



Algerian *Capparis spinosa* n-BuOH Extract Alleviates Diabetic Neuropathy Induced with Streptozotocin in Rats

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Abstract

Diabetic neuropathy (DN) is the most common diabetes complication. This study aimed to investigate the possible neuroprotective effects of the n-BuOH extract of *Capparis spinosa* (BECS) against DM-neuropathy induced with streptozotocin (STZ) and explore its phytoconstituents. From our results, BECS showed no toxicity against C6 brain cancer cell lines. The treatment of male Swiss albino rats with BECS (200 mg/kg) for a period of 28 days significantly attenuated the adverse effects of STZ-induced diabetes (60 mg/kg). A decrease in blood glucose was noted in the BECS-STZ group, associated with a significant ($p < 0.01$) improvement in serum and brain insulin levels. The BECS-treated group showed a significant ($p < 0.01$) improvement in serum C-peptide, serum AGE and serum CK-BB levels. BECS was also successful in restricting HbA1c in STZ-treated rats. Furthermore, a resurrection of neuronal membrane integrity was manifested in the BECS+STZ group, as evidenced by increased brain LDH activity and reduced brain MDA level. Treatment of STZ-treated rat with BECS was also able to significantly ($p < 0.01$) regulate markers of brain dysfunction (AChE and BChE activities and glutamate, dopamine levels). Additionally, treatment of rats with BECS efficacy restored the GSH level and GSH-system enzymes towards normal levels. Regarding the levels of (IL-6, TNF- α , and NO) produced by diabetogenic effects of STZ, the BECS successfully regulated these inflammatory mediators. From all these results, the neuroprotective effect of BECS could be due to its richness in polyphenols identified by LC-MS/MS (both positive and negative modes) analysis, mainly chlorogenic acid and rutin, as well as the synergistic interactions of all contents.

Keywords: Diabetic Neuropathy, Streptozotocin, *Capparis spinosa*, LC-MS/MS analysis, Oxidative stress, Inflammation.

1. Introduction

Diabetes mellitus (DM) is now the biggest health problem in the world despite the understanding of the pathogenesis of DM, existing drugs have only a temporary antidiabetic effect and have failed to completely prevent the progression of these anomalies [1]. Diabetes mellitus is caused by the breakdown of carbohydrates, proteins and lipids, characterized by increased blood glucose levels, resulting from inadequate or insufficient production of insulin [2]. Diabetic neuropathy (DN) is the most common diabetes complication and its prevalence ranges from 40 % to 50 % of patients with diabetes [3]. Previous studies have shown that uncontrolled blood sugar in long-standing diabetes leads to astroglial abnormalities in certain brain regions that decrease cognitive function, anxiety, memory

impairment, and depression [4, 5]. Insulin resistance in the brain can trigger key pathophysiological events of neurodegenerative disorders such as oxidative stress [6], neuro-inflammation [7], Glycation End Products (AGE) [8], CK-BB release [9], increased C-reactive peptide [10], changes in neurotransmitters and impaired brain repair processes [11]. Clinical approaches have been studied to combat diabetes. Among the most available diabetes medications are the sulfonylurea, metformin and meglitinides, glipizide, rosiglitazone and glimepiride [12]. Unfortunately, the consumption of these synthetic agents could lead to various side effects [13]. These are therefore, bring to the new insights on using the natural plant as a remedy for the cognitive impairment due to diabetes that were safer, and more effective for treating and managing diabetes

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Receive Date: 16 August 2022, Revise Date: 20 September 2022, Accept Date: 19 October 2022

DOI: 10.21608/EJCHEM.2022.156714.6790

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complications, especially in long-term therapy [14]. Indeed, the inhabitants of the world, including the Algerians, believed that eating and consuming fresh medicinal plants could cure diseases and ailments; among these plants was *Capparis spinosa*, which belongs to the family Capparidaceae [15]. Different parts of *Capparis spinosa* are used in the management of various conditions and have been shown to have a wide range of biological activities such as antioxidants [16], and anti-inflammatories [17, 18]. Several experimental and clinical studies have investigated the antidiabetic effects [19-21], and have referred *C. spinosa* as an improvement cognitive impairment agent [22, 23]. The body of data collected over the past decades on the chemical composition of

C. spinosa has led to evidence that the plant is a rich source of bioactive compounds, mainly polyphenolic compounds [24]. However, it has been reported that a difference between these levels of chemical compounds may be related to different geographical locations which may vary the profile of bioactives associated with caper functionalities [25]. Taking into account that leaves and flowers of n-BuOH fraction from *C. spinosa* (BECS) grown in East of Algeria have never been screened for diabetic neuropathy, the present study investigated whether *C. spinosa* attenuates

DM-induced neurodegeneration, and explored its possible underlying mechanisms, particularly, the possible involvement of anti-inflammatory and antioxidant properties. The present study is also an attempt to identify and enrich the knowledge on the variation in the chemical composition of *C. spinosa* (MILA region; Algeria) by using

LC-MS/MS analyses.

2. Materials and methods

2.1. Chemicals

Streptozotocin (STZ), Aluminium chloride (AlCl_3), Folin-Ciocalteu's phenol reagent, quercetin, sodium carbonate (Na_2CO_3), thiobarbituric acid (TBA), high glucose Dulbecco's Modified Eagle's medium (DMEM), trypsin, sulforhodamine B (SRB), rat TNF- α , rat IL-6, ELISA kits were purchased from eBioscience (San Diego, CA USA), reduced glutathione (GSH), glutathione reductase (GR),

5,5-dithiobis-2-nitrobenzoic (DTNB) and 1-chloro-2,4-dinitrobenzene (CDNB), acetylthiocholine iodide (ATCI), Glutamine/Glutamate Determination Kit, Human haemolysate [glycated haemoglobin (HbA [sub]1c)] Kit. Standards for LC-MS/MS analysis (chlorogenic acid, p-hydroxybenzoic acid, kaempferol, gentisic acid, gallic acid, rosmarinic acid, cichoric acid, caffeic acid, 4-hydroxybenzaldehyde, and rutin), were purchased from Extrasynthese (Genay, France). The solvents and/or reagents were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Plant material and extract preparation

Aerial parts of *C. spinosa* (Capparidaceae) were collected in the flowering stage from the area of Mila region; Algeria and authenticated on the basis of Quézel *et al.* [26]. Air-dried aerial parts (leaves and flowers, 500 g) of *C. spinosa* were macerated at room temperature with MeOH-H₂O (80:20, v/v) three times (24 hours for each time). The filtrates were combined, concentrated under reduced pressure, diluted in H₂O (1000 mL). After filtration, the resulting solution was successively extracted with solvents with increasing polarities: chloroform (CHCl_3), ethyl acetate (EtOAc) and

n-butanol (n-BuOH). The organic layers were dried with anhydrous Na_2SO_4 , filtered and concentrated under vacuum at room temperature [27] to obtain CHCl_3 (0.20 g), EtOAc (1.3 g) and n-BuOH (7.71 g) extracts. A part of n-BuOH extract of *C. spinosa* (BECS) was used for the neuroprotective study (This extraction was repeated as needed).

2.3. Phytochemical screening

2.3.1. Phytochemical screening of n-BuOH extract of *C. spinosa* aerial parts

Aerial part of *C. spinosa* was subjected to qualitative tests in order to characterize several chemical groups using standard procedures [28, 29].

2.3.2. Estimation of total phenolic compounds (TPC)

TPC was determined with the Folin-Ciocalteu reagent following the method of Wolfe *et al.* [30], with a slight modification using Gallic acid as standard. Results were expressed as micrograms of Gallic acid equivalents per milligrams of extract (μg GAE/mg).

2.3.3. Determination of total flavonoid content (TFC)

The concentration of flavonoids was achieved using the method described by Ordonez *et al.* [31], by using the aluminium chloride reagent. Results were expressed as μg quercetin equivalents (QE)/ mg extract.

2.3.4. Liquid-chromatography tandem mass spectrometry (LC-MS/MS)

The LC-MS/MS was used for analysis and for indicating the profile of chemical constituents present in the n-BuOH extract and quantified their concentrations in milligrams. For quantitative analysis, a Thermo Scientific Dionex Ultimate 3000 - TSQ Quantum with a Thermo ODS Hypersil column (250×4.6 mm, particle size of $5 \mu\text{m}$) was employed. The injection volume was 20 μL , where water with 0.1% formic acid and methanol were used in mobile phase as eluent A & B respectively. At 40 °C, the

flow rate was 0.7 mL/min. The gradient program was fixed as follow: 0-1 min, 100 % A, 5-20 min, 95 % A, 1-22 min, 5 % A, 25 min, 5 % A, 30 min 0 % A. Plant extract was prepared and analyzed at a concentration of 1 mg/mL, while the entire time procedure took 30 min. For each component, the correlation between peak area and concentration was found to be linear from 0.5 to 10 mg/L and the linearity was determined using 6 points linear regression analysis. Three replicates are used per point in a linear plot. The correlation coefficients (R² values) for all studies were found to be ≥ 0.99 . For ionization of the substances, electrospray ionization (ESI) source of the mass spectrometer was operated in both positive and negative modes. The spray voltages for positive and negative polarities were 4000 and 2500 V, respectively. Compound limit of detection (LOD) and limit of quantification (LOQ) values are determined using the LC-MS/MS technique. According to Eurachem Guide (Second Edition, 2014), LOD and LOQ were obtained by measuring reagent blanks spiked with low concentrations of analyte. A 0.1 ppm spike was added to blank solution. The standards used for LC-MS/MS analysis were chlorogenic acid, sisoric acid, p-hydroxybenzoic acid, kaempferol, gentisic acid, quercetin, gallic acid, rosmarinic acid, caffeic acid and rutin.

2.4. Cytotoxicity assay

-Sulforhodamine B (SRB assay)

The human brain cancer cells (C6) was used for the cytotoxicity studies, C6 astroglial cell line was obtained from the American Type Culture. The procedures described by Vichai and kirtikara [32] were followed. The method described here has been optimized for the toxicity screening of extract to adherent cells in 96 well formats. After an incubation period, cell monolayer is fixed with 10 % (wt/vol) TCA and stained for 30 min, after which the excess dye is removed by washing repeatedly with 1 % (vol/vol) acetic acid. The protein-bound dye is dissolved in 10 mM Tris base solution for OD determination 510 nm using a microplate reader. The results are linear over a 20-fold range of cell numbers and the sensitivity is comparable to those of fluorometric methods. IC₅₀ values can be derived using curve-fitting methods with statistical analysis software or IC₅₀ calculation software.

2.5. Antidiabetic studies

-Animals and experimental design

Sixty male Swiss albino rats weighing 180-210 g obtained from the Animal Research and Service Centre, Brothers Mentouri University, Constantine, were used in the study. All the animals were kept under standard laboratory conditions (temperature 25

± 2 °C and 12 h light/12 h dark cycle). They were fed with standard rodent pellet diet and water *ad libitum*. The *in vivo* experimental protocol was approved by the Institutional Project Committee (D01N01UN250120190002). The experimental procedures adopted in this study were in strict compliance with the Guidelines for Reporting Animal Research [33].

2.5.1. Induction of diabetes mellitus

Development of diabetes was confirmed by polydipsia, polyuria and by determining glucose concentrations three days after intraperitoneal (ip) injection of STZ (dissolved in 0.1 M citrate buffer, pH 4.5) at a dose of 60 mg/Kg [34]. Rats with a blood glucose level of 250 mg/dL or above were considered to be diabetic; the diabetes was confirmed by measuring fasting blood glucose levels from a tail vein using an Accu-Chek Glucometer.

The rats were divided into five groups comprising twelve animals in each group as follows:

Group 1 (control-group): served as control, received an ip injection of an equal volume of citrate buffer, after two hours, the rats were administered with 4 mL/Kg of distilled water, once daily for 28 days, continuously.

Group 2 (BECS-group) treated orally with BECS (200 mg/kg b.w.28 days), after vehicle injection.

Group 3 (diabetic-group): diabetic rats, received 4 mL/Kg of distilled water, once a day for 28 days, continuously

Group 4 (STZ+metformin-group): diabetic rats, administered orally with metformin (200 mg/kg b.w.), once daily for 28 days, continuously.

Group 5 (STZ+BECS-group): diabetic rats, orally administered with BECS (200 mg/kg b.w.), once daily for 28 days, continuously.

At the end of the experimental period, the rats were euthanized by decapitation for brain tissue collection. Their brains were quickly removed out on an ice-cold plate, washed with ice cold phosphate-buffered saline solution (pH 7.4), weighed and stored at -80 °C until further biochemical studies. The blood sample was centrifuged 3000 x g for 15 minute at 4 °C, serum was collected and stored at until analyses.

2.5.2. Assay of Advanced Glycation End Products (AGE) in serum

The amount of blood plasma AGEs was determined following the method described by Putta and Kilari [35]. Plasma was diluted in a 1:50 ratio with PBS (pH 7.4). Bovine serum albumin preparation (1 mg/mL of 0.1 N NaOH) was used as a standard. The level of AGEs in plasma was determined by measuring the fluorescence at an excitation wavelength of 370 nm and emission wavelength of 440 nm. The fluorescence intensity of the samples was expressed as AU per mg of protein.

2.5.3. Biochemical diabetic parameters

Glycation of haemoglobin (GlycHb), serum insulin, brain insulin, plasma C-peptide, plasma CK-BB levels, TNF- α , IL-6, were evaluated using Enzyme Immunoassay Kit, according to the manufacturer's instructions.

