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RESEARCH ARTICLE

Sandalwood Oil Neuroprotective Effects on Middle Cerebral Artery Occlusion Model of Ischemic Brain Stroke

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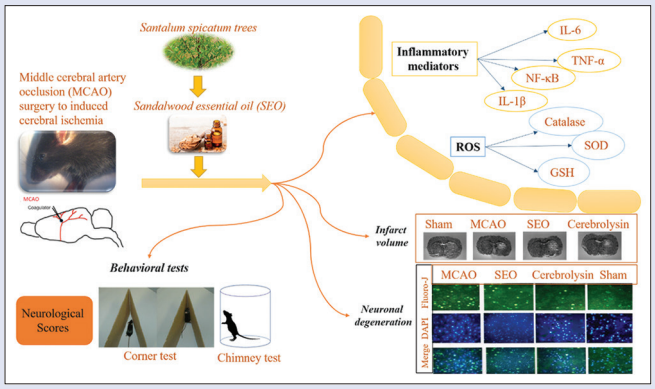
ABSTRACT

Background: Cerebral injury is a common health crisis that may affect the patient lifestyle or cause death. Although many drugs involved in the treatment of such cases and relieving symptoms, there is no precise cure. Accordingly, the importance of protection against the insult could be understood, especially when natural products can be a part of the protective agent. **Objectives:** The target of the current study was to inspect the pharmacological actions of sandalwood essential oil (SEO) through oxidative stress as well as inflammation pathways in mice exposed to middle cerebral artery occlusion (MCAO) surgery as a reproducible mean to generate focal brain ischemia. **Materials and Methods:** Ischemic animals were treated with sandalwood oil and were compared to cerbrolysin actions, atypical neuroprotective medication. Infarct volume, measured via sliver staining, as well as several behavioral tests including neurological deficits scores was measured to evaluate sandalwood oil defensive effects. Neural degeneration, measured via Fluoro-Jade B staining, levels of different inflammatory mediators including interleukin (IL)-1 β, IL-6, tumor necrosis factor-α, and nuclear factor-kappa β, and antioxidants enzymes activities containing glutathione, superoxide dismutase, and catalase were assessed. **Results:** Sandalwood oil exposure improved the neurological deficits as well as behavioral experiments and diminished infarct volume and neural degeneration in ischemic mice. In addition, oxidative stress overproduction and inflammation cascade were significantly suppressed subsequent to SEO treatment. **Conclusion:** These results suggest that sandalwood oil abstains protective effects through deterring oxidative stress and inflammation cascade in cerebral ischemia.

Key words: Brain ischemia, cerbrolysin, cerebral injury, santalaceae, *Santalum spicatum*

SUMMARY

- Sandalwood oil exposure amended the neurological deficits as well as behavioral tests and moderated infarct volume and neural deterioration in middle cerebral artery occlusion ischemic mice. In addition, oxidative stress overproduction and inflammation cascade were significantly overwhelmed following SEO treatment. These outcomes advocate that sandalwood oil abstains shielding properties through deterring oxidative stress and inflammation cascade in cerebral ischemia.



Abbreviations used: SEO: Sandalwood essential oil; MCAO: Middle cerebral artery occlusion; IL-1 β: Interleukin-1 β; IL-6: Interleukin-6; TNF-α: Tumor necrosis factor-α; NF-κB: Nuclear factor-kappa β; GSH: Glutathione; SOD: Superoxide dismutase; ER: Estrogen receptor; HSV: Herpes simplex virus; DAPI: 4′,6-diamidino-2-phenylindole, dilactate; SIS: Silver infarct staining; FJ-B: Fluoro-jade B; GST: Glutathione S-transferase.

