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Brassica oleracea Extracts Prevent Hyperglycemia in Type 2 Diabetes Mellitus

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ABSTRACT: This study investigated the protective effect of extracts from flowers of *Brassica oleracea* L. var. *italica* Plenck on type 2 diabetes mellitus and its associated disorders. Three different doses of each extract (petroleum ether, ethanol, and aqueous) were administered orally for 42 days. Biochemical parameters, behavioral studies, and histological studies were measured at different periods. Mortality was found to be nil up to 2,000 mg/kg. Statistically significant ($P<0.001$) improvement in serum glucose level was observed in the groups receiving 400 mg/kg of petroleum ether, aqueous, or ethanol extracts compared with the negative control group. Insulin level was decreased by aqueous extracts, whereas lipid profiles were improved by aqueous and ethanol extracts. A reduction in transfer latency was observed in treatments of all three extract types. Ethanol extract treatment (400 mg/kg) showed maximum percentage inhibition in a lipid peroxidation assay. Additionally, the aqueous and ethanol extract treatments markedly reduced tumor necrosis factor-α, interleukin-6, and glycosylated hemoglobin levels. Histological results showed that high doses of extracts alleviated the damages induced by type 2 diabetes mellitus in various organs and bones. Based on the results of this study, it can be concluded that *B. oleracea* has the potential to alleviate type 2 diabetes mellitus.

Keywords: *Brassica oleracea*, caffeic acid, cytokines, dexamethasone, glucosinolates

INTRODUCTION

An increase in reactive oxygen species and dysfunction of antioxidant enzymes may lead to the development of chronic diseases such as diabetes mellitus, cancer, atherosclerosis, nephritis, rheumatism, ischemia, cardiovascular diseases, and neurodegenerative disorders like Alzheimer's and Parkinson's diseases. Various studies suggest that a high intake of fruits and vegetables provides a good source of phytonutrients that reduce the risk of chronic diseases (Boeing et al., 2012). Diabetic-osteoporosis and diabetes with Alzheimer's disease are two new complications identified as associated with type 2 diabetes mellitus (Dreher, 2018). Additionally, both diabetes-osteoporosis and diabetic Alzheimer's occur when oxidative stress is present (Liguori et al., 2018). A positive link between type 2 diabetes mellitus and hip fracture due to osteoporosis has been reported in the Asian population with chronic diabetes mellitus in various epidemiological studies (Abdulameer et al., 2018). Furthermore, patients with type 2 diabetes mellitus are more prone to memory impairment than those without diabetes (Mittal and Katare, 2016; Gupta et al., 2020).

Presently, various insulin preparations (Jacob et al., 2018) and a wide array of antidiabetic agents are available for the treatment of diabetes. Nevertheless, managing diabetes mellitus using medicines without side effects remains a challenge. There are several options available for alternative therapy either from herbal plants or nutraceuticals, which can be used as an add-on therapy for long-

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term management of type 2 diabetes mellitus (Gupta et al., 2011; Gupta et al., 2013; Sharma et al., 2020).

Nutraceuticals, functional foods, and dietary supplements are gaining special attention as adjuvant therapies or dietary sources in the prevention of various disorders (Berberich and Hegele, 2018). *Brassica*, or cruciferous vegetables, are an important vegetable food source that includes cabbage, cauliflower, broccoli, and kohlrabi. One study has shown that cruciferous vegetables act as a good source of natural antioxidants and can produce an antidiabetic effect (Li et al., 2018). Additionally, there has been an increase in the popularity and consumption of vegetable *Brassica* species because of their nutritional value (Sanlier and Guler Saban, 2018). *Brassica oleracea* L. var. *italica* Plenck (Brassicaceae) is popularly known as 'broccoli' or 'calabrese' (Gray, 1982) and contains various nutrients and health-promoting phytochemicals such as polyphenols (hydroxycinnamic acid esters, quercetin glucosides, kaempferol, lutein, zeaxanthin, and α-tocopherol), along with high concentrations of selenium and glucosinolates, particularly glucoraphanin and isothiocyanate sulforaphane (Jahangir et al., 2009). Because of its nutraceutical properties, the present investigation was undertaken to evaluate the protective effect of *B. oleracea* against metabolic complications associated with type 2 diabetes mellitus.

MATERIALS AND METHODS

Animals

Adult male and female albino Wistar rats (200～250 g) were procured from an approved animal house at the National Institute of Pharmaceutical Education and Research (Mohali, Punjab, India). The animals were housed under standard temperature (24～28°C) and relative humidity (60～70%) conditions with a 12:12 light-dark cycle. The animals were fed the special diet (Reeves, 1997) and purchased from Khadya Bhandar (Ambala, India) given water *ad libitum* throughout the experiment.

Ethical approval

The study was approved by the Institutional Animal Ethics Committee (MMCP/IEC/12/01-R1). The experiment was conducted as per the guidelines issued by the Committee for Control and Supervision of Experiments on Animals under the Ministry of Animal Husbandry, Government of India.

Drugs and chemicals

Dexamethasone sodium phosphate was a gift from Strides Pharma Science Ltd. (Bangalore, India). Metformin was obtained as a gift sample from Ind Swift Pharmaceutical Ltd. (Parwanoo, India). All other reagents and chemicals used in the study were of analytical grade. All drugs were dissolved in distilled water and other suitable solvents for oral administration.

Plant material

B. oleracea inflorescences (flower, bud, and stem of the whole plant) were collected from a local agricultural market. The plant was identified and authenticated by the National Institute of Science Communication and Information Resources (NISCAIR), New Delhi, India (NISCAIR/RHMD/Consult/-2011-12/1988/288). The fresh plant parts were washed with distilled water to remove debris, dried at 35～40°C for 10 days, and pulverized using an electric grinder. The powder obtained was passed through sieve No. 60 and used for further extraction.

Preparation of *B. oleracea* phytochemical extracts

Phytochemical extraction was performed on the dried powder of *B. oleracea* inflorescences (3 kg) in a Soxhlet apparatus using petroleum ether and ethanol consecutively for 72 h each. The last trace of solvent was removed via vacuum drying (Anroop et al., 2005). Crude aqueous extract of these dried powder materials (1 kg) was freshly prepared separately by maceration for 24 h. The extracts were stored between 3°C to 4°C until further use.

Preliminary phytochemical screening

Preliminary phytochemical tests were performed on the n petroleum ether, ethanol, and aqueous extracts using standard protocols to identify the possible nature of its chemical contents (Harborne, 1998; Evans, 2009; Khalil et al., 2015).

Profiling of phenolic components in different fractions of *B. oleracea* inflorescences via validated high-performance thin-layer chromatography (HPTLC) and high-performance liquid chromatography (HPLC)

A validated HPTLC method was used to quantify the metabolites in the aqueous, ethanol, and petroleum ether extracts of *B. oleracea* dried inflorescences. The chromatography was performed on a 20 cm×10 cm precoated silica gel F_{254} HPTLC plates using caffeic, benzoic, *p*-coumaric, and *trans*-cinnamic acids as marker compounds. The markers, along with the different sample extracts, were applied using a CAMAG Automatic TLC Sampler 4 (CAMAG, Muttenz, Switzerland) to the HPTLC plate. A CAMAG Automated Developing Chamber 2 (CAMAG) was used to develop the HPTLC plate under controlled conditions (Hossain and Ismail, 2009). The developed plate was dried and derivatized by spraying with *p*-anisaldehyde reagent and scanned by a computerized CAMAG TLC Scanner 3 (CAMAG) using visionCATS software version 2.5.18072.1 (CAMAG).

For HPLC analysis, 0.5-g fresh mass of broccoli (flow-

er, bud, and stem of plant) was homogenized in 5 mL of 100% ethanol and then centrifuged at 12,298 *g* for 20 min at room temperature (25°C) to prepare the ethanol extract. Subsequently, chlorophyll was removed from the broccoli samples by treating with a 1:1:1 ratio of sample, chloroform, and water, vortexing, and centrifuging (12,298 *g*) to collect the upper layer of supernatant for HPLC analysis. Similarly, extraction was performed using 5 mL of 100% petroleum ether to prepare the petroleum ether extract. The extracts were filtered through a 0.45-μm syringe filter before injection. HPLC analysis of broccoli was performed on a Waters C18 column (Sunfire, 5 μm, 250 mm×4.6 mm) using a Waters-HPLC system (Waters Corporation, Milford, MA, USA). Peaks were identified by comparing their retention time and ultraviolet (UV)-spectra to a caffeic acid standard. Chromatograms were monitored with a photodiode array detector at a wavelength of 310 nm. An isocratic solvent system of 1 mM trifluoroacetic acid in water : methanol [32:68 (v/v)] with a flow rate of 1.0 mL/min for 15 min was used to elute the caffeic acid.

Acute toxicity test

The median lethal dose (LD_{50}) of extracts (petroleum ether, ethanol, and aqueous) in albino male Wistar rats was determined according to the Organization for Economic Cooperation Development guidelines (OECD, 2016). There were 12 groups of rats, with each group comprising eight rats (male : female=4:4). Each extract was given orally at varying doses of 5, 50, 300, 500, and 2,000 mg/kg to different groups for 28 days. The rats were observed individually for acute toxicity signs and behavioral changes 1 h post-dosing and at least once daily for 28 days. The bodyweight of the rats was monitored weekly. On the 29th day, after overnight fasting, one rat of each group was anesthetized with ether, and blood samples for hematological and biochemical analysis were collected into microfuge tubes (2 mL). Necroscopy was performed for each group, and the weight of the organs was recorded.

