PREEMPTIVE EFFECT OF *PANAX GINSENG* EXTRACT ON SENSORIMOTOR DYSFUNCTION IN EXPERIMENTALLY INDUCED MIDDLE CEREBRAL ARTERY OCCLUSION-REPERFUSION INJURY MODEL OF ISCHEMIC STROKE.

MUFZALA SHAMIM AND NAZISH IQBAL KHAN

Department of Physiology, University of Karachi, Karachi, Pakistan
Correspondence author email: mufzalashamim@yahoo.com

خلاصہ

اسٹروک(فالج) اور اکی نقائص کے ساتھ ساتھ اموات اور مستقل معذوری کی ایک اہم وجہ ہے۔ حالیہ تحقیق کا مقصد فالج کے بعد ہونے والی دماغی خلیوں کی حیاتی تبدیلیوں پر پناکس جنسنگ عرق کے استعمال کے حفاظتی اثرات کو درمیانی سیر یبرل آرٹری آکلوژن/ ریپرفیوژن ماڈل میں جانچا گیا۔

اس مقصد کیلئے ہم عمر و ہم جنس ۲۰±۲۰۰ گرام جسمانی وزن کے وسٹار چوہوں کو بلا لحظہ ترتیب گروپ ۱ (کنٹرول)، گروپ ۲ (شیم)، گروپ ۳ (MCAO/R, stroke) اور گروپ ۴ (MCAO+PGE treated) میں تقسیم کیا گیا۔ گروپ ۴ کے چوہوں کو بذریعہ منہ ۱۰ ملی لیٹر پناکس جنسنگ عرق ۳۰ دن تک پلایا گیا۔ ایک مہینہ کے بعد درمیانی سیر یبرل آرٹری آکلوژن/ ریپرفیوژن سرجری گروپ ۴ کے چوہوں پر کی گی۔ تجرباتی مدت کے اختتام پر گروپس میں اسکیمیک فالج سے متعلق حسیاتی و عصبی تبدیلیوں کو دماغی خلیوں کے حیاتیاتی امتحان سے جانچا گیا جس میں (a) neurological deficit score, corner turn, vibrissae-evoked forelimb placing ipsilesional (b) contralesional, foot-fault test اور wire-hanging امتحانات شامل تھے۔

پناکس جنسنگ عرق کے علاج نے درمیانی سیر یبرل آرٹری آکلوژن/ ریپرفیوژن نقائص کے ساتھ ہونے والی دماغی خلیوں کی حرکات میں مستقل اور اہم بہتری ظاہر کی جو کہ ND ipsilesional forelimb placement, foot faults, corner turn & score, contralesional اور wire hanging کے امتحان کے نتائج سے ظاہر ہوئی۔

اسکیمیک ریپرفیوژن سے متعلق دماغی خلیوں کے نقائص کے خلاف پناکس جنسنگ عرق کے استعمال نے اہم حفاظتی اثرات مرتب کیئے۔

Abstract

Present study aims to investigate the protective effects of *Panax ginseng* extract (PGE) following middle cerebral artery occlusion/reperfusion (MCAO/R) model in relation to post-stroke neurobehavioral changes.

Age and sex-matched *Wistar* rats of body weight 200 ± 20g were randomly divided as *group I*- (control group), *group II* (sham group), *group III* (MCAO/R, stroke group) and *group IV* (MCAO+PGE treated group). Group IV animals were orally administered with *panax ginseng* extract 10mL/day for thirty days. After one month MCAO/R surgery was employed to group IV animals. At the end of experimental period, all groups were tested for ischemic stroke-related sensorimotor changes via battery of neurobehavioral tests including neurological deficit score, corner turn test, vibrissae-evoked forelimb placing test (a) ipsilesional limb and (b) contralesional limb placement, foot-fault test and wire-hanging maneuver.