2.5.4. Effect of BECS on diabetic neuropathy

- Brain homogenate preparation

An aliquot of brain tissue was homogenized in 0.1 M phosphate buffered saline (pH 7.4) containing KCl (1.15 %) to give a final concentration of 10 % weight/volume (w/v). The homogenate was first centrifuged first at $800 \times g$ for 5 min at 4 °C, and then at $10,000 \times g$ for further 10 min at 4 °C to estimate antioxidant status and LDH activity.

2.5.4.1. Measurement of brain malondialdehyde (MDA) level

The levels of MDA were measured spectrophotometrically at 535 nm using the method of Ohkawa *et al.* [36]. The amount of MDA was calculated using a molar extinction coefficient of $1.56 \times 10^5 \text{ M}^{-1}\text{cm}^{-1}$. The results were expressed as nmol/mg protein.

2.5.4.2. Determination of LDH activity in rat brain

The total reaction mixture (3 mL) contained 1 mL of 200 mM Tris-HCl buffer (pH 7.4), 0.15 mL of 100mM KCl, 0.15 mL of 50 mM sodium pyruvate, 0.20 mL of 2.4 mM NADH and supernatant of homogenate. A decrease in extinction ($6220 \text{ M}^{-1}\text{cm}^{-1}$) at $\lambda_{\text{max}} = 340 \text{ nm}$ for 2 min at 25°C was measured and result was expressed in U/mg protein (U: μM NADH oxidized/min/mg protein) [37].

2.5.5. Effect of BECS on neurochemical markers

2.5.5.1. Determination of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activities

In the STZ-rat brain, AChE activity was determined based on the methods of Ellman *et al.* [38] adapted for a microtiter plate format. Briefly, for each assay data point, 50 μL of 3 mM DTNB, 50 μL rat brain homogenate, 35 μL of 50 mM Tris/HCl pH 8.0. The assay was initiated by the addition of 25 μL of 15 mM ATCI, with production of 5-thio-2-nitrobenzoate anion read at 412 nm every 30 sec for 10 min using a microplate reader AChE activity was calculated using molar extinction coefficient of chromophore ($1.36 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}$). Results were expressed as $\mu\text{mol} / \text{mg}$ protein, while the activities of BChE in the brain were analyzed following a method described by Jońca *et al.* [39].

2.5.5.2. Estimation of dopamine content

The quantitative determination of dopamine levels was carried out fluorometrically according to the method of Ciarlone [40]. Apart of the frozen brain samples were taken out and thawed on the day of experiment. An aliquot of brain tissues was homogenized with acidified n-butanol to give 10 % weight/volume (w/v) homogenate. The method is based on estimation of dopamine fluorophors formed after oxidation by iodine at excitation/emission wave lengths of 320/375 nm. The results were expressed as ng/mg tissue.

2.5.5.3. Determination of L-glutamate content

Equal volumes of brain homogenate and 1.2 M perchloric acid were mixed, centrifuged at $600 \times g$ at 4 °C for 15 min. The supernatant was used according to Lund [41]. Glutamine/glutamate determination Kit was used for spectrophotometric measurement of L-glutamate via enzymatic deamination of L-glutamine and dehydrogenation of L-glutamate with conversion of NAD⁺ to NADH. Spectrophotometric assessment was done at 340 nm and results were expressed in nmoL/mg tissue.

2.6. Measurement of glutathione-metabolizing enzymes

Reduced glutathione (GSH) levels estimation was based on the GSH reaction with DTNB producing a yellow colored complex estimated at 412 nm according to the method of Sedlak and Hanus [42]. The GSH levels were calculated using an extinction coefficient of $13600 \text{ mol}^{-1}\text{cm}^{-1}$, the values were expressed as nmoL/mg protein. Glutathione-S-transferase (GST) activity was assayed by quantifying the conjugate glutathione resulting from the conjugation of CDNB with GSH following the method of Habig *et al.* [43]. GST activity was monitored at 340 nm for 3 min. The enzyme activity was expressed as units/mg protein; one unit of GST activity was defined as μmoles CDNB conjugate formed/min/mg protein using a molar extinction coefficient of $9.6 \times 10^3 \text{ M}^{-1}\text{cm}^{-1}$. Glutathione peroxidase (GPx) activity was determined by the method of Rotruck *et al.* [44] based on the degradation of H₂O₂ in the presence of GSH. Enzyme activity was expressed as units/mg protein; one unit of GPx activity was defined as nmoles GSH/mg protein. Glutathione reductase (GR) activity was tested by measuring NADPH oxidation at 340 nm using oxidized glutathione as a substrate [45]. GR was estimated, and the results were expressed as units/mg protein (U: μmol NADPH oxidized/min). The protein estimation in brain tissue was assayed by Lowry method [46] using bovine serum albumin as standard.

2.7. Measurement of nitrite content in rat brain

A mixture of 0.5 mL of Greiss reagent (equal volumes of 1% sulphanilamide in 3 M HCl and 0.1%

N-1-Naphthyl ethylene diamine dihydrochloride in water) and 0.1 mL supernatant was incubated at room temperature in the dark for 10 min, and absorbance was measured at 548 nm. Total nitrite content ($\mu\text{M}/\text{mg}$ of protein) was determined from standard curve of sodium nitrite (10-100 μM) [47].

2.8. Statistical analysis

Results were expressed as the mean \pm standard deviation (SD). Data were analyzed using a one-way analysis of the variance test (One-way ANOVA) followed by Honest significant difference test (HSD) of Tukey used as post hoc test to compare significance between groups at $p < 0.05$ and $p < 0.01$, using Open stat 2014 program. $n=12$ for in vivo studies. Values of $p < 0.05$ was regarded as significant.

3. Results and Discussion

3.1. Phytochemical studies

3.1.1. Preliminary phytochemical screening of *n*-BuOH extract of *Capparis spinosa* aerial parts

Preliminary phytochemical screening of aerial part of *C. spinosa* revealed the presence of a wide range of natural compounds such as alkaloids, saponins, terpenoids, phenolic compounds, anthocyanins, flavonoids, Glycosides, tannins and sterols (Table 1). Our findings are in agreement with those reported by Yang *et al.* [48] and Rajesh *et al.* [49].

Table 1

Phytochemical profile of *n*-BuOH extract of *Capparis spinosa* aerial parts (BECS)

Chemical groups	Aerial parts	Chemical groups	Aerial parts
Alkaloids	+	Anthocyanins	+
Glycoside	+	Phenolic compounds	+
Terpenoid	+	Flavonoids	+
sterols	+	Saponins	+
		Tannins	+

“+” Presence

3.1.2. Polyphenol and flavonoid contents

Table 2.

Polyphenol and flavonoid contents of *n*-BuOH extract of *Capparis spinosa* aerial part (BECS)

Extract	TPC (μg GAE/ mg extract)	TFC (μg QE/ mg extract)
BECS	1000.44 \pm 6.48	568.27 \pm 5.80

BECS : *n*-BuOH extract of *Capparis spinosa*.

Results are expressed as means \pm SD deviation of three measures. Total phenolics (TPC) is expressed as μg Gallic acid equivalents/mg of extract; Total flavonoids (TFC) are expressed as μg Quercetin equivalents/ mg of extract.

Total phenol content varies with different plant types and parts, growth condition and stage, extraction method, and other factors [50]. *C. spinosa* has been reported for its richness in secondary metabolites, in particular flavonoids and phenolic acids [24]. Despite that, *C. spinosa* has been previously studied with an exclusive attention to ethanolic, hydro-ethanolic, methanolic, chloroform, ethyl acetate and aqueous extracts [51-56], no studies have been carried out on the *n*-BuOH fraction. In the present results, phenolic and flavonoid contents were recorded in *n*-BuOH plant extract from *C. spinosa* with values of 1000.44 \pm 6.48 μg GAE/mg extract of TP and 568.27 \pm 5.80 μg QE /mg extract of TF (Table 2), which seems ranged between (140.86 \pm 0.62 to 1670.1 \pm 62.2 μg GA-Eq/g of TP and 230.50 \pm 0.76 μg Q-Eq/g to 530.8 \pm 8.9 μg Eq Rutin/g of TF when recorded in different fractions of Algerian *C. spinosa* [16, 57]. In fact, the variation between TP and TF results could be due to many factors such as harvest time and place, climate, geographical conditions, plant organ used, extraction method, solubility and the degree of plant maturation [50, 58].

3.1.3. LC-MS/MS analysis

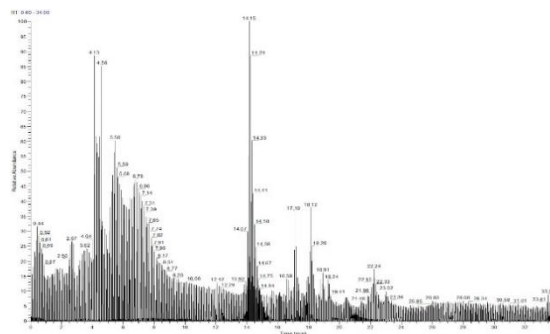


Figure 1: LC-MS/MS profile of *n*-BuOH extract of *Capparis spinosa* aerial parts (BECS)

Table 3.

LC-MS/MS analysis of the phenolic constituents of *n*-BuOH extract of *Capparis spinosa* (BECS).

N°	Name	MS/MS ions studied				LOD	LOQ	RT	mg phenolic/Kg Extract
		Parent	Product	CE	Polarity				
1	p-Hydroxybenzoic acid	137,9	66,6	38	-	0,243	0,519	14,64	313,392
			94,6	17	-				
2	Genticic acid	153,7	109,5	21	-	0,026	0,039	13,87	150,402
			80,5	25	-				
3	Gallic acid	169,7	126,2	16	-	0,058	0,091	10,11	132,519
			135,2	27	-				
4	Cafeic acid	179,7	136,2	18	-	0,042	0,058	15,26	159,115
			153	33	+				
5	Kaempferol	286,97	165	28	+	0,188	0,447	21,92	36,519
			152,1	23	-				
6	Quercetin	301	179,9	20	-	0,141	0,181	20,51	487,543
			152,1	23	-				
7	Chlorogenic acid	353,4	192,1	21	-	0,051	0,072	14,25	5659,158
			134,3	44	-				
8	Rosmarinic acid	359,18	162,2	20	-	0,029	0,050	17,86	25,049
			136,3	48	-				
9	Skoric acid	473,1	180,1	29	-	0,090	0,237	15,21	278,486
			300,6	38	-				
10	Rutin	609,37	301,7	34	-	0,022	0,034	18,04	2037,19
			301,7	34	-				

Several phytochemical studies that were performed on BECS, revealed differences, among phenolic contents. In our results, the LC-MS/MS profile of BECS is illustrated in (Table 3 and Figure 1). Several compounds were detected; only ten phenolic compounds were identified in the BECS by comparing their retention times with those of available commercial standards. The most abundant was chlorogenic acid (5659.158 mg/kg extract) and rutin (2037.19 mg/Kg), followed by quercetin (487.543 mg/Kg), *p*-hydroxy benzoic acid (313.392 mg/Kg), sisoric acid (278.486 mg/Kg), caffeic acid (159.115), gentisic acid (150.402 mg/Kg), gallic acid (132.519 mg/Kg), kaempferol (36.519 mg/Kg) and rosmarinic acid (25.05 mg/Kg). In agreement with our findings, several studies have noted that rutin is the most abundant in *C. spinosa*. Our results corroborate those obtained by Boudries *et al.* [51], reported the presence of five common products and other different compounds in which rutin had the highest concentration in the ethanol extract from flower buds of *C. spinosa* (Bejaia region; North Algeria). Mollica *et al.* [20] reported that methanolic extract from the leaf and buds of *C. spinosa* contains a large amount of flavonoids in which rutin has been determined as a major flavonoid along with other different compounds. On the other hand, Stefanucci *et al.* [25] and Mollica *et al.* [59] also found that rutin was as predominant phenolic compound in *C. spinosa* buds harvested from different areas. In general, different compounds such as quercetin, kaempferol, rutin (quercetin-3-*O*-rhamnoglucoside), kaempferol-3-*O*- β -rutinoside (kaempferol-3-*O*-rhamnoglucoside), kaempferol-3-rhamnosyl-rutinoside, have been reported in many parts of the *C. spinosa* plant, such as leaves, seeds, berries and flower buds [60-63] at different concentrations. Despite the fact that *C. spinosa* are generally known for their richness in rutin, the specific phenolic composition of *C. spinosa* extracts is dependent on several factors, which may be due to the used technique, the type of solvents, and the origin of samples, as has been previously reported for many species [56, 62, 63].

3.2. Cytotoxicity assay

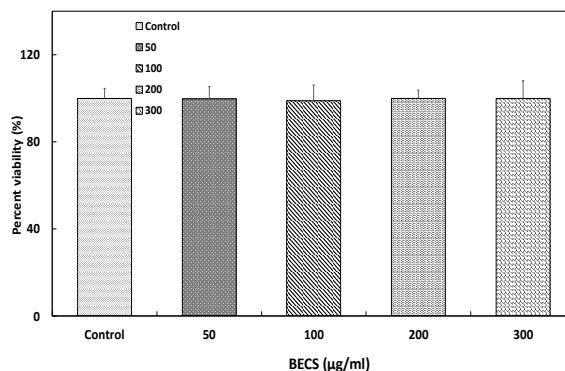


Figure 2: The cytotoxicity of the *n*-BuOH extract of *Capparis spinosa* aerial parts (BECS) against human brain cancer cell lines C6 (SRB assay).

