Ameliorative effects of 6-gingerol in cerebral ischemia are mediated via the activation of antioxidant and anti-inflammatory pathways

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Received November 10, 2022; Accepted February 1, 2023

DOI: 10.3892/br.2023.1608

Abstract. Focal ischemia occurs when an embolus or thrombus occludes an artery, causing the rapid obstruction of cerebral blood flow. Although stroke represents a main cause of disability and mortality in developing countries, therapeutic approaches available for this condition remain very limited. The aim of the present study was to examine the effects of the phytochemical, 6-gingerol, on the brain infarct volume, neuronal loss and on the oxidative stress parameters, cyclo-oxygenase-2 (COX-2) and interleukin (IL)-6, in an animal model of focal ischemic stroke. Male Wistar rats, weighing 250-300 g, were divided into the following six groups: i) The control; ii) right middle cerebral artery occlusion (Rt.MCAO) + vehicle; iii) Rt.MCAO + piracetam; iv) Rt.MCAO + 6-gingerol (6-Gin) at 5 mg/kg body weight (BW); v) Rt.MCAO + 6-Gin at 10 mg/kg BW; and vi) the Rt.MCAO + 6-Gin at 20 mg/kg BW group. The rats in each group received the vehicle or piracetam or 6-gingerol intraperitoneally for 7 days following Rt.MCAO. The brain infarct volume, neuronal loss and alterations in antioxidant and anti-inflammatory levels were assessed in the cortex and hippocampus. The results revealed that the brain infarct volume, malondialdehyde level and the density ratio of COX-2 and IL-6 to β-actin were significantly decreased following treatment with 6-gingerol. In addition, neuronal density and superoxide dismutase activity in the cortex and hippocampus were increased. On the whole, the findings of the present study suggest that 6-gingerol exerts antioxidant and anti-inflammatory effects *in vivo*, which effectively ameliorate the brain damage induced by focal cerebral ischemic strok

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Key words: stroke, middle cerebral artery, 6-gingerol, antioxidant, anti-inflammatory

Introduction

Focal ischemia occurs when an embolus or thrombus occludes an artery, causing the rapid obstruction of cerebral blood flow (CBF). There is increasing evidence to indicate that stroke is a main cause of disability and mortality in developing countries (1). Moreover, the global economic burden of stroke on health care is enormous. At present, stroke accounts for ~34% of total global healthcare-related costs (2). In addition, effective therapeutic strategies for stroke remain very limited. Novel treatment options are thus required.

Normally, the average CBF for an adult is ~50 ml/100 g/min, which is crucial for brain function (3). In focal infarcts, the CBF decreases to <10 ml/100 g/min, causing irreversible neuronal damage (4). Folioing the onset of complete ischemia, the levels of adenosine triphosphate (ATP) are depleted, disrupting ionic homeostasis. Radical species then form and these are involved in mediating damage. Cells have enzymatic and non-enzymatic defense mechanisms to protect themselves from this type of damage (5). Enzymatic mechanisms include neutralization by superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT). Previous studies have demonstrated that increasing the levels of these antioxidant enzymes can mitigate brain damage caused by ischemic stroke (5-7).

Neuronal inflammation and apoptosis are also induced by the oxidative stress described above (8). Arachidonic acid derivatives and prostaglandins are the major inflammatory mediators. With the onset of ischemia, the levels of intracellular calcium increase, with the consequent activation of phospholipase. The metabolites of arachidonic acid are the most critical contributors to the pathophysiology of ischemic stroke. A number of studies have found that interleukin (IL)-1 and IL-6 are also mediators of ischemic damage (9-11). Cyclooxygenase-2 (COX-2) is also a mediator of inflammation (12,13), and there is accumulating evidence to indicate that COX-2 suppression can attenuate ischemic injury following middle cerebral artery occlusion (14). Herbal bioactive components exhibit anti-inflammatory, antioxidant and anti-apoptotic properties that may have therapeutic potential against the neuronal injury caused by ischemic stroke.

The main phytochemical present in ginger (*Zingiber officinale*; Zingiberaceae family) is 6-gingerol. This compound exhibits a variety of pharmacological properties, including anti-inflammatory, antioxidant (15,16), anti-apoptotic (17) and anticancer activities (18). Additionally, a previous study demonstrated that 6-gingerol significantly reduced the infarct volume and brain damage following ischemia/reperfusion injury by inhibiting NLR family pyrin domain containing 3 inflammasome-induced inflammation and neuronal apoptosis (19). However, the effects of 6-gingerol against focal ischemic stroke-induced brain damage have not yet been evaluated, at least to the best of our knowledge. Thus, the present study examined the effects of 6-gingerol on the brain infarct volume, neuronal loss and on the oxidative stress parameters, COX-2 and IL-6, in rats following focal ischemic stroke.

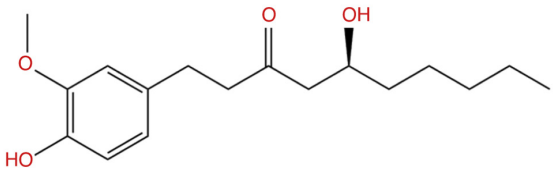

Figure 1. Chemical structure of 6-gingerol.

Materials and methods

Test treatments. The test compound, 6-gingerol (98.7% purity; chemical structure illustrated in Fig. 1), was purchased from Chengdu Biopurify Phytochemicals Ltd. Piracetam, the positive control, was obtained from Glaxosmithkline (Thailand) Ltd. and DMSO, the vehicle, was obtained from Thermo Fisher Scientific, Inc. (product code: D/4121/PB15).

