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RESEARCH ARTICLE

Potential of Black Pepper Extract (Piper Nigrum L) as Antidiabetic and Anticholesterol in Alloxan and Propylthiouracil (PTU) Induced Wistar Rats

Gerfan Patandung[1] , Elly Wahyudin[2*] , Ika Yustisia[3] , Peter Kabo[4] , Yulia Yusrini Djabir[5] , Sulfahri[6] , Zulfiah[7]

[1] Master of Biomedical Sciences, Postgraduate Program, Hasanuddin University, Makassar, South Sulawesi, Indonesia
Jalan Perintis Kemerdekaan KM 11, Makassar, South Sulawesi, 90245, Indonesia
[2] Department of Pharmacy, Faculty of Pharmacy, Hasanuddin University, Makassar, Indonesia
Jalan Perintis Kemerdekaan KM 11, Makassar, South Sulawesi, 90245, Indonesia
[3] Master Program of Biomedical Science, Graduate School Hasanuddin University, Makassar, South Sulawesi, Indonesia
Jalan Perintis Kemerdekaan KM 11, Makassar, South Sulawesi, 90245, Indonesia
[4] Faculty of Medicine, Hasanuddin University, Makassar, Indonesia
Jalan Perintis Kemerdekaan KM 11, Makassar, South Sulawesi, 90245, Indonesia
[5] Faculty of Pharmacy, Hasanuddin University, Makassar, Indonesia
Jalan Perintis Kemerdekaan KM 11, Makassar, South Sulawesi, 90245, Indonesia
[6] Faculty of Mathematics and Natural Sciences, Hasanuddin University, Makassar, Indonesia
Jalan Perintis Kemerdekaan KM 11, Makassar, South Sulawesi, 90245, Indonesia
[7] Politeknik Sandi Karsa, Makassar, Indonesia
Jalan Bung No.37, Tamalanrea Jaya, Makassar, South Sulawesi, 90245, Indonesia

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***Corresponding Author:**

ellywahyudins@gmail.com

ABSTRACT

Uncontrolled blood sugar and cholesterol levels affect hypercholesterolemia and diabetes mellitus as a significant risk factor for cardiovascular disease, which is the leading cause of death in the world. Treatment of hypercholesterolemia and diabetes mellitus using synthetic drugs has not been able to achieve the expected therapeutic targets, and their long-term use shows side effects and complications in various organs. Black pepper plants (Piper Nigrum L) with piperine alkaloids, cavicin, essential oils, methyl-pyrolin, phenolic acid, and flavonoids can reduce cholesterol and blood sugar levels. This study is to determine the potential of black pepper extract (Piper Nigrum L) as a complementary therapy to reduce cholesterol and blood sugar levels. Methods. This study was conducted experimentally using a post-test-only control group design, carried out April 20 - June 20, 2024, at Biopharmaca and Biopharmaceutical Laboratory, Hasanuddin University Makassar, South Sulawesi, Indonesia. The research samples were male Wistar rats aged 2-3 months divided into five groups Glucose-induced alloxan for 14 days and given 25%, 50%, and 75% black pepper extract. 5 PTU-induced cholesterol group for 14 days and given 25%, 50%, and 75% black pepper extract for 7 days. This study showed high consecutive glucose levels in the first positive group, 75% extract group, 50% extract group, 25% extract group, and negative group. At the same time, the high consecutive cholesterol is the first Positive group, 25% extract group, 50% extract group, 75% extract group, and negative group. From the data and the discussion results, it can be concluded that the percentage of black pepper extract treatment decreased. The highest glucose level was a dose of 25%, while the highest cholesterol level was a dose of 75%.

INTRODUCTION

Female white rats (Rattus norvegicus) are one of the experimental animals widely used by researchers who apply preclinical experimental methods. To determine the effect of a substance used and utilized by humans, it is necessary to conduct research in the laboratory. This study used Wistar rats because they are easily maintained, adaptable, and available in large quantities. Breeding, maintenance, and use are easy and relatively cheap (Tello-Palma, E. et al., 2022). The advantages of using female rats are that this animal model has a database that is relevant to humans so that it is easy to interpret, the life cycle of female rats is relatively short, and the cost and maintenance process is cheaper and more accessible. In addition, female rats can be used as a model to see the picture of acute and chronic human diseases. This is due to the completeness of the organs of biochemical and metabolic mechanisms and the nutritional needs of female rats being relatively close to those of humans (Mukhtar S. et al., 2021). Rats also have better resistance to disease than other test animals. Changes in anatomical shape and behavior in rats are more accessible to observe, so if there is a disability, it is easy to recognize and observe (Aini, Q. et al., 2023. Cakir, M. U. et al., 2022).

