

Ameliorative Effect of *Teucrium polium* Extract on γ -radiation Toxicity in Brain of Albino Rats

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THE IMPAIRMENT to the healthy brain tissue is a crucial factor limiting the application of radiation therapy in patients with nervous system neoplasms. *Teucrium polium* (TP) L. (family Lamiaceae), a wild-growing flowering plant, has beneficial curative properties. The flavonoids of TP have many valuable biological activities. This study aims at investigating the ameliorative effect of TP extract on γ -radiation toxicity in brain of albino rats.

Thirty adult male albino rats were used. Ionizing irradiation was performed by whole body exposure of rats to 8Gy. TP was administered by intragastric tube in a dose of 200mg/kg body weight. Animals were divided into five groups: Group I (control), Group II (TP), Group III (γ -irradiated), Group IV (TP+ γ -irradiated) and Group V (TP+ γ -irradiated+TP). The action of TP extract was investigated after one week post irradiation by measuring serum liver and renal functions, and oxidative stress parameters in brain tissue. Moreover, serum S100B and brain-derived neurotrophic factor (BDNF) in brain tissue were measured, in addition to histopathological examination.

TP at a dose of 200mg/kg body weight did not have hepatotoxic or nephrotoxic effects. TP extract significantly ameliorated γ -radiation-induced brain damage by improving radiation-induced oxidative stress. The role of the extract was confirmed by improvement of the levels of S100B in the serum and BDNF in the brain as well as amelioration of histopathological changes induced by radiation. The administration of TP before and after irradiation was more effective than administration only before irradiation. These results revealed that the administration of TP extract might ameliorate γ -radiation-induced brain injury by the attenuation of oxidative stress, the regulation of BDNF and the suppression of S100B.

Keywords: *Teucrium polium*, γ -radiation, brain, oxidative stress, S100B, Brain-derived neurotrophic factor.

Introduction

Exposure of the central nervous system (CNS) to ionizing radiation occur during a number of clinical situations; radiotherapy remains a major treatment modality for primary and metastatic neoplasms located in the CNS (Caruso et al., 2013). Exposure of the spinal cord and the brain is frequently unavoidable in the radiotherapeutic management of tumors located nearby the CNS such as cancers of head and neck. In addition, there is a growing radiation application in management

of other brain disorders as epilepsy (Régis et al., 1999). Radiation-induced brain injury (RIBI) is still a prominent and serious side effect in spite of current advances in radiation delivery and planning procedures, and the neuroprotective therapies for RIBI are insufficient. Therefore, preclinical studies are crucial to find an active radio-neuroprotector and unravel its possible mechanisms. Oxidative stress as well plays a key role in radiation-induced cell damage, chiefly through generation of reactive oxygen species (ROS) (Mao et al., 2012).

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Oxidative stress happens when the balance between ROS and antioxidants is disturbed. A high level of polyunsaturated fatty acids contained in the brain making it more liable to oxidative damage which is an important mechanism of RIBI. In some preceding studies, ionizing radiation induced significant oxidative stress levels in neuron cells, characterized by the increase of ROS and malondialdehyde (MDA) in addition to the reduction of antioxidants including reduced glutathione, catalase and superoxide dismutase (SOD) (Rola et al., 2007; Huang et al., 2012 and Xin et al., 2012). ROS scavengers were illustrated to be effective in ameliorating radiation-induced neuronal injury (Xin et al., 2012 and Lu et al., 2015). In previous animal studies, ionizing radiation also appeared to provoke hippocampal neuronal apoptosis correlated to the learning and memory shortfalls (Huo et al., 2012; Xin et al., 2012; Hassan et al., 2013; Zhang et al., 2014 and Dong et al., 2015).

Brain-derived neurotrophic factor (BDNF) is a neurotrophin which facilitates neurogenesis, neuroregeneration, neuroprotection, synaptic plasticity, cell survival, as well as formation, retention, and recall or memory in hippocampus and frontal cortex (Bekinschtein et al., 2008).

S100B has been involved in the cell cycle progression regulation and differentiation throughout microtubule and intermediate filament assembly, and its expression is predominantly occurred in the astrocytes cytoplasm (Donato, 2001). The protein was firstly defined by Moore (1965) and after that, the variation of S100B gene has been associated with various neurological illnesses including Alzheimer's, amyotrophic lateral sclerosis and epilepsy (Otto et al., 1998; Chaves et al., 2010 and Calik et al., 2013). More importantly, S100B has emerged as a marker of the neurological damage acute phase. Following acute brain injury, astrocytes release the protein and if the blood brain barrier is compromised, it can be detected in serum. S100B, moreover, acted as a prognostic marker in outcome prediction after traumatic brain injury (TBI) and large volume cerebral infarction (Rainey et al., 2009; Egea-Guerrero et al., 2013 and Thelin et al., 2013). Additionally, an elevated serum level of S100B has been illustrated in ischemic stroke and is concomitant with worse outcome after a stroke (Weglewski et al., 2005 and Kaca-Orynska et al., 2010).

The medical use of plants dates back to ancient times. *Teucrium polium* (TP) L. (Lamiaceae) has been used for over two hundred years in traditional medicine owing to its diuretic, tonic, diaphoretic, antispasmodic, antipyretic and cholagogic properties (Galati et al., 2000). Furthermore, the plant owns hypoglycemic, hypolipidemic, insulinotropic, antinociceptive, antioxidant, and anti-inflammatory properties (Gharaibeh et al., 1988; Tariq et al., 1989; Rasekh et al., 2001; Abdollahi et al., 2003; Couladis et al., 2003; Esmaeili & Yazdanparast, 2004 and Mousavi et al., 2012). TP also affects the cardiovascular system (Niazmand et al., 2011), diminishes body weight (Suleiman et al., 1988), and protects against hepatotoxicity induced by acetaminophen (Kalantari et al., 2013). It has been demonstrated that TP extract possesses anti-amnesic properties in a mouse model of amnesia induced by scopolamine (Orhan & Aslan, 2009). Hence, TP may be a herbal alternative for memory enhancement (Hasanein & Shahidi, 2012).

In light of these beneficial effects, the aim of this study is to investigate the ameliorative effect of TP extract on γ -radiation toxicity in brain of albino rats.

Materials and Methods

Preparation of plant extract

TP plant species were collected in June 2014 from South Sinai, Egypt and aerial parts were air-dried. Plants were identified and voucher specimens were deposited in the Herbarium of Saint Katherine Protectorate, Egypt. The collection was carried out under the permission of Saint Katherine Protectorate for scientific purposes and official permission was granted from the National Research Center, Egypt. The aerial parts (100g) of TP were powdered and extracted with CH_2Cl_2 -MeOH (1:1) at room temperature. The filtrate solvents extract was concentrated in vacuum using rotary evaporator to obtain a crude extract.

High-performance liquid chromatography (HPLC)

HPLC equipment

HPLC analysis was performed using an Agilent 1260 series. The separation was carried out using C18 column (4.6mm x 250mm i.d., 5 μ m).

Chemicals

The standards chemicals such as gallic acid,

chlorogenic acid, catechin, caffeine, rutin, caffeic acid, syringic acid, pyrocatechol, coumaric acid, vanillin, ferulic acid, naringenin, propyl gallate, 4',7-dihydroxy isoflavone, quercetin, and cinnamic acid were purchased from Sigma Chemical Co. and HPLC-grade solvents such as tri-fluoro-acetic acid, acetonitrile, and water were purchased from Merck (Germany).

