Chronic Pre-Treatment of Kombucha Tea Protects Brain Injury induced by Ischemia/Reperfusion in Global Brain Ischemia

Mehran Mesgari-Abbasi  
Tabriz University of Medical Sciences Drug Applied Research Center

Fatemeh Eskandari Vaezi  
Tabriz University of Medical Sciences Drug Applied Research Center

Fezzeh Hossienzadeh (✉ Hossenzadeh_fez@yahoo.com)  
Tabriz University of Medical Sciences Drug Applied Research Center  
https://orcid.org/0000-0001-6443-4260

Research Article

**Keywords:** Antioxidant, Cerebral ischemia, Kombucha tea, Brain edema, Neurobehavioral

**Posted Date:** March 29th, 2022

**DOI:** https://doi.org/10.21203/rs.3.rs-1482869/v1

**License:** ☝ Ⓥ This work is licensed under a Creative Commons Attribution 4.0 International License.  
Read Full License
Abstract

Cerebral ischemia is an important factor in developing neurological damage without appropriate medical treatment. Kombucha tea (KT) is a fermented beverage that produces many compounds with antioxidant power with potential health effects. This study was performed to evaluate the effect of pre-treatment of kombucha tea on brain edema, neurobehavioral, and oxidative stress in the global brain ischemia model. Adult male Wistar rats were divided into four groups include; the sham, the ischemic, and the KT-treated-ischemic groups. KT was administrated two weeks before inducing transient global cerebral ischemia at two different dosages (one and two ml/kg/day, gavage). The global brain ischemia was induced by blocking the common carotid arteries for one hour, followed by 24 hours of reperfusion. At the end of reperfusion, neurobehavioral studies were done. Brain edema was determined by dry-wet methods. Malondialdehyde (MDA), total antioxidant capacity (TAC), glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT) levels were measured in the serum and the brain tissue. Our results showed that pre-treatment of KT significantly reduced brain edema and MDA levels in the blood and the brain. It significantly increased the total TAC and GPx levels in the blood and the brain in ischemic rats treated with KT. The CAT activity significantly increased in the blood, but it didn't significantly change the brain. KT of pre-treatment significantly ameliorated neurobehavioral defects in the ischemic rats. Our data demonstrated that pre-treatment of KT maybe protect the brain against ischemic-reperfusion injury through a decrease in the production of lipid peroxidation and an increase in the capacity of antioxidants defense.

Introduction

Cerebral ischemia is one of the most important reasons for disability and death worldwide (Lee et al. 2018). Ischemia leads to beginning a complex set of molecular events such as calcium overload, excitatory toxicity, oxidative stress in neurons and endothelial cells of cerebral arteries, which causes neuronal damage, the permeability of the blood-brain barrier, formation of cerebral edema (Xing et al. 2012; Shah and Abbruscato 2014), and also causes motor and functional defects (Rao and Balachandran 2002). Several pieces of evidence suggest the role of oxidative stress in the pathophysiology of brain ischemia/reperfusion injury (Allen and Bayraktutan 2009; Shirley 2014). In the ischemic phase, reactive oxygen species (ROS) are produced due to reducing conditions. Also, in the reperfusion phase of cerebral ischemia, neuronal injury aggravates due to increased free radical production (Saraf et al. 2010). Free-radical and reactive oxygen species (ROS) oxidize several essential cellular components, mainly membrane phospholipids, proteins, mitochondrial, and nucleotides leading to impair cell function (Pizzino et al. 2017; Chen et al. 2011). Also, mitochondrial damage may play a role in delayed cell death during cerebral ischemia and reperfusion (Fiskum 2000). In addition, increasing ROS production during ischemia/reperfusion causes discharge or damage antioxidant defense, free radicals accumulation, and the overproduction of malondialdehyde (MDA) (Rao and Balachandran 2002; Iadecola and Alexander 2001; Gawel et al. 2004). Antioxidant defenses include enzymatic parts such as glutathione peroxidase and non-enzymatic compounds such as phenolics that attenuate ROS formation.
and remove free radicals (Lobo et al. 2010). The effects of free radical damage are controlled by the balance between their production and removal rate through these defense systems in the physiological condition (Halliwell 1994). It has been well documented that antioxidants reduce the permeability of the blood-brain barrier, cerebral edema formation, and neuronal damage in ischemic and hypoxic conditions (Shirley et al. 2014; Margail et al. 2005; Haghnejad et al. 2017). Therefore, one of the necessities of research in neurology and pharmacology is using supplements and food products as pre-treatment, such as antioxidant agents that reduce the complications of a stroke.

