

# Hepatoprotective effect of medicinal plant in nanoemulsions form

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## ABSTRACT

**Introduction:** Clove extract (CE) and grape seed oil (GSO) are medicinal plant compounds. Clove (*Syzygium aromaticum*) and grape (*Vitis vinifera*) are widely recognized botanical species with established therapeutic benefits. This study aims to assess the hepatoprotective effect of nanoemulsions containing CE and GSO in acute liver injury induced by carbon tetrachloride (CCl<sub>4</sub>) in rats (*Rattus norvegicus*).

**Materials and Methods:** Twenty laboratory mice with criteria were healthy, male, and weighing around 250 mg. Liver damage was induced in experimental animals using carbon tetrachloride (CCl<sub>4</sub>) on days 20 and 21 of treatment. The experimental animals were divided into four groups, the group 1 (G1) was a negative control group which was not induced and given standard feed, the group 2 (G2) was a positive control group which was induced and given standard feed, the 3 (G3) was induced and given CE nanoemulsion pretreatment and GSO formula A, the group 4 (G4) was induced and given pretreatment of nanoemulsion CE and GSO formula B. Giving nanoemulsion according to the group for 21 consecutive days. Next, on day 22, serum and liver tissue samples were taken to assess the condition of the liver tissue.

**Results:** The study's findings demonstrated that the administration of nanoemulsions containing CE and GSO exhibited a dose-dependent reduction in acute liver injury induced by CCl<sub>4</sub>. Liver function based on levels of serum *aspartate aminotransferase* (AST) and *alanine aminotransferase* (ALT) has a significant difference in group 2 compared to group 4 with a P value of 0.006 and 0.01. The liver histopathology showed a healing process in the group treated with clove and grape nanoemulsion therapy. In group 4 there was no vacuole degeneration, reduced inflammatory cell infiltration, and increased neovascularization compared to group 2.

**Conclusion:** The hepatoprotective effect of nanoemulsions containing CE and GSO was observed to have antioxidant, antibacterial, and anti-inflammatory properties resulting in significant improvements in serum biomarkers related to hepatotoxicity and histopathological analysis of liver injury.

## KEYWORDS:

Hepatoprotective, liver injury, nanoemulsion

## INTRODUCTION

Injury to the liver can be caused by the consequences of oxidative stress, which arises when oxidants and antioxidants are not balanced. Hepatocyte proteins, lipids, and DNA are significantly impacted by reactive oxygen species (ROS) and reactive nitrogen species. These processes lead to defects in the liver's structure and function.<sup>1</sup> The size and anatomical positioning of the liver contribute to the persistent challenge posed by liver injuries in the context of achieving favorable treatment outcomes. The necessity of surgical intervention should be primarily determined by clinical characteristics, with particular emphasis on the assessment of hemodynamic condition. In this study, we endeavored to elucidate our methodology for addressing hepatic injuries. Furthermore, we emphasized the importance of careful management, considering the elevated mortality rates linked to liver trauma interventions.<sup>2,3</sup> Routine and excessive administration of analgesic and antipyretic drugs leads to hepatotoxicity.<sup>4,5</sup> Liver injury serves as a prevalent pathophysiological foundation for several liver diseases, with prolonged liver injury often playing a significant role in initiating liver fibrosis, cirrhosis, and hepatocellular carcinoma (HCC).<sup>6,7</sup>

The research that has been done extensively supports the employment of herbal medicines as a prominent avenue for developing innovative pharmaceutical substances to address serious diseases. Numerous research indicates that many plant species include phytoconstituents, including glycosides, saponins, flavonoids, steroids, tannins, alkaloids, terpenes, and other compounds that possess pharmacological properties. Clove, scientifically referred to as *Syzygium aromaticum*, is a conventional spice that has been historically employed for food preservation and contains a range of therapeutic attributes. *S. aromaticum* exhibits a high abundance of sesquiterpenes, monoterpenes, hydrocarbons, phenolic compounds, and several other phytochemicals. The composition of clove extract (CE) primarily consists of three crucial phytochemical compounds, namely eugenyl acetate, eugenol, and -caryophyllene. Multiple investigations have demonstrated that eugenol possesses antiviral, antifungal,

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anticancer, antiseptic, antidepressant, antispasmodic, and antibacterial attributes, rendering it efficacious against a diverse range of pathogenic bacteria.<sup>8–12</sup> The efficacy of clove essential oil (*Syzygium aromaticum*, L.) in inhibiting the growth of many pathogenic microbes has been extensively established.<sup>13,14</sup>