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INTRODUCTION

Ischemic cerebrovascular disease arises when stenosis or obstruction occurs in one of the major arteries that supply blood to the brain.[1] The mechanisms of cerebral injury are complicated and interrelated. Important steps in the pathogenesis of cerebral injuries include a series of an oxidative-inflammatory cascade, which is triggered by the interaction between oxidative stress and inflammatory response.[2] Inflammatory agents, including tumor necrosis factor-α (TNF-α), interleukin-1 β (IL-1 β), IL-6, and nuclear factor-kappa β (NF-κB), induces profound inflammation as well as free radicals upsurge leads to an irretrievable inflammation and oxidative stress situation producing cell death and cerebral ischemic injuries.[3]

Cerebrolysin is consumed clinically for cerebral ischemia treatment.[4] Cerebrolysin produces a dose-dependent effect; doses of ≥2.5 ml/kg display enriched functional consequence, whereas 5 ml/kg compacts infarct size.[4,5] The mechanism through which cerebrolysin act is via boosting neurogenesis in the ischemic brain via phosphoinositide-3-kinase/Akt pathway, prompting brain progenitor cell proliferation.[5]

Sandalwood essential oil (SEO) is extracted from *Santalum spicatum* trees, family Santalaceae.[6] The Food and Drug Administration permitted

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SEO as a natural flavoring factor for human ingesting.[7] SEO chemical constitution has been considered comprehensively; at least 300 chemical ingredients have been recognized, of which the sesquiterpenes such as α-santalol and β-santalol are utmost plentiful.[6] *S. spicatum* is recognized for the presence of other sesquiterpenes alcohols in relatively lower concentrations such as α-bisabolol, farnesol, and lanceol. Other sesquiterpenes such as santalenes and curcumenes are found in a lesser extent.[8] Sandalwood oil and its main component possess little oral and dermal toxicity in laboratory animals.[7] SEO retains various favorable pharmacological properties and utilizes for centuries in conventional folk remedy for the management of diverse human diseases. Sandalwood oil shows a broad spectrum of medicinal activities including chemopreventive,[9] antihyperglycemic,[10] antioxidant,[11] antiviral,[11,12] anti-inflammatory, antianxiety, anti-stress, and antidepression.[13]

α-santalol prompts both extrinsic as well as intrinsic trails of apoptosis with caspase-8 and caspase-9 stimulation, demonstrating robust antineoplastic outcome against breast cancer cells.[14] Its another mechanism of action inhibits the breast cancer cells via targeting Wnt//β-catenin pathway.[15] It deters not only breast cancer growth but also prostate cancer cell growth as it was proven that α-santalol prompted apoptotic cell death and attenuated caspase-3 causing inhibition in the human prostate cancer cells growth.[16] In addition, SEO demonstrated antiviral action against herpes simplex virus 1 and 2 *in vitro* via dose-dependent inhibition of viral replication.[12]

As for the anti-inflammatory effects, it was reported that sandalwood seed oil inhibited diverse pro-inflammatory elements, for instance, PGF2α, PGE2, LTB4, TNF-α, and IL1 β.[13]

However, SEO's effects on the brain ischemia have still to be elucidated. Therefore, the leading purpose of this study is to illuminate SEO impact on brain ischemia and examine its properties on oxidative stress as well as the inflammation cascade in mice suffer from cerebral injury.

MATERIALS AND METHODS

Materials

Avertin (2,2,2-tribromoethanol) and 4',6-diamidino-2-phenylindole, dilactate were acquired from Sigma Aldrich, while Fluoro-Jade staining was obtained from Chemicon (USA). Mouse IL-6 (Cat. No. M6000B), TNF-α (Cat. No. MTA00B), and mouse IL-1 β (Cat No. MLB00C) ELISA kits were obtained from R and D Systems (USA). NF-κB (Cat no. ab176648), reduced glutathione (GSH) (Cat. No. ab142044), superoxide dismutase (SOD) (Cat. No. ab65354), and catalase (Cat. No. ab83464) kits were bought from Abcam Co., Ltd.

Plant material

Australian sandalwoods (*S. spicatum*, family Santalaceae), dried heartwood, were gathered from the local markets in Al Ahsa, Eastern region, Kingdom of Saudi Arabia, in January 2018. Expert taxonomists from King Saud University and Egyptian Agricultural museum recognized the heartwood. The isolated essential oil was exposed to gas chromatography–mass spectrometry fingerprint investigation (data not shown) according to Du, *et al.*,[17] and the results specified that the isolated oil was definitely the sandalwood (*S. spicatum*, family Santalaceae) essential oil after matching the key components such as α- and β-santalol, farnesol, and curcumene-12-ol.