Kombucha tea (KT) is a sour-tasting fermented beverage made from black or green tea fermentation with sugar and a symbiotic colony of bacteria and yeast (Dutta and Gachhu 2007; Malbasa et al. 2011). The fermentation process produces many compounds with antioxidant power, such as organic acids, vitamins such as E and C, polyphenols, and active enzymes with potential health effects (Kapp and Sumner 2019; Jakubczyk et al. 2020; Jayabalan et al. 2007; Vijayaghavan et al. 2000; Dufresne and Farnsworth 2000). Polyphenols modify various enzymes activity and cell receptors for defense against ROS-induced oxidative stress (Tsao 2010). Numerous studies have shown that the beneficial effects of kombucha tea, such as anti-inflammation, anti-cancer, immunity improvement, and neuroprotection, may be related to its antioxidant properties (Dufresne and Farnsworth 2000; Tsao 2010; Pauline et al. 2001; Kabiri and Setorki 2016). In addition, previous studies have suggested evidence for the antioxidant properties of kombucha tea in nephrotoxicity and hepatotoxicity, diabetic, gastric ulceration models, and the experimental model of MS (Gharib 2009; Banerjee et al. 2010; Bhattacharya et al. 2011; Bhattacharya et al. 2013; Haghmorad et al. 2021). There is also evidence that KT has neuroprotective and anti-edematous properties in the focal brain ischemia model (Kabiri and Setorki 2016). Also, it has been shown that the administration of KT decreases lipid oxidation and DNA fragmentation (Malbasa et al. 2011).

Considering the facts mentioned above about kombucha tea, in the current study, we studied the effects of pre-treatment of KT on brain edema, oxidative stress, motor and functional defects in the global cerebral ischemic model.

**Materials And Methods**

**Animals**

Adult male Wistar rats (250-300 g) were obtained from Tabriz University of Medical Sciences animal laboratory and maintained in standard cages in a controlled environment with a temperature of 22-24° C, a humidity of 40–60%, a 12-hour cycle of dark and light, and free access to water and food.

**Experimental Groups**

The fifty-six rats were randomly divided into four experimental groups (n=14), including:

**Sham:** The group orally received distilled water as a vehicle (one ml/kg/ day, gavage) for 14 days
Ischemic: The group orally received the vehicle (one ml/kg/day, gavage) for 14 days before global brain ischemia induction.

KT-1: The group orally received Kombucha tea (one ml/kg/day, gavage) for 14 days before global brain ischemia induction

KT-2: The group orally received Kombucha tea (2 ml/kg/day, gavage) for 14 days before global brain ischemia induction.

This study used 28 rats to assess brain edema, and 28 rats were used for neurobehavioral and biochemical studies.

**Preparation of Kombucha tea (KT) fermented**

The green tea (12 g/L; Lahyjan tea, Iran) and one hundred grams of sugar were added to one liter of distilled water and were boiled for 15 minutes in a sterile glass jar. Then the solution allowed cooling to room temperature for 60 min. The fermentation was carried out by incubating with freshly grown kombucha mat in the sterile glass jars at (28 ± 1) °C for 8-10 days. Then, the medium was centrifuged at 3000 rpm for 30 minutes and then stored at -20°C for future use (Sai Ram et al. 2000).

**Global cerebral ischemia induction**

All the experimental groups, on day-15, were occluded both common carotid arteries by a micro clamp as previously explained. In brief, rats were anesthetized by chloral hydrate (400 mg/kg, i.p.), and the right and left common carotid arteries (CCA) were exposed and occluded by micro clamps for 60 minutes. Then the micro clamps were slowly removed after an hour of ischemia to perfuse for 24 hours. The sham group was subjected to a similar surgical procedure except for occlusion of common carotid arteries. After the surgical, rats were kept in individual cages for 24 hours with food and water access *ad libitum* (Saraf et al. 2010).

