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Therapeutic potential of *Raphanus sativus* var. L ethyl acetate fraction in hyperlipidemic and hyperglycemic wistar rats (*Rattus norvegicus*)

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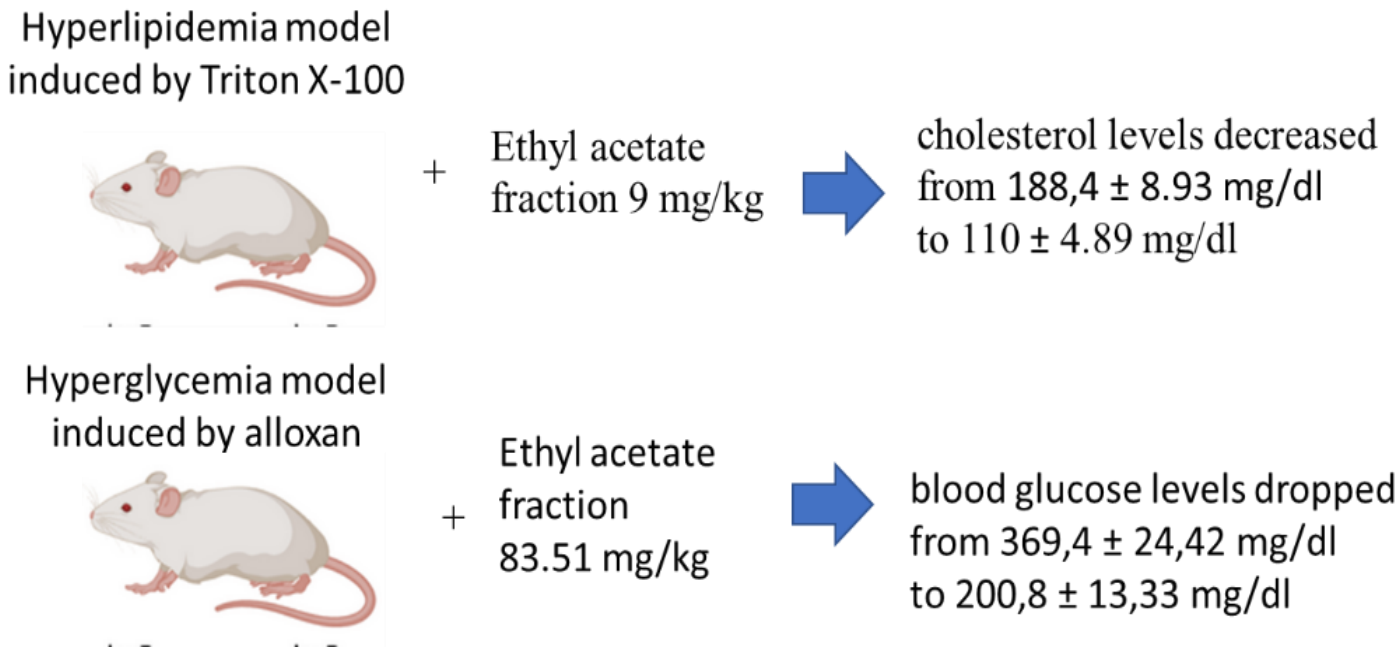
ABSTRACT

Cardiovascular diseases (CVD), primarily driven by hyperlipidemia and hyperglycemia, represent a significant global health burden. Effective treatments for these conditions are often inaccessible, particularly in low-income populations, which underscores the importance of exploring alternative therapeutic approaches. This study investigates the anti-hyperlipidemic and anti-hyperglycemic effects of the ethyl acetate fraction from *Raphanus sativus* var. L. (white radish) in male Wistar rats. The fraction demonstrated significant cholesterol-lowering effects, reducing total serum cholesterol levels in hyperlipidemic rats from 216.4 ± 8.08 mg/dl to 110 ± 4.89 mg/dl. Additionally, it lowered blood glucose levels in hyperglycemic rats from 446.6 ± 46.19 mg/dl to 335 ± 69.31 mg/dl. These findings suggest that white radish extract, particularly the ethyl acetate fraction, holds potential as a natural therapeutic agent for managing hyperlipidemia and hyperglycemia.



Keywords: Anti-hyper colesterol, Anti-hyperglycaemic, Ethyl acetate fraction, *Raphanus sativus* var. L.

