Snakefruit *(Salacca zalacca (Gaerth.)Voss)* vinegar with mixed culture fermentation and lipid regulation: A study on CCl_4-induced male rats

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Abstract

Carbon tetrachloride (CCl_4) is a hepatotoxic compound that disrupts lipid metabolism, increasing total cholesterol, triglycerides, and LDL while decreasing HDL levels. Snakefruit vinegar contains bioactive compounds with potential lipid-lowering and hepatoprotective effects. This study evaluated the effects of single-culture and mixed-culture fermented snakefruit vinegar on lipid profiles and liver weight in male Wistar rats induced with CCl_4. A post-test-only control group design was used with 28 male Wistar rats divided into four groups. The first group served as a healthy control, while the second received CCl_4 (0.5 ml/kg BW) without treatment. The third and fourth groups were induced with CCl_4 and administered single-culture or mixed-culture fermented snakefruit vinegar (0.75 ml/kg BW) via gavage for 28 days. CCl_4 was injected intraperitoneally during the last seven days. Parameters measured included total cholesterol, triglycerides, LDL, HDL, and liver weight. One-Way ANOVA followed by Duncan's Multiple Range Test (DMRT) was used for statistical analysis. Results showed that single-culture fermented snakefruit vinegar more effectively reduced total cholesterol and triglycerides than mixed-culture fermentation. Both fermentation types lowered LDL, but neither significantly increased HDL. Liver weight was higher in the mixed-culture group, suggesting greater hepatoprotection. In conclusion, snakefruit vinegar shows promise for managing dyslipidemia and protecting the liver from CCl_4 toxicity. Single-culture fermentation was more effective in improving lipid profiles, while mixed-culture fermentation had a stronger hepatoprotective effect. Further research is needed to elucidate the underlying mechanisms.

1. Introduction

The liver is a vital organ that plays a crucial role in metabolism, detoxification, and lipid regulation within the body. However, exposure to toxic substances such as carbon tetrachloride (CCl_4) can cause significant liver damage. CCl_4 is a well-known hepatotoxic compound widely used in research to induce liver injury in experimental animal models. Its exposure leads to lipid peroxidation, inflammation, and lipid imbalance, which negatively impact overall liver function. The hepatotoxicity of CCl_4 is characterized by the formation of trichloromethyl radicals (CCl_3•), which trigger lipid peroxidation, cellular necrosis, and inflammation in liver tissues (Elmeligy et al., 2019; Liu et al., 2018). Therefore, effective intervention strategies are needed to protect the liver from the hepatotoxic effects of CCl_4.

One promising approach that has gained attention is the use of vinegar as a hepatoprotective agent and lipid regulator. Fermented vinegar contains various bioactive compounds, including organic acids, phenolics, and antioxidants, which can benefit liver health. The fermentation process significantly influences the functional characteristics of vinegar and the profile of its bioactive compounds. This transformation occurs in stages, where carbohydrates are converted into alcohol by yeast and subsequently oxidized into acetic acid by acetic acid bacteria (Li-chun et al., 2021; Seo et al., 2022). Several studies have demonstrated that vinegar consumption can help reduce blood lipid levels, enhance antioxidant activity, and reduce inflammation in metabolic disorders. Both synthetic acetic acid vinegar and nipa vinegar have been found to reduce fat accumulation in the liver and lower serum cholesterol

levels in high-fat diet-induced obese rats. These effects are linked to the downregulation of the SREBP1 gene, which plays a role in lipid metabolism (Beh et al., 2017; Mohamad et al., 2017).

Among various types of vinegar, snakefruit vinegar (*Salacca zalacca* (Gaerth.) Voss) has shown promising potential. Snakefruit is rich in bioactive compounds such as flavonoids, tannins, and dietary fiber, which support lipid metabolism and liver health (Mazumdar et al., 2019; Zubaidah et al., 2017). Furthermore, snakefruit vinegar produced through mixed-culture fermentation can generate more complex secondary metabolites, enhancing its biological activity. This fermentation process increases the levels of acetic acid, phenolic compounds, and antioxidants (Pratiwi et al., 2025). Snakfruit vinegar potentially improving its efficacy in protecting the liver and regulating lipid levels.

Lipid metabolism disorders are one of the primary effects of CCl_4 exposure. This toxic compound causes elevated levels of total cholesterol (TC), triglycerides (TG), and LDL, while reducing HDL levels in the bloodstream. The main mechanisms involved in these disturbances are oxidative stress and inflammation, which disrupt enzyme balance in lipid metabolism. CCl_4 is metabolized in the liver by cytochrome P450 enzymes, producing trichloromethyl radicals ($CCl_3\cdot$). These radicals initiate lipid peroxidation, impairing normal lipid metabolism (Dutta et al., 2018; Iqbal et al., 2020). Studies on CCl_4-treated rats have shown significant increases in TC, TG, LDL, and VLDL levels, along with **a** marked decrease in HDL, reflecting impaired liver function and metabolism.