**Cerebral edema evaluation**

All rats (n=28) were sacrificed using overdose sodium pentobarbital (100 mg/kg, i.p) after twenty-four hours of global ischemia. The brain was carefully separated, and also the olfactory bulb and pons were removed, then the brain was weighted to estimate its wet weight (WW). Next, it dried at 110 °C for 24 hours to assign its dry weight (DW). After that, brain water content (BWC) was evaluated by the following formula: \((WW−DW)/WW×100\); (Hossienzadeh et al. 2013).

**Preparation of Tissue Homogenates**

At the end of the experiments, the animals (n=28) were deeply anesthetized with overdose sodium pentobarbital (100 mg/kg, i.p). The blood samples were collected from the heart and used to determine SOD, MDA, GPx, and CAT activities in the blood, and then brains were immediately removed and kept at -70°C. Subsequently, tissue samples were homogenized in a 1.15% cold KCl solution. Next, supernatants
after centrifuge were used for determining the total protein levels through the Bradford method by bovine serum albumin as standard (Bradford 1976). After that, it was used to assess MDA, SOD, GPx, and CAT activities in the brain. Also, the results were normalized by brain tissue extract protein.

**Assessment of oxidative stress biomarkers**

According to the manufacturer’s protocol, the TAC, GPx, SOD, and CAT of the blood serum and tissue samples were assessed using commercial kits (Randox, Italy). The thiobarbituric acid reactive substances (TBARS) technique was used to determine MDA levels (Uchiyama and Mihara 1978).

**Neurobehavioral test**

Neurological, cognitive, and motor functions tests did on the 15th day before induction of global brain ischemia and the sixteenth day, 24 hours after reperfusion before sacrifice.

**Neurological deficit score**

Neurological deficit was evaluated 24 hours after ischemia as described previously in four categories (Saraf et al. 2010).

Score 0: No neurological deficit

Score 1: Flexion of the contralateral forelimb and the wrist and adduction of the shoulder

Score 2: Reductions of resistance to pushing to the lateral side

Score 3: Movements of the circle to the ipsilateral side

**Hanging wire test (HWT)**

The animals were evaluated by measuring the ability to grasp and the strength of the forelimb via a Hanging wire test before induction ischemia and 24 hours after it (Saraf et al. 2010). Briefly, rats hung through their front limbs from a metal wire (80 cm long, 7 mm in diameters) 45 cm high from the floor. The duration of the delay in the fall of the animal was recorded. The cut-off period of the experiment was two minutes.

**Elevated plus maze test (EPM) for memory retention**

During training experiments, rats were placed on the plus-maze platform at the open arm end; the face of the rats was away from the center. The time of movement of the rat from the open arm and entering in the closed arm with four claws was documented as the transfer latency time (TL). In this method, the acquisition index is named the transfer latency (TL) during the training period, whereas retention latency, an index of retrieval, is called the transfer latency (TL) after 24 h ischemia (Saraf et al. 2010). The cut-off period of the experiment is 90 seconds.
Statistical Analysis

Data showed as the means ± SEM. One-way analysis of variance (ANOVA) test compared the differences between the groups followed by Tukey’s test or LSD as post hoc analyses. The nonparametric Kruskal-Wallis’s test compared brain edema between groups, followed by Mann–Whitney test. The significant level was \( P < 0.05 \). SPSS software 13.0 was used for the statistical analysis of data.

Results

The effect of pre-treatment of KT on brain edema

Brain edema was evaluated via brain water content (BWC) assessment. The global brain ischemia significantly increased BWC in the ischemic group compared to the sham group \(( P < 0.01 \)) . Pre-treatment of KT at doses (one and 2 ml/kg/day, gavage) for 14 days in the KT-1 and the KT-2 groups significantly reduced BWC compared with the ischemic group \(( P < 0.01 )\); (Figure.1). Further, two doses (one and 2 ml/kg) of KT showed no dose-dependent protection on brain edema.

The effect of pre-treatment of KT on blood serum and brain MDA level

The global brain ischemia significantly increased blood serum MDA levels in the ischemic group compared to the sham group \(( P < 0.001 )\). Pre-treatment of KT at doses (one and 2 ml/kg, gavage) for 14 days in the KT-1 and KT-2 groups significantly decreased blood serum MDA levels compared to the ischemic group, respectively \(( P < 0.001 ; P < 0.05 )\); (Figure.2A). Brain MDA levels significantly increased in the ischemic group compared to the sham group \(( P < 0.01 )\). It significantly decreased MDA levels in the KT-1 and the KT-2 groups compared to the ischemic group \(( P < 0.01 )\); (Figure.2B).

