Journal of Diabetes Mellitus, 2022, 12, 252-262
https://www.scirp.org/journal/jdm
ISSN Online: 2160-5858
ISSN Print: 2160-5831

Acetylsalicylic Acid Administered in Patients with Type 2 Diabetes Mellitus and Its Effect on the Antioxidant Enzyme System

Sara Pascoe-González[1], Karina Celia Morgado-Castillo[2], Maria Guadalupe Ramos-Zavala[1], Ernesto Germán Cardona-Muñoz[1], Fernando Grover-Paez[1], María Claudia Espinel-Bermúdez[2], Sandra Ofelia Hernández-González[2*]

[1]Instituto de Terapéutica Experimental y Clínica (INTEC), Departamento de Fisiología, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Guadalajara, México
[2]Unidad de Investigación Biomédica 02, Unidad Médica de Alta Especialidad, Hospital de Especialidades, Centro Médico Nacional de Occidente del Instituto Mexicano del Seguro Social, Guadalajara, México
Email: *dra_sandy2003@yahoo.com.mx

How to cite this paper: Pascoe-González, S., Morgado-Castillo, K.C., Ramos-Zavala, M.G., Cardona-Muñoz, E.G., Grover-Paez, F., Espinel-Bermúdez, M.C. and Hernández-González, S.O. (2022) Acetylsalicylic Acid Administered in Patients with Type 2 Diabetes Mellitus and Its Effect on the Antioxidant Enzyme System. *Journal of Diabetes Mellitus*, **12**, 252-262.
https://doi.org/10.4236/jdm.2022.124020

Received: October 4, 2022
Accepted: November 14, 2022
Published: November 17, 2022

Abstract

Type 2 diabetes mellitus and its complications are associated with oxidative stress and the depletion of antioxidant defenses. The influence of acetylsalicylic acid on reversing the decrease in antioxidants, insulin resistance, glucose homeostasis, and inflammatory cascade can help prevent diabetes complications. **Purpose:** The aim of the study was to evaluate the effect of acetylsalicylic acid on the antioxidant enzymatic system in patients with diabetes. **Methods:** A randomized, double-blind, placebo-controlled clinical trial was carried out in 21 patients of both sexes with Type 2 diabetes for less than five years at the time of diagnosis, without pharmacological treatment, and randomly selected. Acetylsalicylic acid (300 mg) was administered orally for three months to the study group (n = 11) compared to the placebo control group (n = 10). Before and after the intervention, anthropometric and metabolic measurements were taken, fasting plasma glucose, glycated hemoglobin A1c, lipid profile panel, glutathione peroxidase, superoxide dismutase, catalase, and antioxidant capacity/activity were determined; values are presented as mean ± standard deviation. Intra- and intergroup differences were tested by Wilcoxon signed rank and Mann-Whitney U test, respectively; p-value ≤ 0.05 was considered statistically significant. **Results:** The acetylsalicylic acid group showed a decrease in weight (85.6 ± 19.3 vs. 84.1 ± 19.0 kg p = 0.01), cholesterol (205.9 ± 16.6 vs. 186.0 ± 23.2 mg/dL p = 0.02), and glycated hemoglobin A1c (7.8% ± 0.9% vs. 7.0% ± 0.7% p = 0.02). The placebo group exhibited reduction in weight (76.1 ± 14.9 vs. 74.9 ± 15.0 kg p = 0.04), glycated

hemoglobin A1c (6.9% ± 0.6% vs. 6.2% ± 0.4% p = 0.004), and total antioxidant capacity (4.1 ± 0.5 vs. 4.8 ± 0.3 mmol/L p = 0.002). **Conclusion:** The administration of acetylsalicylic acid did not modify the antioxidant enzyme system.