This is just a preliminary toxicity testing of the studied BECS. The cytotoxicity of the BECS was performed against human brain cancer cell lines C6, (Figure2). From the results, it is clear that BECS showed no toxicity against C6 brain cancer cell lines. Several data are currently available regarding the potential adverse effects of *C. spinosa*. Literature reports suggested that *C. spinosa* was safe, in a 2-month clinical trial; diabetic patients given *C. spinosa* fruit extract (400 mg three times a day) showed no signs of adverse effects [64]. An acute *in vivo* toxicity study of methanolic extracts of fruits and root barks of *C. spinosa* at oral doses ranging from 500 to 5000 mg/kg body weight showed no renal or hepatic toxicity[65]. These findings were in agreement with those found by Karanayil *et al.*[66], who reported an LD50 superior to 5000 mg/kg for *C. spinosa*.

3.3. Antidiabetic studies

Table 4.

Effect of *n*-BuOH extract of *Capparis spinosa* aerial parts (BECS) on diabetic parameters in brain of STZ-treated rats

	Glucose plasma level(mg/dl)	plasma insulin level (pg/dl)	Brain insulin (pg/L)	C-peptide (ng/mL)	GlycHb (%)	PlasmaAGE (AU/mg protein)
Control	98.75±5.3	65.75±4.3	59.6±4.2	1.14±0.25	3.5±0.8	1.09±0.01
BECS	96.98±4.8	66.80±4.8	58.7±4.1	1.12±0.11	3.8±0.2	1.05±0.02
STZ	368.75±10 ^{***}	39.33±3.18 ^{***}	42.3±3.8 ^{***}	0.53±0.01 ^{***}	14.23±1.8 ^{***}	2.11±0.01 ^{***}
STZ+Metf	166.3±6.3 ^{***EE} (74.98 %)	60.1±4.1 ^{***EE} (78.61 %)	56.51±1.08 ^{***EE} (79.76 %)	1.02±0.2 ^{***EE} (80.32 %)	9.96±1.02 ^{***EE} (75.43 %)	1.2±0.08 ^{££} (89.21 %)
STZ+BECS	180.2±4.8 ^{***EE§§} (69.83 %)	58.22±5.2 ^{***EE} (73.16 %)	54.3±4.2 ^{***EE§§} (74.56 %)	0.9±0.01 ^{££} (65.69 %)	10.4±1.04 ^{***EE} (69.65 %)	1.33±0.05 ^{***EE} (75.49 %)

Values are mean ± SD, (n = 12),

*: Comparison of groups vis controls; (ns: no significant); (**p*<0.05); (***p*<0.01).

¥: Comparison of groups vis BECS; (¥*p*<0.05); (¥¥*p*<0.01).

£: Comparison of groups vis STZ-group; (£*p*<0.05); (££*p*<0.01).

§: Comparison between STZ + Met-group and STZ + BECS-group; (§*p*<0.05); (§§*p*<0.01).

Values in parentheses indicate percent protection. The % of protection is calculated as: $100 \times (\text{values of STZ}) - \text{values of samples} / (\text{values of STZ}) - \text{values of control}$.

3.3.1. Effect of BECS on blood glucose level in diabetic rats

In our study, the induction of diabetes was confirmed by elevated fasting blood glucose levels [67]. The level of blood glucose of STZ-rats reached 368.75 ± 10 mg/dL throughout the 28 days experimental period, compared to the control group (98.75 ± 5 mg/dL) (Table 4). The results of the current study indicated that the administration of BECS (200 mg/kg b.w.) for a period of 28 days significantly alleviated (69.83 %) the adverse effects of STZ-induced diabetes. The decrease in blood glucose of BECS was similar to that of standard antidiabetic drug metformin, (74.98 %). In experimental diabetes, different parts of the *C. spinosa* plant have been shown to be very effective in controlling blood glucose [54, 68]. The possible mechanism by which BECS mediates its antihyperglycemic action may be through potentiation of pancreatic insulin secretion from islet β cells or through enhancement of blood glucose transport to peripheral tissue [69].

3.3.2. Effect of BECS on serum and brain insulin levels and plasma C-peptide level

STZ has been used as an inducing agent to induce diabetes mellitus by causing selective damage to pancreatic insulin-generating β cells [70]. In the central nervous system (CNS), insulin is involved not only in regulating glucose output, but also in maintaining neuronal survival and development [71]. Disorders of the insulin signaling pathway in the periphery and brain have been implicated in diabetes [72]. Therefore, insulin deficiency may play a driving role in the development of diabetic encephalopathy in the STZ-induced animal model. In the present study, induction of diabetes with STZ, resulted in approximately 1.67-fold reduction in serum insulin level (39.33 ± 3.18 pg/dL; $p < 0.01$) and in brain insulin (42.3 ± 3.8 pg/dL; $p < 0.01$) compared to control rats (65.75 ± 4.3 pg/dL; $p < 0.01$) and (59.6 ± 4.2 pg/dL; $p < 0.01$) respectively (Table 4). *C. spinosa* treated - group showed a significant ($p < 0.01$) improvement in the serum and brain insulin (73.16 %; 74.56 %; $p < 0.01$) respectively, similar to that of metformin (78.61 %; 79.76 %; $p < 0.01$) respectively (Table 4). In STZ diabetic-group, the induced pathological lesion in islets of langerhans of pancreas was resulted in low insulin secretion from β cells in comparison to that of the normal control group. Insulin has been reported to have the ability to cross the blood-brain barrier and is abundantly detected in the brain [73]. It should be mentioned that the amount of insulin in the CSN is much lower than the amount of insulin in the serum; although their levels are correlated, we therefore know that the most important source of insulin to the

brain is its transport across the blood-brain barrier [74]. Long-term DM can disrupt the structure of the blood-brain barrier resulting in vascular damage [75]. From the current study we speculate that *C. spinosa* treatment look like the effect of metformin that may crosses the blood-brain barrier fast [76]. C-peptide which secreted by pancreatic cells [77] is one of the factors reflecting insulin deficiency [78]. The lower levels of C-peptide and decreased beta cell function have been linked to greater levels of glucose variability [79], therefore, C-peptide is a useful for assessing pancreatic beta cells function [80, 81]. As shown in (Table 4), induction of diabetes with STZ resulted in approximately 2.15-fold reduction in serum C-peptide level (0.53 ± 0.01 ng/mL) compared to control rats (1.14 ± 0.25 ng/mL). Serum C-peptide levels reflect the absolute amount of endogenous insulin secretion [82]. The BECS-treated group showed significant ($p < 0.01$) improvement in the serum C-peptide (60.65 %) as compared to metformin-treated rats with (80.32 %) (Table 4). Preliminary phytochemical screening of aerial part of *C. spinosa* revealed the presence of a wide range of natural compounds such as alkaloids, saponins that have been mentioned as ant diabetic agents, in diabetic rats or clinical trials [83, 84]. Interestingly, the possible synergistic interactions of the phenolic compounds contained in BECS, mainly quercetin, rutin, 4-hydroxybenzoic, and chlorogenic acid may be beneficial [85].

3.3.3. Effect BECS on AGEs and HbA1c levels

AGEs and glycated hemoglobin are other markers of oxidative stress and subsequent protein damage under DM conditions. Excess glucose availability promotes increased production of AGEs [86]. AGEs which have been considered as possible biomarkers for diagnosing diabetes complications, are generated by a non-enzymatic reaction of glucose using amino groups on protein amino acids during diabetes [87]. Hyperglycemia which is a clinical feature of poorly controlled diabetes mellitus, results in increased protein glycation resulting in structural and functional alteration of proteins, including hemoglobin [88]. In our study, we observed an increase (2.11 ± 0.1 AU/ mg protein) in the AGEs level under the STZ-treatment compared with the control group (1.09 ± 0.01 AU/ mg protein) (Table 4). BECS showed a significant ($p < 0.01$) improvement in the serum AGE levels (75.49 %) as compared to metformin (89.21 %). The amount of glycated haemoglobin (% GHb) was also increased (14.23 ± 1.8 %; 4.06-fold) compared with the control group (3.5 ± 0.8 %). In the present study, the HbA1c level was significantly elevated in diabetic rats, which is in agreement with previous studies [89, 90]. Nonetheless, BECS significantly lowered (69.65 %; $p < 0.05$) HbA1c compared to metformin (75.43 %; $p < 0.05$). The high level of HbA1c is due to the

persistently high blood glucose level, which leads to hemoglobin glycation [91]. These results suggest that BECS may prevent formation of Amadori products [92] that can lead to protein oxidation and indicate that the overall blood glucose level is controlled, which must be due to improvement in insulin secretion as noted above. Moreover, BECS could directly decrease the formation of glycated hemoglobin, possibly due to antioxidant activity [93]. These findings are also, alongside the study Huseini *et al.* [64] reported that patients who took 1200 mg *C. spinosa* fruit extracts daily for 2 months had a significantly lower glycosylated hemoglobin levels. Interestingly, the anti-AGE effect of BECS could be due to its richness in polyphenols identified by LC-MS/MS analysis, in particular the most abundant and potent agents reported; chlorogenic acid and rutin which have all been reported to significantly inhibit glucose-mediated protein modification, the primary inhibitors of AGEs [94, 95].

3.4. Effect of BECS on neurodegeneration

3.4.1. Effects of BECS on LDH activity, on MDA and CK-BB Levels.

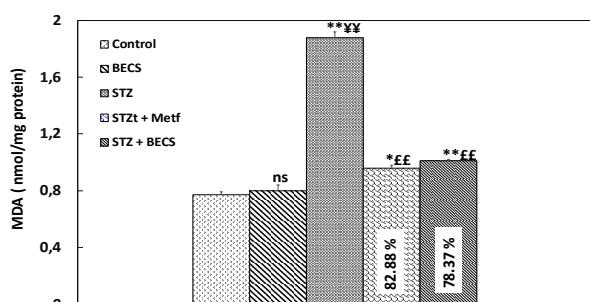


Figure 3: The effect of *n*-BuOH extract of *Capparis spinosa* aerial parts (BECS) (200 mg/Kg) on MDA level in the brain of STZ treated -group.

Values are mean \pm SD, (n = 12),

: Comparison of groups vis controls; (ns: no significant); (p <0.05); (** p <0.01).

‡: Comparison of groups vis BECS; (‡ p <0.05); (‡‡ p <0.01).

£: Comparison of groups vis STZ-group; (£ p <0.05); (££ p <0.01).

§: Comparison between STZ + Metf-group and STZ + BECS-group; (§ p <0.05); (§§ p <0.01).

Values in box indicate percent protection. The % of protection is calculated as: 100 x (values of STZ)-values of samples/ (values of STZ)-values of control.

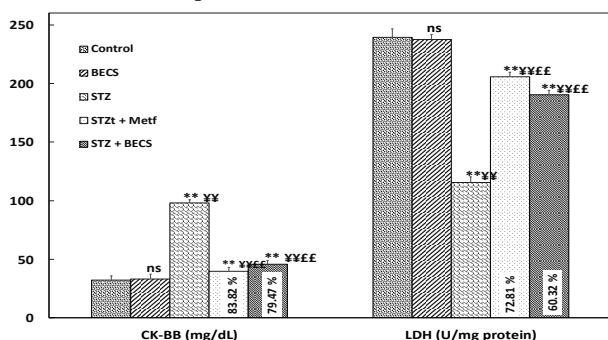


Figure 4: The effect of *n*-BuOH extract of *Capparis spinosa* aerial parts (BECS)(200 mg/Kg) on CK-BB level and LDH activity in the brain of STZ- treated group

Values are mean \pm SD, (n = 12),

: Comparison of groups vis controls; (ns: no significant); (p <0.05); (** p <0.01).

‡: Comparison of groups vis BECS; (‡ p <0.05); (‡‡ p <0.01).

£: Comparison of groups vis STZ-group; (£ p <0.05); (££ p <0.01).

§: Comparison between STZ + Metf-group and STZ + BECS-group; (§ p <0.05); (§§ p <0.01).

Values in box indicate percent protection. The % of protection is calculated as: 100 x (values of STZ)-values of samples/ (values of STZ)-values of control.

Figure 3, showed that the level of brain MDA was significantly (P <0.01) increased in STZ-diabetic rats by about 2.44 fold. LDH is associated with adverse clinical outcomes of traumatic brain injury. Neurons are largely dependent on lactate as the main energy substrate which also promotes neuroprotection [96, 97]. However, LDH activity was decreased approximately 2.06 times, compared to healthy rats (Figure 4). STZ-treated rats showed a reduction in brain LDH activity, which signified a loss of cell viability. Our results support the results of Kumar and Bansal [98], who found that central administration of STZ impaired neural coherence evident by increased brain LDH activity. LDH leakage may be due to the oxidation of PUFAs making them more hydrophilic, which changes the structure of the membrane [99]. These PUFAs are rich in the brain and cause oxygen free radical origin. Increase in ROS level results in elevated MDA level causes death of neurons by oxidizing various components of the cellular system [100]. Treatment with the BECS (200 mg/kg) decreased significantly (P <0.01) the level of MDA up to 78.37 %; and restored (60.97 %) LDH activity as compared to metformin (82.88 %; 72.81 %) respectively (Figure 3, 4). The increase in brain LDH activity seen in the BECS+STZ group may manifest the resurrection of neuronal membrane integrity. CK-BB found in astrocytes plays a role in energy transfer in tissues with high-energy requirement such as brain [101]. As shown in Figure 4, induction of diabetes with STZ resulted in approximately 3.05-fold reduction in serum CK-BB level compared to control rats, *C. spinosa*-treated group showed significant improvement (p <0.01) serum CK-BB (79.47%) compared to metformin (83.82%) (Figure 4). It has been reported that serum CK-BB levels can increase in various brain injury cases, and trigger neurodegenerative events that lead to neuronal losses [102, 103].

