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Anti-diabetic activity of different oils through their effect on arylsulfatases

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Abstract

Background: Diabetes mellitus (DM) is characterized by the overproduction of the reactive oxygen species which affects the integrity of the lysosomal membrane affecting lysosomal enzymes. The effect of these species is blocked by some natural products as antioxidants. In the current study, groups of normal and streptozotocin (STZ)-induced diabetic rats were treated by Nigella sativa (NS), olive and canola oils and subjected to the study of arylsulfatases as a model of lysosomal enzymes. The aim of the present study is to investigate the effect of STZ-induced diabetes on arylsulfatases in presence and absence of NS, olive and canola oils.

Methods: Different groups of rats were induced by STZ, treated with different oils and compared to their corresponding control group. All groups were subjected for the assays of blood glucose, insulin, catalase and arylsulfatases. A comparative kinetic study of arylsulfatses was performed to detect the alteration of catalytic characterization.

Results: The results demonstrated that diabetes causes a significant elevation in the level of hepatic arylsulfatase B and a significant reduction of hepatic catalase as an antioxidant enzyme. NS and olive oils returned catalase and arylsulfatase B activities back near to normal by fixing their catalytic properties. Furthermore, the maximum velocity of arylsulfatases A and B was significantly elevated in the induced diabetes, whereas their K_m values were significantly changed. The treatment of diabetic rats by NS and olive oils reduced the degree of significance.

Conclusion: Diabetes induces significant alterations of the catalytic characters of arylsulfatases and some oils decrease this alteration through an antioxidant-mediated effect.

Keywords: Diabetes, Arylsulfatases, Kinetic parameters, Catalase, Oxidative stress

Background

DM is a chronic metabolic disorder that presents a major worldwide health problem. DM is characterized by complete or partial deficiencies in insulin production and/or insulin action coupled with chronic hyperglycemia and disruption in metabolism [1]. In DM, oxidative stress represents an imbalance between the production of free radicals and the ability of the antioxidant defense mechanisms to detoxify them [2,3]. On the other hand, STZ is an antibiotic produced by Streptomycetes achromogenes and is used to stimulate insulin-dependent DM [4].

ASA (EC 3.1.6.8) and ASB (EC 3.1.6.9) are lysosomal enzymes, which are involved in the metabolism of glycoconjugates. ASA is known as cerebroside-3-sulfohydrolase

and it desulfates the galactose-3-sulfate residues in cerebroside sulfate and other sulfated galactolipids [5]. An increase in the enzyme concentration was found in the body fluids of patients with cancer of various organs [6]. ASB desulfates the non-reducing terminal N-acetylgalactosamine-4-sulfate residues that are present in glycosaminoglycan [7]. ASB activity is increased in different tumors tumors [5,8-11]. DM is characterized by the overproduction of ROS which affects the lysosomal membrane integrity [12]. ROS were found to decline on increasing the antioxidant enzymes [13] and antioxidant activity of some natural products [14]. It was found that antioxidant therapy can be subdivided into vitamins and supplements, plants and their active ingredients and drugs with antioxidant potential [14-18]. Also, it was found a diverse effect of different oils such as Nigela sativa [19,20] and olive [21-25] oils in DM. It was reported that both NS and olive oils have antidiabetic and antioxidant

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activities [19,21,26,27]. Taken together, the present research is conducted to investigate the effect of STZ-induced diabetes on arylsulfatases in presence and absence of NS, olive and canola oils. The enzymatic activity of arylsulfatases is assayed in both liver and serum in all groups and the catalytic parameters of ASA and ASB were determined to detect any induced catalytic change of these enzymes in diabetes.

Materials and methods

Experimental animals

Sixty four male albino Sprague–Dawley rats of 4–6 weeks old with an approximate body mass of 200 g were obtained from the animal house of the Faculty of Science, Beirut Arab University. Animals were housed in a poly carbonate cages (8 animals/cage) under standardized laboratory conditions with a twelve hour day –night cycle, temperature of 25°C, and humidity of 45% to 60%. Animals had access to tap water and to standard food diet ad libitum. The animal protocols were approved by the regional ethical committee (BAU Ethics Committee).

Chemicals and oils

STZ, DE-52, insulin ELISA kit and *p*-NCS salt were purchased from Sigma-Aldrich (Beirut office Lebanon). *p*-NCS was prepared as a substrate reagent for ASA ASB as previously explained [28]. CAT assay kit was purchased from Biodiagnostic, Diagnostic and Research Reagents, Giza, Egypt.

Rats treatment

Eight groups of male rats were prepared, each includes 8 rats. Four of these groups were treated with STZ to induce diabetes in them at a dosage of 50 mg/kg as previously described [4]. Some groups were treated by NS or olive or canola oils. The different groups were classified as non-diabetic rats (control), STZ-induced diabetic rats (GI), control treated with NS oil (GII), diabetic treated with NS oil (GIII), control treated with Olive oil (GIV), diabetic treated with Olive oil (GV), control treated with canola oil (GVI) and diabetic treated with canola oil (GVII). A dose of 0.2 ml/kg/day of NS, olive and canola oils were given daily by i.p injection for 4 weeks.