Keywords

Type 2 Diabetes Mellitus, Acetylsalicylic Acid, Oxidative Stress, Glucose, Glycated Hemoglobin A1c

1. Introduction

Advanced glycation end-products (AGEs) are generated in the diabetic milieu due to chronic hyperglycemia and enhanced oxidative stress [1]. Type 2 diabetes mellitus (T2DM), a hydrocarbon disorder of increased incidence worldwide, is also known as a state of oxidative stress in which there is an imbalance between excessive formation and insufficient removals of highly reactive molecules, such as reactive oxygen species (ROS) and reactive nitrogen species (RNS). Among these highly reactive molecules, two well-known species for their relationship to the endothelium are superoxide ion (O_2^-) and its counterpart, nitric oxide (NO^-) [2]. In this regard, oxidative stress plays a decisive role in the development of complications in T2DM [3]. Excessive oxidant activity is mechanically related to various etiologies of insulin resistance and cardiovascular disease [4]. Many sources of oxidative stress exist in T2DM, including enzymatic, non-enzymatic, and mitochondrial pathways. Mainly, increased oxidative stress occurs due to various factors [5] and the most dominant is the autoxidation of glucose, resulting in the development of free radicals [6]. The interaction of advanced glycation end products with their cellular receptors promotes the intracellular production of free radicals and lowers intracellular levels of antioxidants [7]. It is also known that β-cells have relatively low levels of free radical detoxifying and redox-regulating enzymes, such as superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase, and total antioxidant capacity (TAC) [8]. Nonetheless, low-dose acetylsalicylic acid (ASA) therapy of 150 mg per day combined with fluoxetine in patients with depression has shown a statistically significant decrease in copper-zinc superoxide dismutase (SOD1), catalase (CAT), GPx, and malondialdehyde (MDA) antioxidant enzyme activities, as well as a statistically significant increase in total antioxidant status (TAS) [9].

In experimental diabetes, low-dose ASA therapy is a standard therapeutic approach for the prevention of long-term vascular complications [10]. In this context, several mechanisms of action have been proposed for ASA: 1) Scavenging of hydroxyl radical and chelation of transition metals; 2) Upregulation of nitric oxide; 3) Increased syn-thesis of lipoxins; 4) Inhibition of neutrophil oxidative burst; 5) Inhibition of NF-κB and AP-1 protein kinases; 6) Inhibition of lectin-like oxidized LDL receptor-1 [11], suggesting that ASA can prevent some of the late

complications of diabetes by lowering glucose concentration and probably inhibiting glycation by acetylation of protein amino groups [12]. Thus, increasing the antioxidant system may be a useful target for intervention strategies based on reversing the negative impact of glucolipotoxicity in diabetes. Therefore, the overall aim of this study was to evaluate the effect of ASA on the antioxidant enzymatic system in patients with type 2 diabetes.

2. Materials and Methods

A randomized, double-blind, controlled clinical study was conducted in adult patients (35 to 50 years old) with T2DM for less than five years at the time of diagnosis. All participants attending the research unit were residents of the Guadalajara Metropolitan Area (GMA), Mexico, with the same demographic, social, and economic characteristics. The inclusion criteria were patients of any sex with T2DM without pharmacological treatment, body mass index (BMI) between 25.0 and 35.0 kg/m^2, stable body weight within three months before the start of the study, glycated hemoglobin A1c (HbA1c) levels between 6.5 and 9.0% and without a history of thyroid, liver, or kidney disease. The non-inclusion criteria were patients with recognized hypersensitivity to ASA, history of intake of dietary supplements and/or antioxidants, smoking or drug abuse six months prior to the study, history of bleeding, peptic acid disease or gastric ulcer, and pregnant or lactating women during the study period.

All anthropometric measurements and vital signs, as well as the procedures for the interrogation, physical examination, and integration of the clinical history, were performed by universally accepted methods and under the Regulation of the General Health Law of the United Mexican States on the Provision of Medical Care Services [13], and under the ethical principles for pharmacological research in humans detailed in the latest revision of the International Conference on Harmonization [14]. The study was reviewed and approved by the local Bioethics and Research Committee of the Department of Physiology of the University Center for Health Sciences, University of Guadalajara (No. 14.012011), and complied with the principles of the Declaration of Helsinki. Trial registration: ClinicalTrial.gov NCT03341117, retrospectively registered. Written informed consent was obtained from all volunteers.

Before the pharmacological intervention and three months after, tests were performed at 8:00 a.m. following an overnight fast of 10 - 12 h. Weight and height were recorded with the subjects wearing light clothing and without shoes. Height was measured and rounded off to the nearest centimeter with the subjects standing and using a calibrated scale. BMI was calculated as weight (kg) divided by height (m^2). The patients in standing position obtained the waist and hip circumference measurements. The waist circumference was measured immediately above the iliac crest and the hip circumference; the hip circumference was taken at the point yielding the maximum circumference over the buttocks with the tape held on a horizontal plane and feet positioned close together.