Snakefruit vinegar fermented with a mixed culture is believed to have great potential in lipid regulation and liver protection. The underlying mechanisms of its effects **include** enhancing enzyme activity in lipid metabolism, inhibiting lipid peroxidation, which leads to dyslipidemia, and increasing HDL levels while reducing LDL and triglycerides. Vinegar consumption has demonstrated strong potential in modulating lipid profiles in CCl_4-induced liver damage models in rats. By reducing total cholesterol and triglyceride levels while increasing HDL, vinegar acts as a protective agent against lipid dysfunction associated with hepatotoxicity (Jasim & Ghadhban, 2024; Oumeddour et al., 2019; Ousaaid et al., 2021).

Thus, studying the effects of snakefruit vinegar on lipid regulation and liver protection **in** CCl_4-induced animal models is essential. This research aims to evaluate the effects of mixed-culture fermented snakefruit vinegar on lipid regulation in male rats exposed to CCl_4. The findings are expected to provide new insights into the potential use of snakefruit vinegar as a functional agent for managing dyslipidemia and liver damage caused by toxic exposure. Therefore, this study contributes to the development of fermented food-based approaches for the prevention and treatment of liver diseases.

2. Materials and Methods

2.1 Materials

Carbon tetrachloride (CCl_4,≥99.0%) Merck (Germany) is used for the induction of liver damage, dissolved in corn oil. 6-week-old male Wistar rats weighing an average of 150–200 g. Kit reagen untuk pengujian Serum kit uji profil lipid. Carbon tetrachloride (CCl_4, ≥ 99.0%) from Merck (Germany) was used to induce

liver damage, dissolved in corn oil. Male Wistar rats, aged six weeks and weighing an average of 150–200 g, were used in the study. A serum test kit was used to analyze the lipid profile.

2.2 Methods

2.2.1 Snakefruit vinegar fermentation

Snakefruit vinegar was produced by fermenting *Salacca zalacca* fruit using *Saccharomyces cerevisiae* 3004 (2%) for single-culture fermentation (SCV) and a combination of *Saccharomyces cerevisiae* 3004 (2%) with *Lactobacillus plantarum* 0026-CCRC 10069 (2%) for mixed-culture fermentation (MCV) during the alcoholic fermentation stage, following the method described by (Pratiwi et al., 2025). The snakefruit juice was obtained by crushing snakefruit and mixing it with water in a 1:2 ratio. The mixture was then filtered and supplemented with 12.5% sucrose, 200 mg/L sodium bisulfite, and 0.2% diammonium hydrogen phosphate. The mixture was pasteurized at 70°C for 15 minutes, divided into two containers, and inoculated with the respective starter cultures. Alcoholic fermentation was conducted for seven days at room temperature under anaerobic conditions, followed by acetic acid fermentation for 14 days at room temperature using *Acetobacter aceti* 0016-IFO 3283 (4%) under aerobic conditions.

2.2.2 Animals experiment

Twenty-eight six-week-old male Wistar rats, each weighing 150–200 g, were randomly divided into four treatment groups as follows:

K- (Negative Control): Healthy rats given only distilled water and normal feed.

K+ (Positive Control): Rats induced with CCl_4 and given distilled water.

SCV: Rats induced with CCl_4 and given **single-culture fermented snakefruit vinegar.**

MCV: Rats induced with CCl_4 and given **mixed-culture fermented snakefruit vinegar.**

Snakefruit vinegar was administered **orally gavage** at a dose of **0.75 ml/kg** daily for **28 days** in groups SCV and MCV. **CCl_4 injection** was performed via intraperitoneal injection during the **last seven days** (days 21–28) at a dose of **0.5 ml/kg bw**, diluted in corn oil. All rats were sacrificed **24 hours after the final treatment**. This study followed a **post-test-only control group design** and received ethical clearance under **approval number 0342/EC/KEPK/UNUSA/2023**.

2.2.3 Lipid profile analysis

Serum was obtained from blood collected directly from the heart on day 29 and centrifuged at 3,000 rpm for 10 minutes. Total cholesterol and HDL were analyzed using the CHOD-PAP method, triglycerides were measured using the GPO-PAP method, while LDL levels were determined using the direct precipitation method or calculated using the Friedewald equation (Zubaidah et al., 2014).