PGE treatment showed consistent and significant ($P<0.05$) improvement in neurobehavioral activities following MCAO/R injury as expressed by significant reduction in ND score, significant improvement in contralesional & ipsilesional forelimb placement, foot faults, corner-turn and wire hanging maneuver.

PGE consumption has significant protective effect against ischemia-reperfusion associated neuronal injury as assessed by recovery of neurobehavioral functions.

Introduction

Globally, stroke is the leading cause of death and permanent disabilities accompanied with sensory deficits, motor malfunctioning such as poor motor coordination and paralysis, cognitive impairments, perceptive disturbances and emotional ailments (de Diego, *et al.*, 2013; Go and Lee, 2016) affecting around 15 million people per year with nearly 6 million fatalities. Among other forms of stroke, ischemic stroke is the most prevalent one, accounts for ≈87% of all type of stroke cases (Benjamin, *et al.*, 2017). According to several MCAO (middle cerebral artery occlusion) model of ischemic stroke, brain territories that usually get affected with ischemic insult are higher cortical regions (Wang, *et al.*, 2007; Wen *et al.*, 2017) striatum or sensorimotor cortex, hippocampus and associated areas (Kerr and Tennant, 2014; Schallert, 2006).

The principal molecular mechanisms that involve in the pathogenesis of ischemic stroke are oxidative stress, inflammation, excitotoxicity, and apoptosis, causing lipid peroxidation and reactive oxygen species (ROS) generation which ultimately results in mitochondrial dysfunction related critical ATP depletion and its consequences (Ban *et al.,* 2012; Mehta, *et al.,* 2007; Shin, Park, & Kim, 2006). Post-stroke, patients suffered a vast array of sensorimotor impairments depending on affected brain region such as damage to higher cortical regions results in memory instabilities and other related cognitive deficits, therefore, post-stroke assessment of neurological functions outcome is highly significant as it allows to assess the extent of the neuronal damage. In this regard, several animal studies demonstrate MCAO associated behavioral deficits (Lipsanen and Jolkkonen, 2011). According to National Research Council (US), alterations in brain chemical or electrical activities change animal behavior, therefore, relative animal models can be used to understand disturbed neurological mechanisms that develop as a consequence of brain injury, disease, genetic modifications, exposure to toxic substances or therapeutic side effects (Research, 2003).

Recombinant tissue plasminogen activator (r-tPA) is the only food & drug administration (FDA) approved therapeutic drug for ischemic stroke that should be administered within first 2 hours following a stroke hence less than 4% patients get assistance from r-tPA (Bae *et al.,* 2013; Ginsberg, 2009; Wardlaw *et al.,* 2012).

Panax Ginseng is a popular ancient medicinal herb used in the alternative medicinal system since years. Ginsenosides (a group of saponins) are the principal bioactive agents responsible for ginseng's pharmacological properties. Ginsenosides possess several health benefits and marked anti-oxidative, anti-inflammatory, anti-apoptotic, hypoglycemic, antianxiety, anti-depressive and hypotensive properties (Ong *et al.,* 2015). Ginseng also modulates various pathophysiological aspects of neuropsychiatric, neuro-developmental and neurodegenerative disorders. Several research studies also reveal ginseng's beneficial properties regarding cognitive impairments and immunity (Kim, *et al.,* 2013; Rastogi, *et al.,* 2015). In view of this context present study aimed to investigate the protective neurobehavioral effects of *Panax Ginseng* aqueous extract consumption in MCAO/R animal models via battery of neurobehavioral tests & composite scoring scale.

Material and Methods

Ethical Guidelines

Study protocol conforms to the *Guide for the Care and Use of Laboratory Animals*, National Research Council, US

Animal Model

A total of 36 female Wistar rats with average body weight of 200±20g (age 5-6 weeks) were used for the study. Animals were purchased from animal care facility of International Center of Chemical and Biological Sciences, University of Karachi, Karachi, Pakistan. All animals were allowed to acclimatize for one week prior experimentation in the animal house of Department of Physiology, University of Karachi. During the study period, animals were provided with standard laboratory diet and water *ad libitum*.