Experimental design

Animals were divided into 12 groups with eight rats each. Group 1 (normal control) was given oral saline. Group 2 was treated with dexamethasone sodium phosphate [10 mg/kg, subcutaneous (s.c.)] and served as a negative control. Group 3 was administered dexamethasone sodium phosphate and metformin (200 mg/kg once daily) (Oliveira et al., 2016) and served as a standard control. Besides dexamethasone sodium phosphate injection, Groups 4～6, Groups 7～9, and Groups 10～12 were treated once daily with 100, 200, or 400 mg/kg of petroleum ether extract, ethanol extract, or aqueous extract of *B. oleracea*, respectively. In Groups 2～12, insulin resistance in the form of type 2 diabetes mellitus was induced in overnight-fasted rats by administering freshly prepared dexamethasone sodium phosphate (10 mg/kg s.c.) until the end of 42nd day. To maintain a diabetic state, dexamethasone sodium phosphate was injected for three consecutive days per week (Wego et al., 2019). The diabetic state was confirmed as a slight increase in serum blood glucose levels after being administered dexamethasone sodium phosphate injection. The dose of dexamethasone sodium phosphate was based on preliminary work, which showed that insulin tolerance was induced within a week and tolbutamide (10 mg/kg) (Chao et al., 2018) failed to lower the hyperglycemia levels. Rats with insulin resistance were applied as a model of non-insulin-dependent diabetes mellitus (type 2 diabetes mellitus), and homeostasis model assessment for insulin resistance (HOMA-IR) was calculated by the following formula:

HOMA-IR=
fasting insulin (μU/mL)×fasting glucose (mmol/L)/22.5

All the rats received their relevant assigned treatment daily and specific diet for 42 days (Gupta et al., 2010).

Biochemical estimations

Blood samples were drawn at weekly intervals on the 0, 7th, 14th, 21st, and 42nd days. The rats were anesthetized with ether, and blood samples were collected by retro-orbital puncture. Approximately 30-μL serum was then separated for the estimation of glucose, triglyceride, high-density lipoprotein cholesterol (HDL-c), and low-density lipoprotein cholesterol (LDL-c) levels. Serum insulin level was also estimated by a radioimmunoassay kit purchased from Stat Diagnostics (Linco Research Inc., Mumbai, India). The cytokine levels [tumor necrosis factor (TNF)-α and interleukin (IL)-6] were determined on the 40th day by the sandwich enzyme-linked immunosorbent assay method (Arokoyo et al., 2018) with a commercially available kit from Fisher Thermo Scientific Co. (Rockford, IL, USA). Glycosylated hemoglobin levels were also estimated using a standard technique (Arokoyo et al., 2018).

Body weight

Rats from each group were weighed at different intervals (0, 7th, 14th, 21st, and 42nd days) on a digital electronic balance for small animals.

Lipid peroxidation assay of pancreas and brain

Lipid peroxidation was assessed on the 21st day by measuring the thiobarbituric acid (TBA) reactivity of malondialdehyde as an end product of fatty acid peroxidation. For this purpose, approximately 0.2 mL of cells or plasma was suspended in a combination of 0.8 mL of phosphate-buffered saline and 0.025 mL of butylated hydroxytoluene

(88 mg/10 mL absolute alcohol). Then, 30% of trichloroacetic acid (0.5 mL) was added. Afterward, the tubes were vortexed and then allowed to stand on ice for at least 2 h. The tubes were centrifuged at 2,000 *g* for 15 min. For each tube, approximately 1 mL of supernatant was transferred to a new tube, and then, 0.25 mL of 1% TBA in 0.05 N sodium hydroxide was added. The tubes were then mixed and kept in a boiling water bath for 15 min, and the concentration of the malondialdehyde-TBA complex was assessed as described earlier (Konieczka et al., 2014). The TBA reactive substance (TBARS) values were expressed as nmol/mg protein.

Assay of superoxide dismutase (SOD) of pancreas and brain

SOD was assayed on the 21st day utilizing the technique based on inhibition of the formation of nicotinamide adenine dinucleotide, phenazine methosulfate, and amino blue tetrazolium formazan. A single unit of the enzyme was expressed as 50% inhibition of nitroblue tetrazolium reduction/min/mg/protein (Pari and Latha, 2004).

Assay of glutathione peroxidase (GPx) of pancreas and brain

GPx was determined on the 21st day by the method of Ellman (1959). Supernatant (1 mL) was treated with Ellman's reagent (0.5 mL) and phosphate buffer (3.0 mL; 0.2 M, pH 8.0). The absorbance was read at 412 nm. GPx activity was expressed as μg of GPx consumed/min/mg protein, and reduced glutathione were expressed as mm/mg of tissue.

Elevated plus-maze

An elevated plus-maze served as an exteroceptive behavioral model to evaluate rodent learning and memory. The testing procedure for learning and memory was performed as previously described (Ellman, 1959). The apparatus consisted of two open arms (16 cm×5 cm) and two enclosed arms (16 cm×5 cm×12 cm). The arms extended from a central platform (5 cm×5 cm), and the apparatus was elevated to a height of 25 cm from the floor. On the first day, each rat was placed at the end of an open arm, facing away from the central platform. Transfer latency was defined as the time taken by the rat with its four legs to move into one of the enclosed arms. Transfer latency was recorded on the first day. If the animal did not enter into one of the enclosed arms within 90 s, then it was pushed gently into one of the two enclosed arms, and transfer latency was assigned as 90 s. The rat was allowed to explore the maze for 10 s and then returned to its home cage. Retention of this learned task was examined 24 h after the first-day trial.

Histological studies

At the end of the experiment (45th day), three animals from each group were sacrificed under anesthesia using pentobarbital sodium (60 mg/kg) (Nair et al., 2018); the pancreas was removed, cut into small fragments, and fixed overnight in freshly prepared Zamboni's fixative solution. Representative fragments were taken from the tail of the pancreas. They were dehydrated in an ethanol series, cleared in xylene, and embedded in paraffin wax. Sections 7-μm thick were cut by microtome and transferred onto microscopic slides, which were dried at 55°C for 30 min to enhance section attachment. Forbrain and femur bone sections, samples were isolated and immediately washed with ice-cold saline solution. A portion of tissue was fixed in a 10% neutral formalin fixative solution. After fixation, tissues were embedded in paraffin wax, solid sections were cut at 5 μm, and the sections were stained with hematoxylin and eosin.

Statistical analysis

The data were expressed as mean±standard deviation. The statistical significance between means was analyzed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. $P<0.05$ was considered statistically significant.

RESULTS

Phytochemical screening

The qualitative analyses showed that all three extracts of *B. oleracea* contained major active constituents such as flavonoids, alkaloids, terpenoids, steroids, tannins, and glycosides at different concentrations, whereas saponins and amino acids were found additionally in the aqueous ex-

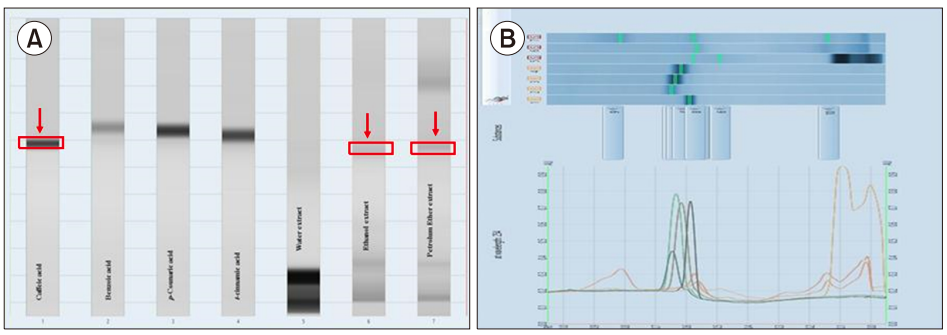

Fig. 1. High-performance thin-layer chromatography chromatogram with assigned peak acquired by 254 nm wavelength absorption densitometric scan of *Brassica oleracea* L. extracts. (A) Different bands of sample extracts and standard compound and (B) chromatogram of sample extracts and standard compounds.

tract.

Phenolic components profiling by HPTLC and HPLC

Validated HPTLC was applied for quantitative analysis of the biomarker caffeic acid in different extract solvents (Fig. 1). Caffeic acid was present in the ethanol and petroleum ether extracts and absent in the aqueous extract. The R_f value of caffeic acid was 0.587, and the ethanol and petroleum ether extracts had R_f values of 0.563 and 0.574, respectively. HPLC analysis confirmed the presence of caffeic acid in the purified ethanol and petroleum ether extract but was absent in aqueous extract. The peak of caffeic acid was identified by comparing retention time and the UV-spectrum of standard caffeic acid (Fig. 2).

Toxicity studies

Acute oral toxicity studies showed no mortality or any major behavioral changes with the extracts given at all doses tested. Rodents given the highest dose of the petroleum ether extract (2,000 mg/kg) showed slight inactiveness and slow movement, but no significant changes were observed in body weight (data not shown).