2.2.4 Total cholesterol analysis

Serum was separated through centrifugation at 3,000 rpm for 10 minutes. The isolated serum was then mixed with CHOD-PAP reagent, which contains cholesterol esterase hydrolase (CEH), cholesterol oxidase (CHOD), and peroxidase (PAP), forming a red quinoneimine complex. The intensity of the red color was measured using a spectrophotometer at a wavelength of 500 nm. The absorbance value obtained was compared to the absorbance of the standard cholesterol solution using the following formula:

$$Total\ cholesterol\ (\mathrm{mg/dL}) = \frac{Sample\ Absorbance}{Blanko\ Ansorbance}\ x\ Standart\ concentration$$

2.2.5 Triglyceride analysis

Triglyceride levels were measured using the enzymatic GPO-PAP (Glycerol-3-Phosphate Oxidase) method. The obtained serum was mixed with GPO-PAP reagent, which contains lipase, glycerol kinase, glycerol-3-phosphate oxidase, and peroxidase, forming a red complex that was measured using a spectrophotometer at a wavelength of 500 nm. Triglyceride levels were determined using the following formula:

$$Triglyceride\ (\mathrm{mg/dL}) = \frac{Sample\ Absorbance}{Blanko\ Ansorbance}\ x\ Standart\ concentration$$

2.2.6 Low Density Lipoprotein (LDL) analysis

LDL levels in the blood were determined using the direct precipitation method or calculated using the Friedewald formula as follows:

$$LDL\ (\mathrm{mg/dL}) = Total\ cholesterol - \left(HDL + \frac{Triglyceride}{5}\right)$$

2.2.7 High Density Lipoprotein (HDL) analysis

HDL levels were analyzed using the CHOD-PAP method, where serum was mixed with CHOD-PAP reagent, producing a color change that was measured using a spectrophotometer at a wavelength of 500 nm. HDL levels were calculated using the following formula:

$$HDL\ (\mathrm{mg/dL}) = \frac{Sample\ Absorbance}{Blanko\ Ansorbance}\ x\ Standart\ concentration$$

2.3 Statistical analysis

Data were analyzed using One-Way ANOVA ($p < 0.05$) to determine significant differences among groups, followed by a post-hoc analysis using Duncan's Multiple Range Test (DMRT) with SPSS 27.0.

3. Results and discussion

3.1 Total cholesterol after intervention

Based on Fig. 1a, total cholesterol levels varied across the treatment groups. The negative control group (K-), consisting of healthy rats without any treatment, had the highest total cholesterol levels compared to the other groups. Meanwhile, the CCl_4-induced group (K+) showed a slight decrease in total cholesterol levels compared to the K- group, suggesting that CCl_4 induction did not have a significant impact on total cholesterol levels in the experimental rats. In the group treated with SCV after CCl_4 induction, a significant reduction in total cholesterol was observed compared to the negative control group (K-). This indicates that single-culture fermented snakefruit vinegar has the potential to lower cholesterol levels in rats with CCl_4-induced liver damage. In contrast, the group that received mixed-culture fermented snakefruit vinegar (MCV) did not show a significant difference compared to the negative control group (K-), indicating that mixed-culture fermentation did not have the same cholesterol-lowering effect as single-culture fermentation. These findings suggest that single-culture fermented snakefruit vinegar is more effective in reducing total cholesterol levels than mixed-culture vinegar, possibly due to differences in the metabolite composition produced during fermentation.

Carbon tetrachloride (CCl_4) is known as a hepatotoxic compound that induces oxidative stress and liver damage in various experimental studies, particularly in rats. The impact of CCl_4 on increased total cholesterol levels and altered lipid profiles has been widely studied, demonstrating disturbances in lipid metabolism. Administration of CCl_4 has been shown to significantly increase total cholesterol levels. Exposure to CCl_4 results in a significant rise in total cholesterol levels compared to the control group, indicating lipid metabolism disorders due to its toxic effects on the liver (Ifidon & Oriakhi, 2018). Lipid metabolism disruption caused by CCl_4 is closely related to liver damage, as the liver plays a key role in lipid production and regulation. The toxic effects of CCl_4 are triggered by the formation of reactive oxygen species (ROS), which accelerate lipid peroxidation and liver cell damage. CCl_4 can induce oxidative stress, leading to mitochondrial dysfunction and disturbances in lipid metabolism (Hamed et al., 2016). Lipid peroxidation caused by CCl_4 affects not only cholesterol levels but also causes imbalances in the liver lipid profile (Azlina et al., 2016).

The effects of vinegar on total cholesterol levels in CCl_4-induced rats have been the focus of several recent studies. Administration of apple cider vinegar has been shown to significantly reduce total cholesterol levels in Wistar rats (Okoye & Porolo, 2019). These findings suggest that vinegar has the potential to protect against hyperlipidemia, particularly in liver damage caused by CCl_4. Vinegar consumption may provide protective effects against liver damage induced by CCl_4. It can reduce serum transaminase enzyme levels and lipid peroxidation markers, indicating improvements in liver function and lipid metabolism (Tang et al., 2023). This suggests that vinegar may help mitigate the negative effects of CCl_4, potentially stabilizing cholesterol levels. The administration of vinegar to hyperlipidemic rats has been found to reduce total cholesterol levels through a mechanism involving the reduction of oxidative stress, supported by vinegar's antioxidant properties (Karakci et al., 2023).