Experimental Protocol

Animals were randomly divided into following groups.

Experimental Group I- (Control: n=9): Received normal lab diet and water for consecutive 30 days.

Experimental Group II- (Sham-operated: n=9): Received normal lab diet for 30 days. Animals were then anesthetized to expose left common carotid artery and external carotid artery without any ligation or occlusion. Behavioral tests were assessed after 24 hours of sham operation.

Experimental Group III- (MCAO/R: n=9): Received normal lab diet for 30 days. At day 31st MCAO/R surgery was employed to this group.

Experimental Group IV- (MCAO+ PGE treated: n=9): Together with normal lab diet, animals of this group orally (gavage) received 10 ml PG extract (≈ 4.3g ginsenoside) per day for 30 days (modified from the study of Ban *et al.,* 2012). MCAO/R surgery was performed at 31st day. Sensorimotor changes were assessed following 24 hours of reperfusion.

Middle Cerebral Artery Occlusion/Reperfusion (MCAO/R) Model

Focal cerebral ischemia was induced via Intraluminal monofilament model of MCAO/R method as previously described by Memezawa and colleagues (1992) and revised by Chiang *et al.,* 2011(Memezawa *et al.,* 1992). Briefly, following successful anesthesia, the left common carotid artery and the external carotid artery were exposed. Silicon coated surgical monofilament was inserted into ECA and threading forward into the internal carotid artery until the tip occludes the origin of the middle cerebral artery. Filament was removed after 15 minutes of MCAO following reperfusion of 24 hours. **N**eurological impairment was evaluated after 24 hours of MCAO induction via composite score and other sensorimotor tests (Bederson *et al.,* 1986; Encarnacion *et al.,* 2011).

***Panax Ginseng* Extract (PGE) Preparation**

Panax ginseng roots was purchased from local market of Karachi (Karachi, Pakistan). PG aqueous extract was prepared by decoction method as described by (Ban *et al.*, 2012). Prepared PG extract contains ≈0.43 gram active ginsenosides in per ml of extract, measured spectrophotometrically by the method of Kevers & colleagues at the 520nm wavelength (Kevers *et al.*, 2017).

Assessment of Neurological Function via Neurobehavioral Tests

At the end of the experimental period, all groups were tested for MCAO/R associated sensorimotor changes via a battery of neurobehavioral tests including neurological deficit score (NDS), corner turn test, vibrissae-evoked forelimb placing test [(a) ipsilesional limb placement & (b) contralesional limb placement], foot-fault test and wire-hanging maneuver.

Animals were handled and trained by experimenters, all behavioral tests were conducted during light phase and approximately at the same time, every day.

Neurological Deficit Score

In the present study neurological deficit scoring used to evaluate ischemic stroke-related neurological abnormalities as reported by Yamamoto *et al.*, (1988) & De Ryck *et al.*, (1989; Roulston, 2017). ND scoring comprised of three neurological tests: (1) body twisting (2) forelimb flexion, and (3) balance test. Scoring for all tests was based on a set of pre-determined criteria as described by (Roulston, 2017).

Scoring: $ND\ Score = \ test\ 1\ score + \ test\ 2\ score + \ test\ 3\ score$

Neurologic functions were rated on a scale of 0 to 9. The scores for each test were added to calculate total neurological deficit with a highest possible score of 9, indicating maximal neurological deficit, and the lowest score of 0, for normal animals with no impairment/deficit. ND score was compared between post-stroke (test group) and pre-stroke (control group) so that each animal act as its own control.

Corner Turn Test

Corner turn test is a sensorimotor test based on animal's ability to turn/move in a specific direction depending on the magnitude of injury hence besides sensorimotor evaluation, the test is also helpful in revealing postural asymmetries (Lekic, *et al.*, 2012).