Levels of blood glucose, insulin, lipid, and glycosylated hemoglobin

Long-term administration of dexamethasone sodium phosphate led to a 75% elevation of fasting serum glucose levels, which was maintained throughout the 42 days. Significant improvement in blood glucose levels was observed in groups treated with 400 mg/kg of *B. oleracea* aqueous (32.31%), ethanol (19.62%), and petroleum ether (18.65%) extracts from days 0 to 7 (Table 1). The aqueous extract at all doses showed a significant reduction (P<0.001) in the glucose level from the 14th to 45th days, whereas the ethanol and petroleum ether extracts showed hypoglycemic activity at higher doses (200 and 400 mg/kg) compared with the negative control group. Additionally, the lipid profiles indicated that both the

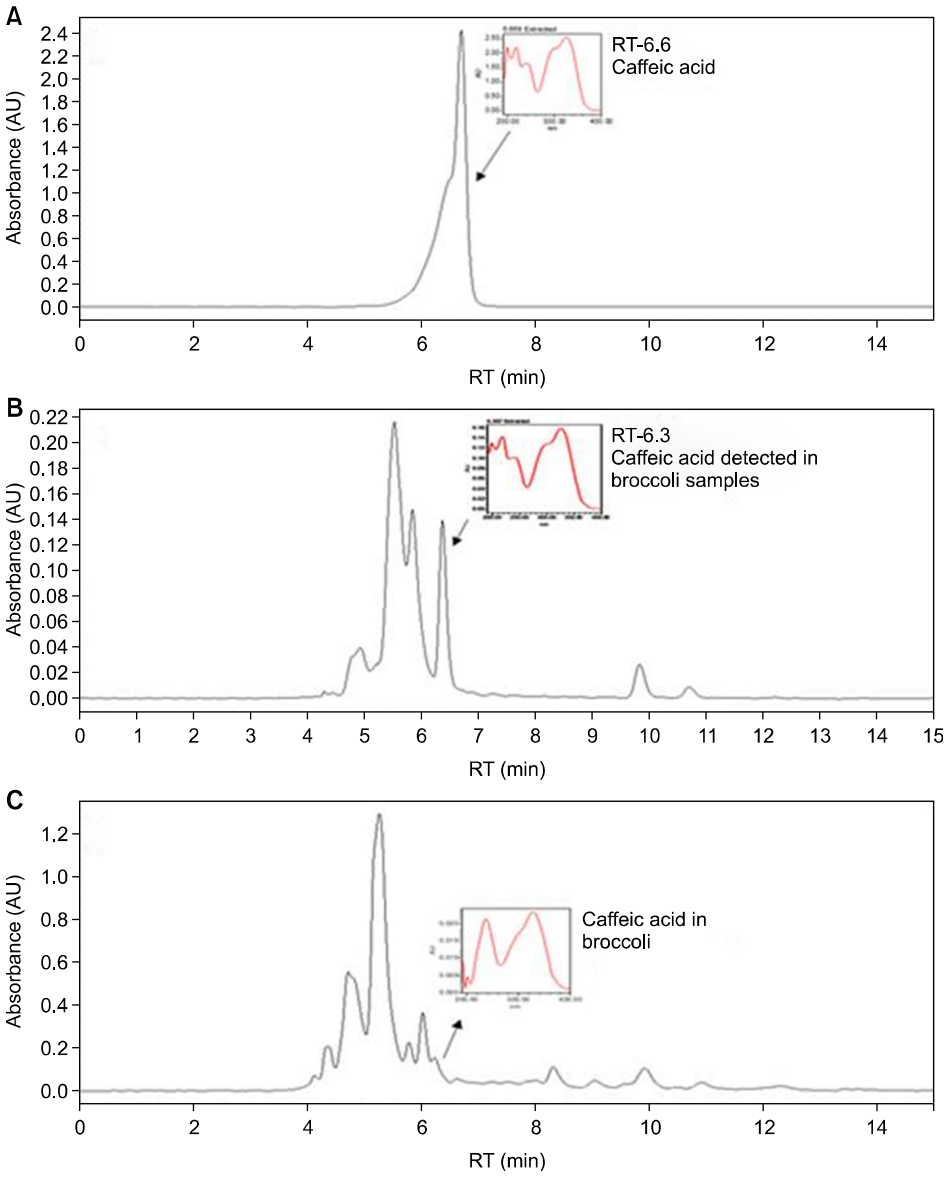

Fig. 2. High-performance liquid chromatography analysis of *Brassica oleracea* L. ethanol extract. (A) Aqueous extract, (B) ethanol extract, and (C) pet ether extract. RT, retention time.

Table 1. Serum glucose level after treatment with various extracts of *Brassica oleracea* L. at different intervals in rats with type 2 diabetes mellitus (unit: mg/dL)

Group	0 day	7th day	14th day	21st day	42nd day
Normal control	87.45±6.16***	83.11±7.75***	81.58±5.13***	89.33±9.11***	80.65±3.17***
Negative control	345.15±0.77	341.40±28.65	355.42±32.19	358.17±37.81	348.60±43.98
DM + metformin (200 mg/kg)	355.18±51.79	279.72±38.16*###	183.17±22.58***###	99.05±12.19***###	75.27±4.13***###
DM + PEE (100 mg/kg)	362.11±33.68	344.33±46.24	347.71±32.12	326.21±77.11ns	299.13±38.57
DM + PEE (200 mg/kg)	387.76±46.17	318.17±39.75##	298.21±12.29***###	255.71±14.78***###	221.17±22.11***###
DM + PEE (400 mg/kg)	371.43±43.18	302.13±24.19*#	243.11±26.11***###	197.16±23.18***###	143.44±23.77***###
DM + EE (100 mg/kg)	362.8±12.84	345.13±44.12	325.16±17.18	291.27±43.28#	253.65±40.02***###
DM + EE (200 mg/kg)	385.54±30.19	338.16±49.11	287.61±55.14*##	233.14±29.28***###	184.24±28.18***###
DM + EE (400 mg/kg)	377.1±29.31	303.11±13.48*#	232.11±41.78***###	186.19±42.68***###	137.18±47.14***###
DM + AE (100 mg/kg)	373.23±40.11	355.03±39.19	296.21±33.78*##	220.29±15.01***###	201.09±17.03***###
DM + AE (200 mg/kg)	393.32±14.48	296.14±40.23*###	212.28±17.16***###	154.71±14.78###	128.14±15.18***###
DM + AE (400 mg/kg)	378.50±51.23	256.21±27.19***###	201.17±64.16**###	152.41±29.12***###	123.14±23.14***###

The values are presented as mean±SD for each group (n=8).
Statistical analysis of data was performed using one-way ANOVA followed by Tukey's multiple range test.
*P<0.05, **P<0.01, and ***P<0.001 compared with negative control group and #P<0.05, ##P<0.01, and ###P<0.001 compared with day 0.
DM, type 2 diabetes mellitus; PEE, petroleum ether extract; EE, ethanol extract; AE, aqueous extract; ns, not significant compared with control group.

ethanol and aqueous extract treated groups showed significant improvement in LDL-c and triglyceride levels (Table 2). It was also observed that 200 and 400 mg/kg of the aqueous extract markedly elevated the HDL-c level compared with the negative control group. Furthermore, serum insulin level was restored to normal when treated with aqueous extracts (200 and 400 mg/kg). Bodyweight was reduced (P<0.05) on the 45th day in groups treated with 400 mg/kg of ethanol extract, 100 mg/kg of aqueous extract, and metformin compared with the negative control group (Table 2). Additionally, glycosylated hemoglobin levels significantly decreased when treated at a high dose of 400 mg/kg ethanol (7.54±1.10 mg/g) or aqueous extracts (7.20±1.40 mg/g) relative to the negative control group (10.04±1.32 mg/g) (Table 2).

Cytokine parameters

TNF-α and IL-6 levels (Table 3) were higher in the negative control group than in the treated groups. The aqueous (100, 200, and 400 mg/kg) and ethanol (200 and 400 mg/kg) extract significantly lowered TNF-α and IL-6 levels (P<0.05), whereas no changes were observed when treating with the petroleum ether extract.

Antioxidant parameters

Antioxidant assays revealed interesting results with all three extracts (petroleum ether, ethanol, and aqueous) at 400 mg/kg in both the pancreas and brain compared

Table 2. Effect of various extracts of *Brassica oleracea* L. on serum lipid profile, insulin, and hemoglobin levels at the 42nd day in type 2 diabetes mellitus (unit: mg/dL)

Group	HDL-c	Triglycerides	LDL-c	Insulin	Hb (mg/g)
Normal control	42.13±8.10***	92.29±12.15***	97.19±15.90***	17.03±6.40**	5.33±1.14***
Negative control	18.10±4.50	219.52±18.11	198.18±36.30	35.45±12.11	10.04±1.32
DM + metformin (200 mg/kg)	41.91±7.30***	104.6±12.15***	70.11±10.80***	19.30±7.70*	6.32±1.10***
DM + PEE (100 mg/kg)	22.04±5.40	190.77±22.87	176.13±14.98	23.66±9.66	9.91±1.34
DM + PEE (200 mg/kg)	27.18±6.20	189.27±22.65	170.12±14.77	26.55±10.04	9.03±1.20
DM + PEE (400 mg/kg)	20.10±5.80	191.62±24.55	164.20±13.23*	22.95±8.45	10.02±1.30
DM + EE (100 mg/kg)	31.43±8.00	145.33±15.22***	156.33±11.76*	21.05±9.33ns	8.40±1.03
DM + EE (200 mg/kg)	30.04±4.70	132.12±11.23***	132.19±9.54***	22.86±7.88	8.61±1.20
DM + EE (400 mg/kg)	39.10±6.34***	124.27±10.12***	100.05±8.10***	22.13±8.05	7.54±1.10**
DM + AE (100 mg/kg)	22.62±10.32	155.44±13.54***	142.49±11.99***	22.24±12.67	8.27±1.00
DM + AE (200 mg/kg)	39.52±7.70***	130.46±15.55***	144.16±12.45***	19.14±11.99*	7.87±1.02*
DM + AE (400 mg/kg)	41.77±11.60***	117.12±9.50***	104.54±10.41***	19.01±4.90*	7.20±1.40***

The values are presented as mean±SD for each group (n=8).
Statistical analysis of data was performed using one-way ANOVA followed by Tukey's multiple range test.
*P<0.05, **P<0.01, and ***P<0.001 compared with the negative control group.
HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; Hb, hemoglobin; DM, type 2 diabetes mellitus; PEE, petroleum ether extract; EE, ethanol extract; AE, aqueous extract; ns, not significant compared with control group.