The effect of pre-treatment of KT on serum and brain TAC level

Induction of global brain ischemia significantly decreased serum TAC levels in the ischemic group \(( P < 0.05 )\). Pre-treatment of KT at doses (one and 2 ml/kg/day, gavage) for 14 days significantly increased serum TAC levels in the KT-1 and the KT-2 groups compared to the ischemic group \(( P < 0.001 )\); (Figure.3A). Also, Fig. 5A showed that the serum TAC levels significantly increased in the KT-1 and the KT-2 groups compared to the sham group, respectively \(( P < 0.01 , P < 0.05 )\); (Figure.3A). Brain TAC levels significantly decreased in the ischemic group compared to the sham group \(( P < 0.01 )\), as well as brain TAC levels in the KT-1 and the KT-2 groups significantly increased compared to the ischemic group \(( P < 0.05 )\); (Figure.3B). Further, two doses (one and 2 ml/kg) of KT showed no dose-dependent protection on the TAC levels.

The effect of pre-treatment of KT on serum and brain GPx levels

Induction of global brain ischemia significantly decreased serum GPx levels in the ischemic group \(( P < 0.01 )\). Pre-treatment of KT at doses (one and 2 ml/kg/day, gavage) for 14 days significantly increased serum GPx levels in the KT-1 and KT-2 groups compared to the ischemic group \(( P < 0.01 )\); (Figure.4A). Brain GPx levels in the ischemic group significantly decreased compared to
the sham group ($P<0.01$), as well as brain GPx levels in the KT-1 and KT-2 groups significantly increased compared to the ischemic group, respectively ($P<0.01$, $P<0.05$); (Figure.4B). Further, two doses (one and 2 ml/kg) of KT showed no dose-dependent protection on the GPx levels.

**The effect of pre-treatment of KT on serum and brain SOD levels in global ischemia**

Serum and brain SOD levels did not significantly change by induction global brain ischemia or pre-treatment KT at a dose (one and 2 ml/kg/ day, gavage) for 14 days ($P>0.05$); (Table 1).

**The effect of pre-treatment of KT on brain and serum CAT levels**

The serum catalase (CAT) levels did not significantly change by induction of global brain ischemia in the ischemic group($P>0.05$). Pre-treatment of KT at doses (one and 2ml/kg/ gavage/day, gavage) for 14 days significantly increased serum CAT levels in the KT-1 and KT-2 groups compared to the ischemic groups respectively ($P<0.05$; $P<0.01$). The brain catalase levels significantly changed in the ischemic group compared to the sham group ($P<001$), but pre-treatment of KT at doses (one and 2 ml/kg/ /day, gavage) for 14 days didn't significantly change brain CAT levels in the KT-1 and the KT-2 groups compared to the ischemic groups($P>0.05$). (Table 1).

**The effect of KT pre-treatment on the neurological deficit score**

The neurological deficit score in the ischemic group was significantly severe at 24 hours after ischemia compared to the sham group ($P<0.01$). Pre-treatment of KT at doses (one and 2 ml/kg/day, gavage) for 14 days significantly improved the neurological deficit score in comparison to the ischemic group ($P<0.05$); (Table 1).

**The effect of pre-treatment of KT on the motor function**

The motor function tests showed a significant decrease in the fall-off latencies in HWT in the ischemic group compared to the sham group ($P<0.001$). Pre-treatment of KT at doses (one and 2 ml/kg/day, gavage) for 14 days significantly improved motor function compared to the ischemic group ($P< 0.01$); (Figure.5 ).

**The effect of pre-treatment of KT on the memory deficit**

Results of the cognitive functions showed no significant difference in the acquisition phase (TL) before ischemia during the training phase ($P>0.05$); (Figure.6A). Retention (TL) significantly increased in the ischemic group after 24 hours compared to the sham group ($P< 0.001$). Pre-treatment of KT at doses (one and 2 ml/kg/day, gavage) for 14 days effectively improved the cognitive deficit compared to the ischemic group, respectively ($P<0.001$; $P<0.01$); (Figure.6B). Further, two doses (one and 2 ml/kg) of KT didn't show significant and dose-dependent protection on the neurological, motor, and cognitive deficits.