Chromatographic analysis of phenolic compounds

The mobile phase consisted of water (A) and 0.02% tri-fluoro-acetic acid in acetonitrile (B) at a flow rate 1ml/min. The mobile phase was programmed consecutively in a linear gradient as follows: 0min (80% A); 0–5min (80% A); 5–8min (40% A); 8–12min (50% A); 12–14min (80% A) and 14–16min (80% A). The injection volume was 10 μ l for each of the sample solutions. The temperature of column was maintained at 35°C. HPLC chromatograms were detected using a photo diode array UV detector at wavelength (280nm) according to absorption maxima of analyzed compounds. Each compound was identified by its retention time and by spiking with standards under the same conditions. The quantification of the sample was done by the measurement of the integrated peak area and the content was calculated using the calibration curve by plotting peak area against concentration of the respective standard sample. The data were reported with convergence limit in triplicate (Fig. 1).

Experimental animals

Thirty adult male albino rats, weighing 180–200g, were obtained from the National Center for Radiation Research and Technology (NCRRT), Egyptian Atomic Energy Authority, Cairo, Egypt. The animals were kept in a well-ventilated animal house in isolated cages, and were maintained on a standard diet and provided water *ad libitum*. Before the beginning of the experiment, rats were kept under surveillance for one week to be acclimatized. They were maintained at a room temperature of 22 \pm 5°C under 12hr light dark cycles.

Experimental design

Rats were randomly divided into five groups (each of six rats): Group I (control), Group II (TP), Group III (γ -irradiated), Group IV (TP + γ -irradiated) and Group V (TP + γ -irradiated + TP). The irradiated rats were whole body exposed to acute single dose of γ -irradiation (8Gy). Groups II, IV and V received TP (200ml/kg body weight,

orally) (Mousavi et al., 2015) dissolved in saline for 7 days, then Groups IV and V were exposed to γ -irradiation in the 8th day, one hour after γ -irradiation, Group V continued to receive TP for another 7 days. All rats were sacrificed 24hr after the last dose of TP. The investigation complies with the Guide for Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH) and the study protocol was approved by the ethical committee of the NCRRT.

Irradiation processing

Irradiation processing was carried out at the NCRRT using a Canadian Gamma Cell-40, (¹³⁷Cs). Animals were submitted to whole body γ -irradiation at a dose level of 8Gy delivered at a dose rate of 0.4319Gy/min.

Samples collection and preparation

Animals were anaesthetized with urethane after overnight fasting then sacrificed. Blood samples were obtained by heart puncture. Serum was separated by centrifugation at 3000rpm and stored at -20°C until analysis. The brain was excised and a suitable weight of brain tissues (0.5g) was homogenized in 5ml of ice-cold 0.1M phosphate buffer saline (pH 7.4), then the homogenate was centrifuged and the supernatant was collected for further biochemical analysis. Brain tissue specimens were fixed in 10% buffered formalin solution for the histopathological investigation.

Biochemical procedures

Serum hepatic transaminases enzymes (alanine transaminase (ALT) and aspartate transaminase (AST)) activities as well as renal function (urea and creatinine) were assessed according to standard methods using obtainable commercial kits (Spectrum diagnostics, Cairo, Egypt). Lipid peroxidation was determined by quantifying MDA levels in tissue homogenates according to the method illustrated by Yoshioka et al. (1979). Also, activity of SOD in tissue homogenates was assessed according to Minami & Yoshikawa (1979) method. The total antioxidant capacity (TAC) in brain tissue was measured using Randox total antioxidant status kit (UK) according to Miller et al. (1993). S100B and BDNF protein levels in serum and brain tissue respectively were quantified using a commercially existing enzyme-linked immunosorbent assay (ELISA) kit (My Biosource, USA) according to the manufacturer's protocol.

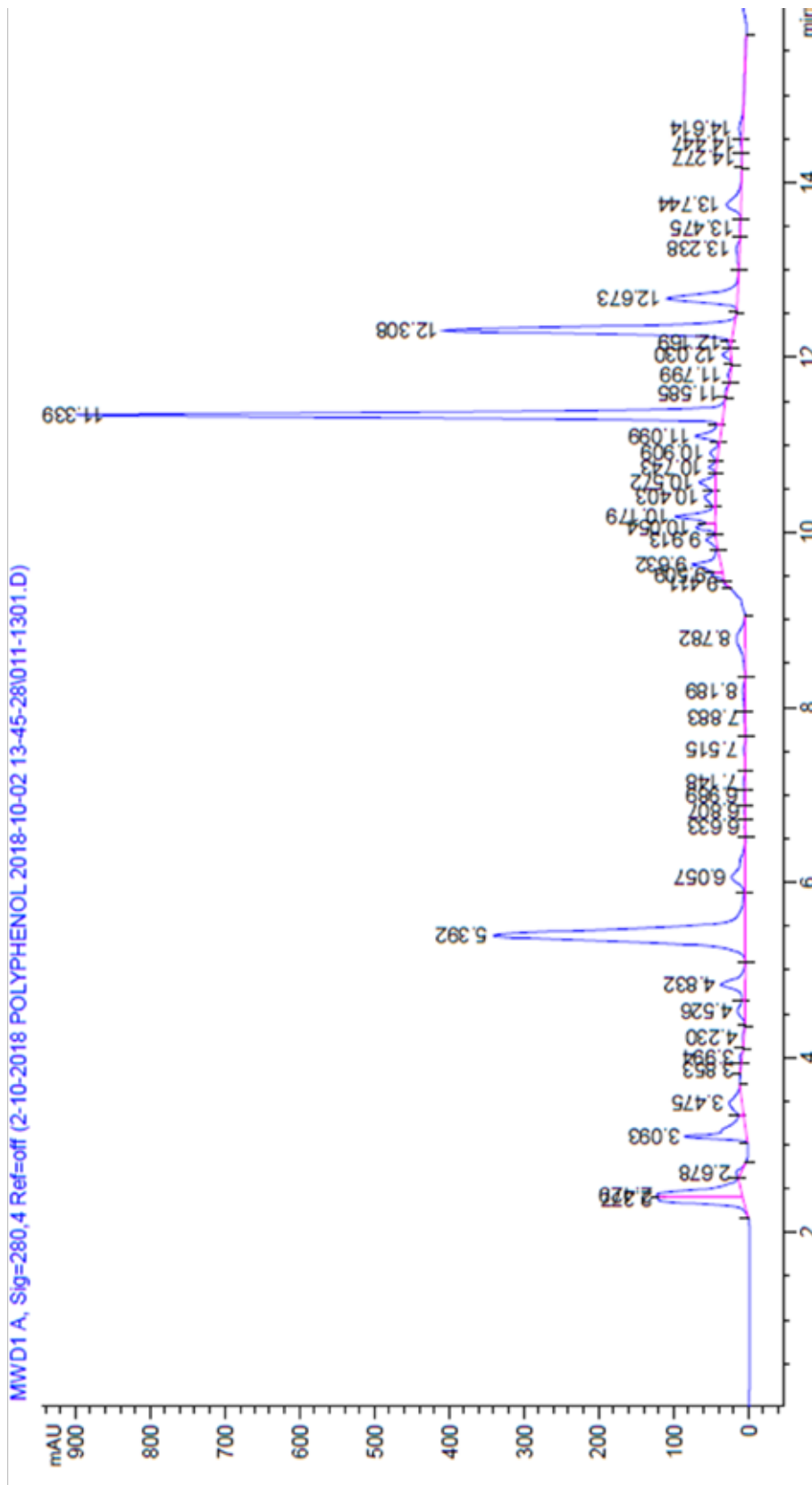


Fig. 1. Typical HPLC chromatogram of TP extract [CH₂Cl₂:MeOH (1:1)]

Histopathological examination

For the histopathological investigation, specimens of brain tissue were fixed in 10% buffered formalin solution followed by dehydration, clearing and embedding in paraffin. Paraffin sections of 5 μ m thickness were sliced and stained routinely with haematoxylin and eosin reagent, according to Bancroft & Stevens (1996) and examined with light microscope.

