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NEUROAMELIORATIVE EFFECTS OF BERRY EXTRACTS IN ALZHEIMER INDUCED RATS

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ABSTRACT

This study was designed to evaluate the neuro-ameliorative effect of red (*Morus rubra*) and white berry (*Morus alba*) extracts against Alzheimer´s disease (AD) in male rats. $AlCl_3$ was administered orally to male albino rats (100 mg/kg/day for 6 weeks) while, berry extracts (300 mg/kg/day for six weeks) was administered orally post $AlCl_3$ administration. The data showed markedly elevation in lipid peroxide, apoptotic marker enzyme (caspase-8) and calmodulin (CaM) levels while, reduced glutathione (GSH) level and antioxidant enzyme; paraoxanase-1 (PON1) were reduced in AD rats when compared with normal control one. The treatment of rats with $AlCl_3$ induced AD was associated with increase in the 8-OHdG/2-dG ratio and decreased the expression of DHCR24 and FKBP1B genes. However, the treatment of AD-induced rats with red and white berry extracts exhibited low 8-OHdG/ 2-dG ratio and increase the expression of the DHCR24 and FKBP1B genes. The results suggested the attenuated role of berry extracts which could be attributed to the inhibition of reactive oxygen species (ROS), increase in the antioxidant enzymes activity as well as inhibition in the occurrence of apoptotic related enzymes such as caspases-8.

KEYWORDS: **Red berry, White berry, Alzheimer´s disease, Inflammation, Apoptosis, Reactive oxygen species**

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INTRODUCTION

Several neurological studies have shown that regular flavonoid rich fruit intake is associated with delayed Parkinson's disease (PD) and Alzheimer´s disease (AD).[1,2] Data from *in vivo* studies suggested that among the sources of antioxidants, phytochemicals in berry fruits (anthocyanin and caffeic acid) have a beneficial role in brain aging and neurodegenerative disorders because of their anti-oxidative, anti-inflammatory and anti-proliferative properties.[3] It has been found that oxidative stress and inflammation appear to be involved in brain aging and in AD.[4] Thus, increasing the consumption of antioxidants may be effective in preventing or reducing the deleterious effects of aging and behavior.[5] Additionally, the neuro-protective effects of berry extracts were demonstrated by many scholars.[6,7] Neuro-inflammatory processes in the brain are believed to play a crucial role in the development of neurodegenerative diseases, especially due to the increased production of reactive oxygen species (ROS).[8] Brain tissues have a high content of lipids which it rapidly oxidized under high stress conditions to generate ROS.[9] Obviously, the exposure of oxidative stress (OS) stimulates the activation of compensatory responses. Unfortunately, both enzymatic and non-enzymatic antioxidant defenses seem to be impaired in AD patients.[10] Thus, due to low activity of antioxidant defense systems, the brain is susceptible to oxidative stress more than other organs.[11] The calcium hypothesis of AD invokes the disruption of calcium signaling as the underlying cause of neuronal dysfunction and ultimately apoptosis. As a primary calcium signal transducer, calmodulin (CaM) responds to cytosolic calcium fluxes by binding to and regulating the activity of target CaM-binding proteins (CaMBPs).[12] Calcium/calmodulin-dependent kinase II (CaMKII) is a remarkably complex protein kinase which has a principle role in plasticity of synapsis and memory formation. CaMKII has also been suggested to be a tau kinase. CaMKII dysregulation may therefore be a modulator of toxicity in AD; a dementia characterized by aberrant calcium signaling, synapse and neuronal loss, and impaired memory. There is evidence for CaMKII dysregulation in Alzheimer's patients which contributed to neurodegeneration and memory impairment.[13] On the other hand, AD is concerned with several conditions causing the excessive production of ROS: mitochondrial dysfunctions, Aβ-related microglial activation and inflammation. One of the important issues in AD is inflammation. In fact, this neurodegenerative disorder is characterized by an uncontrolled inflammatory activation of microglial cells. The peroxisome proliferator-activated receptor gamma (PPAR-γ) is a regulator of the inflammatory processes which exerts anti-inflammatory properties as well as paraoxanase anti-inflammatry and antioxidant enzyme.[14,15] It was suggested that PON1 may confer protection against inflammatory and oxidative challenge which, in turn, plays a key-role in the onset and progression of dementia.[16] It is intriguing to hypothesize that in mild cognitive impairment patients decreased levels of PON-1 may result in a weaker defense against reactive species, which in turn leads to the formation of a high amount of lipoperoxidation by-products.[17] Several molecular markers are associated with the degenerative disorders pathway inhibition such as FKBP1B and DHCR2 genes. FKBP1B is a gene encoding binding protein (BP) for the immunosuppressive drug. The protein encoded by this gene is a member of the immunophilin protein family, which play a role in immunoregulation and basic cellular processes involving protein folding and trafficking.[18] Additionally, 24-dehydrocholesterol reductase gene (DHCR24) is gene encodes a flavin adenine dinucleotide (FAD)-dependent oxido-reductase which catalyzes the reduction of the delta-24 double bond of sterol intermediates during cholesterol biosynthesis. Reduced expression of the gene occurs in the temporal cortex of AD patients.[19] The neuro-protective effects of many polyphenols rely on their ability to cross the blood-brain barrier and directly scavenge pathological concentrations of reactive oxygen and nitrogen species and chelate transition metal ions.[20] Different polyphenolic compounds were shown to have scavenging activity and the ability to activate key antioxidant enzymes in the brain, thus breaking the vicious cycle of OS and tissue damage.[21] There is a growing interest in the potential of natural polyphenols in berries to improve memory, learning and general cognitive abilities.[22,23] Preclinical evidence has indicated that flavonoids may exert powerful actions on mammalian cognitive function and may reverse age-related declines in memory and learning. These beneficial effects are mainly in demand in preventing against brain damage, such as ischemic and neurodegenerative diseases, reducing neuronal apoptosis, and improving memory, learning and cognitive functions.[24] Therefore, the present study aimed to investigate the ameliorative effect of red berry in AD induced rats against oxidative stress, inflammation, apoptosis, calmodulin, DNA adducts formation and alteration in the expression of neurodegenerative related genes.

