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Neuroprotective effect of chlorogenic acid on Traumatic Brain Injury in rats

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ABSTRACT :

Background: Traumatic brain injury is a leading cause of death and disability worldwide. To prevent harm from spreading beyond the initial injury, novel and effective therapies are required. The purpose of this study was to see if chlorogenic acid has a neuroprotective impact on traumatic brain injury in rats.

Methods: The rats were randomly separated into six groups: the control group (received vehicle saline 2ml I.P.), the TBI group (no treatment), CGA 50 mg/kg, and CGA 100 mg/kg. and standard drug Donepezil 5mg/kg. TBI was induced by Feeney's weight-drop technique. Motor coordination, spatial learning, and memory were evaluated using the Rota rod test and the Morris water maze test, respectively. Biochemical markers such as lipid peroxidation (LPO), reduced Glutathione (GSH) and TBARS were estimated after 14 days. Histopathological study of rat brain was also done after specific time.

Results: Pretreatment with CGA (50mg/kg and 100mg/kg) significantly reduced MDA levels while increasing SOD activity and GSH levels 14 days after TBI. These findings suggested that CGA may reduce excessive ROS generation and boost anti-oxidant abilities to prevent the progression of secondary brain damage caused by TBI in rats.

Conclusion: Our study found that pretreatment with CGA can help reduce TBI-induced brain damage in rats, including neurological deficiency, cerebral oedema, hippocampal neurone loss, and delayed neuronal death. CGA has also been shown to lessen the level of oxidative stress. We hypothesise that CGA's antioxidative and antiapoptotic characteristics may provide neuroprotection against TBI in rats. Moreover, CGA could significantly improve the impairment of learning and cognitive function after TBI in terms of long-term therapeutic effect. This study confirmed that CGA could be a potential therapeutic strategy for the treatment of traumatic brain injury.

Introduction

Traumatic brain injury (TBI) remains a serious health concern in the United States and around the world, often with long-term implications that reduce quality of life and cause chronic cognitive impairment (Dewan et al., 2018). Every year, an estimated 1.4 million persons in the United States suffer from TBI, and more than 5 million people live with TBI-related disability, costing $56 billion (Thurman et al., 1999). TBI is an epigenetic risk factor for Alzheimer's and Parkinson's disease. As a result, TBI is a serious health risk as well as a huge economic burden. TBI occurs when an external mechanical force causes brain damage, which can result in temporary or permanent deficits in cognitive, physical, and psychosocial functioning. TBI is a leading cause of disability and mortality worldwide, particularly in young adults and the elderly. It can be mild, moderate, or severe, with mild accounting for approximately 80% of cases (Temkin et al., 1990). However, even mild TBI (mTBI) can cause devastating effects (McMahon et al., 2014; Ganti et al., 2019). It is incorrect to think that when physical force is given to the head, the repercussions are less severe. Some patients experience significant and long-term symptoms after mTBI, including headaches, dizziness, and memory problems (Prince and Bruhns, 2017; Hoffer and Balaban, 2019; Lu et al., 2020). Many TBI patients suffer from cognitive loss, behavioral issues, headaches, and vision impairments, which limit their ability to work, socialize, and fully engage in daily life.

Survivors of TBI are at increased risk for the development of severe, long-term psychiatric disorders. Prevalence of any psychiatric illness in the first year after the injury has been observed at a rate of 49% following moderate to severe TBI and 34% following mild TBI, compared to 18% in those without TBI. Early diagnosis, appropriate treatment, and rehabilitation are crucial for minimizing damage and improving outcomes for those affected by TBI (Fann et al., 2004).

Pharmacologic therapies play important roles in mild to severe TBI. There are several pharmacologic therapies recommended by guidelines, which have proven efficacies and well-documented safety profiles for use in acute and post-acute TBI patients. Post acute treatment of TBI mainly consist of antidepressants, Anitpsychotics, CNS stimulants, Rivastigmine, Donepezil, BZDs, Amantadine, L-Dopa, Bromocriptine, Prazosin, B-Blockers etc . An

ongoing concern is a high incidence and high mortality of TBI that needs to be solved urgently during the perinatal period. Therapeutic options to manage the neurodegeneration, enhanced oxidative stress and inflammation in TBI are limited.