Blood pressure and heart rate were measured after a period of rest of at least 5 minutes in a sitting position and determined using OMRON HEM-781INT digital equipment.

Venous blood was obtained while subjects were in a supine position in a quiet room. Blood was allowed to clot for 30 min at room temperature and then centrifuged to obtain a serum aliquot. Another sample was collected in a tube with ethylenediaminetetraacetic acid to obtain a plasma aliquot. The first aliquot was immediately used for the measurement of serum glucose, total cholesterol (TC), HDL-cholesterol (HDL), and triglycerides (TG). Serum glucose was determined by the glucose oxidase technique by (Boehringer Mannheim GmbH, Mannheim, Germany), with an intra- and inter-assay coefficient of variation < 3%. The lipid profile (TC, HDL, and TG) was measured enzymatically (Ortho-Clinical Diagnostics, Johnson &Johnson Company, Rochester, NY, USA) with an intra- and inter-assay coefficient of variation < 2%, LDL cholesterol (LDL) was estimated by the Friedewald formula (LDL = TC − HDL − TG/5) and VLDL cholesterol (VLDL) with the TG/5 equation. The second aliquot was placed at −80˚C to perform the following tests: glutathione peroxidase activity kit by the colorimetric method (Catalog No. ADI-900-158, Enzo); superoxide dismutase assay (Item no. 706002, Cayman Chemical Company); fluorometric catalase detection (Catalog No. ADI-907-027, Enzo), and antioxidant assay (CS0790 SIGMA by BioTek Synergy HT Multi-Mode Microplate Reader and Microplate Data Collection with the Analysis Software GEN 5). The values were inferred previously from standardized curves. Calculations relevant to the adequacy of the results were performed according to the insert instructions. To determine HbA1c levels, ion-exchange high-performance liquid chromatography was carried out (Bio-Rad Laboratories, Hercules, CA) with an intra-assay coefficient of variation of 2.8% and 3.5% and an inter-assay coefficient of variation of <3.0%.

Nutrition and Exercise: each participant received medical nutrition therapy from a registered dietician at the beginning of the study. Subjects were not prescribed a specific exercise regimen.

Simple randomization with a 1:1 concealed allocation was performed using a table of random numbers. After randomization, 11 patients received 300 mg of ASA once daily, and ten patients received a placebo (calcined magnesia) in the same pharmacological presentation for 90 days. The ASA dose was determined not to alter renal uric acid reabsorption.

Statistical Analysis

The sample size was calculated by the mean of a formula for clinical trials [15] with a statistical confidence of 95%, a statistical power of 80%, an SD for GPx of 0.7 U/mol [9], and an expected difference of at least 1.5 SD of GPx between groups, obtaining a total of 9 patients for each group that included 20% of expected loss. Results are presented as mean ± standard deviation (SD). The inference analysis was performed with a Chi-squared test. Intra and intergroup dif-

ferences were calculated using the Wilcoxon signed-rank and Mann-Whitney U tests, respectively; data were analyzed with SPSS-20.0 software (SPSS Inc. Chicago, IL), and a $p \leq 0.05$ was considered significant.

3. Results

The selection and screening of patients that met the inclusion criteria were performed throughout the protocol, and a total of 21 patients were included in the statistical analysis (Figure 1).

All patients who were eligible for enrollment completed the 90 days of the pharmacological intervention, including 9 women and 1 man in the control group and 8 women and 3 men in the acetylsalicylic acid group. No statistically significant differences by gender distribution were found ($p = 0.343$). Likewise, no significant differences in age between groups (48.8 ± 4.7 vs. 50.9 ± 8.3 years; $p = 0.468$) were detected.

After the pharmacology intervention in the ASA group, there were significant differences in weight (85.6 ± 19.3 vs. 84.1 ± 19.0 kg, $p = 0.01$), total cholesterol (205.9 ± 16.6 vs. 186.0 ± 23.2 mg/dL, $p = 0.02$), and A1C (7.8% ± 0.9% vs. 7.0% ± 0.7%, $p = 0.02$). In the placebo group, significant differences were found in weight (76.1 ± 14.9 vs. 74.9 ± 15.0 kg, $p = 0.04$), A1C (6.9% ± 0.6% vs. 6.2% ± 0.4%, $p = 0.004$), BMI (30.97 ± 3.9 vs. 30.57 ± 3.9, $p = 0.05$), and TAC (4.1 ± 0.5 vs. 4.8 ± 0.3, $p = 0.002$) (Table 1), in addition, no significant changes were found

