FACETS
a multidisciplinary open access science journal

RESEARCH ARTICLE

Neuroprotective effect of *Panax ginseng* extract against cerebral ischemia–reperfusion-injury-induced oxidative stress in middle cerebral artery occlusion models

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Abstract

The present study investigated the in vivo neuroprotective role of *Panax ginseng* extract (PGE) pretreatment against transient cerebral ischemia in a middle cerebral artery occlusion (MCAO) model. Rats were randomly divided as follows: group I, control; group II, sham-operated; group III, where animals were subjected to MCAO surgery; and group IV, where animals were orally administered 10 mL PGE per day (200 mg/kg of body weight per day) for 30 d followed by MCAO induction at day 31. Following 24 h of reperfusion, blood and tissue (brain, liver, and kidney) samples were collected for biochemical and histopathological examination. Biochemical testing included lipid profile, liver enzymes, kidney function tests, C-reactive protein (CRP), lactate dehydrogenase (LDH), glucose, and total protein estimation. Tissue antioxidants (catalase, superoxide dismutase, and glutathione) were assessed in brain, liver, and kidney tissues. MCAO-induced histopathological changes were also examined in the tissues. Pretreatment with PGE showed significant improvement in tissue antioxidant status in brain, liver and kidney tissues. PGE treatment maintains plasma lipid profile, liver enzymes, kidney function, and CRP, LDH, and glucose levels. Histologically, monocytes and macrophage infiltration were observed in the tissues of MCAO animals, whereas PGE treatment preserved tissue architecture and minimal monocyte infiltration. PGE supplementation showed a neuroprotective effect against ischemia–reperfusion injury by effectively increasing endogenous antioxidant enzyme activity.

Key words: antioxidants, cerebral, ginseng, ischemia–reperfusion, middle cerebral artery occlusion, neuroprotective, oxidative stress

Citation: Shamim M and Khan NI. 2019. Neuroprotective effect of *Panax ginseng* extract against cerebral ischemia–reperfusion-injury-induced oxidative stress in middle cerebral artery occlusion models. FACETS 4: 52–68. doi:10.1139/facets-2018-0025

Handling Editor: Peter Zahradka

Received: July 30, 2018

Accepted: January 4, 2019

Published: March 7, 2019

Published by: Canadian Science Publishing

Introduction

Stroke is a multifactorial debilitating disorder, and is the fourth leading cause of mortality and the leading cause of disability accompanied by cognitive impairments (Lopez-Valdes et al. 2014; Nomani et al. 2017). According to the World Health organization (WHO) statistics, every year 15 million people globally suffer a form of stroke (WHO 2018).

Ischemic stroke is the most prevalent type of stroke (87% of all type of stroke cases), and results from the thromboembolic occlusion of cerebral vessels, which initiates ischemic cascade and subsequent tissue injury (Benjamin et al. 2017). In ischemic cascade, energy and oxygen deprivation generate reactive oxygen species (ROS), followed by glutamate excitotoxicity, intracellular calcium accumulation, and inflammation (Fluri et al. 2015). Reperfusion of ischemic tissue augments ROS production (causing reoxygenation injury of nervous tissue) and neuroinflammation (Bang 2016). Several experimental studies have shown that neuronal cell death within an ischemic lesion is associated with oxidative stress. ROS (including hydrogen peroxide, nitric oxide, hydroxyl free radicals, and superoxide anion) (Lee and Won 2014; Jung et al. 2016) destroy cellular structures such as lipids, nucleic acid, proteins, membrane channels and receptors, and redox-sensitive enzymes, which eventually causes neuronal cell death in ischemic lesions. In addition, ROS result in leakage of cytochrome C from mitochondria, which, in turn, initiates the caspases that further worsen the neuronal death after ischemia and reperfusion injury (Lee and Won 2014; Jung et al. 2016).

To date, recombinant tissue plasminogen activators (r-tPA) are the only US food and drug administration approved drug; however, <4% patients with stroke receive assistance from r-tPA due to its narrow therapeutic time window and cerebral hemorrhage risk (Yip and Demaerschalk 2007; Tajiri et al. 2014). Therefore, prevention and effective management of ischemic stroke is the most important therapeutic challenge.

Antioxidants are long-recognized to ameliorate ROS-induced ischemic injury in biological systems. Plants, including vegetables, fruits, and herbs are rich source of antioxidants and are renowned to counter ischemic injury by enhancing or maintaining the body's antioxidant status (Bae et al. 2013).

Panax ginseng is a popular medicinal herb that is frequently used in traditional medicine systems. Ginsenosides (triterpene saponins) are the principal bioactive constituents of ginseng (Zhang et al. 2008). Several investigational studies revealed the therapeutic potentials of ginseng including antioxidative, antiapoptotic, antiinflammatory, antihyperglycemic, cardio-protective, antihypertensive, anti-anxiety, antidepressive, and hypotensive properties (Ong et al. 2015), as well as modulation of neurodegenerative disorders (Ong et al. 2015).

In the present study, we investigated the neuroprotective effects of *Panax ginseng* extract in the management of ischemia–reperfusion injury to neuronal tissues in middle cerebral artery occlusion (MCAO) rat models.

Materials and methods

Panax ginseng extract

Dried roots of fully matured (6 years old) *Panax ginseng* (PG) were purchased commercially from the local herbal store in Karachi, Pakistan. PG aqueous extract was prepared using a modified decoction method as described by Ban et al. (2012) and Lo et al. (2015). In brief, 2 g of ginseng roots were steeped in 100 mL of boiling distilled water for 60 min. Boiling volume was maintained by constantly adding water. The extract was filtered and stored in a clean dark glass bottle at 4 °C for 1 week. Prepared PG extract (PGE) contains approximately 0.43 g of active ginsenosides per gram of ginseng, measured spectrophotometrically by the method of Kevers et al. (2004) at a wavelength of 520 nm.

PGE dose

The dose of *Panax ginseng* administered was 200 mg/kg of body weight per day (equal to 86.32 mg ginsenosides in 10 mL of extract).