Study design. A total of 90 healthy male Wistar rats (weighing 250-300 g, 8 weeks old, from the Northeastern Laboratory Animal Center, Khon Kaen University, Khon Kaen, Thailand) were randomly divided into six groups (n=15 per group) as follows: i) The control; ii) right middle cerebral artery occlusion (Rt.MCAO) + vehicle; iii) Rt.MCAO + piracetam at 250 mg/kg body weight (BW); iv) Rt.MCAO + 6-gingerol (6-Gin) at 5 mg/kg BW; v) Rt.MCAO + 6-Gin at 10 mg/kg BW; and vi) the Rt.MCAO + 6-Gin at 20 mg/kg BW group. The animals in all the groups received their treatments by intraperitoneal (i.p.) injection once daily for 7 consecutive days following Rt.MCAO. All rats were housed in groups of five in standard metal cages, maintained under standard conditions with a 12-h on/off light/dark cycle, relative humidity controlled at ~30-60%, and a temperature controlled at 23±2˚C. The rats had *ad libitum* access to water and commercial pellets (24 h/day). The piracetam at 250 mg/kg BW and 6-gingerol at 5, 10 and 20 mg/kg BW were selected based on previous research by the authors, preliminary studies and literature reviews (6,16,20). Moreover, as previously reported, the median lethal dose of gingerol (i.p. administration) has been determined in mice and rats. The dose required to kill half the members of a tested population following a specified test duration value (LD50) has been reported to be ~58.1 mg/kg BW (21). Thus, the doses used in the present study were noted to be non-toxic. The infarct volume was examined for 5 rats in each group using 2,3,5-triphenyltetrazolium chloride (TTC) staining. Another 5 rats per group were used to measure cerebral cortex and the hippocampus neuronal density using cresyl violet staining. The remaining 5 animals per group were used to examine the malondialdehyde (MDA) levels and SOD activities in the cortex and hippocampus mitochondria using biochemical assays. IL-6 and COX-2 expression levels were also measured in the cortex and hippocampus of the rats treated with those doses of 6-gingerol that produced optimum changes in infarct volume, neuronal density and oxidative stress markers (Fig. 2). Two replicates were performed for each test.

Model of Rt.MCAO. All animals were fasted overnight, but were allowed free access to water before their surgery. The animals were anesthetized using isoflurane (5% for induction and 1-3% for maintenance) delivered in 100% oxygen. The model of focal ischemia was established by the permanent intraluminal occlusion of the right middle cerebral artery, as previously described (22). Briefly, a 4-0 silicone-coated monofilament (USS DG^{TM} Division of United States Surgical; Tyco Healthcare Group LP, Norwalk, CT, USA) was inserted into the internal carotid artery ~17 mm or until a slight resistance was detected. The wound was then sutured and 10% povidone iodine solution was applied at the incision site for antiseptic postoperative care. In the sham operation, all the arteries were exposed as described above, but monofilament insertion was not performed. The criteria for humane endpoints was defined as the inability to move, wound infection following surgery, a weight loss of >20%, dehydration, dyspnea, progressive pain, lack of response to external stimuli and bleeding from any orifice. However, all animals in the present study survived to the end of the study period (8 days).

Determination of brain infarct volume. At the end of the study period, the rats were anesthetized with thiopental sodium (80 mg/kg BW; i.p. administration) prior to cardiac perfusion with cold normal saline solution. The brains were then removed from the skull and the 2-mm-thick coronal sections were stained with 2% TTC (MilliporeSigma) in normal saline for 30 min at 37˚C. Images were then obtained using a digital camera and the infarct volume was determined using Image J® software (version 1.53e, National Institutes of Health). The infarct volumes were then calculated using the formula described in a previous study by the authors (6).

Cresyl violet staining for neuronal density determination. Serial coronal sections of the cortex and hippocampus (30-μm-thick) were stained with cresyl violet acetate solution (MilliporeSigma) for 16 min at 60˚C to determine the neuronal density. Regions of the cortex and hippocampus (CA1, CA2 and CA3) were then examined using an Olympus light microscope (model CX23; Olympus Corporation) at x40 magnification. Images of the cortex and hippocampus at stereotaxic co-ordinates, selected as described in a previous study by the authors (23), were used to measure neuronal density. This was achieved by blinded analysis, and the data are expressed as a percentage of the control.

Isolation of brain mitochondria for biochemical assays. Following the completion of perfusion, brain tissues from