MATERIALS AND METHODS

Chemicals

Donepezil was purchased from Sigma Co (USA) and aluminum chloride ($AlCl_3$) from BDH Laboratory Supplies, Poole (UK).TRIzol reagent was bought from Invitrogen (Germany). The reverse transcription and PCR kits were obtained from Fermentas (USA). SYBR Green Mix was purchased from Stratagene (USA).

Preparation of berry extracts

Red and white berry fruits were obtained from the local market, grinded and extracted three times with ethanol 70%. The extracts were filtered through filter paper and each filtrate was concentrated under vacuum using Rotary evaporator (Heidolph-Germany) till dryness.[25,26] The crude extracts were obtained and stored at 4 °C for further investigations.

Biological experiment

Animals

Male albino rats (180-200 g) were obtained from Central Animal House, National Research Centre (NRC). Animals were acclimatized to the laboratory conditions at room temperature prior to the experimentation. Animals were kept under standard conditions of a 12 h light/dark cycle with food and water in plastic cages with soft bedding. All the experiments were carried out between 9.00 and 15.00 h. The protocol was approved by the NRC Ethics Committee Guidelines for the use and care of animals (Ethical approval no: 0113345).

Drug and treatment schedule

$AlCl_3$ was dissolved in drinking water at the beginning of the experiment and administered in a dose of 100 mg/kg to rats daily for 6 weeks.[27] Donepezil tartrate (5 mg/kg b.wt./day) diluted in ultra pure water daily for 6 weeks.[28] Animals were randomized into seven groups (seventy adult male albino rats) based on their body weight. Each group had ten numbers of animals. The groups were as follows:

Group one: Normal control untreated rats.
Group two: Normal control rats treated with red berry ethanolic extract.
Group three: Normal control rats treated with white berry ethanolic extract.
Group Four: Serving as AD rats, where rats were orally administered with $AlCl_3$.
Group five: AD rats daily orally treated with red berry ethanolic extract for 6 weeks in a dose of 300 mg /Kg b.wt.[29]
Group six: AD rats orally administered daily with white berry ethanolic extract for 6 weeks in a dose of 300 mg /Kg b.wt.[29]
Group seven : AD rats orally treated daily with standard drug (donepezil at a dose of 5 mg/kg b.wt.).

Brain tissue sampling and preparation

At the end of the experiment, the rats were fasted overnight, subjected to anesthesia with diethyl ether and sacrificed. The whole brain of each rat was rapidly dissected, washed with isotonic saline and dried on filter paper. The brain was weighed and homogenized in ice-cold medium containing 50 mMTris/HCl and 300mM sucrose at pH 7.4 to give a 10 % (w/v) homogenate.[30] This homogenate was centrifuged at 1 400 × g for 10 min at 4 °C. The supernatant was stored at -80 °C and used for biochemical analyses that included OS biomarker (lipid hydroperoxide), non-enzymatic antioxidant level (GSH), apoptotic marker (caspase-8), anti-inflammatory (PON1) enzyme activity and calmodulin level. The ethical conditions were applied such that the animals suffered no pain at any stage of the experiment and the study was approved by the Ethics Committee of the National Research Centre. Animals were disposed of in bags provided by the Committee of Safety and Environmental Health, NRC.

Biochemical analyses

Determination of lipid peroxide

Lipid peroxidation products represented by malondialdehyde (MDA) were evaluated by the method of Satoh.[31] Using thiobarbituric acid (TBA) and measuring the reaction product spectrophotometrically at 534 nm.

Determination of GSH

Brain GSH was measured colorimetrically according to the method of Moron et al.[32] This method is based on determination of the relatively stable yellow color when 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) is added to sulfhydryl compounds which can be measured at 503 nm.

Determination of caspase-8

The activity of caspase enzyme in the brain tissue homogenate is measured, according to the method of Thompson.[33] The cells that are suspected or have been induced to undergo apoptosis are first lysed to collect their intracellular contents. The cell lysate can then be tested for protease activity by the addition of a caspase-specific peptide that is conjugated to the color reporter molecule p-nitroaniline (pNA). The cleavage of the peptide by the caspase releases the chromophore pNA, which can be quantitated spectrophotometrically at a wavelength of 405 nm. The level of caspase enzymatic activity in the cell lysate is directly proportional to the color reaction.

Determination of PON1 activity and calmodulin level

PON1 was determined in serum samples by measuring the hydrolysis of paraoxon using a spectrophotometric method .Serum (5 µl) was added to freshly prepared tris-NaOH buffer (0.26 M, pH 8.5) containing 0.5 M NaCl,1.2 mM paraoxon and 25 mM calcium chloride. After thirty seconds of incubation at 37 °C, the liberation of p-nitrophenol (ε= 18.053 M -1cm-1) was followed at 405 nm for six minutes (at 54 second intervals). One unit of activity is defined as the amount of enzyme producing 1µ mol of p-nitrophenol per minute[34] where the activity of calmodulin as an activator of cAMP phosphodiesterase was assayed by spectrophotometric assay method according to Garg et al.[35] Where, AMP was hydrolyzed by 5'-nucleotidase and the adenosine formed was measured by spectrophotometry at 265 nm.

Measurement of 8-Hydroxy-2-deoxyguanosine (8-OHdG) and 2-deoxyguanosine (2-dG) by HPLC

DNA was extracted from brain tissues by homogenization in buffer containing 1% sodium dodecyl sulfate, 10 mM Tris, 1 mM EDTA (pH 7.4), and an overnight incubation in 0.5 mg/ml proteinase K at 55°C. Homogenates were incubated with RNase (0.1 mg/ml) at 50°C for 10 min and extracted with chloroform/isoamyl alcohol. The extracts were mixed

with 3 M sodium acetate and two volumes of 100% ethanol to precipitate DNA at -20°C. The samples were washed twice with 70% ethanol, air-dried for 15 min, and dissolved in 100 µl of 10 mM Tris/1 mM EDTA (pH 7.4). DNA digestion was performed according to Patel et al.[36] The adduct 8-OHdG was measured with high-performance liquid chromatography (HPLC) equipped with a CoulArray system (Model 5600). Analytes were detected on two coulometric array modules, each containing four electrochemical sensors attached in series, which allows identification targets based on reduction potential. UV detection was set to 260 nm. HPLC was controlled and the data acquired and analyzed using CoulArray software. The mobile phase was composed of 50 mM sodium acetate/5% methanol at pH 5.2. Electrochemical detector potentials for 8-OHdG and 2-dG were 120/230/280/420/600/750/ 840/900 mV, and the flow rate was 1 ml/min.