Blood Glucose assay

The blood glucose level was measured 3 days and 4 weeks of STZ-PI and oil treatment, respectively. Glucose levels in all groups were measured by means of a glucometer by using accu-check strip and the detected color reflects the glucose concentration in mg/dl [29].

Insulin assay

Insulin is assayed in the pancreatic homogenate of animals of all groups by Sigma-Aldrich insulin ELISA kit.

Sampling

Oils treatment occurred within 4 weeks, after which the rats were sacrificed, blood samples collected by cardiac puncture and centrifuged within heparinized tubes at 3000 r.p.m for 20 min to obtain serum samples. Both serum and liver were collected and stored at −80°C until use. The removed hepatic tissues are homogenized in 9 volumes of ice-cold 10 mM Tris–HCl buffer, pH 7.4 containing Triton X-100 and 0.25 M sucrose using a Brinkman homogenizer. The homogenate was centrifuged at $500 \times g$ and 4°C for 20 min [30]. The obtained supernatant was subjected to enzymes assay.

Enzyme assay

CAT activity was assayed as previously described [31]. One unit CAT is defined as that decomposes 1.0 μM of H_2O_2/min at 25°C and pH 7. For arylsulfatases, one unit of enzyme activity is defined as one nmol of *p*-nitrocatechol liberated per an hour under these assay conditions [30]. The incubation mixture was incubated at 37°C and the reaction, stopped after 30 min by adding 1 M NaOH and measured for the absorbance at 515 nm.

Determination of kinetic parameters

The liver homogenate was collected from Control, GI, GIII and GV and then subjected for purification for the kinetic studies. ASA and ASB were purified as previously described [30]. Time courses of the enzymes were performed to determine the values of initial velocities at different substrate concentration ranges (2.0 – 10.0 mM and 10–50 mM for ASA and ASB, respectively) at the above mentioned assay conditions. The values of K_m and V_{max} of ASA and ASB were determined from Lineweaver-Burk plots.

Protein assay

The protein is assayed as described previously [32] using bovine serum albumin as a standard.

Statistical analyses

All the data are represented as mean ± S.E. The statistical analysis was carried out using the student's *t*-test (SPSS version 16). All experiments were run on three occasions for reproducibility, and all assays were done in triplicate. Values were considered statistically significant when $p < 0.05$.

Results

STZ injection induces diabetes leading to an increase in BGL more than 120 mg/dl. BGLs were measured in the different groups 3 days PI of STZ/oil treatment and 4 weeks PI of STZ/oil treatment (Figure 1), respectively. All data were compared to the control group as a reference group. The control group showed a BGL range of
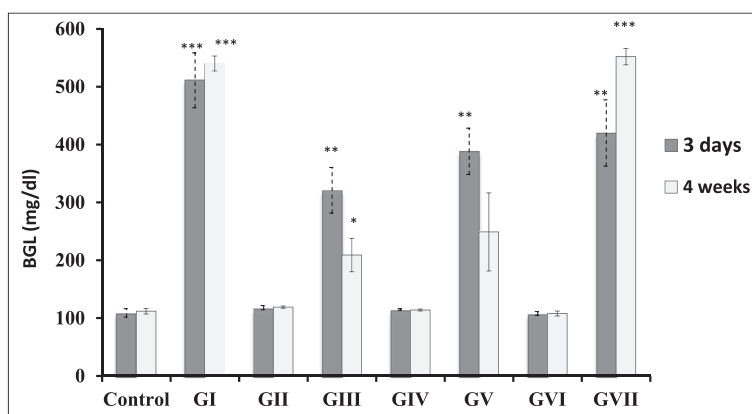
Figure 1 Blood glucose levels in the different groups 3 days and 4 weeks PI of STZ and oil treatment. Significant difference for all groups is compared to the control represented by *p < 0.05, **p < 0.01, ***p < 0.001 and non-significant at p > 0.05.

109 ± 7 - 112 ± 5 mg/dl. BGL in GI group showed 4.7 and 4.8 fold - increases, respectively with a significant increase (p < 0.001) compared to the control. BGL in GII, GIV and GVI groups showed a non-significant change (p > 0.05) compared to the control. BGL in GIII, GV and GVII groups showed a significant increase compared to the control (p < 0.05). Insulin level was measured in the pancreatic tissues to assess the effect of the oil treatment on damaged pancreatic tissues in STZ-treated rats. The diabetic group (GI) showed a significant decrease (4.0 fold) in insulin compared to control (p < 0.001). Insulin level showed a non-significant change in GII, GIV and GVI groups (p > 0.05) and a significant increase in GIII, GV and GVII groups (p < 0.05) compared to the control.