In along with cloves, grape seeds have been extensively utilized in the healthcare industry. Grape seed oil (GSO) possesses a notable abundance of phenolic compounds, fatty acids, and vitamins, rendering it of considerable commercial significance within the medicinal, cosmetic, and food sectors. The suggestion of utilizing it as a consumable oil has also been put out, mostly due to its favorable sensory attributes. GSO possesses advantageous attributes for human health, including anti-inflammatory, cardioprotective, antibacterial, and anticancer qualities. Additionally, it has the potential to interact with several cellular and molecular pathways. The observed impacts have been attributed to the elements found in GSO, including tocopherol, linolenic acid, resveratrol, quercetin, procyanidins, carotenoids, and phytosterols.<sup>15,16</sup>

Carbon tetrachloride (CCl<sub>4</sub>) therapy can cause steatosis in mice, which is a model for both alcoholic and non-alcoholic fatty liver disease in humans. Compared to animals treated with CCl<sub>4</sub>, proanthocyanidins successfully reduced lipid buildup, liver injury, and DNA damage while also restoring antioxidant enzyme levels. Further research indicated that proanthocyanidins reduced CYP2E1 production in the liver, preventing the first step in free radical synthesis from CCl<sub>4</sub>. Proanthocyanidins isolated from grape seeds were administered orally to reduce CCl<sub>4</sub>-induced hepatic steatosis and liver damage by lowering oxidative stress and inhibiting cytochrome CYP2E1.<sup>17</sup>

Due to the above, it has been suggested that the combination of cloves and grape seeds exhibits considerable promise. The nanoemulsion preparation mixture has been extensively refined due to its exceptional ability for absorption. They improve the solubility and bioavailability of medications that are weakly water soluble by creating a large interfacial area for drug dissolution with their small droplet size, which is typically between 20 and 200 nanometers. Additionally, because nanoemulsions can encapsulate both hydrophilic and hydrophobic medications. Nanoemulsions are regarded as thermodynamically stable isotropic systems, in which the input energy is produced mechanically or physicochemically, and the two immiscible liquids mix to form a single phase that aids emulsifying agents like cosurfactants and surfactants.<sup>18,19</sup>

This study aims to analyze the hepatoprotective effect of nanoemulsion containing CE and GSO induced by carbon tetrachloride (CCL<sub>4</sub>) in animal models. Hepatotoxic agents commonly used to induce liver damage are acetaminophen, galactosamine, acetaminophen, thioacetamide, and azoxymethane.<sup>20</sup> In this study, CCL<sub>4</sub> was used, since it is a potent hepatotoxic, nephrotoxic, and prooxidant agent that is commonly used to induce hepatotoxicity in experimental animals. It is also a model that is frequently used to screen for the hepatoprotective activity of medications and natural products. Histopathological analysis and the quantity of cytoplasmic enzymes released into the bloodstream are

typically used to determine the severity of injury to the liver.<sup>21</sup> The CCl<sub>4</sub>-induced hepatotoxicity process occurs due to biotransformation into trichloromethyl free radicals (CCl<sub>3</sub>) or trichloroperoxy radicals (CCl<sub>3</sub>O<sub>2</sub>-) produced by the mixed-function cytochrome P450 oxygenase system of the endoplasmic reticulum, which causes oxidative stress and membrane damage.<sup>22</sup>

Determining the architectural alteration is one of the most important aspects of the histological evaluation of liver biopsies in the context of liver disease.<sup>23</sup> The mitigation of liver damage caused by CCl<sub>4</sub> has long been regarded as an indication of liver protective action, as the alterations associated with CCl<sub>4</sub>-induced liver damage are similar to those seen in acute viral hepatitis. In this work, attenuation of carbon tetrachloride-induced hepatotoxicity is an excellent technique to evaluate the liver protective capabilities of CE and GSO.<sup>24</sup> This study aims to assess the hepatoprotective effect of nanoemulsions containing CE and GSO on the level of acute liver damage using histopathological evaluation and increased levels of certain enzymes released into the circulation induced by carbon tetrachloride (CCl<sub>4</sub>).

## MATERIALS AND METHODS

### *Plants and nanoemulsions preparation*

Clove (*Syzygium aromaticum* L.) samples were obtained from the agricultural region of West Java, Indonesia. The clove samples were analyzed at the Bandungense Herbarium, School of Life Technology (SITH), Bandung Institute of Technology Indonesia. The process of plant determination is carried out by identifying the shape, color, and texture of plant parts. The color and texture of both the front and back sides of the plant sample contain deterministic parameters to obtain a unique optimum combination of features that maximize the identification rate. The plant database is created from commonly used front and back scan images of plants. Plants are classified based on a combination of shape and dimensions. The GSO used in this study was sourced from Tabanan Regency, Bali, Indonesia. An examination using Gas Chromatography-Mass Spectrometry (GC-MS) was conducted to ascertain the precise composition of the GSO.

