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EFFECT OF COMBRETUM INDICUM LEAVES ON LIPID PROFILE AND HEMATOLOGY OF MICE INDUCED WITH ALLOXAN

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Abstract

Diabetes has become one of the main causes of death throughout the world recent years. Diabetes symptoms include high blood glucose and hyperlipidemia. An rise in blood glucose can produce an increase in the amount of leukocytes and erythrocytes in the hematology profile. Consequently, this study was to ascertain how extract Combretum indicum leaves affected the lipid profile and hematological of mice given alloxan. The study sample included 36 male mice divided into six groups: normal control (KN) without treatment, negative control (K-) with alloxan treatment, positive control (K+) with alloxan and glibenclamide treatment, P1 with alloxan treatment and Combretum indicum extract dose 100 mg/kgBW, P2 with alloxan treatment and Combretum indicum extract dose 200 mg/kgBW, and P3 with alloxan treatment and Combretum indicum extract dose 400 mg/kgBW. After the mice hyperglycemia on day 3rd, the therapy was continued. The treatment lasted for 20 days. On days 10 and 20, hematological and lipid profile measures were performed. Combretum indicum leaves have the ability to lower total cholesterol, LDL and triglyceride with ascalate HDL levels on 20 days of treatment. In addition, it markedly raised the percentage of lymphocytes and neutrophils with escalate the quantities of lymphocytes and neutrophils in alloxan induced mice. So, in aloxan-induced mice, this extract can reduce the effects of diabetes.

Keywords: Blood Glucose; Hematology; Hyperglycemia; Hyperlipidemia

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INTRODUCTION

Health is a vital part of our lives. There are various technological advances in health development, but they are not in line with the community's behavior and consumption patterns (Susanti *et al*., 2021). Previous research has shown that people who consume excessive amounts of sugar are 3.9 times more likely to develop diabetes mellitus (DM). *The International Diabetes Federation* (IDF) reports that there are now an estimated 537 million diabetics worldwide. This number is expected to increase to 643 million in 2030 and 783 million in 2045. DM may present with hyperglycemia (IDF, 2021).

The main transport medium in the body is blood, which performs a variety of very important physiological functions, such as providing extra nourishment and delivering gases like oxygen and carbon dioxide. Additionally, it has a variety of immune cells that protect the body from different pathogenic conditions. Metabolic products are transported to and from different parts of the circulatory system by blood. Blood components interact with modified biochemical and tissue products upon entry, influencing their functional abilities (Hajam *et al*., 2020). Several research efforts confirmed the correlation between the metabolic indicators signifying insulin resistance and the hematological parameters related to systemic inflammation (Cho *et al*., 2023). DM can lead to various complications of other serious diseases such as vascular disease (atherosclerosis), heart disease, stroke, erectile dysfunction, kidney failure, and damage to the nervous system (Maryama *et al*., 2021).

Based on previous research, it is known that hyperglycemia causes increased erythrocytes, *Mean Corpuscular Hemoglobin* (MCH), and *Mean Corpuscular Hemoglobin Concentration* (MCHC) (Maryama *et al*., 2021). Hemoglobin, *Hematocrit* (HCT), *Mean Corpuscular Volume* (MCV), MCH, dan MCHC levels all decrease in diabetics. Along with a rise in platelet count, diabetes also causes a reduction in lymphocytes and the total *White Blood Cell Count* (WBCC) (Hajam *et al*., 2020). DM is often treated with chemical drugs or modern drugs developed by doctors (Faoziyah & Rahmah, 2019).

Nowadays, more people choose traditional medicine for their health problems. This is because it is cheaper and reduces the side effects of chemical drugs (Prayitno & Mukti, 2018). *Combretum indicum* is a plant with various pharmacological uses anti-hyperlipidemic, insecticide, antiviral, antidiabetic, and antibacterial. Extracts from the leaves of the *Combretum indicum* contain antioxidants in the form of alkaloids,

flavonoids, saponins, phenols, steroids, and tannin. Much research has been done on using *Combretum indicum* leaf extract to lower cholesterol (Majumder *et al*., 2022; Pertiwi *et al*., 2024). Some of these studies have shown a reduction in blood cholesterol levels in hyperlipidaemic mice following the administration of *Combretum indicum* extract (Kulshreshtha *et al*., 2018). However, research on the effect of *Combretum indicum* leaf extract on lipid profile in alloxan-induced mice regarding hematological profile is not widely reported. Thus, further research is needed to determine the efficacy of *Combretum indicum* leaf extract on the lipid profile of diabetic mice and to determine its effect on the hematological profile of diabetic mice.

