ASSESSMENT OF EFFECTS OF NANOMATERIALS IN FENNEL (*Foeniculum vulgare* Miller) SEED ON THE CLOTH DISSOLUTION AFTER SPONTANEOUSLY STROKE OF MALE MOLE RAT (*Spalax leucodon*) IN MUŞ, TURKEY

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The purpose of this study was to evaluate effects of nanomaterials (α-thujone,α-pinene, camphene, sabinene, myrcene, α-phellandrene, *p*-cymene,limonene,1,8 cineole, β-ocimene,γ-terpinene, fenchone, camphor, terpinen-4-ol, methylchavicol, *cis* anethole, anisaldehyde ,*trans*-anethole,germacrene d,tetradecanoic acid, hexadecanoic acid,tetradecane; 1,2- benzenedi carboxylicacid,dioctylester, pentadecane, pentacosane,hexadecanol, hexacosane, octadecanol, octadecanone and extracts of fennel (*Foeniculum vulgare* Miller) seed on the cloth dissolution after stroke in male mole rat. Mole rats were the average weight of 345 grams. Limonene,1.8-cineole, sabinene, β-ocimene, camphor,terpinen-4-ol, terpinen-4-ol,methylchavicol, tetradecanoic acid,hexadecanoic acid, tetradecane, hexadecanol and octadecanol and 35-65 per cent of the water extracts of the fennel seed were increased significantly the dissolution of cloth ($p< 0.01$) and other nanomaterials in funnel seeds were increased significantly ($p<0.05$). These results are new results for the antithrombotic agents in the cerebral ischemia.

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1. Introductıon

Some people get transient attacks of brain stroke recover completely. They need good medical attention to prevent repeated attacks. Some gets brain infarct due to blood clots, such patients develop brain stroke over a period of few hours after initial alarming signals. Expert's care is needed to take care of emboli and clots. Some suffer from long standing high blood pressures and suddenly the blood vessel bleeds giving sudden and complete brain stroke in no time. Different patients react differently to brain stroke. A lot depends on the person's personality and his education and his belief about self and life and the family support. Depression in brain stroke patients is largely because of unexpected limitations in life arising from lost movement control and not necessarily from stroke pathology. Therefore solution lies in solving the problem of movement and not in antidepressant drugs. Relatives also play a role in recovery of brain stroke [1-24]. Some relatives, who are half informed or misinformed, sometimes get carried away by quacks and their hollow promises and do more damage to the patient than one can gauge. Some relatives are extremely responsible and look after the patient in the right direction under the guidance of expert and are a good support not only to the patient but a support also to the rehabilitation team. In most cases, cerebral ischemia is the result of a thrombotic event in the circulation outside the brain. In consequence, the current therapeutic approaches aim to improve the thrombotic pathology and the underlying cardiovascular defects. Following occlusion of cerebral blood vessels, thrombolysis within a very narrow time window is by now the only approved and effective intervention. Acute cerebral ischemia is the perfect example for the discrepancy between experimental splendour and

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clinical failure of novel neurobiological procedures. Numerous reasons account for this discrepancy. For example, animals undergoing cerebral artery occlusion are healthy in the cardiovascular system; the experimental intervention occurs before the occlusion or within a narrow time frame; substances can be applied intrathecally; many experimental drugs are not monitored for putative side effects, and finally it remains to be elucidated which experimental model reflects the clinical neuropathology following stroke [1-35]. In addition, the application of one drug might not be sufficient to rescue the ischemic neuroparenchym which suffers from multifactorial damage such as coincident inflammation, apoptosis, necrosis, withdrawal of trophic factors, excitotoxicity or breakdown of membrane potentials. In consequence, the optimal therapeutic regimen might comprise a `cocktail` of different compounds. The test of such a `cocktail`, however, is a nightmare for pharmaceutical companies and clinical stroke units. Numerous scientific and experimental studies have been performed to rescue neuronal death following ischemia and or to improve the neurological outcome. Many drugs which act on specific targets involved in the presumed ischemic cascade have been tested in clinical trials in the last decade. So far, none of these drugs was shown to be effective in improving outcome after acute cerebral ischemia [1-40].

