2 Diabetes. *Molecules (Basel, Switzerland)*, *25*(9), 2224.
64. Rayego-Mateos, S., Morgado-Pascual, J. L., Opazo-Ríos, L., Guerrero-Hue, M., García-Caballero, C., Vázquez-Carballo, C., Mas, S., Sanz, A. B., Herencia, C., Mezzano, S., Gómez-Guerrero, C., Moreno, J. A., & Egido, J. (2020). Pathogenic Pathways and Therapeutic Approaches Targeting Inflammation in Diabetic Nephropathy. *International journal of molecular sciences*, *21*(11), 3798.

Table 1. Anti-inflammatory activity of an aqueous extracts of antidiabetic mixture and standard drug diclofenac

Group of animals		Vo	Vt			
			1 hour	2 hours	3 hours	6 hours
Control group	ΔV	46.6±1.2	57.8±1.2	61.4±1.3	67.3±1.5	82.6±1.8
Aqueous extract of antidiabetic mixture, 9 mL/kg	ΔV	45.3±1.1	56.0±2.7*	62.0±2.2	66.2±1.3	68.1±1.9*
	E, %		4.5	12.8	1.0	36.7
Aqueous extract of antidiabetic mixture, 12 mL/kg	ΔV	46.2±1.3	55.1±1.7*	57.2±3.1*	64.8±2.6	66.7±2.0*
	E, %		20.5	25.7	10.1	43.1
Aqueous extract of antidiabetic mixture, 25 mL/kg	ΔV	45.4±1.4	55.2±2.2*	57.1±2.0*	60.9±2.1*	66.4±1.4*
	E, %		12.5	21.0	25.1	41.6
Diclofenac, 8 mg/kg	ΔV	45.8±1.4	50.2±2.8*	50.0±4.1*	57.9±4.3*	60.7±2.9*
	E, %		60.7	69.6	41.5	58.6

Values are expressed as mean ± SEM from 6 rats; *$p<0.05$ with respect to Control group.