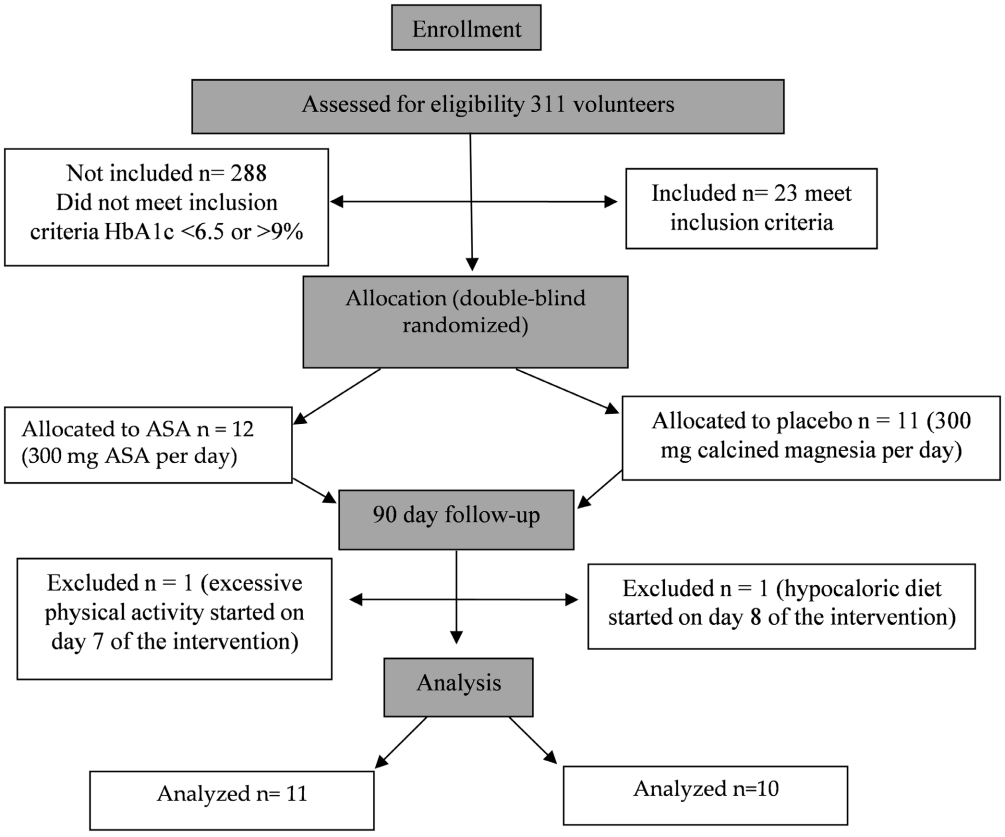

Figure 1. Trial profile.

Table 1. Characteristics before and after the intervention in the study groups.

	PLACEBO			ASA		
	Basal	**Final**	***p***	**Basal**	**Final**	***p***
Weight (kg)	76.1 ± 14.9	74.9 ± 15.0	0.04*	85.6 ± 19.3	84.1 ± 19.0	0.01*
BMI (kg/m^2)	30.97 ± 3.9	30.57 ± 3.9	0.05*	30.98 ± 3.3	30.79 ± 3.1	0.06
Waist (cm)	101.2 ± 12.5	99.8 ± 11.9	0.061	103.2 ± 13.2	102.8 ± 11.7	0.459
Hip (cm)	109.7 ± 11.4	107.8 ± 9.1	0.139	110.9 ± 9.1	109.6 ± 8.6	0.117
Waist/hip ratio	0.92 ±.07	0.93 ±.05	0.508	0.92 ±.05	0.92 ±.05	0.646
BPS (mm/Hg)	122.0 ± 14.4	121.7 ± 11.5	0.859	126.3 ± 12.7	125.9 ± 12.8	0.742
BPD (mm/Hg)	84.0 ± 7.9	82.6 ± 5.1	0.250	78.3 ± 9.3	79.5 ± 10.6	0.401
Glucose (mg/dl)	119.8 ± 11.8	120.1 ± 21.3	0.94	142.1 ± 62.3	130.4 ± 24.8	0.83
HbA1c (%)	6.9 ± 0.6	6.2 ± 0.4	0.00*	7.8 ± 0.90	7.0 ± 0.7	0.02*
TC (mg/dl)	193.1 ± 32.4	179.4 ± 18.9	0.10	205.9 ± 16.60	186.0 ± 23.2	0.02*
TG (mg/dl)	200.2 ± 96.0	167.9 ± 87.1	0.23	195.1 ± 75.30	153.4 ± 55.4	0.07
HDL (mg/dl)	43.5 ± 14.2	43.5 ± 10.9	0.73	46.5 ± 7.50	50.0 ± 14.1	0.96
LDL (mg/dl)	115.6 ± 26.9	116.5 ± 18.5	0.85	120.5 ± 35.80	119.8 ± 19.2	0.45
VLDL (mg/dl)	42.9 ± 24.2	38.5 ± 18.6	0.43	40.6 ± 23.90	40.9 ± 17.1	0.92
Uric Acid (mg/dl)	5.2 ± 1.0	5.3 ± 1.1	0.67	5.6 ± 1.2	5.6 ± 1.1	0.80

