Original article

Amelioration of streptozotocin-induced neurotoxicity in rats by a standardized extract of *Cydonia oblonga* seeds

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Abstract

Background: Globally, medicinal plants are widely used to treat ailments due to their safety, accessibility and efficacy. ***Objectives*:** The objective of this study was to determine *in vivo* neuroprotective effect of methanolic extract of seeds of *Cydonia oblonga*against streptozotocin-induced diabetes in rats, as this research has not been reported previously. ***Methods*:** Methanolic extract (100, 150 and 200 mg/kg b.w) was administered for 21 days to diabetic rats. Total protein content, glutathione-S-transferase (GST) specific activity, total glutathione level (tGSH), catalase activity (CAT), malondialdehyde (MDA) level, superoxide dismutase (SOD) level and acetylcholinesterase (AChE) activity were determined in the brain homogenate. ***Results:*** *C. oblonga* seeds extract showed a significantly (*p < 0.05) increased in GST activity, tGSH level, CAT activity and level of SOD. While MDA levels and AChE activity were decreased in comparison with streptozotocin-treated group. Histopathological examination also showed a marked improvement in the morphology of brain tissues. ***Conclusion:*** It is concluded that the methanolic extract of *C. oblonga* seeds has the potential to protect the brain against streptozotocin-induced neurotoxicity by reducing oxidative stress and is beneficial for cognitive improvement in diabetic patients.

Keywords: *Cydonia oblonga*; Neurotoxicity;Acetylcholineesterase; Streptozotocin, Oxidative stress

Bangladesh Journal of Medical Science Vol. 21 No. 04 October'22 Page : 848-857
DOI: https://doi.org/10.3329/bjms.v21i4.60284

Introduction

Cydonia oblonga Mill is also known as Bahee Dana, Quince, Safarjal and Strythion. It comes under the family *Rosaceae* and is famous for its therapeutic, dietary and decorative usages. The chemical study of the plant depicts that it contains pectin, flavonoids and phenolic phytochemicals along with volatile and essential oils. *C. oblonga* fruit contains minerals like calcium,sodium, phosphorus and potassium. The plant is an enriched source of proteins, carbohydrates, lipids andvitamin C.*Cydonia oblonga* is also a resource of valuable phytochemicals, including tannins, alkaloids, glycosaponins, polyphenols and flavonoids. Traditionally, *Cydonia oblonga* is widely used to treat different disorders such as inflammation, bacterial infections, hypertension and diabetes.[1]

Diabetes mellitus (DM) is one of the most prevalent non-communicable disorders that results in hyperglycemia, hyperlipidemia and glycosuria. DM is also presented with severe complications included

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nephropathy, retinopathy, neurotoxicity, skin diseases, depression and dementia. According to the World Health Organization (WHO), DM prevalence is increasing exponentially around the globe for the last two decades. The occurrence of DM in 2017 is 425 million and it is predicted that DM cases tend to be amplified up to 629 million by 2045.[2] Type 1 diabetes mellitus (T1DM) is described as a total insufficiency of insulin due to the loss of pancreatic β cells. The loss of pancreatic β cells can be occurred due to many reasons including viral attack, toxic chemical agents or by autoimmune antibodies.[3] Streptozotocin (STZ) is a diabetogenic chemical that stimulates pancreatic β cells injury and induces hyperglycemia. The STZ induced diabetes triggers the formation of free radicals that leads to the oxidative stress. This oxidative stress causes severe nervous system damageand ensues acute neurotoxicity i.e. speech disorder, weaken memory, cognitive impairment, coma and eventually death.[4, 5] Further, levels of the oxidative stress indicators,i.e. protein, glutathione-S-transferase (GST), glutathione reductase (GR), total glutathione (tGSH), catalase (CAT) malondialdehyde (MDA), acetylcholinesterase (AChE) and superoxide dismutase (SOD) are also altered in STZ induced diabetes. The estimation of these biomarkers is quite crucial in order to evaluate the effects of various pharmacological active compounds and their association with diabetes mellitus. Currently, researchers givemore concentration on natural remedies to cure hyperglycemia and reduce oxidative stress with minimum side effects.[6]

The aim of the current study is to investigate *in vivo* neuroprotective potential of *C. oblonga* methanolic extract against streptozotocin induced diabetes in rats. As previously such research was not found through an extensive literature study.