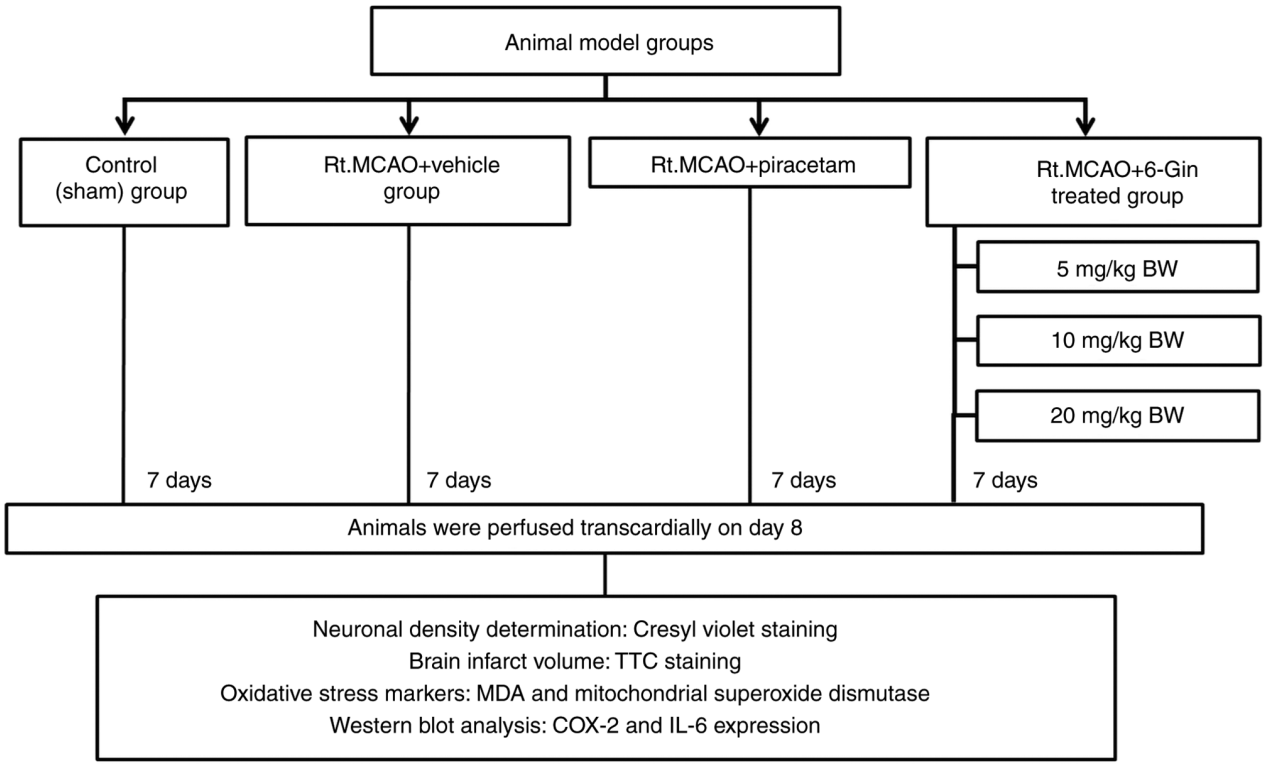

Figure 2. Schematic diagram of the experimental design use in the present study. Rt.MCAO, right middle cerebral artery occlusion; BW, body weight; TTC, 2,3,5-triphenyltetrazolium chloride; MDA, malondialdehyde; COX-2, cyclooxygenase-2; IL-6, interleukin 6.

the cerebral cortex and hippocampal regions were isolated and prepared for mitochondrial extraction, using a protocol described in a previous study by the authors (24). The brain tissues were stored at -80˚C until use.

Protein determination. The method described in the study by Lowry *et al* (25) was used to determine the mitochondrial protein concentrations in the brain areas aforementioned, using bovine serum albumin (MilliporeSigma) as a standard.

Determination of the MDA level. The lipid peroxidation product, MDA, was used as an indicator of oxidative stress. Its levels were measured using the thiobarbituric acid (MilliporeSigma) reaction in all samples, according to the method described in the study by Ohkawa *et al* (26). The results are reported as nmol/mg protein mitochondria.

Determination of SOD activity. A SOD assay kit from MilliporeSigma (19160-1K-F) was used to determine the SOD activity. Data are expressed as U/mg protein mitochondria.

Western blot analysis. At the end of the study period, COX-2 and IL-6 expression levels were measured in the cortex and hippocampus of the rats using western blot analysis as described in a previous study by the authors (22). The brain tissues were homogenized in lysis buffer (cat. no. 87792, Thermo Fisher Scientific, Inc.) and the method of Lowry *et al* (25) was used to determine the total protein concentrations. Equal quantities of protein (40 μg protein) were subjected to 10% SDS-polyacrylamide gel electrophoresis, transferred to a Hybond-P (PVDF) membrane (Cytiva) and then incubated with rabbit monoclonal anti-COX-2 (1:1,000, cat. no. ab179800, Abcam), mouse monoclonal anti-IL-6 (1:2,000, cat. no. ab9324, Abcam) and rabbit monoclonal anti-β-actin (1:5,000, cat. no. AC026, ABclonal Biotech Co., Ltd.) antibodies at 4˚C overnight. The membranes were then incubated with anti-rabbit (1:2,000, cat. no. AS063, ABclonal Biotech Co., Ltd.) or anti-mouse (1:2,000, cat. no. 12-349, MilliporeSigma) secondary antibodies for 1 h at room temperature. The immunoreactive proteins on the blots were visualized using chemiluminescent substrate (Supersignal West Pico; Pierce; Thermo Fisher Scientific, Inc.). The density of the COX-2 and IL-6 bands were normalized to β-actin, and the protein expression was calculated using a ChemiDoc™ MP imaging system with Image Lab software (version 6.0.0 build 25, Bio-Rad Laboratories Inc.).

Statistical analysis. The data are expressed as the mean ± the standard error of the mean. Statistical analysis was assessed using one-way analysis of variance (ANOVA), followed by a Tukey's post hoc test using SPSS® software (version 25, SPSS-IBM Inc.). A P-value <0.05 was considered to indicate a statistically significant difference.