One of the key mechanisms behind vinegar's cholesterol-lowering effect is its ability to regulate lipid metabolism. Vinegar may enhance liver efficiency in fat metabolism, possibly by increasing the activity of enzymes involved in lipid breakdown. Additionally, vinegar contains acetic acid, which is known to inhibit the activity of enzymes responsible for cholesterol synthesis. Dietary acetic acid consumption has been shown to reduce serum total cholesterol levels in rats fed a high-cholesterol diet (Zubaidah et al., 2017). This inhibition of cholesterol synthesis is particularly important in cases of liver damage caused by CCl_4, as liver function in processing fats is impaired under these conditions.

Vinegar also possesses antioxidant properties that help reduce oxidative stress induced by CCl_4. The oxidative stress caused by CCl_4 exposure leads to the production of reactive oxygen species (ROS), which can exacerbate liver damage and increase lipid peroxidation (Hussein et al., 2018). In addition to its antioxidant effects, vinegar can also influence inflammatory pathways activated during liver injury caused by CCl_4. This inflammatory response typically involves the release of pro-inflammatory cytokines, which can further impair liver function and lipid metabolism. Studies indicate that vinegar can reduce the expression of inflammatory markers, suggesting its potential to decrease inflammation caused by CCl_4 toxicity (Shen et al., 2016). This anti-inflammatory effect may help restore normal lipid metabolism and lower total cholesterol levels.

3.2 Triglyceride after intervention

According to Fig. 1b, the displayed triglyceride levels (mg/dL) demonstrate significant differences among the treatment groups. The negative control group (K-), consisting of healthy rats without any treatment, exhibited relatively low triglyceride levels, with no statistically significant difference compared to the treatment groups (SCV and MCV). In contrast, the CCl_4-induced group (K+) showed a significant increase in triglyceride levels compared to the K- group ($p < 0.05$). This elevation indicates that CCl_4 induction can disrupt lipid metabolism, as evidenced by increased triglyceride concentrations in the bloodstream. These findings align with previous studies demonstrating that exposure to CCl_4, a known toxic compound, significantly elevates triglyceride levels in Wistar rats, further indicating an imbalance in lipid homeostasis (Abu et al., 2022; NF, 2021).

In the treatment groups receiving SCV and MCV following CCl_4 induction, triglyceride levels declined compared to the K + group. However, the reduction was not statistically significant compared to the negative control group (K-). These results suggest that the administration of snakefruit vinegar, regardless of fermentation type, has the potential to lower elevated triglyceride levels resulting from CCl_4 induction. Overall, the findings indicate that CCl_4 exposure leads to an increase in triglyceride concentrations, reflecting lipid metabolism dysfunction due to its toxicity. However, snakefruit vinegar—whether fermented using a single or mixed culture—was able to restore triglyceride levels to near-normal conditions, suggesting its protective role in lipid metabolism regulation.

The mechanism by which CCl_4 influences triglyceride levels in rats is associated with its hepatotoxic effects, which disrupt normal lipid metabolism. CCl_4 is metabolized in the liver by cytochrome P450 enzymes, generating reactive metabolites such as trichloromethyl radicals (CCl_3) and trichloromethyl

peroxyl radicals (CCl_3O_2) (Ibrahim et al., 2020; Verma et al., 2023). These reactive species trigger lipid peroxidation, leading to cellular damage and impaired hepatic function, which plays a crucial role in lipid metabolism regulation. CCl_4 exposure induces a significant increase in serum triglyceride levels through multiple interrelated mechanisms. One major contributing factor is oxidative stress, which is characterized by increased lipid peroxidation byproducts such as malondialdehyde (MDA) (Elghareeb et al., 2023). Additionally, CCl_4 downregulates the activity of lipoprotein lipase (LPL), an enzyme responsible for hydrolyzing triglycerides within lipoproteins, and its inhibition results in triglyceride accumulation in the bloodstream (Emmanuel et al., 2022).