Animals

All study protocols were performed in accordance with the *Guide for the Care and Use of Laboratory Animals*, National Institutes of Health (NIH) (National Research Council (US), Committee for the Update of the Guide for the Care and Use of Laboratory Animals, and Institute for Laboratory Animal Research 2011) and reviewed and approved by Departmental Research Committee (DRC), Department of Physiology, University of Karachi.

A total of 36 age- and sex-matched *Wistar* albino rats weighing 220 ± 20 g were purchased from the animal care facility of the International Center of Chemical and Biological Sciences, University of Karachi (Karachi, Pakistan). Animals were housed in groups of two animals per cage in a well-ventilated animal house and maintained under a natural 12:12 h light/dark cycle with a constant temperature of 23 ± 2 °C. Throughout the study period, animals were provided with standard laboratory diet and water ad libitum.

Middle cerebral artery occlusion (MCAO) surgery

Following the standard animal care guidelines, animals were subjected to transient MCAO via monofilament method as described by previous studies (Park et al. 2010; Chiang et al. 2011). In brief, anesthesia was induced with ketamine hydrochloride (50 mg/kg) with xylazine (5 mg/kg) via intraperitoneal administration and monitored accordingly. For MCAO induction, a midline incision was made on the neck to expose the left common carotid artery (LCCA), external carotid artery (ECA), and internal carotid artery (ICA). A silicon-coated monofilament (4-0, 0.37 tip diameter) was introduced into LCCA bifurcation via small incision, then the monofilament was gently advanced 16–18 mm distal of the ICA until the monofilament occluding the origin of the middle cerebral artery (MCA) resulted in transient ischemia with subsequent brain infarction in the MCA territory. The filament was removed after 15 min for reperfusion. The neck incision was sutured, and the animal was kept in a nursing box under a maintained temperature of 36.5 ± 0.5 °C until complete recovery from anesthesia and then returned to home cage. Animals with successful MCAO-induced ischemic stroke resulted in weakness and flaccidity on the right side of body (Hata et al. 1998; Chiang et al. 2011; Guven et al. 2015).

Sham surgery

Sham-operated animals underwent the same MCAO surgical procedure without any suture insertion/ligation or MCA occlusion.

Experimental protocol

After a 1 week acclimatization period animals were randomly assigned to one of the following four groups ($n = 9$ for each group). *Group I:* control group, which received a standard laboratory rodent diet throughout the study. *Group II:* sham-operated group, which received a standard laboratory diet for 30 d following the sham protocol. *Group III:* MCAO group, for which stroke was induced via the MCAO procedure. *Group IV:* MCAO + PGE treated group, which together with the normal lab diet animals were orally (via gavage) administered 10 mL PGE per day (200 mg/kg of body weight per day), following a method modified from Ban et al. (2012). At day 31 animals were subjected to MCAO for 15 min followed by 24 h of reperfusion.

Blood and tissue sampling

At the end of the experimental protocol (24 h after MCAO) all animals were euthanized to collect blood (serum/plasma was separated) and organs (brain, liver and kidney) and stored at −80 °C. These materials were then processed for histopathological and biochemical assays.

Tissue preparation for histopathology

For histopathological assay, brain (coronal section), liver, and kidney sections (2–3 mm thick) were immersed in 10% formaldehyde phosphate buffer solution overnight (24 h). After fixation, tissues were embedded in paraffin, sliced into 5 µm thick sections, stained with routine hematoxylin-eosin, and observed under light microscope (Palipoch and Punsawad 2013). Histological assessments of the slides were performed by an independent blind observer.

A Quantitative method was used to examine the histopathological changes in the brain, liver, and kidney tissues. Each tissue section was graded as absent (−), slight (+1), mild (+2), moderate (+3), and severe (+4), according to the severity of the structural alterations.

Tissue homogenization

Tissue samples were homogenized in ice-cold saline to obtain 10% (*w*/*v*) homogenates according to the methods described by previous studies by Khan et al. (2012) and Palipoch and Punsawad (2013) to assess tissue antioxidants (catalase, superoxide dismutase, and glutathione) status in brain, liver, and kidney tissues.

Biochemical assays

Plasma total cholesterol (TC) was estimated by the CHOD PAP enzymatic end point method using an enzymatic kit (Global, UK). Plasma triglyceride (TG) concentration was determined by the GPO-PAP enzymatic endpoint method using an enzymatic kit (Global, UK). Plasma low density lipoprotein-C (HDL-C) concentration was estimated by the phosphotungstate precipitation method using an enzymatic kit (Global, UK). Plasma low density lipoprotein-C (LDL-C) level was calculated by Friedewald's formula (Friedewald et al. 1972). Plasma very low density lipoprotein-C (VLDL-C) levels were calculated using the formula mentioned by Bairaktari et al. (2005). Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) concentration and alkaline phosphatase (ALP) were estimated according to the International Federation of Clinical Chemistry recommended manual RX MONZA method using an enzymatic kit (Randox, UK). Serum urea concentration was determined by the modified Berthelot colorimetric method using an enzymatic kit (Global, UK). Estimation of serum creatinine levels was performed by Jaffe's (two-point) method using an enzymatic kit (Biogene Diagnostics, USA). Concentration of serum uric acid was determined by the PAP method using a uric acid assay kit (Global, UK). Serum levels of blood urea nitrogen (BUN) were calculated by the formula described by Lamb et al. (2006). Plasma total protein (TP) was assessed by the Biuret method using an enzymatic kit (Spinreact, Spain). Serum C-reactive protein (CRP) levels were assessed by the Particle Enhanced Turbid Metric Immunoassay method using a BioLatex kit (Conformidad Europea, Spain). Serum lactate dehydrogenase (LDH) levels were estimated using a colorimetric enzymatic kit (Biotech, USA). Plasma glucose concentration was determined by the Enzymatic-Colorimetric GOD-PAP in vitro method using an enzymatic kit (Global, UK).

Tissue antioxidants

Recommended procedures were used to assess tissue catalase (CAT), superoxide dismutase (SOD), and glutathione (GSH) activity levels, as briefly described below.

