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Is genistein neuroprotective in traumatic brain injury?

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HIGHLIGHTS

• Genistein reduced the development of brain edema in a model of brain injury.

• Increase of ICP was suppressed by administration of genistein in a model of brain injury.

· Disturbance of neurobehavioral function improved following genistein administration in a model of brain injury.

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ABSTRACT

The concerns about negative consequences of estrogen therapy have led to introduce other strategies to obtain estrogen's benefits in the brain. The present study tests the hypothesis that a major isoflavone of soy; genistein with estrogen-like activity can be neuroprotective in traumatic brain injury (TBI). The male Wistar rats were randomly divided to four groups: sham, TBI, vehicle and genistein. The TBI was induced by Marmarou method. The brain edema and the disruption of blood–brain-barrier (BBB) were evaluated 48 h post-TBI. Genistein (15 mg/kg) or dimethyl sulfoxide (DMSO) was injected i.p., twice after TBI. The intracranial pressure (ICP), the motor performance, and the beam-walk task (WB) were determined before trauma, on trauma day (D0), and first (D1) and second (D2) days post-TBI. Genistein inhibited a development of brain edema and a BBB permeability in TBI animals. An increase of ICP induced by TBI was suppressed by genistein on D1 and D2 times. Genistein improved a motor disorder induced by TBI, on D1 and D2 times. Also an increase of traversal time in WB task was suppressed by genistein in TBI animals, on D1 and D2 times. The results of this study demonstrated that genistein can be neuroprotective in TBI. Genistein inhibited the disruption of BBB, the brain edema and the increase of ICP, and the disturbance of neurobehavioral performance in TBI.

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1. Introduction

Traumatic brain injury (TBI) is the third most common cause of death in the world [1]. TBI accounts for 52% of the trauma-induced deaths and is therefore a leading cause of death among trauma patients [2]. On average, 39% of patients with a severe TBI die from their injury [3]. In Iran, road-traffic crashes, which are a main source of brain injuries, cause disability to more than 300,000 persons each year [4].

Traumatic brain injury (TBI) immediately causes both the primary damage and the secondary damage. The primary damage is induced due to a direct mechanical damage of the brain, resulting in the immediate and irreversible neuronal death [5]. The secondary damage is evolved by a large number of cellular, molecular, and biochemical cascades, resulting in the avoidable neuronal death for hours after TBI.

* Corresponding author. *E-mail address:* mkhaksari@kmu.ac.ir (M. Khaksari). One such cascade is an inflammatory response in an injured brain [6]. Applying treatment strategies before an occurrence of permanent neuronal damage in TBI is very important.

Previous studies have demonstrated an anti-inflammatory and neuroprotective effect of estrogen in TBI [7–9]. Soy phytoestrogens have been introduced as an alternative to hormone replacement therapy, due to the deleterious effects of estrogen [10]. Soy isoflavones, such as genistein and daidzein have phytoestrogen properties [11,12] and can act as an estrogen receptor modulator or act through a non-estrogen receptor pathway [13].

Both ovariectomized female and male rats with a soy diet have shown a significant reduction in infarct volume, neurological defect, and apoptosis following cerebral ischemia [14,15]. Genistein has similar protective effects in both sexes in cerebral ischemia [16,17]. Genistein is also found in lupine (*Lupinus*), alfalfa (*Medicago sativa*), chickpea, and some legumes (Leguminosae) [18,19]. Genistein has anti-inflammatory and antioxidant properties [20]. Our recent study showed that a soy diet resulted in preventing of the blood–brain barrier (BBB) permeability and the increase of intracranial pressure (ICP), and the improvement of neurologic performance, in TBI animals [21].

Genistein at low dose reduces the neuronal apoptosis and damage induced by thapsigargin [25], oxidative stress [22], and glutamate excitotoxicity [23]. But genistein at high dose may induce a neuronal apoptosis [24]. In addition, chronic administration of genistein reduces a brain lesion after experimental stroke and delays the onset of disability and mortality in a model of amyotrophic lateral sclerosis [19]. However chronic treatment of genistein at high doses may induce cytotoxicity and apoptosis in a rat brain [25].