Results

Effects of 6-gingerol on brain injury in rats subjected to Rt.MCAO. The present study measured the brain infarct volume using TTC staining following treatment of the rats with 6-gingerol. Rats undergoing permanent occlusion (Rt. MCAO) and who received the vehicle exhibited a significantly increased infarct volume (P<0.05) compared to the rats in the

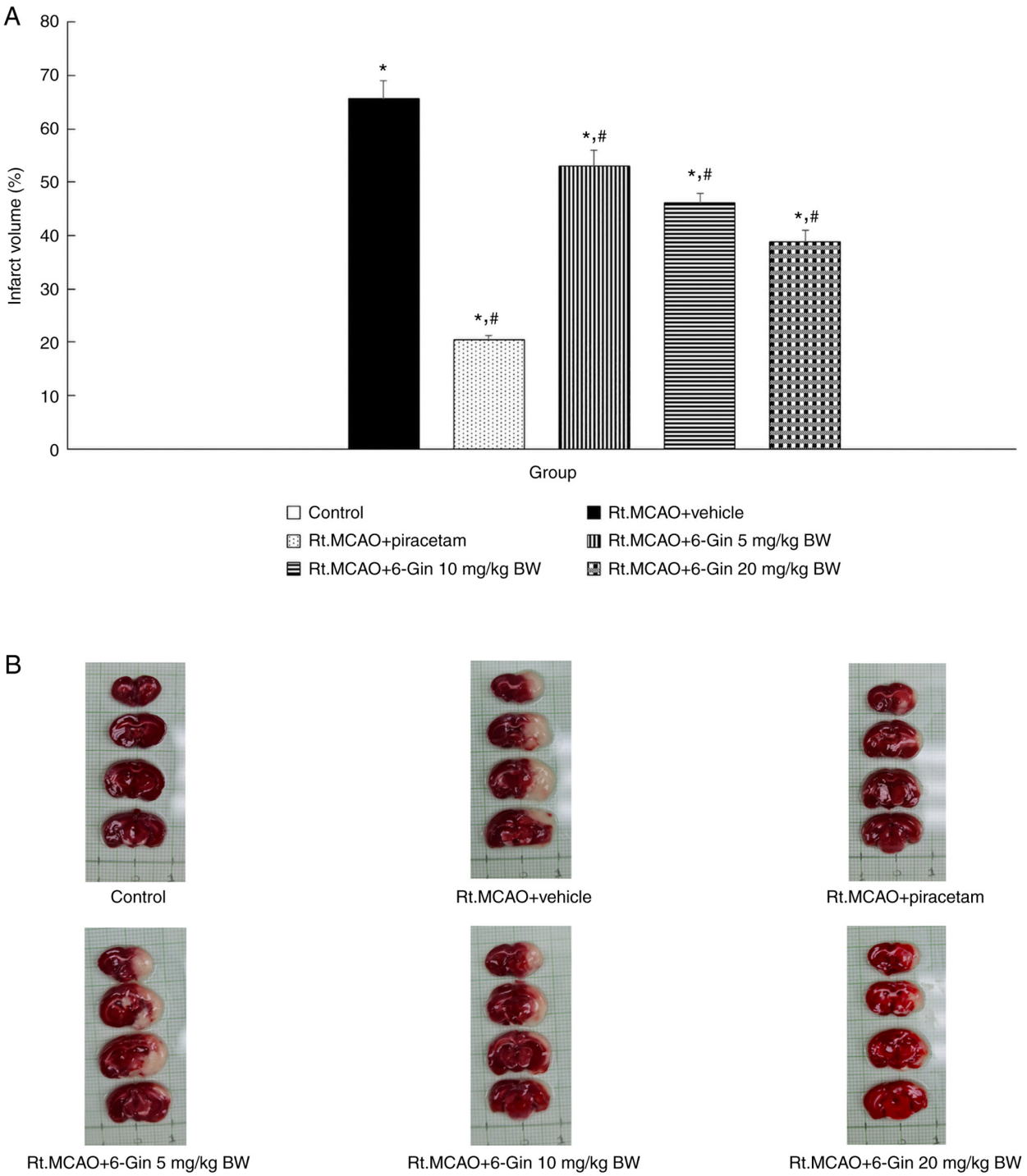

Figure 3. Effects of 6-gingerol on brain infarct volume. (A) Bar graph illustrating the effects of 6-gingerol on brain infarct volume in rats with cerebral ischemia. Data are presented as the mean ± SEM (n=5). *P<0.05, compared to the control; #P<0.05, compared to the Rt.MCAO + vehicle group. (B) Representative rat brains stained using 2,3,5-triphenyltetrazolium chloride following focal cerebral ischemia. The red area represents normal tissue, while the white area is the infarcted region. Rt.MCAO, right middle cerebral artery occlusion; BW, body weight; Gin, 6-gingerol.

control group (Fig. 3). However, the rats treated with piracetam and 6-gingerol exhibited a marked decrease in their infarct volumes compared to the vehicle-treated ischemic stroke group (P<0.05).

Effects of 6-gingerol on neuronal damage in the cortex and hippocampus of rats subjected to Rt.MCAO. Cerebral ischemia induced major neuronal damage in the cortex and all subregions of the hippocampus (CA1, CA2 and CA3). The Rt.MCAO + vehicle (DMSO) group exhibited a significant decrease in neuronal survival in the cortex or CA1, CA2 or CA3 regions of the hippocampus compared to the control. By contrast, piracetam (at a dose of 250 mg/kg BW) and 6-gingerol (at doses of 10 and 20 mg/kg BW) markedly reduced ischemic stroke-induced neuronal loss within the cortex and all subregions of the hippocampus (P<0.05) compared to the Rt.MCAO + vehicle group (Fig. 4). At the lower dose 5 mg/kg BW), 6-gingerol did not lead to any significant difference in