Chlorogenic acid (CGA, 5-O-caffeoylquinic acid) is a significant polyphenol found in Coffea canephora, Coffea arabica L., and Mate (Ilex paraguariensis A. StHil. Clinical investigations found that drinking coffee with high amounts of CGA reduced mental tiredness and headaches, improved mood-related processes (Cropley et al. 2012), and improved brain ageing (Johnston et al. 2003). Evidence suggests that CGA has neuroprotective (Ahn et al. 2011; Heitman and Ingram 2016; Nabavi et al. 2017), neurotrophic (Ito et al. 2008), anti-oxidative (Sato et al. 2011), and anti-inflammatory (Hwang et al. 2014) properties. Previous in-vivo investigations using CGA demonstrated that it boosted dopaminergic neuron survival (Shen et al. 2012), reduced anxiety, improved motor function (Bouayed et al. 2007), and improved spatial learning and memory (Han et al. 2010). CGA co-therapy with tissue plasminogen activator (tPA) extended the latter's therapeutic window (Lapchak 2007). It decreased oxidative stress and neuroinflammation in MPTP-treated mice (Singh et al., 2018). CGA has been shown to protect rats from focal cerebral ischemia-reperfusion injury via regulating nerve growth factor, inflammatory factor, and hypoxia factor levels (Miao et al. 2017). Furthermore, our earlier research have indicated that CGA successfully reaches the brain in higher concentrations when supplied via the intranasal method as opposed to the intravenous approach. (Kumar et al. 2018). There are currently no approved medicines for controlling oxidative stress, inflammation, or having a neuroprotective effect in TBI. Despite numerous research confirming the neuroprotective effect of CGA, the exact mechanisms of CGA's neuroprotective potential in TBI have yet to be investigated. As a result, the current study was carried out to examine the neuroprotective impact of CGA in traumatic brain damage. The current study also used Donepezil (an acetylcholinesterase inhibitor), which was originally developed as an Alzheimer's disease treatment. Donepezil dramatically up-regulates nicotinic acetylcholine receptor (nAChR) and activates protective cascades that confer neuroprotection and prevent apoptotic neuronal death in vitro (Takada et al., 2003) and in vivo (Fujiki et al., 2005; Meunier et al., 2006). In the current investigation, we investigated the effects of donepezil on neurodegeneration and behavioral deficits caused by concussive head injury as a reference substance in the rat brain following CGA therapy.

Materials & Methods

Animals and agents

Wistar rats weighing 250-300 g were employed in the current study.

Animal studies were conducted after getting approval from the Institutional Animal Ethical Committee (IAEC) (IAEC No. 1407/PO/ReBiBt/S/11/CPCSEA).The study was performed as per regulations of CPCSEA. The rats were fed a standard laboratory pellet diet and given free access to water. The animals were acclimatized to laboratory settings for one hour each day for seven days before the trials began. All of the tests were carried out between 9 a.m. and 12 p.m., in accordance with the Committee for the Purpose of Control and Supervision on Animal tests Guidelines. Groups of five animals were used in all sets of experiments. The animals were fasted overnight before use. The doses of test drugs were administered orally with the help of an oral cannula fitted on a tuberculin syringe.

Induction Of Experimental Traumatic Brain Injury

Wistar albino rats weighing 200-250g were exposed to TBI via the weight drop method. The weight loss gadget is a novel adaptation of Feeney's weight loss paradigm. It comprises of a metal flatbed to hold the rats, a head pin to immobilize the rat's head, and a seat belt to secure the rat's body during the treatment. At the front end of the flat bed, there is a projectile support holder in the shape of a tube that is attached to a transparent glass tube 25 cm long. The transparent glass tube was outfitted with a long ruler measuring centimeters to drop the weight onto the rat's head from a particular height. The glass tube can be spun 360 degrees to change the position of the weight put on the rat's head based on the study requirements.