The neuroprotective effect of estrogen in TBI animals has been suggested in our previous studies [7–9], and our recent study demonstrated that a soy diet can be neuropreventive in TBI [21], and also the estrogenlike activity of soy isoflavones has been indicated [11,12]. Therefore in this research, the neuroprotective effect of genistein, as an isoflavone of soy, in diffuse and severe experimental TBI was assessed. With this aim, we tested the effect of genistein on the brain edema by measuring brain water content, the BBB permeability by measuring brain leakage of Evans blue, the ICP by digital recording, and the neurobehavioral outcome by evaluating motor and vestibulomotor function.

2. Materials and methods

2.1. Animals and animal groups

The study was executed in accordance with a protocol approved by an ethical committee (No. EC/KNRC/92–23) in Kerman University of Medical Sciences, in accordance with internationally approved guidelines for the animal use and care, as indicated in the European community guidelines (EU Directive of 2010; 010/63/EU) or US guidelines (NIH publication #85-23, revised in 1985).

The male Wistar rats, were purchased just after weaning (3-weeks after birth), and received a soy-free diet [21] to avoid interference due to soy. Animals were housed in a light (on 7:00 a.m. to 7:00 p.m.) and a temperature (21 ± 1 °C) controlled environment with a food and water available ad libitum and allowed to grow for 15–17 additional weeks.

Rats were then randomly divided to sham (control), TBI, vehicle of genistein (Veh) and genistein (Gen) groups (n = 12 in each group). Six rats in each group were used for determining BBB permeability and other six rats for recording ICP and evaluating neurobehavioral outcome.

Genistein (Sigma, USA) (15 mg/kg) [26] was dissolved in dimethyl sulfoxide (DMSO) and injected through intraperitoneal, once 30 min and again 24 h [26] after TBI in the genistein group. DMSO was injected than genistein in the vehicle group.

2.2. Surgery (induction of TBI)

All animals were intubated before surgery. In all groups except sham group, diffuse TBI was induced by Marmarou method using a TBI induction device (made by Dept. of Physiology and Pharmacology, Kerman University of Medical Sciences) in animals anesthetized with ketamine (60 mg/kg) and xylazine (10 mg/kg). TBI was induced as we have previously described in detail [7]. Briefly, a severe TBI was induced by falling a weight 450 g, from a 2 m height onto a metal disk (stainless steel, 10 mm in diameter, 3 mm thick) attached to the animal's skull. After an induction of TBI, the rats were connected to a respiratory pump (TSA animal respiratory compact, Germany). In the sham group, all stages of TBI induction were performed except falling weight on the skull.

2.3. Determination of brain edema

The brain edema was determined by measuring brain water content as we have previously described in detail [7]. Briefly, the brain of anesthetized animals was removed 48 h after TBI, and the brain sample was weighed (wet weight). The brain sample was placed in an incubator (Memmert, Germany) at 60 °C for 72 h, and then was reweighed (dry weight). The percentage of water in each sample was then calculated using a formula; $(100 \times [(wet weight - dry weight) / wet weight])$.

2.4. Determination of BBB permeability

Blood-brain-barrier (BBB) disruption was determined by measuring a brain leakage of Evans blue (EB) dye injected and using a spectrophotometer as we have previously described in detail [27]. Briefly, 48 h after trauma, 20 mg/kg Evans blue dye 2% (1 ml/kg) was injected via a jugular vein, under an anesthesia to 50 mg/kg thiopental. One hour after injection, the thorax was opened and descending aorta was clipped. Then, an isotonic saline solution was infused into the left ventricle for 20 min, simultaneous with bilaterally cutting jugular vein to remove intravascular Evans blue dye. Next, the brain was removed and weighed, followed by homogenization. Then, it was placed in a solution containing 6 ml of sodium sulfate 1% + 14 ml acetone on a shaker for 24 h. In the next step, 1 ml of the supernatant liquid and 1 ml of trichloroacetic acid were mixed and then were centrifuged at 2000 cycle/min for 10 min. Then, an Evans blue absorbance of the supernatant liquid was measured at 620 nm by a spectrophotometer (Pharmacia Biotech, Germany). The amount of dye leakage was quantified as micrograms per gram brain tissue.