3.4.2. Effect of BECS on neurochemical markers: AChE, BChE, dopamine and glutamate levels

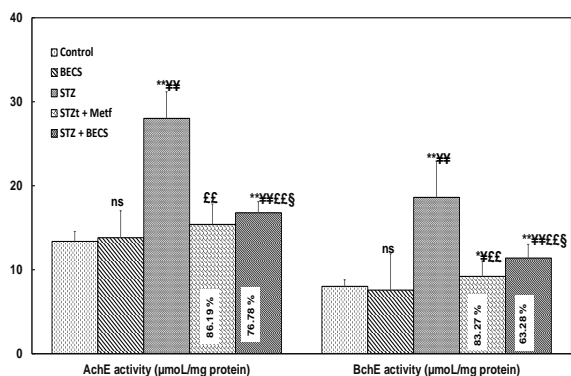


Figure 5a: The effect of *n*-BuOH extract of *Capparis spinosa* aerial parts (BECS), (200 mg/Kg) on AchE activity and BchE activity in the brain of STZ-treated group.

Values are mean \pm SD, (n = 12),

: Comparison of groups vis controls; (ns: no significant); (p <0.05); (** p <0.01).

¥: Comparison of groups vis BECS; (¥ p <0.05); (¥¥ p <0.01).

£: Comparison of groups vis STZ-group; (£ p <0.05); (££ p <0.01).

§: Comparison between STZ + Met-group and STZ + BECS-group; (§ p <0.05); (§§ p <0.01).

Values in box indicate percent protection. The % of protection is calculated as: 100 x (values of STZ)-values of samples/ (values of STZ)-values of control.

The development of diabetic encephalopathy is correlated with persistent hyperglycemia and generation of ROS, which cause cerebral angiopathy as well which cause cerebral angiopathy as well as neuron and glial cell abnormalities in brain tissue [104]. The manifestations of these disorders in diabetic patients include; alterations in neurotransmission and cognitive deficit [105]. Insulin has been reported to exert some effects on several neurotransmitters involved in memory formation, such as the acetylcholine line [106]. AChE and BChE are two major forms of cholinesterase implicated in the pathogenesis of neurological disorders [107]. In the present study, no significant change (p >0.05) was noted when compare to the normal control, BECS and metformin groups in the BChE and AChE activities, STZ administration to rat induced marked brain impairment, as evidenced by significant (p <0.01) elevation of AChE (28 ± 3.2 $\mu\text{mol/mg}$ protein) and BChE (18.6 ± 4.3 $\mu\text{mol/mg}$ protein) levels and in the cerebral tissue of STZ-group, when compared to the control group (13.35 ± 1.2 $\mu\text{mol/mg}$ protein; 7.99 ± 0.8 $\mu\text{mol/mg}$ protein) respectively. BECS treatment (200 mg/kg) or metformin treatment (200 mg/kg) daily for 28 days, significantly (p <0.01) inhibited (63.18 % or 83.27 %) the BChE and AChE (76.70 % or 86.19 %) activities in the brain

respectively, (Figure 5a). As mentioned above in this study, STZ exhibited an insulin deficiency, therefore, the stimulatory effect of insulin actions on protein synthesis may be disrupted, thus leading to neurological disorders associated with cognitive dysfunction [108]. The STZ gives rise to a significant increase in the AChE activity, an indicative of declining cholinergic system. Our conclusion above confirms the hypothesis of Suzuki and Clayton [109], mentioning that oxidative brain damage is a long-term complication, therefore it could lead to a decrease in the efficiency of cholinergic neurotransmission, due to the decrease levels of acetylcholine in the synaptic cleft of diabetic rats brain. High cholinesterase activity leads to non-degradation of ACh; a neurotransmitter that improves cognitive functions and strengthens nerve and gland cells in the brain [10]. The observed cholinesterase inhibition exhibited by BECS or metformin could prevent the rapid breakdown of ACh, making it more available for transmission of nerve impulses through neurons. A study by Mollica *et al.* [59] revealed the *in vitro* cholinesterase inhibitory activities of *Capparis*. In accordance with our hypothesis, some researchers have reported that rutin is an effective AChE and BChE inhibitor [110, 111].

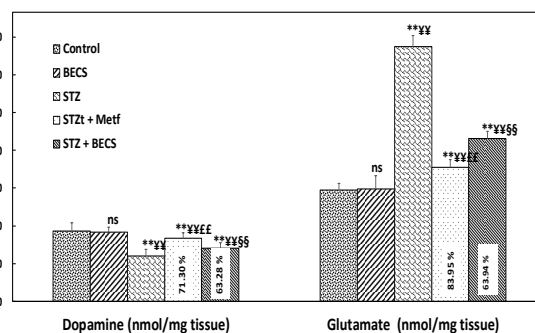


Figure 5b: The effect of *n*-BuOH extract of *Capparis spinosa* aerial parts (BECS), (200 mg/Kg) on Dopamin and Glutamate levels in the brain of STZ-treated group.

Values are mean \pm SD, (n = 12),

: Comparison of groups vis controls; (ns: no significant); (p <0.05); (** p <0.01).

¥: Comparison of groups vis BECS; (¥ p <0.05); (¥¥ p <0.01).

£: Comparison of groups vis STZ-group; (£ p <0.05); (££ p <0.01).

§: Comparison between STZ + Met-group and STZ + BECS-group; (§ p <0.05); (§§ p <0.01).

Values in box indicate percent protection. The % of protection is calculated as: 100 x (values of STZ)-values of samples/ (values of STZ)-values of control.

An earlier study had shown that MAO is a major source of oxidative stress and that its increased

activity causes dopamine deficiency in the brain [112]. Dopamine is an important neurotransmitter that is widely distributed in the central nervous system and has been explored as a biomarker in metabolic and neurological disorders [113]. Glutamate is a major cause of neuronal cell death in a number of different neurodegenerative diseases [114]. Changes in glutamic acid concentrations seem to be of interest, as this amino acid plays a role as a neurotransmitter in the brain and is involved in the brain pathogenesis [115]. A contributing factor in many such conditions is excessive glutamate release, and subsequent glutamatergic neuronal stimulation, which leads to increased ROS production, excitotoxicity and neuronal damage [116]. In the present study, we noticed that STZ treatment depleted the level of cerebral DA (35.91 ± 4.2 ng/mg tissue); ($P < 0.01$) compared to control group (55.81 ± 6.5 ng/mg tissue) (Figure 5, b), but increased the glutamate level (202.8 ± 10.6 nmol/mg tissue; ($P < 0.01$) in cerebral tissues at the end of the study, as compared with control group (88.33 ± 5.4 nmol/mg tissue) (Figure 5 b). Treatment with BECS resulted in significant increase in DA level (67.73 % $P < 0.01$) and decrease in glutamate (63.94 %; $P < 0.01$), metformin-treatment resulted in similar effects (71.3 %; 83.95 %) respectively (Figure, 5 b). The increased inhibition of AChE and BChE activities, increased DA level and decreased glutamate after treatment with 200 mg/kg BECS extract, could be attributed to the significant amounts of its phytochemical composition, especially polyphenolic compounds (Table 2), which have been reported to exhibit cholinesterase inhibiting activities. This is consistent with the report by Rahimi *et al.* [22] which shows *C. spinosa* (100 and 300 mg/kg) as a modulator of behavioral and biochemical parameters, Mohebbi *et al.* [23] demonstrated the effects of *C. spinosa* on amyloidogenesis-related genes in A β -injected rat, Turgut *et al.* [117] showed that 100 and 200 mg/kg of *C. spinosa* improve cognitive impairments by D-galactose.

3.5. Effect of BECS on Glutathione and Glutathione-metabolizing enzymes in STZ-animals

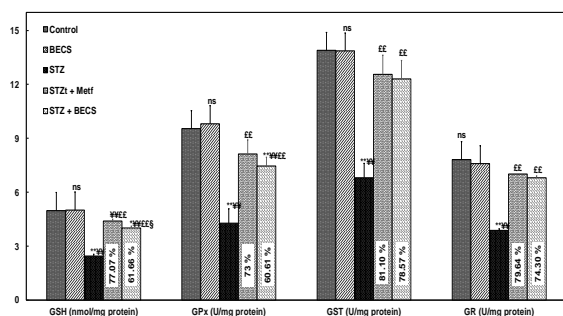


Figure 6: The effect of *n*-BuOH extract of *Capparis spinosa* aerial parts (BECS)(200 mg/Kg) on Glutathione-metabolizing enzymes in the brain of STZ-treated group.

Values are mean \pm SD, (n = 12),

: Comparison of groups vis controls; (ns: no significant); ($p < 0.05$); (** $p < 0.01$).

‡: Comparison of groups vis BECS; (‡ $p < 0.05$); (‡‡ $p < 0.01$).

£: Comparison of groups vis STZ-group; (£ $p < 0.05$); (££ $p < 0.01$).

§: Comparison between STZ + Met-group and STZ + BECS-group; (§ $p < 0.05$); (§§ $p < 0.01$).

Values in box indicate percent protection. The % of protection is calculated as: $100 \times (\text{values of STZ}) - \text{values of samples} / (\text{values of STZ}) - \text{values of control}$.

Streptozotocin is an unstable molecule that accumulates in pancreatic β -cells and breaks down into carbonium radicals; highly reactive carboxylic radicals produce direct and indirect toxic effects on pancreatic islet cells by increasing the ROS formation [118]. Several investigations have shown that STZ enters the pancreatic β -cells by glucose protein-2 transporter and disrupts the balance between antioxidant and oxidant systems damaging the insulin-producing islet β -cells and inducing the progression of diabetes [119, 120]. Reduction of endogenous antioxidant defense system can promote free radical formation, degradation of GSH-dependent antioxidant defense system and sequential alteration of cellular redox balance [121]. In the current study, STZ-treatment clearly depleted brain GSH level and reduced the GSH-related enzymes as evidenced by the decline in the GSH, GST, GR, and GPx levels ((Figure 6). Treatment of rats with 200 mg/kg of BECS efficacy restored the GSH level and GSH-system enzymes towards normal levels. A marked response (61.66 %) of GSH was observed in the brain of BECS-STZ treated rats as compared to metformin treatment (70.07 %). Treatment with *C. spinosa* reversed (60.61 %) GPx, (78.57 %) GST, and GR (74.30 %) levels. The efficacy of BECS was comparable to that of metformin in restoring GPx (73 %), GST (81.10 %) and GR (79.64 %) (Figure 6).

Glutathione which is a tripeptide of glutamate, cysteine and glycine, occurring in neurons at concentrations of 0.2–2 mM [122], is a major antioxidant and redox regulator in cells [123]. In addition to its essential roles in redox homeostasis, it functions as cofactors for a multitude of enzymes [124]. Persson *et al.* [125] showed that glutathione synthesis was directly related to microglial glutamate uptake and the release of glutamate metabolites. Barger *et al.* [126] showed that the depletion of glutathione levels due to oxidative stress is linked to microglial glutamate release. And that in addition to

its support of antioxidant function, the glutathione cycle also serves as a reservoir of intracellular neural glutamate [127]. Several studies have hypothesized that glutathione synthesis is directly dependent on glucose metabolism, which is correlates with a decrease in the content of glutamate precursor of glutathione precursor [128, 129]. Another possible explanation for the loss of brain GSH, is that glutamate affects its level, where the high extracellular concentration of glutamate blocks the uptake of cysteine (an essential amino acid for sustained glutathione synthesis). Consequently, this blockade eventually decreases glutathione concentration [130]. Our above findings on glutamate levels under STZ treatment, confirm that depletion of glutathione levels under oxidative stress may be related to microglial glutamate release. The restoration effect of the BECS on the glutathione, and glutathione-metabolizing enzymes, could be due to the antioxidant properties of the compounds contained in BECS that enhance the ROS elimination. The over expression of the brain glutathione antioxidant system in BECS-treated rats suggests that this potential antioxidant defense may be reactivated by Plant phenolic compounds such as flavonoid [131]. The whole body of data collected in the past decades on the chemical composition of *C. spinosa*, has led to evidence that the plant is a rich source of bioactive compounds, mostly polyphenolic compounds [21]. Moreover, as mentioned above in the current study from the LC-MS/MS data, BECS is rich in many different phenolic antioxidants, among which the most abundant are; chlorogenic acid, rutin and a small amount of other compounds (quercetin, *p*-hydroxy benzoic acid, caffeic acid, gentisic acid, gallic acid, kaempferol and rosmarinic acid; which have property of antioxidant activities [132-134].