Gene expression analysis

Extraction of total RNA and cDNA synthesis

Brain tissues of male rats were used to extract the total RNA using TRIzol® Reagent (Invitrogen, Germany) Kit. The isolation method was carried out according to the manufacturer's instructions of the above Kit. Approximately 50 mg of the brain tissues were mixed with some drops of liquid nitrogen and homogenized in 1 ml of TRIzol® Reagent in autoclaved mortar. Afterwards, total RNA was dissolved and preserved in diethylpyrocarbonate (DEPC)-treated water up to use. To assess the RNA yield and purity of the total RNA, RNAse-free DNAse I (Invitrogen, Germany) was used to digest DNA contamination. A small drop of isolated RNA was examined photospectrometrically at 260 nm. The purity of total RNA was determined between 1.8 and 2.1 to be good purified when it examined by photospectrometer at the 260/280 nm ratio. To avoid RNA damaging, aliquots of RNA were prepared after isolation for either reverse transcription reaction or otherwise for storing at -80°C up to use. To synthesize the complementary DNA (cDNA) isolated RNA from brain tissues was reverse transcribed into cDNA. The reaction volume was carried out in 20 µl. The reaction volume was prepared according to the instructions of the RevertAid^{TM} First Strand cDNA Synthesis Kit (MBI Fermentas, Germany). The reverse transcription (RT) reaction was performed for 10 min at 25°C. Afterwards, the tubes of the reaction were put in thermo-cycler machine for 60 min at 42°C, and then the reaction was terminated for 5 min at 99°C. The PCR products containing the cDNA were kept at -20°C up to use for DNA amplification.[37,38]

Quantitative Real Time-PCR (qRT-PCR)

A StepOne Real-Time PCR System (Applied Biosystem, USA) was used to assess the copy of the cDNA of male rats to detect the expression values of the tested genes. To perform the PCR reaction, a volume of 25 µl of reaction mixtures was prepared containing 12.5 µl of SYBR® green (TaKaRa, Biotech. Co. Ltd.), 0.5 µl of 0.2 µM forward and reverse primers, 6.5 µl DNA-RNA free water, and 2.5 µl of the synthesized cDNA. The cDNA was propagated using reaction program consisted of 3 steps. In the first step, the PCR tubes was incubated at 95°C for 3 min. In the second step, the reaction program consisted of 50 cycles. Each cycle of them consisted of 3 sub-steps: (a) 15 sec at 95°C; (b) 30 sec at 60°C; and (c) 30 sec at 72°C. In the third step, the reaction program consisted of 71 cycles. The first cycle of them started at 60°C for 10 sec and then the followed cycles increased about 0.5°C every 10 sec up to 95.0°C. A melting curve of the reaction was performed for each qRT-PCR termination at 95°C to assess the quality of the primers. To verify that, the reaction of the qRT-PCR does not have any contamination PCR tubes containing non template control were used. The sequences of specific primer of the genes used are listed in Table (1). The relative quantification of the target genes to the reference (β-Actin) was determined by using the $2^{-\Delta\Delta CT}$ method**.**

Statistical Analysis

Data were analyzed by one way analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS) program, version combined with co-state computer program and least significant difference (LSD) to compare significance between groups, where unshared letters are significant at P ≤0.05.

RESULTS

Effect of red and white berry extracts on lipid peroxide and GSH levels

Table (2) revealed an insignificant difference in lipid peroxide and GSH level in red and white berry extracts treated normal control rats comparing with untreated control one. AD rats demonstrated significant increase in lipid peroxide level with percentage reached to 130.15%. While, significant decrease in GSH level was detected (70.87%), compared to normal control rats. Treatment of AD induced rats with red and white berry extracts showed significant increase in lipid peroxide level with percentages of improvement 118.09 and 109.54%, respectively. While, red and white berry extracts showed significant decrease in GSH level with improvement percentages 55.17 and 54.60%, respectively. Reference drug recorded amelioration percentages 123.14 and 58.35%, for lipid peroxide and GSH levels respectively.

Effect of red and white berry extracts on caspase-8 enzyme activity

From the manipulated data (Table 3), it can be deduced, insignificant change in caspase-8 activity in normal rats treated with red and white berry extracts as compared to untreated control rats. AD induced rats declared significant increase in caspase-8 activity with percentage 130.09%. Treatment of AD induced rats with red berry and standard drug showed insignificant difference in enzyme activity. While, white berry administered to AD rats demonstrated significant increase in caspase-8 activity with amelioration percent 101.80%.

Effect of white and red berry extracts on PON1 activity and calmodulin level

The results presented in Table (4), clearly indicated significant increase in PON1 activity in normal rats treated with red berry (9.62%), however, insignificant change in normal rats treated with white berry extract was detected as compared to untreated normal control one. While, insignificant change was detected in calmodulin level in normal rats treated with red and white berry extracts comparing with normal control one. Regarding to AD rats, significant inhibition was recorded in antioxidant enzyme PON1 activity (27.36%) as compared to normal control rats. However, CaM level showed significant increase with percentage 186.32%. Treatment of AD induced rats with red berry extract exhibited insignificant difference in PON1 enzyme activity. While, CaM level showed significant increase with amelioration percent 47.37%. In addition, white berry extract treated AD rats declared significant inhibition in PON1 activity, while significant increase in CaM level with percentages of amelioration 14.41 and 110.53%, respectively. Furthermore, treatment of AD induced rats with donepezil standard drug revealed insignificant change in PON1 activity while, significant increase in CaM level with amelioration percent 108.07%.