Based on the comparison of GCMS analysis results of grape seed oil, it can be seen that grape seed oil contains fatty acids in the form of Hexadecanoic acid (CAS) Palmitic acid, 9-Octadecenoic acid (Z)-, methyl ester (CAS) Methyl oleate, Tetradecanoic acid (CAS) Myristic acid (table 1). Palmitic acid is a saturated fatty acid that also shows antibacterial activity against gram-positive and gram-negative bacteria. The production of clove flower ethanol extract involves utilizing the maceration process. Initially, a mass of 300 grams of simplicia was measured. The extraction process was conducted using the maceration technique, employing a solvent consisting of 96% ethanol. The ratio of simplicia to solvent was maintained at 1:10, and the extraction duration spanned three days. Subsequently, the filtrate is gathered and subjected to evaporation utilizing a rotating vacuum evaporator, maintaining a temperature below 50°C. This is then succeeded by concentrating the resulting extract by applying a water bath at a temperature of 60°C till it attains the state of a concentrated clove extract.

The composition analysis of the extracted portions of clove Phytochemical screening aims to determine the content of compounds contained in both the simple and extract that will produce pharmacological effects.

The nanoemulsion formulation was prepared through the precise measurement of each constituent. The ethanol extract derived from clove flowers was dissolved in ethanol of analytical grade with the assistance of ultrasonic equipment. The dissolved extract is afterward combined with grape seed oil, tween 80, and PEG 400. The mixture is then stirred using a magnetic stirrer on a hotplate, operating at 750 revolutions per minute for 15 minutes. The stirring process is conducted at a temperature of 40°C to ensure homogeneity. The water phase, specifically distilled water, should be gradually incorporated into the mixture while employing a magnetic stirrer operating at a speed of 750 revolutions per minute (rpm) for 30 minutes at 40°C. Subsequently, the sample was subjected to sonication for 60 minutes, with the application of plastic wrap to ensure proper containment. Several nanoemulsion formulas were made, then the two best formulas were taken for further testing, namely formulas A and B with clove extract content of 25% and 50% respectively.

#### *Histopathology assessment*

A biopsy of the rat's liver tissue was performed then the sample was soaked in 10% formol salt solution for 24 hours to fix it. These samples were then immersed in a 10% formol saline solution for 24 hours to fix them. The samples underwent a washing process using tap water, followed by a series of dilutions with several types of alcohol (methyl, ethyl, and 100% ethyl) to remove moisture. The specimens underwent a rinsing process using xylene, followed by embedding in paraffin at a temperature of 56 °C within a hot air oven for 24 hours. The tissue blocks, composed of paraffin and beeswax, were prepared for sectioning using a sled microtome at a thickness of 4 µm. The tissue slices that were acquired were carefully placed onto glass slides. These slides were then subjected to a deparaffinization process to remove the paraffin wax. Subsequently, the sections were stained using hematoxylin and eosin stain, a commonly used method for routine examination. The stained slides were then observed using a Leica light microscope. Analysis by assess the score of Hepatocyte vacuolar degeneration, Fatty change in hepatocytes, Fibroblastic cells proliferation, number of inflammatory cells, and neovascularisation. Histoscore from 0-3 (nill, mild, moderate, severe).

#### *Blood sampling and biochemical evaluation*

Blood samples were collected at the final stage of the experiment. They were retrieved from the cardiovascular veins of each rat using fine capillary. Serum samples were utilized to examine several biochemical parameters, explicitly focusing on liver function tests. The activities of hepatic enzymes, specifically alanine transaminase (ALT) and aspartate transaminase (AST), were examined.

#### *Experimental animals*

Healthy adult male rats (*Rattus norvegicus* Strain) of approximately the three months of age, weighing around 250 gram, were purchased from Animal Laboratory Bandung. The animals were maintained in a controlled

environment with a 12-hour light-dark cycle, a relative humidity of 50 ± 5%, and an ambient temperature of 25 ± 2 °C. Rats received a standard pellet diet and water ad libitum.

