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Neuroprotective effects of Alpha-pinene nanophytosome on oxidative damage induced by carbon tetrachloride (CCl_4)

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Introduction

Damage caused by CCl_4 has been revealed in various organs including liver, brain, lungs and kidneys (Zaib & Khan, 2014). CCl_4 is metabolized to trichloromethyl radical (CCl_3•) by cytochrome P450 in the endoplasmic reticulum of liver cells and quickly reacts with oxygen to form trichloromethylproxyl radical (CCl_3OO•) (Altinoz *et al*, 2018). Studies have shown that CCl_4 induces oxidative stress by causing lipid peroxidation in addition to changing the antioxidant defense of the brain (Okoro *et al*., 2019).

Alpha-pinene ($C_{10}H_{16}$) is a natural bicyclic hydrocarbon with neuroprotective, antioxidant, anti-inflammatory, anti-tumor and antimicrobial effects (Allenspach & Steuer, 2021). But due to low bioavailability, the use of this compound is limited. Phytosomes have higher antioxidant activity compared to normal plant extracts (Jain et al., 2009). Nanophytosome is one of the newest nanocarriers and a fast way to absorb plant nutrients (Ghanbarzadeh et al., 2016). They can improve the antioxidant activities of bioactive compounds (Al-Askar *et al*., 2017). Therefore, the aim of this study is to investigate the effects of Alpha-pinene nanophytosome

on lipid peroxidation level, antioxidant enzymes and glutathione level in brain damage induced by CCl_4 in male Wistar rats.

Materials & Methods

In this study, 35 male Wistar rats were randomly divided into 5 groups: Control group: comprised of untreated animals. APNP group: the animals received oral gavage of 50 mg/kg of APNP for 14 consecutive days. CCl_4 group: animals were administered intraperitoneal injection of CCl_4 dissolved in olive oil at a 1:1 ratio (twice a week) for two weeks. The treatment groups received CCl_4 (twice a week) and oral gavage of AP and APNP (50 mg/kg/) for 14 consecutive days. Two hours after the last gavage, the rats were decapitated and 200 mg of brain tissue was homogenized in 3 ml of 0.4 M sodium phosphate buffer (pH=7.4). Then the samples were centrifuged at 13000 rpm for 20 minutes. The supernatant was separated and used to measure antioxidant activities. The CAT and SOD activities were applied following the method of Genet *et al*., (2002). GPx enzyme activity was measured according to the method of Sharma and Gupta, (2002). Fukuzawa and Tokumurai, (1976) method was used to measure GSH level. The MDA level was examined based on Esterbauer and Cheeseman, (1990).

Results & discussion

The levels of CAT and SOD enzymes activity in the CCl_4 group showed a significant decrease ($p<0.01$) compared to the control group. The treatment group with Alpha-pinene nanophytosome 50 mg/kg caused a significant increase ($p<0.05$) compared to the CCl_4 group. But, the treatment with Alpha-pinene in the same dose could not cause a significant increase compared to the CCl_4 group.

The level of GPx enzyme activity in the CCl_4 group showed a significant decrease ($p<0.001$) compared to the control group. In the group treated with Alpha-pinene and Alpha-pinene

nanophytosome 50 mg/kg, a significant increase was observed ($p<0.001$) compared to the CCl_4 group.

In line with the studies of Altinoz *et al*. in (2018), CCl_4 injection decreased the activity of antioxidant enzymes SOD, CAT and GPx in the rat brain. Our results showed that treatment with Alpha-pinene nanophytosome could effectively increase the activity of antioxidant enzymes SOD, CAT and GPx. Based on the results of this research, Shriram *et al*. (2022) showed that the phytosomal formulation of curcumin can increase the activity of antioxidant enzymes in the toxicity caused by CCl_4 in the liver and kidney due to its higher bioavailability and more antioxidant properties than pure curcumin.

The level of GSH in the CCl_4 group decreased significantly ($p<0.001$) compared to the control group. The treatment group with Alpha-pinene and Alpha-pinene nanophytosome 50 mg/kg showed a significant increase compared to the CCl_4 group ($p<0.01$) and ($p<0.001$) respectively.

The level of MDA in the CCl_4 group increased significantly ($p<0.001$) compared to the control group. The treatment group with Alpha-pinene and Alpha-pinene nanophytosome 50 mg/kg decreased significantly ($p<0.01$) and ($p<0.001$) compared to the CCl_4 group.

Also, based on the results of this research, chronic injection of CCl_4 increases the level of MDA and decreases the level of GSH. Depletion of GSH increases the sensitivity of the brain to oxidative stress and redox imbalance. Consistent with our results, the study of Ritesh *et al.* (2015) It showed that the level of GSH decreased and the level of MDA increased after CCl4 injection. Although Alpha-pinene nanophytosome could more effectively increase the level of GSH and decrease the level of MDA in the brain of rats exposed to CCl_4. Based on the results of this research, Baradaran *et al*. (2015) showed that the phytosomal formulation of silymarin

could improve the levels of these two parameters more effectively than silymarin. Similar to previous reports, we used a novel drug delivery system to overcome these barriers as well as the low bioavailability of essential oils and increase efficacy in the brain. These effects are probably attributed to the antioxidant activity of Alpha-pinene nanophytosome. Therefore, our results prove the superiority of Alpha-pinene nanophytosome over pure alpha-pinene due to its better bioavailability and solubility.

Conclusion

The findings of this research showed that chronic injection of CCl_4 for 14 days decreased antioxidant indexes and increased oxidative stress in the brain. Treatment with Alpha-pinene and Alpha-pinene nanophytosome caused a significant reduction in oxidative stress and brain damage due to its antioxidant properties. Also, the healing effects of Alpha-pinene nanophytosome due to its high solubility and bioavailability and greater permeability of the blood-brain barrier compared to Alpha-pinene are significantly higher in the brain damage model induced by CCl_4. Therefore, it can be a possible candidate for brain injury management.

*Keywords**:** brain damage, antioxidant enzymes, carbon tetrachloride, Alpha-pinene nanophytosome*

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Declaration of conflict of interest: *The authors declare no conflict of interest related to this study.*

Statement of Ethics

All experimental procedures were conducted in compliance with the regulations stipulated by the Bioethics Committee (IR.UMZ.REC.1401.069) of the University of Mazandaran Research Vice-Chancellor.