2.5. Measurement of ICP

The animal head was fixed in a stereotaxic instrument, as the head was located at a midsagittal plane, and the anterior–posterior point was placed at about a midpoint between the occipital crest and the lambda suture. A 20-gauge needle connected to a pressure transducer through a polyethylene short tube, was connected to a recording system (AD Instruments, Australia) and inserted into the cisterna magna. There was an initial increase and then a sudden decrease of resistance during insertion of a needle, due to the needle insertion to dura mater and cistern magna respectively [28]. The ICP of all groups was recorded before trauma, on trauma day (D0), and first (D1) and second (D2) days post-TBI.

2.6. Neurobehavioral performance

2.6.1. Evaluation of motor function

As we described previously, the motor function was evaluated according to a motor score of veterinary coma scale (VCS) and expressed as a range from 1 to 8 [21,29] (Table 1). A higher score indicates a better function, and a lower one indicates a worse function. The assessment of motor function was performed by an experienced person blinded to the experimental condition, before trauma, and on D0, D1 and D2 times of post-TBI.

Та	ble	21

Motor score scale of neurologic outcome.

	Variable	Score
Motor function	Normal movement Mildly drowsy with spontaneous, purposeful movements Lethargic, unable to stand, but maintains sternal recumbency Lethargic, withdraws to pinch, and lifts head with attention to visual stimuli; no sternal recumbency Withdraws or pedals to pinch Spontaneous pedaling	8 7 6 5 4 3
	Extensor posturing (spontaneous or to stimuli) Flaccid to stimuli	2 1

2.6.2. Evaluation of vestibulomotor function

The task of beam-walk (BW) was used for evaluating the finer components of vestibulomotor function and coordination. The modified BW task was devised by Feeney and colleagues [30], consisting of training the animals using a negative-reinforcement paradigm to escape a bright light and a white noise by traversing an elevated narrow beam $(2.5 \times 100 \text{ cm})$ and entering a dark goal box placed on the opposite end (using an instrument made by the Dept. of Physiology, Kerman University of Medical Sciences). A task of BW was assessed by recording the time traversed to a beam as well as the distance traversed. The scoring criterion for distance traversed is based on a rating scale from 0 to 5, where 0 indicates inability to move from a starting point, 1–4 correspond to the traveled segments of 20, 40, 60, or 80 cm from a starting point, respectively, and 5 indicates a traveled entire length of the beam (100 cm). Animals were trained prior to TBI or sham surgery (i.e., traverse entire length of the beam under 5 s). "A distance traveled in the task of BW was when rats fell from the beam on a foam or traveled the entire length of the beam and arrived to the dark box without the time limitation."

The task of BW was evaluated before trauma, and on D0, D1 and D2 times of post-TBI consisting of three trials for any time evaluated. Data for each time were a mean of three trials. The results were obtained using a camera and a software (video tracking, Borjesanat Company, Tehran, Iran) by an experienced person blinded for the experimental condition.

2.7. Statistical analysis

Data were expressed as mean \pm SEM. The normality of data was checked by using the Shapiro–Wilk's W test. Because of interaction between the groups and the times in the evaluation of ICP, and vestibulomotor and motor function, the comparison of groups in each time was analyzed using one-way analysis of variance (ANOVA), the same as for an evaluation of the permeability of BBB and the brain edema. Tukey's test was used for post hoc analysis. The significant difference was accepted at p < 0.05.