Fennel (*Foeniculum vulgare* Miller) is an annual, biennial or perennial plant, depending on the variety, belonging to Apiaceae family and is native to the Mediterranean area. It has been cultivated and introduced into many regions outside that zone; it is grown commercially in some of them, such as Russia, India, China and Japan. Fennel has been known, since antiquity, as a medicinal and aromatic herb, commonly used to flavour liqueurs, breads, fishes, salads and cheeses. The drug consists of the dry, ripe, whole cremocarps and mericarps (commonly called seeds), which contain 1–4% (v/w) of volatile oil whose major constituents are the phenylpropanoid derivative *trans*-anethole and fenchone.The oil is used as an ingredient of cosmetic and pharmaceutical products for its balsamic, cardiotonic, digestive, lactogogue and tonic properties. The essential oil of aromatic herbs has usually been isolated either by hydrodistillation or extraction with classical solvents such as hexane or ethanol, as traditional spice-processing methods. These techniques present serious drawbacks: Low extraction efficiency, long extraction time, toxic residual solvent in the products and deterioration of the thermally sensitive materials. Much work has recently been done on the composition of fennel essential oil obtained by traditional techniques [30-43].

In this study, effects of nanomaterials in fennel (*Foeniculum vulgare* Miller) seed on the cloth dissolution after spontaneously stroke of male mole rat (*Spalax leucodon*) were evaluated.

2. Materıals and methods

Fennel seeds were purchased from the herbal seller in Malatya,Turkey and chemicals related fennel seeds were purchased commercially (Sigma, Merck). All of the fennel chemicals were prepared as 25 per cent aqueous solutions after preliminiary tests. Nanomaterials (α-thujone, α-pinene,camphene,sabinene,myrcene,α-phellandrene, p -cymene, limonene, 1.8-cineole,β-ocimene, γ-terpinene,fenchone,camphor,terpinen-4-ol, methylchavicol,cis-anethole, anisaldehyde, trans-anethole,germacrene-d,tetradecanoic acid,hexadecanoicacid,tetradecane; 1,2-benzenedicarboxylicacid,dioctylester, pentadecane, pentacosane, hexadecanol, hexacosane, octadecanol,octadecanone) and extracts of fennel (*Foeniculum vulgare* Miller) seed on the cloth dissolution after stroke were tested in the field. Many factors can influence the amount of essential oil in aromatic herbs, e.g., climate and environmental conditions, season of collection, age of plants and, for fennel especially, the stage of ripening of the fruits. The seeds were air-dried and stored in double layer paper bags at ambient temperature, protected from direct light until further analysis. Seeds were ground in an electric grinder and later 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60 and 65 per cent of the water extract was prepared to test on cloth. Sixty male mole rats (*Spalax leucodon*) were collected randomly in the fields plowed with tractors between Muş and Bingöl city, Turkey. Mole rats were the average weight of 345 grams. In the result of this process, heads of the mole rats had crushed through the tractor plow.Thus, The vast majority of the animals were paralyzed. Because, all of the animals under the soil was crushed. Living the animals with this trauma were used in the study area. Fennel extract and fennel seed chemicals were

injected to these animals paralyzed spontaneously by crushing in the field. Funnel extracts (all volumes % 0.01ml) and funnel seed constituents (all volumes %0.01 ml) were carried out intra cerebral injection once a day and three days period for all animals in the field. Because , more injections has not made any changes on clot dissolution in the brain when injections were made three days. All of animals were marked by a leather blue paint in the study fileds and whole animals were relased in same ecological fields. Thus, animals were allowed to heal in the natural environment. We established that animals were fed with tubers of orchids species [*Orchis tridentata* and *Orchis anatolica* (Orchidaceae)] underground. Recent works have reported isolation of anthocyanins, stilebnoids and triterpenoids from orchids. Orchinol, hircinol, cypripedin, jibantine, nidemin and loroglossin are some important phytochemicals reported from orchids. Also, these constituents of orchid tubers can effect to healing on stroke, because orchid tubers were found in whole animal holes.Therefore, effects of funnel nanomaterilas and chemicals of orchid tubers were considered together. After two weeks, animals were collected one by one from the holes and they were decapited under 50 mg/kg intra periotonal pentobarbital anesthesia and their brains (cerebrum and cerebellum) were analysed blood clots in the paralyzed area and all vessels and brain tissues [1-43].