Statistical analysis

Data were reported as means \pm standard error (SE). All results were statistically analyzed by one way analysis of variance (ANOVA) followed by a post hoc, least significant difference (LSD) test using Statistical Package for Social Science (SPSS) program version 15. Results were considered statistically significant when P value < 0.05.

Results

Identification and quantification of phenolic compounds in TP extract

The HPLC chromatogram of CH₂Cl₂-MeOH (1:1) extract of the aerial parts of TP showed the presence of gallic acid, chlorogenic acid, catechin, caffeine, caffeic acid, ellagic acid, coumaric acid, vanillin, ferulic acid, naringenin, propyl gallate, 4',7-dihydroxy isoflavone, quercetin, and cinnamic

acid as presented in Table 1. The structures of these compounds were illustrated in Fig. 2.

Effect of TP extract on liver and renal functions

Compared to control group, serum ALT and AST activities in γ -irradiated rats showed a significant increase (P \leq 0.001). Moreover, γ -radiation induced a significant increase in serum urea and creatinine levels (P \leq 0.001) (Table 2).

Compared to γ -irradiated group, TP extract administration before γ -radiation induced a significant reduction in serum activities of ALT and AST (P \leq 0.01 and P < 0.05, respectively), and serum levels of urea and creatinine (P \leq 0.001). In addition, TP extract treatment for additional one week after γ -radiation further reduced these parameters (P \leq 0.001 and P \leq 0.01, for ALT and AST respectively; P \leq 0.001, for urea and creatinine) compared to γ -irradiated Group (Table 2).

Effect of TP extract on oxidative stress parameters

The exposure to γ -radiation caused a significant increase of MDA levels in brain tissue compared to control group (P \leq 0.001). Further, SOD activities and TAC levels in brain tissue markedly decreased in γ -irradiated rats (P \leq 0.001) (Table 3).

TABLE 1. Determination of polyphenols in *Teucrium polium* extract CH₂Cl₂:MeOH (1:1) by using the proposed HPLC method (wavelength 280nm).

Compounds	Retention time	Peak area	Conc. (μ g/ml)
Gallic acid	3.093	545.72	35.94
Chlorogenic acid	3.475	365.60	24.18
Catechin	3.853	35.77	6.12
Caffeine	3.994	39.90	1.18
Caffeic acid	4.832	270.35	9.18
Syringic acid	5.239	0.00	0.00
Rutin	5.539	0.00	0.00
Pyrocatechol	5.741	0.00	0.00
Ellagic acid	6.633	10.25	0.74
Coumaric acid	7.515	23.09	0.53
Vanillin	8.189	58.55	2.09
Ferulic acid	8.782	203.05	4.43
Naringenin	9.328	0.00	0.00
Propyl gallate	10.179	394.57	9.87
4',7-Dihydroxy isoflavone	10.401	182.46	4.86
Quercetin	10.571	165.16	13.81
Cinnamic acid	11.100	189.18	1.60

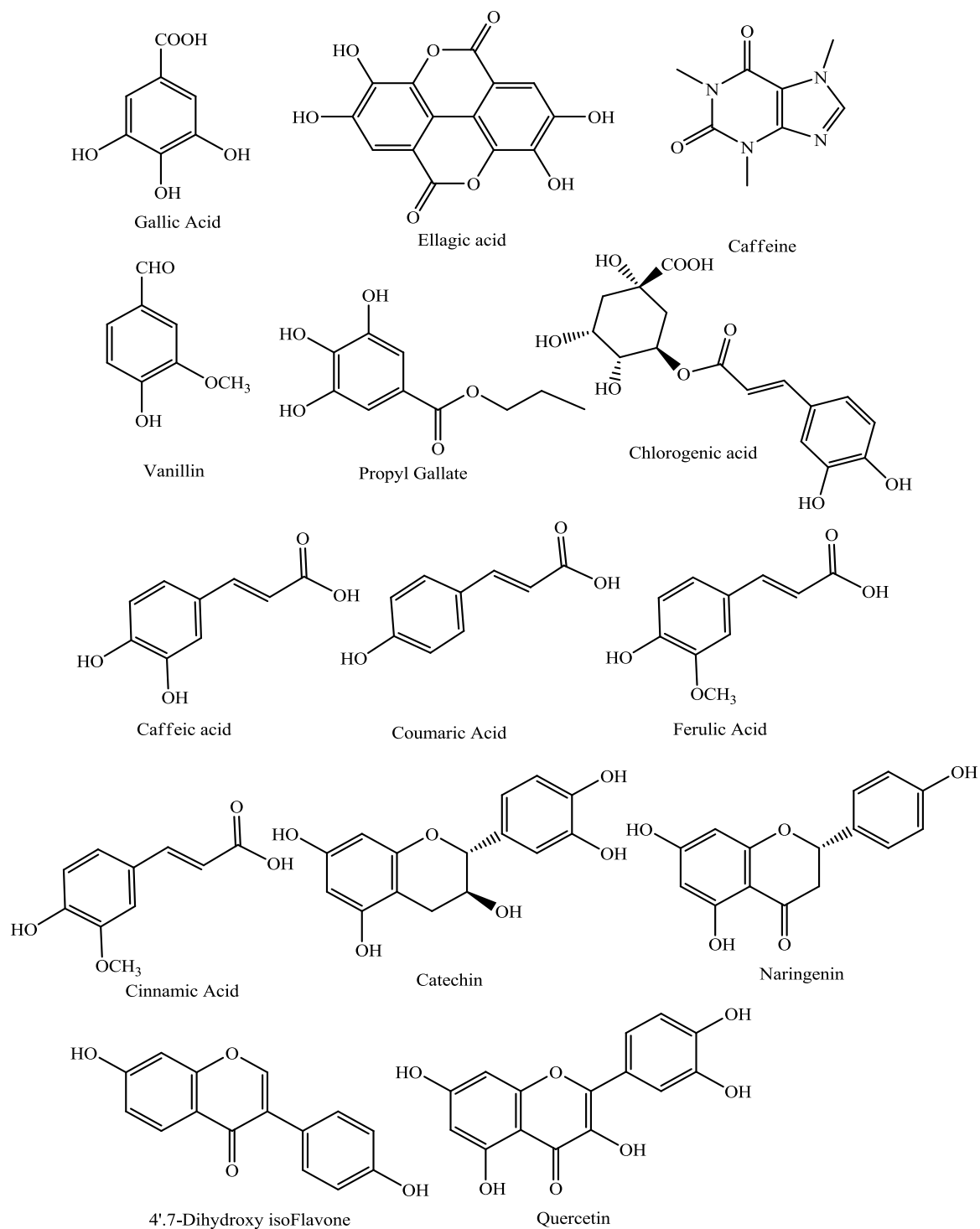


Fig. 2. Structure of compounds determined in TP extract CH_2Cl_2 :MeOH (1:1) by using the proposed HPLC method.