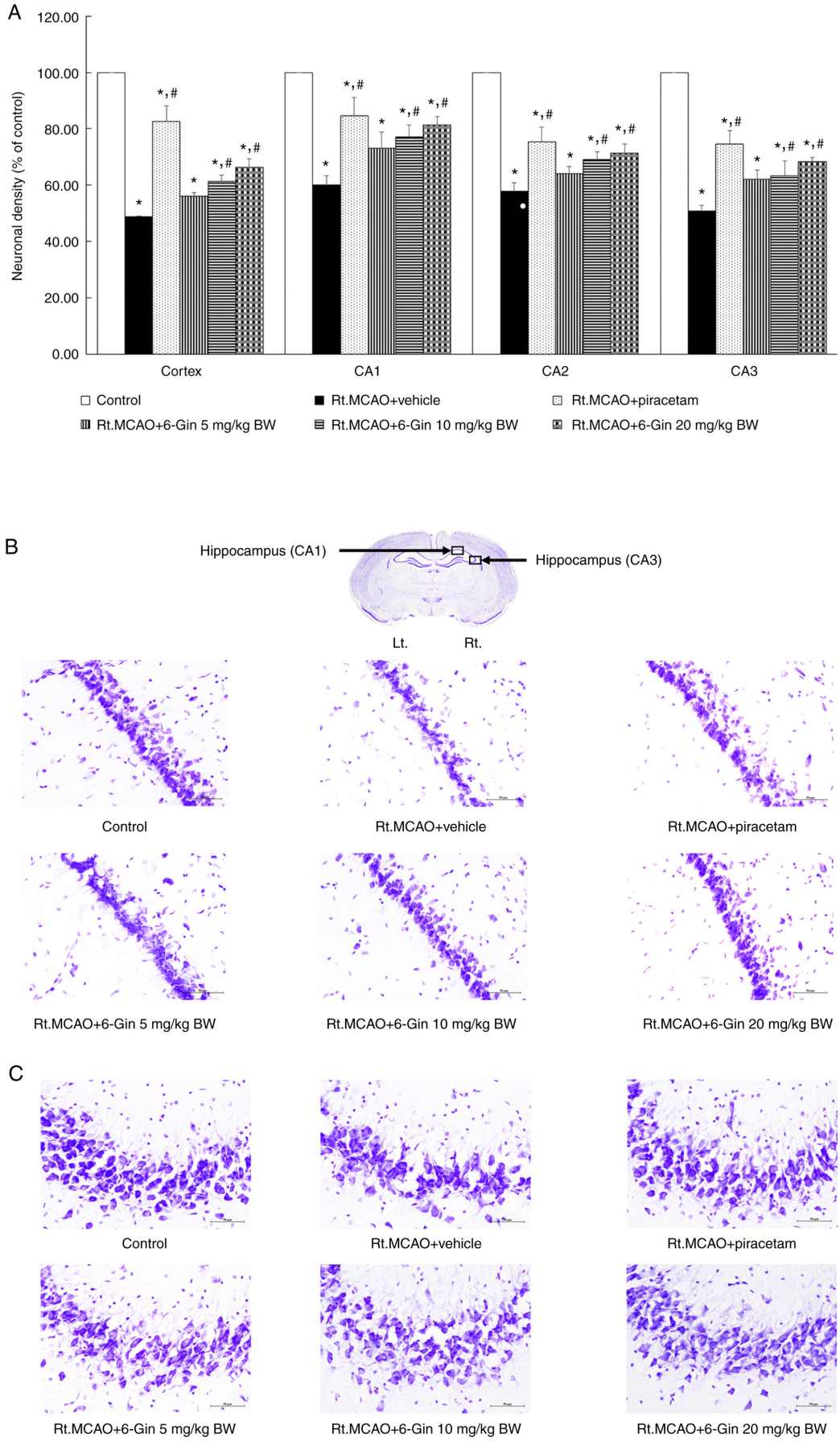

Figure 4. Effects of 6-gingerol on neuronal damage in the cortex and hippocampus. (A) Graph illustrating neuronal density in the cortex and CA1, CA2 and CA3 regions of the hippocampus. (B and C) Images of Nissl-stained rat sections of CA1 and CA3 taken at x40 magnification. Data are presented as the mean ± SEM (n=5). *P<0.05, compared to the control; #P<0.05, compared to the Rt.MCAO + vehicle group. Rt.MCAO, right middle cerebral artery occlusion; BW, body weight; Gin, 6-gingerol.