3. Results

3.1. Brain edema: brain water content

The brain edema of different groups is shown in Fig. 1. TBI resulted in an increase in brain water content ($79.3\% \pm 0.34$) in comparison with



Fig. 1. The effect of genistein on the brain edema determined by the brain water content, 48 h post-severe traumatic brain injury (TBI) (n = 6 in each group). Data are presented as mean \pm SEM. ***P < 0.001: TBI and Veh groups vs. sham group. ###P < 0.001: Gen group vs. TBI group. ^{\$\$\$\$}P < 0.001: Gen group vs. Veh group. Gen: genistein; Veh: vehicle.

the sham group (77.1% \pm 0.14), 48 h post-TBI (p < 0.001). Brain edema was not significantly different between TBI group and vehicle group (79.51% \pm 0.28). TBI-induced brain edema was reversed by administration of genistein (77.12% \pm 0.24) (p < 0.001). The brain water content of genistein group was not significantly different with that of sham group.

3.2. BBB permeability: EB dye content of brain

BBB permeability of different groups is shown in Fig. 2. TBI increased the brain content of EB dye ($25.1 \pm 3.6 \mu g/g$ tissue) in comparison with sham group ($3.7 \pm 0.61 \mu g/g$ tissue), 48 h post-TBI (p < 0.001). The BBB disruption was not significantly different between TBI group and vehicle group ($23.7 \pm 2.6 \mu g/g$ tissue). The administration of genistein reversed ($9.6 \pm 1.8 \mu g/g$ tissue) TBI-induced BBB disruption (p < 0.01). The brain content of EB dye in genistein group was not significantly different with that of sham group.

3.3. ICP

The ICP of different groups, before trauma, and on D0, D1 and D2 times after TBI, is shown in Fig. 3. Before trauma, ICP was not statistically different among the groups. TBI resulted in an increase of ICP on D0 (7.16 \pm 0.54 mm Hg), D1 (9.66 \pm 0.49 mm Hg) and D2 (9.33 \pm 0.61 mm Hg) times after TBI in comparison with sham group (3.16 \pm 0.16, 2.83 \pm 0.16, 3.5 \pm 0.22 mm Hg; respectively) (p < 0.001). The ICP of TBI group was not significantly different with that of vehicle group in any time. Although, genistein did not eliminate TBI-induced increase of ICP on D0 and D1 (9 \pm 0.85, 5.33 \pm 0.61 mm Hg; respectively) times but, eliminated that on D2 time (4.5 \pm 0.5 mm Hg) (p < 0.001). TBI-caused increase of ICP in the genistein group on D1 time was less than that on Do time in comparison with the sham group. The ICP was not significantly different between the sham group and genistein group on D2 time.

3.4. Neurobehavioral performance

3.4.1. Motor function of VCS

The motor performance in different groups, before trauma, and on D0, D1 and D2 times, is shown in Fig. 4. Before trauma, the score was not statistically different among the groups. TBI decreased motor score on D0, D1 and D2 times (2 ± 0.36 , 6.33 ± 0.33 , 6.50 ± 0.42 ;



Fig. 2. The effect of genistein on the permeability of blood–brain barrier determined by the brain leakage of Evans blue dye, 48 h post-severe traumatic brain injury (TBI) (n = 6 in each group). Data are presented as mean \pm SEM. ***P < 0.001: TBI and Veh groups vs. sham group. *#P < 0.01: Gen group vs. TBI group. ^SP < 0.05: Gen group vs. Veh group. Gen: genistein; Veh: vehicle.



Fig. 3. The effect of genistein on the intracranial pressure, before trauma, on trauma day (D0), and first (D1) and second (D2) days post-severe traumatic brain injury (TBI) (n = 6 in each group). Data are presented as mean \pm SEM. ***P < 0.001: TBI and vehicle groups vs. sham group on D0, D1 and D2 time; genistein group vs. sham group on D0 time. ##*P < 0.001: genistein group vs. TBI group on D1 and D2 times. SSP < 0.001: genistein group vs. sham group on D1 and D2 times. *P < 0.05: genistein group vs. sham group on D1 and D2 times. *P < 0.05: genistein group vs. sham group on D1 time. Gen: genistein; Veh: vehicle.

respectively) (p < 0.001, p < 0.01, p < 0.01; respectively) in comparison with sham group (8 ± 0, 8 ± 0, 8 ± 0; respectively). The motor score of TBI group was not significantly different with that of vehicle group in any time. Although, genistein could not recover motor function on D0 time (1.67 ± 0.33) (p < 0.001) but, recovered motor function on D1 and D2 times (7.83 ± 0.16, 7.83 ± 0.16; respectively) in comparison with TBI and vehicle groups (p < 0.01). The motor score was not significantly different between sham group and genistein group on D1 and D2 times.