TABLE 2. Effect of *Teucrium polium* extract on liver and renal functions against irradiation.

Parameters	Group I	Group II	Group III	Group IV	Group V
ALT (U/L)	21.00±1.73	22.67±1.76	50.00±6.66 ^{a***b***}	28.67±2.03 ^{c**}	24.33±2.85 ^{c***}
AST (U/L)	22.67±2.03	24.33±2.03	49.33±6.74 ^{a***b***}	33.67±2.60 ^{c*}	28.33±2.40 ^{c**}
Urea (mg/dl)	41.00±2.31	38.67±2.03	75.33±2.03 ^{a***b***}	47.00±2.08 ^{b*c***}	42.33±2.03 ^{c***}
Creatinine (mg/dl)	0.15±0.02	0.15±0.01	0.86±0.08 ^{a***b***}	0.41±0.06 ^{a*b*c***}	0.38±0.08 ^{a*b*c***}

Group I (control), group II (TP), group III (γ -irradiated), group IV (TP + γ -irradiated) and group V (TP + γ -irradiated + TP). n= 6 rats per group.

a: Significance vs control; b: Significance vs TP; c: Significance vs γ -irradiated

* : P< 0.05; ** : P≤ 0.01; *** : P≤ 0.001

TABLE 3. Effect of *Teucrium polium* extract on oxidative stress parameters in brain against irradiation.

Parameters	Group I	Group II	Group III	Group IV	Group V
MDA (U/mg protein)	9.80±0.38	8.90±0.67	44.17±7.47 ^{a***b***}	19.30±3.46 ^{c***}	16.67±2.07 ^{c***}
SOD (U/mg protein)	2.65±0.28	3.10±0.21	0.75±0.15 ^{a***b***}	2.03±0.19 ^{b**c***}	2.07±0.32 ^{b*c***}
TAC (U/mg protein)	25.90±1.33	29.37±1.85	13.70±1.87 ^{a***b***}	20.93±1.86 ^{b**c*}	21.97±1.26 ^{b*c**}

Group I (control), group II (TP), group III (γ -irradiated), group IV (TP + γ -irradiated) and group V (TP + γ -irradiated + TP). n= 6 rats per group.

a: Significance vs control; b: Significance vs TP; c: Significance vs γ -irradiated

* : P< 0.05; ** : P≤ 0.01; *** : P≤ 0.001

TP extract administration before γ -radiation resulted in a significant reduction in MDA levels (P≤ 0.001), and a significant increase in SOD activities (P≤ 0.01) and TAC levels (P<0 .05) compared to γ -irradiated control group. Moreover, TP extract treatment for additional one week after γ -radiation further reduced MDA levels (P< 0.001) and increased SOD activities (P≤ 0.01) and TAC levels (P≤ 0.01) compared to γ -irradiated control group (Table 3).

Effect of TP extract on S100B and BDNF

Serum S100B levels in γ -irradiated rats showed a significant increase compared to control group

(P≤ 0.01). Additionally, γ -radiation induced a significant decrease in BDNF levels in brain tissue (P≤ 0.001) (Table 4).

Compared to γ -irradiated group, TP extract administration before γ -radiation caused a significant reduction in serum S100B levels (P< 0.05), and a significant increase in brain BDNF levels (P≤ 0.001). Likewise, TP extract treatment for additional one week after γ -radiation further reduced S100B levels (P< 0.05) and increased BDNF levels (P≤ 0.001) compared to γ -irradiated control group (Table 4).

TABLE 4. Effect of *Teucrium polium* extract on serum S100B and brain BDNF against irradiation.

Parameters	Group I	Group II	Group III	Group IV	Group V
S100B (ng/ml)	15.83±1.37	14.17±1.12	64.10±17.49 ^{a**b**}	33.10±1.99 ^{c*}	28.67±6.73 ^{c*}
BDNF (ng/mg protein)	115.73±2.01	116.50±3.39	60.03±5.38 ^{a***b***}	91.03±2.72 ^{a**b***c***}	101.87±4.86 ^{a*b*c***}

Group I (control), group II (TP), group III (γ -irradiated), group IV (TP + γ -irradiated) and group V (TP + γ -irradiated + TP). n= 6 rats per group.

a: Significance vs control; b: Significance vs TP; c: Significance vs γ -irradiated

* : P< 0.05; ** : P≤ 0.01; *** : P≤ 0.001

Histopathological examination

For supplementary description of the brain injury induced by γ -radiation and the effect of TP extract administration, histopathological examination of brain tissue was done. No histopathological alteration and normal histological structure of the brain tissue were recorded in control and TP groups (Fig. 3 a, b; Fig. 4 a, b; Fig. 5 a, b; Fig. 6 a, b and Fig. 7 a, b). In contrast, histological examination of brain tissue from γ -irradiated animals revealed nuclear pyknosis and degeneration in most of the neurons associated with congestion in the blood vessels in cerebral cortex (Fig. 3 c, d). In regard to subiculum in hippocampus, irradiated rats exhibited nuclear pyknosis and degeneration in some of the neurons (Fig. 4 c). In the same group fascia dentata and hilus in the hippocampus also displayed nuclear pyknosis and degeneration in some of the neurons (Fig. 5 c). Striatum of irradiated animal brains presented multiple focal eosinophilic plaques (Fig. 6 c). In addition, the histopathological examination of cerebellum revealed nuclear pyknosis and degeneration in some of the Purkenji cells (Fig. 7 c). TP extract administration before irradiation reverse radiation-induced injury in cerebral cortex (Fig.

3 e). However, the group of rats treated with TP extract for additional one week after γ -radiation still had nuclear pyknosis and degeneration in the neurons in cerebral cortex (Fig. 3 f). Subiculum in hippocampus showed normal histopathological findings in the two groups either administered TP extract before γ -radiation or treated with TP extract for additional one week after γ -radiation (Fig. 4 d, e). Nuclear pyknosis and degeneration were detected in some neurons in TP extract administrated group before irradiation (Fig. 5 d) and in some few neurons in group treated with TP extract for additional one week after γ -radiation (Fig. 5 e) in fascia dentata and hilus in the hippocampus. TP extract administration before radiation restored striatum portion (Fig. 6 d). On the other hand, nuclear pyknosis and degeneration in some few neurons in striatum portion were showed in rats treated with TP extract for additional one week after γ -radiation (Fig. 6 e). There were no histopathological alterations and normal histological structure of the neurons in cerebellum of the brain of either TP extract administrated rats before γ -radiation or rats treated with TP extract for additional one week after γ -radiation (Fig. 7 d, e).