3. Results

Limonene, 1.8 Cineole, sabinene, β-Ocimene, Camphor,Terpinen-4-ol, Terpinen-4-ol, Methylchavicol, Tetradecanoic acid, Hexadecanoic acid,Tetradecane, Hexadecanol and Octadecanol and 35-65 per cent of the water extracts of the fennel seed were increased significantly the dissolution of cloth ($p<0.01$) and other nanomaterials in funnel seeds were increased significantly ($p<0.05$). In this study, results were shown that both funnel extracts and funnel constituents have effected significantly on the cloth dissolution ($p< 0.05$ and $p< 0.01$). Findings of this study were shown that Figures 1-4.

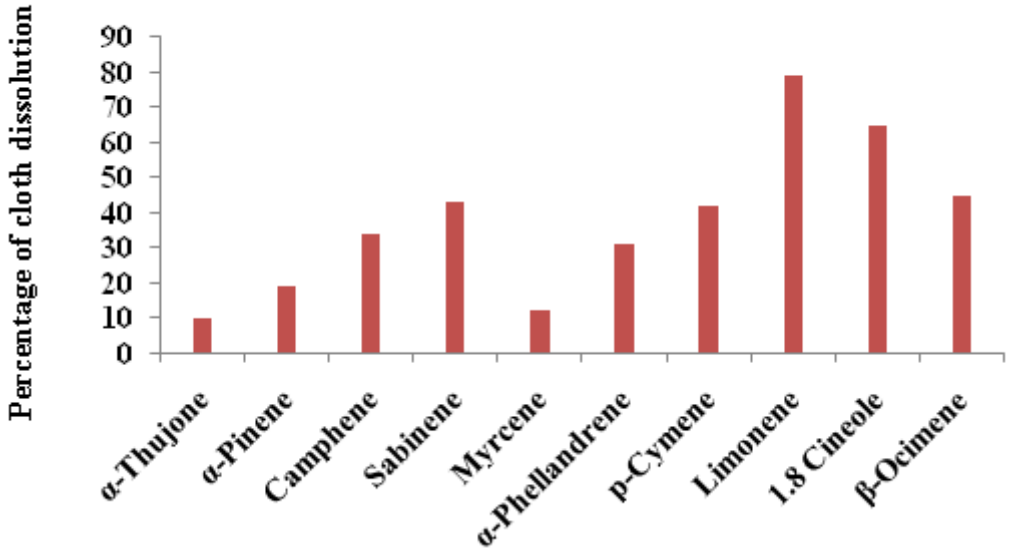

Fig . 1. Effects of funnel constituents on cloth dissolution.