3.4.2. Vestibulomotor function: BW (traversal time)

The traversal time of BW task of different groups, before trauma, and on D0, D1 and D2 times, is shown in Fig. 5. Before trauma, the time was



Fig. 4. The effect of genistein on the motor function evaluated by motor score of veterinary coma scale, before trauma, on trauma day (D0), and first (D1) and second (D2) days post-severe traumatic brain injury (TBI) (n = 6 in each group). Data are presented as mean \pm SEM.***P < 0.001: TBI, vehicle and genistein groups vs. sham group on D0 time. **P < 0.01: TBI and vehicle groups vs. sham group on D1 and D2 times. **P < 0.01: genistein group vs. TBI group on D1 and D2 times. **P < 0.01: genistein group vs. TBI group on D1 and D2 times. **P < 0.01: genistein group vs. vehicle group on D1 and D2 times. **P < 0.01: genistein group vs. vehicle group on D1 and D2 times. **P < 0.01: genistein group vs. vehicle group on D1 and D2 times. **P < 0.01: genistein group vs. vehicle group on D1 and D2 times. **P < 0.01: genistein group vs. vehicle group on D1 and D2 times. **P < 0.01: genistein group vs. vehicle group on D1 and D2 times. **P < 0.01: genistein group vs. vehicle group on D1 and D2 times. **P < 0.01: genistein group vs. vehicle group on D1 and D2 times. **P < 0.01: genistein group vs. vehicle group on D1 and D2 times. **P < 0.01: genistein group vs. vehicle group on D1 and D2 times. **P < 0.01: genistein group vs. vehicle group on D1 and D2 times. **P < 0.01: genistein group vs. vehicle group on D1 and D2 times. **P < 0.01: genistein group vs. *



Fig. 5. The effect of genistein (Gen) on the time (s) to traverse an elevated narrow beam, before trauma, on trauma day (D0), and first (D1) and second (D2) days post-severe traumatic brain injury (TBI) (n = 6 in each group). Data are presented as mean \pm SEM. ***P < 0.001: TBI group vs. sham group on D1 time. **P < 0.01: vehicle group vs. sham group on D0, D1 and D2 times; TBI group vs. sham group on D2 time. #*P < 0.01: genistein group vs. TBI group vs. sham group on D0 time. **P < 0.01: genistein group vs. Sham group on D0 time. **P < 0.01: genistein group vs. Sham group on D0 time. **P < 0.01: genistein group vs. Sham group on D0 time. **P < 0.01: genistein group vs. Sham group on D0 time. **P < 0.05: genistein group vs. vehicle group on D1 time. Sen: genistein; Veh: vehicle.

not statistically different among the groups as, all rats reached the goal box in approximately 5 s. TBI increased traversal time in all times after trauma (11.55 \pm 1.35, 11.38 \pm 0.81, 10.27 \pm 0.68 s; respectively) in comparison with sham group (6.27 \pm 0.62, 5.66 \pm 0.32, 4.88 \pm 0.18 s; respectively) (p < 0.05, p < 0.001, p < 0.01; respectively). There was no significant difference between TBI group and vehicle group in any time. Although, genistein could not reduce traversal time on D0 time (10.88 \pm 0.95 s) but, reduced traversal time on D1 and D2 times (6.55 \pm 0.48, 7.27 \pm 0.39 s; respectively). The motor score of genistein group was not significantly different with that of sham group on D1 and D2 times.