According to the World Health Organization (WHO, 2020), Diabetes is a chronic metabolic disease characterized by elevated blood glucose (or blood sugar) levels, which has an impact on serious diseases such as heart, blood vessels, eyes, kidneys, and nerves, About 830 million people worldwide have diabetes, the majority living in low- and middle-income countries. More than half of people with diabetes receive no treatment (WHO. 2023). Both the number of people with diabetes and the number of people with untreated diabetes have continued to increase over the past few decades. Based on Riskesdas data, the prevalence of diabetes in 2020, the International Diabetes Federation (IDF) has recorded 537 million adults (aged 20 - 79 years) or a percentage of 1 in 10 people living with diabetes worldwide. The prevalence of Diabetes Mellitus in South Sulawesi is 1.6 percent. DM diagnosed by a doctor or based on symptoms amounted to 3.4 percent. The prevalence of diabetes in Pinrang District (2.8%), Makassar City (2.5%), North Toraja District (2.3%), and Palopo City (2.1%). The prevalence of diabetes diagnosed by a doctor or based on symptoms was highest in Tana Toraja District (6.1%), Makassar City (5.3%), Luwu District (5.2%) and North Luwu District (4.0%). Diabetes Mellitus is a disease related to glucose levels in the blood and is referred to as a silent killer, and some call it the mother of disease. The chronic complications of diabetes mellitus in Indonesia consist of neuropathy 60%, coronary heart disease 20.5%, diabetic ulcer 15%, retinopathy 10%, and nephropathy 7.1% (Rusli, R. et al., 2024).

Cholesterol is a fatty substance that circulates in the blood, is produced by the liver, and is needed by the body. Still, excess cholesterol will cause problems, especially in the blood vessels of the heart and brain. Blood contains 80% cholesterol produced by the body, and 20% comes from food (Kartika R. et al., 2023). Low cholesterol levels will cause the cerebrovascular endothelium to become fragile, making it more susceptible to microaneurysms, which are the main pathological findings in Intracerebral Hemorrhage, which is one of the causes of stroke (Rais et al. et al., 2024).

According to WHO data, in 2022, deaths due to high cholesterol disorders amounted to 4.4 million people; in Indonesia, it increased by 28% per year. In Indonesia, there is an increase in the number of older adults from 18 million people (7.56%) in 2010 to 25.9 million people (9.7%) in 2019 and can be expected to continue to increase where in 2035, it will be 48.2 million people (15.77%). Meanwhile, the proportion of older adults in North Sumatra reached 7.25 percent of the total population in 2017. According to the Ministry of Health or Kemenkes (2019), Indonesia began to enter the aging population period, where an increase followed an increase in life expectancy in the number of elderly. This condition shows that over the past year in North Sumatra, the proportion of older adults has increased significantly, from 6.96 percent in 2016 to 7.25% percent in 2017, or an increase of 0.29 percent (BPS Sumut 2017). Based on basic health research (Riskesdas) conducted by the Ministry of Health, the stroke rate in Indonesia is 10.9%. The data shows that Yogyakarta Special Region (DIY) Province has the highest stroke rate at 14.6%. Strokes in South Sulawesi province have a prevalence rate of 10.6%, which generally attacks the age group 75 years and over and mainly attacks men. The increase in the elderly population will be a burden if older people have health decline problems (Kemenkes R1, 2019).

According to WHO, about one-third of patients do not know how to take their medication immediately after leaving the facility, and although half of patients are instructed on how to take their medication, the remaining 80% are often administered by unqualified medical personnel. In addition, 20-50% of medications are administered without labels. Unwarranted drug abuse can negatively affect patients and result in treatment optimization by increasing treatment costs and harm. Efforts are made to prevent irrational drug use by evaluating drug use.