3.6. Effect of BECS on NO and pro-inflammatory cytokines in the brain

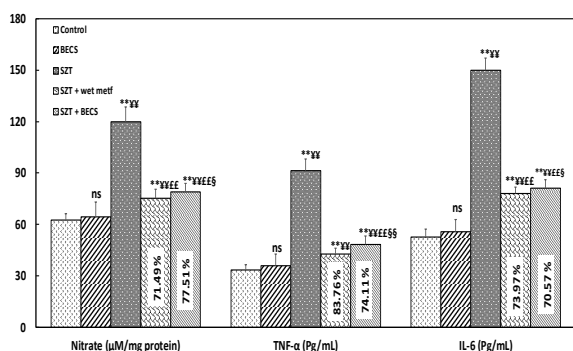


Figure 7: The effect of *n*-BuOH extract of *Capparis spinosa* aerial parts (BECS)(200 mg/Kg) on inflammatory parameters in the brain of STZ-treated group.

Values are mean \pm SD, (n = 12),

: Comparison of groups vis controls; (ns: no significant); (p <0.05); (** p <0.01).

¥: Comparison of groups vis BECS; (¥ p <0.05); (¥¥ p <0.01).

£: Comparison of groups vis STZ-group; (£ p <0.05); (££ p <0.01).

§: Comparison between STZ + Met-group and STZ + BECS-group; (§ p <0.05); (§§ p <0.01).

Values in box indicate percent protection. The % of protection is calculated as: 100 x (values of STZ)-values of samples/ (values of STZ)-values of control.

DM propagation is associated with a chain of molecular events, including inflammation which has been shown to be a key factor in this disease [135]. Chronic hyperglycemic conditions could affect the neuronal metabolism of astrocytes and microglia which have been shown to elaborate inflammatory cytokines in the brain [136]. The diabetogenic and cytotoxic effects of STZ are associated with the production of free radicals causing oxidative damage to cells and also coupled with propagation of diabetes-linked autoimmunity [137, 138]. High reactivity of ROS exerts toxic effects on pancreatic acinar cells and is linked to the production of inflammatory mediators [139]. There is a strong body of evidence that hyperglycemia induces the activation of microglia and astrocyte which correlates with increased expression of inflammatory and oxidative stress markers such as TNF- α , INOS, ROS, and other pro-inflammatory cytokines [140]. TNF- α is one of the first pro-inflammatory biomarkers to be associated with the pathogenesis of insulin resistance and T2D-related glucose abnormalities and plays an important role in ROS production [141]. IL-6 is also produced in microglia, astrocytes and neurons, and it plays a pivotal role in various CNS functions such as induction and modulation of astrocyte reactivation, pathological inflammation and neuroprotection [142]. To examine if the BECS treatment exerted an anti-inflammatory effect, we analyzed the relative abundance of some pro-inflammatory cytokines such as IL-6 and TNF- α , (Figure 7). Regarding the levels of IL-6 and TNF- α , it was found that the STZ-group had a significantly (p <0.01) high level (149.8 ± 7.1 ; 91.41 ± 6.8 pg/mL) compared to normal control levels (p <0.01) 52.6 ± 4.8 ; 33.38 ± 3.1 pg/mL; (p <0.01) respectively (Figure 7). Meanwhile, treatment of diabetic rats with BECS or metformin significantly (p <0.01), reduced IL-6 (70.57 %) and TNF- α , (74.11 %) levels as compared to metformin-rats (73.97 %; 83.76 %; p < 0.01) respectively. Our results corroborate those mentioned, increased of IL-6, TNF- α have been reported in T2DM [143, 144]. In addition to the mechanisms mentioned above, another important mechanism by which β cells are partially destroyed is the formation of nitric oxide free radicals [145]. It has been proposed that the NO molecule

contributes to STZ-induced DNA damage and causes excess NO production through the pathological activation of iNOS which results in neurodegeneration and memory loss [146]. The treatment of diabetic rats with either BECS or metformin significantly ($p < 0.01$), reduced NO (77.51 %; 71.49 %) levels respectively. Nevertheless, excessive nitrite levels in brain leads to neurodegeneration [147] and it also potentiates NO production in astrocytes [145]. Interestingly, our findings showed that the groups receiving the 200 mg/kg dose of BECS extract had lower levels of inflammatory cytokines (IL-6 and TNF- α) throughout the brain. In this regard, *C. spinosa* has been reported to be used as therapeutic traditional medicine to relieve various ailments [148, 149] such as rheumatism, rheumatoid arthritis and gout [150]. These results were consistent with other studies related to the anti-inflammatory effect of different types of *C. spinosa*, Moutia et al. [151] showed that aerial part of *C. spinosa* (100 and 300 mg/kg) up-regulates the gene expression of an anti-inflammatory cytokine interleukin (IL)-4, Mohebbali et al. [23] revealed that *C. spinosa* down-regulated the genes involved in inflammation. In the current study, the anti-inflammatory effect of BECS could be due to its richness in polyphenols identified by LC-MS/MS analysis. The most abundant and potent agents reported were chlorogenic acid and rutin, have been reported to be the major anti-inflammatory agents [152]. Possible synergistic interactions of all contained phenolic compounds have shown anti-inflammatory properties.

Conclusion

Altogether, the results presented here demonstrate, for the first time, that treatment with *n*-BuOH extract of *Capparis spinosa* (BECS) can prevent the DM-neuropathy induced with STZ, that could be mostly attributed to different mechanisms, such as reducing the blood glucose, improvement in the serum and brain insulin, the serum C-peptide, and the serum CK-BB levels. BECS was also successful in restricting HbA1c, resurrection of neuronal membrane integrity through restoration of LDH, attenuation of markers of brain dysfunction (AChE and BChE activities; glutamate level) and increased dopamine level. Moreover, the effectiveness of the BECS treatment restored GSH level and GSH-system enzymes. And successfully regulated the diabetogenic production of the inflammatory mediators IL-6, TNF- α , NO and MPO. From all of these results, the neuro-protective effect of BECS could be due to its richness in polyphenols identified by LC-MS/MS analysis, mainly chlorogenic acid and rutin, as well as the synergistic interactions of all the constituents.

Abbreviations

DN: Diabetic neuropathy, STZ: streptozotocin, BECS: *n*-BuOH extract of *Capparis spinosa*, AGE: Glycation End Products, LC-MS/MS: Liquid-chromatography tandem mass spectrometry, SRB: Sulforhodamine B, Met :metformin CK-BB: Creatine Kinase BB, TNF- α : *Tumor Necrosis factor*, IL-6: Interleukin-6, MDA: malondialdehyde, HbA1c: *hemoglobin A1c*, AChE : Aetylcholinesterase , BChE: Butyrylcholinesterase , LDH: Lactate dehydrogenase ,GST: Glutathione-S-transferase, GPx: Glutathione peroxidase ,GR:Glutathione reductase, MAO: Monoamine oxidase, NO: Nitric Oxide

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding and Acknowledgements

We thank Algerian government for financial support. The project was funded by the Ministry of Higher Education and Scientific Research of Algeria. Grant n°: D01N01UN250120190002

4. References

- [1] Lotfy, M., Adeghate, J., Kalasz, H., Singh, J., & Adeghate, E. Chronic complications of diabetes mellitus: a mini review. *Curr Diabetes Rev*, 13(1), 3-10, 2017, doi.org/10.2174/1573399812666151016101622
- [2] Giannoulaki, P., Kotzakioulafi, E., Chourdakis, M., Hatzitolios, A., & Didangelos, T. Impact of *Crocus sativus L.* on metabolic profile in patients with diabetes mellitus or metabolic syndrome: A systematic review. *Nutrients*, 12(5), 1424, 2020, doi.org/10.3390%2Fnu12051424
- [3] Figueroa-Romero, C., Sadidi, M., & Feldman, E. L. Mechanisms of disease: the oxidative stress theory of diabetic neuropathy. *Rev Endocr Metab Disord*, 9(4), 301-314, 2008, doi.org/10.1007/s11154-008-9104-2
- [4] Liu, S. Y., Chen, L., Li, X. C., Hu, Q. K., & He, L. J. *Lycium barbarum* polysaccharide protects diabetic peripheral neuropathy by enhancing autophagy via mTOR/p70S6K inhibition in Streptozotocin-induced diabetic rats. *J Chem Neuroanat*, 89, 37-42, 2018, doi.org/10.1016/j.jchemneu.2017.12.011
- [5] Shinohara, M., & Sato, N. The roles of apolipoprotein E, lipids, and glucose in the pathogenesis of Alzheimer's disease. *Diabetes Mellitus*, 85-101, 2019, doi.org/10.1007/978-981-13-3540-2_5
- [6] Sivitz, W. I., & Yorek, M. A. Mitochondrial dysfunction in diabetes: from molecular mechanisms to functional significance and therapeutic opportunities. *Antioxid Redox*

- Signal*, 12(4), 537-577, 2010, doi.org/10.1089/ars.2009.2531
- [7] Gratas-Delamarche, A., Derbré, F., Vincent, S., & Cillard, J. Physical inactivity, insulin resistance, and the oxidative-inflammatory loop. *Free Radic Res*, 48(1), 93-108, 2014, doi.org/10.3109/10715762.2013.847528
- [8] Perez Gutierrez, R. M., & de Jesus Martinez Ortiz, M. Beneficial effect of *Azadirachta indica* on advanced glycation end-product in streptozotocin-diabetic rat. *Pharm Biol*, 52(11), 1435-1444, 2014, doi.org/10.3109/13880209.2014.895389
- [9] Vázquez, M. D., Sánchez-Rodríguez, F., Osuna, E., Diaz, J., Cox, D. E., Pérez-Cárceles, M. D., ... & Pounder, D. J. Creatine kinase BB and neuron-specific enolase in cerebrospinal fluid in the diagnosis of brain insult. *Am J Forensic Med Pathol*, 16(3), 210-214, 1995, doi.org/10.1097/00000433-199509000-00004.
- [10] Sonkusare, S., Srinivasan, K., Kaul, C., & Ramarao, P. Effect of donepezil and lercanidipine on memory impairment induced by intracerebroventricular streptozotocin in rats. *Life Sci*, 77(1), 1-14, 2005, doi.org/10.1016/j.lfs.2004.10.036
- [11] Hamed, S. A. Brain injury with diabetes mellitus: evidence, mechanisms and treatment implications. *Expert Rev Clin Pharmacol*, 10(4), 409-428, 2017, doi.org/10.1080/17512433.2017.1293521
- [12] Dai, Y., & A Kamal, M. Fighting Alzheimer's disease and type 2 diabetes: pathological links and treatment strategies. *CNS Neurol Disord Drug Targets*, 13(2), 271-282, 2014, doi.org/10.2174/18715273113126660134
- [13] Adler, B. L., Yarchoan, M., Hwang, H. M., Louneva, N., Blair, J. A., Palm, R., ... & Casadesus, G. Neuroprotective effects of the amylin analogue pramlintide on Alzheimer's disease pathogenesis and cognition. *Neurobiol Aging*, 35(4), 793-801, 2014, doi.org/10.1016/j.neurobiolaging.2013.10.076
- [14] Salazar-García, M., & Corona, J. C. The Use of Natural Compounds as a Strategy to Counteract Oxidative Stress in Animal Models of Diabetes Mellitus. *Int J Mol Sci*, 22(13), 7009, 2021, doi.org/10.3390/ijms22137009
- [15] Nabavi, S. F., Maggi, F., Daglia, M., Habtemariam, S., Rastrelli, L., & Nabavi, S. M. Pharmacological effects of *Capparis spinosa* L. *Phytother Res*, 30(11), 1733-1744, 2016, doi.org/10.1002/ptr.5684
- [16] Arrar, L., Benzidane, N., Krache, I., Charef, N., Khennouf, S., & Baghiani, A. Comparison between polyphenol contents and antioxidant activities of different parts of *Capparis spinosa* L. *Pharmacogn Commun*, 3(2), 70, 2013, doi.org/10.5530/pc.2013.2.14
- [17] Zhou, H., Jian, R., Kang, J., Huang, X., Li, Y., Zhuang, C., ... & Wu, X. Anti-inflammatory effects of caper (*Capparis spinosa* L.) fruit aqueous extract and the isolation of main phytochemicals. *J Agric Food Chem*, 58(24), 12717-12721, 2010, doi.org/10.1021/jf1034114
- [18] Zhu, X., Yang, Y., Gao, W., Jiang, B., & Shi, L. *Capparis spinosa* alleviates DSS-induced ulcerative colitis via regulation of the gut microbiota and oxidative stress. *J Evid Based Complementary Altern Med*, 2021, 2021, doi.org/10.1155/2021/1227876
- [19] Kazemian, M., Abad, M., reza Haeri, M., Ebrahimi, M., & Heidari, R. Anti-diabetic effect of *Capparis spinosa* L. root extract in diabetic rats. *Avicenna J Phytomed*, 5(4), 325, 2015.
- [20] Mollica, A., Zengin, G., Locatelli, M., Stefanucci, A., Mocan, A., Macedonio, G., ... & Novellino, E. Anti-diabetic and anti-hyperlipidemic properties of *Capparis spinosa* L.: in vivo and in vitro evaluation of its nutraceutical potential. *J Funct Foods*, 35, 32-42, 2017, doi.org/10.1016/j.jff.2017.05.001
- [21] Vahid, H., Rakhshandeh, H., & Ghorbani, A. Antidiabetic properties of *Capparis spinosa* L. and its components. *Biomed Pharmacother*, 92, 293-302, 2017, doi.org/10.1016/j.biopha.2017.05.082
- [22] Rahimi, V. B., Rajabian, A., Rajabi, H., Vosough, E. M., Mirkarimi, H. R., Hasanpour, M., ... & Askari, V. R. The effects of hydro-ethanolic extract of *Capparis spinosa* (*C. spinosa*) on lipopolysaccharide (LPS)-induced inflammation and cognitive impairment: evidence from in vivo and in vitro studies. *J Ethnopharmacol*, 256, 112706, 2020, doi.org/10.1016/j.jep.2020.112706
- [23] Mohebali, N., Shahzadeh Fazeli, S. A., Ghafouri, H., Farahmand, Z., MohammadKhani, E., Vakhshiteh, F., ... & Sanati, M. H. Effect of flavonoids rich extract of *Capparis spinosa* on inflammatory involved genes in amyloid-beta peptide injected rat model of Alzheimer's disease. *Nutr neurosci*, 21(2), 143-150, 2018, doi.org/10.1080/1028415X.2016.1238026
- [24] Germano, M. P., De Pasquale, R., D'angelo, V., Catania, S., Silvari, V., & Costa, C. Evaluation of extracts and isolated fraction from *Capparis spinosa* L. buds as an antioxidant source. *J agric food chem*, 50(5), 1168-1171, 2002, doi.org/10.1021/jf010678d
- [25] Stefanucci, A., Zengin, G., Locatelli, M., Macedonio, G., Wang, C. K., Novellino, E., ... & Mollica, A. Impact of different geographical locations on varying profile of bioactives and