INTRODUCTION

Cardiovascular disease (CVD), a leading cause of mortality globally, is characterized by the narrowing or blockage of blood vessels, leading to potentially life-threatening conditions such as heart attacks, angina, and stroke [15]. A significant contributing factor to CVD is hyperlipidemia, a condition marked by elevated levels of lipids, primarily cholesterol and triglycerides, in the blood [8]. Hyperglycemia, characterized by persistently high blood glucose levels, is also a significant and independent risk factor for CVD, often exacerbating the effects of hyperlipidemia and increasing the risk of complications [16]. The World Health Organization (WHO) highlights the alarming global prevalence of both conditions. In 2020, the WHO estimated that over 1 billion adults aged 60 and above had elevated cholesterol levels, representing a substantial portion of the global population. Furthermore, the WHO reports a similarly staggering prevalence of diabetes and associated hyperglycemia [19]. This combined prevalence of dyslipidemia and hyperglycemia underscores the substantial global health burden of these conditions, with significant implications for both developed and developing nations[17]. The escalating prevalence of both hyperlipidemia and hyperglycemia necessitates the exploration of effective and accessible treatment strategies.

Modern medical treatments for hyperlipidemia, hyperglycemia, and associated CVDs are often expensive and inaccessible to many, particularly in low-income populations. This highlights the urgent need for alternative therapeutic approaches, including the investigation of traditional medicines derived from medicinal plants. The WHO actively promotes research into the safety and efficacy of herbal remedies [20]. Among the promising candidates is *Raphanus sativus* L., commonly known as white radish, whose root has demonstrated significant anti-hyperlipidemic and anti-hyperglycemic properties. Studies have shown that white radish extracts possess notable cholesterol-lowering activity, attributed to the presence of bioactive compounds such as flavonoids, saponins, and pectin, which can inhibit the synthesis of HMG-CoA reductase [1-9]. This enzyme plays a crucial role in cholesterol biosynthesis, making its inhibition a key mechanism for cholesterol reduction. Furthermore, the flavonoid content of white radish is believed to enhance insulin sensitivity and contribute to the reduction of blood glucose levels [9-18], suggesting a potential role in managing hyperglycemia. This dual action – addressing both hyperlipidemia and hyperglycemia – makes white radish a particularly attractive candidate for further investigation.

Previous research has indicated the efficacy of white radish extracts in lowering blood glucose levels in diabetic models [4, 11, 21]. However, further investigation into the anti-hyperlipidemic and anti-hyperglycemic properties of specific fractions, such as the ethyl acetate fraction, of white radish extract, particularly in *in vivo* models, remains limited. The lack of comprehensive studies focusing on the ethyl acetate fraction and its efficacy in managing both hyperlipidemia and hyperglycemia in a controlled *in vivo* setting necessitates further research.

This study aims to address this gap by investigating the anti-hyperlipidemic effects in Triton X-100-induced hyperlipidemic rats and the anti-hyperglycemic effects in alloxan-induced hyperglycemic rats of the ethyl acetate fraction of the root extract of *Raphanus sativus* var. L (white radish) in male *Rattus norvegicus*. The research will determine the effective dose for lowering total cholesterol and blood glucose levels. This research explores the potential of white radish as a traditional medicine for both hyperglycemia and hyperlipidemia, contributing to the development of safer and more effective herbal therapies for these prevalent and debilitating conditions. The findings will contribute valuable data to the growing body of knowledge on the therapeutic potential of traditional medicine in addressing the global burden of CVD, hyperlipidemia, and hyperglycemia.guidelines [14, 15].

MATERIALS AND METHODS

Prior to commencing, the study obtained ethical approval from the Animal Research Ethics Committee of Ahmad Dahlan University in Yogyakarta (no. 022402015) and Universitas Padjadjaran (no. 415/UN6.KEP/EC/2024) to ensure that all procedures were conducted ethically. The white radish plant was authenticated through a determination test at the Biology Laboratory of Padjadjaran University to verify the authenticity of the sample. The aim of this identification was to classify the white radish plant (*Raphanus sativus* L.). The research was conducted at the Pharmacology Laboratory of Muhammadiyah University of Bandung, which is equipped with facilities supporting pharmacological and experimental studies. The study commenced in February 2024 and concluded in May 2024, lasting approximately four months. This in vivo experimental study used male Wistar rats (Rattus *norvegicus*) as subjects.