As shown in Figure 2, the specific activity of CAT in the eight groups is illustrated for the liver tissue and serum, respectively. The enzyme in control group showed specific activities of $(5.1 \pm 0.04) \times 10^{-3}$ and $(1.652 \pm 0.07) \times 10^{-3}$ U/mg, respectively. The enzyme in GI showed a value of $(2.1 \pm 0.06) \times 10^{-3}$ and $(0.31 \pm 0.03) \times 10^{-3}$ U/mg, respectively with a significant decrease (p < 0.01) compared to control. The enzyme in GII, GV and GVI groups displayed a non-significant change (p > 0.05) compared to control. In addition, the enzyme in GIII showed a value of $(4.6 \pm 0.121) \times 10^{-3}$ U/mg with a significant decrease (p = 0.023) and $(1.037 \pm 0.16) \times 10^{-3}$ U/mg and with a non-significant change (p = 0.119), respectively compared to the control. The enzyme In GIV displayed a significant decrease (p = 0.037) and a non-significant change (p = 0.191), respectively compared to control. The enzyme in GVII group displayed a significant decrease (p < 0.05) compared to control.

The results in Figure 3a represent the hepatic ASA and ASB specific activity in the eight groups after oil treatment. The control group showed an ASA and ASB specific activity of 85.25 ± 5.14 and 194 ± 11.57 U/mg for ASA and ASB, respectively. ASA and ASB in GI group

showed values of 1.5 (non-significant) and 1.6 fold-increase (significant), respectively compared to control, whereas the values in GII group were non-significantly changed compared to the control. The enzymes in GIII, GV and GVI groups showed a non-significant change of compared to the control, whereas the values in GIV group were non-significantly decreased compared to control. The enzymes in GVII showed a significant increase compared to control.

For serum ASA and ASB, the control group showed an ASA and ASB specific activity of 4.5 ± 0.86 and 10.76 ± 1.975 U/mg, respectively. The enzymes in GI and GVI groups displayed a non-significant change compared to control. ASA and ASB in GII group displayed a significant increase and a non-significant change, respectively with respect to control. These enzymes in GIII group displayed a non-significant change and a significant increase, respectively compared to control. In GIV, GV and GVII groups, the enzymes displayed a significant increase and a non-significant change, respectively compared to control (Figure 3b).

The initial velocity versus substrate concentrations for ASA and ASB from the investigated group showed a hyperbolic behavior and the initial rates of these enzymes increase on elevating the substrate concentrations (Figure 4 a & b). The values of the kinetic constants V_{max} and K_m of arylsulfatases increased after the induction of diabetes. These values are summarized in Table 1 for the hepatic enzymes from control and treated rats. The purified ASA from the control group shows a V_{max} value of 22.8 ± 1.03 nmol/min/mg protein and a K_m value of 1.68 ± 0.011 mM. The enzyme from GI group shows a significant increase of V_{max} value and a significant decrease of K_m value compared to control. The enzyme from GIII and GV groups shows a non-significant increase of V_{max} and K_m values compared to control. The purified ASB from the control group shows a V_{max}
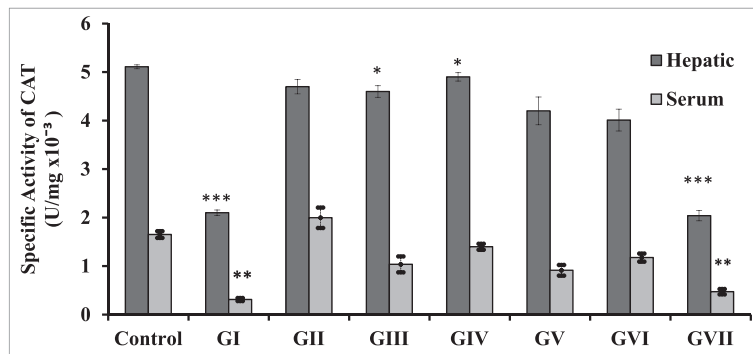
Figure 2 Specific activity of hepatic and serum CAT (U/mg) 4 weeks PI of STZ and oil treatment. Significant difference for all groups is compared to the control represented by *p < 0.05, **p < 0.01, ***p < 0.001 and non-significant at p > 0.05.

value of 33.07 ± 0.33 nmol/min/mg protein and a K_m value of 11.373 ± 0.089 mM. The enzyme from GI, GIII and GV groups shows significance increase ($p < 0.05$) of V_{max} and K_m values compared to control.