Catalase assay

Tissue catalase activity was estimated using the method reported by Sinha (1972). Briefly, the assay consisted of tissue homogenate, 0.05 mol/L PO_4 buffer (pH 7.0), and 1 mL of 0.2 mol/L H_2O_2. This mixture was added to 2 mL of 5% dichromate acetic acid solution and then boiled (10 min) and cooled. Absorbance of the mixture was measured spectrophotometrically at 570 nm (Spectrophotometer UV-120-01, Shimadzu, Kyoto, Japan). CAT activity was expressed as µmol/g of tissue.

Superoxide dismutase assay

Tissue SOD activity was measured using the Kono method (Kono 1978). Briefly, the total reaction mixture consisted of 0.5 mL of 90 µm nitroblue tetrazolium (NBT), 1.3 mL of EDTA–Na_2CO_3, 0.1 mL of 20 mmol/L hydroxylamine hydrochloride solution (pH 6.0), and 0.1 mL of Triton X-100 EDTA solution (pH 10). The NBT reduction reaction rate was measured by recording per minute changes in absorbance using a UV spectrophotometer at 560 nm (Shimadzu, Kyoto, Japan). 0.1 mL of the tissue homogenate was then added and the percentage inhibition of the NBT reduction rate was again recorded. SOD activity was presented as units per gram (U/g) of tissue.

Glutathione assay

Tissue glutathione activity was determined by the procedure reported by Carlberg and Mannervik (1985). Briefly, the reaction assay mixture consisted of 0.1 mL of tissue homogenate, 1.5 mL (50 mmol/L, pH 7.6) of potassium phosphate buffer, 0.3 mL of 10% bovine serum albumin (BSA), 0.35 mL of 0.8 mmol/L NADPH, and 0.1 mL of 30 mmol/L oxidized glutathione. The optical density (absorbance) was recorded on a kinetic spectrophotometer PRIM 500 for 5 min at 25 °C. Tissue glutathione activity level was expressed as units per gram (U/g) of tissue.

Statistical analysis

Results were expressed as mean ± SEM. Statistical analysis was performed using SPSS 16.0 software. Intergroup differences were tested with ANOVA followed by post hoc *t* test. $p < 0.05$ was considered statistically significant.

Results

Body weight changes

Significant ($p < 0.05$) decreases in the body weight of animals from the MCAO group were observed with stroke induction compared with the control group. Thirty day administration of *Panax ginseng* extract showed a protective effect on body weight change in animals from the MCAO + PGE group; however, this change was not significant ($p > 0.05$; $p = 0.775$) compared with the control group (**Table 1**).

Effect of PGE treatment on biochemical parameters

Effect of PGE on lipid profile

The changes in plasma lipid profile are shown **Table 1**. Thirty days of ginseng supplementation decreased plasma TC (5%) and LDL (12.3%), with a 5.4% increase in HDL concentration in the MCAO + PGE treated animals compared with animals of the MCAO group. However, no significant ($p > 0.05$) change was observed in plasma TG and VLDL levels.

Effect of PGE on liver enzymes

Table 1 shows the changes in serum liver enzymes. An increase in liver enzymes was observed in MCAO animals after stroke compared with controls. Thirty days of ginseng pre-stroke treatment significantly decreases ($p < 0.05$) serum AST and ALP levels, whereas the decrease in serum ALT was not statistically significant ($p > 0.05$) when compared with the MCAO group. Ginseng supplementation maintained liver enzymes MCAO + PGE animals.

Effect of PGE on kidney parameters

When compared with MCAO animals, plasma urea and BUN levels were significantly decreased ($p < 0.05$) in animals that underwent PGE treatment before stroke (MCAO + PGE). Plasma uric acid and creatinine levels also decreased but non-significantly ($p > 0.05$) compared with the MCAO group (**Table 1**).

Table 1. Changes in body weight, plasma lipid profile, liver enzymes, kidney parameters, LDH, CRP, and glucose level among experimental groups.

Parameters	Control ($n = 9$)	Sham ($n = 9$)	MCAO ($n = 9$)	MCAO + PGE treated ($n = 9$)
Body Weight (g)	222.778 ± 5.873	199 ± 7.356*	189.75 ± 4.761***/NS	191.625 ± 4.366***/NS/NS
TC (mg/dL)	132.576 ± 7.738	127.245 ± 7.358NS	128.215 ± 7.190NS/NS	121.797 ± 4.054NS/NS/NS
TG (mg/dL)	65.725 ± 7.356	62.778 ± 6.934NS	60.871 ± 1.986NS/NS	64.329 ± 1.866NS/NS/NS
HDL (mg/dL)	51.358 ± 2.626	52.785 ± 3.602NS	42.638 ± 1.240*/*	44.928 ± 1.815NS/NS/NS
LDL (mg/dL)	68.072 ± 5.353	61.903 ± 8.549NS	73.012 ± 7.666NS/NS	64.002 ± 4.111NS/NS/NS
VLDL (mg/dL)	13.145 ± 1.471	12.555 ± 1.386NS	12.563 ± 0.415NS/NS	12.865 ± 0.373NS/NS/NS
ALT (IU/L)	20.137 ± 3.01	23.978 ± 0.678NS	25.135 ± 0.988NS/NS	23.779 ± 1.694NS/NS/NS
AST (IU/L)	34.171 ± 1.945	36.333 ± 2.752NS	40.105 ± 2.015NS/NS	30.16 ± 3.246NS/NS/*
ALP (IU/L)	42.72 ± 3.174	43.434 ± 2.491NS	51.466 ± 3.174NS/NS	41.535 ± 2.24NS/NS/*
Urea (mg/dL)	33.233 ± 0.718	35.233 ± 0.864NS	49.02 ± 3.19***/**	36.671 ± 3.693NS/NS/*
Uric Acid (mg/dL)	2.537 ± 0.553	2.739 ± 0.882NS	4.13 ± 0.981NS/NS	3.796 ± 1.921NS/NS/NS
Creatinine (mg/dL)	0.315 ± 0.033	0.34 ± 0.041NS	0.552 ± 0.047***/**	0.401 ± 0.054NS/NSNS
BUN (mg/dL)	15.529 ± 0.335	16.464 ± 0.404NS	22.906 ± 1.49***/**	15.216 ± 1.51NS/NS/***
LDH (U/L)	682.295 ± 16.106	696.213 ± 15.523NS	711.078 ± 26.244NS/NS	681.163 ± 26.904NS/NS/NS
CRP (mg/dL)	9.575 ± 0.169	10.107 ± 0.416NS	10.914 ± 0.735NS/NS	10.251 ± 0.259NS/NS/NS
Glucose (mg/dL)	113.743 ± 5.266	110.836 ± 8.648NS	112.97 ± 13.76NS/NS	105.881 ± 7.049NS/NS/NS