Materials

This section outlines the materials and equipment used in this study. The equipment included stirring rods, beaker glasses, blenders, glass funnels, Erlenmeyer flasks, measuring cylinders, a Sinocare glucometer, disposable syringes, rat cages, volumetric flasks, mortars, gloves, animal feeding and drinking probes, a rotary vacuum evaporator, and a water bath. These tools facilitated various stages of the experiment, including extract preparation and animal testing. The materials involved a range of chemical and biological substances. The chemicals included alloxan, concentrated ammonia (NH_4OH), distilled water, acetic anhydride ($(CH_3CO)_2O$), concentrated hydrochloric acid (HCl), concentrated sulfuric acid (H_2SO_4), 96% ethanol, $FeCl_3$ (ferric

chloride), glibenclamide, magnesium (Mg), Na-CMC, and reagents such as Mayer's, Wagner's, and Dragendorff's reagents for phytochemical screening. Wistar male rats were used as test animals, along with mouse feed, magnesium powder, simvastatin tablets (0.18 mg), and white radish (*Raphanus sativus* L.) roots.

Extraction and Fractionation

The extraction method used in this study was maceration, where the white radish root was cut, washed, dried, and ground into a powder to obtain the simplicia. This was then extracted using 96% ethanol for five days, producing a concentrated extract. After extraction, fractionation was performed to isolate active compounds using n-hexane and ethyl acetate solvents. The resulting fractions were evaporated using a rotary evaporator and water bath.

Animal Experimental Design

In the experimental phase, male Wistar rats (*Rattus norvegicus*) aged 2-3 months, weighing between 180-195 grams, were used. Prior to the treatment, the rats were acclimatized for 7 days to adjust to the experimental environment. During the acclimatization period, their health was monitored, and their body weight was recorded. The rats were divided into five groups, each consisting of five rats. The groups included a negative control (K-), positive control (K+), and three groups receiving different doses of the white radish extract (D1, D2, D3). Prior to treatment, the rats were fasted for 12 hours, and observations were made every three days over a 7-day period following alloxan induction. The rats were grouped into five treatment groups, with five rats in each group. Before the experiment began, the rats were acclimatized for 7 days with food and water provided. The day before treatment, the rats were fasted for 12 hours to increase their susceptibility to hyperglycemia and hyperlipidemia induction.

Antihyperglycemic Activity Assay

Before being induced with alloxan, the test animals were fasted for 12 hours but were allowed access to water. This was to make them more susceptible to hyperglycemia compared to non-fasted animals. Alloxan monohydrate was then administered via intraperitoneal injection at a dose of 20 mg/200 g body weight to the Negative Control (K-), Positive Control (K+), and treatment groups (D1, D2, D3, with low, medium, and high doses, respectively), each consisting of 5 rats. After injection, the rats were given food and water as usual. Blood glucose levels were measured on days 0, 3, 5, and 7 post-induction to confirm persistent hyperglycemia, with a fasting blood glucose above 140 mg/dL indicating hyperglycemia. Rats were considered normal if their glucose levels were between 50-135 mg/dL. Three days after alloxan induction, the test substance was administered according to the treatment groups, starting after another 12-hour fasting period. Observations were conducted for 7 days, with blood samples taken on days 0, 3, 5, and 7. Blood was collected by cleaning the rat's tail with 70% alcohol, making a small incision on the tail tip, and using a glucometer to measure glucose levels. Blood was applied to a glucose test strip in the glucometer, and after a 10-second wait, the glucose concentration was recorded in mg/dL.

Anti-hyperlipidemic Activity Assay

In the experiment, blood was taken from rats one day before fasting and inducing them with Triton X-100. The cholesterol levels were found to be at normal levels, as indicated by a reading of LO (low) on the cholesterol autocheck tool, which corresponds to a cholesterol level around 100 mg/dL. This aligns with the typical cholesterol range for rats, which is between 70-120 mg/dL under normal conditions. After measuring the normal cholesterol levels, the rats were fasted for 18 hours to avoid food interfering with the testing. After the fasting period, the rats were induced with Triton X-100 at a dose of 20 g per 200 g body weight, as this dose is known to increase total cholesterol and triglycerides within 72 hours. The induction was done via intraperitoneal injection (IP) for faster absorption and to target the liver, where it would metabolize and raise cholesterol levels. This was checked again after 3 days to assess the cholesterol increase. Simultaneously, to induce hyperlipidemia, the rats were fed a high-fat diet for 7 consecutive days. This high-fat diet aimed to elevate blood lipid levels, particularly total cholesterol, triglycerides, and LDL. After 7 days of the high-fat diet, blood lipid levels were measured to confirm the onset of hyperlipidemia. The high-fat diet consisted of materials such as corn oil and egg yolk, which are sources of both saturated and unsaturated fats that increase lipid levels. For the hyperlipidemia test, the rats were first given a high-fat diet for 7 consecutive days to increase blood lipid levels, including total cholesterol, triglycerides, and LDL. The high-fat diet included sources such as corn oil and egg yolk, which contain both saturated and unsaturated fats, contributing to increased lipid levels. After inducing hyperlipidemia, the white radish extract was administered with the aim of reducing elevated lipid levels. The test substance for antihyperlipidemia was administered daily following the 7 days of high-fat diet feeding, with lipid measurements taken on days 0, 3, 5, and 7 to monitor reductions in cholesterol and triglyceride levels.