Discussion

A significant elevation in BGL in the diabetic rats compared to control was reported [33]. The BGL in GV group showed a decrease that is still non-significantly increased compared to that of control group. This implies

that some oils were useful in improving BGL in DM. Similar studies showed a significant decrease in BGL after olive [21] and NS oils treatment [33] in diabetic rats. The decrease in BGL in NS oil-treated diabetic rats may be attributed to a decrease of gluconeogenesis in liver [34,35]. It is well known that prolonged hyperglycemia favors the permanence of excessive hepatic glucose output due to an increase of liver gluconeogenesis in DM caused by over expression of glucose-6-phosphatase enzyme [36]. The canola oil treatment was
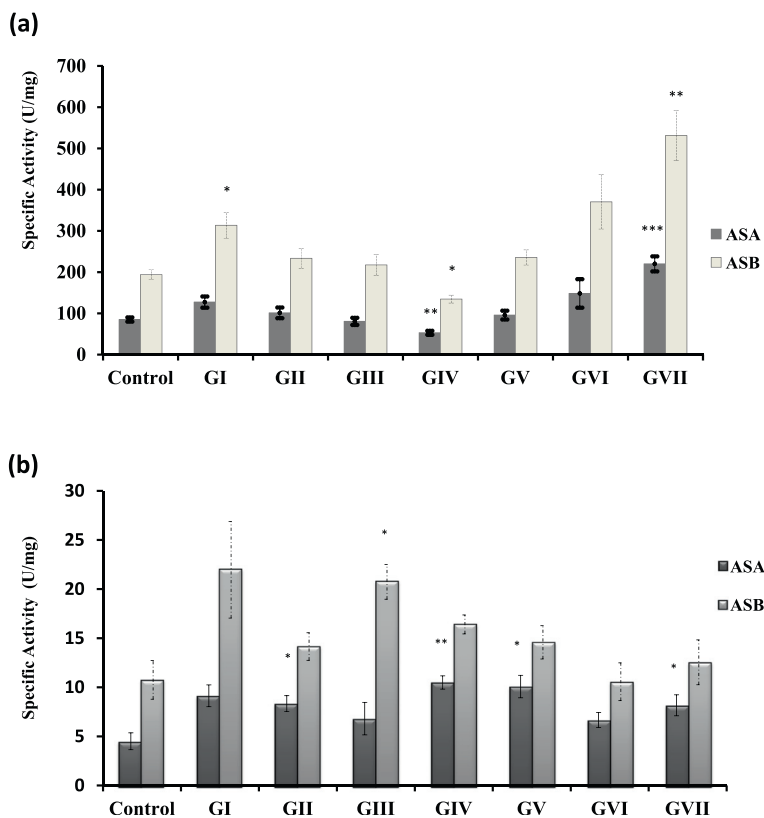

Figure 3 Specific activities of hepatic (a) and serum (b) ASA & ASB in all groups 4 weeks PI of STZ and oil treatment. Significant difference for all groups is compared to the control represented by *p < 0.05, **p < 0.01, ***p < 0.001 and non-significant at p > 0.05.
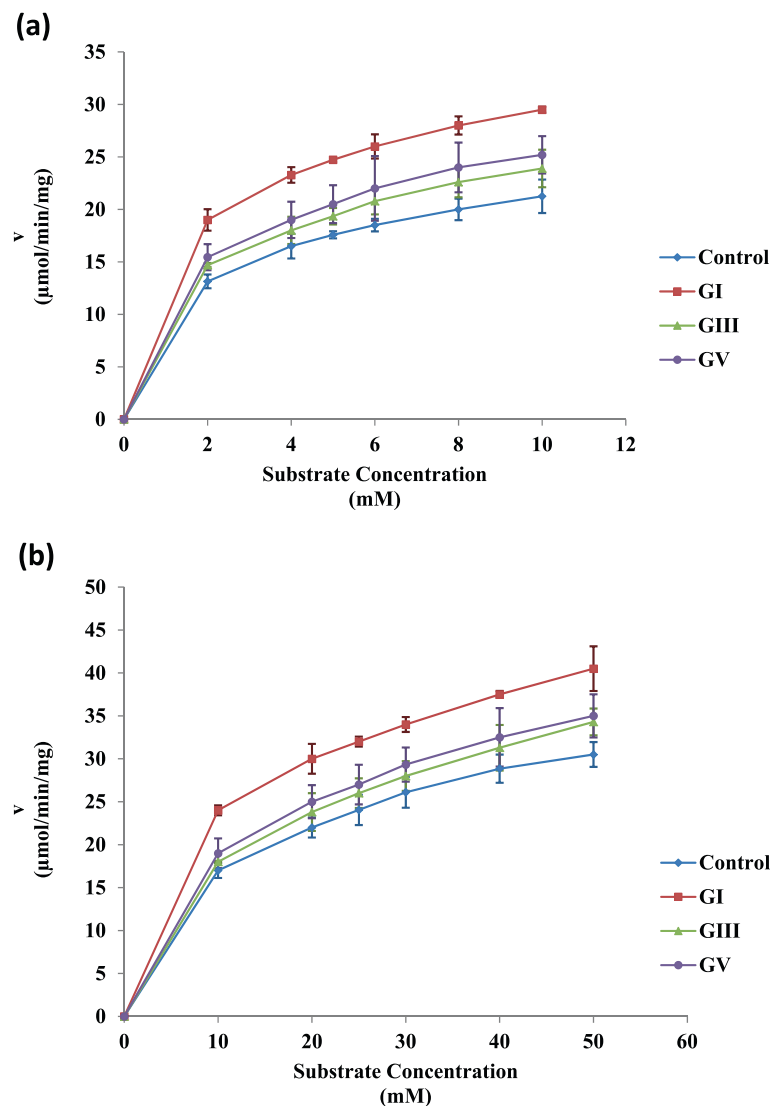
Figure 4 Effect of different substrate concentrations on the initial velocities of hepatic ASA (a) and ASB (b) from the control, GI, GIII and GV groups.