Note: Values are presented as mean ± SEM. Significant difference between experimental groups by *t* test are: *$p < 0.05$, **$p < 0.01$, ***$p < 0.005$. NS, non-significant, compared with control/compared with sham/compared with MCAO group. MCAO, middle cerebral artery occlusion; PGE, *Panax ginseng* extract; TC, total cholesterol; TG, triglyceride; HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; BUN, blood urea nitrogen; LDH, lactate dehydrogenase; CRP, C-reactive protein.

Effect of PGE on LDH, CRP, and glucose

Table 1 shows the effect of PGE treatment on other biochemical parameters from various experimental groups. A non-significant ($p > 0.05$) increase was observed in serum LDH and CRP in MCAO animals when compared with animals in the control and sham groups. Ginseng extract treatment slightly decreased the LDH and CRP levels in the MCAO + PGE group ($p > 0.05$).

No statistically significant change was observed in plasma glucose level among the control, sham, and MCAO groups. No significant difference in plasma glucose level was observed between the PGE-treated group and the control group ($p > 0.05$) (Table 1).

Effect of PGE treatment on tissues (brain, liver, and kidney) weight, total protein, and antioxidants (catalase, superoxide dismutase, and glutathione)

Effect of PGE on brain antioxidant levels

A significant increase in brain weight was observed in MCAO animals compared with the control group ($p < 0.05$). PGE treatment showed a slight but non-significant increase in the brain weight of MCAO + PGE animals when compared with animals in the MCAO group ($p > 0.05$). No changes were observed in tissue total protein levels in MCAO animals when compared with control,

sham-operated, and MCAO + PGE treated groups. Tissue CAT levels were found to decrease ($p > 0.05$) in MCAO animals compared with control and sham-operated groups. Thirty days of ginseng extract administration showed an increase ($p > 0.05$) in tissue CAT level when compared with MCAO, control, and sham-operated groups. The brain tissue SOD and glutathione levels were observed to decrease ($p > 0.05$) in MCAO animals when compared with the control and sham-operated groups. Ginseng treatment showed an increase ($p > 0.05$) in brain SOD and glutathione levels in MCAO + PGE treated animals when compared with the MCAO group (Table 2).

Effect of PGE on liver antioxidant levels

Table 3 shows the effect of ginseng pretreatment on liver antioxidant activities. A significant ($p < 0.05$) decrease in liver tissue CAT activity was observed in MCAO animals compared with the control and sham groups. In contrast, PGE pretreatment showed a marked increase in tissue CAT activity in MCAO + PGE treated animals when compared with MCAO ($p < 0.005$), sham ($p < 0.05$), and control group ($p < 0.005$) animals. Liver tissue SOD activity was found to decrease in MCAO animals when compared with control ($p > 0.05$) and sham group ($p < 0.05$) animals. Per day 10 mL ginseng supplementation for 30 d increased tissue SOD activity level in the MCAO + PGE treated group when compared with the MCAO ($p < 0.005$), sham ($p < 0.05$), and control ($p < 0.005$) groups. Glutathione activity was reduced in the liver tissue of MCAO animals compared with the control ($p < 0.01$) and sham ($p < 0.05$) groups. PGE supplementation increased GSH activity level in

Table 2. Brain tissue antioxidant status among experimental groups.

Parameters	Control ($n = 9$)	Sham ($n = 9$)	MCAO ($n = 9$)	MCAO + PGE treated ($n = 9$)
Organ weight (g)	1.378 ± 0.077	1.543 ± 0.041NS	1.661 ± 0.023*/NS	1.703 ± 0.049**/*/NS
Total protein (g/dL)	4.788 ± 0.228	4.762 ± 0.122NS	4.829 ± 0.046NS/NS	4.448 ± 0.114NS/NS/*
Tissue catalase (µmol/g of tissue)	19.564 ± 1.387	19.135 ± 0.959NS	18.686 ± 0.802NS/NS	20.303 ± 0.425NS/NS/NS
Tissue SOD (U/g of tissue)	65.995 ± 2.626	63.924 ± 2.107NS	58.842 ± 1.984NS/NS	61.553 ± 0.376NS/NS/NS
Tissue glutathione (U/g of tissue)	0.061 ± 0.002	0.05766 ± 0.001NS	0.055 ± 0.002NS/NS	0.0611 ± 0.002NS/NS/NS

Note: Values are presented as mean ± SEM. Significant difference between experimental groups by *t* test are: *$p < 0.05$; **$p < 0.01$; *** $p < 0.005$. NS, non-significant, compared with control/compared with sham/compared with MCAO group. MCAO, middle cerebral artery occlusion; PGE, *Panax ginseng* extract; SOD, superoxide dismutase.

Table 3. Kidney antioxidant status among different experimental groups.