Data Analysis

The data obtained from the study were analyzed using One-Way ANOVA to compare the total cholesterol, triglyceride, LDL, and blood glucose levels among groups. If the ANOVA results indicated significant differences, the analysis was followed by the Least Significant Difference (LSD) test to determine which groups differed significantly. All data from the measurements of blood glucose and lipid levels were analyzed using relevant statistical software. With a rigorous study design and standardized procedures, this research aims to evaluate the potential of white radish extract in lowering blood glucose, cholesterol, and lipid levels in rats induced with diabetes and

hyperlipidemia.

RESULTS

The result of phytochemical screening (Table 1) indicates that ethyl acetate fraction *Raphanus sativus* var L ethanol extract contain the sample contains several beneficial secondary metabolites, while steroids and triterpenoids are not present.

Table 1: Phytochemical Screening Results

Secondary Metabolites	Results
Alkaloids	+
Flavonoids	+
tanin	+
Steroid and Triterpenoid	-
Saponin	+

Table 2: Effect of *Ethyl acetate fraction Raphanus sativus var L ethanol extract* on serum total cholesterol in triton-induced hyperlipidemic rats

Group	Treatment	Blood Cholesterol (mg/dl, mean ± SEM)			
		0 h	3 h	7 h	14 h
I	Normal Control (vehicle-Na-CMC 1%)	187,4 ± 7.53	196,2 ± 6.87**	209,6 ± 6.65**	216,4 ± 8.08**
II	Simvastatin 0.9 mg/kg	185 ± 8.15	153,4 ± 5.81**	125 ± 4.58**	103,4 ± 2.7**
III	Ethyl acetate fraction 2.25 mg/kg	182,8 ± 9.36	178.2 ± 7.59*	164,6 ± 4.15**	138,4 ± 5.31**
IV	Ethyl acetate fraction 4.5 mg/kg	187,2 ± 6.83	172,6 ± 7.6**	154,6 ± 9.2**	129,6 ± 4.56**
V	Ethyl acetate fraction 9 mg/kg	188,4 ± 8.93	162,4 ± 11.08**	132 ± 8.74**	110 ± 4.89**

Table 3: Effect of Ethyl acetate fraction *Raphanus sativus* var. L ethanol extract on blood glucose in Alloxan-induced Hyperglycemic rats

Group	Treatment	Blood Glucose (mg/dl, mean ± SEM)			
		0 h	3 h	5 h	7 h
I	Normal Control (vehicle-Na-CMC 1%)	446,6 ± 48,87	446,6 ± 49,23	447,8 ± 48,33	446,6 ± 46.19
II	Glibenclamide 2 mg/kg	390 ± 25,96	343,2 ± 36,35*	300,4 ± 37,92**	256 ± 40,81**
III	Ethyl acetate fraction 83.51 mg/kg	369,4 ± 24,42	305 ± 24,53**	256,2 ± 27,16**	200,8 ± 13,33**
IV	Ethyl acetate fraction 167.02 mg/kg	305,2 ± 60,62*	262,4 ± 52,66**	231,4 ± 52,54**	195,2 ± 52,49**
V	Ethyl acetate fraction 334.1 mg/kg	422 ± 73,03	388,2 ± 68,03	360,6 ± 67,62*	335 ± 69,31*

The ethyl acetate fraction of *Raphanus sativus* var. L ethanol extract demonstrated a significant reduction in serum cholesterol levels in hyperlipidemic rats, decreasing total cholesterol to 110 ± 4.89 mg/dl 14 hours post-treatment, compared to the control group with diet-induced hyperlipidemia, which had a total cholesterol level of 216.4 ± 8.08 mg/dl (Table 2). Additionally, the fraction significantly lowered serum glucose levels in hyperglycemic rats, reducing blood glucose to 335 ± 69.31 mg/dl 7 hours post-treatment, compared to the diet-induced hyperglycemic control group with a blood glucose level of 446.6 ± 46.19 mg/dl. The standard antihyperlipidemic agent, simvastatin (0.9 mg/kg body weight), also effectively reduced elevated serum lipid levels, lowering total cholesterol to 103.4 ± 2.7 mg/dl at the 7 hours post-treatment. Similarly, the standard antihyperglycemic agent, glibenclamide, reduced blood glucose to 256 ± 40.81 mg/dl 7 hours post-treatment, although this level remained higher than that observed in groups III and IV (Table 3).