Animal was placed halfway between alley (corner) of a box with face towards the corner. As the rat approaches the corner, both side vibrissae were stimulated simultaneously hence the intact animal reared upward & forward and turned 180º to either left or right randomly while animal with unilateral lesion such as in MCAO models, animal preferentially turned around in ipsilateral (left) direction, leading with non-impaired limb and displaying asymmetry in corner turning. Each animal was tested for 10 trials and the side chosen for turning was noticed. Turning without rearing were not scored. Finally, results were expressed as % left (ipsilateral) turns compared to a total number of turns (Ye, *et al.*, 2011).

Scoring: $\%\ CT\ score = \ [(Number\ of\ left\ turns)\ \div\ (Number\ of\ right\ +\ left\ turns)] \times 100$

Vibrissae-Evoked Forelimb Placing Test

Forelimb placing test used to evaluate the extent of nervous tissue damage associated with focal ischemic brain injuries. This test allows the evaluation of forelimb sensorimotor integration ability following brain injuries as rodents use their vibrissae to gain surrounding information (Hua *et al.*, 2002). Experimenter holds the animal by torso to allow all four limbs hang freely. Forelimb testing was assessed by brushing the vibrissae on the corner edge of a table. Animals without any brain damage elicit a response of placing forelimb ipsilateral to vibrissae evoked side while animals with damage to the motor system, such as in MCAO, elicit impairment in paw placement. Each animal was tested for 10 trials and vibrissae evoked each forelimb (ipsilateral & contralateral limb) placement was noted.

***Ipsilesional Forelimb Placement*:** While restraining animal's contralateral paw, experimenter brushes the vibrissae against table corner edge. Number of successful ipsilateral limb placement was recorded. 10 trials were performed on each lateral side.

***Contralesional Forelimb Placement*:** Ipsilateral limb was restrained, number of successful forelimb placement, contralateral to whisker brushed against table edge were recorded.

The test was scored by calculating the percentage of ipsilateral and contralateral limb placement response (Hua *et al.*, 2002; Schallert, 2006).

Scoring: $\%\ successful\ placement\ score = \ [(Paw\ Placement)\ \div\ (10\ trials)] \times 100$

Foot-Fault Test

Foot-fault test used to assess sensorimotor functioning and limb placing deficits during locomotion in ischemic stroke rodent models. This task can also use to study the post-stroke rehabilitation effects (Encarnacion *et al.*, 2011). Animal was trained to walk across elevated, leveled horizontal ladder with openings. Non-brain damaged animals learn precise paw placement during a walk while the animals with brain damage elicit paw misplacement, every time limb slipped from opening a "foot-fault" is recorded.

Scoring: Individual foot faults for both ipsilateral and contralateral limb were noted, compared with total number of steps taken to calculate foot fault index (FFI) as reported by Bland *et al.*, (2000).

Scoring: $FFI = [(contralateral\ footfault\ - ipsilateral\ footfault) \div (total\ steps)]$

A score of 0 indicates no asymmetry, negative score represents ipsilateral deficit while positive score exhibit contralateral deficit (Bland *et al.*, 2000).

Wire Hanging Maneuver:

Wire hanging maneuver is a motor functioning test to assess rodent's grip strength, grasping skills and sensorimotor coordination. Animals with focal brain ischemia exhibit poor performance in the wire-hanging task because of the impaired grip of contralateral limb. In the present study, we used modified wire hanging task as described by Balkaya and colleagues 2013. In this task animal was suspended on a wire 60 cm above a cushion. Animals were trained to hold the wire with forepaws only, for that reason animal's hind limbs were gently concealed with adhesive tape (Balkaya, *et al.*, 2013; Canazza, *et al.*, 2014).

Scoring: Wire holding time (time to drop-off the wire) was noted. Increase wire holding time indicates better motor endurance/grip strength.

Statistical Analysis

Data management and statistical analysis was done using SPSS 16.0 software package. Values were presented as mean ± standard error of mean (SEM). Values in percentage (%) presented as value ± standard deviation (SD). Statistically significant differences among different experimental groups were evaluated by one-way analysis of variance (ANOVA) and $P<0.05$ was taken as the expected level of significance.