Parameters	Control ($n = 9$)	Sham ($n = 9$)	MCAO ($n = 9$)	MCAO + PGE treated ($n = 9$)
Left kidney weight (g)	0.457 ± 0.054	0.638 ± 0.061NS	0.669 ± 0.081NS/NS	0.656 ± 0.019*/NS/NS
Right kidney weight (g)	0.480 ± 0.062	0.653 ± 0.06NS	0.676 ± 0.082NS/NS	0.675 ± 0.017NS/NS/NS
Total protein (g/dL)	4.826 ± 0.092	4.624 ± 0.134NS	3.996 ± 0.178**/*	4.5 ± 0.13NS/NS/NS
Tissue catalase (µmol/g of tissue)	23.161 ± 1.394	22.872 ± 1.002NS	20.406 ± 0.508NS/NS	24.616 ± 0.587NS/NS/***
Tissue SOD (U/g of tissue)	64.924 ± 3.078	64.08 ± 2.858NS	60.77 ± 1.245NS/NS	65.402 ± 1.103NS/NS/*
Tissue glutathione (U/g of tissue)	0.072 ± 0.006	0.0816 ± 0.007NS	0.05 ± 0.002*/**	0.086 ± 0.001NS/NS/***

Note: Values are presented as mean ± SEM. Significant difference between experimental groups by *t* test are: *$p < 0.05$; **$p < 0.01$; ***$p < 0.005$. NS, non-significant, compared with control/compared with sham/compared with MCAO group. MCAO, middle cerebral artery occlusion; PGE, *Panax ginseng* extract; SOD, superoxide dismutase.

Table 4. Liver antioxidant status among different experimental groups.

Parameters	Control ($n = 9$)	Sham ($n = 9$)	MCAO ($n = 9$)	MCAO + PGE treated ($n = 9$)
Organ weight (g)	4.8 ± 0.19	4.976 ± 0.28NS	4.705 ± 0.138NS/NS	5.083 ± 0.366NS/NS/NS
Total protein (g/dL)	5.073 ± 0.165	5.27 ± 0.184NS	4.283 ± 0.244*/*	4.823 ± 0.481**/***/NS
Tissue catalase (μmol/g of tissue)	3.766 ± 0.078	3.835 ± 0.232NS	3.098 ± 0.189*/*	4.676 ± 0.075***/*/***
Tissue SOD (U/g of tissue)	11.943 ± 0.379	13.021 ± 0.236*	11.125 ± 0.251NS/***	13.866 ± 0.176***/*/***
Tissue glutathione (U/g of tissue)	6.866 ± 0.341	6.558 ± 0.222NS	5.072 ± 0.437**/*	7.94 ± 0.28*/*/***

Note: Values are presented as mean ± SEM. Significant difference between experimental groups by *t* test are: *$p < 0.05$; **$p < 0.01$; ***$p < 0.005$. NS, non-significant, compared with control/compared with sham/compared with MCAO group. MCAO, middle cerebral artery occlusion; PGE, *Panax ginseng* extract; SOD, superoxide dismutase.

MCAO + PGE animals when compared with the MCAO ($p < 0.005$), sham ($p < 0.05$), and control ($p < 0.05$) groups (Table 3).

Effect of PGE on kidney antioxidant levels

The CAT activity was slightly decreased ($p > 0.05$) in kidney tissues of animals in the MCAO group compared with the control and sham groups. Per day oral supplementation of ginseng extract significantly increased the tissue CAT activity in the MCAO + PGE group compared with the MCAO group ($p < 0.005$). Tissue SOD level was decreased ($p > 0.05$) in the MCAO group compared with the control and sham groups. Ginseng treatment significantly increased SOD activity in the kidney tissue of MCAO + PGE animals when compared with MCAO animals ($p < 0.05$). A significant decrease in tissue glutathione activity was observed in animals of the MCAO group compared with the sham and control groups ($p < 0.05$). PGE treatment showed a marked increase in tissue GSH levels in MCAO + PGE treated animals compared with the MCAO group ($p < 0.005$) (Table 4).

Effect of PGE treatment on tissue (brain, liver, and kidney) histology

Histopathological changes in brain tissue

Histopathological examination revealed cellular disintegration and swelling together with marked polymorphonuclear leucocyte (PMNL) degranulation with focal monocyte and macrophage infiltration in the brain tissue of MCAO rats. Compared with MCAO group brain tissue, ginseng-treated (MCAO+PGE) rat brains displayed minimal cellular swelling and less monocyte and macrophage infiltration, whereas no PMNL degranulation was observed (Table 5; Fig. 1).

Table 5. Histopathological index of brain in control, sham, MCAO, and *Panax ginseng* extract (PGE) treated groups.

Pathology type	Control ($n = 9$)	Sham ($n = 9$)	MCAO ($n = 9$)	MCAO + PGE treated ($n = 9$)
Cellular disintegration	—	—	+2	—
Cellular swelling	—	+1	+2	+1
Monocyte infiltration	—	+1	+2	+1
Macrophage infiltration	—	+1	+3	+1
PMNL degranulation	—	+1	+3	—

Note: Scale: (−) absent; (+) present; (+1) minimal; (+2) mild; (+3) moderate; (+4) severe. MCAO, middle cerebral artery occlusion; PGE, *Panax ginseng* extract; PMNL, polymorphonuclear leucocyte.