3.4.3. Vestibulomotor function: BW (distance traveled)

The score of distance traveled in BW task of different groups, before trauma, and on D0, D1 and D2 times, is shown in Fig. 6. Before trauma,



Fig. 6. The effect of genistein on the distance traveled score along an elevated narrow beam, before trauma, on trauma day (D0), and first (D1) and second (D2) days post-severe traumatic brain injury (TBI) (n = 6 in each group). Data are presented as mean \pm SEM. *P < 0.05: Veh and TBI groups vs. sham group on D0 time. Gen: genistein; Veh: vehicle.

a significant difference of the score was not observed among the groups as, all rats traversed an entire length of beam for a maximum score of 5. A reduction of the score was showed in TBI and vehicle groups in comparison with sham group, only on D0 time. There was no significant difference between sham group and genistein group on D0 time. There was no significant difference among groups on D1 and D2 times.

4. Discussion

In the present study, for the first time, the neuroprotective effect of genistein, as a soy isoflavone was evaluated in an animal model of diffuse TBI. In this survey, the administration of genistein reduced brain edema, BBB permeability and ICP, and improved the disturbance of neurobehavioral performance in TBI animals.

Although it is known that 17β-estradiol has the neuroprotective effect in brain injuries [7,8,31], but the usage of estrogen increases a risk of breast cancer. Phytoestrogens have been introduced as an alternative to hormonal replacement therapy, because phytoestrogens have the estrogenic-like effects without a risk of breast cancer. Phyto-estrogens are natural molecules derived from plants that can act as an estrogen receptor agonist [15] or an antagonist [32] in the nervous system. Phytoestrogens are found in some products, such as soy [33] which contains genistein and daidzein among other isoflavones [34]. The structure of soy isoflavones (genistein, daidzein, glycitein), is similar to endogenous estrogens. It is known that isoflavones act as an estrogen receptor modulator or act through a non-estrogen receptor pathway [13,35].

A damage in the central nervous system results in neuroinflammatory responses including, the formation of brain edema, the disruption of BBB [36], the elevation of ICP [8] and the acute increase of pro-inflammatory cytokines [9].

Studies have shown that the brain water content and the content of Evans blue dye in the brain following TBI are significantly high in comparison with control group, 48 h post-trauma [37,38], in accordance with our present findings. Administration of genistein resulted in inhibiting the brain edema development and the BBB disruption. The effect of genistein on the disruption of BBB in this study is in agreement with the report of protection of the BBB after cerebral ischemia in soyfed animals [21,39]. Also, an antiedema effect of isoflavones in cerebral ischemia/reperfusion [26,40] agrees with an antiedema effect of genistein in this study. Ma et al. showed that a protective effect of soy on the permeability of BBB is mediated by a reduction of endothelial hypoxia-inducible factor 1-alpha (HIF1- α) and vascular endothelial growth factor (VEGF) [39]. It is known that an elevation of free radicals [41] and a development of inflammatory mechanisms [41,42] have a role in the BBB disruption and the brain edema development. The scavenging [26] and antiinflammatory effects of genistein [43] have been demonstrated. Therefore it is proposed that the inhibitory effects of genistein in the development of brain edema and the disruption of BBB probably are mediated via antioxidant and antiinflammatory effects as well as vascular protective alterations.

An increase of ICP for 48 h post-TBI has been reported in other studies [27,44] that is a confirmation for a time course of the increase of ICP in the present study. We showed that an increase of ICP was initiated in the first hour of trauma, and persisted for 48 h. Our present research indicated that genistein could not eliminate an increase of ICP on the trauma day (first hour). Although genistein weakened an increase of ICP on the first day of post-trauma, but inhibited that on the second day of post-trauma, as the level of ICP was not different between genistein group and sham group in this time. The reasons of an increase of ICP in TBI could be hypoxia [45], a reduction of cerebral blood flow [46], cerebral edema [47], ischemic injury, a hyperperfusion in the early stages after TBI [48], and changes in the expression of aquaporin-4 [49]. Our recent study reported a reduction of ICP along a reduction of vasogenic edema following TBI by dietary soy as is seen

in present research [21]. Therefore it is supposed that genistein may decrease ICP following a decrease in brain edema. Estrogen prevents increasing ICP post-TBI by its antioxidant effect [50]. Since, soy isoflavones have an antioxidant effect [51], this effect is also deemed as another action mechanism of genistein in suppressing an increase of ICP. Since genistein did not castrate an increase of ICP on the day of trauma but castrated that on days post-trauma, it seems that action mechanism of genistein on ICP may be mediated by a decrease in brain edema than an antioxidant effect, although it is needed to research for confirmation.