The use of natural materials, in this case, plants as medicine, has proliferated worldwide, including in Indonesia, and there is a thought of returning to nature. One of the plants commonly used as a treatment is the fruit of Black Pepper (Piper et al.). Piperine is the main component and efficacious substance contained in black pepper fruit, which has activity as an antidiarrheal and aphrodisiac. Black pepper also contains phenolic amides, phenolic acids, and flavonoids that are antioxidants (Riansyah F. et al., 2024). Flavonoids can neutralize acid digestive fluids and release toxins in the body to reduce nausea and vomiting (Ikhlas. E.N. et al., 2023). Pepper fruit suspension can be used to lower cholesterol in people with hypertension. Black pepper contains, among others, piperine alkaloids (5.3-9.2%), civic (up to 1%) and methyl-pyrolin; essential oil (1.2- 3.5%); fat (6.5-7.5%); starch (36-37%) and crude fiber (± 14%) (Khaerunnisa et al. l., 2024). The main compound in pepper, piperine, has various pharmacological activities, including antioxidants, anti-inflammatories, antidepressants, carminatives, analgesics, antithyroid, anticholesterol, antitumor, anti-asthma, anticholesterol, antidiabetes, hepatoprotective, anti-arthritic, anti-mycobacterial, and increasing fertility (Kinanthi, P. et al. l., 2021).

Empirically and research has known the content of black pepper is used by people around Walenrang sub-district, Luwu Regency, for various treatments such as lowering blood sugar levels and hypercholesterolemia, but currently, research on the use of black pepper extract in reducing blood sugar levels induced by ALOKSAN and PTU-induced cholesterol in Wistar rats. Based on the description above, the researchers conducted a study on the effect of reducing blood sugar and cholesterol levels of black pepper extract (Piper nigrum L) in Wistar rats induced by ALOKSAN and PTU with Strip and GOD-PAP / CHOD-PAP methods.

The evaluation stage must be carried out to assess whether the current best evidence used to determine the therapy provided can optimally benefit patients and minimize risks. In this stage, a search can be made for the latest evidence that may have different results from previous treatment decisions. This step is also done to ensure that the intervention that will be given has more benefits than the number of risks incurred (Yonanda, 2022). One strategy to manage blood glucose levels is to use medication rationally. Rational medication ensures patients get safe, cost-effective drugs tailored to their clinical needs.

Based on the problem description above, a study was conducted on the suitability of using Diabetes Mellitus drugs in Wistar males as test animals. This research aims to analyze the suitability of black pepper extract in reducing glucose and cholesterol levels. Research that obtained the value of glucose and cholesterol levels in experimental animals was conducted in several preclinical and clinical trials. However, information on glucose levels induced by Alloxan and cholesterol levels induced by Propylthiouracil (PTU) in male Wistar using black pepper extract has not been found, so the administration of black pepper extract to male Wistar who have been induced with Alloxan and PTU with toxic doses is essential to be analyzed to determine the levels of glucose and cholesterol of male Wistar experimental animals.

MATERIALS AND METHODS MATERIALS

Maceration equipment analytical balance, measuring cup, glass jar, stirring rod, funnel, flask, filter paper, electric stove, measuring flask, Erlenmeyer, drinking water bottle, 1.0 ml injection syringe, 5ml oral syringe, White rat cage, Animal scales, Oral sonde, Glucometer (Easy et al. acid/GCU measuring instrument), Hemolyzer (Hematology Analyzer), Stopwach/hour. Black pepper extract, white Wistar rats, feed, Alloxan, Propylthiouracil (PTU), NaCMC, Simvastatin 2mg®, Glibenclamide 2mg® distilled water, Alcohol 96%, Easy Touch Glucose and Cholesterol Strips.

Experimental Design

The research design used is a True Experiment with experimental design in the form of a posttest-only control group design, with research samples used as control and treatment groups randomly selected from a specific population. This study was conducted April 20 - June 20, 2024, at the Biopharmaca and Biopharmaceutical laboratory of Hasanuddin University Makassar (South Sulawesi, Indonesia) after obtaining a recommendation for ethical approval 419/UN.17.8/kP.06.07/2024 from the Pharmaceutical Research Ethics Committee of Hasanuddin University Makassar.