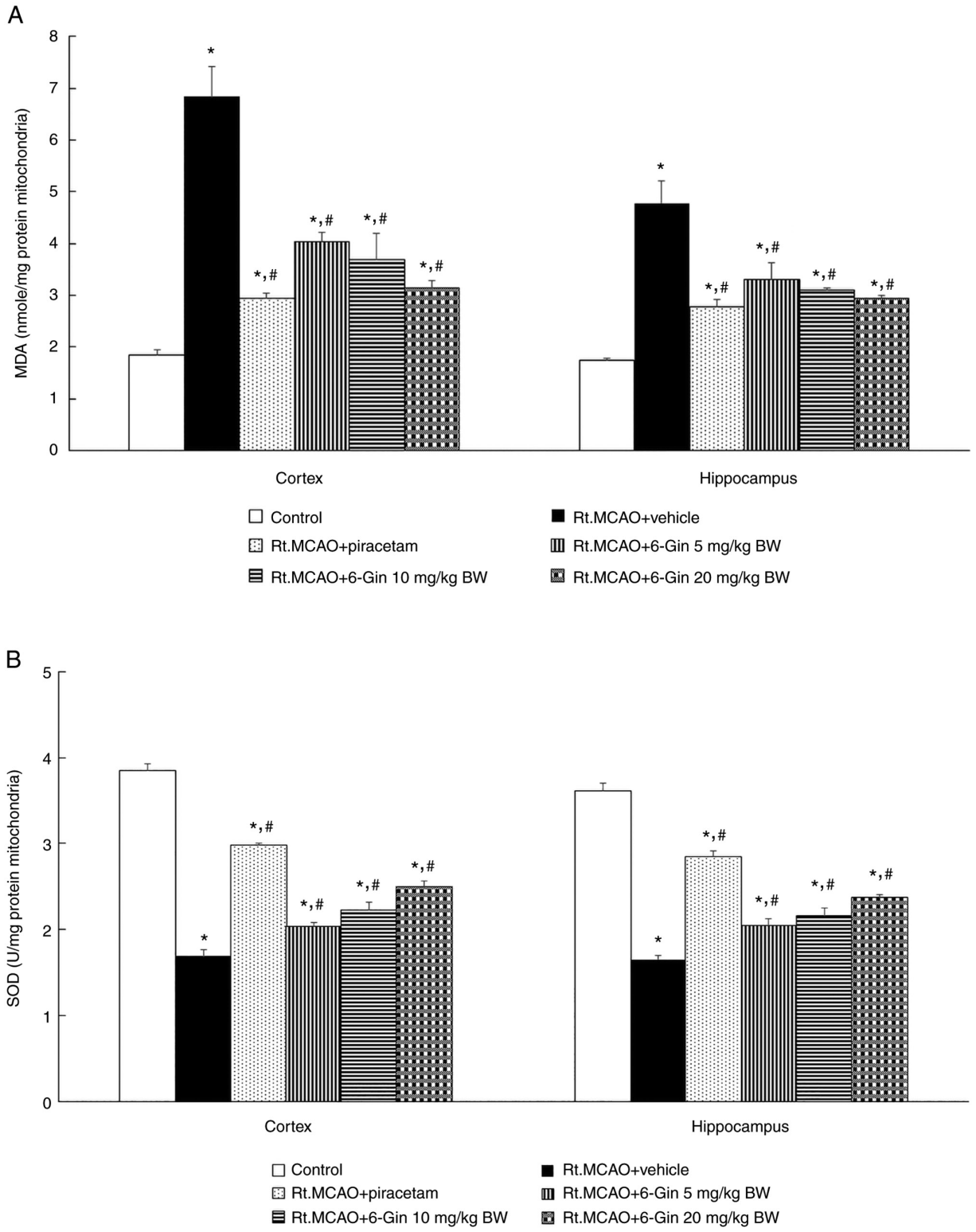

Figure 5. Effects of 6-gingerol on cerebral ischemia-induced oxidative stress in the cortex and hippocampus mitochondria. Treatment with 6-gingerol significantly decreased the (A) MDA levels and (B) SOD activity. Data are expressed as the mean ± SEM (n=5). *P<0.05, compared to the control; #P<0.05, compared to the Rt.MCAO + vehicle group. Rt.MCAO, right middle cerebral artery occlusion; BW, body weight; Gin, 6-gingerol; MDA, malondialdehyde; SOD, superoxide dismutase.

neuronal damage in all areas compared to the Rt.MCAO + vehicle group.

Effects of 6-gingerol on oxidative stress in the mitochondria from the cortex and hippocampus of rats subjected to Rt.MCAO. As oxidative stress plays a key role in the pathogenesis of ischemic stroke, the present study also examined the effects of 6-gingerol on oxidative stress markers, including MDA levels and activity of the scavenging enzyme, SOD, in the mitochondria. Rats that underwent permanent Rt.MCAO