DISCUSSION

The results of the study indicate that the ethyl acetate fraction of *Raphanus sativus* var. L ethanol extract contains several beneficial secondary metabolites, including alkaloids, flavonoids, tannins, and saponins, but lacks steroids and triterpenoids. Phytochemical Composition of white radish roots has been investigated by previous studies. One study investigating ethyl acetate-soluble compounds the glucosinolate and isothiocyanate concentrations across different varieties of white radish, specifically the *Miyashige* and *Ping Pong* varieties, reveals significant differences between the sprouts and mature taproots [7]. For both varieties, the glucosinolate concentrations in the sprouts were substantially higher than in the mature taproots. For instance, the *Miyashige* sprouts contained 133.9 μmol/g (± 19.8) of glucosinolates, while the taproots only had 21.0 μmol/g (± 1.9). This suggests that the sprouts of white radish varieties, regardless of specific cultivar, tend to have a higher concentration of glucosinolates than their mature taproots. The *Miyashige* sprouts had a concentration of 36.3 μmol/g (± 6.1), which was significantly higher than the 4.2 μmol/g (± 0.5) found in the taproots [7]. These findings align with the broader trend that sprouts generally contain more bioactive compounds, such as glucosinolates and isothiocyanates, than the mature taproots. The analysis of glucosinolates in mature taproots revealed a range of compounds with varying concentrations depending on the radish variety. Glucoraphenin, however, made up less than 10% of the total glucosinolates. The white radish varieties Miyashige and Ping Pong contained significant levels of glucoraphasatin, with concentrations of 14.0 ± 1.1 and 15.5 ± 1.7 μmol/g dry weight, respectively [7].

In vivo assay result of the current investigation shows that the extract demonstrated significant effects on hyperlipidemic and hyperglycemic rats. It reduced serum cholesterol and blood glucose levels. The standard treatments simvastatin and glibenclamide also reduced cholesterol and glucose levels, respectively, but the extract showed comparable or superior effects in certain measures. The results show similar trends compared to prior studies [10, 11,21]

Both the antihypercholesterolemic and antihyperglycemic effects of white radish might be due to glucosinolates, phytochemicals primarily found in cruciferous vegetables, have been extensively studied for their potential health benefits [5]. Both preclinical and clinical research suggests that glucosinolates and their metabolites, such as isothiocyanates, exert various effects, including anti-inflammatory, antioxidant, and chemo-protective properties. These

compounds have been linked to positive outcomes in conditions like hyperglycemia, diabetes, hypertension, and dyslipidemia [12, 14].

Sulforaphane, a prominent isothiocyanate derived from cruciferous vegetables, has shown anti-inflammatory effects by activating Nrf2 and inhibiting NF-κB. Research indicates that isothiocyanates such as raphasatin and sulforaphane may help prevent or mitigate complications associated with glycemic control [3]. For example, studies on hyperglycemic mice have demonstrated that sulforaphane can prevent diabetes-induced hypertension and cardiac dysfunction. Human studies have also shown benefits, such as a 13% reduced risk of type 2 diabetes with high cruciferous vegetable intake in a meta-analysis [2]. Antiinflammation action of sulforaphane has been proven by investigation of its effect on reducing the formation of atherosclerotic lesions in rabbits [6-13].

CONCLUSION

The ethyl acetate fraction of *Raphanus sativus* var. L (radish) ethanol extract was found to contain beneficial secondary metabolites like alkaloids, flavonoids, tannins, and saponins, but not steroids or triterpenoids. In animal studies, the extract significantly reduced both cholesterol and blood glucose levels— at a dose of 9 mg/kg, total cholesterol was reduced from 216.4 to 110 mg/dl, and glucose from 446.6 to 335 mg/dl.. Its glucose-lowering effect even surpassed that of the standard drug, glibenclamide. These findings highlight its potential as a natural treatment for hyperlipidemia and hyperglycemia. However, more research—including clinical trials and mechanism studies—is needed to confirm safety, identify active compounds like isothiocyanates and glucosinolates, and explore other possible therapeutic applications.

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Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this research.

Ethics Statement

Prior to commencing, the study obtained ethical approval from the Animal Research Ethics Committee of Ahmad Dahlan University in Yogyakarta (no. 022402015) and Universitas Padjadjaran (no. 415/UN6.KEP/EC/2024) to ensure that all procedures were conducted ethically.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

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