The samples in this study were female rats aged 2-3 months with a total sample of 50 male white rats divided into 5 Glucose groups induced by alloxan for 14 days and given 25%, 50%, and 75% black pepper extract. Moreover, five groups of cholesterol-induced PTU were given for 14 days, and 25%, 50%, and 75% black pepper extract were given for 7 days. Each group consisted of 5 male rats. The negative control group was given Na CMC 150-200 mg/kgBB, and the positive control group was given Simvastatin for cholesterol treatment and Glibenclamide for glucose treatment. In contrast, the black pepper extract treatment group was 25%, 50%, and 75%. The study's final results were data on glucose and total cholesterol levels in each group measured by strip technique and hematology analyzer (GOD-PAP for glucose and CHOD-PAP for cholesterol levels).

The data obtained were analyzed statistically using computer software. Measurement of Hemoglobin levels by taking blood from the medial chants into the retro-orbital sinus as much as one cc in all groups by researchers in the Biopharmaca laboratory, then blood samples were screened to test Glucose levels using a Glucometer Strip (Easy touch) and Cholesterol levels using Cholesterol strips (Easy touch) and using a Hematology Analyzer at the Makassar Public Health Laboratory Center. Then, the data obtained was analyzed using the SPSS version 22 program. The quantitative data obtained was analyzed using SPSS and the Kruskall-Kawallis statistical test.

Statistical Analysis of Blood Glucose Levels

The first data analysis was done with a normality test using the Sapiro-Wilk test on Glucose and Cholesterol level data. If the data obtained is usually distributed, the mean data is displayed, but if the data obtained is not normally distributed, the data displayed is the median data. Furthermore, data analysis was carried out using the Kruskall-Wallis test to see if there were differences in glucose and cholesterol levels between groups. The Kruskall-Wallis test is an alternative to the One-way ANOVA test when one or all data distributions are not normally distributed or homogeneous.

Blood glucose levels in the GOD-PAP pretest group, strip induction group, strip posttest, and GOD-PAP have a P-value>0.05, so it can be concluded that blood glucose levels are typically distributed. While the pretest strip group was given 25% and 75% black pepper extract, the GOD-PAP induction group with 50% extract shows that the significance value is smaller than the p-value <0.05, so it is concluded that blood glucose levels are not normally distributed.

Table 1. Distribution of blood glucose levels

Group		Mean Rank	Glucose Level (mg/dL) Mean±SD	P-Value*
Pretest	Strip	5.80	107.72±14.662	0.754
	GOD-PAP	5.40	105.68±18.542	0.916
Induction	Strip	4.10	153.08±29.707	0.141
	GOD-PAP	3.20	151.72±25.124	0.016
Posttest	Strip	3.00	125.72±36.244	0.009
	GOD-PAP	3.00	121.48±31.753	0.009
Difference	Strip Difference	3.00	-27.3600±23.15866	0.009
	GOD-PAP Difference	3.00	-30.2400±22.07427	0.009

Based on table 1. shows the difference in glucose levels of Wistar males in each group. The positive group is Glibenclamide, the opposing group is Na CMC with a dose of 1%, and the Black Pepper Extract Group with doses of 25%, 50%, and 75%.

Before treatment, the value of blood glucose levels in the pretest strip group (0.754) GOD-PAP (0.916), induction strips (0.141) had a p-value of

>0.05, so it is concluded that "there is no significant difference" compared to the GOD-PAP induction group (0.016), the post-test strip, and GOD-PAP groups with a P-Value of 0.009 <0.05, which means "there is a significant difference" with the value of blood glucose levels.

Table 2: Kruskal-Wallis test analysis of blood glucose levels between groups.

Rank				
	Group	**n**	**Mean Rank**	**P-Value***
Pretest Strip	Positive	5	14.80	
	Negative	5	11.50	0.209
	25% Extract	5	15.60	
	50% Extract	5	16.40	
	75% Extract	5	6.70	
	Total	25		
GOD-PAP Pretest	Positive	5	15.30	
	Negative	5	11.70	0.271
	25% Extract	5	15.10	
	50% Extract	5	15.90	
	75% Extract	5	7.00	
	Total	25		
Induction Strip	Positive	5	18.10	0.064
	Negative	5	11.80	
	25% Extract	5	15.90	
	50% Extract	5	13.90	
	75% Extract	5	5.30	
	Total	25		
GOD-PAP induction	Positive	5	21.60	0.006