Experimental designs

Thirty male Wistar rats (aged 2 months, weighing 200–250 g) were housed in cages in the Laboratory. The environment was maintained with a light-dark cycle of 12:12 h, a room temperature of 26–31 °C, and a humidity level of 70%–90%. Rats were placed in separate cages between groups. Rat food pellets and tap water were provided ad libitum. Rats were randomly separated into six groups: control (received vehicle saline 2ml I.P.), TBI (no treatment), CGA 50 mg/kg, and CGA 100 mg/kg and standard drug Donepezil 5mg/kg I.P. Intraperitoneal CGA (Sigma-Aldrich, USA, Cat. #3878-1G) injection was administered daily for 14 days. Intraperitoneal saline injection for group C was administered in equivalent amounts. The Morris water maze test was conducted before termination to assess the improvement of memory function and the rotarod test was performed to evaluate impairment of motor coordination and balance. Biochemical markers such as lipid peroxidation (LPO), reduced Glutathione (GSH) and TBARS were estimated after 14 days. Histopathological study of rat brain was also done after specific time. The statistical analysis consisted of a one-way analysis of variance, followed by Dunnett's post-test. P-values ≤ 0.05 were considered statistically significant.

Evaluation of Neurologic Outcome

All sensorimotor and cognitive tests were performed by a researcher who was blinded to the treatments.

Morris Water Maze (MWM) Test

The Morris water maze test was used to evaluate learning and memory (Morris, 1984). It is based on the natural ability of an animal to escape on a hidden platform placed in the circular pool. The Morris water maze apparatus consisted of a circular pool (150 cm diameter, 45 cm height) filled with water (28 + 2°C) to a depth of 30 cm. The water was made opaque by adding a white-coloured non-toxic dye. Two equal threads were fixed at the rim of pool at right angles to each other, dividing the tank into four equal quadrants (q1, q2, q3 and q4).

A white platform (10 cm2) was placed in the target quadrant (q4) 1 cm below the water's surface and remained in place during the session. All the animals were subjected to acquisition trials from day 10-15, each animal being subjected to four consecutive trials every day with a gap of 5 minutes.

During the trials, the animal was placed in a quadrant facing the pool's wall and given 120 seconds to identify the submerged platform. If the animal found the platform within 120 seconds, it was permitted to stay there for 20 seconds; otherwise, it was directed to find the platform after 120 seconds and allowed to stay for 20 seconds. During the retrieval trial, which allowed the animal to search the pool for 120 seconds, the time spent in the target quadrant (TSTQ) was used as a parameter to evaluate retrieval. The time it took the animal to find the hidden platform was referred to as escape latency time (ELT), which was an indicator of acquisition/learning.

4.5 Behavioral testing

After 1 week, all rats were subjected to the following behavioral tests.

4.5.1. Rotarod test

The rotarod test was performed to evaluate impairment of motor coordination and balance. In brief induction of TBI, the rats were first acclimated to the rod (3 cm diameter). On the day of the test, the rats were placed on the rod and the rotation speed was started at 4 rpm and then gradually increased to 40 rpm within 5 min. Each rat was given 3 trials, and the length of time each rat stayed on the rod was recorded. In this experiment, animals were trained/acclimated to the Rotarod for 3 consecutive days (9 trials total) prior to induction of TBI. Post-injury, animals were re-tested on days 1, 3, and 5 to assess sensorimotor performance and balance improvements over time (Hamm et al., 1994).

Statistical analysis

The data was analyzed using one-way analysis of variance (ANOVA) and Dunnett's post hoc test, with a probability value of P<0.05 indicating statistical significance.

Results

Chlorogenic Acid and Donepezil Rescued TBI-Induced Spatial Learning and Memory Impairment

The Morris water maze (MWM) test was used to assess learning and memory in the current investigation. A significant decrease in escape latency time (ELT) of control group animals between days 1 and 4 reflects normal memory acquisition, whereas an increase in time spent in target quadrant (TSTQ) Q4 to find the missing platform during the retrieval trial on day 5 of MWM demonstrates normal memory retrieval.

The control group mice showed a significant decrease in ELT on day 15 compared to day 14, indicating that the animals have normal learning abilities. Furthermore, the control group animals spent considerably more TSTQ searching for the missing platform than the other quadrants (Q1, Q2, and Q3) during the retrieval trial of the MWM test (Day 15). This demonstrated normal memory retrieval. TBI-induced mice had significantly reduced learning ability and memory capacity, as shown by a substantial difference in day 14 ELT and no increase in TSTQ as compared to control group animals. Animals treated with donepezil (5 mg/kg, p.o.) had a significantly lower day 14 ELT and higher TSTQ than the TBI group. This demonstrates that therapy with donepezil increased learning ability and memory capacity by shielding the animals from TBI-induced impairment. Chrologenic acid (50mg/kg and 100mg/kg, p.o.) treated rats showed a dose-dependent decrease in day 14 ELT and increase in TSTQ compared to TBI-induced and donepezil-treated animals. The results are shown in Table 1 and Figure 1.