Isolation of sandalwood essential oil

Three hundred grams of dried *S. spicatum* heartwood were cut, pulverized into powder, and exposed to hydrodistillation agreeing with Nautiyal.[18] The recovered volatile fraction was dried via anhydrous sodium sulfate. The obtained essential oil was retained in dark containers at 4°C until further procedures.

Animals

C57BL/6 mice (20–25 g) were acquired from Theodor Bilharzias Center, Egypt. Throughout the research, mice were retained in optimized, nutritious conditions. All investigates including surgery procedures, neurological scores performances, behavioral tests, and infarct volume determination were performed randomly.

Ethical statement

All animal investigational techniques were permitted by the Animal Research Ethics Committee at Zagazig University, Egypt, and were completed in harmony with the Guidelines for the Ethical Conduct for Animals handling in research, Zagazig University, Egypt.

Experimental design

Animals were haphazardly dispersed into four main groups ($n = 10$): sham group, ischemic (middle cerebral artery occlusion [MCAO])-operated group, and ischemic group managed with either sandalwood oil SEO (100 mg/kg, i.p.) or cerebrolysin (7.5 mg/kg, i.p.)[4] 30 min before and 24 h after middle cerebral artery occlusion (MCAO) surgery. Sandalwood oil dose was chosen on a preliminary trial on a group of mice that administered the oil (data not shown).

Generation of cerebral ischemia

MCAO surgery was executed following the previously described procedure.[19] After 48 h, Avertin-anesthetized animals were perfused via the heart with Ringer's solution. Brains, cautiously removed, were kept at −80°C and then 20–30 μm thickness segments were obtained via cryostat (SLEE medical GmbH, Mainz, Germany) to be utilized for the infarct volume measurements via silver staining technique and neuronal damage quantification through Fluoro-Jade staining. The levels of the various inflammatory mediators and antioxidants enzyme activities were distinguished using ELISA kits within the ischemic hemisphere homogenate.

Behavioral tests

Three behavioral tests were performed. First, neurological scores in which mice were evaluated for neurological inadequacy and recorded as earlier described in the study by Jia *et al.*[1] Neurological deficits were evaluated following 1 day of treatment postsurgery and were scored on a 5-point scale. Scoring was executed as follows: 0 – no default; 1 – minor defect, shown as not able to extend left forepaw; 2 – modest defect, shown as rotating to the left; 3 – severe defect, shown as falling to the left; and 4 – serious deficit. Second, to evaluate motor coordination before and after the MCAO-induced brain ischemia, the Chimney test was performed as described formerly in the study by Schaar *et al.*[20] Finally, to measure the sensorimotor deficit, the Corner test was utilized.[20]

Infarct volume detection

Silver infarct staining was carried out to measure the dead areas as defined previously in study by Vogel *et al.*[21] Using HP scan jet scanner, brain sections were scanned, and the infarction areas were quantified via ImageJ® program (Scion Corporation). The amended infarct area was calculated as mentioned in the study by Wali *et al.*[22] to cancel the brain edema aspect: (right hemisphere + silver deficit) – left hemisphere = infarct volume.

Neuronal deterioration determination

Fluoro-Jade B-stained slides, a technique described formerly in the study by Li *et al.*,[23] were visualized using fluorescent microscope (Leica,

Germany) and were inspected through ImageJ® software (LOCI at the University of Wisconsin-Madison, USA).

Measurement of inflammatory mediators and antioxidants

Inflammatory intermediators including IL-1 β, IL-6, TNF-α, and NF-κB and antioxidants enzymes including GSH, SOD, and catalase activities ELISA kits were measured in ischemic brain hemisphere homogenates following the manufacturer's protocol.

Statistical investigation

Data were presented as mean ± standard error of the mean. One-way analysis of variance tailed with Tukey's *post hoc* test consuming GraphPad Prism software version 6 (San Diego, CA, USA) was used to determine the statistical investigation. Statistical significance level was established to be at $P < 0.05$.