	Negative	5	11.40	
	25% Extract	5	14.70	
	50% Extract	5	13.10	
	75% Extract	5	4.20	
	Total	25		
Posttest Strip	Positive	5	22.20	0.000
	Negative	5	5.50	
	25% Extract	5	18.60	
	50% Extract	5	12.30	
	75% Extract	5	6.40	
	Total	25		
GOD-PAP Posttest	Positive	5	23.00	0.000
	Negative	5	5.80	
	25% Extract	5	17.80	
	50% Extract	5	11.80	
	75% Extract	5	6.60	
	Total	25		
Strip Difference	Positive	5	23.00	0.010
	Negative	5	7.20	
	25% Extract	5	12.20	
	50% Extract	5	9.90	
	75% Extract	5	12.70	
	Total	25		
GOD-PAP Difference	Positive	5	22.40	0.013
	Negative	5	6.60	
	25% Extract	5	12.70	
	50% Extract	5	10.10	
	75% Extract	5	13.20	
	Total	25		
Kruskal Wallis Test Grouping Variable: Group				

Based on the Results of the Kruskal-Wallis Test data, the pretest group Strip (0.209) GOD-PAP (0.271), and Induction group Strip (0.064) shows that the P-Value>0.05, so it is stated that "there is no significant difference" on Glucose levels before treatment and after alloxan induction treatment and administration of 25%, 50%, 75% black pepper extract. At the same time, the GOD-PAP Induction group (0.006), Posttest Strip group (0.000) GOD-PAP (0.000), and Difference Strip (0.010) GOD-PAP (0.013) have a p-value <0.05 so it is stated that there is a significant difference in glucose levels after being treated with alloxan induction, administration of 25%, 50%, and 75% black pepper extract. Because there is a significant difference, it can be stated that there is an effect of giving black pepper extract at doses of 25%, 50%, and 75% on blood glucose levels of male Wistar rats that have been induced by alloxan.

Cholesterol Levels

The results showed that the significance value of cholesterol levels in the CHOD-PAP pretest group and CHOD-PAP induction group had a P-value> 0.05, so it could be concluded that cholesterol levels were usually distributed. While the pretest and induction strip groups were given 75% black pepper extract, the positive strip postest group, the positive CHOD-PAP postest group, and those given 50%

black pepper extract showed that the significance value was smaller than the p-value <0.05, so it was concluded that cholesterol levels were not normally distributed.

Table 3. Cholesterol Level Distribution

Group		Mean Rank	Cholesterol Level (mg/dL) Mean±SD	P-Value*
Pretest	Strip	5.70	95.88± 10.109	0 .834
	CHOD-PAP	6.60	93.48± 10.552	0 .249
Induction	Strip	5.70	101.32± 10.447	0.834
	CHOD-PAP	5.50	102.92± 8.190	1.000
Posttest	Strip	4.10	89.32± 14.150	0.142
	CHOD-PAP	3.60	88.88± 13.252	0.047
Difference	Strip Difference	3.00	-12.0000± 10.18986	0.009
	CHOD-PAP Difference	3.00	-14.0400± 11.35591	0.008
a. Mann-Whitney Test b. Grouping Variable: Cholesterol				

Based on table 3. shows the difference in male Wistar cholesterol levels in each group. The positive group is given Simvastatin; the opposing group is given Na CMC with a dose of 1%; and the Black Pepper Extract Group with doses of 25%, 50%, and 75%. Cholesterol levels based on the Mann Whitney test, the pretest strip group (0.834) CHOD-PAP (0.249), induction strip (0.834) CHOD-PAP (1.000), post-test strip (0.142) has a p-value>0.05 so it is concluded that "there is no significant difference" compared to the CHOD-PAP post-test group with a P-Value of 0.047 <0.05 which means "there is a significant difference" with the value of Cholesterol levels.

Table 4. Kruskal-Wallis test analysis of blood cholesterol levels between groups.