#### *Experimental design*

A total of twenty mice were utilised in a research investigation aimed at examining the potential protective properties of CE and GSO against hepatotoxicity induced by CCl<sub>4</sub> in rats. The animals were randomly allocated into four groups of equal size, each consisting of five rats. The animals were then subjected to the following treatments:<sup>25,26</sup>

- i. Group 1: serving as the negative control group, consisted of rats that were provided with a typical synthetic food for 3 weeks.
- ii. Group 2: positive control group, were administered a regular meal and intramuscularly injected with CCl<sub>4</sub> (twice) through split injection at a dosage of 1 mL/kg (body weight) on the last two days of 21 days experimental period.
- iii. Group 3: treatment group formula A, rats were administered a conventional meal and intramuscularly injected with CCl<sub>4</sub> (twice) using a split injection technique at 1 mL/kg (body weight) on the last two days of 21 days experimental period and they were orally administered nanoemulsion CE and GSO formula A at a 1 mL/kg dosage, for three weeks.
- iv. Group 4: treatment group formula B, rats were administered a conventional meal and intramuscularly injected with CCl<sub>4</sub> (twice) using a split injection technique at 1 mL/kg (body weight) on the last two days of 21 days experimental period and they were orally administered nanoemulsion CE and GSO formula B at a 1 mL/kg dosage, for three weeks.

#### *Statistical analysis*

The statistical analysis was performed using the GraphPad Prism software, specifically GraphPad 9 Software. The statistical significance of the data was evaluated by employing a one-way analysis of variance (ANOVA), followed by doing Tukey test as a post-hoc test to identify which particular means of several groups differ significantly from one another. A P-value below the threshold of 0.05 (P < 0.05) was considered to have statistical significance.

#### *Ethics statement*

The experimental procedures pertaining to the animals and their welfare were carried out in accordance with the ARRIVE 2.0 guidelines for Laboratory Animals in Research, as authorised and disseminated by the World Health Organisation. The Ethical Committee of the Faculty of Medicine at Unisba Bandung, Indonesia, granted clearance (102/KEPK-Unisba/V/2023) for the procedures applied and the welfare of the animals involved in the study.

## **RESULTS**

#### *Histological examination*

Histopathological examination is still the gold standard in assessing liver damage. The liver is susceptible to many toxins and pollutants such as CCl<sub>4</sub>. Exposure to CCl<sub>4</sub> can cause the formation of ROS and cause liver damage. The liver histopathology showed a healing process in the group treated with clove (*Syzygium aromaticum*) and grape (*Vitis vinifera*)

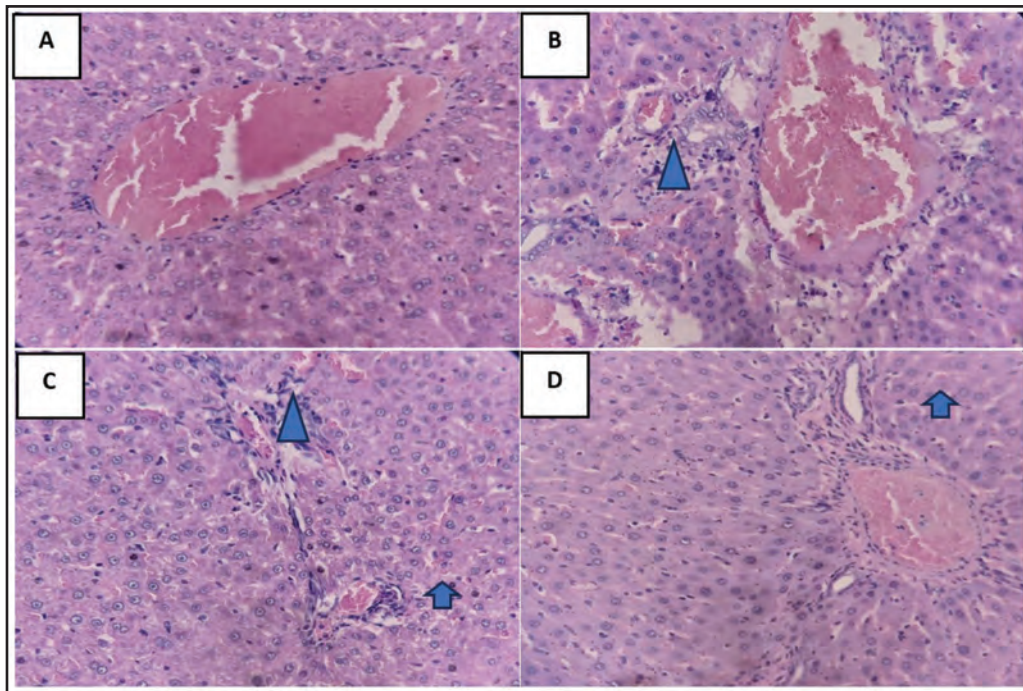
**Table I: Analysis of Gas Chromatography-Mass Spectrometry**

Compounds	GCMS (%)
Hexadecanoic acid (CAS) Palmitic acid	63.65
9-Octadecenoic acid (Z)-, methyl ester (CAS) Methyl oleate	5.03
Tetradecanoic acid (CAS) Myristic acid	31.32

**Table II: The score of histological changes observed in the liver tissue of the experimental groups**

Component	PC	NC	FA	FB
Hepatocyte vacuolar degeneration	+	-	-	-
Fatty change in hepatocytes	+	-	-	-
Fibroblastic cells proliferation	-	-	+	+
Inflammatory cell infiltration in the portal area	++	-	+	-
Neovascularisation	-	-	+	++

PC: positive control, NC: Negative control.  
 FA: Nanoemulsion formula A, FB: Nanoemulsion formula B  
 +++ Severe, ++: Moderate, +: mild, -: nil.