Materials and Methods

Plant collection, authentication and extraction

Cydonia oblonga seeds were collected from a local market located inLahore, Pakistan. The part used (seeds) was authenticated by Prof Dr. Zaheer-ud-Din Khan, a taxonomist at the department of Botany, Government College University (GCU), Lahore, Pakistan. He issued a voucher number (GC.Herb. Bot.2902). A specimen of the voucher was deposited at the Herbarium Department of GCU, Lahore. Seeds of *C. oblonga* were ground to powder. The maceration method was used for the preparation of methanolic extract of *C.oblonga*.

Chemicals

1-Cloror-2,4-dinitrobenzene (CDNB), potassium dichromate, potassium sodium tartrate, pyrogallol, sodium hydroxide, 1,1,3,3-Tetramethoxy propane and 5,5'-dithio-bis-[2-nitrobenzoic acid] (DTNB) were supplied by Sigma-Aldrich. Copper sulfate, dipotassium hydrogen phosphate, glacial acetic acid, hydrochloric acid and methanol were taken from Merck. Acetylcholine iodide was provided by Alfa Aesar.

Standardization of extract

The standardization of the methanolic extract of *C. oblonga* was done in order to determine the concentration of total flavonoids, total polyphenols, total polysaccharides, total protein and glycosaponins in the extract.

Total flavonoids

The method described by Chang was adopted for the quantification of flavonoids in the extract with a smallvariation in the testing protocol. The quercetin was used as a standard. Different dilutions of the quercetin (20, 80, 120, 10 and 40 µg/ml) were made withinmethanol. The stock solution (1mg/1ml) of the extractwas prepared in methanol. Then, aluminum nitrate and potassium acetate were added in 200 µl of both sample and standard,followed bymixing and incubated for 45 minutes. Afterward, absorbance was determined at 415 nm. A calibration curve was plotted for the determination of total flavonoids (expressed as mg/g of quercetin).[7]

Total polyphenols

The Slinkard prescribed methodwas utilized for the quantification of total polyphenols in extract with slight alterations. The gallic acid was used as a standard and its 1mg/1ml stock solution was prepared in methanol. Then, different dilutions of gallic acid (80, 20, 120, 10 and 40 µg/ml) were made with methanol. The stock solution (1mg/1ml) of the extractwas prepared in methanol. Folin-Ciocâlteu (FC) reagent and sodium carbonate were mixed with 200 µl of each sample and standard in separate test tubes and incubated at room temperature in the dark for 2 hours. Next, the absorbance of each sample and standard was determined at 760 nm. A calibration curve was plotted for the determination of total polyphenol (expressed as mg/g of gallic acid).[8]

Carbohydrates

50 mg of the extract was taken in centrifuge tubes then hot ethanol was added and vortexed for 2 minutes.

Centrifugation was done and supernatant was collected. Then, its extraction was carried out with hydrochloric acid and distilled water in a ratio of 1:1. Centrifugation was again done and thesupernatant was collected. The extraction process was repeated two times and the supernatants obtained were pooled together. In the nextstep, 100 µl of this was taken in a separate test tube and the final volume was made up to 1 ml with distilled water. 4 ml of the anthrone reagent was added and vortexed. This mixture was heated ina water bath for 8 minutes then cool down at room temperature. Finally, green color was produced and its absorbance was determined by using UV-visible spectrophotometer at 630 nm. Glucose was taken as a standard and a calibration curve of glucose was plotted.[9]

Total protein

The total protein content of the sample was determined by a method that was devised by Lowery with minor modifications. 50 mg of the extract was mixed in distilled water then 5 drops of the Triton-X were added. After centrifugation, the supernatant was taken in separate test tubes and the volume was made up to 1ml with distilled water. Then, 3ml of the reagent C was added to each test tube. The reagent C was prepared by mixing of 50 ml of reagent A (2 % of the Na_2CO_3 in 0.1 N solution of NaOH) and 1ml of reagent B (0.5 % of the Copper sulphate in 1% of potassium sodium tartrate). Then200 µl of the FC reagent was added and vortexed. Subsequently, incubation was done for 30 minutes in dark and absorbance was measured at a wavelength of 600 nm. Bovine serum albumin standard curve was plotted for the determination of total protein content in the extract.[10]

