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ASSESMENT OF THE ANTI INFLAMMATORY EFFECT OF CINEOL ON CEREBRAL ISCHEMIA REPERFUSION I/R INJURY

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ABSTRACT

Ischemia and reperfusion in the brain brings a inflammating reaction that is possibly exacerbating primary stages of tissue wound. This study has been undertaken to inspect the potential neuroprotective action of cineol in the improvement of inclusive analytical I/R injury in rat model via interfering with inflammation. Twenty four adult Swiss albino rats had been chosen randomly in groups. For 1st group (sham group), rats endured the similar surgical and anesthesia processes as the controller group with no bilateral common carotid artery occlusion (BCCAO). While for 2nd control group (induced-untreated), rats endured half hour of global cerebral ischemia via BCCAO and then one hour of reperfusion. For 3rd group (Control – Vehicle), as controller collection but rats are taken day-to-day the vehicle of 1.8-cineol (10 mL/kg of 2% Tween 80 solution), p.o., then anesthesia and surgery with BCCAO for half hour followed by reperfusion for 1 hr were done. For 4th Group (1,8-cineol treated group), the rats received 1,8-cineol. The dose of 1,8-cineole was 100 mg/kg per oral (po) 1 h before induction ischemia. Then, anesthesia and surgery with BCCAO for 30 min, followed by reperfusion for 1 hr were done. Compared with the sham group, levels of IL-6 and TNF-α increased significantly ($p < 0.05$). The study results disclose that pretreating process with cineol possibly will enhance the overall cerebral ischemia-reperfusion injury by anti-inflammatory influence. We concluded that inflammatory cytokines are involved in global cerebral ischemia due to BCCAO effect. Cerebral ischemia reperfusion injury can be modified by cineol via its anti inflammatory effect.

KEYWORD: IL6, TNF-α, inflammation, cerebral ischemia/reperfusion injury, Cineol.

INTRODUCTION

Cerebral stroke represents an imperative and disastrous incidence that classified as the 2nd death reason in the population. The episode strokes number and stroke-related deaths was continued gradually augmented during past two decades.[1] Cerebral stroke stands for the unexpected inception of focal neurological function loss owing to infarction or outflow in the pertinent fragment of the vital nerve organism, while ischemic stroke represents seventy percent of all stroke incidents.[2] The optimal ischemic stroke treatment is well-timed recanalization of the causing vein followed by revascularization of the pertinent brain area to salvage the peri-infarct neurons.[3] Nevertheless, ischemic/reperfusion (I/R) injury decreases the therapeutic influence of this particular treating. For that reason, an effective method to stop I/R injury is needed for stroke patients to ensure a healthier consequence.

The injury of cerebral I/R is a difficult procedure that includes some steps of three main neurons pathways: excitotoxicity, oxidative stress, and inflammation.[4]

Stroke is a serious leading cause of death that causes financial burden, especially in low-income and middle-income countries.[5] Ischemic stroke accounts for 75% of all stroke patients.[6] Within a certain time window, thrombolysis is thought to be the most effective treatment method, but many people cannot arrive at hospital within 4.5–6.0 hours, therefore systemic recombinant tissue plasminogen activator is limited.[7] Due to developments in pathophysiological stroke research, different mechanisms provide varied treatment opportunities.[8]

Oxidative stress and mitochondrial dysfunction have life-threatening effects in (I/R) injury. 1,8 cineol is an antioxidant that lessens the stress of oxidative and preserves mitochondrial action, probably performing as a protection of I/R injury. In this study, the defensive influence of a likely 1,8 cineol is explored.