Intragroup differences were tested by Wilcoxon signed-rank test. *p ≤ 0.05. BMI: body mass index, BPS: blood pressure systolic; BPD: blood pressure diastolic, HbA1c: glycated hemoglobin A1c, TC: total cholesterol, (TG): triglycerides, HDL: HDL-cholesterol, LDL: LDL cholesterol, VLDL-C: VLDL cholesterol (VLDL-C).

Table 2. Final comparison between groups.

Antioxidant enzymes	Placebo	ASA	*p*
GPx (nmol/min/ml)	129.29 ± 407.76	77.95 ± 175.14	0.908
SOD (U/mg)	0.878 ± 4.29	−0.742 ± 1.72	0.654
Catalase (U/mL)	−0.456 ±0.924	−0.323 ±0.72	0.099
TAC (nmol/mL)	−0.677 ±0.583	−0.142 ±0.99	0.072

Intergroup differences (delta) were tested by Mann-Whitney U test. Data are the mean ± standard deviation. GPx: glutathione peroxidase catalase, SOD: superoxide dismutase TAC: total antioxidant capacity.

in antioxidant enzymes between the placebo and ASA groups (Table 2). It should be noted that patients did not show any adverse events after each assigned treatment.

4. Discussion

Vascular health, including maintaining adequate levels of free radicals and preventing endothelial dysfunction, is an essential concern for T2DM patients. The

available data support the concept that targeting inflammation pathways may represent a valuable option for tackling cardiometabolic complications of T2DM.

Studies with ASA administration in experimental diabetes have shown controversial results. For instance, a decreased glutathione peroxidase activity in ASA-treated non-diabetic animals and an increased ASA-induced G6PDH activity were recorded in both diabetic and non-diabetic rats. While glycation due to diabetic hyperglycemia and ASA-mediated acetylation had very similar effects on the activities of different enzymes, the non-enzymatic modification by either glucose or ASA may be a common mechanism of the observed convergence [10].

The purpose of the current clinical trials is to translate previous preclinical findings by suggesting the clinical efficacy of salicylates on various antioxidant mechanisms, mainly NF-κB, involved in the pathogenesis of the inflammatory response and disruption of inducible nitric oxide synthase (iNOS), which increases nitric oxide (NO) [16], to improve antioxidant systems by reducing oxidative stress and cellular damage associated with diabetes complications.

Differences in gender distribution of study groups (intragroup) were not considered as a covariate due to the lack of statistical significance; it is also well known that in terms of the pharmacokinetics of ASA, gender does not influence the absorption rate nor the volume of distribution, however, it only affects elimination kinetics, which is longer in women [17]. The pH level is more relevant for the renal excretion of salicylic acid and its metabolites [18].

Nevertheless, differences in weight (intergroup) could be significant since changes in regional blood flow, modification of plasma proteins and/or tissue components, the volume of distribution, renal and hepatic clearance mechanisms, the activity of some phase II enzymes, and CYP450, modify adipose tissue. As a result, obesity could contribute to a faster bio-inactivation of aspirin inside and outside the liver since it is a highly lipophilic molecule; however, both intervention groups in this study had a mean BMI (Grade 1 obesity), so the clinical impact of the aspirin dose related to weight should be tested in trials with a more significant number of patients with different degrees of obesity [19] [20].