The triglyceride-lowering mechanism of vinegar in CCl_4-induced rats involves multiple pathways, primarily lipid metabolism regulation, antioxidant activity, and anti-inflammatory effects. One of the key bioactive components of vinegar, acetic acid, plays a significant role in lipid metabolism. Acetic acid has been shown to reduce serum triglyceride (TG) and total cholesterol (TC) levels by inhibiting the activity of 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR), a key enzyme in cholesterol biosynthesis (Son et al., 2017). This inhibition not only decreases triglyceride production but also enhances fatty acid utilization, thereby contributing to reduced triglyceride levels in circulation. Additionally, vinegar has been reported to enhance lipoprotein lipase (LPL) activity, which facilitates the breakdown of triglycerides within lipoproteins. Regular supplementation with coconut vinegar in rats fed a high-cholesterol diet has been found to significantly reduce serum triglyceride levels, suggesting that vinegar may improve LPL activity and accelerate triglyceride metabolism (Malakul et al., 2022). This effect is particularly crucial in cases of CCl_4-induced liver damage, where LPL activity may be impaired.

The antioxidant properties of vinegar also play a vital role in mitigating oxidative stress, thereby protecting hepatocytes from damage caused by CCl_4 exposure. This protective effect helps maintain lipid metabolism homeostasis and prevents excessive triglyceride accumulation in the liver and bloodstream (Ousaaid et al., 2020). Furthermore, vinegar has been shown to modulate inflammatory pathways by downregulating cytokine expression, thereby reducing inflammation caused by CCl_4 toxicity and stabilizing lipid metabolism. Ultimately, this contributes to decreased triglyceride levels (Shen et al., 2016). In summary, vinegar reduces triglyceride levels in CCl_4-induced rats through multiple mechanisms, including lipid synthesis inhibition, enhanced triglyceride breakdown, antioxidant activity, and inflammation modulation.

3.3 Low Density Lipoprotein (LDL) after vinegar intervention

Figure 1c illustrates LDL levels across the treatment groups. The negative control group (K-), consisting of healthy rats without intervention, exhibited low LDL levels, with no significant difference compared to the SCV group and the MCV group. However, in the CCl_4-induced group (K+), LDL levels increased significantly compared to the other groups ($p < 0.05$). This elevation indicates that CCl_4 exposure disrupts lipid metabolism, contributing to an increase in circulating LDL cholesterol.

Meanwhile, the SCV and MCV groups, which received snakefruit vinegar treatment following CCl_4 induction, exhibited a significant reduction in LDL levels compared to the K + group, with values returning

to levels comparable to the negative control group (K-). These findings suggest that snakefruit vinegar, whether fermented using a single or mixed culture, has a lipid-lowering effect on the elevated LDL levels caused by CCl_4 exposure. Based on these results, it can be concluded that CCl_4 induction significantly increases LDL concentrations. However, the administration of snakefruit vinegar effectively suppresses LDL levels, indicating its potential hypolipidemic and protective effects on lipid metabolism.

The effect of CCl_4 on LDL levels in rats is mainly attributed to its hepatotoxicity, which disrupts normal lipid metabolism and leads to dyslipidemia. CCl_4 affects LDL concentrations by impairing hepatic lipid metabolism, leading to increased triglyceride and cholesterol synthesis from acetate, followed by alterations in lipoprotein transport and metabolism, including LDL (Ifidon & Oriakhi, 2018). Additionally, CCl_4 exposure has been linked to endoplasmic reticulum stress, resulting in a significant increase in LDL cholesterol levels, along with elevated triglyceride and very low-density lipoprotein (VLDL) cholesterol levels (NF, 2021). The production of reactive oxygen species (ROS) induced by CCl_4 triggers oxidative stress, disrupting LDL receptor function in the liver, which plays a crucial role in LDL clearance from circulation (NF, 2021). Furthermore, CCl_4-induced liver injury promotes the release of pro-inflammatory cytokines, which can further disrupt lipid metabolism and accelerate LDL accumulation (Jasim & Ghadhban, 2024).

The acetic acid content in vinegar contributes to the reduction of triglyceride (TG) and total cholesterol (TC) levels in serum by inhibiting 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR), a key enzyme in cholesterol biosynthesis. This inhibition not only reduces LDL synthesis but also enhances fatty acid utilization, thereby decreasing LDL levels in circulation (Son et al., 2017). The LDL-lowering effect of vinegar is also attributed to the presence of phenolic compounds, such as chlorogenic acid, which has been shown to inhibit LDL oxidation—one of the primary factors in the development of atherosclerosis. Moreover, apple cider vinegar consumption has been reported to enhance antioxidant enzyme activity, protecting against LDL oxidation and supporting cardiovascular health (Seydim et al., 2016). Additionally, vinegar exhibits anti-inflammatory properties, which contribute to lipid level reduction. CCl_4-induced liver injury is often associated with an inflammatory response, characterized by the release of pro-inflammatory cytokines. Although evidence suggests that vinegar can modulate inflammatory pathways, research specifically linking vinegar consumption to reduced pro-inflammatory cytokine expression in the context of CCl_4 toxicity remains limited (Wang et al., 2023).