Table 1: Effect of Chlorogenic Acid and Donepezil on TBI-Induced Spatial Learning and Memory impairment using Morris Water Maze test

Treatment group*	**Dose (mg/kg)**	**Mean[n] escape latency time (sec) ± S.D.**	
		Day 14	**Day 15**
Sham group	Vehicle	30.01 ± 5.02	29.14 ± 1.02*a
TBI	3	75.25 ± 2.58	65.33 ± 2.19a
Donepezil hydrochloride	5	35.18 ± 4.14	30.14 ± 2.30*
CGA	50	44.15 ± 2.48	42.14 ± 1.87*a
CGA	100	33.20 ± 3.78	31.10 ± 2.10*

n=5; The data is expressed as Mean ± S.D.; **P*<0.05 *vs* TBI; a*P*<0.05 *vs* Chlorogenic acid; one way ANOVA followed by Student-Newman-Keul's test.

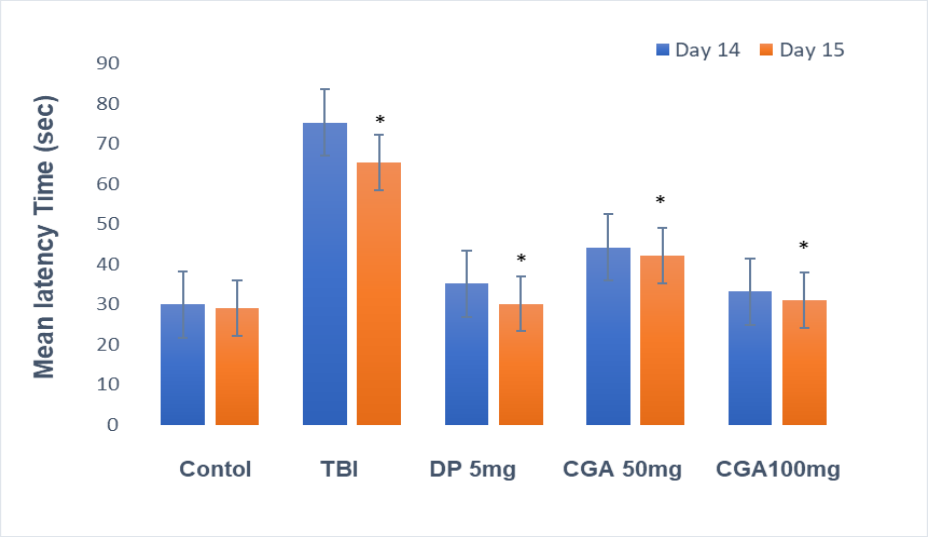

Figure 1: The effect of chlorogenic acid on escape latency time in TBI rats was investigated using the Morris Water Maze test.

CGA reversed the motor deficits in TBI induced rats

After 14 days, the TBI-induced rats exhibited more severe symptoms than the control group. The rota rod test results showed that TBI-induced rats spent less time on the rod, and CGA treatment helped to increase this reduction.

Histopathological studies

The stained histological sections of the hippocampal and cortical areas of the brain (figure 5) of the control group exhibited well-defined neuronal region in the hippocampus and normal neurons in cortex. TBI-induced rats reflected extreme neurodegeneration in cortex and hippocampus regions of rat brain. As observed in Figure 4, groups treated with donepezil and Chlorogenic acid protected neurons and demonstrated moderate congestion relative to the disease group.

Hippocampus

The control group's hippocampus area displayed normal cornus Ammonis and dentate gyrus organization (Fig. 2a). The TBI-induced group demonstrated abundant degenerating cells in the CA3 and CA4 regions, as well as the presence of microglia cells (Fig 2b). The histological examination also revealed that the rat hippocampus treated with Donepezil showed fewer degenerating cells within the CA3 and CA4 regions of the hippocampus when compared to TBI (Fig. 2c). Chlorogenic acid (50 and 100 mg) exerted a protective action, revealing that the rat hippocampus appeared normal as that of Rivastigmine in maintaining the hippocampus structure (Fig. 2d and 2e).