Total glycosaponins

The procedure developed by Hussain opted for the quantification of total glycosaponins in the extract with slight changes. The extract was refluxed with methanol for 30 minutes and filtered. Afterward, this filtrate was concentrated on a water bath andthen added in acetone for precipitation. These precipitates were dried at 100 °C and weighed. The total content of glycosaponins was calculated by the following formula.[11]

$$\text{Glycosaponins (\%)} = \frac{\text{Weight of the precipitate}}{\text{Weight of the sample}} \times 100$$

In-vivo evaluation of streptozotocin-induced neurotoxicity in rats

Animals

Male Sprague-Dawley (SD) rats having a weight range 130 – 160 g were used for this *in vivo* study and were procured from the University of Health Sciences (UHS) Lahore. Guidelines of the Punjab University College of Pharmacy (PUCP) Animal Ethics Committee were followed for the handling of animals and the voucher number assigned was AEC/PUCP/1099. Rats were housed in plastic cages having steel mesh top in the animal house of the PUCP under optimum environmental conditions. Before performing this research, rats were kept under observation and provided with adequate diet and water.

Experimental design

Fifty-four male Sprague Dawley rats were divided into nine groups. Four rats (n=6) were included in each group. Group I: Control, given only distilled water for 21 days. Group II, III & IV, different doses (100, 150 and 200 mg/kg) of methanolic extract were administered for 21 days. Group V (Diabetic control), Streptozotocin 50 mg/kg administered i.p once for the induction of diabetes. Group VI, VII, VIII & IX STZ induced Diabetic groups were given 100, 150 and 200 mg/kg of the extract and Vit. C 100 mg/kg orally for 19 days. Rats were sacrificed on the 20th day, their brains were removed and stored at -80°C till further experiments.[12]

Assay of glutathione s-transferase (GST)

First, the brain sample was homogenized in a phosphate buffer (pH 7.4) then centrifuged at 12,500 × *g* for 20 minutes at 4°C in order to prepare post mitochondrial supernatant (PMS). The concentration of protein in PMS was determined by the Lowry method as described previously. The effect of *C. oblonga* seed extract on GST specific activity was determined by the Habig method with a little adjustment in procedure. The optimized protein concentration of PMS was used with 30mM GSH and 30mM 1-chloro-2, 4-dinitrobenzene as a substrate in 100mM potassium phosphate buffer (pH 6.5). The absorbance of the conjugated substrate was read at 340nm on UV/Vis spectrophotometer for 5 minutes at room temperature.[13]

Following equation was used to calculate the GST specific activity

$$\text{GST specific activity} = \frac{\text{change in absorbance per minute } (\Delta A/\text{min})}{\text{Extinction coefficient} \times \text{Protein Concentration (mg/ml)}}$$

Assessment of the total glutathione (tGSH)

The total glutathione level in brain tissues of the rats was quantified by the Sedlak method after a few amendments in testing steps. The brain tissue was weighed and phosphate buffer was added in it. Subsequently, the brain tissue was homogenized and trichloroacetic acid was added in order to precipitate it. It was then centrifuged for 40 minutes and the supernatantwas separated. Next, Tris-HCl buffer was added tothe supernatant followed by DTNB. In the final step, methanol was added to all of the test tubes. The blank was prepared by the same method by adding phosphate buffer in place supernatant. All the contents were vortexed and incubated for 30 minutes at 37 ^{0}C. In due course, yellow color appeared and its absorbance was quantified at a wavelength of 412 nm.[14]