deleterious in which a significant increase in BGL of GVII group occurs compared to the control group despite its health benefits listed previously [37,38]. It was found that canola oil-rich diets cause insulin resistance thus favoring type 2 DM and omega-6 fatty acid which is one of the important content of canola was confirmed to mediate type 2 DM [39,40].

The results of the current study showed that hepatic and serum CAT activity in oil-untreated diabetic rats decreases significantly compared to the control group.

Table 1 Kinetic parameters of ASA and ASB from control, GI, GIII and GV where the whole parameters are compared to control and GI groups

Enzymes	Km				Vmax			
	Control	GI	GIII	GV	Control	GI	GIII	GV
ASA	1.68 ± 0.01	1.48 ± 0.008 b	1.72 ± 0.015 y	1.768 ± 0.01 y	22.8 ± 1.03	29.5 ± 0.29 a	25.66 ± 0.88	27.3 ± 1.06
ASB	11.37 ± 0.089	8.5 ± 0.28 b	13.1 ± 0.208 b,z	12.37 ± 0.186 a,z	33.33 ± 0.33	45.15 ± 0.15 c	39.66 ± 0.66 a,x	39.75 ± 0.75 a,x

Superscript letters represented by a, b & c indicate a significant variation compared to control group and x, y & z compared to diabetic group at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively. No superscript letter is an indication for non-significant variation at $p > 0.05$. Vmax is expressed in nmol/min/mg and Km is expressed in mM.

CAT activity in hepatic tissue of GIII group has decreased significantly compared to the control group, while that of GV showed a non-significant decrease compared to the control group. CAT activity in sera for each of GIII and GV was non-significantly decreased compared to the control group but their activities were greater than that of GI group. This is an evidence that both oils improve the antioxidant defense mechanisms. In an agreement of these results, it was found that NS seed extract has antioxidant activity due to the flavonoids which quenchers O_2^- [41]. Also, NS seed extract-treated diabetic rats showed a significant increase in the hepatic and renal CAT activity [41]. Moreover, it was confirmed that NS oil might play a role against the enzymatic alterations in the liver caused by *Schistosoma mansoni* infection by improving the host immunity due to the NS oil antioxidant activity [27]. However, it was found that the olive oil hydrophilic fraction has an antioxidant potential and a significant increase in the hepatic CAT activity in rats subjected to herbicide treatment [42]. Another study also suggests that the olive oil phenolic compounds have an antioxidant activity [26]. Therefore, we suggest that the effect of NS and olive oils in the current study may be attributed to their antioxidant action. On the other hand, canola oil was not able to correct the oxidative stress produced in DM. A reported study that agrees to this finding reveals that the canola oil decreases the antioxidant status in hypertensive rats [43].

It was previously reported that STZ-induced diabetes has an effect on the cellular glycosaminoglycan (GAG) content and STZ-induced diabetes alters the synthesis and catabolism of GAG [44]. Also, it was found that the sulfation of heparan sulfate is reduced in the liver of STZ-induced diabetic rats [45]. It was reported that sulfatides are required for normal insulin secretion [46] and the promotion of proinsulin folding [47]. Moreover, it was proven that sulfatides reduce the incidence of diabetes in non-obese diabetic mice compared to control [48]. These findings support the data of ASA and ASB in the present study. Accordingly, the elevation of both hepatic ASA and ASB may be attributed to the alteration of the modification of GAG sulfation and sulfatides content, respectively.

In fact, lysosomal membrane permeability occurs in cell death process which also favors the amplification of the death signaling. One of the essential factors that contribute in the lysosomal membrane permeability is ROS formation. In response to the increased ROS, increased amounts of H_2O_2 can penetrate into lysosome. The acidic environment of the lysosomal lumen enhances the reduction of iron and the generation of $OH^{\bullet}$ that stimulates the peroxidation of membrane lipids leading to the outflow of lysosomal constituents into cytosol [49]. Therefore, it could be suggested that the elevation in the hepatic ASA and ASB activities may be related to lysosomal membrane permeabilization mediated by oxidative stress, which is one of the predominating features in DM.