Results

Prior to MCAO induction, behavioral analyses of all experimental animals showed normal behavior patterns.

Neurological Deficit Sore: Post-stroke 2nd day i.e. 24 hours after MCAO induction all animals display typical stroke-induced behavioral deficit characterized by significantly increased ND score and impaired neurological performances. MCAO group exhibit elevated ND scores when compared with pre-ischemic (control) group ($p=0.00001$) and with sham group ($p<0.005$; $p=0.0000007$). For 30 days daily supplementation of PGE showed consistent and significant ($p<0.05$) improvement in neurobehavioral activities following MCAO/R injury characterized by significant reduction in ND score in *MCAO+PGE* treated group as compared to MCAO untreated group $p=0.0001$ (Table I).

Corner Turn Test: In corner turn test, the percentage of left i.e. ipsilateral turns were observed in order to examine animals' preferential reliant limb. Animals with unilateral brain damage display asymmetry in corner turn test while intact animal turn randomly either in left or right direction as also exhibit by our data. Control animals took an approximately equal number of right & left turns during experimental session hence showed no corner turn asymmetries. Average left turns 53.3% ±0.852 and right turns 46.7% ±0.816 (Table I). However, animals from MCAO group displayed a significant increase in ipsilateral left turns (71.66%±0.752) when compared with control ($p=0.002$) and sham groups ($p=0.002$) demonstrating animal's reliance on non-impaired (left) limb and showing corner turn asymmetry. 1-month PGE treatment exhibit decrease in % left turns in *MCAO+PGE* treated group (63.3%±1.03) when compared with MCAO group ($p>0.05$) (Table I).

Vibrissae-Evoked Forelimb Placing Test: Animals were tested for two versions of Vibrissae evoked forelimb placing test. (a) Same side (Ipsilesional) forelimb placement & (b) Cross-midline (contralesional) forelimb placement test. Percent successful limb placement was noted whereas lower scores (%) indicates greater impairment. MCAO induction results in significant forelimb placement deficit compared with all other groups. MCAO group exhibit significantly more impaired contralesional forelimb placement (13.3%±1.2) than in same-side ipsilateral forelimb placement (27.1%±0.75) compared to pre-ischemic ($p<0.005$) and sham groups ($p<0.005$). Per day oral administration of PG extract for 30 days showed significant improvement in contralesional (61.5%±0.75; $p<0.005$) & ipsilesional (75%±0.54; $p<0.005$) forelimb placement in *MCAO+PGE* treated group as compared to MCAO untreated group (Table I).

Foot Fault Test: In foot fault test number of foot faults for forelimb and hind limb were recorded and scored as foot fault index (FFI). Animals from control and sham group score zero (FFI=0) for both forelimb and hind limb whereas MCAO group exhibit impaired foot fault task (Table I). Animals from MCAO group scored significantly higher FFI for hind limb ($FFI_{Hindlimb}$=0.15; p=0.01) than forelimb ($FFI_{Forelimb}$=0.1; p>0.05) compared with pre-ischemic control group. Daily PG extract treatment improves animals' performance in foot fault test as indicated by a reduction in foot fault index. There was a stable improvement in foot fault index (forelimb & hind limb) in *MCAO+PGE* treated group $FFI_{Hindlimb}$=0.084 and $FFI_{Forelimb}$=0.05 demonstrating stroke affected contralateral hind limb deficit is more compared to contralateral forelimb, however, the differences were non-significant (p>0.05) (Table I).