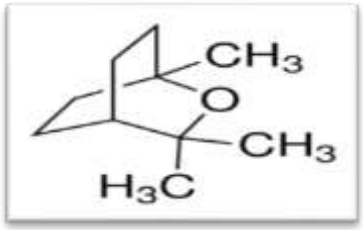
1,8 cineol, in contrast to I/R injury by defending mitochondrial dysfunction *through* the upregulation ofanti inflammatory IL10 and inhibit pro inflammatory mediatore like IL-6 and TNF-alpha.[9]

1,8-Cineole is conventionally consumed as a food flavor agent, whereas it had demonstrated to have a numerous pharmacological actions involving anticancer, anti-microbial, antioxidation and anti-inflammatory.[10-16] It was extensively adopted for recovery of

rheumatism, cough, bronchial asthma, and septic-shock-associated pathologies, in addition to pharmaceutical arrangements as in nasal spray, external applicant, disinfectant and analgesic.[17-19]

1,8-cineole is the monoterpenoid and is the foremost constituent of the elemental oil gotten from leaves of *Eucalyptus globulus* L.(RR) Eucalyptus oil (EO) and its main constituent, 1,8-cineole, has antimicrobial properties in contradiction of various bacteria, with viruses, methicillin-resistant Staphylococcus aureus (MRSA), mycobacterium tuberculosis and fungi (including Candida). Remarkably for an antimicrobial matter, there are likewise resistant-stimulatory, anti-inflammatory, antioxidant, analgesic, and spasmolytic properties. For the white blood cells, macrophages and monocytes are mostly influenced, particularly with augmented phagocytic action. Vapor inhalation or oral route applications have benefits for both non-purulent and purulent respiratory difficulties as in chronic obstructive pulmonary disease (COPD) asthma, and bronchitis.[20]

It was suggested that 1,8-cineole is a robust *in vitro* inhibitor of the relief of cytokines concerned in flight lane inflammation. It repressed arachidonic acid metabolism and production of TNFα, IL-1β, LTB_4 and TXB_2 in humanoid blood monocytes.[17] A different analysis also realized that 1,8- cineole exhibited antinociceptive features in mice and rats.[21]



Cineole

The permeation improving structure of 1,8-cineole had been recommended for increasing lipid fluidity)[22]

MATERIALS AND METHODS

Animals

A total of twenty four adult albino rats weighting (200-250 g), had been obtained from Animal Resource Center, the National Center for Drug Control and Researches. The rats had lodged in the animal house of pharmacy faculty / Kufa University in a room in which illumination was controlled for 12 hr on and 12 hr off). Hotness centigrade was kept at

(25±1°C) and humidity was kept at (60–65%). The rats obtained typical chow nutrition with water. The Animal Investigation Committee(AIC) office of Kufa university approved the experimental protocol.

Preparation of 1,8-Cineole

1,8-Cineole wase purchased from Sigma Chemical Co., St. Louis, MO. In order to conduct the experimentations, 1,8-cineole had been liquefied in 2% Tween80 gotten from Merck Co. in Germany.

Experimental groups

Subsequent to seven days of adaptation, the rats had been divided randomly into four equal groups (each of 6). The 1st Group was sham group: The rats had been exposed to the similar clinical processes like other groups but without BCCAO. For 2nd group, control ischemic reperfused group: The rats experienced anesthesia and surgery with BCCAO for half hour and after that reperfusion for sixty minutes excluding drugs. For 3rd group (control vehicle group), rats received daily the vehicle of 1.8-cineol, (10 mL/kg of 2% Tween 80 solution), p.o. (23) for three days before surgery, then anesthesia and surgery with BCCAO for half hour. Followed by reperfusion for 1 hrn for the 4th group (1,8-cineol treated group), the rats received 1,8-cineol. The dose of 1,8-cineole was 100 mg/kg per oral (po) one hour before induction ischemia.[23]

Induction of global brain ischemia

Each rat underwent general anesthesia by intraperitoneal (iP) ketamine &xylazine(80mg/kg&5mg/kg intraperitoneally)[23] Within few min, the rat became unconscious, then placed in supine position and exposed to light source to keep it worm. After that a midline ventral small skin incision in the neck was made and the paratracheal muscles and fascia were splitted an pulled by stay sutures to expose the trachea and all carotid arteries had been isolated from vagal nerves, then subjected jointly and obstructed by using atraumatic micro clamps and clamped for half hour. The clamp was taken out when reperfusion and ischemia was tolerable to have effect for one hour.[24]