The determination of the V_{max} and K_m for each of ASA and ASB allows the demonstration of the catalytic alterations in these enzymes that occur under the pathological circumstances. Since the kinetic behavior of both ASA and ASB is hyperbolic in Figure 4, these enzymes have the normal kinetic behavior and do not display the allosteric characterization [50]. The K_m values for ASA and ASB from the diabetic group showed a significant decrease in comparison to that of the control group suggesting that there is a change in the substrate affinity for the enzyme [50-52]. It was found previously that a slight change in the ASA affinity results from *Schistosomiasis* and this paralleled the non-significant change of the activity [12]. Accordingly, the obtained data suggests that the significant changes in the activity that occur in diabetes are related to the alterations in the catalytic site and binding affinity for both ASA and ASB. It was previously reported that ASA and ASB are modified by sialylation and phosphorylation in some tumors [53-55] and ASB activity was changed due to its phosphorylated by cAMP-dependent protein kinase in lung cancer [9,10]. Accordingly, we suggest a regulatory mechanism may be involved in diabetes and may lead to a changed behavior of arylsulfatases.

Conclusion

The present work demonstrates that both NS and olive oils are capable of improving the diabetic complications at the enzymatic level. Their mechanistic action was suggested to be attributed to an antioxidant activity. Conversely, canola oil showed an adverse effect in relation to the diabetic complications and enzymatic alterations.

Authors' contributions
Author MB designed the study, wrote the protocol and supervised the work. Author RS carried out all laboratories work and performed the statistical analysis. Author MB managed the analyses of the study. Author MB wrote the first draft of the manuscript. Author MB managed the literature searches and edited the manuscript. Both authors read and approved the final manuscript.