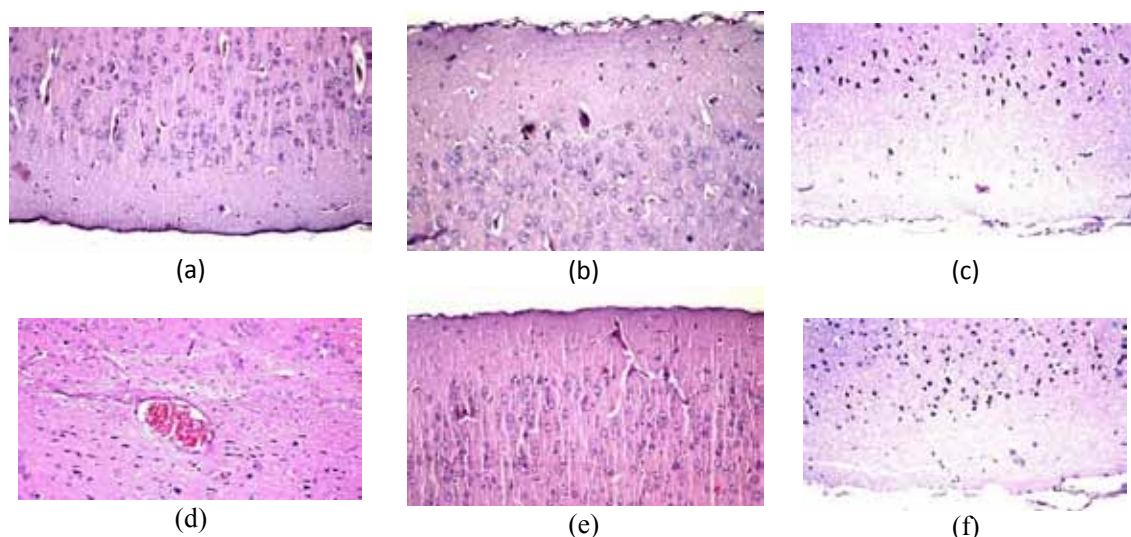


Fig. 3. Histopathological findings of cerebral cortex: (a) There were no histopathological alteration and normal histological structure of the neurons, (b) There were no histopathological alteration and normal histological structure of the neurons, (c & d) Nuclear pyknosis and degeneration were detected in most of the neurons (c) associated with congestion in the blood vessels (d), (e) There were no histopathological alteration and normal histological structure of the neurons, (f) There were nuclear pyknosis and degeneration in the neurons [where, (a) control group, (b) TP group, (c) and (d) γ -irradiated group, (e) TP + γ -irradiated group and (f) TP + γ -irradiated + TP group].

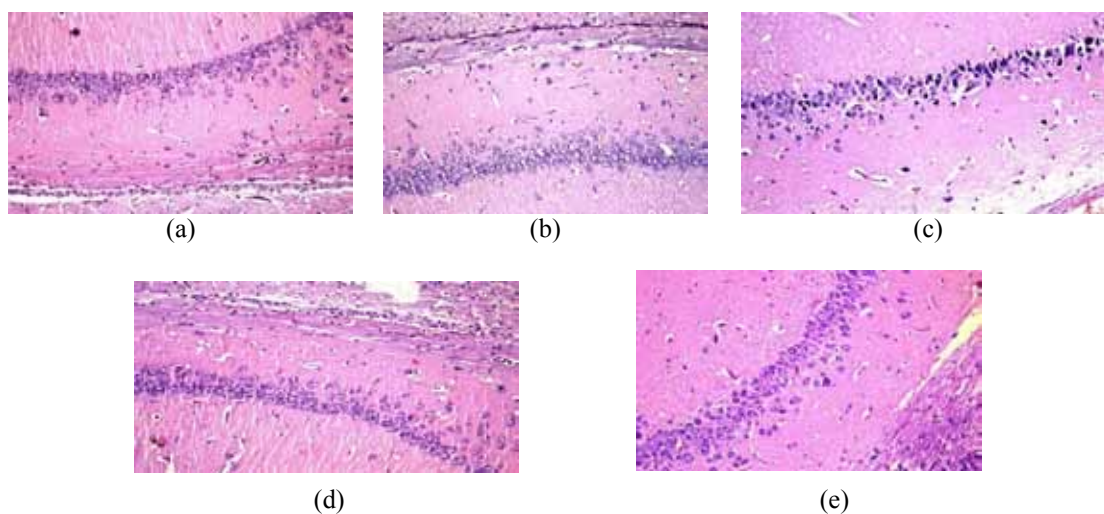


Fig. 4. Histopathological findings of subiculum in the hippocampus: (a) There were no histopathological alteration and normal histological structure of the neurons, (b) There were no histopathological alteration and normal histological structure of the neurons, (c) Nuclear pyknosis and degeneration were recorded in some of the neurons, (d) There were no histopathological alteration and normal histological structure of the neurons, (e) There were no histopathological alteration and normal histological structure of the neurons [where, (a) control group, (b) TP group, (c) γ -irradiated group, (d) TP + γ -irradiated group and (e) TP + γ -irradiated + TP group].

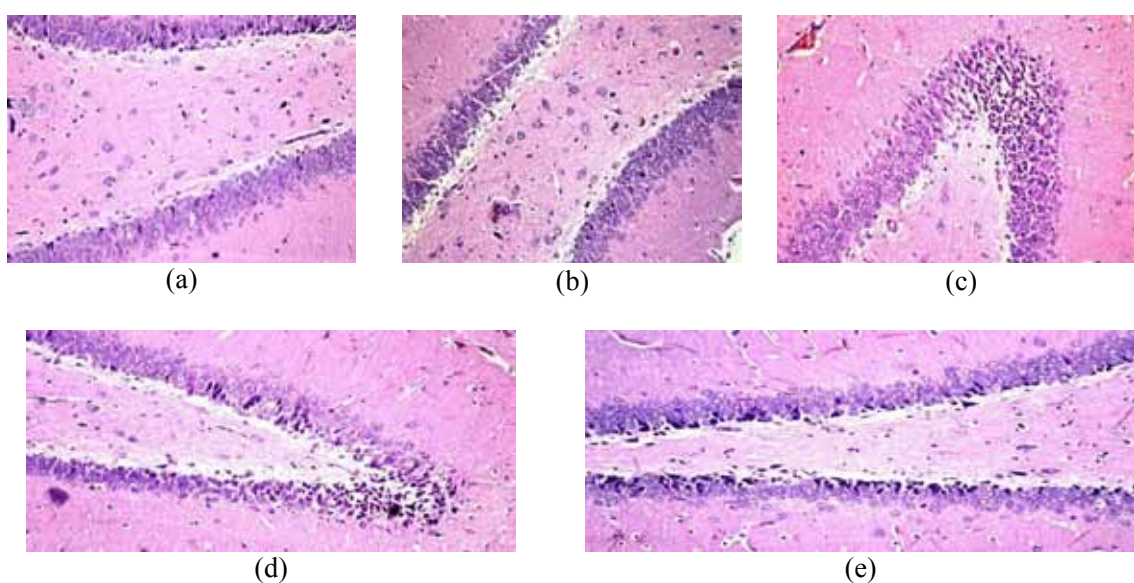


Fig. 5. Histopathological findings of fascia dentata and hilus in the hippocampus: (a) There were no histopathological alteration and normal histological structure of the neurons, (b) There were no histopathological alteration and normal histological structure of the neurons, (c) Some of the neurons showed nuclear pyknosis and degeneration, (d) Nuclear pyknosis and degeneration were detected in some neurons, (e) Some few neurons showed nuclear pyknosis and degeneration [where, (a) control group, (b) TP group, (c) γ -irradiated group, (d) TP + γ -irradiated group and (e) TP + γ -irradiated + TP group].