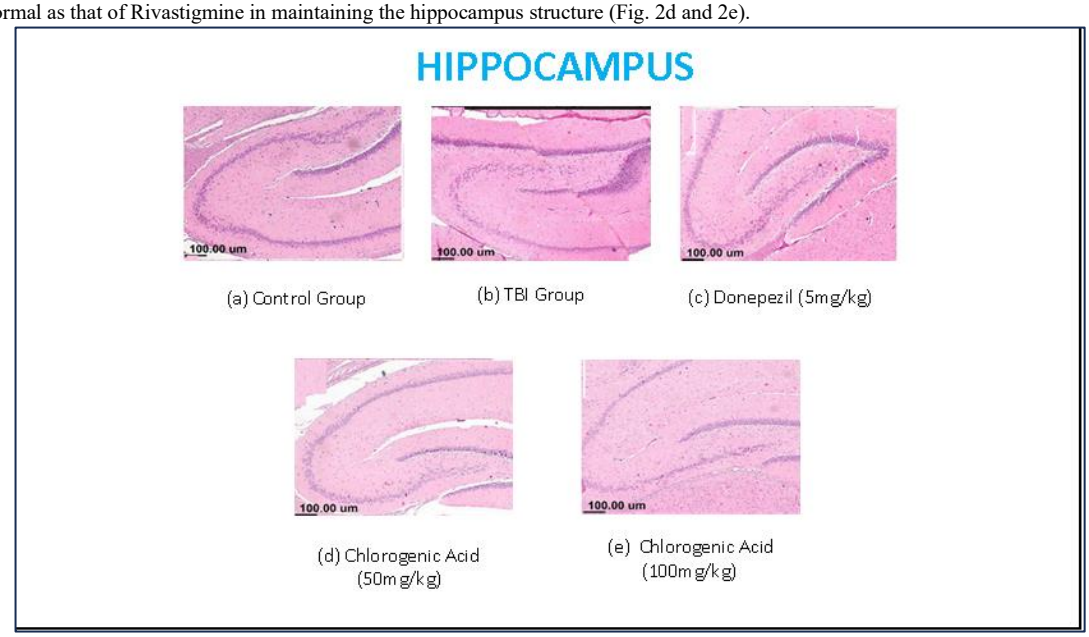

Figure 2: Hippocampus region, (H&E) stained

(a) The control group has a normal hippocampal structure, (b) while the TBI group has lower cell density and degenerating dark neurons in the hippocampus area. C) Standard Donepezil 5mg medication showed fewer degenerating cells than the TBI group. (d) The chlorogenic acid

50mg and 100mg groups appeared to have normal hippocampal structure when compared to the TBI group.

Cerebral cortex

The control group has a normal histological architecture of the cerebral cortex, as illustrated in Fig. 3a. On the other hand, the TBI group had a wide spectrum of histological abnormalities, as seen in Fig. 3b. Perivascular hemorrhages surrounding blood vessels (particularly those in the deep cortex) and thickened their walls (cerebral angiopathy). Perivascular lymphocytic aggregations were commonly observed in the cerebral cortex. Other portions revealed focused, tiny astrocyte aggregations. As seen in Fig. 3c, Donepezil treatment reduced neurotoxicity. The cerebral cortex appeared normal, with the exception of a few patients who had minute hemorrhages in the deep cortex.

In comparison to the usual Donepezil group, rats in groups given 50 mg and 100 mg of chlorogenic acid showed normal histological architecture of various brain regions. (Fig. 3d and 3 e).