**Discussion**
Our studies showed new evidence that the chronic pre-treatment of KT alleviated brain edema formation following brain ischemia and reperfusion. These results agree with other studies showing anti-edematous properties of KT in focal brain ischemia and colitis models (Kabiri and Setorki 2016; Pakravan et al. 2019). Studies have shown that in pathological situations such as ischemia, the balance between the production and removal of oxidants is impaired (Birben et al. 2012). Oxidants are produced during ischemia mainly by damaged mitochondria and inflammatory responses (Lehner et al. 2011). Several evidence demonstrated that oxidants play essential roles in developing ischemic injuries, such as the blood-brain barrier disruption and brain edema formation (Lehner et al. 2011; Valko et al. 2007). Therefore, to reveal the mechanism of the anti-edematous properties of pre-treatment of KT, the present study evaluated the effects of KT pre-treatment on oxidative stress in the blood and brain in the global brain ischemic condition. Our results showed that pre-treatment of KT decreased MDA levels, an indicator of lipid peroxidation, and significantly increased total antioxidant capacity in the blood and the brain. Moreover, our study showed that pre-treatment of KT significantly elevated the activity of GPx in both brain and blood and only increased CAT activity in the blood.

Our findings effectively showed antioxidant properties and GPx replacement capacity after ischemic stroke in both blood and brain in the chronic pre-treatment of KT. According to our results, other studies showed KT attenuates oxidative stress markers and enhances antioxidant defense in the liver and kidney against radiation toxicity. In addition, KT ameliorates tertiary butyl hydroperoxide-induced oxidative stress and cell death in the liver (Bhattcharyya et al. 2011; Gharib 2009). Also, only a study documented that KT protects the brain from focal ischemia-induced oxidative stress through free radical scavenging and anti-lipid peroxidative effects (Kabiri and Setorki 2016). Interestingly, our data showed that although pre-treatment of KT increased brain total antioxidant capacity (TAC), it significantly can't be effective in increasing superoxide dismutase (SOD) and catalase (CAT) activation in the brain. This discrepancy may be related to the SOD amount and low CAT activation in the rat brain (Nakano 1990; Shohami et al. 1997). Among other organs, the brain is more vulnerable to oxidative stress because of producing an abundance of oxidants, mainly superoxide and hydrogen peroxide, and its low antioxidant defenses (Chen et al. 2011; Lee et al. 2020). According to these explanations, maybe the brain tissue, in addition, GPx, the vital brain's antioxidant defense, uses different antioxidant defenses versus oxidative stress such as coenzyme Q, α-tocopherols, α-lipoic acid, and ascorbate (8, Lee et al. 2020; Warner et al. 2004; Hayes et al. 1989; Galkina 2013). Our observations suggested that the anti-edematous property of KT pre-treatment may be partly related to decreasing lipid peroxidase and increasing TAC and GPx levels in ischemic rats. In addition, we prepared KT with green tea in this study. It has been reported that kombucha fermented with green tea has high-level free-radical scavenging for neutralizing hydroxyl radicals and superoxide anions (Fu et al. 2014).

On the other hand, studies have been shown that KT fermented contains alpha-tocopherol (vitamins E), ascorbic acid (vitamin C), polyphenols, flavonoids, and other compounds that have potent antioxidant properties. Its protective mechanism may also be related to these antioxidant components (Vina et al. 2014, 30).
In the second part of our study, we have determined the effect of KT pre-treatment on cognitive, motor, and behavioral deficits. Studies have shown that the damage to the neuronal tissue by overproduction of free radicals during I/R caused cognitive, motor, and behavioral deficits accompanied by brain edema (Martinez et al. 1997). Our studies showed that pre-treatment of KT at doses (one and 2 ml/kg/day, gavage) for 14 days reverse post-ischemic memory disturbance, neuro deficit score, and grip strength of the forelimbs in the global brain ischemia. These results noticeably suggest a protective effect of KT. Hence, the protective effect of KT observed in our study may be related to the free radical scavenging, antioxidant activity, and inhibition of lipid peroxidation. Also, Kombucha tea contains many possibly active compounds that can exert their effect at multiple levels within the brain (Vina et al. 2014; Bhattacharya et al. 2011). Therefore, the impacts of KT pre-treatment on improving memory, learning, cognitive function, and neurological defects may be partly attributed to the active compounds such as flavonoids and polyphenols on the brain with potent antioxidant properties (Bhattacharya et al. 2011, Jayabalan et al. 2014; Spencer 2008; Spencer 2010). Recent evidence has indicated that dietary flavonoids have neuroprotective actions, including neuroprotection against neurotoxin-induced damage and neuroinflammation suppression, increasing brain blood flow, promoting neurogenesis, and improving memory and cognitive function (Spencer 2008; Spencer 2010).