**Fig. 1:** (A) Group 1: No histopathological changes were seen; (B) Group 2: There is vacuolar generation of hepatocytes, fatty changes in hepatocytes, and many inflammatory cells (▲); (C) Group 3: The presence of neovascularisation (↕) in the portal vein, accompanied with limited infiltration of inflammatory cells (▲), was seen in the portal area; (D) Group 4: The increase of neovascularisation (↕) in the portal vein was seen in the portal area. (H&E.X40)

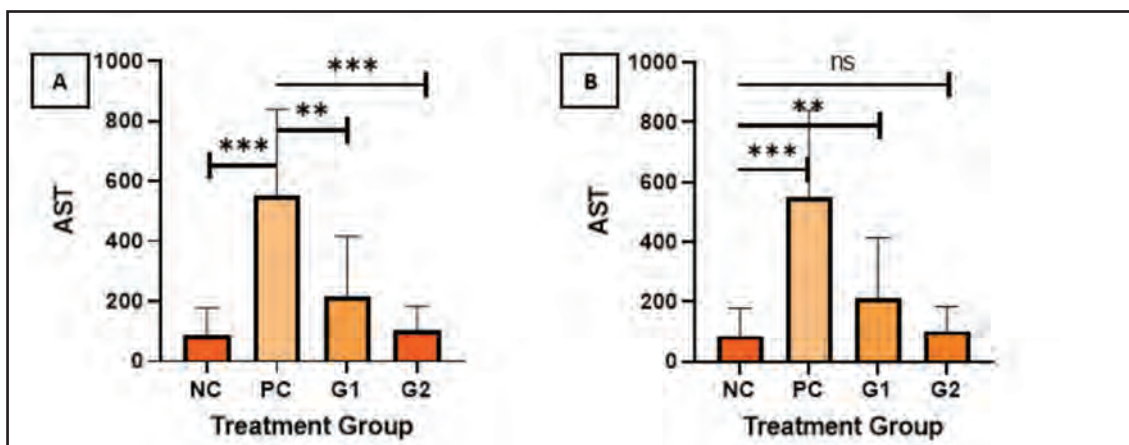
nanoemulsion therapy. In group 4 (nanoemulsion formula B) there was no vacuole degeneration, reduced inflammatory cell infiltration, and increased neovascularization compared to group 2 (Fig. 1, Table IV).

*Liver function*

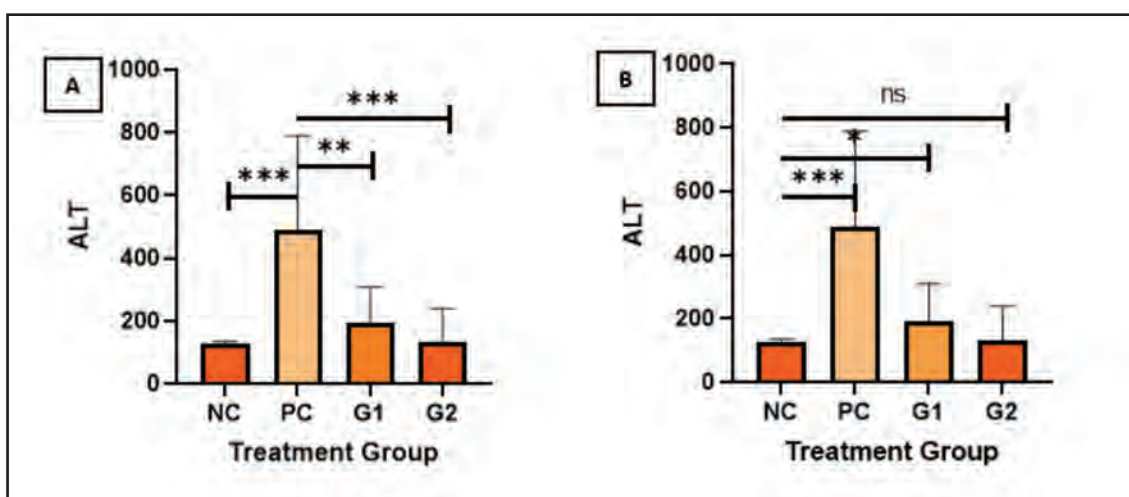
To investigate liver function, serum levels of AST and ALT were examined, with results as depicted in Figures 2 and 3. These findings indicate that the positive control group of mice induced with CCl4 experienced a significant increase in AST and ALT levels compared with the negative control group. However, administration of nanoemulsion before treatment succeeded in reducing the increase in blood AST