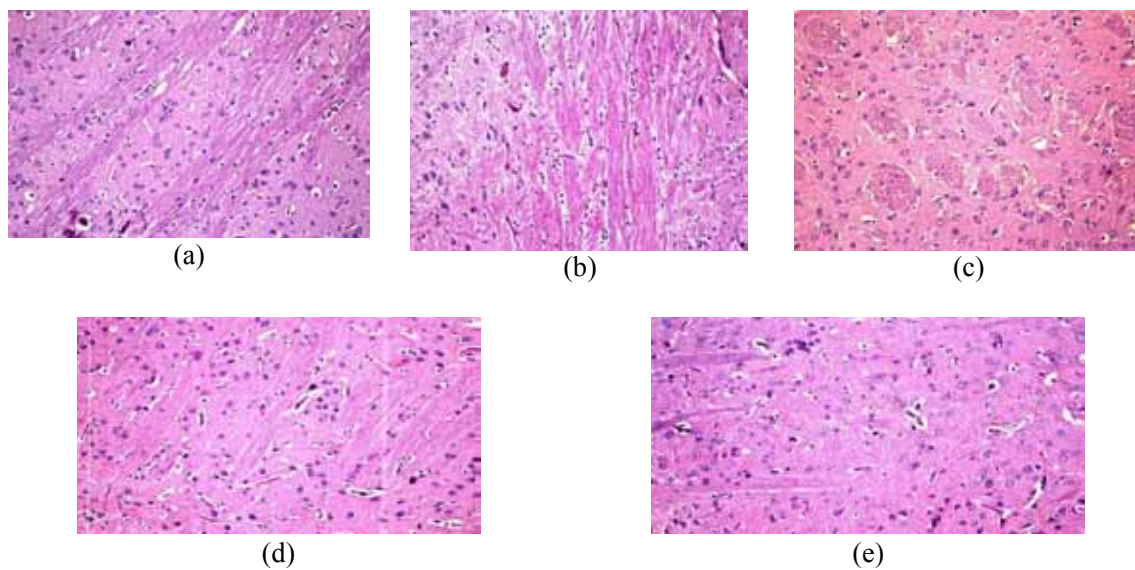


Fig. 6. Histopathological findings of striatum: (a) There were no histopathological alteration and normal histological structure of the neurons, (b) There were no histopathological alteration and normal histological structure of the neurons, (c) Multiple focal eosinophilic plaques were showed, (d) There were no histopathological alteration and normal histological structure of the neurons, (e) There were nuclear pyknosis and degeneration in some few neurons [where, (a) control group, (b) TP group, (c) γ -irradiated group, (d) TP + γ -irradiated group and (e) TP + γ -irradiated + TP group].

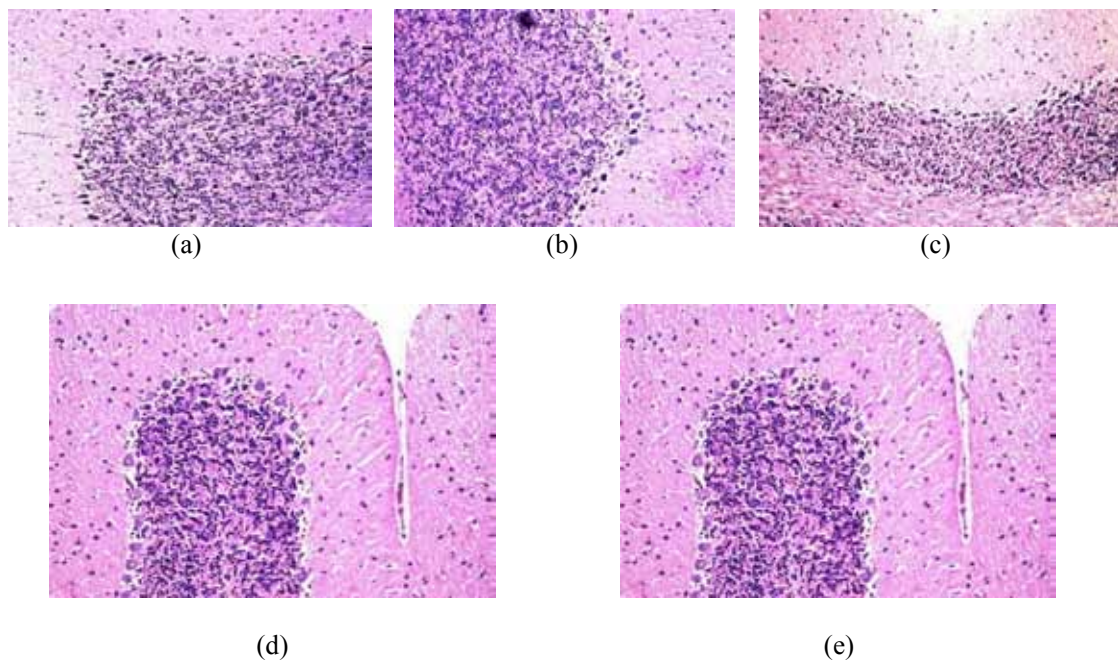


Fig. 7. Histopathological findings of cerebellum: (a) There were no histopathological alteration and normal histological structure of the neurons, (b) There were no histopathological alteration and normal histological structure of the neurons, (c) There were nuclear pyknosis and degeneration in some of the Purkenji cells, (d) There were no histopathological alteration and normal histological structure of the neurons, (e) There were no histopathological alteration and normal histological structure of the neurons [where, (a) control group, (b) TP group, (c) γ -irradiated group, (d) TP + γ -irradiated group and (e) TP + γ -irradiated + TP group].

Discussion

Radiation treatment is an important therapeutic choice in the management of various cancers. Normal healthy tissues are also commonly affected during this treatment option (Demirel et al., 2011). A comprehensive understanding of the molecular and cellular processes underlying radiobiological responses is presently developing (Calabrese, 2015) and there is still ambiguity surrounding the nature of biological responses to various doses of ionizing radiation, chiefly within the situation of the complicated and multifaceted CNS or newly born differentiating cells that integrate into the hippocampal network, manifesting as longer term functional shortfalls (Parihar & Limoli, 2013). Inflammation, protection, defense and repair of neurobiological mechanisms comprise of networks of cells and molecular mediators that react to alterations in homeostasis (Zhang et al., 2016).

A recent published data meta-analysis suggests an overall significant effect of oxidative injury in response to ionizing radiation, mostly on the brain cells. Interestingly, there was a significant heterogeneity in effect sizes among species and cell types crosswise the body (Einor et al., 2016), underlining the huge variability of responses to ionizing radiation. Ionizing radiation leads to a cascade of events that activates microglia, the brain's innate immune effect or cells, which exhibit striking morphological and functional plasticity in response to insults which start to release chemokines and pro-inflammatory cytokines which may cause oxidative stress in the brain. These immune cells infiltrated into the brain tissue and in turn produce ROS which trigger more microglia and more immune cells that can increase the oxidative stress level (Ballesteros-Zebadúa et al., 2012). Cooperatively, activated microglia can act through mechanisms such as the nuclear factor kappa B pathway to release ROS, reactive nitrogen species and pro-inflammatory cytokines such as tumor necrosis factor- α , interleukin (IL)-1 β and IL-6, or through mitogen-activated protein kinase signaling pathways that trigger NADPH to establish neuroinflammation (Han & Choi, 2012; Yuste et al., 2015; Sharma & Nehru, 2016 and Ye et al., 2016).