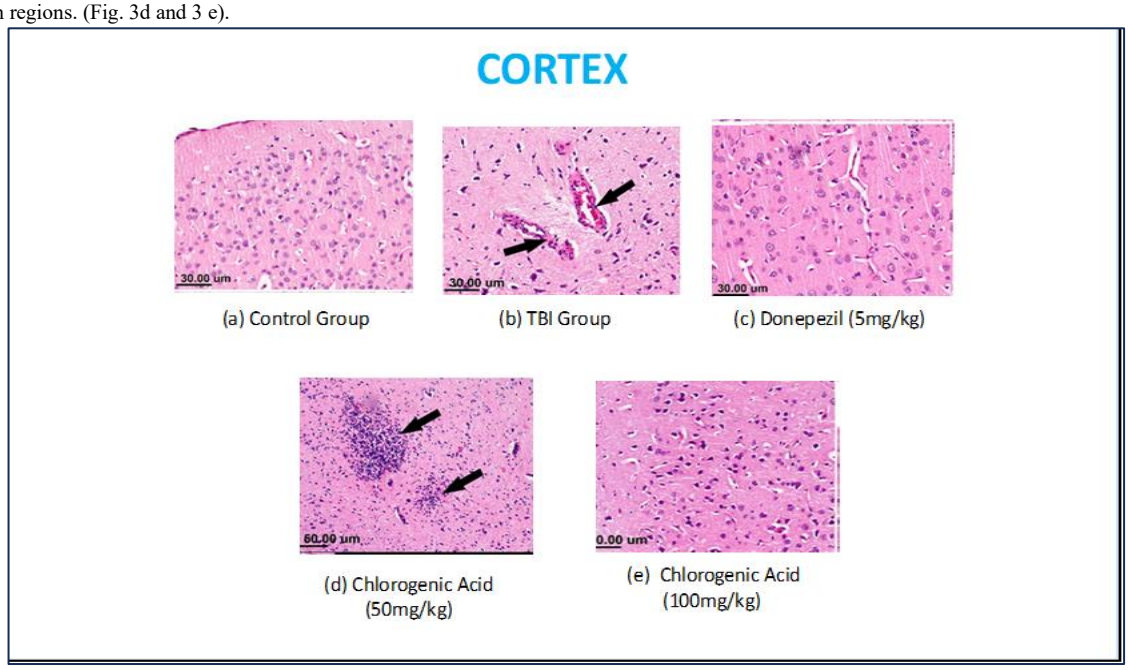

Figure 3: Cortex region, (H&E) stained

(a) Control group, normal anatomy of the cortex (b) TBI Group - perivascular bleeding (arrow), deep cortex with thicker vessel walls, astrocyte infiltrations, and neuronal degeneration. © Standard Donepezil 5mg medication results in normal brain and endothelial capillary growth. (d) The chlorogenic acid 50mg and 100mg groups appeared to have normal cortical structure when compared to the TBI group.

Biochemical estimations

Effects of treatment on MDA level

Chlorogenic acid and Donepezil significantly decreased MDA level in the brain tissue of TBI rats. Figure 5 shows that TBI rats had significantly higher MDA levels in their brains compared to the control group (# $p < 0.01$). Table 3 shows that MDA activities significantly decreased after 24th hours of Donepezil 5mg and high dose of Chlorogenic acid (50 mg/kg and 100 mg/kg) compared with the vehicle treatment ($p < 0.01$) after14 days.

Effects of treatment on SOD level

Chlorogenic acid and donepezil dramatically raised the SOD levels in the brain tissue of TBI rats. TBI rats showed significantly lower SOD activity compared to the control group ($p < 0.01$) (Table 3). Donepezil (5mg/kg) and Cholrogenic acid (50mg and 100mg) significantly increased SOD levels after 24 hours as compared to the vehicle treatment ($p < 0.01$).

Effects of treatment on GSH level

Chlorogenic acid and donepezil significantly enhanced GSH levels in the brain tissue of TBI rats. TBI in rats resulted in lower GSH levels. Table 2 demonstrates that Chlorogenic Acid (50mg and 100mg/kg) and Donepezil (5 mg/kg) significantly raised GSH levels in TBI rats' brain tissues compared to the vehicle group ($p<0.05$).

Table 2. The effects of Chlorogenic Acid and Donepezil therapy on brain antioxidant enzyme activity in TBI-induced rats after 14 days.

Groups	Dose (mg/kg)	TBARS	GSH	SOD
Control	Vehicle	3.70 ± 0.64*[a]	65.20 ± 1.28*[a]	1.75 ± 0.07*[a]
TBI		7.20 ± 1.21[a]	25.50 ± 0.52[a]	0.50 ± 0.04[a]
Donepezil	5	5.75 ± 1.12*	38.68 ± 1.75*	1.55 ± 0.08*
Chlorogenic Acid	50	4.90 ± 1.12*[a]	45.35 ± 2.50*[a]	1.60 ± 0.03*[a]
Chlorogenic Acid	100	4.40 ± 0.54*	54.40 ± 1.70*	1.68 ± 0.03*

n=5; The data is expressed as Mean ± S.D.;
*P<0.05 *vs* control; aP<0.05 *vs* Donepezil;
one way ANOVA followed by Student-Newman-Keul's test.