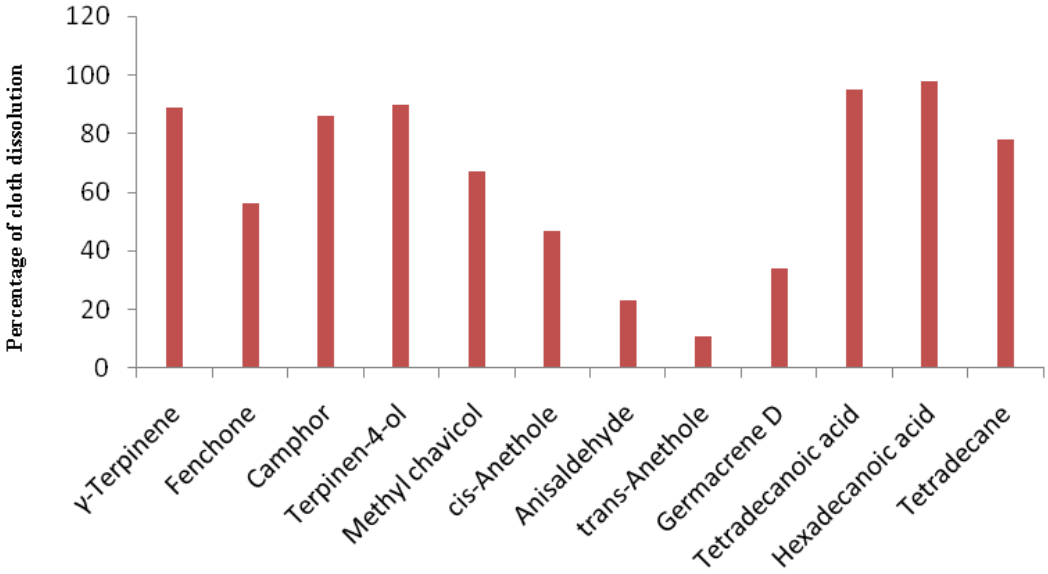

Fig. 2. Effects of funnel constituents on cloth dissolution

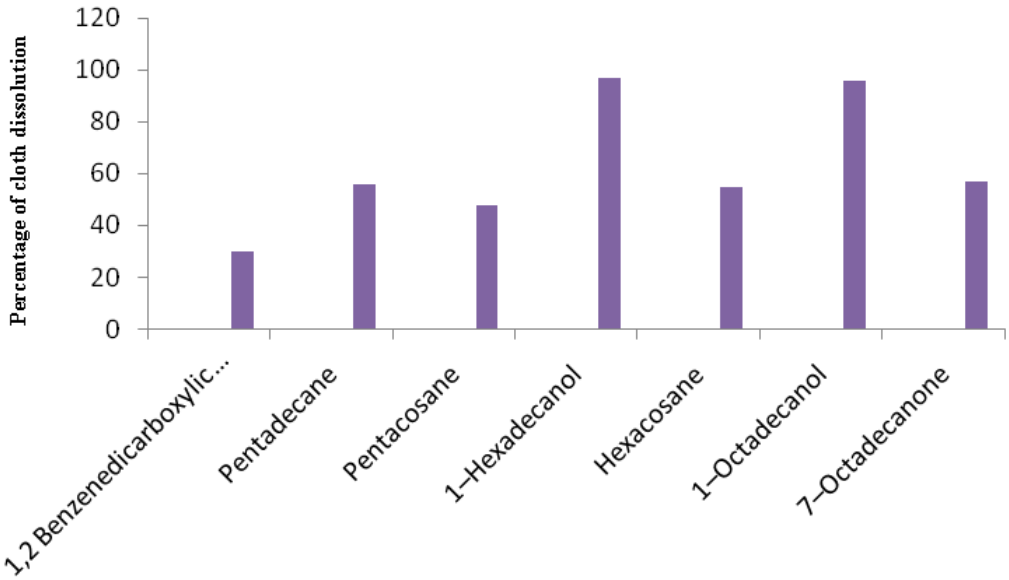

Fig. 3. Effects of funnel constituents on cloth dissolution

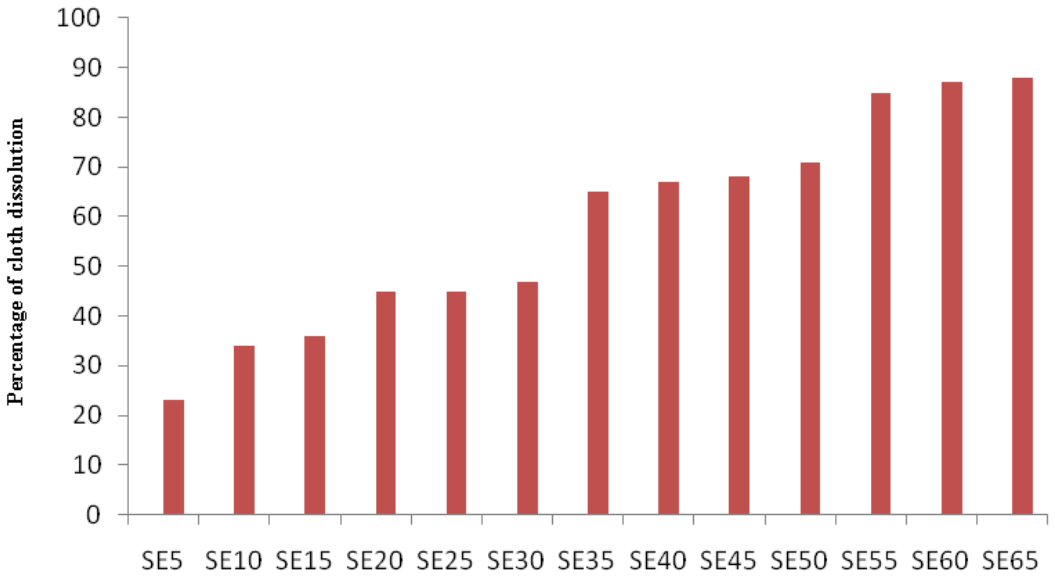

Fig 4. Effects of the water extracts of the fennel seed on cloth dissolution. SE: Seed Extract (%0.01 ml dosage and mg/100ml concentration).