RESULTS

Sandalwood oil and cerebrolysin effects on behavioral tests

Figure 1 shows the alteration in behavioral test prompted by treatment with sandalwood oil SEO (100 mg/kg, i.p.) and cerebrolysin (7.5 mg/kg, i.p.), which cause a significant decline in neurological deficits scores accompanying MCAO surgery [Figure 1a]. Chimney test offers an approach evaluating rodent's spontaneous forelimb use and is often used to demonstrating the motor system injury in a stroke model.[20] The Chimney test results showed that the ischemic operated group was significantly slower in time than sham-operated group. However, SEO and cerebrolysin considerably amended the time required by the mice to exit the tube on days 1 and 2 related to ischemic days 1 and 2, respectively [Figure 1b].

Corner test, a sensorimotor functional assessment, is dependable tool for recognizing and quantifying sensorimotor and postural irregularities, thus providing an approach of spotting contralateral deficits and ipsilateral turning biases.[20] Left MCAO ischemic animals exhibited a substantial rise in the right turn percentage related to the same mice prior to MCAO. Figure 1c shows that the management of SEO and cerebrolysin substantially lessened the right turn proportion.

Sandalwood oil and cerebrolysin effects on infarct volume and neuronal degeneration

Brain infarct size was measured as a sign of the ischemic brain impairment extent following MCAO surgery and subsequently cerebral ischemia. SEO and cerebrolysin with the above-mentioned doses significantly lessened the infarct volume reaching 16.17 ± 1.4 and 12.6 ± 1.2 mm^3 compared to the ischemic group reaching 26.83 ± 1.9 mm^3, as shown in Figure 2a. Sliver staining images which are taken from the ischemic and treated group show the live (gray color) and dead ischemic (whitish color) brain areas, as illustrated in Figure 2a. Figure 2b and c demonstrates that SEO and cerebrolysin significantly deterred the neuronal deterioration as specified by Fluoro-Jade fluorescence, and the images are shown in Figure 2.

Sandalwood oil and cerebrolysin effects on the inflammatory mediators

The usage of SEO (100 mg/kg, i.p.) and cerebrolysin (7.5 mg/kg, i.p.) significantly diminished different inflammatory mediators as IL-1 β reaching 76.83 ± 7.13 and 100.33 ± 4.13 pg/ml compared to the ischemic group 151.17 ± 6.7. Furthermore, for IL-6, SEO and cerebrolysin significantly decreased the elevated IL-6 level accompanying with cerebral ischemia reaching 79.33 ± 5.88 and 56 ± 4.85 pg/ml compared to 114.33 ± 5.71 pg/ml, respectively [Figure 3]. In addition, cerebral ischemia resulted in an increase in TNF-α level reaching 403.33 ± 32.62, while the treatment with SEO and cerebrolysin caused a significant decrease reaching 363.83 ± 18.24 and 279 ± 11.38 pg/ml, respectively. Finally, NF-κB levels were significantly elevated in the ischemic group, while the treatment with either SEO or cerebrolysin significantly lessened NF-κB.