Evaluation of the catalase (CAT) activity

The catalase activity in brain tissue was estimated by the Sinha method[16] with a slight adjustment in procedure protocol. The test was performed in triplicates. Firstly, phosphate buffer was added to all the test tubes then hydrogen peroxide and brain homogenate were added respectively. Next, all of the test tubes were vortexed. Later, 1 ml of this mixture was taken in separate test tubes and a reagent was added that resulted in production of the blue-colored precipitates. This reagent was prepared by mixing of potassium dichromate and glacial acetic acid in a ratio of 1:3. The reaction mixture was vortexed then heated at 100^{0}C for 10 minutes. These blue colored precipitates were changed to the green-colored solution. Lastly, absorbance was measured at a wavelength of 570 nm on a spectrophotometer.[15]

Estimation of the superoxide dismutase (SOD) activity

The superoxide dismutase level in brain tissue of rats was estimated by a method described by Magnani with a slight alteration. The brain homogenate was prepared in phosphate buffer and test was performed in three replicates. 1 ml of Tris-buffer was added in all of the sample test tubes then homogenate was added. In the control, distilled water was used in place of the brain homogenate. Then pyrogallol solution was added just before the determination of the absorbance. The absorbance was determined at a wavelength of 420 nm.[16] The results were calculated by applying the following formula

% Inhibition of pyrogallol autoxidation = A test /A control × 100

SOD activity (U/ml) = % Inhibition of pyrogallol autoxidation/ 50

Analysis of malondialdehyde (MDA) activity

The Okhawa's method was adopted for the determination of the MDA level in brain tissue with minor changes. Brain tissues were homogenized in potassium chloride solution and the test was performed by making three replicates of each sample. Sodium lauryl sulfate, acetic acid and thiobarbituric acid were added in test tube respectively. Then distilled water and homogenate were added. Test tubes were incubated at 98^{0}C for 1 hour, cooled and after that n-butanol was added. Reaction mixture was vortexed, then centrifuged for 30 minutes and absorbance was measured at a wavelength of 532 nm.[17]

Estimation of neuronal acetylcholinesterase (AChE) activity

The brain samples of each group were homogenized in phosphate buffer. The test was performed in triplicates. To each of the test tubes containing brain homogenate, phosphate buffer (pH 8) and DTNB were added. Then the reaction mixture was mixed well and absorbance was recorded at 570 nm using UV-visible spectrophotometer. After that acetyl thiocholine iodide (20 µL) which is used a substrate was added to the reaction mixture and absorbance was recorded at an interval of 2 minutes to find the change in absorbance per minute.[18]

The following equation was used to determine the level of AChE.

$$R = 5.74 \times 10^{-4} \times A/CO$$

Where

R is the rate of substrate hydrolyzed/mg/min, A is the change of absorbance per minute and CO is original concentration of the tissue.

Histopathological examination

The tissue sections of the brain were blotted dry and washed with normal saline. These sections were preserved in10 % formalin. Later, tissues were fixed in paraffin and cut into thethinnerslices (5 µm) by using the microtome. For permanent fixation of the tissue, slides were placed in the oven for 10 – 15 hours at 58ºC. Then, Hematoxylin and Eosin (H and E) staining was done. H & E stain blue color to the nucleus and pink or red color to protein, intracellular membrane and extracellular fibers. All the sections were examined using light microscope.

The criteria for neurotoxicity included liquefactive necrosis, vacuolization, pyknosis or loss of nuclei and infiltration of inflammatory cells. Each specimen was scored from none too severe. Histological examination and scoring wereperformed and assessed by an independent histopathologist, non-related to this study.[19]

Statistical analysis

Graph pad Prism version 5 was used for statistical analysis. One way ANOVA and Dunnett's test was employed for making comparison between different groups. Results were expressed in mean and standard deviation (Mean ± SD) and *p < 0.05 was considered statistically significant.

Ethical clearance

Guidelines of the Punjab University College of Pharmacy (PUCP) Animal Ethics Committee were followed for the handling of animals and voucher number assigned was AEC/PUCP/1099.

Results

Standardization of the extract

The results of the extract standardization (Table I)are; total flavonoids (76.70mg/g) > carbohydrates (68.46 mg/g) > total protein content (68.10 mg/g) > total polyphenols(33.08 mg/g). The total glycosaponins was found to be 7.20 %.