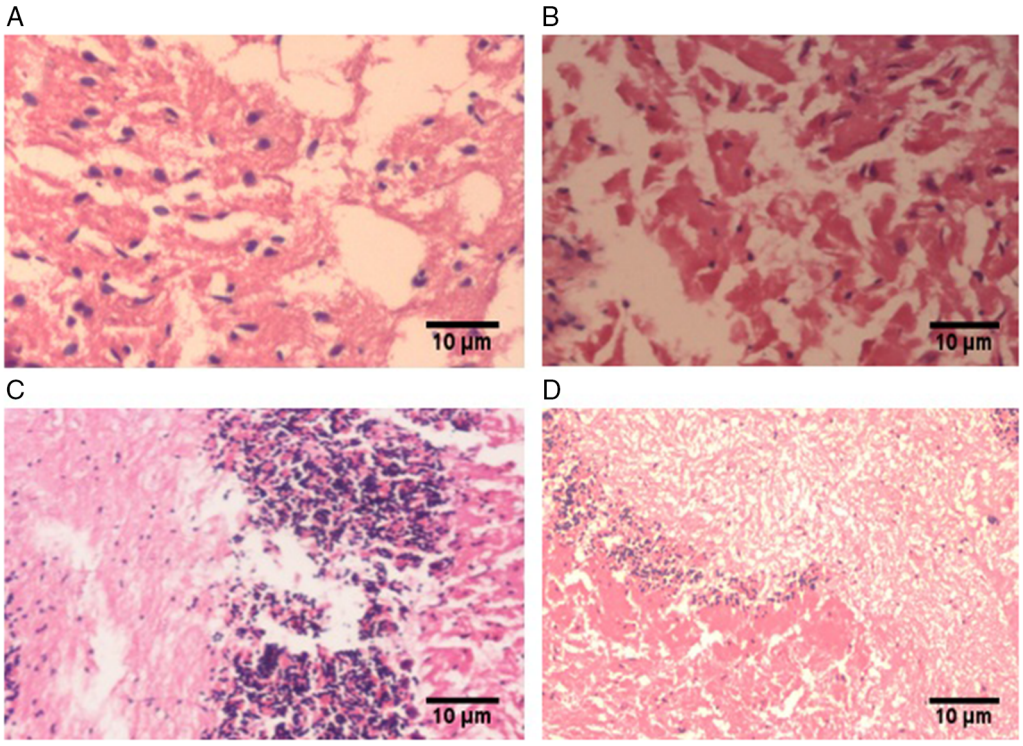

Fig. 1. Histology of brain tissue from different experimental groups. (A) Brain tissue from control group, (B) brain tissue from sham-operated group, (C) brain tissue from MCAO group, (D) brain tissue from MCAO + PGE group. MCAO, middle cerebral artery occlusion; PGE, *Panax ginseng* extract.

Histopathological changes in liver tissue

Histological examination of liver tissue from control animals revealed normal intact tissue architecture without any inflammatory signs. Liver from MCAO group animals showed moderate and focal neutrophil and PMNL infiltration. Hepatic tissue from the MCAO + PGE treated group showed preserved tissue architecture with minimal neutrophil and PMNL infiltration (Table 6; Fig. 2).

Table 6. Histopathological index of liver tissue in control, sham, MCAO, and *Panax ginseng* extract (PGE) treated groups.

Pathology type	Control ($n = 9$)	Sham ($n = 9$)	MCAO ($n = 9$)	MCAO + PGE treated ($n = 9$)
Cytoplasmic vacuolization	—	—	—	—
Nuclear condensation	—	—	—	—
Neutrophil infiltration	—	—	+2	+1
PMNL infiltration	—	—	+2	+1
Inflammatory changes	—	—	—	—

Note: Scale: (+1) minimal; (+2) mild; (+3) moderate; (+4) severe. MCAO, middle cerebral artery occlusion; PGE, *Panax ginseng* extract; PMNL, polymorphonuclear leucocyte.

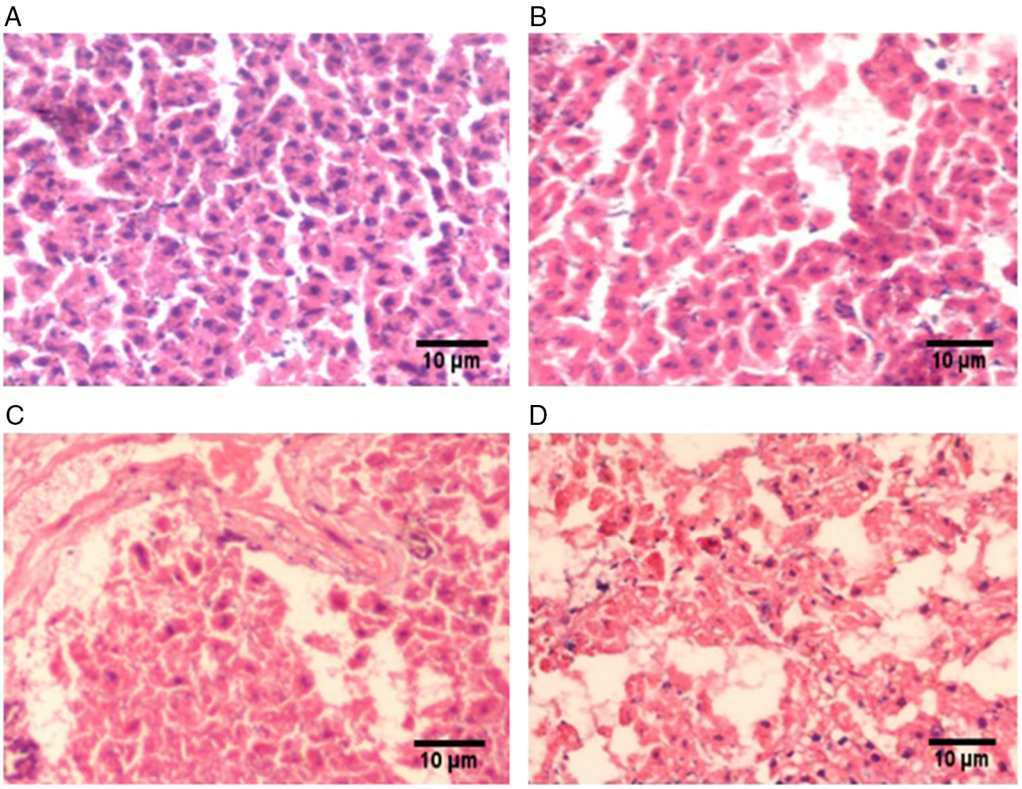

Fig. 2. Histology of liver tissue from different experimental groups. (A) Liver tissue from control group, (B) liver tissue from sham-operated group, (C) liver tissue from MCAO group, (D) liver tissue from MCAO + PGE group. MCAO, middle cerebral artery occlusion; PGE, *Panax ginseng* extract.

Histopathological changes in kidney tissue

Normal tissue histology was observed in animals in the control and sham-operated groups. Histopathological changes in kidney sections from MCAO animals showed moderate neutrophil and macrophage infiltration. Kidney sections from animals in the MCAO + PGE treated group showed minimal neutrophil infiltration (**Table 7**; **Fig. 3**).

Table 7. Histopathological index of kidney tissue in control, sham, MCAO, *Panax ginseng* extract (PGE) treated groups.

Pathology type	Control ($n = 9$)	Sham ($n = 9$)	MCAO ($n = 9$)	MCAO + PGE treated ($n = 9$)
Neutrophil infiltration	—	—	+2	+1
Monocyte/macrophage infiltration	—	—	+2	+1
Mesangial proliferation	—	—	—	—
Interstitial inflammation	—	—	—	—

Note: Scale: +1, minimal; +2, mild; +3, moderate; +4, severe. MCAO, middle cerebral artery occlusion; PGE, *Panax ginseng* extract.