Our results were consistent with other studies regarding the changes found in CT and HbA, possibly given by the glycation of proteins through the acetylation of free amino groups of said proteins [12] [21], an effect attributed to salicylates or hyperglycemia, and thus, the change in HbA was observed in both groups. Weight reduction was attributed mainly to the adoption of lifestyle changes performed by patients due to their discipline during the trial since this change cannot be attributed to the direct mechanism of salicylates.

Most ASA assays have been shown to improve glucose metabolism as well as markers that mediate inflammation at high doses (3.0 to 7.0 g/d); however, its clinical applicability is limited because, in these trials, patients reported side effects like tinnitus and gastrointestinal bleeding, which generated the reduction of the standard dose, and in combination with the diet, the results of ASA on glucose were reduced. Patients treated with doses of 500 mg/d for two years increased total antioxidant capacity but also had to be under tolerability surveil-

lance derived from the intervention [14]. Finally, oxidative stress and thromboembolic complications are reduced with 100 mg/d of ASA as adjunctive therapy in hypertensive or infarcted patients measured by the reduction of 2,3- and 2,5-dihydroxybenzoic acids, which justifies its prolonged use [22]. Yet, at 300 mg/d doses in diabetic patients without cardiovascular disease, these changes were not achieved, but it was well tolerated, and glucose decreased clinically. However, some studies have measured inhibition of urinary excretion of 8-isoPGF2α and 11dhTxB2 without finding significant changes with placebo or ASA intake in patients with poor glycemic control or non-ASA responders. This suggests that oxidative stress can maintain platelet function independently of the inhibition of the COX-1 pathway and/or increase the systemic generation of thromboxane from non-platelet sources [23] [24].

ASA was found to work as an acetylating agent with many beneficial effects on the vascular endothelium beyond platelet inhibition. The maximum plasma concentration of ASA reaches one mg/L within half an hour of 100 mg of ASA and 3 mg/L of 300 mg of ASA, taken orally. These doses do not inhibit COX-2 since COX-1 inhibition is the net pharmacological effect. Because of the irreversible action of ASA (*i.e.*, acetylation), the duration of its impact is determined by target protein resynthesis [25] [26]. The activation of the prostanoid receptor on platelets leads to platelet activation and further amplification of Thromboxane A2 synthesis and release; this promotes inflammation and, thus, oxidative stress.

Furthermore, continuous signaling via TP receptors leads to the downstream generation of more ROS. In clinical practice, the preventive effect of ASA is achieved via the inhibition of platelet activation and aggregation due to the inhibition of Thromboxane A2 synthesis. Apparently, one can also anticipate breaking the vicious cycle of TxA2-mediated platelet activation, oxidative stress, vascular inflammation, eNOS uncoupling, and reduced NO bioavailability with TxA2 inhibition [27].

Although ASA blocks one pathway, activation by other mechanisms may be present and could interfere with the potential protective effect of ASA against oxidative stress. Despite this, the mechanisms of action of ASA are yet not fully elucidated.

We believe that further research involving low doses of ASA with anti-inflammatory potential and fewer adverse effects in the prevention of cardiovascular complications by T2DM patients should be continued for more extended administration periods and possibly with the comparison between responders and non-responders as well as ASA and its derivatives like lysine acetylsalicylate in patients resistant to ASA [28] [29].

5. Conclusion

The administration of acetylsalicylic acid did not modify the antioxidant enzyme system. Total antioxidant capacity changed only in the placebo group, which may be associated with changes in lifestyle or the chronicity of the disease. Both

groups presented changes in weight and glycated hemoglobin A1c.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Khalid, M., Petroianu, G. and Adem, A. (2022) Advanced Glycation End Products and Diabetes Mellitus: Mechanisms and Perspectives. *Biomolecules*, **12**, Article No. 542. https://doi.org/10.3390/biom12040542

[2] Darenskaya, M.A., Kolesnikova, L.I. and Kolesnokov, S.I. (2021) Oxidative Stress: Pathogenic Role in Diabetes Mellitus and Its Complications and Therapeutic Approaches to Correction. *Bulletin of Experimental Biology and Medicine*, **171**, 179-189. https://doi.org/10.1007/s10517-021-05191-7

[3] Zatalia, St. R. and Sanusi, H. (2013) The Role of Antioxidants in the Pathophysiology, Complications, and Management of Diabetes Mellitus. *The Indonesian Journal of Internal Medicine*, **45**, 141-147.