- associated functionalities of caper (*Capparis spinosa* L.). *Food Chem Toxicol*, 118, 181-189, 2018,doi.org/10.1016/j.fct.2018.05.003
- [26] Qu  zel, P., Santa, S., & Schotter, O. Nouvelle flore de l'Algerie et des regions desertiques meridionales-v. 1-2, 1962.
- [27] Zater, H., Huet, J., Fontaine, V., Benayache, S., St  vigny, C., Duez, P., & Benayache, F. Chemical constituents, cytotoxic, antifungal and antimicrobial properties of *Centaurea diluta* Ait. subsp. algeriensis (Coss. & Dur.) Maire. *Asian Pac J trop med*, 9(6), 554-561, 2016,doi.org/10.1016/j.apjtm.2016.04.016
- [28] Harborne JB. *Phytochemical Methods. A guide to modern techniques of plant analysis*, 3rd Ed. Chapman and Hall, London, pp235, 1998.
- [29] Khandelwal KR. Preliminary phytochemical screening, in: *Practical Pharmacognosy Techniques and Experiments*. 8th ed. Nirali Publication, Pune, 149-156, 2001.
- [30] Wolfe, K., Wu, X., & Liu, R. H. Antioxidant activity of apple peels. *J Agric Food Chem*, 51(3), 609-614, 2003,doi.org/10.1021/jf020782a
- [31] Ordonez, A. A. L., Gomez, J. D., & Vattuone, M. A. Antioxidant activities of *Sechium edule* (Jacq.) Swartz extracts. *Food chem*, 97(3), 452-458, 2006,doi.org/10.1016/j.foodchem.2005.05.024
- [32] Vichai, V., & Kirtikara, K. Sulforhodamine B colorimetric assay for cytotoxicity screening. *Nat Protoc*, 1(3), 1112-1116, 2006, doi.org/10.1038/nprot.2006.179
- [33] Kilkenny, C., Browne, W., Cuthill, I. C., Emerson, M., & Altman, D. G. Animal research: reporting in vivo experiments: the ARRIVE guidelines. *Br J Pharmacol*, 160(7), 1577, 2010,doi.org/10.3390/ani4010035
- [34] Oghbaei, H., Asl, N. A., Sheikhzadeh, F., & Alipour, M. R. The effect of regular moderate exercise on miRNA-192 expression changes in kidney of streptozotocin-induced diabetic male rats. *Adv Pharm Bull*, 5(1), 127, 2015, doi.org/10.5681%2Fapb.2015.018
- [35] Putta, S., & Kilari, E. K. A review on methods of estimation of advanced glycation end products. *World J Pharm. Res*, 4(9), 689-699, 2015.
- [36] Ohkawa, H., Ohishi, N., & Yagi, K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem*, 95(2), 351-358, 1979,doi.org/10.1016/0003-2697(79)90738-3
- [37] Deshmukh, R., Sharma, V., Mehan, S., Sharma, N., & Bedi, K. L. Amelioration of intracerebroventricular streptozotocin induced cognitive dysfunction and oxidative stress by vinpocetine—a PDE1 inhibitor. *Eur J Pharmacol*, 620(1-3), 49-56, 2009,doi.org/10.1016/j.ejphar.2009.08.027
- [38] Ellman, G. L., Courtney, K. D., Andres Jr, V., & Featherstone, R. M. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem pharmacol*, 7(2), 88-95, 1961,doi.org/10.1016/0006-2952(61)90145-9
- [39] Jo  ca, J.,   uk, M., Wasa  , B., Janaszak-Jasiecka, A., Lewandowski, K., Wielgomas, B., ... & Jasiecki, J. New insights into butyrylcholinesterase activity assay: serum dilution factor as a crucial parameter. *PLoS One*, 10(10), e0139480, 2015,doi.org/10.1371/journal.pone.0139480
- [40] Ciarlone, A. E. Further modification of a fluorometric method for analyzing brain amines. *Microchem J*, 23(1), 9-12, 1978,doi.org/10.1016/0026-265X(78)90034-6
- [41] Lund, P. UV-method with glutaminase and glutamate dehydrogenase. *Methods of Enzymatic Analysis*, Vol. VIII, 357, 1984.
- [42] Sedlak, J. Changes of glutathione and protein bound SH-groups concentration in rat adrenals under acute and repeated stress. *Endocrinol Exp*, 16(2), 103-109, 1982.
- [43] Habig, W. H., Pabst, M. J., & Jakoby, W. B. Glutathione S-transferases: the first enzymatic step in mercapturic acid formation. *J Biol Chem*, 249(22), 7130-7139, 1974,doi.org/10.1016/S0021-9258(19)42083-8
- [44] Rotruck, J. T., Pope, A. L., Ganther, H. E., Swanson, A. B., Hafeman, D. G., & Hoekstra, W. Selenium: biochemical role as a component of glutathione peroxidase. *Science*, 179(4073), 588-590, 1973,doi.org/10.1126/science.179.4073.588
- [45] Moss, D. W., & Butterworth, P. J. *Enzymology and medicine*. Pitman Medical, 1974.
- [46] Classics Lowry, O., Rosebrough, N., Farr, A., & Randall, R. Protein measurement with the Folin phenol reagent. *J biol Chem*, 193(1), 265-75, 1951.
- [47] Berkels, R., Purol-Schnabel, S., & Roesen, R. Measurement of nitric oxide by reconversion of nitrate/nitrite to NO. In *Nitric Oxide Protocols* (pp. 1-8), Humana Press, 2004.
- [48] Yang, T., Liu, Y. Q., Wang, C. H., & Wang, Z. T. Advances on investigation of chemical constituents, pharmacological activities and clinical applications of *Capparis spinosa*. *Zhongguo Zhong yao za zhi = China journal of Chinese materia medica*, 33(21), 2453-2458, 2008.
- [49] Rajesh, P., Latha, S., Selvamani, P., & Kannan, V. R. Phytochemical screening and toxicity studies on the leaves of *Capparis sepiaria* Linn.(Capparidaceae). *JBCP*, 1(1), 2010,

- [50] Burri, S. C., Ekholm, A., Håkansson, Å., Tornberg, E., & Rumpunen, K. Antioxidant capacity and major phenol compounds of horticultural plant materials not usually used. *J Funct. Foods*, 38, 119-127, 2017, doi.org/10.1016/j.jff.2017.09.003
- [51] Boudries, H., Nabet, N., Chougui, N., Souagui, S., Loupassaki, S., Madani, K., & Dimitrov, K. Optimization of ultrasound-assisted extraction of antioxidant phenolics from *Capparis spinosa* flower buds and LC-MS analysis. *J Food Meas Charact*, 13(3), 2241-2252, 2019, doi.org/10.1007/s11694-019-00144-1
- [52] Farhangi, A., Mohammadi, A., Zamani, F., Hemati, H., Nosrati, F., Nazari, N., & Nazari, H. Antidiabetic activity of water extract of *Capparis spinosa* on STZ-induced diabetics Rat. *J Adv Pharm Educ Res*, 9(S2), 149, 2019.
- [53] Rajhi, I., Hernandez-Ramos, F., Abderrabba, M., Ben Dhia, M. T., Ayadi, S., & Labidi, J. Antioxidant, Antifungal and Phytochemical Investigations of *Capparis spinosa* L. *J Agric*, 11(10), 1025, 2021, doi.org/10.3390/agriculture11101025
- [54] Assadi, S., Shafiee, S. M., Erfani, M., & Akmal, M. Antioxidative and antidiabetic effects of *Capparis spinosa* fruit extract on high-fat diet and low-dose streptozotocin-induced type 2 diabetic rats. *Biomed Pharmacother*, 138, 111391, 2021, doi.org/10.1016/j.biopha.2021.111391
- [55] Saleem, H., Khurshid, U., Sarfraz, M., Ahmad, I., Alamri, A., Anwar, S., ... & Ahemad, N. Investigation into the biological properties, secondary metabolites composition, and toxicity of aerial and root parts of *Capparis spinosa* L.: An important medicinal food plant. *Food Chem Toxicol*, 155, 112404, 2021, doi.org/10.1016/j.fct.2021.112404
- [56] Kirkan, B., Ceylan, O., SARIKÜRKCÜ, C., & Bektas, T. E. P. E. Phenolic profile, antioxidant and enzyme inhibitory activity of the ethyl acetate, methanol and water extracts of *Capparis spinosa* L. *Int J Sec Metabolite*, 8(4), 337-351, 2021, doi.org/10.21448/ijsm.981149
- [57] Boumerfeg, S., Ameni, D., Adjad, M., Djarmouni, M., Khennouf, S., Arrar, L., & Baghiani, A. Antihymolytic and Antioxidant Effects of Medicinal Plant *Capparis spinosa* L. *J Life Sci*, 6(6), 637, 2012.
- [58] Rajhi, I., Ben Dhia, M. T., Abderrabba, M., Ouzari-Hadda, I., & Ayadi, S. Phytochemical screening, in vitro antioxidant and antibacterial activities of methanolic extracts of *Capparis spinosa* L. different parts from Tunisia. *J Mater Environ Sci*, 10(3), 234-43, 2019.
- [59] Mollica, A., Stefanucci, A., Macedonio, G., Locatelli, M., Luisi, G., Novellino, E., & Zengin, G. Chemical composition and biological activity of *Capparis spinosa* L. from Lipari Island. *S Afr J Bot*, 120, 135-140, 2019, doi.org/10.1016/j.sajb.2018.02.397
- [60] Wiese, S., Wubshet, S. G., Nielsen, J., & Staerk, D. Coupling HPLC-SPE-NMR with a microplate-based high-resolution antioxidant assay for efficient analysis of antioxidants in food-Validation and proof-of-concept study with caper buds. *Food Chem*, 141(4), 4010-4018, 2013, doi.org/10.1016/j.foodchem.2013.06.115
- [61] Tagnaout, I., Zerkani, H., Mahjoubi, M., Bourakhouadar, M., Alistiqsa, F., Bouzoubaa, A., & Zair, T. Phytochemical study, antibacterial and antioxidant activities of extracts of *Capparis spinosa* L. *Int J Pharmacogn Phytochem Res*, 8(12), 1993-2005, 2016.
- [62] Jiménez-López, J., Ruiz-Medina, A., Ortega-Barrales, P., & Llorent-Martínez, E. J. Phytochemical profile and antioxidant activity of caper berries (*Capparis spinosa* L.): Evaluation of the influence of the fermentation process. *Food Chem*, 250, 54-59, 2018, doi.org/10.1016/j.foodchem.2018.01.010
- [63] Aksay, O., Selli, S., & Kelebek, H. LC-DAD-ESI-MS/MS-based assessment of the bioactive compounds in fresh and fermented caper (*Capparis spinosa*) buds and berries. *Food Chem*, 337, 127959, 2021, doi.org/10.1016/j.foodchem.2020.127959
- [64] Huseini, H. F., Hasani-Rnjbar, S., Nayebi, N., Heshmat, R., Sigaroodi, F. K., Ahvazi, M., ... & Kianbakht, S. *Capparis spinosa* L. (Caper) fruit extract in treatment of type 2 diabetic patients: a randomized double-blind placebo-controlled clinical trial. *Complement Ther Med*, 21(5), 447-452, 2013, doi.org/10.1016/j.ctim.2013.07.003
- [65] Meddour, A., Yahia, M., & Hambaba, L. Safety evaluation and analgesic studies of defatted methanol extract of *Capparis spinosa* L. (Capparidaceae) fruits and roots bark in albino wistar rats. *J Biol Res - Boll della Soc Ital di Biol Sper*, 92(1), 2019, doi.org/10.4081/jbr.2019.7456
- [66] Karanayil R, S., Barij N, S., & Aiyolu, R. (2011). Protective effects of *Capparis zeylanica* linn. leaf extract on gastric lesions in experimental animals. *Avicenna J Med Biotech*, 3, 31-5, 2011.
- [67] Song, F., Xue, Y., Dong, D., Liu, J., Fu, T., Xiao, C., ... & Li, Z. Insulin restores an altered corneal epithelium circadian rhythm in mice with streptozotocin-induced type 1 diabetes. *Sci Rep*, 6(1), 1-9, 2016, doi.org/10.1038%2Fsrep32871