Some of these studies indicate a decrease in blood cholesterol levels with the administration of *Combretum indicum* leaf extract in hyperlipidemic mice (Kulshreshtha *et al*., 2018). However, research related to the effect of *Combretum indicum* leaf extract on the lipid profile of alloxan-induced mice regarding hematological profiles has not been widely reported. Therefore, further research is needed to determine the efficacy of *Combretum indicum* leaf extract on lipid profile and its effect on hematological profile.

RESEARCH METHODS

Materials

This study use 36 male mice aged 6-8 weeks and weighing 20-30gr were prepared, *Combretum indicum* leaves, 96% methanol, Glibenclamide, NaCMC 1%, Eppendorf tube, hematocrit capillary, Alloxan, EDTA. The research was conducted out at April – May 2022 in the Animal Physiology, Developmental, and Molecular Laboratory. Faculty of Mathematics and Natural Sciences, Mulawarman University. The study was approved by the Health Research Ethics Committee of Medical College of Mulawarman University (approval no.75/KEPK-FK/VI/2022).

Preparation of Leaf Extract and Glibenclamide

Combretum indicum leaf samples are rinsed and dried to slightly reduce their water content. After drying, the simplisia was cut up, mashed, and weighed up to 200 grams. The simplisia powder was then placed in a maceration container, and 2 L of 96% methanol was added until all samples were submerged. After submerge, it was properly sealed and left for three times 24 hours, with occasional stirring. After that, filter and separate the residue from the filtrate (Rosmiati & Fernando, 2017). The filtrate is then evaporated using a rotary evaporator, heated in a water bath to make a thick

extract, and stored in an airtight container.

Glibenclamide was provided at a dose of 0.65 mg/gBW (Iryani *et al*., 2017). Glibenclamide tablets containing up to 5 mg were coarsely powdered before being suspended and homogenized with up to 1 ml of 1% *Carboxymethyl Cellulose Sodium* (Na CMC) solution, which was gradually added until homogenous to 60 ml.

Alloxan Induction and Experiment Protocol

Alloxan induction was performed after the mice had been fed for 16-18 hours. Male mice were induced with alloxan intraperitoneally on the first day at a dose of 125 mg/kgBW, and their blood glucose levels were tested three days later for diabetes analysis in mice (Azizah & Qomariyah, 2022). Mice have suffered hyperglycemia. After experiencing hyperglycemia, mice were grouped into six groups: normal control (KN) without treatment, negative control (K-) with alloxan treatment, positive control (K+) with alloxan and glibenclamide treatment, P1 with alloxan treatment and *Combretum indicum* extract dose 100 mg/kgBW, P2 with alloxan treatment and *Combretum indicum* extract dose 200 mg/kgBW, and P3 with alloxan treatment and *Combretum indicum* extract dose 400 mg/kgBW. Each dose of the extract is given orally.

The treatment lasted for 20 days. On days 10 and 20, hematological and lipid profile measures were performed (Wismaya, 2019).

Parameter measurement and data analysis.

Blood was taken from the retroorbital sinus using a hematocrit capillary. The blood was then collected into two Eppendorf tubes. First, the blood was placed in Eppendorf tube I with EDTA for hematological profile assessment using a hematology analyzer. The blood in Eppendorf tube II was then centrifuged to extract serum for lipid profile analysis. After the data was collected, it was analyzed and tested using Duncan's additional test.

RESULTS AND DISCUSSION

Lipid Profile

Based on the results, the lipid profile obtained in the test animals is shown in Table 1. The average quantity of cholesterol, triglycerides, and LDL acquired on days 10 and 20 reveals no variations overall. On day 20, the average lipid profile numbers increased. The average results on day 20 for cholesterol, triglyceride, and LDL profiles revealed no difference. However, on day 20, the average number of HDL in groups K +, P1, and P3 differed from the other groups.