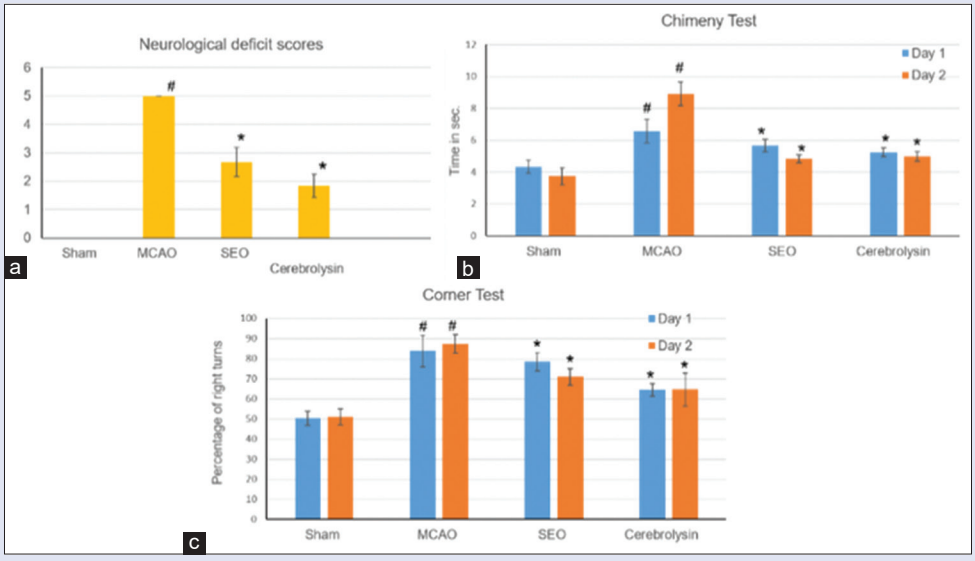

Figure 1: Behavioral tests alterations prompted by the treatments with Sandalwood oil Sandalwood essential oil (100 mg/kg, i.p.) and cerebrolysin (7.5 mg/kg, i.p.) on cerebral ischemia induced via MACO in mice on: (a) Neurological deficit scores, (b) Chimney test, (c) Corner test. Data are expressed as mean ± standard error of the mean, $n = 10$. #significantly different from the sham operated group, *significantly different from the MACO ischemic group at $P < 0.05$ using analysis of variance followed by Tukey's *post hoc* test

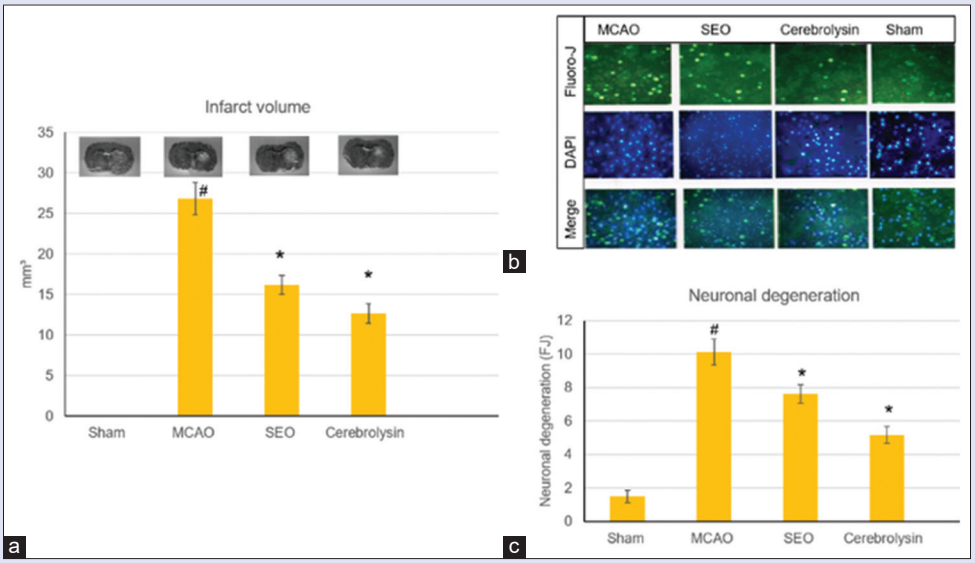

Figure 2: Infarct volume and neuronal degeneration prompted by the treatments with sandalwood oil (100 mg/kg, i.p.) and cerebrolysin (7.5 mg/kg, i.p.) on cerebral ischemia induced via MACO in mice: (a) infarct volume and (b and c) neuronal degeneration. Data are expressed as mean ± standard error of the mean, $n = 10$. #significantly different from the sham-operated group, *significantly different from the MACO ischemic group at $P < 0.05$ using analysis of variance followed by Tukey's *post hoc* test