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References
1. Duckworth WC: Hyperglycemia and cardiovascular disease. *Curr Atheroscler* 2001, **3**:383–391.
2. Cross CE, Halliwell B, Borish ET: Oxygen radicals and human disease. *Ann Intern Med* 1987, **107**:526–545.
3. Low PA, Nickander KK, Tritschler HJ: The roles of oxidative stress and antioxidant treatment in experimental diabetic neuropathy. *Diabetes* 1997, **46**:S38–S42.
4. Ganda OP, Rossi AA, Like AA: Studies on streptozotocin diabetes. *Diabetes* 1976, **25**:595–603.
5. Boyland E, Wallaee DM, Williams DC: The activity enzymes sulfatases and β-glucuronidase in the urine, serum and bladder. *Br J Cancer* 1955, **9**:62–79.
6. Laidler P, Kowalski D, Silberring J: Arylsulfatase A in serum from patients with cancer of various organs. *Clin Chim Acta* 1991, **204**:69–78.
7. Rockfor K, Gary M, John E: Purification, molecular weight, amino acid and subunit composition of arysulfatase A from human liver. *Arch Biochem Biophys* 1976, **177**:525–538.
8. Morgan LR, Somueel MS, Thomas W, Krementy ET, Meeker W: Arylsulfatase B in colorectal cancer. *Cancer* 1975, **36**:2337–2345.
9. Gasa S, Balbaa M, Nakamura M, Yonemori H, Makita A: Phosphorylation of human arylsulfatase B by cAMP-dependent protein kinase: different sites of phosphorylation between normal and cancer tissues. *J Biol Chem* 1987, **262**:1230–1238.
10. Balbaa M, Gasa S, Makita A: Alteration of protein kinase isozymes in transplantable human lung cancer with special reference to the phosphorylation of arylsulfatase B. *Biochem Biophys Res Commun* 1988, **150**:163–169.
11. Dzialoszynski LM, Gniot-Szulzycka J: The clinical aspects of arylsulphatase activity. *Clin Chim Acta* 1967, **15**:381–386.
12. Johansson AC, Appelqvist H, Nilsson C, Kagedal K, Roberg K, O¨llinger K: Regulation of apoptosis-associated lysosomal membrane permeabilization. *Apoptosis* 2010, **15**:527–540.
13. Nishikawa T, Edelstein D, Du XL, Yamagishi S, Matsumura T, Kaneda Y, Yorek MA, Beebe D, Oates PJ, Hammes HP, Giardino I, Brownlee M: Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. *Nature* 2000, **404**:787–790.
14. Chanwitheesuk A, Teerawutgulrag A, Rakariyatham N: Screening of antioxidant activity and antioxidant compounds of some edible plants of Thailand. *Food Chem* 2005, **92**:491–497.
15. Penckofer S, Schwertz D, Florczak K: Oxidative stress and cardiovascular disease in type 2 diabetes: the role of antioxidants and prooxidants. *J Cardiovasc Nurs* 2002, **16**:68–85.
16. Naziroglu M, Butterworth P: Protective effects of moderate exercise with dietary vitamin C and E on blood antioxidative defense mechanism in rats with streptozotocin-induced diabetes. *Can J Appl Physiol* 2005, **30**:172–185.
17. Larkins N, Wynn S: Pharmacognosy: phytomedicines and their mechanisms. *Vet Clin North Am Small Anim Pract* 2004, **34**:291–327.
18. Amer M, El-Habibi ES, El-Gendy A: Effects of Trifolium alexandrium extracts on streptozotocin-induced diabetes in male rats. *Ann Nutr Metab* 2004, **48**:343–347.
19. Zaoui A, Cherrah Y, Alaoui K, Mahassine N, Amarouch H, Hassar M: Effects of Nigella sativa fixed oil on blood homeostasis in rat. *J Ethnopharmacol* 2002, **79**:23–26.
20. Houghton PJ, Zarka R, de Las HB, Hoult JRS: Fixed oil of Nigella sativa and derived thymoquinone inhibit eicosanoid generation in leukocytes and membrane lipid peroxidation. *Planta Med* 1995, **61**:33–36.
21. Al Jamal AR, Ibrahim A: Effects of olive oil on lipid profiles and blood glucose in type2 diabetic patients. *Int J Diabetes Metab* 2011, **19**:19–22.
22. Gorinstein S, Leontowicz H, Lojek A, Leontowicz M, Ciz M, Krzeminski R, Gralak M, Czerwinski J, Jastrzebski Z, Trakhtenberg S, Grigelmo-Miguel N, Soliva-Fortuny R, Martin-Belloso O: Olive oils improve lipid metabolism and increase antioxidant potential in rats fed diets containing cholesterol. *J Agric Food Chem* 2002, **5**:6102–6108.
23. Mangas-Cruz MA, Fernández-Moyano A, Albi T, Guinda A, Relimpio F, Lanzón A, Pereira JL, Serrera JL, Montilla C, Astorga R, García-Luna PP: Effects of minor constituents (non-glyceride compounds) of virgin olive oil on plasma lipid concentrations in male Wistar rats. *Clin Nutr* 2001, **2**:211–215.
24. Franconi F, Coinu R, Carta S, Urgeghe PP, Ieri F, Mulinacci N, Romani A: Antioxidant effect of two virgin olive oils depends on the concentration and composition of minor polar compounds. *J Agric Food Chem* 2006, **54**:3121–3125.
25. Fabiani R, Rosignoli P, De Bartolomeo A, Fuccelli R, Servili M, Montedoro GF, Morozzi G: Oxidative DNA damage is prevented by extracts of olive oil, hydroxytyrosol, and other olive phenolic compounds in human blood mononuclear cells and HL60 cells. *J Nutr* 2008, **138**:1411–1416.
26. Cicerale S, Conlan XA, Sinclair AJ, Keast RSJ: Chemistry and health of olive oil phenolics. *Crit Rev Food Sci Nutr* 2009, **49**:218–236.
27. Mahmoud MR, El-Abhar HS, Saleh S: The effect of Nigella sativa oil against the liver damage induced by Schistosoma mansoni infection in mice. *J Ethnopharmacol* 2002, **79**:1–11.
28. Baum H, Dodgson KS, Spencer B: The assay of arylsulphatases A and B in human urine. *Clin Chim Acta* 1959, **4**:453–455.
29. Hönes J, Müller P, Surridge N: The technology behind glucose meters: test strips. *Diabetes Tech Therapeut* 2008, **10**:S10–S26.
30. Balbaa M, El-Kersh M, Mansour H, Yacout G, Ismail M, Malky A, Bassiouny K, Abdel-Monem N, Kandeel K: Activity of some hepatic enzymes in schistosomiasis and concomitant alteration of arylsulfatase B. *J Biochem Mol Biol* 2004, **37**:223–228.
31. Aebi H: Catalase in vitro. *Methods Enzymol* 1984, **105**:121–126.
32. Doumas BT, Bayse DD, Carter R, Peters T Jr, Schaffer R: A candidate reference method for determination of total protein in serum. I. Development and validation. *Clin Chem* 1981, **27**:1642–1650.
33. Abdelmeguid NE, Fakhoury R, Kamal SM, Al Wafai RJ: Effects of Nigella sativa and thymoquinone on biochemical and subcellular changes in pancreatic b-cells of streptozotocin-induced diabetic rats. *J Diabetes* 2010, **2**:256–266.
34. Al-Awadi F, Fatania H, Shamte U: The effect of a plants mixture extract on liver gluconeogenesis in streptozotocin induced diabetic rats. *Diabetes Res* 1991, **18**:163–168.
35. Fararh KM, Shimizu Y, Shiina T, Nikami H, Ghanem MM, Takewaki T: Thymoquinone reduces hepatic glucose production in diabetic hamsters. *Res Vet Sci* 2005, **79**:219–223.
36. Massillon D, Barzilai N, Chen W, Hu M, Rossetti L: Glucose regulates in vivo glucose-6-phosphatase gene expression in the liver of diabetic rats. *J Biol Chem* 1996, **271**:9871–9874.
37. Wakamatsu D, Morimura S, Sawa T, Kida K, Nakai C, Maeda H: Isolation, identification, and structure of a potent alkyl-peroxyl radical scavenger in crude canola oil, canolol. *Biosci Biotech Biochem* 2005, **69**:1568–1574.
38. Bucher HC, Hengstler P, Schindler C, Meier G: N-3 polyunsaturated fatty acids in coronary heart disease: a meta-analysis of randomized controlled trials. *Am J Med* 2002, **112**:298–304.
39. Heredia FP, Larque E, Portillo MPP, Canteras M, Zamora S, Garaulet M: Age-related changes in fatty acids from different adipose depots in rat and their association with adiposity and insulin. *Nutrition* 2008, **24**:1013–1022.
40. Costa CA, Carlos AS, dos Santos Ade S, Monteiro AM, Moura EG, Nascimento-Saba CC: Abdominal adiposity, insulin and bone quality in young male rats fed a high-fat diet containing soybean or canola oil. *Clinics (Sao Paulo)* 2011, **66**:1811–1816.
41. Kaleem M, Kirmani D, Asif M, Ahmed Q, Bano B: Biochemical effects of *Nigella sativa* L seeds in diabetic rats. *Indian J Exp Biol* 2006, **44**:745–748.
42. Nakbi A, Tayeb W, Grissa A, Issaoui M, Dabbou S, Chargui I, Ellouz M, Miled A, Hammami M: Effects of olive oil and its fractions on oxidative stress and the liver's fatty acid composition in 2,4-Dichlorophenoxyacetic acid-treated rats. *Nutr Metab (Lond)* 2010, **7**:80. doi: 10.1186/1743-7075-7-80.
43. Papazzo A, Conlan X, Lexis L, Lewandowski P: The effect of short-term canola oil ingestion on oxidative stress in the vasculature of stroke-prone spontaneously hypertensive rats. *Lipids Health Dis* 2011, **10**:180. doi:10.1186/1476-511X-10-180.
44. Mohanam S, Bose SM: Influence of streptozotocin- and alloxan-induced diabetes on the metabolism of glycosaminoglycans. *Acta Diabetol Lat* 1984, **21**:203–210.