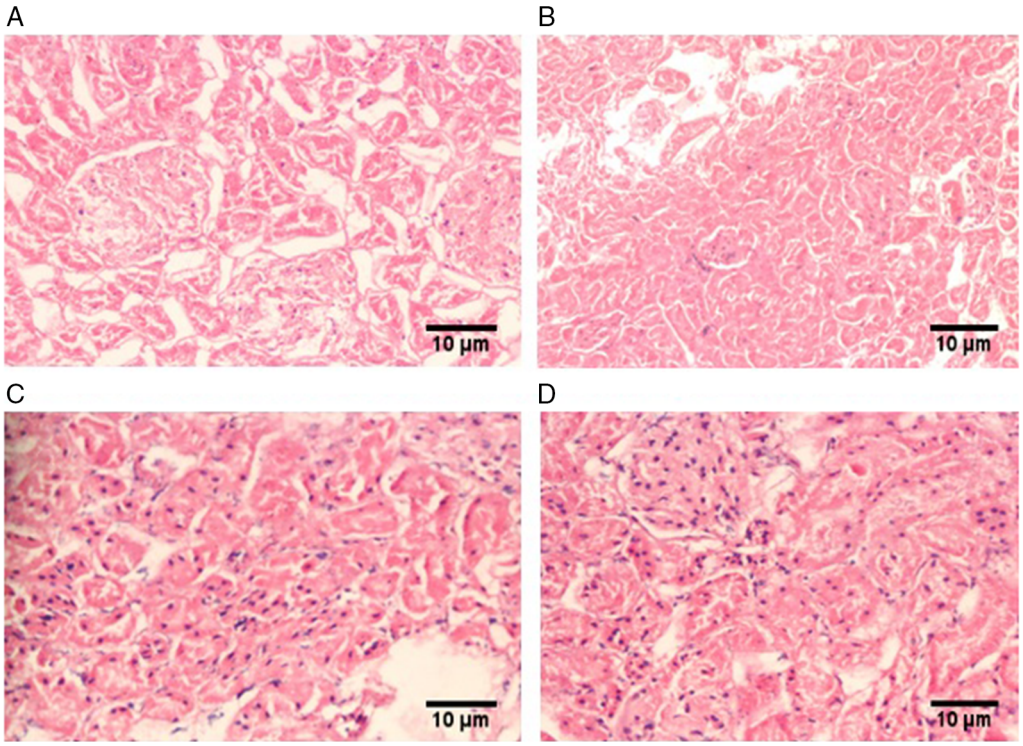

Fig. 3. Histology of kidney tissue from different experimental groups. (A) Kidney tissue from control group, (B) kidney tissue from sham-operated group, (C) kidney tissue from MCAO group, (D) kidney tissue from MCAO + PGE group. MCAO, middle cerebral artery occlusion; PGE, *Panax ginseng* extract.

Discussion

Stroke is a leading cause of death and disability around the globe (Feigin et al. 2015). During ischemic stroke loss of cerebral blood flow shuts down hemodynamic, metabolic, and biochemical processes in affected brain territory causing brain damage and loss of brain function. Reperfusion following ischemic insult aggravates the neuronal necrosis (Li and Gao 2017). Among other pathological mechanisms, chronic increase in oxidative stress (ROS) and loss of antioxidant enzyme reserves are the most deleterious outcomes of ischemic stroke (Bocci and Valacchi 2015). MCAO is a well-known rodent model to reproduce ischemic-stroke-associated brain damage to study stroke phenomena and to evaluate the efficacy of novel neuroprotective therapies (Trueman et al. 2017). With the purpose of neutralizing oxidative stress, increased antioxidant intake would be an attractive therapy against ischemic diseases. Herbs and herbal extracts are widely used to cure diseases in traditional medicinal systems, as herbs are long-recognized to possess powerful therapeutic properties such as antioxidant, antitumor, and antiinflammatory activities (Paur et al. 2011). The aim of the present study was to investigate the protective effect of *Panax ginseng* extract pre-treatment against MCAO-induced cerebral ischemia.

Thirty days of PGE administration pre-stroke-treatment showed hypolipidemic effects of ginseng in MCAO + PGE rats, non-significantly decreased plasma TC (5%) and LDL (12.3%), with 5.4% increase plasma HDL concentration; however, a non-significant increase was observed in plasma TC and VLDL levels in MCAO + PGE treated animals compared with the MCAO untreated group. The results of the present study are in accordance with those of a previous study by Delui et al. (2013), who reported non-significant changes in the plasma lipid profile up until 4 weeks of ginseng

extract supplementation. However, after 8 weeks of ginseng consumption the plasma lipid profile (TC, TG, and LDL) was significantly reduced, whereas plasma HDL levels were maintained at baseline value. According to Lee et al. (2013), ginseng saponins increase cholesterol secretion in bile acid and upregulate LDL receptors, thus decreasing plasma lipid concentration. The results of our study are consistent with these previous studies, although further investigation is needed with similar doses of PGE in experimental animals for a longer duration.

In the present study, per day ginseng treatment for 1 month showed a positive effect on liver health as exhibited by a significant decrease ($p < 0.05$) in serum ALT (24.8%), ALP (19.3%) levels, and a 5.4% decrease ($p > 0.05$) in serum AST level. In agreement with our data, a study on hypercholesterolemic rats showed a significant decrease in serum ALT, AST, and ALP levels with 40 d of ginseng (1 g/kg BWT) supplementation, compared with high-fat-fed rats. This ginseng-mediated hepatoprotective effect accentuates ginsenoside antioxidant activity including the degradation of ROS and lipid peroxyl radicals (Uluısık and Keskin 2016).