- [68] Mahmoodi, M., Karimi, M., Hoseini, F., Heydari, R., Salehi, M., & Yousefi, A. Effect of hydroalcoholic extract of *Capparis spinosa* fruit on blood sugar and lipid profile of diabetic and normal rats. *Zahedan J Res Med Sci*, 15(11), 2013.
- [69] Layam, A., & Reddy, C. L. K. Antidiabetic property of spirulina. *Diabetes Croat*, 35(2), 29-33, 2006.
- [70] Zhang, Y., Wu, L., Ma, Z., Cheng, J., & Liu, J. Anti-diabetic, anti-oxidant and anti-hyperlipidemic activities of flavonoids from corn silk on STZ-induced diabetic mice. *Molecules*, 21(1), 7, 2015, doi.org/10.3390/molecules21010007
- [71] Eidi, A., Eidi, M., & Esmaeili, E. Antidiabetic effect of garlic (*Allium sativum* L.) in normal and streptozotocin-induced diabetic rats. *Phytomed*, 13(9-10), 624-629, 2006, doi.org/10.1016/j.phymed.2005.09.010
- [72] Hallschmid, M., & Schultes, B. Central nervous insulin resistance: a promising target in the treatment of metabolic and cognitive disorders?. *Diabetologia*, 52(11), 2264-2269, 2009, doi.org/10.1007/s00125-009-1501-x
- [73] Zhou, X., Zhang, F., Hu, X., Chen, J., Wen, X., Sun, Y., ... & Song, Y. Inhibition of inflammation by astaxanthin alleviates cognition deficits in diabetic mice. *Physiol Behav*, 151, 412-420, 2015, doi.org/10.1016/j.physbeh.2015.08.015
- [74] Strachan, M. W. J., Frier, B. M., & Deary, I. J. Type 2 diabetes and cognitive impairment. *Diabet Med: a journal of the British Diabetic Association*, 20(1), 1-2, 2003, doi.org/10.1046/j.1464-5491.2003.00855.x
- [75] Dai, J., Vrensen, G. F., & Schlingemann, R. O. Blood-brain barrier integrity is unaltered in human brain cortex with diabetes mellitus. *Brain Res*, 954(2), 311-316, 2002, doi.org/10.1016/S0006-8993(02)03294-8
- [76] Łabuzek, K., Suchy, D., Gabryel, B., Bielecka, A., Liber, S., & Okopień, B. Quantification of metformin by the HPLC method in brain regions, cerebrospinal fluid and plasma of rats treated with lipopolysaccharide. *Pharmacol Rep*, 62(5), 956-965, 2010, doi.org/10.1016/S1734-1140(10)70357-1
- [77] Bowman, P., McDonald, T. J., Shields, B. M., Knight, B. A., & Hattersley, A. T. Validation of a single-sample urinary C-peptide creatinine ratio as a reproducible alternative to serum C-peptide in patients with Type 2 diabetes. *Diabet Med*, 29(1), 90-93, 2012, doi.org/10.1111/j.1464-5491.2011.03428.x
- [78] Gottsäter, A., Ahmed, M., Fernlund, P., & Sundkvist, G. (1999). Autonomic neuropathy in Type 2 diabetic patients is associated with hyperinsulinaemia and hypertriglyceridaemia. *Diabet Med*, 16(1), 49-54. doi.org/10.1046/j.1464-5491.1999.00001.x
- [79] Hope, S. V., Knight, B. A., Shields, B. M., Strain, W. D., Hattersley, A. T., Choudhary, P., & Jones, A. G. Low c-peptide is associated with high glycaemic variability and hypoglycaemia in insulin-treated patients with type 2 diabetes. *Diabet Med*, 33(144), 18, 2016.
- [80] Sari, R., & Balci, M. K. Relationship between C peptide and chronic complications in type-2 diabetes mellitus. *J Natl Med*, 97(8), 1113, 2005.
- [81] Saisho, Y. β -cell dysfunction: Its critical role in prevention and management of type 2 diabetes. *World J diabetes*, 6(1), 109, 2015, doi.org/10.4239%2Fwjcd.v6.i1.109
- [82] Leighton, E., Sainsbury, C. A., & Jones, G. C. A practical review of C-peptide testing in diabetes. *Diabetes Ther*, 8(3), 475-487, 2017, doi.org/10.1007/s13300-017-0265-4
- [83] Choudhary, M. I., Adhikari, A., Rasheed, S., Marasini, B. P., Hussain, N., & Kaleem, W. A. Cyclopeptide alkaloids of *Ziziphus oxyphylla* Edgew as novel inhibitors of α -glucosidase enzyme and protein glycation. *Phytochem lett*, 4(4), 404-406, 2011.
- [84] Zheng, T., Shu, G., Yang, Z., Mo, S., Zhao, Y., & Mei, Z. Antidiabetic effect of total saponins from *Entada phaseoloides* (L.) Merr. in type 2 diabetic rats. *J ethnopharmacol*, 139(3), 814-821, 2012, doi.org/10.1016/j.jep.2011.12.025
- [85] Patel, D. K., Kumar, R., Laloo, D., & Hemalatha, S. Diabetes mellitus: an overview on its pharmacological aspects and reported medicinal plants having antidiabetic activity. *Asian Pac J Trop Biomed*, 2(5), 411-420, 2012, doi.org/10.1016/S2221-1691(12)60067-7
- [86] Singh, R., Kaur, N., Kishore, L., & Gupta, G. K. Management of diabetic complications: a chemical constituents based approach. *J Ethnopharmacol*, 150(1), 51-70, 2013, doi.org/10.1016/j.jep.2013.08.051
- [87] Xing, L. V., Gao-Hong, L. V., Guo-Ying, D. A. I., Hong-Mei, S. U. N., & Hui-Qin, X. U. Food-advanced glycation end products aggravate the diabetic vascular complications via modulating the AGEs/RAGE pathway. *Chinese J Natl Med*, 14(11), 844-855, 2016, doi.org/10.1016/S1875-5364(16)30101-7
- [88] Lyons, T. J., & Basu, A. Biomarkers in diabetes: hemoglobin A1c, vascular and tissue markers. *Transl Res*, 159(4), 303-312, 2012, doi.org/10.1016/j.trsl.2012.01.009

- [89] Babukumar, S., Vinothkumar, V., Sankaranarayanan, C., & Srinivasan, S. Geraniol, a natural monoterpene, ameliorates hyperglycemia by attenuating the key enzymes of carbohydrate metabolism in streptozotocin-induced diabetic rats. *Pharm Biol*, 55(1), 1442-1449, 2017, doi.org/10.1080/13880209.2017.1301494
- [90] Muruganathan, U., Srinivasan, S., & Vinothkumar, V. Antidiabetogenic efficiency of menthol, improves glucose homeostasis and attenuates pancreatic β -cell apoptosis in streptozotocin–nicotinamide induced experimental rats through ameliorating glucose metabolic enzymes. *Biomed Pharmacother*, 92, 229-239, 2017, doi.org/10.1016/j.biopha.2017.05.068
- [91] Perumal, V., Manickam, T., Bang, K. S., Velmurugan, P., & Oh, B. T. Antidiabetic potential of bioactive molecules coated chitosan nanoparticles in experimental rats. *Int J Biol Macromol*, 92, 63-69, 2016, doi.org/10.1016/j.ijbiomac.2016.07.006
- [92] Chatterjea, M. N., & Shinde, R. Textbook of medical biochemistry. New Delhi: Jaypee Brothers, 2002.
- [93] Pari, L., & Saravanan, R. Antidiabetic effect of diasulin, a herbal drug, on blood glucose, plasma insulin and hepatic enzymes of glucose metabolism in hyperglycaemic rats. *Diabetes Obes Metab*, 6(4), 286-292, 2004, doi.org/10.1111/j.1462-8902.2004.0349.x
- [94] Khan, M., Liu, H., Wang, J., & Sun, B. Inhibitory effect of phenolic compounds and plant extracts on the formation of advanced glycation end products: A comprehensive review. *Food Res Int*, 130, 108933, 2020, doi.org/10.1016/j.foodres.2019.108933
- [95] Spagnuolo, L., Della Posta, S., Fanali, C., Dugo, L., & De Gara, L. Antioxidant and antiglycation effects of polyphenol compounds extracted from hazelnut skin on advanced glycation end-products (ages) formation. *Antioxidants*, 10(3), 424, 2021, doi.org/10.3390/antiox10030424
- [96] Li, W., Liu, J., Chen, J. R., Zhu, Y. M., Gao, X., Ni, Y., ... & Ao, G. Z. Neuroprotective effects of DTIO, a novel analog of Nec-1, in acute and chronic stages after ischemic stroke. *Neurosci*, 390, 12-29, 2018, doi.org/10.1016/j.neuroscience.2018.07.044
- [97] Engstrom, M., Schott, U., Nordstrom, C. H., Romner, B., & Reinstrup, P. Increased lactate levels impair the coagulation system—a potential contributing factor to progressive hemorrhage after traumatic brain injury. *J Neurosurg Anesth*, 18(3), 200-204, 2006, doi.org/10.1097/01.ana.0000211002.63774.8a
- [98] Kumar, M., & Bansal, N. Effects of Chronic Lithium Chloride and L-Arginine Treatment on Prevention of Streptozotocin Induced Cognitive Deficits by Ellagic Acid. *Biomed Pharmacol J*, 11(1), 53-65, 2018, doi.org/10.13005/bpj/1347
- [99] Sharma, M. K., Kumar, M., & Kumar, A. Protection against mercury-induced renal damage in Swiss albino mice by *Ocimum sanctum*. *Environ Toxicol Pharmacol*, 19(1), 161-167, 2005, doi.org/10.1016/j.etap.2004.06.002
- [100] Al-Enazi, M. M. Effect of thymoquinone on malformations and oxidative stress-induced diabetic mice. *Pak J Biol Sci: PJBs*, 10(18), 3115-3119, 2007, doi.org/10.3923/pjbs.2007.3115.3119
- [101] Nehlig, A., & Coles, J. A. Cellular pathways of energy metabolism in the brain: is glucose used by neurons or astrocytes?. *Glia*, 55(12), 1238-1250, 2007, doi.org/10.1002/glia.20376
- [102] Sharma, R., Rosenberg, A., Bennett, E. R., Laskowitz, D. T., & Acheson, S. K. A blood-based biomarker panel to risk-stratify mild traumatic brain injury. *PLoS one*, 12(3), e0173798, 2017, doi.org/10.1371/journal.pone.0173798
- [103] Zhou, G., Yan, M., Guo, G., & Tong, N. Ameliorative effect of berberine on neonatally induced type 2 diabetic neuropathy via modulation of BDNF, IGF-1, PPAR- γ , and AMPK expressions. *Dose Response*, 17(3), 1559325819862449, 2019, doi.org/10.1177%2F1559325819862449
- [104] Jing, Y. H., Chen, K. H., Kuo, P. C., Pao, C. C., & Chen, J. K. Neurodegeneration in streptozotocin-induced diabetic rats is attenuated by treatment with resveratrol. *Neuroendocrinol*, 98(2), 116-127, 2013, doi.org/10.1159/000350435
- [105] Duarte, J. M. Metabolic alterations associated to brain dysfunction in diabetes. *Aging and Dis*, 6(5), 304, 2015, doi.org/10.14336%2FAD.2014.1104
- [106] Ly, H., Verma, N., Wu, F., Liu, M., Saatman, K. E., Nelson, P. T., ... & Despa, F. Brain microvascular injury and white matter disease provoked by diabetes-associated hyperamylinemia. *Ann Neurol*, 82(2), 208-222, 2017, doi.org/10.1002/ana.24992
- [107] Kumar, S. Dual inhibition of acetylcholinesterase and butyrylcholinesterase enzymes by allicin. *Indian J Pharmacol*, 47(4), 444, 2015, doi.org/10.4103%2F0253-7613.161274
- [108] Blurton-Jones, M., Kitazawa, M., Martinez-Coria, H., Castello, N. A., Müller, F. J., Loring, J. F., ... & LaFerla, F. M. Neural stem cells