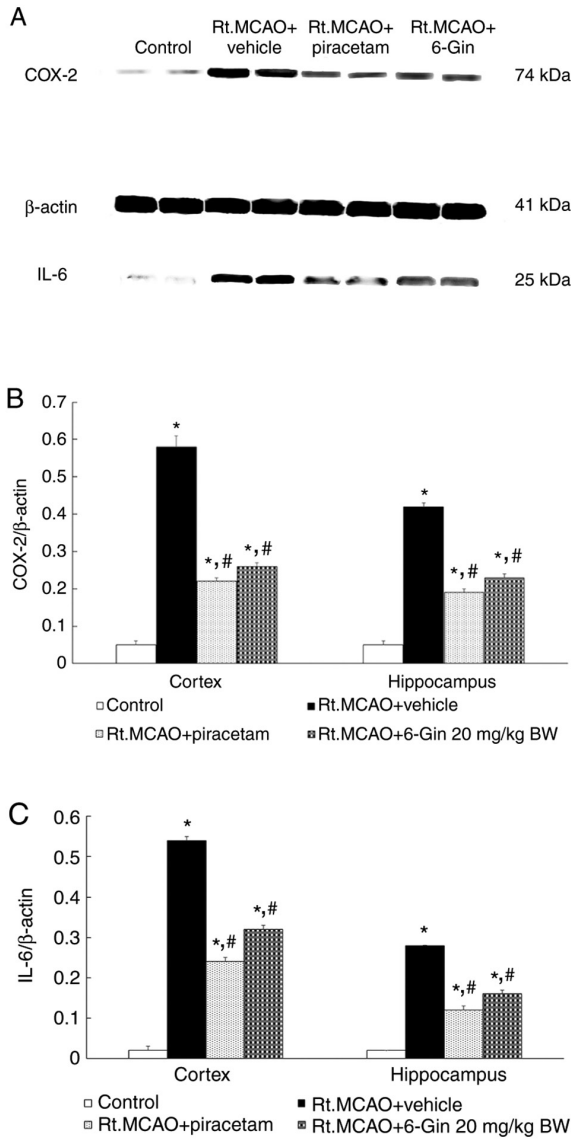

Figure 6. Effects of 6-gingerol on COX-2 and IL-6 expression in the cerebral cortex and hippocampus of rats determined using western blot analysis. (A) Representative western blots of COX-2 (74 kDa) and IL-6 (25 kDa) from the cerebral cortex. β-actin (41 kDa) was used as a loading control. (B) Quantitative analysis of the COX-2 band density normalized to β-actin. (C) Quantitative analysis of IL-6 band density normalized to β-actin. Data are expressed as the mean ± SEM (n=5). *P<0.05, compared to the control; #P<0.05, compared to the Rt.MCAO + vehicle group. Rt.MCAO, right middle cerebral artery occlusion; BW, body weight; Gin, 6-gingerol; COX-2, cyclooxygenase-2; IL-6, interleukin 6.

exhibited increased mitochondrial MDA levels (Fig. 5A) and significantly reduced mitochondrial SOD activities (Fig. 5B) compared to the control group. By contrast, the rats treated with piracetam or 6-gingerol (5, 10 and 20 mg/kg BW) exhibited mitochondrial SOD activities that were significantly less diminished and mitochondrial MDA levels that were significantly less elevated than the Rt.MCAO + vehicle group both in the cerebral cortex and hippocampus (P<0.05; Fig. 5).

Effects of 6-gingerol on COX-2 and IL-6 expression in the cortex and hippocampus of rats subjected to Rt.MCAO. To assess the anti-inflammatory activity of 6-gingerol, the present study measured COX-2 and IL-6 protein expression in the cortex and hippocampus using western blot analysis. As 20 mg/kg BW 6-gingerol led to optimum changes in infarct volume, neuronal density and oxidative stress markers, this dose was selected to investigate the effects of 6-gingerol on COX-2 and IL-6 levels. Bands showing positive immunoreactivities against COX-2, β-actin and IL-6 were detected at 74, 41 and 25 kDa, respectively (Fig. 6A). Treatment with 6-gingerol at a dose of 20 mg/kg BW markedly decreased the density ratios of COX-2 and IL-6 to the β-actin band compared to the Rt.MCAO + vehicle group (P<0.05, Fig. 6B and C).

Discussion

Animal models are widely used to gain understanding of the pathophysiology of cerebral ischemic injury. In such studies, MCAO is one of the most commonly employed surgical procedures to induce ischemic stroke (27). This model, known as the permanent MCAO model, has been developed to mimic human ischemic stroke, and generates cerebral infarction in both cortical and subcortical areas (28). The present study used the MCAO model to investigate 6-gingerol as an experimental therapy. As was expected, cortical and subcortical region infarct volumes were large in rats treated with only DMSO, whereas these volumes were significantly smaller in the rats treated with 6-gingerol and piracetam.

In addition to the infarcts described above, cortical and hippocampal neuronal loss and brain damage are reportedly induced in the MCAO model (29-31), with apoptotic cell death occurring after 24 h (32-34). Data from the present study are in agreement with these previous findings. Herein, the positive control drug, piracetam, which increases CBF, was found to enhance the density of neurons within the cortex and hippocampus. This result is also in agreement with those of previous studies, which reported the nootropic action of this drug and its ability to ameliorate cerebral ischemia-induced brain damage (35-37). In the present study, the experimental treatment agent, 6-gingerol, markedly alleviated ischemic stroke-induced neuronal damage within the cortex and hippocampus regions CA1, CA2 and CA3. Although this has not been previously demonstrated, at least to the best of our knowledge, it does correspond well with other reported activities of 6-gingerol. For example, 6-gingerol has previously been shown to reduce the *in vitro* apoptosis of PC12 cells (38). It has also been previously demonstrated that 6-gingerol protects rats from lipopolysaccharide-induced brain injury by improving the expression of brain-derived neurotrophic factor (BDNF) within the cerebral cortex and hippocampus (20). In another *in vivo* study, 6-gingerol improved the hippocampal levels of BDNF and nerve growth factor in rats exposed to gold nanoparticles (17). Moreover, during pathophysiological processes in the subacute phase of ischemic stroke (up to 7 days following complete ischemia onset), ATP levels are depleted, disrupting ionic homeostasis. Radical species then form and these are involved in mediating damage. Cells have enzymatic and non-enzymatic defense mechanisms to protect themselves from this type of damage (5). In addition, the initiation of the inflammatory response followed by the release of mediators exacerbates the effects of neuronal inflammation and primary damage (39). Therefore, the present study selected the duration of 6-gingerol treatment to be 7 days.

Reactive oxygen species (ROS) contribute to the progression of numerous diseases (40-42), and both ROS and lipid peroxidation (LPO) are known to mediate tissue damage in ischemic stroke. Cells have enzymatic and non-enzymatic defense mechanisms to protect themselves from some of this damage (5). Enzymatic mechanisms include neutralization by SOD, GSH-Px and CAT. Previous studies have found that increasing these antioxidant enzymes can mitigate brain damage from ischemic stroke (5-7). Another study demonstrated that 6-gingerol is a potent antioxidant, with the potential to treat and prevent chronic diseases (43). As mitochondrial dysfunction and SOD deficiency contribute to increased neuronal death, increased numbers of superoxide anions and increased cerebral infarction (44,45), the present study examined the effects of 6-gingerol on LPO (by examining the MDA levels) and SOD activity. The results revealed that both the positive control, piracetam, and the experimental treatment agent, 6-gingerol (5, 10 and 20 mg/kg BW), decreased the LPO product and increased SOD activity in cortical and hippocampal mitochondria compared to the Rt.MCAO + vehicle group. Several lines of evidence demonstrate that 6-gingerol has antioxidant, anti-inflammatory (15,16), anti-apoptotic (17) and anticancer properties (18). In addition, a 6-gingerol rich fraction from *Z. officinale* has been shown to prevent acrylonitrile-induced cerebral cortex damage partly via its antioxidant and anti-inflammatory activities (46).