Polyphenols neutralize free radicals that lead to promoting lipid peroxidation (Jayabalan et al. 2008). In addition, they are involved in preventing various diseases associated with oxidative stress, such as neurodegenerative problems (Tsao 2010). Also, they modify different enzymes activity and cell receptors for defense against ROS-induced oxidative stress (Tsao 2010). Therefore, as established in the present study, increasing the antioxidant capacity and decreasing the lipid peroxidation with pre-treatment of KT may protect the brain versus oxidative stress injury after brain ischemic stroke. According to our results, part of the beneficial effects of pre-treatment of KT in reducing brain edema and improving behavior tests may be related to its development in decreasing the production of lipid peroxidation and increasing antioxidant defense. Although we did not study active compounds of KT, the non-enzymatic antioxidant, in the present study, also, it seems that the protective effect of KT may be in part related to non-enzymatic antioxidants such as flavonoids and polyphenolic. More experiments are required to elucidate these possibilities.

Conclusion

Our findings showed that global brain ischemia partly induces brain edema and behavior defects by augmenting lipid peroxidation and diminishing antioxidant defenses. The pre-treatment of KT ameliorates brain ischemia-induced damage, maybe via anti-lipid peroxidative, free radical scavenging, and antioxidant properties in the blood and brain. Therefore, it can be used as a natural antioxidant supplement against brain ischemia-induced injury. However, more studies are needed to clarify its mechanism of action.

Declarations
Funding declaration

This study was financially supported by the Drug Applied Research Centre of Tabriz University of Medical Sciences (Grant no.98/105), and Author F.H has received this research support.

Competing Interests

The authors declare that there is no conflict of interest.

Author contributions

Mehran Mesgari-Abbasi and Fatemeh Eskandari Vaezi performed laboratory experiments and contributed to the analysis. Fezzeh Hossienzadeh conceived and designed the study and wrote the paper. All authors approved the final version of the manuscript.

Data Availability Statement

The datasets of this study are available from the corresponding author upon reasonable request.

Ethics approval

The study was approved by the research and ethics committee of Tabriz University of Medical Sciences in Iran (the ethics code is IR.TBZMED.VCR.REC.1398.457). These experiments were carried out under the Tabriz University of Medical Sciences guidelines for the care and use of laboratory animals.

Acknowledgment

The authors would like to thank Mr. Mahmod Reza Hassanzadeh for his helpful surgery assistance.

References


10.1016/j.neulet.2012.11.062


Tables

Table 1: The effect of induction global brain ischemia and pre-treatment of KT at a dose (one and 2 ml/kg/day, gavage) for 14 days on serum and brain the SOD and the CAT levels, and the neurologic deficits (NS) score.

<table>
<thead>
<tr>
<th>Group names</th>
<th>Sham</th>
<th>Ischemic</th>
<th>KT-1</th>
<th>KT-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum SOD(U/ml)</td>
<td>33.78±4.1</td>
<td>28.95±4.5&lt;sup&gt;as&lt;/sup&gt;</td>
<td>31.32±3.59&lt;sup&gt;ai&lt;/sup&gt;</td>
<td>33.63±4.72&lt;sup&gt;as&lt;/sup&gt;</td>
</tr>
<tr>
<td>Brain SOD(U/mg protein)</td>
<td>26.94±1.78</td>
<td>26.64±1.92&lt;sup&gt;as&lt;/sup&gt;</td>
<td>27.15±1.80&lt;sup&gt;ai&lt;/sup&gt;</td>
<td>28.33±2.04&lt;sup&gt;as&lt;/sup&gt;</td>
</tr>
<tr>
<td>Serum CAT (nmol/min/ml)</td>
<td>15.07±0.18</td>
<td>10.52±1.15&lt;sup&gt;m&lt;/sup&gt;</td>
<td>18.1±2.62&lt;sup&gt;*&lt;/sup&gt;</td>
<td>22.65±3.34&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>Brain CAT (nmol/min/mg protein)</td>
<td>4.79±0.49</td>
<td>2.65±0.78&lt;sup&gt;$&lt;/sup&gt;</td>
<td>3.08±0.11&lt;sup&gt;as&lt;/sup&gt;</td>
<td>3.04±0.25&lt;sup&gt;as&lt;/sup&gt;</td>
</tr>
<tr>
<td>NS score</td>
<td>0.0±0</td>
<td>1.42±0.29&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.43±0.2&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.42±0.2&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data are shown as the mean± SEM; n = 7; Statistical comparisons are made between the ischemic group vs. the sham group and KT-1 and KT-2 groups vs. the ischemic group.

