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RESEARCH ARTICLE

Neuroprotective effect of ginger in the brain of streptozotocin-induced diabetic rats

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SUMMARY

Diabetes mellitus results in neuronal damage caused by increased intracellular glucose leading to oxidative stress. Recent evidence revealed the potential of ginger for reducing diabetes-induced oxidative stress markers. The aim of this study is to investigate, for the first time, whether the antioxidant properties of ginger has beneficial effects on the structural brain damage associated with diabetes. We investigated the observable neurodegenerative changes in the frontal cortex, dentate gyrus, and cerebellum after 4, 6, and 8 weeks of streptozotocin (STZ)-induced diabetes in rats and the effect(s) of ginger (500 mg/kg/day). Sections of frontal cortex, dentate gyrus, and cerebellum were stained with hematoxylin and eosin and examined using light microscopy. In addition, quantitative immunohistochemical assessments of the expression of inducible NO synthase (iNOS), tumor necrosis factor (TNF)-α, caspase-3, glial fibrillary acidic protein (GFAP), acetylcholinesterase (AChE), and Ki67 were performed. Our results revealed a protective role of ginger on the diabetic brain via reducing oxidative stress, apoptosis, and inflammation. In addition, this study revealed that the beneficial effect of ginger was also mediated by modulating the astroglial response to the injury, reducing AChE expression, and improving neurogenesis. These results represent a new insight into the beneficial effects of ginger on the structural alterations of diabetic brain and suggest that ginger might be a potential therapeutic strategy for the treatment of diabetic-induced damage in brain.

1. Introduction

Diabetes mellitus (DM) is one of the most common chronic metabolic disorders leading to complications in multiple organs and systems. These complications often result in either morbidity or mortality (Perkins and Bril, 2005). Diabetic encephalopathy is one of the complications that occur due to gradually developing end-organ damage in the central nervous system (Northam and Cameron, 2013). The damage could be as a result of chronically increased intracellular glucose concentration leading to several structural, neurochemical, and neurodegenerative changes in different regions in the brain including the frontal cortex (Kumar et al., 2008), hippocampus (Pamidi and Satheesha Nayak, 2012), and cerebellum (Hernandez-Fonseca et al., 2009). These pathological changes underpin different cognitive, motor, and neuroendocrine disturbances characterizing diabetic encephalopathy (Rajashree et al., 2011). For instance, diabetes-induced cerebellar dysfunction is associated with seizure generation, motor deficits, and memory impairment (Anu et al., 2010). Defects in hippocampal synaptic plasticity and transmission result in deterioration of learning and memory in diabetic patients (Shingo et al., 2012). Interestingly, accumulating recent evidence indicates that diabetes mellitus is an important risk factor for sporadic Alzheimer's disease (AD) (Vignini et al., 2013).

One of the underlying mechanisms of diabetic neuronal injury is the excessive free radical generation from the auto-oxidation of elevated intracellular glucose levels (Gradinaru et al., 2013). Indeed, several in vitro, experimental, and clinical studies have implicated oxidative stress in the pathogenesis of diabetic complications. Oxidative stress results in depolarization of the inner mitochondrial membrane, release of cytochrome c into the cytosol, and ultimately, induction of caspase mediated apoptosis (Gurpinar et al., 2012). Ginger (*Zingiber officinale*) is widely consumed as a spice for the flavoring of foods. Ginger is reported to have several beneficial pharmacological effects (hypoglycemic, insulinotropic, and hypolipidemic) in experimental animals (Shanmugam et al., 2011) and in humans (Huang et al., 2004). It has been documented that ginger or its extracts possess some pharmacological activities including analgesic (Young et al., 2005), anti-tumor (Habib et al.,

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2008) and anti-oxidant effects (Shanmugam et al., 2010). Anti-oxidants in ginger include gingerols, shogaols and some phenolic ketone derivatives. Anti-oxidant therapy has proved to be remarkably beneficial to remedy reactive oxygen species (ROS)-induced injury in the CNS (Tsakanova et al., 2011). Therefore, antioxidants such as ginger could be promising candidates in ameliorating the pathological sequelae of diabetic encephalopathy.

In this study, we set out to investigate whether ginger has a protective effects on neuropathological changes associated with streptozotocin (STZ)-diabetic brains in rats. Quantified immunohistochemical assessments were conducted to elucidate mechanism(s) of the potential protective effect of ginger. Our results have shown that ginger ameliorated the morphological and neuropathological changes induced by diabetes. Oxidative stress, apoptosis, and inflammation were reduced in the ginger-treated group. In addition, ginger treatment down-regulated astrogliosis, decreased acetylcholinesterase (AChE) expression, and enhanced neurogenesis. Up to the best of our knowledge, this study is the first to demonstrate the protective role of ginger on neuropathological alterations associated with diabetic brain.