Table 3. Effect of various extracts of *Brassica oleracea* L. on cytokine levels in rats with type 2 diabetes mellitus on the 40th day (unit: pg/mL)

Group	TNF-α	IL-6
Normal control	19.64±1.21***	106.39±10.38***
Negative control	58.20±4.81	137.63±11.45
DM + metformin (200 mg/kg)	22.16±2.16***	110.90±10.81***
DM + PEE (100 mg/kg)	59.46±4.96	140.55±12.52
DM + PEE (200 mg/kg)	56.21±4.17	130.38±8.11
DM + PEE (400 mg/kg)	50.38±3.63**	127.31±9.20
DM + EE (100 mg/kg)	52.25±3.55	137.70±8.91
DM + EE (200 mg/kg)	45.22±4.02***	126.82±9.87*
DM + EE (400 mg/kg)	30.06±2.10***	122.35±7.04
DM + AE (100 mg/kg)	35.39±2.96***	120.69±6.45
DM + AE (200 mg/kg)	28.84±1.93***	119.36±8.08*
DM + AE (400 mg/kg)	24.93±1.21***	113.60±6.30**

The values are presented as mean±SD for each group (n=8). Statistical analysis of data was performed using one-way ANOVA followed by Tukey's multiple range test.
*P<0.05, **P<0.01, and ***P<0.001 compared with the negative control group.
TNF-α, tumor necrosis factor-alpha; IL-6, interleukin-6; DM, type 2 diabetes mellitus; PEE, petroleum ether extract; EE, ethanol extract; AE, aqueous extract.

with the negative control group. Among all three extracts, the ethanol extract at 400 mg/kg showed the most significant (P<0.001) results in the pancreas (SOD: 64.12%, GPx: 34.34%, and TBARS: 50.41%) and brain (SOD: 71.38%, GPx: 33.43%, and TBARS: 60.52%) tissues (Table 4).

Behavioral studies

Treatment with either the ethanol or aqueous extract resulted in a statistically significant reduction in transfer latency, indicating tremendous improvement in memory compared with the negative control group (Table 5).

Histopathological results

The cerebral cortexes of the normal control group (Fig. 3A) were normal in shape and comprised three layers: molecular, pyramidal, and polymorphic. The pyramidal layer had normal neuron cells that appeared triangular with large vesicular nuclei. In the negative control group, there were significant changes in the shape of the cerebral cortex with enlarged and pyknotic nuclei and increased cell size. Lymphocytic infiltration and cellular edema were also identified because of inflammatory changes in some parts of the cerebral cortex. Sections revealed a loss of normal architecture, extensive shrinkage, and observed empty areas due to the loss of pyramidal cells (Fig. 3B). Similar changes (Fig. 3D, 3E, and 3G) were also observed in groups treated with specific doses of petroleum ether (100 and 200 mg/kg) and ethanol (100 mg/kg), with no significant improvement and a mild reduction in pyramidal cells observed. Sectional showed the normal histological structure of the three layers with a mild reduction in the pyramidal cells compared with the normal control group. All doses of aqueous, the two higher doses of ethanol extract (200 and 400 mg/kg) and the standard drug-treated groups had remarkable improvement in the regeneration of glial cells, and no inflammatory changes were observed. No histopathological alterations were found in the molecular, pyramidal, and polymorphic layers in the cortex compared with the negative control group (Fig. 3C, 3F, 3H~3L).

Microscopic investigation of pancreas sections of normal control rats showed the normal appearance of islets

Table 4. Effect of different extracts of *Brassica oleracea* L. on level of antioxidant enzymes and lipid peroxidation (TBARS) on rat tissue on the 21st day

Group	SOD (U/mg protein)		GPx (mM/mg protein)		TBARS (nmol/mg protein)	
	Pancreas	Brain	Pancreas	Brain	Pancreas	Brain
Normal control	3.51±0.38***	4.51±0.37***	64.50±2.42***	64.53±3.93***	0.56±0.18***	0.46±0.25***
Negative control	1.13±0.25	1.03±0.18	39.50±2.94	38.83±3.76	2.40±0.30	2.66±0.28
DM + metformin (200 mg/kg)	3.26±0.38***	3.93±0.31***	62.33±3.66***	60.00±2.52***	1.16±0.38***	1.08±0.18***
DM + PEE (100 mg/kg)	1.65±0.56	1.80±0.58**	40.16±3.31	40.66±5.00	2.02±0.11	2.09±0.18***
DM + PEE (200 mg/kg)	2.18±0.53***	2.08±0.22***	46.83±5.54*	44.00±3.79	1.96±0.10*	1.88±0.30***
DM + PEE (400 mg/kg)	2.46±0.58***	2.95±0.10***	56.83±5.38***	53.16±3.31***	1.45±0.36***	1.72±0.22***
DM + EE (100 mg/kg)	2.13±0.20***	2.46±0.41***	43.83±3.40	45.66±5.20	1.93±0.15*	1.81±0.33***
DM + EE (200 mg/kg)	2.13±0.20***	2.73±0.36***	50.66±4.96***	50.66±5.39***	1.45±0.15***	1.76±0.15***
DM + EE (400 mg/kg)	3.15±0.28***	3.60±0.35***	60.16±2.22***	58.33±3.44***	1.19±0.30***	1.05±0.24***
DM + AE (100 mg/kg)	1.88±0.54*	2.18±0.23***	41.50±4.76	42.50±4.08	1.99±0.11	1.89±0.30***
DM + AE (200 mg/kg)	2.06±0.39**	2.33±0.42***	49.50±6.89***	51.50±6.50***	1.61±0.33***	1.76±0.12***
DM + AE (400 mg/kg)	2.83±0.49***	3.33±0.42***	58.66±3.20***	57.16±4.40***	1.3±0.35***	1.13±0.30***

The values are presented as mean±SD for each group (n=8).
Statistical analysis of data was performed using one-way ANOVA followed by Tukey's multiple range test.
*P<0.05, **P<0.01, and ***P<0.001 compared with the negative control group.
SOD, superoxide dismutase; GPx, glutathione peroxidase; TBARS, thiobarbituric acid reactive substance; DM, type 2 diabetes mellitus; PEE, petroleum ether extract; EE, ethanol extract; AE, aqueous extract.

Table 5. Effect of different extracts of *Brassica oleracea* L. on the transfer latency on acquisition day and retention day, at the 38th and 39th day, respectively (unit: s)

Group	Transfer latency	
	Acquisition day	Retention day
Normal control	63.23±3.91***	70.68±6.70***
Negative control	162.58±8.34	169.59±10.03
DM + metformin (200 mg/kg)	67.39±3.66***	24.40±2.81***
DM + PEE (100 mg/kg)	164.64±8.56	160.21±5.25
DM + PEE (200 mg/kg)	182.18±8.97***	167.27±6.07
DM + PEE (400 mg/kg)	101.44±7.23***	79.19±3.99***
DM + EE (100 mg/kg)	142.71±7.15***	138.55±4.51***
DM + EE (200 mg/kg)	119.20±6.22***	92.53±4.57***
DM + EE (400 mg/kg)	65.31±3.40***	46.10±3.04***
DM + AE (100 mg/kg)	99.54±5.96***	37.18±3.49***
DM + AE (200 mg/kg)	81.59±7.63***	53.09±4.71***
DM + AE (400 mg/kg)	49.23±6.13***	24.37±1.86***

The values are expressed as mean±SD for each group (n=8). Statistical analysis of data was performed using one-way ANOVA followed by Tukey's multiple range test.
***P<0.001 compared with the negative control group.
DM, type 2 diabetes mellitus; PEE, petroleum ether extract; EE, ethanol extract; AE, aqueous extract.

of Langerhans (Fig. 4A). Negative control rats (Fig. 4B) had pathological changes of both exocrine and endocrine components. In the pancreas morphology, the acinar and β cells of the pancreas were damaged and shrunken in size. Infiltration of lymphocytes was also seen in comparison with the normal control group. Islets β-cells were almost entirely lost in the negative control group. The pancreatic β cells showed remarkable improvement in the ethanol and aqueous extract treated groups (Fig. 4H~4L) compared with the negative control group. Cellular regeneration was also observed in all treated groups at higher doses.

Femur bones of different extract treated groups (Fig. 5F, 5I, 5K, and 5L) were improved significantly; particularly, the trabeculae and Haversian canal were similar to those of the normal control group (Fig. 5A). Destruction of the medullary cavity (Fig. 5B) was observed within the bone tissue in the negative control group. Negative control rats also displayed a thin trabecular structure of the cancellous bone along with lesser cells. Thus, the negative control rats showed invasion and irregularities in both the outer and inner surfaces of the bone tissue in some places. Regarding trabeculae thickness, all three extracts at 400 mg/kg showed a significant anti-osteopathic effect when compared with the negative control group (Fig. 6). It was also observed that trabecular bones were arranged in a more orderly manner and bone matrix density increased at higher doses.