and ALT activities. The findings of this study indicate that the nanoemulsion formulation, containing CE and GSO, has hepatoprotective properties in the context of acute liver damage caused by CCl4. Liver function based on levels of serum AST and ALT has a significant difference in group 2 (positive control) compared to group 4 (nanoemulsion formula B) with a p-value = 0.01.

Despite both G1 and G2 were given the same standard feed in this study, G1 is a negative control group that does not experience an acute liver injury caused by carbon tetrachloride, whereas G2 is a positive control group that experiences an acute liver injury caused by carbon



**Fig. 2:** Pretreatment of CCl<sub>4</sub>-induced acute liver damage of mice treated with nanoemulsion. AST values were measured 48 hours after CCl<sub>4</sub> injection. (A) G<sub>2</sub> (\*\*\*) shows significant differences compared to the PC group (P=0.006) while G<sub>1</sub> (\*) indicates no significant differences compared to the PC group (P=0.052). (B) G<sub>1</sub> and G<sub>2</sub> (ns) show no significant differences compared to the NC group (P>0.05)



**Fig. 3:** Pretreatment of acute liver damage in rats induced by CCl<sub>4</sub> by administration of nanoemulsion. ALT values were measured 48 hours after CCl<sub>4</sub> injection. (A) G<sub>2</sub> (\*\*\*) showed a significant difference compared to the PC group (p=0.01) while G<sub>1</sub> (\*) showed no significant difference compared to the PC group (p=0.9). (B) G<sub>1</sub> and G<sub>2</sub> (ns) did not show significant differences compared to the NC group (p>0.05)

tetrachloride but does not receive any hepatoprotective agents. This makes figure 2 more clearly illustrate the hepatotoxic effect caused by carbon tetrachloride. G<sub>1</sub> did not exhibit a significant difference from PC, while G<sub>2</sub> had shown a substantial difference.

#### Aspartate aminotransferase

According to the data presented in Figure 2, the group treated with CCl<sub>4</sub> (referred to as PC) exhibited a notable increase in AST activity (551,4 IU/L) as compared to the normal control group (referred to as NC, with a value of 102,8). The groups treated with nanoemulsions exhibited a notable reduction in AST activity, with values of 214 and 112,6, respectively, in comparison to the CCl<sub>4</sub> control group (AST activity of 551,4). This indicates the efficacy of both nanoemulsions in ameliorating the impact of CCl<sub>4</sub> by preserving the hepatocytes' integrity.

#### Alanine aminotransferase

ALT activity observed in the animals subjected to testing exhibited a range of 126,5 to 428 IU/L, as depicted in Figure 3. The PC group had the highest significant value of ALT activity, which was reported as 428 IU/L. The application of nanoemulsions containing CE and GSO has been found to exhibit a substantial reduction in the activity of ALT levels, with values of 192,2 and 170,6 respectively, effectively restoring them to normal levels observed in the control (NC) group, which recorded a value of 126,5.

#### DISCUSSION

This study shows that a medicinal plant, specifically synthesized into nanoemulsions containing CE and GSO, exhibited a hepatoprotective effect, as evidenced by the assessment of liver injury using histopathology examination.

The levels of AST and ALT also demonstrate normal values compared to positive control groups. The aim of this study was to assess the phytochemical composition of this nanoemulsion, as well as its potential to mitigate hepatic damage and toxicity generated by CCl<sub>4</sub> in rats. CCl<sub>4</sub> is a highly potent and widely recognized poisonous substance that elicits liver injury by promoting the production of reactive oxidative stress. This study demonstrates the hepatotoxic effects caused by carbon tetrachloride. G2 has shown significant differences from PC and G1 has no significant differences from PC as shown in figure 2.