Data from our study showed that per day ginseng extract supplementation maintained kidney function in MCAO + PGE treated animals with 25%, 8.2%, 27.3%, and 33.5% reduction in the serum levels of urea, uric acid, creatinine, and BUN, respectively. In parallel to our results, ginseng-mediated nephroprotective effects were also reported by Park et al. (2015) in in vitro and in vivo models of cisplatin-induced nephrotoxicity. According to Park et al. (2015) and Kalkan et al. (2012), nephroprotective effects of ginseng are attributable to antiinflammatory, antioxidant and antiapoptotic properties of ginsenosides.

The presence of endogenous antioxidant enzymes such as CAT, SOD, and glutathione in brain, liver, and kidney protects these tissues against free-radical-induced oxidative damage (Ban et al. 2012). Tissue glutathione effectively neutralizes the hydroperoxides and other free radicals. Within the brain, GSH is the first and major defendant against oxidative damage (Imam and Ali 2000). SOD prevents hydroxyl radical formation by catalyzing superoxide radicals into H_2O_2. This H_2O_2 then detoxifies into molecular oxygen and water by the action of catalase. For brain tissue, CAT is the second line of defense in confronting oxidative damage (Imam and Ali 2000; Ban et al. 2012). As these antioxidants decrease during ischemia–reperfusion, we examined antioxidant enzyme (CAT, SOD, and GSH) status. In the present study, MCAO animals exhibited significant reduction in CAT, SOD, and GSH in brain, liver, and kidney tissues. Ginseng extract treatment effectively restored the antioxidant status in brain, liver, and kidney tissues (**Tables 2**, **3** and **4**). The results of the present study are in accordance with those of Ban et al. (2012),who reported the administration of ginseng extract at a dose of 100 mg/(kg·d) orally for 7 d significantly restored brain CAT, SOD, and glutathione peroxidase (GPx) levels in MCAO models of ischemic stroke. According to Ramesh et al. (2012), fermented ginseng extract supplementation prevented age-linked tissue deterioration as exhibited by a significant increase in cytosolic CAT, SOD, GSH, GPx, and glutathione reductase levels in kidneys, liver, heart, and lungs of aged rats. Ginseng saponins (triterpenoids) inhibit ischemia-induced oxidative damage by detaching Kelch-like enoyl-CoA hydratase (ECH)-associated protein 1 (KEAP1) from Nrf2, thus stimulating the Nrf2 signaling pathway, which reestablishes microglia's stability by upregulating antioxidant response element (ARE) gene expression including glutathione peroxidase (GPx) and SOD that ultimately degrade reactive oxygen species (Rojo et al. 2014; Rastogi et al. 2015).

CRP is an inflammatory marker that is released during acute-phase response. Elevated blood CRP levels are associated with poor disease prognosis. Data from a community-based study showed that elevated CRP levels strongly correlate with a high risk of ischemic stroke (Liu et al. 2014). The results of the present study also showed a 14% increase in serum CRP concentration in MCAO animals compared with controls ($p > 0.05$), with a ginseng treatment CRP level decrease by 6.0% in MCAO + PGE group compared with the MCAO group ($p > 0.05$). Consistent with our results, data from a study by

Costa et al. (2015) on experimental rat models of trinitrobenzenesulfonic-acid-induced intestinal inflammation also showed a decrease in serum CRP levels in rats under Brazilian ginseng treatment. LDH is another indicator of cerebrovascular pathologies. Following a transient ischemic event, increased LDH concentration has been observed, usually as a result of blood–brain barrier disruption, leakage from a cytolytic cell, or increased LDH synthesis in response to vascular damage (Valvona et al. 2016). According to Parakh et al. (2002), a marked increase in serum LDH level was observed in patients of ischemic stroke. In accordance with previous studies, the results of the current study demonstrated an increase (4.2%) in serum LDH levels in MCAO animals compared with controls. Ginseng extract treatment showed a decrease in serum LDH concentration attributable to ginseng antioxidant properties (Kim and Lee 2010). Hyperglycemia is associated with poor clinical outcomes and mortality in ischemic stroke patients (Masrur et al. 2015). Data from the current study showed that per day oral administration of 10 mL ginseng extract maintained plasma glucose level in the MCAO + PGE treated group. Consistent with our data, a study on streptozotocin-induced rats showed a significant reduction in blood glucose level with 8 weeks of ginseng extract supplementation (Moon et al. 2015). Findings from other studies suggest that the hypoglycemic properties of PG are attributable to the enhanced functioning of pancreatic beta cells and decreased insulin resistance (Luo and Luo 2009; Mucalo et al. 2012).

Histological examination of brain tissue from MCAO + PGE treated animals showed that ginseng antioxidant activity protected the brain against oxidative damage, as demonstrated by minimal cellular swelling, monocyte/macrophage infiltration, and no PMNL degranulation compared with MCAO animals. In agreement with the present data, Park et al. (2010) also showed that ginseng administration preserved brain tissue against cerebral ischemia-induced oxidative damage as well as a significant reduction in ischemic infarct volume in ginseng-treated animals. Histological examination of kidney and liver tissue indicated that ginseng supplementation maintained/preserved the normal renal and hepatic histoarchitecture in animals in the MCAO + PGE treated group. Similarly, data from studies by Park et al. (2015) and Miranda-Henriques et al. (2014) showed that the ginseng-mediated nephroprotective and hepatoprotective properties are attributable to ginseng's antioxidant and anti-inflammatory activities.

Conclusion

In brief, the results of the current study revealed that PGE protects neurons against MCAO-induced cerebral ischemic damage attributable to enhanced endogenous enzymatic activities. PGE supplementation also effectively maintains plasma lipid profile, liver enzymes, and kidney parameters at the baseline values. These results suggest that PGE could be a beneficial intervention in the risk management and treatment of ischemic stroke. Moreover, it highlights the clinical application of PGE in the treatment of ischemia–reperfusion tissue damage.

Acknowledgements

The study was partially supported by research grant from the Dean, Faculty of Science's research support program for which authors express their gratitude.

Author contributions

MS and NIK conceived and designed the study. MS performed the experiments/collected the data. MS and NIK analyzed and interpreted the data. MS and NIK contributed resources. MS and NIK drafted or revised the manuscript.

Competing interests

The authors have declared that no competing interests exist.

Data availability statement

All relevant data are within the paper.

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