3.4 High Density Lipoprotein (HDL) after vinegar intervention

Figure 1d illustrates HDL levels across the treatment groups. The negative control group (K-), consisting of healthy rats without treatment, exhibited the highest HDL levels compared to the other groups. The HDL levels in the K- group were significantly different ($p < 0.05$). In the CCl_4-induced group (K+), there was a significant decrease in HDL levels compared to the negative control group. This reduction indicates that CCl_4 induction can cause lipid metabolism disorders, contributing to the decline of high-density lipoprotein (HDL), which plays a crucial role in cardiovascular protection.

Meanwhile, in the SCV and MCV groups, consisting of rats induced with CCl_4 and treated with single-culture and mixed-culture fermented snakefruit vinegar, HDL levels did not show a significant increase compared to the K + group. This suggests that snakefruit vinegar administration was not sufficient to restore HDL levels to those of the healthy group (K-), despite its known antioxidant content, which is associated with lipid metabolism regulation. Based on these findings, it can be concluded that CCl_4 induction significantly decreases HDL levels, potentially increasing the risk of cardiovascular disorders due to the reduced protective effects of HDL. However, the administration of snakefruit vinegar, whether single or mixed culture, did not have a significant effect on increasing HDL levels in rats with CCl_4-induced liver damage.

CCl_4 administration resulted in a decrease in HDL cholesterol levels in rats, indicating that oxidative stress-induced liver dysfunction inhibits HDL synthesis and secretion (Uroko, 2021). CCl_4 disrupts lipid metabolism, particularly the balance among various lipoproteins. It leads to an increase in triglycerides and low-density lipoprotein (LDL) cholesterol, while HDL levels significantly decline (NF, 2021). CCl_4 induces the production of reactive trichloromethyl radicals, which attack cellular lipids and proteins, exacerbating liver damage and further impairing HDL metabolism (Mansoor et al., 2022). Oxidative stress caused by CCl_4 exposure can lead to the downregulation of HDL receptors and apolipoproteins, both of which are essential for optimal HDL function.

The administration of apple cider vinegar in diabetic rats has been shown to significantly increase HDL levels while simultaneously reducing triglycerides and LDL cholesterol (Zubaidah et al., 2017). This suggests that acetic acid can enhance the liver's ability to synthesize HDL while suppressing LDL and triglyceride production. Additionally, vinegar has been found to increase lipoprotein lipase (LPL) activity. Enhanced LPL activity facilitates the conversion of triglyceride-rich lipoproteins into HDL, thereby promoting HDL formation. Coconut vinegar supplementation in rats fed a high-cholesterol diet significantly improved lipid profiles, including an increase in HDL levels (Malakul et al., 2022). This finding suggests that vinegar may strengthen enzymatic pathways involved in HDL synthesis and metabolism.

The presence of phenolic compounds in vinegar, such as chlorogenic acid, has been shown to enhance HDL levels by improving lipid profiles and reducing oxidative stress (Karakci et al., 2023). These compounds help protect HDL from oxidation, enhance its function, and further support its beneficial role in lipid metabolism.

3.5 Liver weight after intervention

Based on the data presented in Table 1, there were significant differences in liver weight among the treatment groups. The negative control group (K-), consisting of healthy rats, exhibited the highest liver weight at 8.20 ± 0.84 g, which was significantly higher than the other groups ($p < 0.05$). In contrast, the positive control group (K+), consisting of rats induced with CCl_4, showed a significant reduction in liver weight to 5.80 ± 0.84 g, indicating that CCl_4 induction led to liver damage, which contributed to a decrease in organ mass. In the SCV treatment group, where rats were administered single-culture

fermented snakefruit vinegar following CCl_4 induction, liver weight increased to 6.20 ± 0.84 g. Although this represented an increase compared to the K + group, the difference was not statistically significant. Meanwhile, in the MCV treatment group, where rats were given mixed-culture fermented snakefruit vinegar, liver weight increased further to 7.00 ± 0.71 g. This value was significantly higher than that of the K + group ($p < 0.05$) and approached that of the negative control (K-). These findings suggest that snakefruit vinegar fermented with mixed cultures was more effective in increasing liver weight in CCl_4-induced rats compared to single-culture fermentation. This difference is likely due to the more complex metabolite composition in mixed-culture fermented vinegar, which may provide enhanced hepatoprotective effects.

Table 1
Liver weight after intervention

Group			Liver weight (g)
K-	=	Negative control	8.20 ± 0.84^{c}
K+	=	Positive control	5.80 ± 0.84^{a}
SCV	=	Rats with CCl_4 induced + single culture snakefruit vinegar	6.20 ± 0.84^{ab}
MCV	=	Rats with CCl_4 induced + mixed culture snakefruit vinegar	7.00 ± 0.71^{b}

Data are shown as means ± standard deviations (n = 5). Significant differences ($p < 0.05$) within the same column are indicated by different letters, based on One-Way ANOVA and DMRT post hoc analysis.