**P<0.01 ,  *P<0.05 ,  $P<0.001 , ns: no significant.

Figures
Figure 1

Brain water content (BWC) in the sham and the ischemic groups received vehicle or kombucha tea (one and 2 ml/kg/day, gavage) for 14 days as pre-treatment. **$P<0.01$ vs. the sham group. ##$P<0.01$ vs. the ischemic group. Data are shown as the mean± SEM; n = 7

Figure 2

The effect of global brain ischemia and pre-treatment of KT (one and 2 ml/kg/day, gavage) on serum and brain MDA Levels; A. Serum MDA levels in the sham and the ischemic groups received vehicle or Kombucha tea (one or 2 ml/kg/day, gavage) for 14 days. ***$P<0.001$ vs. the sham group, ###$P<0.001$ and #P<0.05 vs. the ischemic group. B. Brain MDA levels in the sham and the ischemic groups received
vehicle or Kombucha tea (one and 2 ml/kg/day, gavage) for 14 days **$P<0.01$ vs. the sham group, ##$P<0.01$ vs. the ischemic group. Data are shown as the mean± SEM; n = 7

Figure 3

The effect of global ischemia and KT pre-treatment (one and 2 ml/kg/day, gavage) on serum and brain TAC Levels; A. Serum TAC levels in the ischemic group and ischemic (KT-1, KT-2) groups received vehicle or KT as pre-treatment for 14 days. *$P<0.05$, **$P<0.01$, ***$P<0.001$ vs. the sham group. B. Brain TAC levels in the ischemic group and the ischemic (KT-1, KT-2) groups that received vehicle or KT (one and 2 ml/kg/day, gavage) as pre-treatment for 14 days. **$P<0.01$ vs. the sham group # $P<0.05$ vs. the ischemic group. Data are shown as the mean± SEM; n = 7
Figure 4

The effect of global brain ischemia and pre-treatment of KT (one and 2 ml/kg/day, gavage) on serum and brain GPx Levels; A. Serum GPx levels in the ischemic group and the ischemic (KT-1, KT-2) groups that received vehicle or KT(one and 2 ml/kg) as pre-treatment for 14 days. **P < 0.01, ##P<0.01 vs. the ischemic group. B. Brain GPx levels in the ischemic group and the ischemic (KT-1, KT-2) groups that received vehicle or KT(one and 2 ml/kg) as pre-treatment for 14 days. **P < 0.01 vs. the sham group. ##P< 0.01, #P<0.05 vs. the ischemic group. Data are shown as the mean± SEM; n = 7

Hanging Wire Test

Figure 5

The effect of global ischemia and pre-treatment of KT (one and 2 ml/kg/day, gavage) on motor function in the sham and the ischemic groups that received vehicle or Kombucha tea (one or 2 ml/kg/day, gavage) for 14 days. ###P<0.001 vs. the sham group, ##P<0.01 vs. the ischemic group. Data are shown as the mean± SEM; n = 7
Figure 6

The effect of global brain ischemia and KT pre-treatment (one and 2 ml/kg/day, gavage) on memory deficits. A. Transfer latency (TL) before ischemia during the training phase (acquisition) in the sham and the ischemic groups that received vehicle or Kombucha tea (one or 2 ml/kg/day, gavage) for 14 days (\(P>0.05\)). B. Transfer latency (TL) 24th after ischemia-reperfusion (retention), ***\(P<0.001\) vs. the sham group, ###\(P<0.001\) and ##\(P<0.01\) vs. the ischemic group. Data are shown as the mean± SEM; n = 7