Preparation of samples for IL-6 and TNF-α measurement

Tissue preparation for IL-6 measurement after beheading, the brain had been taken out and cleaned in cold normal saline (0.9% NaCl) to remove any blood or debris and then disfigured on filter paper. Afterward, tissues of brain had normalized in ice-cold 1:10 (w/v) 0.1 M

phosphate buffered saline (PBS) (pH 7.4), having 1× protease inhibitor concoction and 0.2% Triton X-100 for 30 seconds (53), using a high intensity ultrasonic liquid processor. The resultant homogenates had been filtered at 15,000 g for half hour, at 4°C, while supernatants had received and kept at −80°C for IL-6,TNF-α determination consistent with the producer's guidelines by means of enzyme-linked immunosorbent assay (ELISA) kits (Quantikine®/R&D Systems.USA and Uscn. Life Science Inc., USA).

Statistical analysis

Statistics investigations had been achieved using SPSS 17.0 simulator. An expert advice has been checked for the used tests. The whole data are stated as mean ± SE. The change among different groups had been investigated by one-way analysis of variance (ANOVA) and then by various comparison assessments as Post Hoc test employing LSD method. For all tests, P< 0.05 had been taken in consideration to be statistically substantial.

RESULT

Effect of Pro inflammatory markers (IL-6,TNF-α)

At the experiment completion, the level of cerebral IL-6 and TNF-α significantly (P<0.05 increased in control group unlike sham group. The cerebral IL6 level of cineol treated group were considerably (p<0.05) minor than control and control-vehicle group. The cerebral IL-6 magnitudes are shown in figure (1) and Figure(2).

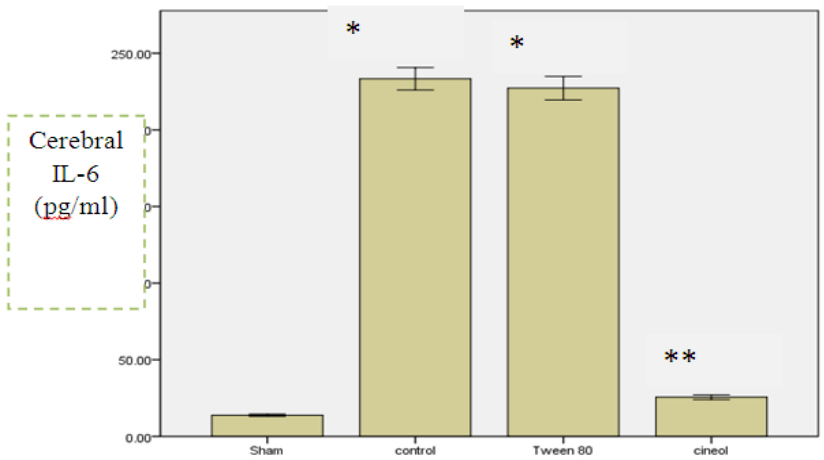


Figure (1): Error bar chart explains the change in mean± SEM magnitudes of cerebral IL-6 level (pg/mg) in 4 investigational sets at the completion of the experimentation using 6 animals in per group. * P < 0.05 vs. sham group, ** P < 0.05 vs. control-vehicle group.