Table I - Standardization of the methanolic extract of *C. oblonga* seeds

Extracts	Total Flavonoids content(mg/g)	Total Polyphenols content (mg/g)	Carbohydrates content (mg/g)	Total Protein content (mg/g)	Total Glycosaponins content (%)
Methanolic extract	76.70 ± 4.69	33.08 ± 3.52	68.46 ± 2.01	68.10 ± 4.32	7.20 ± 0.008

In-vivo evaluation of streptozotocin-induced neurotoxicity in rats

Assay of glutathione s-transferase (GST)

The GST specific activity (Table II) has demonstrated that the methanolic extract (100, 150 & 200mg /kg) co-administered with STZ increased significantly GST specific activity 0.039 ± 0.01, 0.05 ± 0.005& 0.05 ± 0.01respectively as compared to the STZ treated group 0.027 ± 0.01. This change was a dose dependent. However, extract administration showed non-significant effect on GST activity.

Assessment of the total glutathione (tGSH)

The outcomes of tGSHlevel estimation (Table II) exhibited that the methanolic extract (100, 150 & 200mg /kg) co-administered with STZ increased significantly tGSH level 0.902 ± 0.07, 1.14 ± 0.027 & 2.053 ± 0.07respectively as compared to the STZ treated group (0.525 ± 0.040).

Evaluation of the catalase (CAT) activity

The neuronal CAT activity (Table II) showed that the methanolic extract (100, 150 & 200mg /kg) co-administered with STZ increased significantly 0.036 ± 0.08, 0.045 ± 0.01&0.044 ± 0.012respectively as compared to the STZ treated group (0.28 ± 0.01).

Estimation of the superoxide dismutase (SOD) activity

The estimation of SOD level (Table II) in brain illustrated that the methanolic extract (100, 150 & 200mg /kg) co-administered with STZ increased significantly 10.74 ± 0.28, 12.78 ± 0.06 & 14.21 ± 0.16 respectively as compared to the STZ treated group (8.50 ± 0.13).

Analysis of malondialdehyde (MDA) activity

It is evident from the analysis of MDA level (Table II) in brain tissuethat the methanolic extract (100, 150 & 200mg /kg) co-administered with STZ decreased significantly 4.14 ± 0.13, 3.58 ± 0.11 & 2.58 ± 0.16 respectively as compared to the STZ treated group (6.53 ± 0.09).

Estimation of neuronal acetylcholinesterase (AChE) activity

The results of AChE level (Table II) in brain tissue showed that the methanolic extract (100, 150 & 200mg /kg) co-administered with STZ decreased significantly AChE level 7.47 ± 0.42, 5.24 ± 0.15 & 4.95 ± 0.41 respectively as compared to the STZ treated group (9.71 ± 0.13). *C. oblonga* seeds extract showed a dose dependent amelioration effect against STZ induced neurotoxicity.

Taken as a whole, it is obvious from the results that STZ significantly (*p < 0.05) decreased total glutathione level, CAT activity and SOD level, however significantly (*p < 0.05) increased MDA & AchE level in comparison to the control group.

Table II - Effects of *C. oblonga* seeds extract on enzyme activity in STZ induced brain toxicity

Groups	GST activity (µM/min/ mg protein)	tGSH (mM/g tissue)	CAT (µM/g tissue)	MDA (µM/g tissue)	SOD (U/mg)	AChE(µmol/min/ mg)
Control	0.059 ± 0.016	2.530 ± 0.040	0.047 ± 0.017	1.54 ± 0.066	14.74 ± 0.270	4.298 ± 0.063
STZ	0.027 ± 0.006#	0.525 ± 0.040#	0.28 ± 0.014#	6.53 ± 0.092#	8.50 ± 0.134#	9.71 ± 0.126#
COM 100	0.061 ± 0.007	2.483 ± 0.050	0.0465 ± 0.047	1.49 ± 0.067	14.39 ± 0.259	4.27 ± 0.033
COM 150	0.062 ± 0.013	2.500 ± 0.045	0.0447 ± 0.017	1.33 ± 0.184	14.79 ± 0.089	4.29 ± 0.053
COM200	0.049 ± 0.009	2.565 ± 0.044	0.054 ± 0.021	1.07 ± 0.085	14.89 ± 0.268	4.31 ± 0.053
STZ+COM 100	0.039 ± 0.009*	0.902 ± 0.068*	0.036 ± 0.017*	4.14 ± 0.13*	10.74 ± 0.287*	7.47 ± 0.420*
STZ+COM 150	0.049 ± 0.005*	1.148 ± 0.027*	0.045 ± 0.014*	3.58 ± 0.111*	12.78 ± 0.061*	5.235 ± 0.151*
STZ +COM 200	0.056 ± 0.002*	2.053 ± 0.068*	0.044 ± 0.017*	2.58 ± 0.162*	14.21 ± 0.168*	4.952 ± 0.411*
STZ +Vit-C	0.054 ± 0.008*	2.365 ± 0.134*	0.039 ± 0.021*	1.66 ± 0.036*	13.93 ± 0.117*	7.14 ± 0.489*