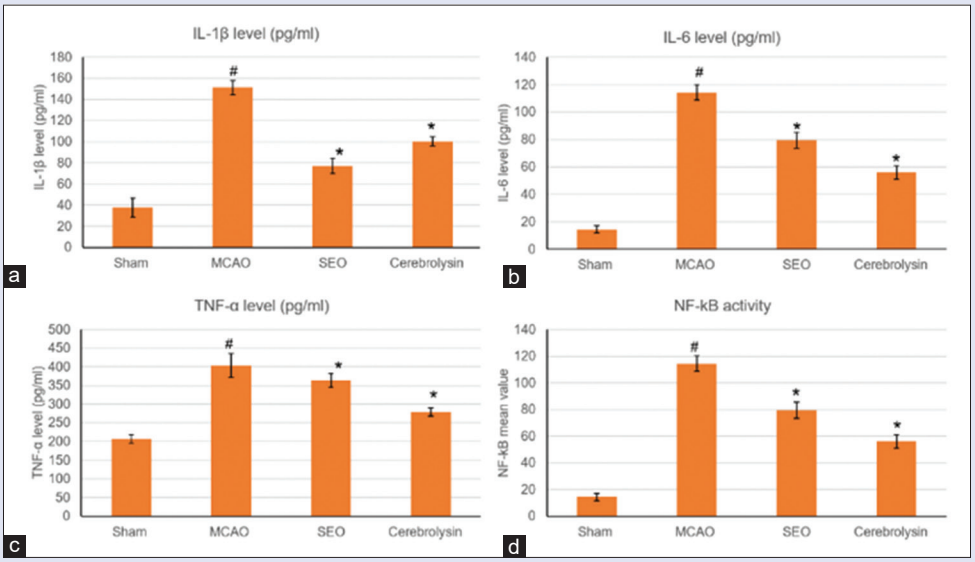

Figure 3: Inflammatory mediators alterations prompted by the treatments with Sandalwood oil sandalwood essential oil (100 mg/kg, i.p.) and cerebrolysin (7.5 mg/kg, i.p.) on cerebral ischemia induced via MACO in mice (a) Interleukin-1 β, (b) Interleukin-6, (c) Tumor necrosis factor-α and (d) Nuclear factor-kappa β. Data are expressed as mean ± standard error of the mean, $n = 10$. #Significantly different from the sham-operated group, *significantly different from the MACO ischemic group at $P < 0.05$ using analysis of variance followed by Tukey's *post hoc* test

Sandalwood oil and cerebrolysin effects on antioxidant enzymes

Antioxidant enzymes activities such as GSH, SOD, and Catalase were declined ($P < 0.05$) significantly in the mice following MCAO-induced brain ischemia when reaching 0.48 ± 0.09, 2.43 ± 0.2 and 2.43 ± 0.2 compared to sham-operated group which results are 1.82 ± 0.10, 5.7 ± 0.2 and 14.33 ± 1.3 respectively. However, treatment with SEO and cerebrolysin with the mentioned doses resulted in a substantial ($P < 0.05$) increase in antioxidant enzymes activity reaching 0.816 ± 0.086 and 1.05 ± 0.12 for GSH and 2.81 ± 0.14 and 3.54 ± 0.22 for SOD and 2.78 ± 0.59 and 7.04 ± 0.78 for Catalase enzymes respectively as revealed in Figure 4.

DISCUSSION

Cerebral infarction and edema are two major pathophysiological deviations detected subsequent to brain ischemia. In most animal studies, potential drug efficiency in brain ischemia is concluded through infarct size measurements. Nevertheless, neuroprotective efficacy is evaluated clinically via neurological function.[20] The current study revealed neurological deficit outcomes improvement as well as reduced brain infarct size via sandalwood oil SEO treatment. Our results granted