Carbon tetrachloride (CCl_4) administration in certain conditions can lead to a reduction in liver weight. CCl_4 is metabolized in the liver by cytochrome P450 enzymes, generating highly reactive free radicals such as trichloromethyl radicals (CCl_3•) (Khan et al., 2020).. These radicals trigger lipid peroxidation, leading to cellular membrane damage and hepatocyte necrosis, which may reduce functional liver mass and contribute to an overall decrease in liver weight, particularly in cases of severe exposure (Farid et al., 2023). The use of vinegar as an intervention for CCl_4-induced liver damage in rats has gained attention due to its potential hepatoprotective effects. Various studies have explored the biochemical and histopathological outcomes of vinegar administration in the context of liver injury caused by CCl_4, a well-known hepatotoxic agent. CCl_4 primarily induces liver damage through the formation of reactive oxygen species (ROS), leading to oxidative stress, lipid peroxidation, and subsequent hepatocyte injury (Abdul Rahman et al., 2022).

The administration of vinegar, particularly those rich in polyphenols and other bioactive compounds, has been shown to mitigate these effects. Fermented sorghum vinegar supplementation in rats treated with CCl_4 significantly reduced liver enzyme levels such as AST and ALT, which are key indicators of liver damage, suggesting that vinegar may help preserve liver function by counteracting CCl_4-induced oxidative stress (Tang et al., 2023). Additionally, vinegar has been recognized for its antioxidant properties, which play a crucial role in protecting liver cells from damage. For instance, Hengshun

aromatic vinegar, which contains various bioactive compounds, has been shown to exert hepatoprotective effects by reducing oxidative stress and inflammation in the liver (Zhu et al., 2020). Coconut water vinegar enhanced recovery from liver damage by improving antioxidant status and reducing inflammation in CCl_4-treated rats (Mohamad et al., 2018). Beyond biochemical improvements, vinegar interventions have also been associated with better histological outcomes. Treatment with cereal vinegar sediment in rats with acute liver injury reduced inflammation and liver fibrosis, indicating protective effects against structural liver damage (Guan et al., 2021). The protective mechanisms of vinegar likely involve inflammation modulation and enhanced antioxidant defenses. Polyphenols present in vinegar have been associated with the inhibition of pro-inflammatory cytokines and the activation of antioxidant enzymes, collectively contributing to the maintenance of liver weight and function in response to CCl_4-induced damage (Guan et al., 2021; Mohamad et al., 2018; Zhu et al., 2020).

Previous research data shown at Table 2, indicate that mixed-culture fermented snakefruit vinegar contains higher levels of phenolic compounds, including caffeic acid, p-coumaric acid, and ferulic acid, compared to single-culture fermented vinegar (Pratiwi et al., 2025). The hepatoprotective effects of phenolic compounds are linked to their ability to enhance antioxidant defense systems. Phenolic compounds activate antioxidant enzyme pathways and inhibit the TGF-β1 signaling pathway, thereby effectively reducing liver injury and fibrosis in CCl_4 models (Lin et al., 2019). This suggests that phenolic compounds not only protect against oxidative damage but also modulate fibrogenic pathways that contribute to liver weight loss due to fibrosis and necrosis. The protective role of phenolic compounds also extends to reducing inflammation associated with CCl_4-induced liver damage (Al-Seeni et al., 2016). Additionally, phenolic compounds may influence liver weight by regulating lipid metabolism. Studies have shown that polyphenol-rich extracts can reduce lipid accumulation and lipid peroxidation in the liver, which are critical factors in maintaining liver weight during CCl_4-induced injury (Lin et al., 2019; Majeed et al., 2020). Furthermore, the modulation of signaling pathways related to apoptosis and inflammation by phenolic compounds contributes to their protective effects against liver weight reduction (Yang & Liu, 2024).

Table 2
Identified chemical compounds using High Performance Liquid Chromatography (HPLC)

Vinegar	Cafeic acid (ppm)	p-Coumaric acid (ppm)	Ferulic acid (ppm)
Single culture fermentation	0.40 ± 0.12	ND	ND
Mixed culture fermentation	6.99 ± 0.19	7.03 ± 0.17	5.71 ± 0.11

4. Conclusions

The findings of this study indicate that the administration of snakefruit vinegar fermented with single and mixed cultures has different effects on lipid profiles and liver weight in male Wistar rats induced with

CCl_4. Single-culture fermented snakefruit vinegar was more effective in reducing total cholesterol and triglyceride levels compared to mixed-culture fermentation. Meanwhile, both types of fermentation were able to lower LDL levels; however, neither showed a significant increase in HDL levels. Additionally, the liver weight of rats receiving mixed-culture fermented snakefruit vinegar was higher than that of the group receiving single-culture fermentation, suggesting that mixed-culture fermentation provides greater hepatoprotective effects. These results indicate that snakefruit vinegar has potential as a natural agent for managing dyslipidemia and protecting the liver from the toxic effects of CCl_4.