Rank				
	Group	n	Mean Rank	P-Value*
Pretest Strip	Positive	5	13.60	
	Negative	5	9.70	0.812
	25% Extract	5	15.40	
	50% Extract	5	13.10	
	75% Extract	5	13.20	
	Total	25		
Pretest CHOD-PAP	Positive	5	9.30	
	Negative	5	6.70	0.070
	25% Extract	5	18.40	
	50% Extract	5	14.80	
	75% Extract	5	15.80	
	Total	25		
Induction Strip	Positive	5	12.60	0.753

	Negative	5	9.40	
	25% Extract	5	15.50	
	50% Extract	5	13.90	
	75% Extract	5	13.60	
	Total	25		
CHOD- PAP induction	Positive	5	12.90	0.992
	Negative	5	11.70	
	25% Extract	5	14.00	
	50% Extract	5	13.30	
	75% Extract	5	13.10	
	Total	25		
Posttest Strip	Positive	5	18.80	0.020
	Negative	5	4.10	
	25% Extract	5	16.40	
	50% Extract	5	13.70	
	75% Extract	5	12.00	
	Total	25		
Posttest CHOD-PAP	Positive	5	18.80	0.012
	Negative	5	3.80	
	25% Extract	5	16.80	
	50% Extract	5	14.50	
	75% Extract	5	11.10	
	Total	25		
Strip Difference	Positive	5	22.80	0.000
	Negative	5	3.70	
	25% Extract	5	17.30	
	50% Extract	5	12.30	
	75% Extract	5	8.90	
	Total	25		
CHOD-PAP Difference	Positive	5	22.80	0.000
	Negative	5	4.20	
	25% Extract	5	17.30	
	50% Extract	5	12.50	
	75% Extract	5	8.20	
	Total	25		

Kruskal Wallis Test

Grouping Variable: Group

Based on the results of the Kruskal-Wallis test data, the Asymp.The sig value of the pretest Strip group (0.812) CHOD-PAP (0.070), the Strip Induction group (0.753) CHOD-PAP (0.992) shows that the P-Value>0.05 so that it is stated that there is no significant difference in Cholesterol levels before and after PTU induction and administration of 25%, 50%, 75% black pepper extract. While the Posttest Strip (0.020) CHOD-PAP (0.012), Difference Strip (0.000) CHOD-PAP (0.000) groups. It has a p-value <0.05, so it is stated that there is a significant difference in cholesterol levels after PTU induction, giving 25%, 50%, and 75% black pepper extract. Because there is a significant difference, it can be stated that there is an effect of giving black pepper extract at doses of 25%, 50%, and 75% on the cholesterol levels of male Wistar rats that PTU has induced.

DISCUSSION

Effect of Alloxan on Blood Glucose Levels of Wistar Males

This study showed a significant difference in Hb levels between the opposing and pretest groups. High glucose levels in the negative group were caused by alloxan induction of 24 mg/kgBB given daily for 7 days to rats, causing oxidative stress. Alloxan induction significantly increases blood glucose levels and causes damaging changes in the pancreas, liver, kidneys, and testes (Gamde et al. l., 2023). Alloxan can trigger the formation of ROS through a cyclic reaction with its reduction product, dialuric acid. ROS formation triggers oxidative stress in experimental animals.

Oxidative stress is considered a state of imbalance between free radicals and antioxidants that arises when there is an imbalance between the formation of reactive oxygen species (ROS) in the body and its ability to neutralize and detoxify harmful molecules (G. et al., 2017). ROS occur naturally, but their accumulation can be a marker of oxidative stress. As a result of increased free radicals, lipid peroxidation of cell membranes increases the end product, namely malondialdehyde (MDA) (Taslim et al. et al., 2023). MDA is a highly toxic carcinogen that causes tissue damage. In diabetic conditions, this imbalance results from hyperglycemia and other metabolic abnormalities, leading to increased production of ROS, disrupting the body's antioxidant defense mechanisms, and resulting in redox imbalance. Thus, the oxidative load aggravates pathophysiological processes associated with diabetes, such as insulin synthesis and dysfunction of pancreatic beta cells responsible for insulin formation (E. et al.., 2024). Alloxan has a significant effect on raising blood sugar levels in rats. Hyperglycemia in rats after alloxan induction is due to alloxan's specific and highly toxic activity that damages pancreatic β cells and causes a lack of insulin production (Wulandari et al., 2024).

Alloxan effectively damages Langerhans islet beta cells, characterized by a reduction in the diameter of Langerhans islet cells and impaired beta cell function so that they are no longer able to increase insulin secretion, which causes an increase in blood glucose levels (Fiorenza et al. et al. l., 2022).