Histopathological examination

Histopathological observation of control (normal) brain tissue of rat showed normal morphology. There are well formed nuclei with intact blood vessels and no infiltration of inflammatory cells, vacuolization and pyknosis of nuclei Figure 1 (A). The Steptozotocin treated group (60mg/kgBW) showed severe liquifactive necrosis with loss of nuclei, enhanced infiltration of inflammatory cells and vacuolization with pyknosis of nuclei and vascular congestion Figure 1 (B).Themethanol extracts *C. oblonga* (100,150 and 200 mg/ kg BW) showed normal brain morphology, there were no signs of necrosis. Neurons with intact nuclei and blood vessels were present, with no morphological changes Figure 1 (C, D, E).The methanolic extract of *C. oblonga* at dose 100 mg/ kg was administered to STZ induced rat, the morphological observation of brain tissue showed moderate degeneration with rare vacuolization and pyknotic nuclei, less necrosis and vascular congestion as shown in Figure 1 (F). The extract of *C. oblonga* at dose 150 mg/ kg was administered to Streptozotocin induced rats, the morphological observation of brain tissue showed moderate degeneration with rare vacuolization and pyknotic nuclei, less necrosis and vascular congestion as shown in Figure 1 (G). The extract of *C. oblonga* at dose 200 mg/ kg was administered to STZ induced rats, the morphological observation of brain tissue showed moderate degeneration with rare vacuolization and pyknotic nuclei, less necrosis and vascular congestion as shown in Figure 1 (H).

Discussion

Streptozotocin (STZ) is a natural nitrosourea compound. It is extensively used to induce insulin-dependent diabetes mellitus in experimental animals due to its toxic effects on islet beta cells. Streptozotocin-induced diabetes depicts a significant example of hyperglycemia-mediated oxidative stress which causes severe nerve damage.[3,4,20] Medicinal plants are widely used across the globe due to their safety, accessibility and efficacy.[21,22] The *Cydonia oblonga* has been employed since centauries to treat different ailments like diarrhea, cough, dysentery, sore throat, constipation, diabetes and bronchitis.[1] The current study showed that the plant contains valuable phytochemicals, i.e. flavonoids, glycosaponins and polyphenols. The major phenolic compound of *C. oblonga* is 5–*O*–caffeoylquinic acid, which showed a reasonable binding affinity for AChE and chlorogenic acid (3–*O*–caffeoylquinic acid) and thus reduces oxidative stress and peroxidation of the lipids.The flavonoids presentin *C. oblonga* extract stabilize the free radicals and thus reduce the neurotoxicity.[1,23,24] The presence of glycosaponins in the plant has indicated that *C. oblonga*possesses anti-tussive, anti-diabetic and antimicrobial properties as many previous scientific reports have documented the beneficial effects of glycosaponins.[25]

Glutathione-S-transferases (GSTs) are a diverse family of Phase II detoxification intracellular enzymes. Cytosolic GSTs play a crucial role in regulating the mitogen-activated protein (MAP) kinase pathway that participates in cell survival and death signals. GST is a protective enzyme that provides shielding against aldehydes in the brain. Reduced levels of GST, glutathione (GSH) and increased level of acetylcholinesterase (AChE) may participate in the pathogenesis of neuron degeneration in dementia.[26]GSH has a multidimensional role in antioxidant defense mechanism. It is a direct scavenger of free radicals as well as a co-substrate for peroxide detoxification by glutathione peroxidases.