Liver injury can be caused by environmental contaminants, such as CCl<sub>4</sub>. The animals exposed to CCl<sub>4</sub> showed various types of alterations like lymphocytes infiltration, edema and congestion.<sup>27</sup> Hepatotoxins, such carbon tetrachloride and paracetamol, are frequently used to induce hepatotoxicity in rats in experiments.<sup>28</sup> The hepatotoxic effects in rats are most indicative of similar hepatotoxic effects in human hepatocytes. Similar concentration-dependent Glutathione depletion, oxidative stress, and mild cytotoxicity are reported in rat and human hepatocytes.<sup>29</sup>

AST and ALT levels correspond well with the severity of acute liver injury generated by carbon tetrachloride during the time span studied, implying that blood AST and ALT levels should be increased in all samples 24 hours after causing acute liver injury with carbon tetrachloride.<sup>30</sup>

According to current literature, it has been suggested that extracts derived from *Syzygium aromaticum* have potential protective properties against these specific problems. As an essential component of metabolic and excretory processes, the liver is consistently responsible for detoxifying substances. Hepatotoxic substances comprise a variety of agents, including viruses, fungal byproducts, bacterial metabolites, minerals, environmental pollutants, and chemotherapeutic drugs. These agents have the potential to generate a wide spectrum of organ dysfunctions. Liver unjury including hepatocellular carcinoma, fibrosis, cirrhosis, and hepatitis, encompass a collection of highly significant medical disorders.<sup>31,32</sup>

Hepatotoxins, including ethanol, acetaminophen, and CCl<sub>4</sub>, have been demonstrated to induce hepatic damage, characterized by diverse levels of hepatocyte degeneration and cellular fatality. Reactive oxygen species (ROS), including superoxide and hydroxyl radicals, have also been associated with carbon tetrachloride toxicity. Based on the available evidence, it can be observed that CCl<sub>4</sub> was frequently used as a hepatotoxin in experimental hepatopathy. The initial stage in a sequence of events that culminates in the oxidation of membrane lipids and subsequent cell death is believed to include the covalent attachment of metabolites of CCl<sub>4</sub>, specifically trichloromethyl-free radicals, to cellular proteins.<sup>33,34</sup>

Carbon tetrachloride (CCl<sub>4</sub>) causes hepatotoxicity in model species because it closely resembles human liver damage. This toxicity results from the activation of several cytochromes, including CYP2E1, CYP2B1, CYP2B2, and perhaps CYP3A, which produce the trichloromethyl radical

(CCl<sub>3</sub>\*). CCl<sub>3</sub>\* can bind to biological molecules such lipids, proteins, and nucleic acids, altering lipid metabolism and resulting in fatty degeneration. It can also combine with DNA to cause liver cancer. When exposed to oxygen, CCl<sub>3</sub>\* degrades into the more reactive CCl<sub>3</sub>OO\*, resulting in lipid peroxidation and membrane damage. At the molecular level, CCl<sub>4</sub> causes the release of various inflammatory cytokines, including TNF- $\alpha$  and NO, which can aid or hinder hepatotoxicity through cellular death.<sup>35</sup>

Drug-induced liver injury (DILI) encompasses a wide array of clinical presentations, from instances of elevated liver enzymes without accompanying symptoms to cirrhosis. The potential for hepatotoxicity could perhaps be mitigated through the utilization of herbal dietary supplements. The liver, the primary organ responsible for detoxification, is susceptible to damage caused ROS, which lead to a depletion of the body's antioxidant reserves. Oxidative stress is a common consequence of chronic liver diseases, as ROS are consistently observed in these conditions.<sup>9</sup>

Eugenol, a fragrant oil derived from cloves, is commonly utilized as a culinary additive to enhance the taste of food and beverages. Additionally, it is a topical herbal medicine for alleviating toothaches and, to a lesser extent, as an oral prescription for gastrointestinal and respiratory conditions. There is currently no evidence to suggest that therapeutic amounts of eugenol are associated with increased blood enzyme levels or clinically observable liver damage. However, it is essential to note that ingesting excessive doses, such as in overdose cases, can lead to significant liver damage. Clove essential oil is composed of eugenol, a volatile phenolic chemical extracted from the buds and leaves of *Eugenia caryophyllata*. It serves as a valuable constituent in several products, where it has been employed in limited quantities within gastronomy, cosmetics, and pharmaceuticals. The derivatives of this substance have been used in medicine as a local anesthetic and antiseptic agent. Eugenol exhibits a diverse range of biological actions, encompassing antioxidant, analgesic, and anti-inflammatory characteristics.<sup>36,37</sup> Shahavi MH., et al.'s study on the evaluation of critical parameters for the preparation of stable clove oil nanoemulsion demonstrated that pulsed ultrasound at the right intervals was more effective than continuous ultrasonication, while the average droplet size of the clove oil nanoemulsion decreased as the work cycle increased.<sup>38</sup>