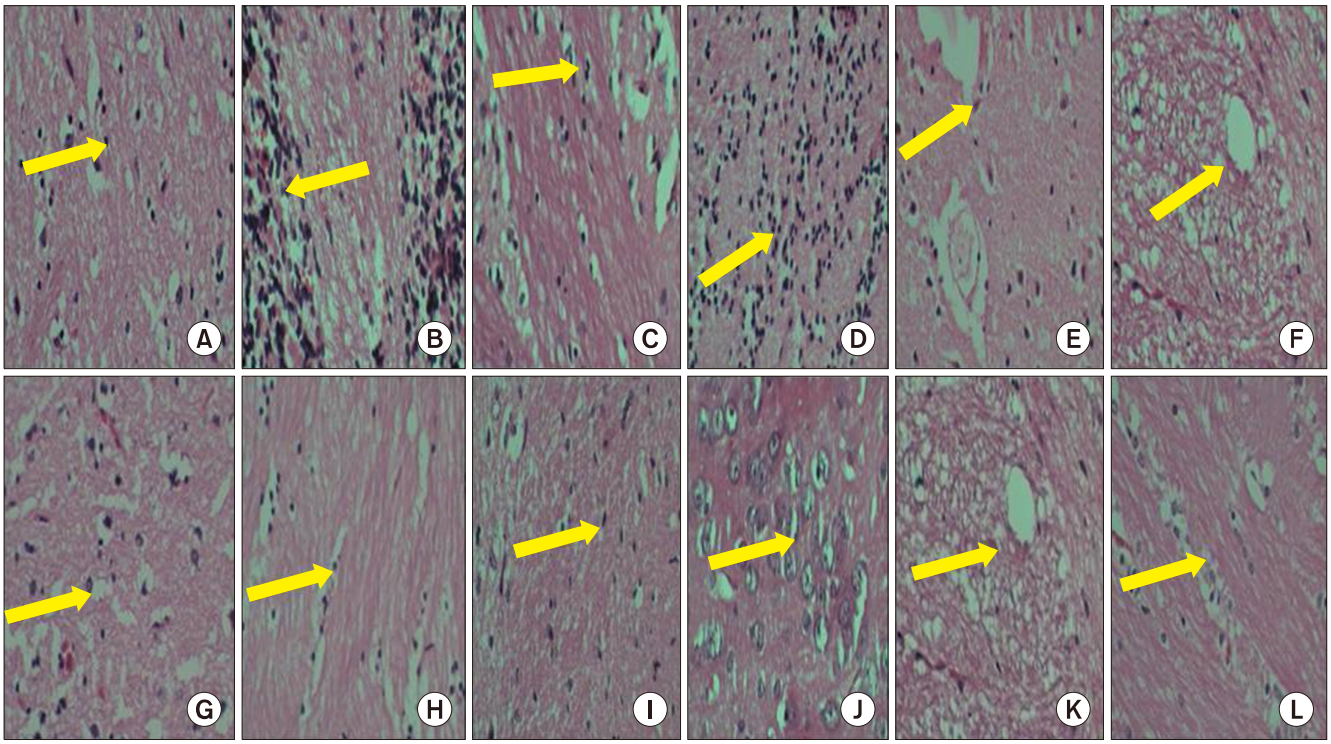

Fig. 3. Light photomicrographs (45× magnification) of rat brain sections from different experimental groups: (A) normal control, (B) negative control, (C) 200 mg/kg of metformin, (D) 100 mg/kg of petroleum ether extracts of *Brassica oleracea* (PEE), (E) 200 mg/kg of PEE, (F) 400 mg/kg of PEE, (G) 100 mg/kg of ethanol extract of *B. oleracea* (EE), (H) 200 mg/kg of EE, (I) 400 mg/kg of EE, (J) 100 mg/kg of aqueous extract of *B. oleracea* (AE), (K) 200 mg/kg of AE, and (L) 400 mg/kg of AE. The arrows indicate neuron cells. (A) The normal control group showed a normal appearance in the cerebral cortex structure. (B, D, E, and G) Negative control and treated groups showed a loss of internal structure. Distorted pyramidal layers revealed degenerative changes and observed shrinkage in pyramidal cells. (C, F, and H~L) Remarkable improvement in the regeneration of glial cells and no inflammatory changes were observed. The pyramidal layer showed the reappearance of normal pyramidal cells.

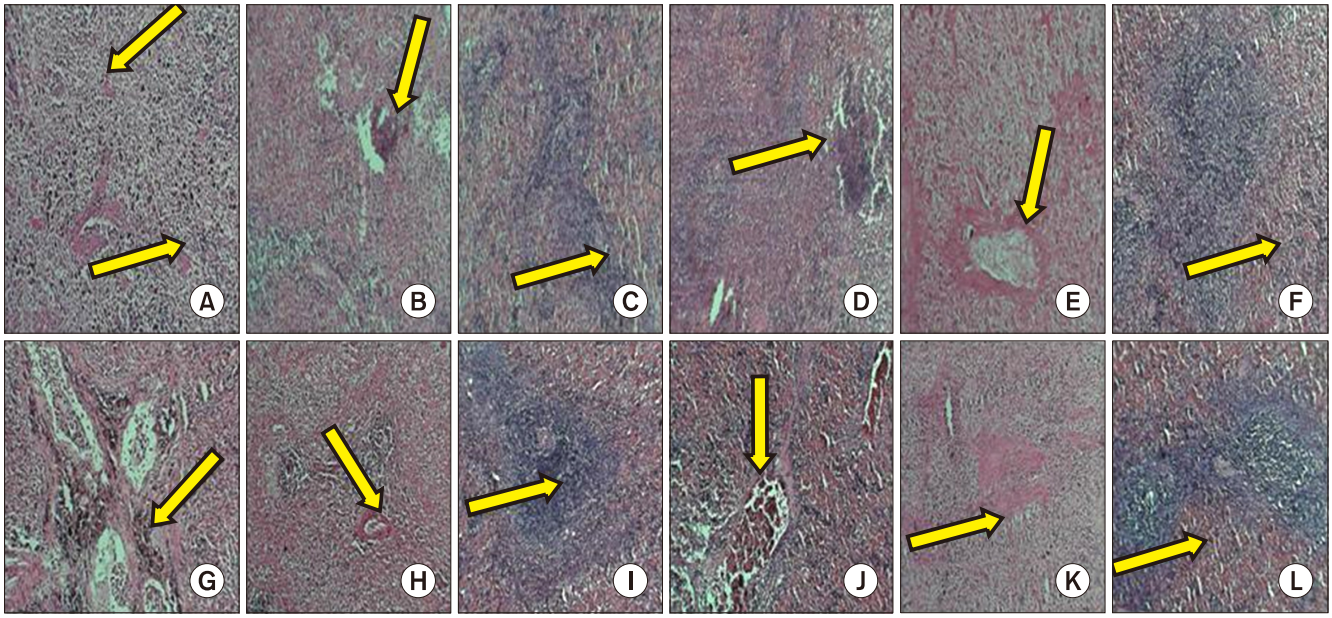

Fig. 4. Light photomicrographs (45× magnification) of rat pancreas sections from different experimental groups: (A) normal control, (B) negative control, (C) 200 mg/kg of metformin, (D) 100 mg/kg of petroleum ether extracts of *Brassica oleracea* (PEE), (E) 200 mg/kg of PEE, (F) 400 mg/kg of PEE, (G) 100 mg/kg of ethanol extract of *B. oleracea* (EE), (H) 200 mg/kg of EE, (I) 400 mg/kg of EE, (J) 100 mg/kg of aqueous extract of *B. oleracea* (AE), (K) 200 mg/kg of AE, and (L) 400 mg/kg of AE. The arrows indicate the appearance of pancreas. (A) The normal control group showed normal architecture of the pancreas. (B) The negative control group showed pathological changes of both endocrine and exocrine components. Acinar and β cells of the pancreas were damaged and shrunken in size. (H~L) Pancreatic β cells showed remarkable improvement and observed regeneration of islets of Langerhans.