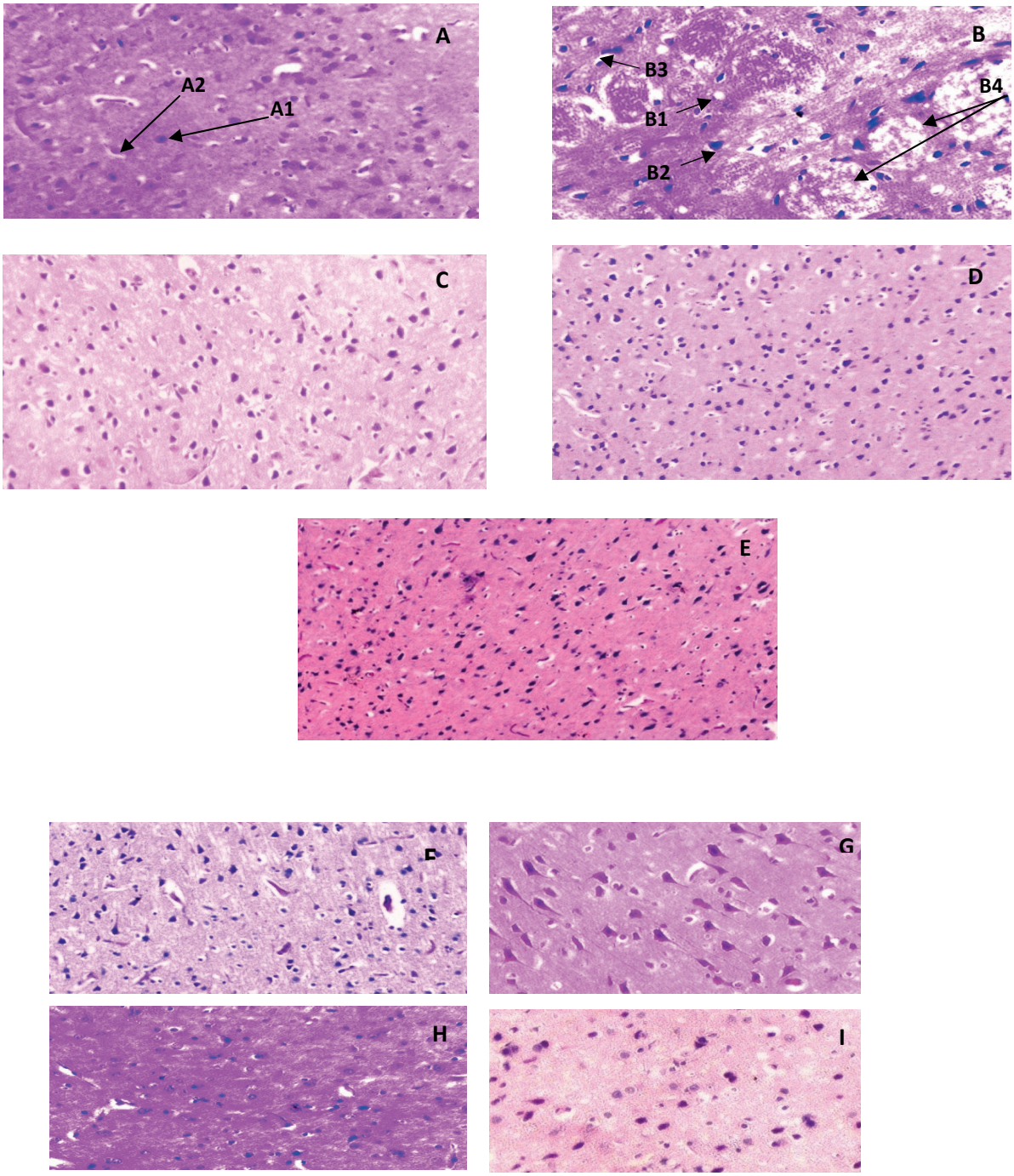

FIGURE 1 - H&E stained light photomicrograph of rat brain of *Cydonia oblonga* extract treated groups, 20X magnifications. **A:** Control brain, **A1**: Well-formed normal nuclei, **A2**: blood vessels, **B:** Streptozotocin (50 mg/ kg BW) treated group, **B1:** vacuolization, **B2**: pyknotic nuclei, **B3:** vascular congestion, **B4:** liquefactive necrosis, **C:** COM(100 mg/ kg BW), **D:**COM (150 mg/ kg BW), **E:** COM (200 mg/ kg BW), **F:** STZ +COM (100 mg/ kg BW), **G**: STZ +COM (150 mg/ kg BW),**H:** STZ +COM (200 mg/ kg BW),**I:** STZ +Vit-C (100 mg/ kg BW

GST catalyze the conjugation reaction in the presence of GSH, as a result, reduces free radicals and ultimately lessens oxidative stress.[27]

The current study results revealed that GST activity, GSH level decreased and acetylcholinesterase level in STZ treated group is significantly ($p < 0.05$) increased as compared to the control group. The methanolic extract groups of *C. oblonga* showed insignificant changes on these. The methanolic extract administration STZ treated groups of *C. oblonga* considerably (*$p < 0.05$) reverse the activity of GST, GSH & AChE level as compared to the STZ treated group. These changes lead to protection against memory deficit due to STZ induced neurotoxicity. The extract treated groups of *C. oblonga* appreciably (*$p < 0.05$) increased catalase (CAT) activity as compared to STZ treated group. This effect may result due to the presence of phenols which prevent the oxidative stress in the brain by scavenging nascent oxygen, H_2O_2 and superoxide species.[28]

The methanolic extract groups of *C. oblonga* showed a slight change in MDA level. The methanolic extract and STZ concomitant treated groups of *C. oblonga* radically (*$p < 0.05$) decreased MDA level as compared to STZ treated group. MDA is a product of the oxidation of unsaturated fatty acid and the contents of which could indirectly reflect the extent of oxidation of body fat and free radicals generation. The flavonoids present in *C. oblonga* inhibited the production of MDA and improved the antioxidant potentials of brain tissue.

The methanolic extract groups of *C. oblonga* showed insignificant change in SOD level. Superoxide dismutase (SOD) is essential antioxidant that can take away the free oxygen radicals and consequently it protects cells. The activity of SOD indirectly reflects the ability to eliminate free oxygen radicals.[29]