Effect of red and white berry extracts on 8-OHdG formation

Assessment of the 8-OHdG generation in brain tissues of AD induced rats' genome following red and white berry extracts treatment is summarized in Figure (1). The results revealed that exposure of male rats with $AlCl_3$ increased significantly the 8-OHdG/2-dG ratio compared with those in control rats. In contrary, the results showed that 8-OHdG/2-dG ratio following treatment of AD rats with red berry extract (300 mg/kg b.wt.) decreased significantly compared with those in AD rats and reached relatively similar to that of the control group. In addition, the treatment of AD rats with white berry extracts decreased significantly the 8-OHdG/2-dG ratio compared with those in AD rats, however, the 8-OHdG/2-dG ratio in AD rats treated with red berry extract was significantly lower than those treated with white berry extract (Figure 1). Moreover, the ratio of 8-OHdG/2-dG generation in AD rats treated with donepezil (5 mg/kg), as reference drug for AD treatment, was also declined significantly decreased significantly compared with those in AD rats (Figure 1).

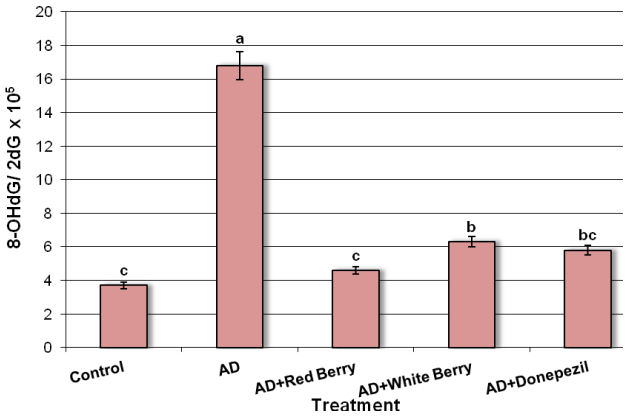

Figure 1

Generation of 8-OHdG in the brain tissues of male rats treated with $AlCl_3$ and berry extracts exposure. DNA damage was expressed as the ratio of oxidized DNA base (8-OHdG) to nonoxidized base (2-dG) in cortex DNA. Data are presented as mean ± SE. ^{a,b,c} followed by different superscripts are significantly different (P≤0.05).

Effect of red and white berry on the expression alteration of AD related genes

The expression values of DHCR24 and FKBP1B genes associated with AD in brain tissues of male rats were quantified by real-time RT-PCR (Figures 2 and 3). The results revealed that exposure of male rats with $AlCl_3$ decreased significantly the expression of DHCR24 and FKBP1B genes compared with those in control rats. The percentage of the mRNA expression of DHCR24 and FKBP1B genes were 32.8% and 22%, respectively compared to control rats (Figures 2 and 3).On the other hand, expression values of DHCR24 and FKBP1B genes increased significantly in AD rats treated with red and white berry extracts as well as with donepezil. The percentage of the mRNA expression of DHCR24 and FKBP1B genes increased to 247.4, 443.9 and 97.8% respectively in AD rats treated with red berry extract compared with those in AD rats (Figures 2 and 3). Also, the percentage of the mRNA expression of DHCR24 and FKBP1B genes increased to 200.4% and 336.6%, respectively, in AD rats treated with white berry extract compared with those in AD rats. Moreover, treatment of AD rats with donepezil increased the percentage of the mRNA expression of DHCR24 and FKBP1B genes to 253.9% and 429.3%, respectively, compared with those in AD rats.

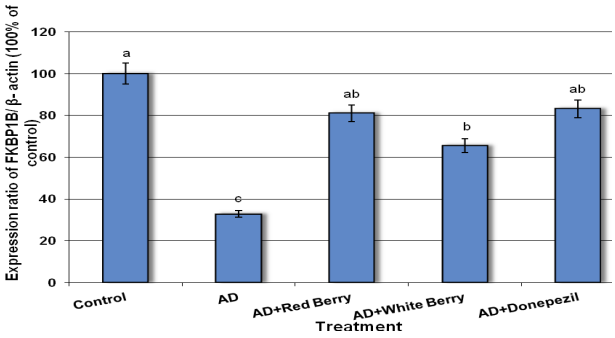

Figure 2

Expression levels of FKBP1B-mRNA in brain tissues of male rats exposed to $AlCl_3$ or/and Berry extracts. Data are presented as mean ± SE. ^{a,b,c,d} followed by different superscripts are significantly different (P≤0.05).

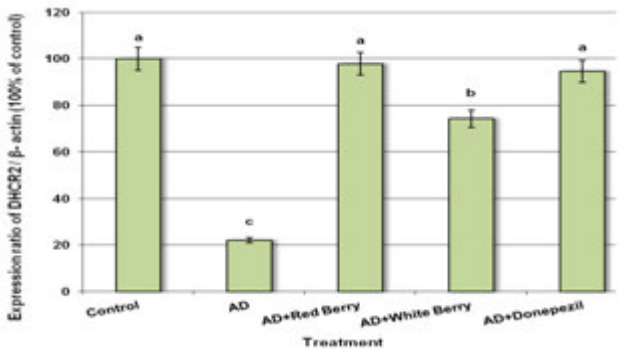

Figure 3

Expression levels of DHCR2-mRNA in brain tissues of male rats exposed to $AlCl_3$ or/and Berry extracts. Data are presented as mean ± SE. ^{a,b,c} followed by different superscripts are significantly different (P≤0.05).

Table 1

Primer sequences used for qPCR

Gene	Primer sequence (5′–3′)	Reference
DHCR24	F: GGGTGTTTGTGTGCCTCTTCC	Hassanzadeh et al.[39]
	R: GCTCCTTCCACTCCCGTACC	
Fkbp1b	F: GCAAGCAGGAAGTCATCAAAGG	Gant et al.[40]
	R: CAGTAGCTCCATATGCCACATCA	
β-actin	F:GGAGATTACTGCCCTGGCTCCTA	Deng et al.[41]
	R: GACTCATCGTACTCCTGCTGCTG	

Where, F: forward primer; R: reverse primer; (*DHCR24*): 24-dehydrocholesterol reductase; (*FKBP1B*): It is a gene encoding the binding protein (BP) for the immunosuppressive drug.