[4] Yaribeygi, H., Sathyapalan, T., Atkin, L.T. and Sahebkar, A. (2020) Molecular Mechanisms Linking Oxidative Stress and Diabetes Mellitus. *Oxidative Medicine and Cellular Longevity*, **2020**, Article ID: 8609213. https://doi.org/10.1155/2020/8609213

[5] Ighodaro, O.M. (2018) Molecular Pathways Associated with Oxidative Stress in Diabetes Mellitus. *Biomedicine & Pharmacotherapy*, **108**, 656-662. https://doi.org/10.1016/j.biopha.2018.09.058

[6] Zatalia, S.R. and Sanusi, H. (2013) The Role of Antioxidants in the Pathophysiology, Complications, and Management of Diabetes Mellitus. *The Indonesian Journal of Internal Medicine*, **45**, 141-147.

[7] Cruz, H.J., Licea, M.E., Hernandez, G.P., Abraham, M.E. and Yanes, Q.M. (2011) Estrés oxidativo y diabetes mellitus. *Revista Mexicana de Patología Clínica y Medicina de Laboratorio*, **58**, 4-15. http://www.medigraphic.com/patologiaclinica

[8] Newsholme, P., Rebelato, E., Abdulkader, F., Krause, M., Carpinelli, A. and Curi, R. (2012) Reactive Oxygen and Nitrogen Species Generation, Antioxidant Defenses, and β-Cell Function: A Critical Role for Amino Acids. *Journal of Endocrinology*, **214**, 11-20. https://doi.org/10.1530/JOE-12-0072

[9] Galecki, P., Szemraj, J., Bieńkiewicz, M., Zboralski, K. and Gałecka, E. (2009) Oxidative Stress Parameters after Combined Fluoxetine and Acetylsalicylic Acid Therapy in Depressive Patients. *Human Psychopharmacology*: *Clinical and Experimental*, **24**, 277-286. https://doi.org/10.1002/hup.1014

[10] Lapshina, E.A., Sudnikovich, E.J., Maksimchik, J.Z., Zabrodskaya, S.V., Zavodnik, L.B., Kubyshin, V.L., Nocun, M., Kazmierczak, P., Dobaczewski, M., Watala, C. and Zavodnik, I.B. (2006) Antioxidative Enzyme and Glutathione-*S*-Transferase Activities in Diabetic Rats Exposed to Long-Term Aspirin Treatment. *Life Sciences*, **79**, 1804-1811. https://doi.org/10.1016/j.lfs.2006.06.008

[11] Baltazar, M.T., Dinis-Oliveira, R.J., Duarte, J.A., Bastos, M.L. and Carvalho, F. (2011) Antioxidant Properties and Associated Mechanisms of Salicylates. *Current Medicinal Chemistry*, **18**, 3252-3264. https://doi.org/10.2174/092986711796391552

[12] Caballero, F., Gerez, E., Batlle, A. and Vazquez, E. (2000) Preventive Aspirin Treatment of Streptozotocin Induced Diabetes: Blockage of Oxidative Status and Revertion of Heme Enzymes Inhibition. *Chemico-Biological Interactions*, **126**, 215-225. https://doi.org/10.1016/S0009-2797(00)00168-X

[13] Diario Oficial de la Federación (DOF) (n.d.) Norma Oficial Mexicana NOM-004-SSA3-2012, Del expediente clinico. https://dof.gob.mx/nota_detalle_popup.php?codigo=5272787

[14] https://www.wma.net/es/policies-post/declaracion-de-helsinki-de-la-amm-principios-eticos-para-las-investigaciones-medicas-en-seres-humanos/

[15] Jeyaseelan, L. and Rao, P.S. (1989) Methods of Determining Samples Size in Clinical Trials. *Indian Pediatr*, **26**, 115-121.