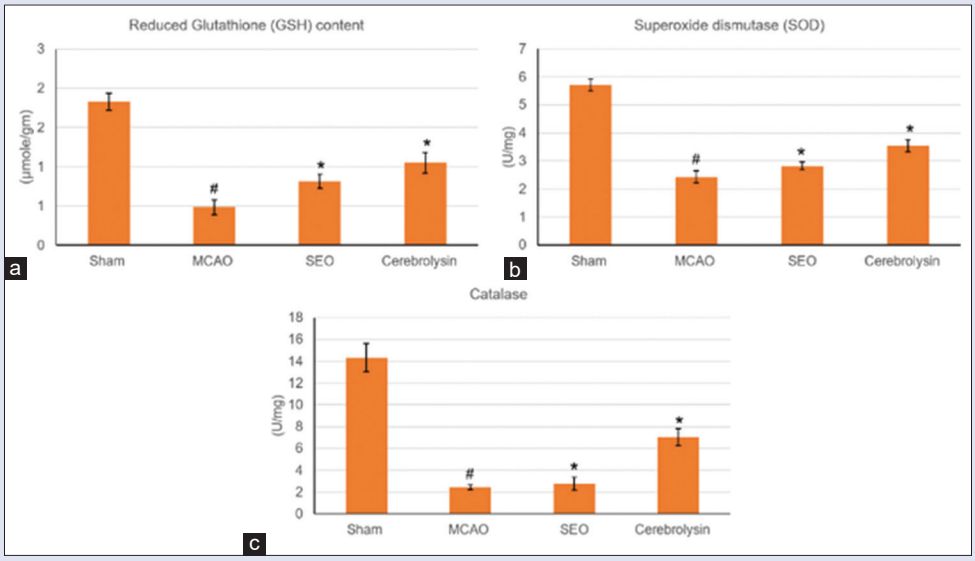

Figure 4: Antioxidant levels alterations prompted by the treatments with sandalwood oil sandalwood essential oil (100 mg/kg, i.p.) and cerebrolysin (7.5 mg/kg, i.p.) on cerebral ischemia induced via MACO in mice (a) Glutathione, (b) Superoxide dismutase and (c) Catalase. Data are expressed as mean ± standard error of the mean, $n = 10$. #significantly different from the sham-operated group, *significantly different from the MACO ischemic group at $P < 0.05$ using analysis of variance followed by Tukey's *post hoc* test

a unique indication of the protective prospective of sandalwood oil in focal cerebral ischemia.

Minimal neuronal degeneration was detected in sham-operated group, but MCAO-induced cerebral ischemia triggered an immediate intensification in neuronal degeneration. The outcomes of the current study discovered augmented neuronal endurance subsequent to sandalwood oil treatment, as directed by degenerated neurons reduction. The present outcomes are in align with prior records, in which SEO wielded a protective response against neurotoxic and proteotoxic stresses in *Caenorhabditis elegans* model.[24]

Since the brain usually exhibits low levels of antioxidative enzymes, it is highly susceptible to reactive oxygen species-induced injury following cerebral ischemia, and consequentially, oxidative stress damage occurs to brain DNA, ending with deteriorated brain-associated cell death.[25] Our outcomes advocated that sandalwood oil mitigated the cerebral ischemic damage via intensifying antioxidant enzymes activities and oxidative stress suppression. This present result corroborates with another study,[10] who proved that the feeding of sandalwood oil escalated hepatic GSH S-transferase activity and sulfhydryl (GSH) levels and that both α-santalol and sandalwood oil stimulate the activities of SOD level.

In the existing investigation, sandalwood oil possessed an anti-inflammatory outcome, as demonstrated by substantially subsiding TNF-α, IL-1 β, IL-6, and NF-κB. Numerous articles proposed a distinct anti-inflammatory influence of sandalwood oil.[26-28] Santalols, as described before, that derived from sandalwood oil have antiproliferative as well as antiinflammatory actions in the skin inflammatory diseases through suppressing epidermal cytokine production efficiently.[26] Furthermore; Sharma *et al.*,[27] showed that sandalwood oil anti-inflammatory action is facilitated through diminishing Phosphodiesterase enzyme (PDE) action and NF-κB inhibition, indicating that sandalwood oil represents a natural therapeutic choice for numerous inflammatory conditions. In addition, SEOs, as well as purified α-santalol and β-santalol, suppress specific pro-inflammatory signaling molecules as revealed by the suppression in lipopolysaccharide-induced generation of the arachidonic acid metabolites in stimulated skin cells.[28]

CONCLUSION

The existent experiment revealed that sandalwood oil compacted infarct size and enriched the neurological function in cerebral ischemic stroke. Sandalwood oil defensive actions were facilitated via amplificating endogenous antioxidant defenses, oxidative stress inhibition in the brain as well as the anti-inflammatory effects. These conclusions advocate that sandalwood oil may have a beneficial agent for the stroke patient management.

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Conflicts of interest

There are no conflicts of interest.

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