Table 2

Effect of red and white berry extracts on lipid peroxide and GSH levels in control, AD and treated rats

Parameters / Groups	Lipid peroxide	GSH
Normal control	9.12±0.11[a]	190.55±9.10[f]
Normal control treated with red berry	8.59±0.13[a]	199.65±5.90[cd]
%Change	5.81	4.78
Normal control treated with white berry	9.15±0.6[a]	195.70±0.80[cd]
%Change	0.33	2.70
AD	20.99±1.01[b]	55.50±2.11[g]
%change	130.15	70.87
AD treated with red berry	10.22±0.98[c]	160.63±10.13[a]
%Change	12.06	15.70
% of improvement	118.09	55.17
AD treated with white berry	11.00±0.83[c]	159.55±11.20[a]
%Change	20.61	16.26
% of improvement	109.54	54.60
AD treated with standard drug	9.76±0.66[a]	178.70±11.23[c]
%Change	7.02	6.22
% of improvement	123.14	58.35

Data are means ± SD of ten rats in each group.
Unshared letters between groups are the significance value at p≤ 0.05.

Table 3
Effect of red and white berry extracts on apoptotic marker; caspase-8 enzyme activity in control, AD and treated rats

Parameter / Groups	Caspase-8 (ng/ml)
Normal control	7.21±1.20[a]
Normal control treated with red berry	6.77±0.90[a]
%Change	6.10
Normal control treated with white berry	6.25±0.80[a]
%Change	13.31
AD	16.59±0.99[b]
%Change	130.09
AD treated with red berry	8.50±1.00[a]
%Change	17.89
% of improvement	112.21
AD treated with white berry	9.25±0.65[c]
%Change	28.29
% of improvement	101.80
AD treated with standard drug	8.62±0.29[a]
%Change	19.55
% of improvement	110.54

Data are means ± SD of ten rats in each group.
Unshared letters between groups are the significance value at p≤ 0.05.

Table 4
Effect of red and white berry extracts on PON1 and calmodulin in control, AD and treated rats

Parameters / Groups	PON1 (U/L)	Calmodulin (ng/ml)
Normal control	210.90±10.20[a]	2.82±0.02[a]
Normal control treated with red berry	230.20±2.50[b]	2.90±0.01[a]
%Change	9.62	1.75
Normal control treated with white berry	222.10±13.10[a]	2.69±0.06[a]
%Change	5.31	5.61
AD	153.20±12.13[d]	8.16±0.95[b]
%Change	27.36	186.32
AD treated with red berry	210.11±13.20[a]	6.81±0.81[c]
%Change	0.38	138.94
% of improvement	26.98	47.37
AD treated with white berry	180.50±21.10[e]	6.00±0.73[c]
%Change	14.41	110.53
% of improvement	12.94	75.79
AD treated with standard drug	190.59±11.50[a]	5.08±0.42[d]
%Change	9.63	78.25
% of improvement	17.73	108.07

Data are means ± SD of ten rats in each group.
Unshared letters between groups are the significance value at p≤ 0.05.

DISCUSSION

This study was conducted to evaluate the ameliorative effect of berry extracts (red and white) against AD. The results of the current study revealed that, $AlCl_3$ induced Alzheimer was associated with increased lipid peroxide level, caspase enzyme activity and CaM level while, GSH level and PON1 activity were deceased. However, on the molecular level, increase in the 8-OHdG/2-dG ratio as indicator parameter of DNA damage was detected. In addition, $AlCl_3$ treatment decreased the expression of DHCR24 and FKBP1B genes. In agreement with the findings, Shati et al.[42] reported that, aluminum (Al) has been proposed as an environmental factor that may affect several enzymes and other biomolecules related to neurotoxicity and AD. The pro-oxidant activity of Al might be interpreted by the formation of Al superoxide semi-reduced radical ions. The promotion of superoxide driven biological oxidation by Al was due to an interaction between the metal and the superoxide radical anion.[43] Under these conditions, high Al exposure could result in its accumulation in the central nervous system (CNS) where it induces the formation of ROS. This would result in an increase in brain oxidative stress and lipid peroxidation that would promote amyloid-β-peptide formation, deposition, and with time AD-like amyloidosis.[44] Al has been reported to enhance peroxidative damage to lipids and proteins and possibly to cause a decrease in GSH and antioxidant enzymes activity.[45] Yousef[46] reported that, Al induces an increase in lipid peroxidation, a decrease in the activity of the antioxidant enzymes and GSH in plasma and tissues of male rabbits. Accordingly, in situations where the generation of free radicals exceeds the capacity of antioxidant defense, oxidative stress may lead to cell membrane degradation, cellular dysfunction and high activity of caspase-8 enzyme indicating apoptosis.[47] Two major reasons may be participating in the high level of CaM observed in the current study that CaM half-life is enhanced in AD cells. The half-life of CaM in AD lymphoblasts was estimated in 22 h, approximately 3 times fold of that of control cells.[48] These values are in consonance with the reported half-life