- improve cognition via BDNF in a transgenic model of Alzheimer disease. *Proc. Natl. Acad. Sci. U. S. A.*, 106(32), 13594-13599, 2009, doi.org/10.1073/pnas.0901402106
- [109] Suzuki, W. A., & Clayton, N. S. The hippocampus and memory: a comparative and ethological perspective. *Curr Opin Neurobiol*, 10(6), 768-773, 2000, doi.org/10.1016/S0959-4388(00)00148-3
- [110] Ademosun, A. O., Oboh, G., Bello, F., & Ayeni, P. O. Antioxidative properties and effect of quercetin and its glycosylated form (Rutin) on acetylcholinesterase and butyrylcholinesterase activities. *Evid Based Complementary Altern Med*, 21(4), 11-17, 2016, doi.org/10.1177/2156587215610032
- [111] Nugroho, A., Choi, J. S., Hong, J. P., & Park, H. J. Anti-acetylcholinesterase activity of the aglycones of phenolic glycosides isolated from *Leonurus japonicus*. *Asian Pac J Trop Biomed*, 7(10), 849-854, 2017, doi.org/10.1016/j.apjtb.2017.08.013
- [112] Maker, H. S., Weiss, C., Silides, D. J., & Cohen, G. Coupling of dopamine oxidation (monoamine oxidase activity) to glutathione oxidation via the generation of hydrogen peroxide in rat brain homogenates. *J Neurochem*, 36(2), 589-593, 1981, doi.org/10.1111/j.1471-4159.1981.tb01631.x
- [113] Morari, M., Marti, M., Sbrenna, S., Fuxe, K., Bianchi, C., & Beani, L. Review Article Reciprocal dopamine-glutamate modulation of release in the basal ganglia. *Neurochem Int*, 33(5), 383-397, 1998, doi.org/10.1016/S0197-0186(98)00052-7
- [114] Lees, G. J. Contributory mechanisms in the causation of neurodegenerative disorders. *Neurosci*, 54(2), 287-322, 1993, doi.org/10.1016/0306-4522(93)90254-D
- [115] Olney, J. W. The toxic effects of glutamate and related compounds in the retina and the brain. *Retina* (Philadelphia, Pa.), 2(4), 341-359, 1982.
- [116] Savolainen, K. M., Loikkanen, J., Eerikäinen, S., & Naarala, J. Interactions of excitatory neurotransmitters and xenobiotics in excitotoxicity and oxidative stress: glutamate and lead. *Toxicol Lett*, 102, 363-367, 1998, doi.org/10.1016/S0378-4274(98)00233-1
- [117] Turgut, N. H., Kara, H., Arslanbaş, E., Mert, D. G., Tepe, B., & Güngör, H. Effect of *Capparis spinosa* L. on cognitive impairment induced by D-galactose in mice via inhibition of oxidative stress. *Turk J Med Sci*, 45(5), 1127-1136, 2015, doi.org/10.3906/sag-1405-95
- [118] Sadek, K. M., Lebda, M. A., Nasr, S. M., & Shoukry, M. *Spirulina platensis* prevents hyperglycemia in rats by modulating gluconeogenesis and apoptosis via modification of oxidative stress and MAPK-pathways. *Biomed Pharmacother*, 92, 1085-1094, 2017, doi.org/10.1016/j.biopha.2017.06.023
- [119] Samarghandian, S., Borji, A., & Hidar Tabasi, S. Effects of *Cichorium intybus* linn on blood glucose, lipid constituents and selected oxidative stress parameters in streptozotocin-induced diabetic rats. *Cardiovasc Hematol Disord -Drug Targets*, 13(3), 231-236, 2013, doi.org/10.2174/1871529x13666131129103139
- [120] Strugała, P., Dzydzan, O., Brodyak, I., Kucharska, A. Z., Kuroпка, P., Liuta, M., ... & Sybirna, N. Antidiabetic and antioxidative potential of the blue Congo variety of purple potato extract in streptozotocin-induced diabetic rats. *Molecules*, 24(17), 3126, 2019, doi.org/10.3390/molecules24173126
- [121] Moskaug, J. Ø., Carlsen, H., Myhrstad, M. C., & Blomhoff, R. Polyphenols and glutathione synthesis regulation. *Am J Clin Nutr*, 81(1), 277-283, 2005, doi.org/10.1093/ajcn/81.1.277S
- [122] Shaw, O. C. 15 Glutathione in the Nervous System: Roles in Neural Function and Health and Implications for Neurological Disease. *Handbook Neurochem Mol Neurobiol: amino acids and peptides in the nervous system*, 2007.
- [123] Dickinson, D. A., & Forman, H. J. Cellular glutathione and thiols metabolism. *Biochem Pharmacol*, 64(5-6), 1019-1026, 2002, doi.org/10.1016/S0006-2952(02)01172-3
- [124] Sedlak, T. W., Paul, B. D., Parker, G. M., Hester, L. D., Snowman, A. M., Taniguchi, Y., ... & Sawa, A. The glutathione cycle shapes synaptic glutamate activity. *Proc National Acad Sci*, 116(7), 2701-2706, 2019, doi.org/10.1073/pnas.1817885116
- [125] Persson, M., Sandberg, M., Hansson, E., & Rönnbäck, L. Microglial glutamate uptake is coupled to glutathione synthesis and glutamate release. *Eur J Neurosci*, 24(4), 1063-1070, 2006, doi.org/10.1111/j.1460-9568.2006.04974.x
- [126] Barger, S. W., Goodwin, M. E., Porter, M. M., & Beggs, M. L. Glutamate release from activated microglia requires the oxidative burst and lipid peroxidation. *J Neurochem*, 101(5), 1205-1213, 2007, doi.org/10.1111/j.1471-4159.2007.04487.x
- [127] Koga, M., Serritella, A. V., Messmer, M. M., Hayashi-Takagi, A., Hester, L. D., Snyder, S. H., ... & Sedlak, T. W. Glutathione is a physiologic reservoir of neuronal glutamate. *Biochem Biophys Res Commun*, 409(4), 596-

- 602, 2011,doi.org/10.1111/j.1471-4159.2007.04487.x
- [128] Chassain, C., Bielicki, G., Durand, E., Lolignier, S., Essafi, F., Traoré, A., & Durif, F. Metabolic changes detected by proton magnetic resonance spectroscopy in vivo and in vitro in a murin model of Parkinson's disease, the MPTP-intoxicated mouse. *J Neurochem*, 105(3), 874-882, 2008,doi/full/10.1111/j.1471-4159.2007.05185.x
- [129] Gao, H. C., Zhu, H., Song, C. Y., Lin, L., Xiang, Y., Yan, Z. H., ... & Li, X. K. Metabolic changes detected by ex vivo high resolution 1H NMR spectroscopy in the striatum of 6-OHDA-induced Parkinson's rat. *Mol Neurobiol*, 47(1), 123-130, 2013,doi.org/10.1007/s12035-012-8336-z
- [130] Schubert, D., & Piasecki, D. Oxidative glutamate toxicity can be a component of the excitotoxicity cascade. *J Neurosci*, 21(19), 7455-7462, 2001,doi.org/10.1523/JNEUROSCI.21-19-07455.2001
- [131] Myhrstad, M. C., Carlsen, H., Nordström, O., Blomhoff, R., & Moskaug, J. Ø. Flavonoids increase the intracellular glutathione level by transactivation of the γ -glutamylcysteine synthetase catalytical subunit promoter. *Free Radic Biol Med*, 32(5), 386-393, 2002,doi.org/10.1016/S0891-5849(01)00812-7
- [132] Elmastas, M., Celik, S. M., Genc, N., Aksit, H., Erenler, R., & Gulcin, İ. Antioxidant activity of an Anatolian herbal tea—*Origanum minutiflorum*: isolation and characterization of its secondary metabolites. *Int J Food Prop*, 21(1), 374-384, 2018,doi.org/10.1080/10942912.2017.1416399
- [133] Sayahi, M., & Shirali, S. (2017). The antidiabetic and antioxidant effects of carotenoids: a review. *Asian J Pharm Res Health Care*, 9(4), 186-191,doi.org/10.18311/ajprhc/2017/7689
- [134] Kalantari, H., Foruozandeh, H., Khodayar, M. J., Siahpoosh, A., Saki, N., & Kheradmand, P. Antioxidant and hepatoprotective effects of *Capparis spinosa* L. fractions and Quercetin on tert-butyl hydroperoxide-induced acute liver damage in mice. *J Tradit Complem Med*, 8(1), 120-127, 2018, doi.org/10.1016/j.jtcme.2017.04.010
- [135] Mokhtari, D., Li, T., Lu, T., & Welsh, N. Effects of imatinib mesylate (Gleevec) on human islet NF-kappaB activation and chemokine production in vitro. *PLoS One*, 6(9), e24831, 2011, doi.org/10.1371/journal.pone.0024831
- [136] Griffiths, M. R., Gasque, P., & Neal, J. W. The multiple roles of the innate immune system in the regulation of apoptosis and inflammation in the brain. *J Neuropathol Exp Neurol*, 68(3), 217-226, 2019,doi.org/10.1097/NEN.0b013e3181996688
- [137] Pari, L., & Sankaranarayanan, C. Beneficial effects of thymoquinone on hepatic key enzymes in streptozotocin–nicotinamide induced diabetic rats. *Life Sci*, 85(23-26), 830-834, 2009,doi.org/10.1016/j.lfs.2009.10.021
- [138] Hectors, T. L. M., Vanparys, C., Van Der Ven, K., Martens, G. A., Jorens, P. G., Van Gaal, L. F., ... & Blust, R. Environmental pollutants and type 2 diabetes: a review of mechanisms that can disrupt beta cell function. *Diabetologia*, 54(6), 1273-1290, 2011,doi.org/10.1007/s00125-011-2109-5
- [139] Marimoutou, M., Le Sage, F., Smadja, J., Lefebvre d'Hellencourt, C., Gonthier, M. P., & Silva, R. D. Antioxidant polyphenol-rich extracts from the medicinal plants *Antirhea borbonica*, *Doratoxylonapetalum* and *Gouania mauritiana* protect 3T3-L1 preadipocytes against H₂O₂, TNF α and LPS inflammatory mediators by regulating the expression of superoxide dismutase and NF- κ B genes. *J Inflamm*, 12(1), 1-15, 2015,doi.org/1-15.10.1186/s12950-015-0055-6
- [140] Bahadar, G. A., & Shah, Z. A. Intracerebral Hemorrhage and Diabetes Mellitus: Blood-Brain Barrier Disruption, Pathophysiology and Cognitive Impairments. *CNS & Neurol Disord-Drug Targets*, 20(4), 312-326, 2021,doi.org/10.2174/1871527320666210223145112
- [141] Imai, Y., Dobrian, A. D., Weaver, J. R., Butcher, M. J., Cole, B. K., Galkina, E. V., ... & Nadler, J. L. Interaction between cytokines and inflammatory cells in islet dysfunction, insulin resistance and vascular disease. *Diabetes Obes Metab*, 15(s3), 117-129, 2013,doi.org/10.1111/dom.12161
- [142] Akash, M. S. H., Rehman, K., & Liaqat, A. Tumor necrosis factor-alpha: role in development of insulin resistance and pathogenesis of type 2 diabetes mellitus. *J Cell Biochem*, 119(1), 105-110, 2018, doi.org/10.1002/jcb.26174
- [143] Akash, M. S. H., Rehman, K., & Chen, S. Role of inflammatory mechanisms in pathogenesis of type 2 diabetes mellitus. *J Cell Biochem*, 114(3), 525-531, 2013,doi.org/10.1002/jcb.24402
- [144] Cruz, N. G., Sousa, L. P., Sousa, M. O., Pietrani, N. T., Fernandes, A. P., & Gomes, K. B. The linkage between inflammation and Type 2 diabetes mellitus. *Diabetes Res Clin Pract*, 99(2), 85-92, 2013,doi.org/10.1016/j.diabres.2012.09.003
- [145] Hamby, M. E., Gragnolati, A. R., Hewett, S. J., & Hewett, J. A. TGF β 1 and TNF α potentiate

- nitric oxide production in astrocyte cultures by recruiting distinct subpopulations of cells to express NOS-2. *Neurochem Int*, 52(6), 962-971, 2008,doi.org/10.1016/j.neuint.2007.10.010
- [146]Szkudelski, T. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. *Physiol Res*, 50(6), 537-546, 2001.
- [147]Shen, H. M., & Pervaiz, S. TNF receptor superfamily-induced cell death: redox-dependent execution. *FASEB J*, 20(10), 1589-1598, 2006,doi.org/10.1096/fj.05-5603rev
- [148]Romeo, V., Ziino, M., Giuffrida, D., Concurso, C., & Verzera, A. Flavour profile of capers (*Capparis spinosa L.*) from the Eolian Archipelago by HS-SPME/GC-MS. *Food Chem*, 101(3), 1272-1278, 2007,doi.org/10.1016/j.foodchem.2005.12.029
- [149]Aliyazıcıoğlu, R., Sahin, H., Erturk, O., Ulusoy, E., & Kolaylı, S. Properties of phenolic composition and biological activity of propolis from Turkey. *Int J Food Prop*, 16(2), 277-287, 2013,doi.org/10.1080/10942912.2010.551312
- [150]Zhang, H., & Ma, Z. F. Phytochemical and pharmacological properties of *Capparis spinosa* as a medicinal plant. *J Nutr*, 10(2), 116, 2018,doi.org/10.3390/nu10020116
- [151]Moutia, M., El Azhary, K., Elouaddari, A., Al Jahid, A., Jamal Eddine, J., Seghrouchni, F., ... & Badou, A. *Capparis Spinosa L.* promotes anti-inflammatory response in vitro through the control of cytokine gene expression in human peripheral blood mononuclear cells. *BMC immunol*, 17(1), 1-12, 2016, doi.org/10.1186/s12865-016-0164-x
- [152]Lee, C. C., Shen, S. R., Lai, Y. J., & Wu, S. C. Rutin and quercetin, bioactive compounds from tartary buckwheat, prevent liver inflammatory injury. *J Funct Foods*, 4(5), 794-802, 2013,doi.org/10.1039/c3fo30389f