Discussion

In the present study, our findings suggest that CGA can be used as a potent neuroprotective agent in TBI. The current study dealt to investigate the effect of chlorogenic acid in TBI induced rats by evaluating its effect on the short term, spatial memory, and motor coordination and balance followed by estimation of antioxidants such as glutathione (reduced), lipid peroxidation and SOD biomarkers using rat brain homogenate. Rat brain histopathology tests were carried out on the cerebral cortex and hippocampal regions. Comparative histological investigations of the hippocampus and cortex areas of mice revealed that CGA (50 mg/kg and 100 mg/kg) had a superior safety profile than other groups and was comparable to the standard group.

Traumatic brain injury (TBI) kills and disables millions of individuals worldwide each year (Finkelstein et al., 2006). Although TBI is a serious health problem worldwide, the ability to improve patient outcomes remains limited due to a lack of effective medicines (Roberts et al., 2012). TBI pathophysiology is generally understood to be caused by both primary brain injuries such as cerebral laceration, contusion, and diffuse axonal damage (DAI), as well as secondary brain injuries such as cerebral ischemia, inflammation, energy failure, oxidative stress, and neuronal death. As a result, preventing subsequent brain injury is the most difficult aspect of TBI treatment. Excessive neuronal calcium entry is well established to contribute significantly to subsequent brain damage after TBI. Furthermore, prior research indicated that reactive oxygen species (ROS) play an important role in the pathophysiology of secondary injury following TBI (Hall and Braughler, 1989; Ikeda and Long, 1990).

TBI-induced excessive ROS production can cause cellular damage and death by oxidizing lipids, proteins, and DNA (Awasthi et al., 1997). As a result, the hunt for a viable treatment based on antioxidant techniques is critical.

Recently, a broader range of natural antioxidants capable of scavenging free radicals and preventing oxidative damage to cells have been investigated for their potential neuroprotective properties (Slemmer et al. 2008). Chlorogenic acid, a natural antioxidant, is an important polyphenolic component of Coffea canephora, Coffea arabica L., and Mate (Ilex paraguariensis A. StHil.). The current study found that among the doses studied, chlorogenic acid at a dose of 100mg/kg had the greatest effect on reducing cortical damage and improving neurological and cognitive function in TBI.

In this work, we employed lipid peroxidation, SOD activity, and GSH levels to assess CGA's influence on oxidative stress. Overproduction of ROS can cause serious harm to cellular activities, such as peroxidizing membrane lipids, oxidizing proteins, and damaging mitochondrial DNA and cytoplasmic RNA (Butterfieldetal.,1997; Mecoccietal.,1994; Nunomuraetal.,1999). MDA, a byproduct of lipid peroxidation caused by the breakdown of polyunsaturated fatty acids, is widely recognized as an indicator of the level of lipid peroxidation. SOD detoxifies O2 into H2O2, which is then scavenged by peroxisomal catalase. GSH, the most important intracellular non-protein thiol, is an essential ROS scavenger. In this investigation, MDA levels were dramatically elevated, while SOD activity and GSH levels were significantly lowered in the TBI group.

Pretreatment with CGA (50mg/kg and 100mg/kg) significantly reduced the elevated level of MDA while increasing SOD activity and GSH levels 14 days after TBI. These findings suggested that CGA may reduce excessive ROS generation and boost anti-oxidant abilities to prevent the progression of secondary brain damage caused by TBI in rats.

In conclusion, our findings suggest that pretreatment with CGA can help reduce TBI-induced brain injury in rats, including neurological impairment, cerebral edema, hippocampal neuron loss, and delayed neuronal death. Meanwhile, we discovered that CGA can lessen the level of oxidative stress. Based on these findings, we propose that CGA's neuroprotective effects against TBI in rats may be achieved through antioxidative and antiapoptotic mechanisms. However, more research is needed to determine the time frame of efficacy for CGA's neuroprotective effect after TBI, as well as the underlying neuroprotective molecular pathways.

Moreover, CGA could significantly improve the impairment of learning and cognitive function after TBI in terms of long-term therapeutic effect. This study confirmed that CGA could be a potential treatment strategy for the treatment of traumatic brain injury.

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