of CaM in rat brain and the rate of CaM degradation which is found to decrease and is directly proportional to intracellular Ca^{2+} levels and ROS status.[48] In addition, the increased levels of CaM were associated with over activation of PI3K/Akt and enhanced proliferation of AD cells.[49] These results are in line with the known role of CaM in regulating cell cycle progression.[50] Apparently there is a reduction in the polyubiquitation of CaM in AD cells, which may contribute to the reduced CaM degradation. Intracellular Ca^{2+} levels and oxygen reactive species content appear to regulate the rate of CaM degradation.[51] Reducing the rate of CaM degradation could be the cellular response to buffer Ca^{2+} overload.[51] previous work indicated that ubiquitination and degradation of CaM *in vitro* show opposite sensitivity to Ca^{2+} and the rate of CaM degradation was found to decrease in response of the presence of Ca^{2+}.[52] It was suggested that ubiquitinated CaM could retain sufficient Ca^{2+} binding capacity to maintain a structure too rigid to be unfolded and directed to the proteasome.[52] On the other hand, treatment with antioxidants also normalized CaM degradation in AD lymphoblasts.[51] It is well known that enhanced ROS generation perturbs Ca^{2+} fluxes.[51] Hence; ROS generation, controlling the rate of CaM degradation which may play an additional role in ROS-induced disruption of Ca^{2+} homeostasis.The present results revealed low PON1 activity in AD induced rats. Although there is some disagreement, a large body of research suggested that low PON-1 activity may be associated with an increased risk of developing late-onset Alzheimer's disease (LOAD) or vascular dementia (VAD).[16,53-55] Lipolactonase activity has recently emerged as the native catalytic activity of PON1 which, in turn, help HDL particles to prevent the accumulation of lipid peroxides in oxidized LDLs.[56] The impairment of PON1 in mild cognitive impairment (MCI) patients could play a role in the derangement of systemic oxidative balance that affects these individuals regardless of their progression to dementia.[16] It must be emphasized that, to a lesser extent, paraoxonase may also contribute to the antioxidant mechanisms of HDLs.[54] This is an important point, as the ability to protect LDLs and HDLs against an oxidative challenge seems to be partially mediated by other anti-atherogenic functions of PON-1 including stimulation of eNOS-dependent NO production with subsequent endothelial anti-inflammatory effects, and enhancement of cholesterol efflux from cholesterol-laden macrophages.[57,58] It was hypothesize that a low level of PON1 activity may reflect a weaker physiological defense against pro-atherogenic processes, thus increasing the risk of VAD onset.[59] Furthermore, the results of the present study suggested that the increase in the level of DNA adducts (8-OHdG/2-dG ratio) and the decrease in the expression of DHCR24 and FKBP1B genes due to $AlCl_3$ treatment could be attributed to ROS formation and decrease the level of enzymatic and non-enzymatic antioxidant. On the other hand, the results of the current study revealed that red and white berry administration reduced lipid peroxide, CaM levels, caspase-8 activity and DNA adducts (8-OHdG/2-dG ratio) while, increased GSH level, PON1 activity and expression of DHCR24 as well as FKBP1B genes associated with Alzheimer pathway inhibition.Kaume et al.[60] revealed that, black berry fruits are well known to be a rich source of antioxidants, rich polyphenols manganese, folate, fibers, cyaniding-3-O-glucoside, vitamin C, salicylate and high tannin. Tavares et al.[61] reported that wild black berries, brigantinus and vagabundus collected from Braganc (northeast region of Portugal) demonstrated attainable neuroprotective effects by reducing intracellular ROS levels, modulating glutathione levels and inhibiting the occurrence of caspases during treatments. These effects protected neuronal cells against oxidative injury, one of the most important features of neurodegeneration. *In vitro* studies have also reported that black berries have potent anti-inflammatory and antiproliferative properties.[62,63] In addition, the antioxidants present in these fruits improved behavioral performance in motor neuron tests in aged rats. The balance and fine motor coordination in cognitive test were also improved in the Morris water maze, demonstrating the measures of spatial working memory and learning.[64] Indeed, the direct effect of berries in the present study on brain tissues inhibiting neurodegenerative disorders, decrease DNA damage and gene expression alterations is not fully understood. However, it could be attributed to the inhibition of ROS production, increase the activity of the antioxidant enzymes as well as inhibiting the occurrence of apoptotic related enzymes such as caspases. It has been found that supplementation of blue berries in adult mice (aged 3 months) improved performance in memory tasks and had a protective effect on DNA damage in the hippocampus and cerebral cortex.[65] Moreover, Barros et al.[65] reported that Reade (*Ericaceae*) berries, increased the protective effects against free radical-induced DNA damage in the brain. These results are reliable with the hypothesis that flavonoids (including anthocyanins) can show beneficial effects on cell signaling and decrease oxidative damage. These results also suggested that flavonoids might directly act on cognitive function, which may help prevent age-related and pathological degenerative processes in the brain.

CONCLUSION

The results of the present study showed that, red and white berry ethanolic extracts of berry have the ability to inhibit ROS production and apoptotic related enzymes that may lead to their neuro-ameliorative effect in Alzheimer's disease.

CONFLICT OF INTEREST

Conflict of Interest declared none.