In the development of ischemic damage, the production of free radicals is markdly elevated, and oxidative stress causes neuronal inflammation and apoptosis (8). Arachidonic acid derivatives and prostaglandins are two of the major inflammatory mediators involved. With the onset of ischemia, intracellular calcium increases, with the consequent activation of phospholipase. Arachidonic acid metabolites are then formed, contributing to the pathophysiological process. A number of studies have found that IL-1 and IL-6 are also inflammatory mediators of ischemic damage (9-11). COX-2 is a key inflammatory mediator in cerebral ischemia and neurodegenerative disorders too (12,13). Recent evidence indicates that the suppression of COX-2 attenuates ischemic injury following MCAO (14). In the present study, significant decreases in the density ratio of COX-2 and IL-6 to the β-actin band were detected in rats subjected to Rt.MCAO receiving 20 mg/kg BW 6-gingerol for 7 days. This is in accordance with the findings of previous studies demonstrating that the inhibition of inflammation can decrease the brain infarct volume in experimental stroke (47) and 6-gingerol can alleviate inflammatory damage by inhibiting the production of pro-inflammatory cytokines (48-51). Consequently, 6-gingerol can attenuate focal cerebral ischemic stroke-induced neuronal injury by suppressing oxidative stress and inflammatory mediators.

In animal studies, it has been demonstrated that the mitochondria are the main source of free radical generation following focal cerebral ischemia. It is possible that mutations in nuclear genes following cerebral ischemia alter the apoptotic process. Gene mutations in mitochondria may be an initiating event that leads to apoptotic neuronal death. Therefore, drugs or other interventions that enhance repair efficiency or reduce apoptosis in the brain offer the possibility of improved outcomes following ischemic stroke. An imbalance between mitochondrial fusion and fission can also enhance free radical generation and has been detected in stroke and other diseases. Mitogen-activated protein kinase (MAPK) is associated with mitochondrial dynamics. Bioinformatics research has demonstrated that some miRNAs are involved in the modulation of MAPK, which is crucial for the alleviation of inflammation and apoptosis in ischemic stroke (52-54). Furthermore, research using rat models of MCAO has demonstrated that reductions in mitofusin (Mfn)1 and Mfn2 induce Ca^{2+} overload mitochondrial Bax translocation (55) and promote neuronal apoptosis. Additionally, Mfn2 has been reported to decrease caspase-3 and increase the Bcl-2/Bax ratio, these two factors reducing cellular susceptibility to apoptosis after cerebral ischemic stroke (54). Further studies are t required in order better understand the effects of 6-gingerol on these pathways and its potential as a supportive treatment for patients with ischemic stroke.

The present study has a few limitations. Firstly, a weakness of the MCAO model is that the filament may not always be inserted far enough to completely occlude the middle cerebral artery. To address this issue, some researchers use laser doppler flowmetry (LDF) to measure the reduction in CBF and confirm successful occlusion. The present study did not use LDF or micro computed tomography (micro-CT), although the authors did always check that the filament was of optimal size and that the point of occlusion was associated with the infarct size after the rats were euthanized. A second limitation is that the present study did not measure LPO or SOD activity in the blood or other tissues. LPO and SOD activity were measured in just the cerebral cortex and hippocampus mitochondria. Lastly, the small number of replicates used in the biochemical assays represents another study limitation. Three or more replicates would have been preferable to two; however, pipetting was performed by an experienced laboratorian and the standard curves had high R^2 values.

In conclusion, 6-gingerol (10 and 20 mg/kg BW for 7 days) exerted antioxidant and anti-inflammatory effects, which effectively reduced brain damage in a rat model of focal cerebral ischemic stroke. However, further investigations are warranted to determine whether any additional mechanism(s) contribute to the ameliorative effects detected in the present study.

Acknowledgements

The authors would like to thank Dr Tim Cushnie (Faculty of Medicine, Mahasarakham University, Mahasarakham, Thailand) for language-editing assistance. As Mahasarakham University does not have any animal housing facility, the animal experiments were performed at the Northeastern Laboratory Animal Center, Khon Kaen University, Khon Kaen, Thailand, which is close to Mahasarakham University.

Funding

The present study received funding from the Mahasarakham University Faculty of Medicine (Med MSU 2/65).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

RK was involved in the study methodology, and in the writing, reviewing and editing of the manuscript. JJ was involved in the conception and design of the study, in funding acquisition, data curation, in the study methodology, as well as in the writing of the original draft and in the writing, reviewing and editing of the manuscript and in project administration. Both authors have read and approved the final manuscript and confirmed the authenticity of all the raw data.

Ethics approval and consent to participate

All animal-related protocols were designed to minimize animal suffering, and were performed according to the approval of the Institutional Animal Care and Use Committee at Khon Kaen University, Khon Kaen, Thailand (Record No. IACUC-KKU-6/65).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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