2. Methodology

2.1. Animals

Male albino rats aged 8–10 weeks at an average weight 150–200 g were maintained in the animal house of the Faculty of Medicine, Menoufia University and were subjected to a 12:12-h daylight/darkness and allowed unlimited access to chow and water. All the ethical protocols for animal treatment were followed and supervised by the animal facilities, Faculty of Medicine, Menoufia University. All studies were approved by The Animal Care and Use Committee of Faculty of Medicine, Menoufia University.

2.2. Experimental design and diabetes induction

Animals were randomly divided into four groups: control, control + ginger, diabetic, and diabetic + ginger. Rats of each of these groups were subdivided into another three subgroups in which rats were sacrificed after 4, 6, and 8 weeks after starting the experiment. Eight rats were used in each group at each time point. In diabetic and diabetic + ginger groups, diabetes was induced by intraperitoneal injections of streptozotocin (STZ, Sigma, St. Louis, MO, USA) administrated at a dose of 60 mg/kg. STZ-induced diabetes is a widely used model of type I diabetes characterized by hyperglycemia. STZ was dissolved in a freshly prepared 0.1 M citrate buffer (pH 4.5). Rats were fasted for 12 h before STZ injection. Fasting blood glucose levels were measured 72 h after STZ induction using blood drawn from the tail plexus of conscious rats. Blood glucose concentrations were tested using the Span Diagnostic kit with Jinque test strips. Rats with blood glucose above 250 mg/dl were considered diabetic.

Control and diabetic groups were provided with standard chow, while control + ginger and diabetic + ginger groups rats were gavage-fed with 500 mg/kg/day of ginger. There was no significant difference between control and control + ginger rats in all the outcomes (fasting blood glucose levels and immunohistological assessments of different markers) at each time point used in the study; therefore, these two groups were pooled in one group (control).

2.3. Histological and immunohistological (IHC) assessments

At the end of each time point studied, each rat was deeply anaesthetized using ketamine (90 mg/kg) and xylazine (15 mg/kg) (i.p.) and decapitated. Each brain was fixed in 10% neutral buffered formalin and embedded in paraffin wax for histological examination. Semi-serial 5 μm-coronal sections (1-in-20 series) were prepared from the frontal cortex, hippocampus, and cerebellar cortex and were dehydrated using ethanol and stained with hematoxylin & eosin (H&E).

For immunohistological staining, paraffin sections (5 μm thick) were deparaffinized in xylene for 1–2 min and then rehydrated in descending grades of ethanol (100%, 95%, and 70% ethanol) two changes 5 min each, then brought to distilled water for another 5 min. Sections were rinsed with PBS, blocked for 30 min in 0.1% H_2O_2 as inhibitor for endogenous peroxidase activity. After rinsing in PBS, sections were incubated for 60 min in blocking solution (10% normal goat serum) at room temperature (RT, 21 °C). The sections were then incubated with the primary antibody (inducible NO synthase (iNOS), 1:500, Labvision; Caspase-3, 1:500, Labvision; tumor necrosis factor (TNF)-α, 1:1000, Labvision; glial fibrillary acidic protein (GFAP), 1:300, Labvision, and Ki67, 1:500, Labvision, and acetylcholinesterase (AChE), 1:300, Novus) at RT for an hour. Sections were rinsed with PBS, followed by 20 min of incubation at RT with secondary biotinylated antibody.

After rinsing the sections in PBS, enzyme conjugate "Streptavidin-Horseradish peroxidase" solution was applied to the sections for 10 min. Secondary antibody binding was visualized using 3,3′-diaminobenzoic acid (DAB) dissolved in PBS with the addition of H_2O_2 to a concentration of 0.03% immediately before use. Finally, sections were PBS rinsed and counterstaining of slides was done using two drops or 100 μl of hemotoxylin. Slides were washed in distilled water until the sections turned blue. Finally, slides were dehydrated in ascending grades of ethanol (70%, 95%, and 100%) for 5 min each and were cleared in xylene and finally coverslipped using histomount mounting solution.

For immunohistological quantitative assessment, five non-overlapping fields (400×) per section were randomly captured by a digital camera (Olympus) in the frontal cortex and the cerebellum, whereas the entire dentate gyral area was analyzed for each brain section for each marker. The number of immunopositive cells in the fields taken from at least three anatomically comparable sections/animal was counted using imageJ software and averaged per field for each animal. The numbers calculated for at least five animals/experimental group were considered for comparison and statistical analyses.

2.4. Statistical analysis

Results were expressed as mean ± SEM and significant differences between groups were evaluated using One way-ANOVA followed by a post hoc Bonferroni test. A level of significance of $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Effects of ginger on glucose blood level

STZ injection resulted in a diabetic syndrome verified by the presence of polydypsia, polyuria, and hyperglycemia in the diabetic animals. Over the 8 weeks after the injection, mean blood glucose levels in STZ-injected rats were significantly higher than the control group ($P < 0.001$). Ginger treatment significantly lowered blood glucose levels in comparison with rats of the diabetic group at all experimental time points. Blood glucose levels are presented in Table 1.

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