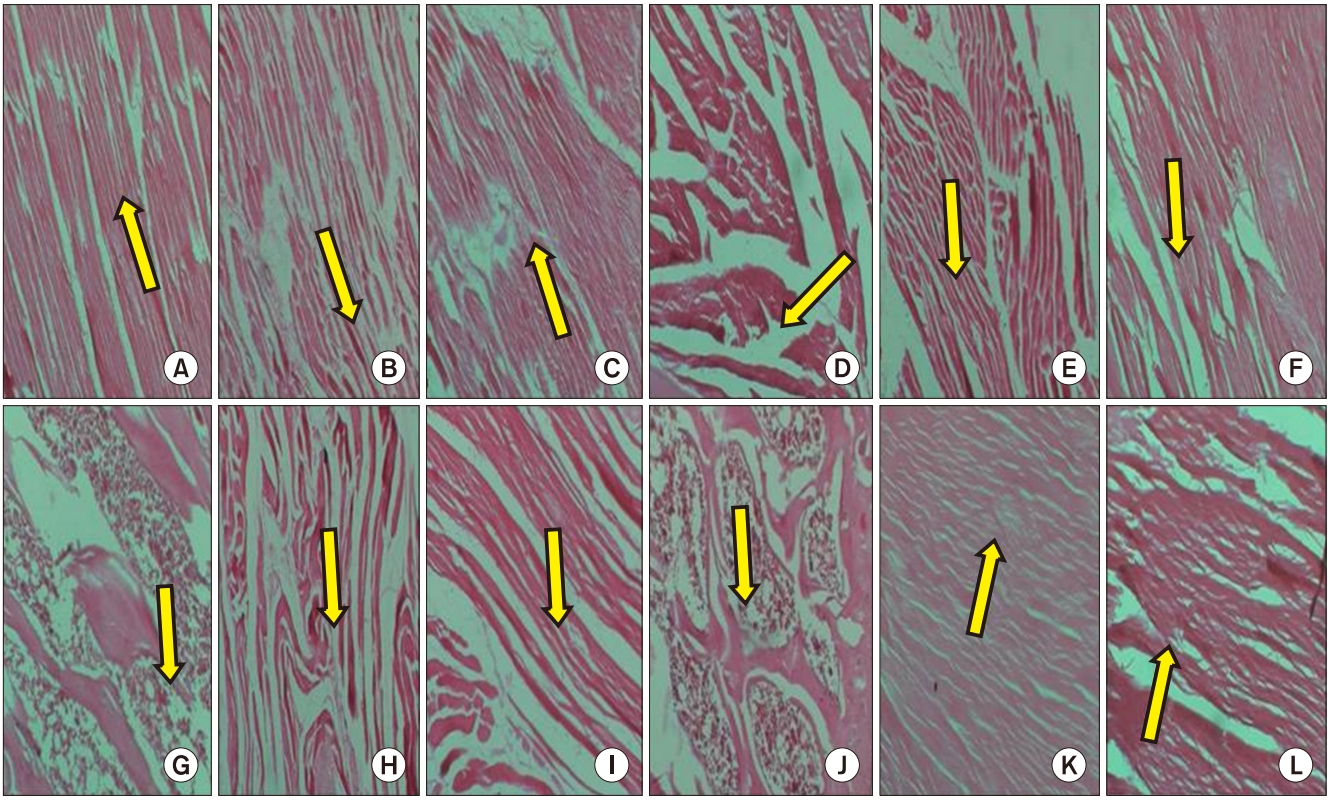

Fig. 5. Light photomicrographs (45× magnification) of rat femur bone sections from different experimental groups: (A) normal control, (B) negative control, (C) 200 mg/kg of metformin, (D) 100 mg/kg of petroleum ether extracts of *Brassica oleracea* (PEE), (E) 200 mg/kg of PEE, (F) 400 mg/kg of PEE, (G) 100 mg/kg of ethanol extract of *B. oleracea* (EE), (H) 200 mg/kg of EE, (I) 400 mg/kg of EE, (J) 100 mg/kg of aqueous extract of *B. oleracea* (AE), (K) 200 mg/kg of AE, and (L) 400 mg/kg of AE. The arrows indicate the bone stucture. (A) The normal control group showed original microstructure in thickness. (B) Negative control showed destruction of the medullary cavity and decreased femoral thickness. (F, I, K, and L) Improvement in femur bones was observed, especially the trabeculae and Haversian canal.

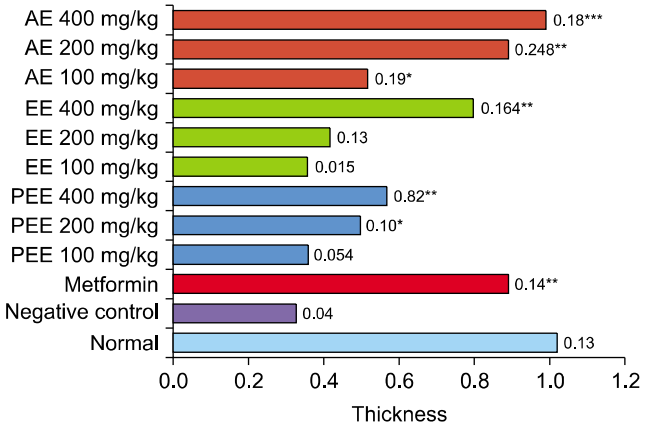

Fig. 6. Femur bone thickness of different groups on the 45th day. The negative control was less thick than the normal control group. Different extracts at high doses showed excellent improvement in thickness relative to the normal control, whereas low doses had decreased thickness. The values are presented as mean±SD for each group (n=8). **P*<0.05, ***P*<0.01, and ****P*<0.001 compared with a negative control group. AE, animals treated with aqueous extract of *Brassica oleracea*; EE, animals treated with ethanol extract of *B. oleracea*; PEE, animals treated with petroleum ether extract of *B. oleracea*; Metformin, animals treated with metformin (200 mg/kg); Negative control, a diseased animal with no treatment; Normal, animals treated with oral saline.

DISCUSSION

Overexposure to free radicals can cause oxidative damage to essential cellular structures that consequently leads to various chronic diseases such as atherosclerosis, cancer, diabetes, rheumatoid arthritis, postischemic perfusion injury, cardiovascular diseases, chronic inflammation, stroke, and other degenerative diseases in humans (Phaniendra et al., 2015; Sun et al., 2018; Forman and Zhang, 2021). Diabetes mellitus is a metabolic disorder that is characterized by higher levels of glucose in the blood and insufficient production or action of insulin (Asmat et al., 2016). Type 2 diabetes mellitus is characterized by insulin resistance and is a serious metabolic disorder in the world (Acharjee et al., 2013). Moreover, insulin resistance induces the release of cytokines like TNF-α and IL-6, which may lead to the development of oxidative stress in the liver and may reduce mitochondrial levels of SOD, and glutathione and increase production of H_2O_2 radicals, leading to lipid peroxidation (Vona et al., 2019). Both longitudinal and cross-sectional studies have shown that insulin resistance is one of the key factors responsible for diabetic-associated complications like osteoporosis and dementia (Zilliox et al., 2016). Furthermore, a study has shown that the association of diabetes with Alzheimer's and osteoporosis is linked to activation of the cytokine network and subsequent release and elevation of different oxidative markers such as TNF-α, IL-6, and IL-18, receptor activator of nuclear factor kappa B (NF-κB), and monocyte chemoattractant protein-1 (Tan et al., 2018).

The discovery of new treatment strategies for the effective therapy of type 2 diabetes mellitus has attracted extensive interest. Indeed, plant-based nutraceutical supplements containing a significant amount of bioactive constituents can provide desirable health benefits for society. Various parts of the plant including leaf, fruit, outer bark, and root are used to enhance memory in traditional Asian medicine. Thus, various research groups are actively studying mouse models of Alzheimer's disease to identify plant extracts that can reduce the severity of this disease with major interest focusing on phytoconstituents, which possess antioxidant activity (Pandey and Rizvi, 2009). Studies suggest that cruciferous edible plants including vegetables are functional foods with some of the best sources of antioxidants (Manchali et al., 2012). Vegetables from the Brassicaceae family are rich in many components beneficial to health, including high levels of glucosinolates (sulforaphane), polyphenolic compounds (anthocyanins, quercetin, kaempferol, hydroxycinnamic acid, and isorhamnetin derivatives), vitamins (C and K1), and dietary essential minerals (Ca, Mg, Na, K, Fe, and Zn) (Li et al., 2018; Huang et al., 2019). Thus, they possess beneficial compounds that can prevent oxidative stress and stimulate the immune system (Kapusta-Duch et al., 2012). Indeed, the leaf of *B. oleracea* is considered as a superfood (Manchali et al., 2012), it contains high levels of well-balanced minerals and vitamins such as β-carotene and amino acids (Šamec et al., 2019). The protective activity of *B. oleracea* against oxidative damage is also reported by Cho et al. (2006). To assess the chemical profile of broccoli, a study has used liquid chromatography-UV diode-array detection-electrospray ionization mass spectrometry to estimate the contents of polyphenols and flavonoids (Vallejo et al., 2004). The present study demonstrated the presence of polyphenols, flavonoids, and triterpenoids in *B. oleracea* flowers. These phytochemical results are consistent with another earlier study (Ahmed et al., 2012). Among different extracts we assessed, caffeic acid was found in the petroleum ether and ethanol extracts by HPTLC analysis. The anti-osteoporotic effect of *B. oleracea* may be due to the presence of lignans (phytoestrogens), which is demonstrated in another study (Wang et al., 2017). The antidiabetic activity of nutraceuticals or natural compounds has also been reported in many studies (Gupta et al., 2011; Gupta et al., 2013; Berberich and Hegele, 2018; Sharma et al., 2020). In the LD_{50} study, no mortality or major behavioral changes were observed at a dose up to 2,000 mg/kg (Shah et al., 2016). In this study, rats were administered dexamethasone sodium phosphate continuously for 3 days per week for 45 days to ensure the induction of insulin resistance. Dexamethasone sodium phosphate induces hyperglycemia, which is reversible as it does not impair insulin sig-

naling despite impairment in insulin action. In a dexamethasone rat model, we observed that the oral administration of *B. oleracea* extracts (ethanol and aqueous) for 45 days reversed the diabetic effect and prevented diabetic osteopathy and diabetic neurodegenerative disorder. Reduction of cholesterol and triglyceride levels were noticed in groups treated with aqueous and ethanol extracts. Additionally, the plant extracts improved lipid profiles in a rat model with insulin resistance. Earlier studies have demonstrated that *B. oleracea* has great potential to decrease the cholesterol level in Japanese populations following administration of 99 g of broccoli sprouts daily for a week (Murashima et al., 2004). Furthermore, long-term administration of these extracts shows significant inhibition in lipid peroxidation activity. In other similar studies, oral administration of broccoli (100 and 200 mg/kg) decreases serum TBARS (Cho et al., 2006), which are important markers of lipid peroxidation. A significant decrease in malondialdehyde and oxidized LDL-c is observed in diabetic patients treated with broccoli powder (10 g/d) (Bahadoran et al., 2011).