*S. aromaticum* exhibits a high abundance of sesquiterpenes, monoterpenes, hydrocarbons, phenolic compounds, and other phytochemicals. The three primary phytochemical constituents in clove oil are eugenyl acetate, eugenol, and  $\beta$ -caryophyllene. The pharmacological properties of *S. aromaticum* have been extensively investigated concerning several pathogenic bacteria, parasites, and microorganisms, such as *Plasmodium*, *Babesia*, *Theileria*, *Herpes simplex*, and hepatitis C viruses. Multiple research investigations indicate that eugenol possesses various beneficial properties, such as antiviral, antifungal, anticancer, antiseptic, antidepressant, antispasmodic, and antibacterial activities. These features enable eugenol to effectively combat pathogenic bacteria, including methicillin-resistant *S. aureus* and *Staphylococcus*

epidermidis. Furthermore, it has been determined that eugenol demonstrates promising lethality against the growth of many parasites, such as *Giardia lamblia*, *Fasciola gigantica*, *Haemonchus contortus*, and *Schistosoma mansoni*. Additionally, eugenol has shown protective effects against hepatotoxicity generated by  $\text{CCl}_4$ .<sup>8</sup>

A review conducted by Gaber ESB, et al., in 2020 investigate the phytochemical composition and biological activity of clove extracts, as well as clove essential oil and its primary active ingredient, eugenol. The study also made use of gas chromatography-mass spectrometry (GC-MS) analysis to uncover novel findings.<sup>8</sup> The radical scavenging activity of clove extract was shown to be greater in comparison to that of honey extracts. Both subjects successfully alleviated the negative effects of oxidative stress and liver damage caused by  $\text{CCl}_4$ .<sup>9</sup> The application of clove extract effectively inhibited the growth of hepatocytes into fibroblasts.<sup>8</sup>

The present investigation demonstrated the synergistic hepatoprotective benefits of a combination of clove and grape seed. The pharmacological effects of the active constituents found in various portions of *V. vinifera*, including hepatoprotective, anticancer, antioxidant, antibacterial, anticancer, and anti-inflammatory properties.<sup>39</sup> The study conducted Abdelsalam, H. M., et al., (2019) states that synergistic therapeutic effect of combining *Vitis vinifera* extract with Silymarin on an experimental cardiorenal damage model can be attributed mostly to the facilitation of the Keap1/Nrf2 signaling pathway. *Vitis vinifera* exhibits a range of mechanisms in its capacity as an antioxidant, antimicrobial, and anti-inflammatory agent. These mechanisms encompass the augmentation of superoxide dismutase, hemeoxygenase-1, and glutathione peroxidase activity, alongside the reduction of glutathione levels and malondialdehyde (MDA) concentrations. Additionally, *Vitis vinifera* activates the factor2/ARE pathway, which is linked to nuclear erythroid2. There exist multiple papers that discuss the possible application of these substances in the prevention of diverse diseases, such as cardiovascular disease, cancer, degenerative diseases, and inflammatory disorders.<sup>40</sup>

Research conducted by Amel et al., in 2016, used grape seed oil against carbon tetrachloride-induced oxidative stress in the liver of  $\gamma$ -irradiated rats shows that rats exposed to  $\text{CCl}_4$  demonstrated increased blood levels of ALT, AST activity, IL-6, and TNF- $\alpha$ . The GSO demonstrated protective benefits against acute liver injury generated by  $\text{CCl}_4$  in rats that were exposed to  $\gamma$ -irradiation. These effects can be related to the potent antioxidant, anti-inflammatory, and anti-apoptotic actions of GSO. The activation of antioxidant enzyme activity. Phenolic chemicals, fatty acids, and vitamins in grape seed oil are valuable to pharmaceuticals. Among grape seed oil's potential interactions with cellular and molecular pathways are its anti-inflammatory, cardioprotective, antibacterial, and anticancer actions, as demonstrated in in vitro investigations. These advantages are caused by the phytosterols, tocopherols, linolenic acid, resveratrol, quercetin, procyanidins, and carotenoids found in grape seed oil.<sup>16</sup>

The limitations of this study include the possibility of wide variations in sensitivity to the hepatotoxic effects of carbon tetrachloride due to differences in the developmental stage and efficacy of cytochrome P450, which are also ultimately species and age dependent. The use of carbon tetrachloride to induce acute liver injury may cause damage to other organs such as the central nervous system and kidneys.  $\text{CCl}_4$  primarily affects the central zone of the liver, which does not correspond to the massive necrosis typically seen in human liver failure. Therefore, the  $\text{CCl}_4$ -induced hepatotoxicity picture in this study may not be fully representative of human liver failure.

## CONCLUSION

The hepatoprotective effect of nanoemulsions containing CE and GSO was observed to have antioxidant, antibacterial, and anti-inflammatory properties resulting in significant improvements in serum biomarkers related to hepatotoxicity and histopathological analysis of liver damage.

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