REFERENCES

1. Alzheimer's Association. Alzheimer's disease facts and figures. Alzheimers and Dementia. 2008;8:110-33.
2. Pandey KB, Rizvi SI. Plant polyphenols as dietary antioxidants in human health and disease. Oxidative Medicine and Cellular Longevity. 2009;2:270-8.
3. Youdim KA, Shukitt-Hale B, Martin A, Wang H, Denisova N, Bickford PC, Joseph JA. Short-term dietary supplementation of blueberry polyphenolics: beneficial effects on aging brain performance and peripheral tissue function. Nutritional Neuroscience. 2000;3:383-97.
4. Casadesus G, Shukitt-Hale B, Joseph JA. Qualitative versus quantitative caloric intake: are they equivalent paths to successful aging?. Neurobiology of Aging. 2002;23:747-69.
5. Mushtaq M, Wani SM. Polyphenols and human health- A review. International Journal of Pharma and Bio Sciences. 2013;4(2):(B)338-60.
6. Basu A, Rhone M, Lyons TJ. Berries: emerging impact on cardiovascular health. Nutrition Reviews. 2010;68:168-77.
7. Rendeiro C, Guerreiro JD, Williams CM, Spencer JP. Flavonoids as modulators of memory and learning: molecular interactions resulting in behavioural effects. Proceedings of the Nutrition Society. 2012;71:246-62.
8. Zheng Z, Lee JE, Yenari MA. Stroke: molecular mechanisms and potential targets for treatment. Current Molecular Medicine. 2003;3:361-72.
9. Lau FC, Shukitt-Hale B, Joseph JA. The beneficial effects of fruit polyphenols on brain aging. Neurobiology of Aging. 2005;26:128-32.
10. Rahman K. Studies on free radicals, antioxidants, and co-factors. Clinical Interventions in Aging. 2007;2:219-36.
11. Uttara B, Singh AV, Zamboni P, Mahajan RT. Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options. Current Neuropharmacology. 2009;7:65-74.
12. O'Day DH, Myre M. Calmodulin-binding domains in Alzheimer's disease proteins: Extending the calcium hypothesis. Biochemical and Biophysical Research Communications. 2004;320(4):1051-4
13. Ghosh A, Giese KP. Calcium/calmodulin-dependent kinase II and Alzheimer's disease. Molecular Brain. 2015;8(78):1-7.
14. Luca M, Luca A, Calandra C. The Role of oxidative damage in the pathogenesis and progression of alzheimer's disease and vascular dementia. Oxidative Medicine and Cellular Longevity. 2015;1-8.
15. Dentesano G, Serratosa J, Tusell JM. CD200R1 and CD200 expression are regulated by PPAR-γ in activated glial cells. Glia. 2014;62(6):982-98.
16. Cervellati C, Romani A, Bergamini CM, Bosi C, Sanz JM, Passaro A, Zuliani G. PON-1 and ferroxidase activities in older patients with mild cognitive impairment, late onset Alzheimer's disease or vascular dementia. Clinical Chemistry and Laboratory Medicine. 2014;53:1049-56.
17. Cervellati C, Trentini A, Romani A, Bellini T, Bosi C, Ortolani B, Zurlo A, Passaro A, Seripa D, Zuliani G. Serum paraoxonase and arylesterase activities of paraoxonase-1 (PON-1), mild cognitive impairment, and 2-year conversion to dementia: A pilot study. Journal of Neurochemistry. 2015; 135(2):395-401.
18. Balbach J, Schmid FX. Proline isomerization and its catalysis in protein folding. In Mechanisms of Protein Folding. 2nd (ed). R.H. Pain editor, Oxford: Oxford University Press; 2000. p.212-37.
19. Fehér A, Juhász A, Pákáski M, Kálmán J, Janka Z. Gender dependent effect of DHCR24 polymorphism on the risk for Alzheimer's disease. Neuroscience Letters. 2012;526(1):20-3
20. Aquilano K, Baldelli S, Rotilio G, Ciriolo MR. Role of nitric oxide synthases in Parkinson's disease: a review on the antioxidant and anti-inflammatory activity of polyphenols. Neurochemical Research. 2008;33:2416-26.
21. Esposito E, Rotilio D, Di Matteo V, Di Giulio C, Cacchio M, Algeri S. A review of specific dietary antioxidants and the effects on biochemical mechanisms related to neurodegenerative processes. Neurobiology of Aging. 2012;23:719-35.
22. Chen L, Xin X, Zhang H, Yuon Q. Phytochemical properties and antioxidant capacities of commercial raspberry varieties. Journal of Functional Foods. 2013;5:508-15.
23. de Souza RV, Pereira PAP, da Silva TLT, Lima LCO, Pio R, Queiroz F. Determination of the bioactive compounds, antioxidant activity and chemical composition of Brazilian blackberry, red raspberry, strawberry, blueberry and sweet cherry fruits. Food Chemistry. 2014;156:362-8.
24. Angeloni C, Pirola L, Vauzour D, Maraldi T. Dietary polyphenols and their effects on cell biochemistry and pathophysiology. Oxidative Medicine and Cellular Longevity. 2012;1-3.
25. Kanthal LK, Dey A, Satyavathi K, Bhojaraju P. GC-MS analysis of bio-active compounds in methanolic extract of *Lactuca runcinata* DC. Pharmacognosy Research. 2014;6(1):58-61.
26. Ravisankar N, Sivaraj C, Seeni S, Joseph J, Raaman N. GC-MS analysis and anticancer activity of methanol extract of leaves of *Hypericum hookerianum* wight & ARN. International Journal of Pharmacy and Pharmaceutical Sciences. 2014;6(5):515-9.
27. Kumar V, Bal A, Gill KD. Aluminum-induced oxidative DNA damage recognition and cell cycle disruption in different regions of rat brain. Toxicology. 2009;264:137-44.
28. Bihaqi SW, Sharma M, Singh AP, Tiwari M. Neuroprotective role of Convolvulus pluricaulis on aluminium induced neurotoxicity in rat brain. Journal of Ethnopharmacology. 2009;124:409-15.
29. Matsumoto M, Hara H, Chiji H, Kasai T. Gastroprotective effect of red pigments in black chokeberry fruit (*Aronia melanocarpa* Elliot) on acute gastric hemorrhagic lesions in rats. Journal of Agricultural and Food Chemistry. 2004;21,52(8):2226-9.