The neurobehavioral performance was evaluated by assessing a motor function using VCS test and a vestibulomotor function using a BW test. In our research, a deterioration of motor function was observed on trauma day, and persisted along 48 h post-trauma, in agreement with the results of other studies [37,44]. In evaluating a vestibulomotor function, we observed that the traversal time increased on trauma day, and continued until 48 h post-trauma, whereas the score of distance reduced only on the trauma day, which is inconsistent with the result of research performed in rats with diffuse TBI [52]. We observed that genistein could not improve motor and vestibulomotor function on the day of trauma, but improved that on the days post-trauma, in accordance with an effect of genistein on ICP. The improvement of neurologic function by genistein has been reported in a model of experimental ischemia [53]. The dietary soy improved the neurobehavioral function in male rats following TBI [21]. The brain edema is a prime cause of a deterioration of neurobehavioral function following TBI [54]. The improvement of neurologic function can occur following a decrease in ICP in a brain injury [27,29]. It is assumed that the decrease in ICP may be an action mechanism of soy in the improvement of neurobehavioral performance. The improvement in neurobehavioral function correlates with the significant cortical and hippocampal tissue preservation [43]. A reduction of brain lesion by genistein administration has been indicated in a model of experimental stroke [19], but this must be assessed in our future research. The beneficial effect of genistein on neurobehavioral performance has not been reported in some studies [24,55] and in the rat brain in vitro [25]. The methodological, strain, sex, dose of consumption of genistein, and brain injury differences can be causes of the discrepancies.

Studies suggest that the neuroprotective effects of genistein are at least in part, mediated by estrogen receptors [56,57]. Although, soy isoflavones may activate both estrogen receptor α and estrogen receptor β , they activate more estrogen receptor β [58], which is highly expressed in neurons and glial cells in the hippocampal formation [59]. Estrogen receptor-independent neuroprotection of genistein can exist via altering the activity of enzymes, such as protein tyrosine kinases leading to affecting the activity of neurotransmitter receptors [60], DNA topoisomerase I and II, and ribosomal S6 kinase [61,62], via a direct interaction with neurotransmitter receptors [63], and finally via the antioxidant properties [22].

The probable cellular and molecular mechanisms of genistein as a neuroprotective factor can be as follows: the reduction of neuronal apoptosis [24], oxidative stress [22], glutamate excitotoxicity [23], the activation of PI3K [60] of [15], and β -amyloid protein. The molecular and additional mechanisms underlying the beneficial effect of genistein in TBI are a subject of future research. It must also be determined whether the effects of genistein are performed via estrogen receptors and also which of the estrogen receptor is involved.

The results of this study suggest that at least in part a preventive effect of dietary soy is mediated via an isoflavone ingredient; i.e. genistein that needs further research and confirmation. The comparison effect of dietary soy to genistein, also a combination of dietary soy and genistein is subject of future research.

We indicated that administration of genistein reduces brain edema, BBB permeability, and ICP and also improves motor and vestibulomotor deficits in TBI. These findings support that genistein can be considered as a neuroprotective agent and a possible alternative to estrogen.

Authors' contributions

ZS: directed the project, TBI induction in animal, carried out the data analysis and interpretations, and prepared the manuscript. MK: supervised and directed the project, and carried out the interpretations. IM and JE carried out the interpretations. NS: carried out ICP recording and neurobehavioral evaluation.

Conflict of interest

The authors declare no conflicts of interest.

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