Certainly, neuroinflammation and oxidative stress have been involved in numerous disorders of CNS, particularly Alzheimer's disease (Jiang

et al., 2016 and Levy Nogueira et al., 2016) and Parkinson's disease (Aquilano et al., 2008; Wang et al., 2013 and Sharma & Nehru, 2016), potentiating cellular damage and pathology. Neuronal injury induced by radiation is one of the most prominent and serious side effects of cranial radiation therapy, which leads to a variety of clinical manifestations (Laack & Brown, 2004). In the same line, the existence of cognitive decline in patients after a clinical high dose of irradiation exposure has led to a research emphasis on the hippocampal microenvironment and its population of mature cells and proliferating progenitor cells in the dentate gyrus subgranular zone. This neurogenesis region is particularly ionizing radiation sensitive (Rola et al., 2008), as mitotic cells are radiosensitive at the cell cycle stages between the G2 and M phase (Marple et al., 2003 and Todorovic et al., 2015). These findings are in agreement with other reports demonstrating that radiation stimulates a premature aging process in the brain and accelerates and/or aggravates the chronic degenerative disorders feature onset for elderly (Dietrich et al., 2008 and Hua et al., 2012).

The curative manipulation of plants dates back to prehistoric eras (Mousavi et al., 2015). Preceding studies illustrated the potential role of different traditional neuroprotective herbal extracts/isolated phytochemicals in prevention of neurodegeneration. Moreover, an evidence exists for the neuromodulatory effects of phytochemicals and suggests that these phytochemicals may induce beneficial outcomes on the vascular system leading to alteration in cerebrovascular blood flow and neuronal function modulation. Therefore, natural products may harmonize very well for the neuronal injury treatment (Li et al., 2003). Since oxidative stress is correlated with dysfunction in mitochondrial and endoplasmic reticulum, which includes apoptosis and protein misfolding in neurons, phytochemical based antioxidants may have neuroregenerative and neuroprotective roles by retreating or reducing cellular damage and by slowing neuronal cell loss progression (Moosmann & Behl, 2002).

In the present study, TP extract exhibits, to certain extent, preventive as well as therapeutic role in brain damage induced by ionizing radiation. In accordance with this, it has been reported that TP protects against memory losses in scopolamine- and diabetes-induced memory impairment models (Orhan & Aslan, 2009; Hasanein &

Shahidi, 2012). The prominent activity of TP in memory augmentation could be related to its terpenic and flavonoid compounds (Bahramikia & Yazdanparast, 2012). In the present study, it was indicated that TP extract significantly reduced lipid peroxidation and increased SOD activity and TAC in the brain tissue. These obvious TP protective effects against oxidative stress observed in this study are consistent with the formerly published reports (Ljubuncic et al., 2006 and Zabihi et al., 2018). In this respect, a TP methanolic extract protected red blood cells against hydrogen peroxide-induced lipid peroxidation (Suboh et al., 2004). In another study, Kadifkova Panovska et al. (2005) proved that the extracts of TP prepared using different organic solvents (diethyl ether, ethyl acetate, and n-butanol) were effective inhibitors of β -carotene oxidation. In an earlier study, it was shown that the extracts prepared from TP suppressed lipid peroxidation *in vitro* (Qabaha, 2013). Also, previous studies indicated that TP prevents oxidative damage in the liver (Panovska et al., 2007), stomach (Mehrabani et al., 2009), and pancreas (Esmaeili et al., 2009). It was suggested that this high antioxidant activity is owing to the phenolic compounds detected in this herb such as hydroxybenzoic acid derivatives, ferulic acid, caffeic acid, and flavonoid derivatives such as luteolin and quercetin (Proestos et al., 2006). In concurrence with this, the results of the current study demonstrated the presence of many phenolic compounds in TP extract.

It has been described that TP (200 and 400mg/kg) prevented the detrimental effects of diabetes on passive avoidance memory, but 100mg/kg of TP did not have any positive effect on the memory deficits induced by diabetes (Hasanein & Shahidi, 2012). On the other hand, in the study of Mousavi et al. (2015) memory deficits were prevented by all three doses of TP (100, 200, and 400mg/kg). Although the exact mechanism of TP in preventing learning and memory deficits is still in debate, Mousavi et al. (2015) suggested that protection against oxidative stress was possibly involved in the learning and memory enhancing properties of the extract since they observed that all three doses of TP significantly reduced lipid peroxidation in the hippocampus and cerebral cortex. In the present study, the chosen dose was 200mg/kg and was effective in our model. Mousavi et al. (2015) also revealed that the high extract dose also increased total concentration of thiol in hippocampal tissues.

In the present study, higher doses were avoided as recommended by Baradaran et al. (2013) and Rasekh et al. (2005). In contrast to the beneficial effects of TP, which were mentioned and seen in the present study and in Mousavi et al. (2015) study, hepatotoxic and nephrotoxic effects of the plant have also been described (Khleifat et al., 2002 and Rafieian-Kopaei & Nasri, 2013) and should be considered before reaching a final conclusion about the efficacy of this plant. In this context, testing of liver and renal functions was performed in the present study to check on these toxic effects and the results illustrated that TP has no hepatic or renal toxicity, but on the contrary of that, it ameliorated the damaging effect of radiation occurred in liver and kidney.

It has been suggested that restoration of the neuronal and synaptic networks in the injured brain is obligatory for the brain functions recovery. It was once trusted that nerve regeneration in the mammalian CNS was irreversible, but recently it has become obvious that damaged neurons regenerate in an active process under occurrence of stimulatory substances such as nerve growth factor and BDNF (Filbin, 2000). In addition to anti-oxidation, TP could alleviate RIBI through regulating BDNF. BDNF was demonstrated to protect the cells against oxidative attack, inhibit apoptosis, and encourage the recovery function of damaged neurons (Grant et al., 2005). Decrease levels of BDNF were observed both in serum and in hippocampus after exposure of brains to ionizing radiation (Forbes et al., 2013 and Oh et al., 2013).

Severe BDNF deficiency was also detected in diabetic neuropathies in brain with depressive behavior through down regulation of levels of peroxisome proliferator-activated receptor gamma (PPAR γ) in the hippocampus (Patel, 2016). Most importantly, Sonic Hedgehog (Shh) is one of the putative signaling molecules, which is implicated in the regulation of CNS polarity and neural patterning (Machold & Fishell, 2002). Shh pathway activation is known to upregulate two important factors, BDNF and vascular endothelial growth factor. Shh signaling attenuates the oxidative stress effect on cortical neurons and has potential role in neurodegenerative disorders (Patel, 2016).