30. Tsakiris S, Schulpis KH, Marinou K, Behrakis P. Protective effect of L-cysteine and glutathione on the modulated suckling rat brain Na^+,K^+-ATPase and Mg^{2+}-ATPase activities induced by the *in vitro* galactosaemia. Pharmacological Research. 2004;49:475-9.
31. Satoh K. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. Clinica Chimica Acta. 1978;90:37-43.
32. Moron MS, Depierre JW, Mannervik B. Level of glutathione, glutathione reductase and glutathion-S-transferase activities in ratlung and liver. Biochemica et Biophysica Acta.1979;582:67-8.
33. Thompson CB. Apoptosis in the pathogenesis and treatment of disease. Science. 1995;267:1456-62.
34. Araoud M, Neffeti F, Douki W, Kenani A, Najjar MF. Development of an automated method for the determination of human paraoxonase1 activity. Asian Biomedicine. 2011;5 (2):217-24.
35. Garg UC, Rai N, Singh Y, Dhaunsi GS, Sidhu GS, Ganguly NK, Bhatnagar R.A spectrophotometric method for calmodulin assay. Biotechniques. 1988;6(4):294-6.
36. Patel M, Liang LP, Roberts LJ. Enhanced hippocampal F2-isoprostane formation following kainite induced seizures. Journal of Neurochemistry. 2001;79(5):1065-9.
37. Khalil WKB, Booles HF. Protective role of selenium against over-expression of cancer-related apoptotic genes induced by o-cresol in rats. Archives of Industrial Hygiene and Toxicology. 2011;62:121-9.
38. El-Baz FK, Aly HF, Khalil WKB, Booles HF, Saad SA. *Jatropha Curcas* repairing effect on adhesion molecules, DNA damage and gene expression alteration in STZ-induced diabetic rats. International Journal of Pharm and Bio Sciences. 2015;6(3):(B)198-214.
39. Hassanzadeh G, Hosseini A, Pasbakhsh P, Akbari M, Ghaffarpour M, Takzare N, Zahmatkesh M. Trimetazidine prevents oxidative changes induced in a rat model of sporadic type of alzheimer´s disease. Acta Medica Iranica. 2015;53(1):17-24.
40. Gant JC, Chen KC, Kadish I, Blalock EM, Thibault O, Porter NM, Landfield PW. Reversal of aging-related neuronal Ca^{2+} dysregulation and cognitive impairment by delivery of a transgene encoding FK506-binding protein 12.6/1b to the hippocampus. Journal of Neuroscience. 2015; 29, 35(30):10878-87.
41. Deng Y, Xu ZF, Liu W, Xu B, Yang HB, Wei YG. Riluzole-triggered GSH synthesis via activation of glutamate transporters to antagonize methylmercury-induced oxidative stress in rat cerebral cortex. Oxidative medicine and cellular longevity. 2012 Aug 22;2012..
42. Shati AA, Elsaid FG, Hafez EE. Biochemical and molecular aspects of aluminium chloride-induced neurotoxicity in mice and the protective role of Crocus sativus L. extraction and honey syrup. Neuroscience. 2011;175:66-74.
43. Exley C. The pro-oxidant activity of aluminum. Free Radical Biology & Medicine. 2004;36:380-7.
44. Pratico D, Uryu K, Sung S, Tang S, Trojanowski JQ, Lee VMY. Aluminum modulates brain amyloidosis through oxidative stress in APP transgenic mice. FASEB Journal. 2002;16:1138-40
45. Julka D, Gill KD. Altered calcium homeostasis: a possible mechanism of aluminium-induced neurotoxicity. Biochim Biophys Acta. 1996;1315:47-54.
46. Yousef MI. Aluminium-induced changes in hemato-biochemical parameters, lipid peroxidation and enzyme activities of male rabbits: protective role of ascorbic acid. Toxicology. 2004;199:47-57.
47. Lucca G, Comim CM, Valvassori SS, Réus GZ, Vuolo F, Petronilho F, Dal-Pizzol F, Gavioli EC, Quevedo J. Effects of chronic mild stress on the oxidative parameters in the rat brain. Neurochemistry International. 2009;54:358-62.
48. Ferrington DA, Krainev AG, Bigelow DJ. Altered turnover of calcium regulatory proteins of the sarcoplasmic reticulum in aged skeletal muscle. Journal of Biological Chemistry. 1998;273:5885-91.
49. Balog EM, Lockamy EL, Thomas DD, Ferrington DA. Site-specific methionine oxidation initiates calmodulin degradation by the 20S proteasome. Biochemistry. 2009;48:3005-16.
50. Rasmussen CD, Means AR. Calmodulin is involved in regulation of cell proliferation. Embo Journal. 1987;6:3961-8.
51. Peng TI, Jou MJ. Oxidative stress caused by mitochondrial calcium overload. Annals of the New York Academy of Sciences. 2010;1201:183-8.
52. Tarcsa E, Szymanska G, Lecker S, O'Connor CM, Goldberg AL. Ca2+-free calmodulin and calmodulin damaged by *in vitro* aging are selectively degraded by 26 S proteasomes without ubiquitination. Journal of Biological Chemistry. 2000;275:20295-301.
53. Pit Y, Zhang L, Chang K, Li B, Guo L, Fang C, Gao C, Wang J, Xiang J, Li J. Lack of an association between Paraoxonase 1gene polymorphisms (Q192R, L55M) and Alzheimer' s disease: a meta-analysis. Neuroscience Letters. 2012;523:174-9.
54. Zengi O, Karakas A, Ergun U, Senes M, Inan L, Yucel D. Urinary 8-hydroxy-2-deoxyguanosine level and plasma paraoxonase 1 activity with Alzheimer's disease. Clinical Chemistry and Laboratory Medicine. 2012;50:529-34.
55. Bednarska-Makaruk ME, Krzywkowski T, Graban A, Lipczynska-Lojkowska W, Bochynska A, Rodo M, Wehr H, Ryglewicz DK. Original article paraoxonase 1 (PON1) gene -108C T and p.Q192R polymorphisms and arylesterase activity of the enzyme in patients with dementia. Folia Neuropathologica. 2013;51(2):111-19.
56. Rosenblat M, Gaidukov L, Khersonsky O, Vaya J, Oren R, Tawfik DS, Aviram M. The catalytic histidine dyad of high density lipoprotein-associated serum paraoxonase-1 (PON1) is essential for PON1-mediated inhibition of low density lipoprotein oxidation and stimulation of macrophage cholesterol efflux. Journal of Biological Chemistry. 2006; 281:7657-65.

57. Besler C, Heinrich K, Rohrer L. Mechanisms underlying adverse effects of HDL on eNOS-activating pathways in patients with coronary artery disease. Journal of Clinical Investigation. 2011;121:2693-2708.
58. Tang WHW, Hartiala J, Fan Y. Clinical and genetic association of serum paraoxonase and arylesterase activities with cardiovascular risk. Arteriosclerosis Thrombosis and Vascular Biology. 2012;32:2803-12.
59. Rohatgi A, Khera A, Berry JD. HDL cholesterol efflux capacity and incident cardiovascular events. The New England Journal of Medicine. 2014;317,2383-93.
60. Kaume L, Howard LR, Devareddy L. The blackberry fruit: a review on its composition and chemistry, metabolism and bioavailability, and health benefits. Journal of Agricultural and Food Chemistry. 2012;60:5716-27.
61. Tavares L, Figueira I, McDougall GJ, Vieira HL, Stewart D, Alves PM, Ferreira RB, Santos CN. Neuroprotective effects of digested polyphenols from wild blackberry species. European Journal of Nutrition. 2013;52:225-36.
62. Wang SY, Jiao H. Scavenging capacity of berry crops on superoxide radicals, hydrogen peroxide, hydroxyl radicals, and singlet oxygen. Journal of Agricultural and Food Chemistry. 2000;48:5677-84.
63. Dai J, Patel JD, Mumper RJ. Characterization of blackberry extract and its antiproliferative and anti-inflammatory properties. Journal of Medicinal Food. 2007;10:258-65.
64. Shukitt-Hale B, Cheng V, Joseph JA. Effects of blackberries on motor and cognitive function in aged rats. Nutritional Neuroscience. 2009;12:135-40.
65. Barros D, Amaral OB, Izquierdo I, Geracitano L, do Carmo Bassols Raseira M, Henriques AT, Ramirez MR. Behavioral and genoprotective effects of Vaccinium berries intake in mice. Pharmacology Biochemistry & Behavior. 2006;84:229-34.