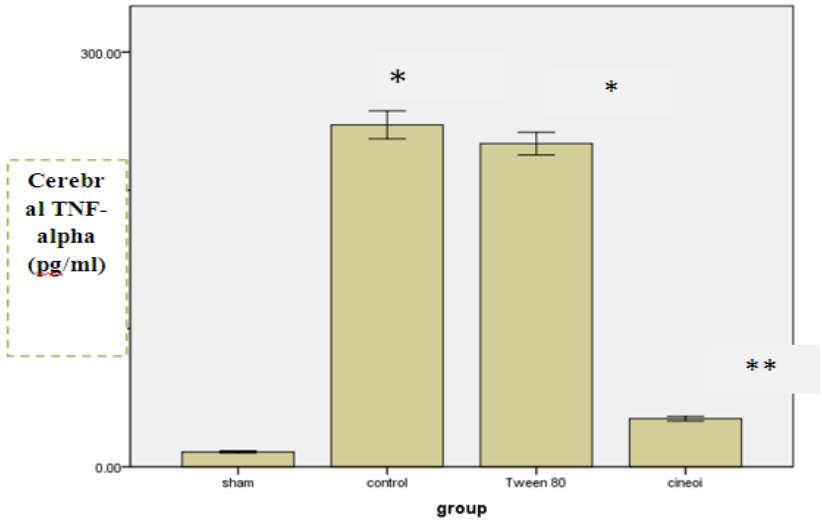


Figure (1): Error bar chart explains the change in mean± SEM magnitudes cerebral TNF-alpha level (pg/mg) 4 investigational sets at the completion of the experimentation using 6 animals per group. * P < 0.05 vs. sham group, ** P < 0.05 vs. control-vehicle group.

DISCUSSION

In this study, a noteworthy increase (P< 0.05) in IL-6 level has been concluded in the control group unlike the sham group.

The transient global cerebral ischemia reperfusion injury cause an extensive growth in the mRNA expression levels of TNF-α and IL-6 in the rat hippocampus.[25] The inflammatory response was initiated after transient cerebral ischemia and the release of inflammatory cytokines such as IL-6 and TNF-α occurred in the brain.[26] In this study, the protective effect of cineol have demonstrated in animal models against I/R injury cineole (eucalyptol or cajeputol) that is a terpene oxide and is a major element of mostly Eucalyptus oils (75%), Psidium (40–60%), rosemary (40%) and other vital oils.[27]

The cineole, a terpenoid oxide existing in various vital oils creates anti-inflammatory and antinociceptive properties. Cineole acts as a *robust in vitro* inhibitor of the cytokines release concerned in airway inflammation. It repressed arachidonic acid metabolism in addition cineole inhibiting cytokine production like TNFα, IL-1β, LTB_4 and TXB_2 in human blood monocytes.[28]

For monoterpene oxide 1,8-cineol, the dynamic component of the medically permitted drug Soledum®, is deep-rooted airway diseases treatment as in bronchitis, chronic obstructive pulmonary disease, chronic sinusitis, and bronchial asthma. While medical investigations

highlight the advantageous properties of 1,8-cineol in recovering inflammation diseases, the molecular action kind stays indistinguishable.

The cineole usually adopted to recover respirational illnesses owing to its secretolytic features and reduce exacerbations in asthma, sinusitis, and chronic obstructive pulmonary illness (COPD) as well as its myorelaxant effects.[29]

Li etal (2014) specified that the 1, 8-cineole has noticeable external tissue breakdown and nucleus cytoplasm focusing or lessening.[30]

1,8-cineol caused reduced amount of nuclear NF-κB p65 and decrease of its target gene IκBα at protein level in humanoid marginal blood mononuclear cells. a new action manner of 1,8-cineol in nuclear NF-κB p65 translocation inhibition through IκBα that reduced proinflammatory NF-κB target genes levels and could consequently extend the clinical field application of this natural drug for considering inflammation diseases recovery.[16]

1,8-cineol expressively reduced the TNF-α and IL-1β levels, and augmented the IL-10 level in lung matters after acute lung injury excited by lipopolysaccharide (LPS). It as well decreased the appearance of toll-like receptor 4 (TLR4), nuclear factor kappa B (NF-κB) p65 and myeloperoxidase action in lung tissues.[31,32]

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