Wire Hanging Maneuver: Results of wire hanging test (Table I) revealed that there was significant difference in average latency to fall among control, sham, MCAO and *MCAO+PGE* treated group. Animals subjected to MCAO/R injury had significantly reduced wire hanging time compared with control (p<0.005) and sham (p<0.005) groups. Daily treatment with ginseng extract for 30 days improve animal's grip in wire hanging maneuver. Data from present study revealed that *MCAO+PGE* treated group exhibit better grip and increase wire holding time i.e. increased latency (25.66± S.E.M. 1.22) when compared with MCAO untreated group (15.5± SEM 1.52 p=0.0004) (Table I)

Table 1: Comparison of Neurobehavioral Tests among Different Experimental Groups under Treatment with Panax ginseng Extract

	Control	Sham	MCAO	MCAO+PGE Treated
Neurological Deficit (ND) Score				
ND Score	0±0	0.833±0.401*	7.833±0.477$^{***/***}$	4.0±0.447 $^{***/***/***}$
Corner Turn Test				
Ipsilateral turns (%)	53.33±0.852	48.33±1.169 ^{NS}	71.66±0.752$^{**/**}$	63.33±1.032 $^{NS/*/NS}$
Vibrissae Evoked Forelimb Placing Test				
Ipsilesional Limb Placement (%)	91.70± 0.408	80±1.095*	21.70± 0.752$^{***/***}$	75±0.547$^{***/NS/***}$
Contralesional Limb Placement (%)	91.70±0.752	88.30±0.752 ^{NS}	13.30±1.21$^{***/***}$	61.70±0.752$^{***/***/***}$
Foot Fault Index (FFI)				
$FFI_{Forelimb}$	0	0^{NS}	0.1±0.040$^{NS/NS}$	0.05±0.020$^{NS/NS/NS}$
$FFI_{Hindlimb}$	0	0^{NS}	0.15±0.061$^{*/*}$	0.084±0.034$^{NS/NS/NS}$
Wire Hanging Maneuver				
Wire Holding Time (seconds)	57.66±0.61	52.66±0.66*	15.5±1.52$^{***/**}$	25.66±1.22$^{**/NS/NS}$

Values in % are presented with ± SD (standard deviation)
Significant difference among various experimental groups by t-test * P< 0.05, **P<0.01, ***P<0.001,
NS= Non-Significant. Compared with MCAO/Compared with PGE Treated

Discussion

Ischemic stroke produces devastating sensory, motor, and cognitive effects. Stroke patients suffer from poor balance and motor coordination, cognitive skills such as memory, attention, and task solving capabilities also reduce significantly. Systemic inflammation and oxidative stress are the key contributors of ischemia associated neuronal damaged and subsequent stroke complications (Nabavi, Sureda, Habtemariam, & Nabavi, 2015). of Reactive oxygen species production plays a key role in the pathogenesis of ischemic stroke. A large number of studies confirms the role of oxidative stress in damaging the anatomical brain regions leading to various sensory and motor deficits.

Similarly, our data also shown ischemic stroke-related neurological impairments in MCAO model group. when compared with control group, animals from MCAO group scored maximum neurological deficit score (NDS=7.833±0.477; P<0.005) and foot fault index ($FFI_{Forelimb}$=0.1 and $FFI_{Hindlimb}$=0.15), MCAO animals had significantly poor grip and memory as exhibit by vibrissae-evoked forelimb placing test (P<0.005), wire-hanging test (15.5 seconds latency to fall, P<0.005) and corner turn test (P=0.003) as compared to control group demonstrating the MCAO/R associated sensorimotor deficits. As described by the study of Rodrigo and colleague (2013), following acute ischemic stroke ROS generation damage cells' macromolecules activating autophagy, necrosis and apoptosis cascades. Additionally, rapid blood flow restoration facilitates tissue oxygenation causing to the second surge of ROS responsible for reperfusion injury (Rodrigo *et al.,* 2013). Another study on MCAO rat models demonstrates that glucose and oxygen deprivation causes neuronal injury in the hippocampus while glutamate-induced injury to cortical neurons following ischemic stroke (Nabavi *et al.,* 2015). Consistent with these studies, data from the present study also validates the MCAO associated neuronal damage leading to functional deficits as assessed by a battery of behavioral tests.