Moreover, STZ induces impaired memory and delayed learning. The histopathological evaluation showed that STZ caused severe degeneration, vacuolization of cytoplasm, loss of nuclei, coagulated necrosis of brain cells and these results are consistent with the previously published studies. The methanolic extract alone groups showed histology similar to normal brain tissue. The group in which the extract was administered parallel to the STZ treated group showed marked improvement with moderate to mild degeneration, less vacuolization, less pyknotic nuclei and congestion. Hence, this research has proved the beneficial effects of methanolic extract of the *C. oblonga* in preventing neurotoxicity and future studies are warranted to ensure further efficacy and safety.[30]

Conclusion

It is concluded that the methanolic extract of *Cydonia oblonga* protects the brain against streptozotocin-induced neurotoxicity in a dose-dependent manner. The study outcomes have shown that the extract is quite helpful to normalize the glucose level with marked improvement in the levels of serum and tissue enzymes. Moreover, a noticeable improvement in cognitive ability and memory was also observed due to a decrease in acetylcholinesterase (AChE) activity. It is recommended that advance investigations should be carried out in order to support the plant use in the management of diabetes and its protection against oxidative stress-induced neurotoxicity. Further, such studies would provide novel insights in drug design and development as well.

Funding

None

Conflict of interest

The authors declare no conflict of interest.

Authors' contributions

RA and DAG conceptualized and designed this study. DAG and AAS conducting the experiments, curated the data and draft the manuscript. RA and KA contributed to interpreting the analyzed data, performed the statistical analysis, provision of resources, structured and edited the manuscript. RA supervised and verified the results of this work. All authors participated in this research equally and approved the final manuscript.

Acknowledgment

The authors would like to acknowledge Punjab University College of Pharmacy, University of the Punjab Lahore Pakistan for kind support and encouragement.

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