4. Discussion

We could not met the investigation concerning with nanomaterials (α-thujone,α-pinene, camphene,sabinene, myrcene, α-phellandrene, p -cymene, limonene,1.8-cineole,β-ocimene, γ-terpinene, fenchone, camphor, terpinen-4-ol, methylchavicol,cis-anethole, anisaldehyde, trans-anethole, germacrene-d, tetradecanoic acid,hexadecanoicacid,tetradecane; 1,2-benzenedicarboxylicacid, dioctylester, pentadecane,pentacosane,hexadecanol,hexacosane, octadecanol,octadecanone) and extracts of fennel (*Foeniculum vulgare* Miller) seed on the cloth dissolution after stroke. Therefore, this study was performed . A stroke or "brain attack" occurs when a blood clot blocks an artery (a blood vessel that carries blood from the heart to the body) or a blood vessel (a tube through which the blood moves through the body) breaks, interrupting blood flow to an area of the brain [1-43]. When either of these things happen, brain cells begin to die and brain damage occurs. When brain cells die during a stroke, abilities controlled by that area of the brain are lost. These abilities include speech, movement and memory. How a stroke patient is affected depends on where the stroke occurs in the brain and how much the brain is damaged. For example, someone who has a small stroke may experience only minor problems such as weakness of an arm or leg. Excitatory amino acids, glutamate and aspartate, are endogenous compounds acting as neurotransmitters in brain, through the activation of three types of ionotropic receptors named after the initially described agonist activating them: N-methyl- D-aspartate (NMDA), alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainic acid receptors. NMDA receptor-associated channels are permeable to Na+, K+ and Ca2+ in a voltage-dependent manner, whereas AMPA and kainic acid receptors are linked to Na+ permeable channels. In addition, glutamate might also activate metabotropic receptors that induce G protein-mediated changes in second messengers. During brain ischemia there is a marked release of glutamate from the brain that can be monitored in plasma and cerebrospinal fluid from patients suffering ischemic stroke. Consistently with these clinical findings, pre-treatment of rats with the NMDA receptor antagonist, MK-801, decreases by 30 % infarct size following middle cerebral arterial occlusion showing that excessive stimulation of these receptors take place during cerebral ischemia, leading to neuronal degeneration. Following glutamate release, there is a marked stimulation of glutamate receptors leading to neuronal degeneration that shows two components: an acute Na+/Cl−-dependent neuronal swelling and delayed Ca2+-mediated cell death where a massive and prolonged Ca^{2+} influx to the cytoplasm and reactive oxygen production has been observed. The mitochondria also represent a potential Ca^{2+} source; however, during resting physiological

conditions mitochondrial Ca^{2+} content is low (about 200 nM). During the last two decades it has become widely accepted that neuronal damage following brain ischemia is due to a perturbation of cellular Ca^{2+} metabolism. Brain ischemia, which compromises the cellular bioenergetic status, leads to cell depolarisation and to a rise in [Ca^{2+}]. When ischemia ends, by reperfusion, the bioenergetic potential usually recovers and ion gradients are restored. However, neuronal damage can be observed following hours or days of reperfusion[1-37].