Effect of Black Pepper on Blood Glucose Levels of Wistar Males

The results showed the significance value of Hemoglobin levels of rats before being induced by the pretest Strip group (0.209) GOD-PAP (0.271), Induction Strip group (0.064) showed that the P-Value>0.05, so it was stated that there was "no significant difference" in Glucose levels before treatment and after alloxan induction treatment and administration of 25%, 50%, 75% black pepper extract. At the same time, the GOD-PAP Induction group (0.006), Posttest Strip group (0.000) GOD-PAP (0.000), and Difference Strip (0.010) GOD-PAP (0.013) have a p-value <0.05 so it is stated that there is a significant difference in glucose levels after being treated with alloxan induction, administration of 25%, 50%, and 75% black pepper extract. Because there is a significant difference, it can be stated that there is an effect of giving black pepper extract at doses of 25%, 50%, and 75% on blood glucose levels of male Wistar rats that have been induced by alloxan.

This study aligns with research conducted by Dludla, P. V et al. l (2023), which states that piperine found in black pepper has been known to have beneficial effects on human health. Starting with preclinical evidence, it has been shown that administering piperine at a 50 mg/kg dose can improve the digestive system while reducing oxidative stress and inflammation in rats. Supported by the results of Du et al.'s 2020 study, it has been stated that piperine reduces blood glucose levels and increases insulin levels in obese or diabetic rats that a high-fat diet has induced.

Effect of PTU on Blood Cholesterol Level of Wistar Males

This study showed a significant difference in cholesterol levels between the opposing and pretest group glucose levels. High cholesterol levels in the negative group were caused by PTU induction of 12.5 mg/kgBB given daily for 14 days to rats, causing oxidative stress. PTU induction significantly decreased thyroid hormone (TH) levels, liver 5'-D1 and antioxidant activity, and TSHR expression also increased cholesterol levels and caused destructive changes in the pancreas, liver, kidneys, and testes (Gamde SM et al., 2023). The increase in total cholesterol was triggered by PTU induction and high-fat feeding in rats. Previous research shows that using high-fat feed for 14 days can increase cholesterol and triglyceride levels in Wistar rats (Lianto et al., 2023). High-fat feeding also triggers an increase in total cholesterol in the blood.

The use of PTU can increase the likelihood of dyslipidemia in rats fed a high-fat diet (Weija et al., 2018). This is supported by the results of previous researchers who stated that the administration of PTU and a high-fat diet can significantly increase cholesterol and triglyceride levels (Sasmita et al., 2023). Side effects of PTU use include increased blood lipid levels and fat deposits in adipose tissue (Wu et al., 2020).

Effect of Black Pepper on Blood Cholesterol Levels of Wistar Males

The results showed a significant value of Cholesterol levels in the pretest Strip group (0.812) CHOD-PAP (0.070), Induction Strip group (0.753) CHOD-PAP (0.992) showed that the P-Value>0.05, so it was stated that there was "no significant difference" in Cholesterol levels before and after PTU induction and administration of 25%, 50%, 75% black pepper extract. While the Posttest Strip (0.020) CHOD-PAP (0.012) and Difference Strip (0.000) CHOD-PAP (0.000) groups have a p-value <0.05, so it is stated that there is a significant difference in cholesterol levels after being induced by PTU, giving 25%, 50%, and 75% black pepper extract. Because there is a significant difference, it can be stated that there is an effect of giving black pepper extract at doses of 25%, 50%, and 75% on the cholesterol levels of male Wistar rats that PTU has induced.

This study's results align with previous research conducted by Duangjai A. et al., 2013 which states that piperine is an active compound in black pepper that can reduce cholesterol absorption by internalizing cholesterol transporter proteins. In line with the research results, piperine is an active compound in black pepper and reduces cholesterol absorption by internalizing cholesterol transporter proteins (Stojanović-Radić Z.et al., 2019). Supported by the research of Octavia MD. Et al. l.. (2023) state that piperine can significantly reduce cholesterol levels in rats given high-fat feed.

Authors' Contributions

GP and EW were involved in the conception and planning of the research, IY and PK performed the data acquisition/collection, YYD and SS calculated the experimental data and performed the analysis, GP drafted the manuscript and designed the figures, and EW aided in interpreting the results. All authors took part in a critical revision of the manuscript.

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