45. Kjellén L, Bielefeld D, Hook M: **Reduced sulfation of liver heparan sulfate in experimentally diabetic rats.** *Diabetes* 1983, **32**:337–342.
46. Buschard K, Høy M, Bokvist K, Olsen HL, Madsbad S, Fredman P, Gromada J: **Sulfatide controls insulin secretion by modulation of ATP-sensitive K (+)-channel activity and Ca(2+)-dependent exocytosis in rat pancreatic beta-cells.** *Diabetes* 2002, **51**:2514–2521.
47. Osterbye T, Jørgensen KH, Fredman P, Tranum-Jensen J, Kaas A, Brange J, Whittingham JL, Buschard K: **Sulfatide promotes the folding of proinsulin, preserves insulin crystals, and mediates its monomerization.** *Glycobiology* 2001, **11**:473–479.
48. Takahashi T, Suzuki T: **Role of sulfatide in normal and pathological cells and tissues.** *J Lipid Res* 2012, **53**:1437–1450.
49. Boya P, Kroemer G: **Lysosomal membrane permeabilization in cell death.** *Oncogene* 2008, **27**:6434–6451.
50. Segel IH: *Biochemical Calculation: how to solve mathematical problems in general biochemistry.* 2nd edition. New York: Whiley; 1976:208–323.
51. Dowd JE, Riggs DS: **A Comparison of Estimates of Michaelis-Menten Kinetic Constants from Various Linear Transformations.** *J Biol Chem* 1965, **240:**863–869.
52. Dixon M, Webb EC: *Enzymes.* 3rd edition. New York: Academic; 1979:860–862.
53. Gasa S, Makita A, Kameya T, Kodama T, Koide T, Tsumuraya M, Komai T: **Arylsulfatases of human lung tumors transplanted into athymic mice: cancer-associated modification of arylsulfatase B variant.** *Eur J Biochem* 1981, **116**:497–503.
54. Gasa S, Makita A: **Phosphorylation on protein and carbohydrate moieties of a lysosomal arylsulfatase B variant in human lung cancer transplanted into athymic mice.** *J Biol Chem* 1983, **258**:5034–5039.
55. Nakamura M, Gasa S, Makita A: **Arylsulfatase A from normal human lung and lung tumor showed different patterns of microheterogeneity.** *J Biochem* 1984, **96**:207–213.