Histopathology of bone and brain images demonstrated a significant improvement in the morphological structure (internal and external) with higher doses of each *B. oleracea* extract. In the elevated plus-maze test, memory was significantly improved by treatment with the aqueous and ethanol extracts. Myricetin is one of the active compounds present in *B. oleracea* which shows neuroprotective ability via anti-amyloid and anti-secretase activity and is responsible for retaining memory (Baptista et al., 2014). Our results are consistent with an earlier study that reports the presence of glucosinolates in broccoli (Prieto et al., 2019).

TNF-α plays an important role in insulin resistance and the vascular inflammation process through multiple actions (da Costa et al., 2016). At low and high concentrations, TNF-α reduces the activity of the insulin-stimulated receptor tyrosine kinase that decreases the expression of insulin receptor substrate-1 through the phosphorylation of serine residue 307, which may affect the downstream signaling (da Costa et al., 2016). Regarding the cytokine levels in our study, *B. oleracea* extracts had an inhibitory effect on the release of TNF-α and IL-6. A study conducted by Ritz et al. (2007) suggests that sulforaphane has the potential to inhibit the production of proinflammatory cytokines. Sulforaphane, a natural isothiocyanate compound present in broccoli plants, has antioxidative and anti-inflammatory activity (Huang et al., 2019). The antidiabetic effect of sulforaphane may be due to activation of the Nrf2 system that binds to the nucleotide sequence of the antioxidative response element that encodes antioxidant enzymes (Fig. 7) including heme oxygenase-1, Mn-SOD, sequestosome 1, nicotinamide adenine dinucleotide phosphate hydrate quinine oxidoreductase 1, GPx, glutathione *S*-transferase A1, and glutamate-cysteine ligase. These enzymes may activate the Nrf2 dependent antioxidant response signaling pathway and also inactivate NF-κB, a key modulator of inflammatory pathways that may lead to the hypoglycemic effect. Sulforaphane can also protect dopaminergic cells by induction of thioredoxin (Bahadoran et al., 2011) and acts as a histone deacetylase inhibitor and peroxisome proliferator-

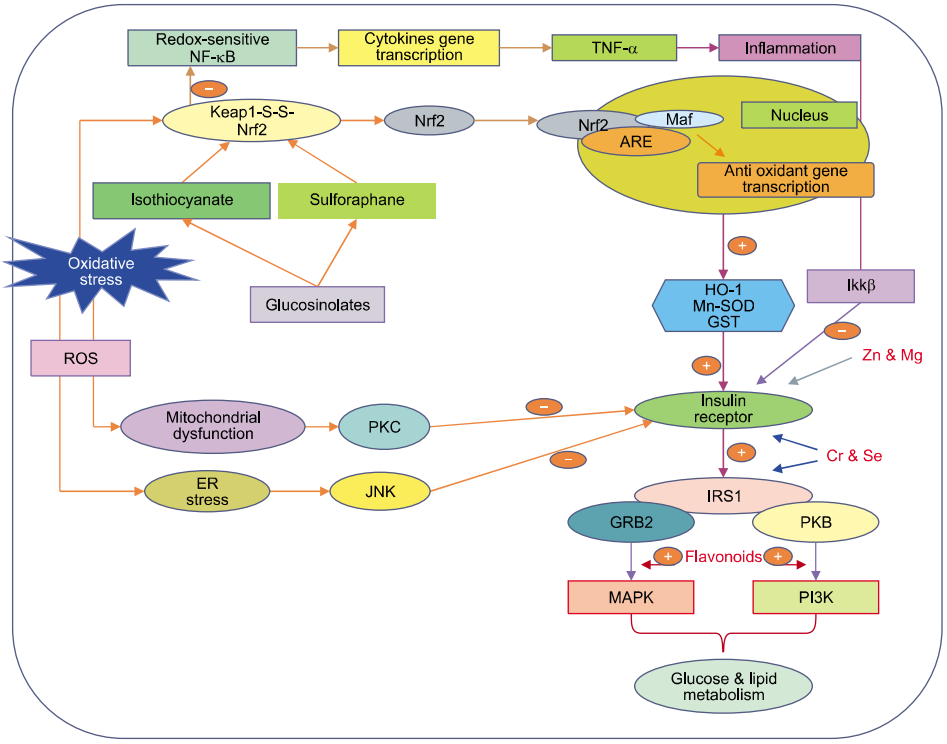

Fig. 7. Possible mechanism of *Brassica oleracea* in the treatment of diabetes complications. The main active compounds isothiocyanate and sulforaphane inhibit oxidation and glucose metabolism via the Nrf2 receptor. NF-κB, nuclear factor kappa B; TNF-α, tumor necrosis factor-α; ARE, antioxidant response element; HO-1, heme oxygenase-1; SOD, superoxide dismutase; GST, glutathione *S*-transferase; ROS, reactive oxygen species; PKC, protein kinase C; ER, endoplasmic reticulum; JNK, c-Jun N-terminal kinase; IRS1, insulin receptor substrate-1; GRB2, growth factor receptor-bound protein 2; PKB, protein kinase B; MAPK, mitogen-activated protein kinase; PI3K, phosphoinositide 3-kinase.

activated receptors modulator, which contributes to lipid metabolism and glucose homeostasis (Hu et al., 2006). Nrf2 activators are also a well-established product available in the market as an antioxidant and a detoxification support formula. Some clinical studies have reported anticancer and antihyperlipidemic activity of broccoli sprouts (10 g/d), which has proven clinically feasible in the treatment of communicable and non-communicable diseases (Wong et al., 1997).

The overall data from the present study demonstrated the potential of ethanol and aqueous extracts of *B. oleracea* against dexamethasone sodium phosphate-induced type 2 diabetes mellitus and its associated disorders. Nevertheless, our study was limited by our ability to isolate and characterize pure compounds from the bioactive extracts. In the future, the bioactive fraction of these extracts should be used to explore the molecular mechanism involved and should be viewed as new candidates for the treatment of diabetes mellitus and its associated disorders.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Concept and design: SG, SB. Analysis and interpretation: SG, SB, ABN. Data collection: SG, SB, ABN, DS. Writing the article: SG, ABN, MD, DM, RD, HEK. Critical revision of the article: SG, ABN, HEK. Final approval of the article: all authors. Statistical analysis: SG, ABN. Obtained funding: none. Overall responsibility: SG, ABN.

REFERENCES

Abdulameer SA, Sahib MN, Sulaiman SAS. The prevalence of osteopenia and osteoporosis among Malaysian type 2 diabetic patients using quantitative ultrasound densitometer. Open Rheumatol J. 2018. 12:50-64.

Acharjee S, Ghosh B, Al-Dhubiab BE, Nair AB. Understanding type 1 diabetes: etiology and models. Can J Diabetes. 2013. 37: 269-276.

Ahmed MF, Rao AS, Ahemad SR, Ibrahim M. Phytochemical studies and antioxidant activities of *Brassica oleracea* L. var. *capitata*. Int J Pharm Pharm Sci. 2012. 4:374-378.

Anroop B, Ghosh B, Parcha V, Vasanti S. Studies on *Ocimum gratissimum* seed mucilage: evaluation of suspending properties. Indian J Pharm Sci. 2005. 67:206-209.

Arokoyo DS, Oyeyipo IP, Du Plessis SS, Chegou NN, Aboua YG. Modulation of inflammatory cytokines and islet morphology as therapeutic mechanisms of *Basella alba* in streptozotocin-induced diabetic rats. Toxicol Res. 2018. 34:325-332.

Asmat U, Abad K, Ismail K. Diabetes mellitus and oxidative stress —a concise review. Saudi Pharm J. 2016. 24:547-553.

Bahadoran Z, Mirmiran P, Hosseinpanah F, Hedayati M, Hosseinpour-Niazi S, Azizi F. Broccoli sprouts reduce oxidative stress in type 2 diabetes: a randomized double-blind clinical trial. Eur J Clin Nutr. 2011. 65:972-977.

Baptista FI, Henriques AG, Silva AM, Wiltfang J, da Cruz e Silva OA. Flavonoids as therapeutic compounds targeting key proteins involved in Alzheimer's disease. ACS Chem Neurosci. 2014. 5:83-92.

Berberich AJ, Hegele RA. Nutraceuticals in 2017: nutraceuticals in endocrine disorders. Nat Rev Endocrinol. 2018. 14:68-70.

Boeing H, Bechthold A, Bub A, Ellinger S, Haller D, Kroke A, et al. Critical review: vegetables and fruit in the prevention of chronic diseases. Eur J Nutr. 2012. 51:637-663.

Chao PC, Li Y, Chang CH, Shieh JP, Cheng JT, Cheng KC. Investigation of insulin resistance in the popularly used four rat models of type-2 diabetes. Biomed Pharmacother. 2018. 101: 155-161.

Cho EJ, Lee YA, Yoo HH, Yokozawa T. Protective effects of broccoli (*Brassica oleracea*) against oxidative damage *in vitro* and *in vivo*. J Nutr Sci Vitaminol. 2006. 52:437-444.

da Costa RM, Neves KB, Mestriner FL, Louzada-Junior P, Bruder-Nascimento T, Tostes RC. TNF-α induces vascular insulin resistance via positive modulation of PTEN and decreased Akt/eNOS/NO signaling in high fat diet-fed mice. Cardiovasc Diabetol. 2016. 15:119. https://doi.org/10.1186/s12933-016-0443-0

Dreher ML. Whole fruits and fruit fiber emerging health effects. Nutrients. 2018. 10:1833. https://doi.org/10.3390/nu10121833

Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys. 1959. 82:70-77.