Declarations

Author Contribution

V.N.P contributed to the conceptualization of the study, methodology design, data analysis, validation as well as writing and editing the main manuscript. C.D.A was responsible for data collection, sample processing, laboratory analysis writing and editing the main manuscript. R.N.K and E.P conducted the literature review, and contributed to the preparation of the results and discussion sections.

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Figures

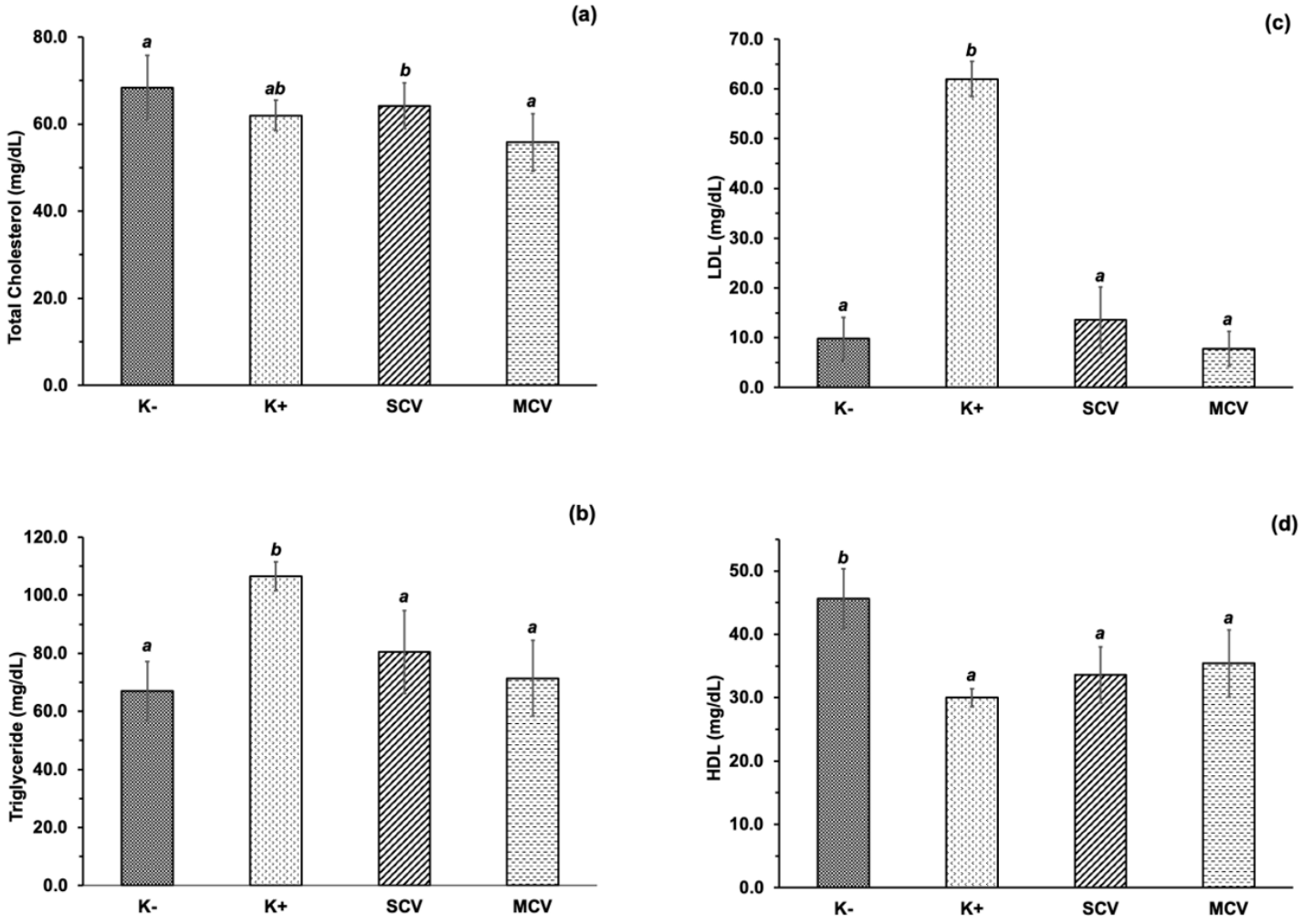

Figure 1

Total cholesterol, triglyceride, LDL, and HDL Level after intervention. Different letters indicate significant ($p<0.05$). SCV= single culture snakefruit vinegar; MCV = mixed culture snakefruit vinegar