On the other hand, evidence has accumulated showing reactive oxygen species generation under excitotoxic or ischemic conditions. It was shown that NMDA toxicity involves both nitric oxide (NO) and reactive oxygen species, *i.e.* superoxide anion radical (O^{2-}), hydrogen peroxide (H_2O_2), and their highly cytotoxic by-product hydroxyl radical (OH). An OH generation also occurred *in vivo* in rat striatum under NMDA or glutamate exposure and during focal or global cerebral ischemia. An increasing amount of evidence has led different authors to propose free radical production as a potential mechanism of brain injury in stroke. An OH generation was actually reported in models of brain ischemia, where increased extracellular concentrations of excitatory amino acids might be the cause of this formation of reactive oxygen species, leading to oxidative stress and subsequent neuronal loss. In fact, evidence has accumulated showing that, when neurones are directly exposed to excitatory amino acids, a reactive oxygen species production occurs possibly through mechanisms involving phospholipase A_2, nitric oxide synthase or xanthine oxidase. For instance, an O_2^- generation was reported in neuronal cultures exposed to glutamate or NMDA. Mepacrine, a phospholipase A_2 inhibitor, could prevent this production, suggesting that polyunsaturated fatty acids released from membranes by this enzyme might be potential precursors of reactive oxygen species *in vivo*.Furthermore, NG-nitro-L-arginine methyl ester, a nitric oxide synthase inhibitor, could reduce OH efflux in rat striatum following NMDA perfusion. It was suggested that, when NO is produced in the presence of O_2^-, both compounds can interact and form a peroxynitrite anion intermediate ($ONOO^-$). The free radical superoxide anion is a product of normal cellular metabolism, produced mainly in mitochondria due to a switch from the normal four-electron reduction of O2 to a one-electron reduction. Superoxide is harmful to the cell because it reduces iron-III to iron-II, and interacts with nitric oxide radical forming the strong oxidant, peroxynitrite. Superoxide is scavenged by superoxide dismutases generating hydrogen peroxide that interacts with transition metal ions to form the hydroxyl radical, the strongest oxidant formed in biological systems. Destructive actions of the hydroxyl radical include alterations of DNA and initiation of chain reactions of lipid peroxidations. Lipid peroxides can interact with either iron-II or iron-III and give rise to alkoxyl and peroxyl radicals, respectively, each of which can initiate new chains of lipid peroxidation. Peroxidized plasmalemmal, endoplasmic reticulum, mitochondrial membrane lipids greatly interfere with cell function. It is vital for the cell to scavenge the peroxides produced and it does this mainly using the selenoenzyme glutathione peroxidase, which, in turn, uses, as cofactor, reduced-glutathione (GSH). The efficiency by which glutathione peroxidase can scavenge peroxides increases with increasing GSH concentration. In other words, relatively small increases in GSH concentration have a marked effect on the ability of glutathione peroxidase to scavenge peroxides. Indeed, increasing intracellular GSH has been demonstrated to increase the ability of cells to scavenge strong oxidants. Conversely, decreasing intracellular GSH results in greater damage following oxidative stress. GSH is also important in the regeneration of ascorbate, which has been used to reduce the vitamin E radical back to vitamin E. Hence, GSH plays a very central role in the ability of cells to manage oxidative stres [11-41].

Changes in Ca^{2+} influx and O_2^- production within the mitochondria prior to cell death are events shared by cells treated with neurotoxic agents such as NMDA, veratridine or β-amyloid. In addition, cell Ca^{2+} overload can also cause mitochondrial failure, that might lead to cell death. Recently, the mitochondria have became the main focus of interest in apoptotic cell death pathways and probably also play a key role in delayed post-ischemic cell death. This is because, during recovery, re-energized mitochondria take up most of the Ca^{2+} that has entered the cell during the insult. This Ca^{2+} uptake activates mitochondrial phospholipases, and seems to trigger increased production of free radicals as well as release of mitochondrial proteins, some of which are proapoptogenic. These factors seem to be released when the mitochondrial membrane permeability is increased by the opening of a large conductance channel: the mitochondrial

permeability transition pore (MPTP). Thus, gradually raising [Ca^{2+}]i followed by mitochondrial Ca^{2+} accumulation and increased free radical production finally leads to irreversible damage to mitochondria, and to the triggering of a cell death program [12-34].

5. Conclusıons

The melting or dissolving process of clot in the brain after stroke is an important problem. It is also used for substances such as glycerol and mannitol. Naochemicals in fennel seeds and seed extracts have made the chemical solvent effects on clotting. Therefore, these nanomaterials and extracts of fennel seed have revealed new findings in the treatment of stroke. Also, vampire bat derived substances (Desmoteplase) are used in the treatment of stroke for the first few days. But, fennel chemicalls are understood that the matter would be more beneficial.

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