Evans WC. Phytochemical screening. In: Evans WC, editor. Trease and Evans' Pharmacognosy. 16th ed. Saunders, London, UK. 2009. p 196-202.

Forman HJ, Zhang H. Targeting oxidative stress in disease: promise and limitations of antioxidant therapy. Nat Rev Drug Discov. 2021. 20:689-709.

Gray AR. Taxonomy and evolution of broccoli (*Brassica oleracea* var. *italica*). Econ Bot. 1982. 36:397-410.

Gupta A, Nair A, Kumria R, Al-Dhubiab BE, Chattopadhyaya I, Gupta S. Assessment of pharmacokinetic interaction of spirulina with glitazone in a type 2 diabetes rat model. J Med Food. 2013. 16:1095-1100.

Gupta S, Hrishikeshvan HJ, Sehajpal PK. Spirulina protects against rosiglitazone induced osteoporosis in insulin resistance rats.

Diabetes Res Clin Pract. 2010. 87:38-43.
Gupta S, Mehla K, Chauhan D, Kumar S, Nair A. Morphological changes and antihyperglycemic effect of *M. Champaca* leaves extract on beta-cell in alloxan induced diabetic rats. Recent Res Sci Technol. 2011. 3:81-87.
Gupta S, Nair A, Jhawat V, Mustaq N, Sharma A, Dhanawat M, et al. Unwinding complexities of diabetic Alzheimer by potent novel molecules. Am J Alzheimers Dis Other Demen. 2020. 35:1533317520937542. https://doi.org/10.1177/1533317520937542
Harborne JB. Phytochemical methods: a guide to modern techniques of plant analysis. 3rd ed. Chapman & Hall, London, UK. 1998. p 21-72.
Hossain MA, Ismail Z. High performance thin layer chromatographic determination of caffeic acid and rosmarinic acid from the leaves of *Orthosiphon stamineus*. Indones J Chem. 2009. 9:137-141.
Hu R, Xu C, Shen G, Jain MR, Khor TO, Gopalkrishnan A, et al. Gene expression profiles induced by cancer chemopreventive isothiocyanate sulforaphane in the liver of C57BL/6J mice and C57BL/6J/Nrf2 (-/-) mice. Cancer Lett. 2006. 243:170-192.
Huang C, Wu J, Chen D, Jin J, Wu Y, Chen Z. Effects of sulforaphane in the central nervous system. Eur J Pharmacol. 2019. 853:153-168.
Jacob S, Morsy MA, Nair A. An overview on the insulin preparations and devices. Indian J Pharm Educ Res. 2018. 52:550-557.
Jahangir M, Kim HK, Choi YH, Verpoorte R. Health-affecting compounds in *Brassicaceae*. Compr Rev Food Sci Food Saf. 2009. 8:31-43.
Kapusta-Duch J, Kopeć A, Piatkowska E, Borczak B, Leszczyńska T. The beneficial effects of *Brassica* vegetables on human health. Rocz Panstw Zakl Hig. 2012. 63:389-395.
Khalil HE, Aljeshi YM, Saleh FA. Authentication of *Carissa macrocarpa* cultivated in Saudi Arabia; botanical, phytochemical and genetic study. J Pharm Sci Res. 2015. 7:497-508.
Konieczka P, Rozbicka-Wieczorek AJ, Więsyk E, Smulikowska S, Czauderna M. Improved derivatization of malondialdehyde with 2-thiobarbituric acid for evaluation of oxidative stress in selected tissues of chickens. J Anim Feed Sci. 2014. 23:190-197.
Li Z, Lee HW, Liang X, Liang D, Wang Q, Huang D, et al. Profiling of phenolic compounds and antioxidant activity of 12 cruciferous vegetables. Molecules. 2018. 23:1139. https://doi.org/10.3390/molecules23051139
Liguori I, Russo G, Curcio F, Bulli G, Aran L, Della-Morte D, et al. Oxidative stress, aging, and diseases. Clin Interv Aging. 2018. 13:757-772.
Manchali S, Murthy KNC, Patil BS. Crucial facts about health benefits of popular cruciferous vegetables. J Funct Foods. 2012. 4:94-106.
Mittal K, Katare DP. Shared links between type 2 diabetes mellitus and Alzheimer's disease: a review. Diabetes Metab Syndr. 2016. 10:S144-S149.
Murashima M, Watanabe S, Zhuo XG, Uehara M, Kurashige A. Phase 1 study of multiple biomarkers for metabolism and oxidative stress after one-week intake of broccoli sprouts. Biofactors. 2004. 22:271-275.
Nair A, Morsy MA, Jacob S. Dose translation between laboratory animals and human in preclinical and clinical phases of drug development. Drug Dev Res. 2018. 79:373-382.
OECD. Test no. 474: mammalian erythrocyte micronucleus test, OECD guidelines for the testing of chemicals, section 4. 2016 [cited 2016 Jul 29]. Available from: https://doi.org/10.1787/9789264264762-en
Oliveira WH, Nunes AK, França ME, Santos LA, Lós DB, Rocha SW, et al. Effects of metformin on inflammation and short-term memory in streptozotocin-induced diabetic mice. Brain Res. 2016. 1644:149-160.
Pandey KB, Rizvi SI. Plant polyphenols as dietary antioxidants in human health and disease. Oxid Med Cell Longev. 2009. 2:270-278.
Pari L, Latha M. Protective role of *Scoparia dulcis* plant extract on brain antioxidant status and lipidperoxidation in STZ diabetic male Wistar rats. BMC Complement Altern Med. 2004. 4:16. https://doi.org/10.1186/1472-6882-4-16
Phaniendra A, Jestadi DB, Periyasamy L. Free radicals: properties, sources, targets, and their implication in various diseases. Indian J Clin Biochem. 2015. 30:11-26.
Prieto MA, López CJ, Simal-Gandara J. Glucosinolates: molecular structure, breakdown, genetic, bioavailability, properties and healthy and adverse effects. Adv Food Nutr Res. 2019. 90:305-350.
Reeves PG. Components of the AIN-93 diets as improvements in the AIN-76A diet. J Nutr. 1997. 127:838S-841S.
Ritz SA, Wan J, Diaz-Sanchez D. Sulforaphane-stimulated phase II enzyme induction inhibits cytokine production by airway epithelial cells stimulated with diesel extract. Am J Physiol Lung Cell Mol Physiol. 2007. 292:L33-L39.
Šamec D, Urlić B, Salopek-Sondi B. Kale (*Brassica oleracea* var. *acephala*) as a superfood: review of the scientific evidence behind the statement. Crit Rev Food Sci Nutr. 2019. 59:2411-2422.
Sanlier N, Guler Saban M. The benefits of *Brassica* vegetables on human health. J Hum Health Res. 2018. 1:104. https://article.scholarena.co/The-Benefits-of-Brassica-Vegetables-on-Human-Health.pdf
Shah MA, Sarker MMR, Gousuddin M. Toxicity study of *Brassica oleracea* var. *italica* extracts in Sprague Dawley (SD) rats. Int J Pharmacogn Phytochem Res. 2016. 8:735-741.
Sharma A, Gupta S, Chauhan S, Nair A, Sharma P. Astilbin: a promising unexplored compound with multidimensional medicinal and health benefits. Pharmacol Res. 2020. 158:104894. https://doi.org/10.1016/j.phrs.2020.104894
Sun MS, Jin H, Sun X, Huang S, Zhang FL, Guo ZN, et al. Free radical damage in ischemia-reperfusion injury: an obstacle in acute ischemic stroke after revascularization therapy. Oxid Med Cell Longev. 2018. 2018:3804979. https://doi.org/10.1155/2018/3804979
Tan BL, Norhaizan ME, Liew WP, Sulaiman Rahman H. Antioxidant and oxidative stress: a mutual interplay in age-related diseases. Front Pharmacol. 2018. 9:1162. https://doi.org/10.3389/fphar.2018.01162
Vallejo F, Tomás-Barberán FA, Ferreres F. Characterisation of flavonols in broccoli (*Brassica oleracea* L. var. *italica*) by liquid chromatography-UV diode-array detection-electrospray ionisation mass spectrometry. J Chromatogr A. 2004. 1054:181-193.
Vona R, Gambardella L, Cittadini C, Straface E, Pietraforte D. Biomarkers of oxidative stress in metabolic syndrome and associated diseases. Oxid Med Cell Longev. 2019. 2019:8267234. https://doi.org/10.1155/2019/8267234
Wang T, Liu Q, Tjhioe W, Zhao J, Lu A, Zhang G, et al. Therapeutic potential and outlook of alternative medicine for osteoporosis. Curr Drug Targets. 2017. 18:1051-1068.
Wego MT, Poualeu Kamani SL, Miaffo D, Nchouwet ML, Kamanyi A, Wansi Ngnokam SL. Protective effects of aqueous extract of *Baillonella toxisperma* stem bark on dexamethasone-induced insulin resistance in rats. Adv Pharmacol Sci. 2019. 2019:8075163. https://doi.org/10.1155/2019/8075163
Wong GY, Bradlow L, Sepkovic D, Mehl S, Mailman J, Osborne MP. Dose-ranging study of indole-3-carbinol for breast cancer prevention. J Cell Biochem Suppl. 1997. 28-29:111-116.
Zilliox LA, Chadrasekaran K, Kwan JY, Russell JW. Diabetes and cognitive impairment. Curr Diab Rep. 2016. 16:87. https://doi.org/10.1007/s11892-016-0775-x