Apart from its documented neurotrophic actions and anti-apoptotic properties, anti-

oxidative effects of BDNF may also contribute considerably to its protective characteristics in various experimental models mimicking neurodegenerative situations, which have gained less attention. In cultured rat's hippocampal neurons, BDNF increases the glutathione reductase and SODs expression levels (Mattson et al., 1995). Furthermore, BDNF may exert its protective effects by regulating superoxide anion homeostasis during an experimental model of temporal lobe status epilepticus (Tsai et al., 2012). Moreover, BDNF also diminishes the level of tyrosine nitration, an indicator for oxidative protein damage (Lee et al., 2009).

Neurons appear capable of reorganizing and repairing connections after primary and secondary brain damage in TBI. The reduction of this progressive neural dysfunction could be achieved using rationally targeted therapies that target mitochondrial damage, proteolysis, and cytoskeletal alteration, or neurotrophic factors such as BDNF that can facilitate reconnection in TBI (Buki & Povlishock, 2006). BDNF is one of the most important neurotrophin factors in the brain, and the hippocampus can potentially recover from dendritic retraction throughout its expression without any neurons discernable loss (Conrad et al., 2008). For instance, induction of BDNF and activation of its intracellular receptor tropomyosin receptor kinase B (TrkB) can produce neural regeneration, reconnection, and dendritic sprouting, and can increase synaptic efficacy (Lipsky & Marini, 2007 and Ola et al., 2014). In addition, low levels of BDNF were correlated with smaller hippocampus and poorer memory, even after controlling the variation related to age (Erickson et al., 2010). Reduced production of BDNF caused by obesity and hyperglycaemia could be harmful to neuronal process and functions leading to neurodegenerative diseases development (Franco-Robles et al., 2014). It was clearly demonstrated that BDNF reduction leading to neuronal atrophy and finally death (Ghademzad et al., 2016).

In the study of Zhang et al. (2016), they found that impairments in BDNF signaling and hippocampal neurogenesis induced by whole body irradiation were well associated with impaired learning and performance of memory as determined by the Morris water maze (MWM) and passive avoidance tests.

The first human TBI study of S100B as a serum biomarker of brain injury assessment was carried out by Ingebrigtsen et al. (1995) in 1995, although increased levels of S100B in cerebrospinal fluid following various neurological disorders had been formerly described in patients by Sindic et al. (1982) in 1982. Afterward, S100B was shown to be sensitive enough to distinguish and measure different traumatic intracranial lesions, including cerebral contusions (Raabe et al., 1998), subdural hematomas and traumatic subarachnoid hemorrhages (Romner et al., 2000), as well as epidural hematomas (Unden et al., 2005). While several reviews now exist, emphasizing S100B role in both mild (Unden & Romner, 2010) and moderate-to-severe TBI (Mercier et al., 2013), new features have progressed in this field for instance the finding of S100B transportation through the newly discovered glymphatic system (Plog et al., 2015), implementation of the Scandinavian guidelines for TBI merging S100B (Unden et al., 2015) and improved kinetic modeling of S100B release from the injured brain (Ercole et al., 2016).

S100 proteins can act both intracellularly as regulators and extracellularly as signaling proteins, they can be secreted or released after cell injury and regulate cell activities as transmitters. The extracellular value of S100B is also quite fascinating because the effect and physiological functions of S100B have been shown to be concentration dependent, where lower concentrations (nanomolar levels) are beneficial and higher concentrations (micromolar levels) are correlated to harmful effects (Rothermundt et al., 2003 and Van Eldik & Wainwright, 2003). Accelerating extracellular S100B levels have been shown to result in neuronal dysfunction or cell death due to an inflammatory response that stimulates astrocytes and microglia to recruit and produce pro-inflammatory cytokines with a subsequent increase of the extracellular levels of calcium and activation of nitric oxide, with harmful effects typically as in ionizing radiation injury (Hu et al., 1997 and Koppal et al., 2001). The diverse effects of S100B have been suggested to depend on the receptor for advanced glycation end-products (RAGE), which is upregulated by S100B levels and may cause pro-inflammatory gene activation (Donato et al., 2013), although much is still unknown about how S100B exerts its biochemical properties.

The results of the present study showed that

S100B increased in RIBI and attenuated by the administration of TP and may be one of the mechanisms of its amelioration of the injury.

Histopathological findings confirmed the biochemical analysis and showed the amelioration effects of TP in different regions of brain.

Conclusion

Administration of TP extract might ameliorate RIBI by the attenuation of oxidative stress, the regulation of BDNF and suppression of S100B. The administration of TP before and after irradiation seems to be more effective than its administration only before irradiation. Therefore, it may be used to maximize the clinical use of radiotherapy in the treatment of various tumors located in or close to the CNS, as well as, in management of other brain disorders without side effects.

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الدور المحسن لمستخلص نبات الجعدة على تسمم الإشعاع الجامي في مخ الجرذان البيضاء

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يعد تلف أنسجة المخ السليمة عاملاً مهماً يحد من تطبيق العلاج الإشعاعي في المرضى الذين يعانون من أورام الجهاز العصبي. نبات الجعدة هو نبات مزهر بري، له خصائص علاجية مفيدة. الفلافونويدز التي يحتوي عليها نبات الجعدة لها العديد من الأنشطة البيولوجية القيمة. تهدف هذه الدراسة إلى دراسة الدور المحسن لمستخلص نبات الجعدة على تسمم الإشعاع الجامي في مخ الجرذان البيضاء.

تم استخدام ثلاثون من ذكور الجرذان البيضاء البالغة. تم إجراء التشيع المؤين من خلال تعريض جسم الجرذان كله لـ 8 جراي. تم إعطاء نبات الجعدة بواسطة أنبوبة معدية بجرعة 200 ملجم/كجم من وزن الجسم. تم تقسيم الحيوانات إلى خمس مجموعات: المجموعة الأولى (الضابطة)، المجموعة الثانية (تم إعطائها نبات الجعدة)، المجموعة الثالثة (المشعة)، المجموعة الرابعة (تم إعطائها نبات الجعدة ثم تشيعها) والمجموعة الخامسة (تم إعطائها نبات الجعدة ثم تشيعها ثم إعطائها نبات الجعدة). تم دراسة تأثير مستخلص نبات الجعدة بعد أسبوع واحد من التشيع عن طريق قياس وظائف الكبد والكلية في مصل الدم، و عوامل الإجهاد التأكسدي في أنسجة المخ. علاوة على ذلك، تم قياس S100B في مصل الدم و BDNF في أنسجة المخ، بالإضافة إلى الفحص الهستوباثولوجي.

و كانت النتائج أن نبات الجعدة عند جرعة 200 ملجم/كجم من وزن الجسم لم يكن له آثار سامة على الكبد أو الكلى. مستخلص نبات الجعدة حسن بشكل ملحوظ تلف المخ الناجم عن الإشعاع الجامي عن طريق تحسين الإجهاد التأكسدي الناجم عن الإشعاع. تم تأكيد دور المستخلص عن طريق تحسين مستويات S100B في مصل الدم و BDNF في المخ وكذلك تحسين التغيرات الهستوباثولوجية الناجمة عن الإشعاع. إعطاء نبات الجعدة قبل وبعد التشيع كان أكثر فعالية من إعطائه فقط قبل التشيع.

كشفت هذه النتائج أن إعطاء مستخلص نبات الجعدة قد يحسن ضرر المخ الناجم عن الإشعاع الجامي عن طريق تخفيف الإجهاد التأكسدي وتنظيم BDNF وتثبيت S100B.