[16] Goldfine, A.B., Silver, R., Aldhahi, W., Cai, D., Tatro, E., Lee, J. and Shoelson, S.E. (2008) Use of Salsalate to Target Inflammation in the Treatment of Insulin Resistance and Type 2 Diabetes. *Clinical and Translational Science*, **1**, 36-43. https://doi.org/10.1111/j.1752-8062.2008.00026.x

[17] Tmavská, Z.T.K. (1983) Sex Differences in the Pharmacokinetics of Salicylates. *European Journal of Clinical Pharmacology*, **25**, 679-682. https://doi.org/10.1007/BF00542358

[18] Elliot Cham, B., Dykman, J.H. and Bochner, F. (1982) Urinary Excretion of Aspirin. *British Journal of Clinical Pharmacology*, **14**, 562-564. https://doi.org/10.1111/j.1365-2125.1982.tb02030.x

[19] Hanley, M.J., Abernethy, D.R. and Greenblatt, D.J. (2010) Effect of Obesity on the Pharmacokinetics of Drugs in Humans. *Clinical Pharmacokinetics*, **49**, 71-87. https://doi.org/10.2165/11318100-000000000-00000

[20] Bordeaux, B.C., Qayyum, R. and Yanek, L.R. (2010) Effect of Obesity on Platelet Reactivity and Response to Low-Dose Aspirin. *Preventive Cardiology*, **13**, 56-62. https://doi.org/10.1111/j.1751-7141.2009.00058.x

[21] Blakytny, R. and Harding, J.J. (1992) Prevention of Cataract in Diabetic Rats by Aspirin, Paracetamol (Acetaminophen) and Ibuprofen. *Experimental Eye Research*, **54**, 509-518. https://doi.org/10.1016/0014-4835(92)90129-G

[22] Coudray, C. and Favier, A. (2000) Determination of Salicylate Hydroxylation Products as an in Vivo Oxidative Stress Marker. *Free Radical Biology and Medicine*, **29**, 1064-1070. https://doi.org/10.1016/S0891-5849(00)00403-2

[23] Ames, P.R., Batuca, J.R., Muncy, I.J., De La Torre, I.G., Pascoe-Gonzales, S., Guyer, K., Matsuura, E. and Lopez, L.R. (2012) Aspirin Insensitive Thromboxane Generation Is Associated with Oxidative Stress in Type 2 Diabetes Mellitus. *Thrombosis Research*, **130**, 350-354. https://doi.org/10.1016/j.thromres.2012.03.025

[24] Lemkes, B.A., Bähler, L., Kamphuisen, P.W., Stroobants, A.K., Van Den Dool, E.J., Hoekstra, J.B., Nieuwland, R., Gerdes, V.E. and Holleman, F. (2012) The Influence of Aspirin Dose and Glycemic Control on Platelet Inhibition in Patients with Type 2 Diabetes Mellitus. *Journal of Thrombosis and Haemostasis*, **10**, 639-646. https://doi.org/10.1111/j.1538-7836.2012.04632.x

[25] Muller, K.A., Chatterjee, M., Rath, D. and Geisler, T. (2015) Platelets, Inflammation and Anti-Inflammatory Effects of Antiplatelet Drugs in ACS and CAD. *Thrombosis and Haemostasis*, **114**, 498-518. https://doi.org/10.1160/TH14-11-0947

[26] Nagelschmitz, J., Blunk, M., Kraetzschmar, J., Ludwing, M., Wensig, G. and Holhfeld, T. (2014) Pharmacokinetics and Pharmacodynamics of Acetylsalicylic Acid after Intravenous and Oral Administration to Healthy Volunteers. *Clinical Pharmacology*: *Advances and Applications*, **6**, 51-59. https://doi.org/10.2147/CPAA.S47895

[27] Dzeshka, M.S., Shantsila, A. and Lip, G.Y.H. (2016) Effects of Aspirin on Endothelial Function and Hypertension. *Current Hypertension Reports*, **18**, Article No. 83. https://doi.org/10.1007/s11906-016-0688-8

[28] Grimaldi, R., Bisi, M., Lonni, E., Beggiato, E., Valpreda, A., Lococo, M.F., Dosio, E., Presutti, D.G., Tagliabue, M. and Gaita, F. (2014) Laboratory Aspirin Resistance Reversibility in Diabetic Patients: A Pilot Study Using Different Pharmaceutical Formulations. *Cardiovascular Drugs and Therapy*, **28**, 323-329. https://doi.org/10.1007/s10557-014-6536-7

[29] Abdin, A.A., Baalash, A.A. and Hamooda, H.E. (2010) Effects of Rosiglitazone and Aspirin on Experimental Model of Induced Type 2 Diabetes in Rats: Focus on Insulin Resistance and Inflammatory Markers. *Journal of Diabetes and Its Complications*, **24**, 168-178. https://doi.org/10.1016/j.jdiacomp.2009.01.005