Ginseng is a popular herb used in complementary and alternative medicine since long. In the present study, we investigate the potential neuroprotective effects of *Panax Ginseng* in the treatment of ischemic stroke associated sensorimotor dysfunctions. We used the MCAO/R model for induction of transient ischemic stroke with reperfusion injury. We use a battery of neurobehavioral tests & composite scoring scale for the assessment of MCAO related neurobehavioral impairment and effect of *Panax ginseng* in the recovery or prevention of these impairments. Ginseng and its bioactive ginsenosides are known to possess powerful antioxidant properties, by preventing ROS production ginsenosides decreases the ischemic injury and improves post-stroke sensory and motor functions.

In the present study, 30 days pre-treatment with ginseng extract produce restorative effects in MCAO+PGE treated groups. As revealed by significantly improved ND and FFI scoring in *PGE treated group* (ND score=4±0.447, P<0.001; $FFI_{Forelimb}$=0.05 and $FFI_{Hndlimb}$=0.084) when compared with *MCAO group*. Similar results also demonstrated by Ban & colleagues study, 100mg/kg of oral ginseng extract administration to MCAO animals significantly improve neurological deficit and corner turn test (Ban *et al.,* 2012). Keeping with previous study Hu *et al.,* (2013) data also shown that pre-treatment of MCAO rats with 10mg ginsenoside per day per kg of body weight decrease poly(ADP-ribose) polymerase-1 with subsequent downregulation of apoptosis-inducing factor's translocation and Nuclear Factor kappa-light-chain-enhancer of activated B cells (NFK-B) p65 subunit suggesting anti-inflammatory and anti-apoptotic actions of ginseng (Hu *et al.,* 2013). Results of our study also showed that daily consumption of 10ml PG extract (≈4.8g ginsenosides) improves the grip and wire holding time in animals of MCAO+PGE treated group. MCAO+PGE treated animals also showed increased wire holding time (25.66 seconds) when compared with animals of MCAO group (15.5 seconds). Ye & colleagues (2011a,b) in their same study on transient stroke mice models also revealed that pretreatment of animal models with 10-50mg ginsenoside significantly improve neurological functioning and survival together with reduced striatal and cortical infarct size, decreased oxidative stress, DNA damage, lipid peroxidation, protein carbonyl formation and with upregulation of endogenous antioxidant enzymes status (Ye *et al.,* 2011a; 2011b). Consistent to the results of present study, another experimental study with per day oral administration of *Panax ginseng* (200mg/kg of body weight) for 15 days to MCAO rats showed decreased ischemia/reperfusion associated neuronal damage as exhibited by enhanced sensorimotor activity with better ND score, % turns in corner turn test, enhanced grip, balance and coordination in Rota-rod test (P<0.05) (Park *et al.,* 2010).

These neuroprotective effects of ginseng are reported to be mainly because of its anti-oxidative, anti-apoptotic and anti-inflammatory properties. Ginseng provides neural protection against ischemic injury by decreasing calcium influx and with upregulation of phosphatidylinositol 3-kinase/AKT, ERK1/2 (extracellular signal regulated kinase ½) and glutamate transporter (GLT-1) signaling pathways. Moreover, ginsenosides by suppressing caspases, apoptosis-inducing factors and by decreasing poly (ADP-ribose) polymerase-1, prevent cytochrome c release, inhibition of nuclear factor kappa B and glycogen synthase kinase-3b thereby extending neural cell survival under ischemic scenarios (Nabavi *et al.,* 2015). Restoration of neural and cognitive functions with per day use of 10ml *Panax* ginseng extract for thirty days in MCAO+PGE treated experimental animals in our study evidence beneficial effects of ginseng on these neuromodulatory pathways.

Conclusion

In conclusion, data from present study indicated that pre-treatment of transient MCAO rats with *Panax ginseng* prevents from ischemia/reperfusion injury associated functional deficit primarily by facilitating neuronal survival and by reducing oxidative stress.

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