Table 1. Lipid profile of *Mus musculus* on days 10 and 20

Parameters	Days	Groups					
		KN	K-	K+	P1	P2	P3
Cholesterol (mg/dL)	10	87± 1.15^{a}	110.3± 4.76^{a}	101.3± 1.43^{a}	93.67± 2.49^{a}	118.67± 1.73^{a}	112± 6.11^{a}
	20	91.3± 2.33^{a}	110.7± 2.18^{a}	101.67± 5.61^{a}	84.67± 1.41^{a}	112± 4.16^{a}	97.67± 12.25^{a}
Triglyserides (mg/dL)	10	67± 5.68^{a}	86± 1.76^{a}	119± 6.54^{a}	164± 8.32^{a}	131± 1.60^{a}	105.67± 7.83^{a}
	20	73.3± 8.98^{a}	96.3± 2.46^{a}	54.66± 2.96^{a}	100.67± 1.42^{a}	106.67± 13.16^{a}	89± 10.01^{a}
HDL (mg/dL)	10	65± 7.63^{a}	69± 1.55^{a}	76.75± 4.67^{a}	69.67± 2.89^{a}	48.3± 2.76^{a}	88.67± 2.60^{a}
	20	57± 8.02^{ab}	66± 7.54^{ab}	75.6± 4.67^{bcd}	80.3± 6.69^{cd}	51.3± 2.91^{a}	90.3± 4.09^{d}
LDL (mg/dL)	10	38± 1.30^{a}	13.00± 2.64^{a}	23.67± 7.79^{a}	23.3± 11.09^{a}	49.67± 22.21^{a}	52.3± 20.17^{a}
	20	41± 6.53^{a}	35.67± 4.74^{a}	31.67± 4.25^{a}	17.67± 4.91^{a}	29.67± 4.17^{a}	38.67± 1.28^{a}

Observation Group: (KN) normal control without treatment; (K-) negative control with alloxan treatment; (K+) as a positive control with alloxan and glibenclamide treatment; P1 treated with alloxan and Combretum indicum leaf extract at a dose of 100 mg/kgBW; P2 with treatment with alloxan and Combretum indicum leaf extract at a dose of 200 mg/kgBW; P3 with alloxan treatment and Combretum indicum leaf extract at a dose of 200 mg/kgBW.

Values are presented as Mean±SE of 3 mice (n=3) followed by different superscript letters (a,b,c,d) in the same row indicates a significant difference in groups with significant ($p \leq 0.05$).

On day 20, the average quantity of LDL increased when compared to the KN, K-, and K+ groups, however the average amount decreased in the P1, P3, and P2 groups. The average amount of HDL in the K+, P1, P2, and P3 groups increased compared to day 10, whereas the K- and KN groups' average HDL levels decreased on day 20.

The observation reveals that the increase in LDL in the body is due to lipolysis, which happens in mice with insulin shortage. The process increases free fatty acids in the body, causing the average amount of cholesterol, triglycerides, and LDL to increase (Pangestuti, 2015). Insulin insufficiency can cause an increase in triglycerides, which inhibits lipolysis in adipose tissue and increases free fatty acids in the plasma (Luo *et al*., 2015). At the end of the examination, the K+ group had a lower average cholesterol, an increase in HDL, but also an increase in LDL. The average results from the treatment groups, P1, P2, and P3, suggest that these doses help lower cholesterol, LDL, and triglycerides while increasing HDL.

Results of this study contrast with Kulshreshtha *et al*., (2018), which found that the dose of methanol extract that reduces cholesterol is 200 mg/kgBW. The dose was given to test animals on a cholesterol diet, and it had nearly the

same impact as the medicine atorvastatin. The discrepancy is attributable to variations in therapy, including the use of alloxan and cholesterol diets in prior trials. A dose of 400 mg/kgBW had a beneficial effect on lipid profiles. This is consistent with Rizkayanti *et al*., (2017) which states that the higher the concentration of extract utilized, the more antioxidant chemicals are included, increasing the potential to decrease free radicals and prevent lipolysis.

Hematology Profile

The hematological profile of test animals is shown in Table 2. The hematology profile includes test measures such as the percentage and number of lymphocytes, MXD (Mixed monocytes, basophils, and eosinophils), neutrophils, erythrocytes, hematocrit, MCV (Erythrocyte index), and platelets. Table 2. of the hematological profile shows that the results acquired are the mean number of lymphocytes, MXD, and neutrophils, lymphocyte%, MXD%, neutrophil%, erythrocytes, hematocrit, MCV, and platelets.

Table 2. Hematology profile of *Mice* on days 10 and 20.

Parameters	Days	Groups					
		KN	K-	K+	P1	P2	P3
Lymphocyte (10^3/µL)	10	54.19± 8.51^{a}	54.4± 1.55^{a}	40.6± 4.49^{a}	69.39± 5.25^{a}	39.78± 2.40^{a}	43.5± 10.42^{a}
	20	37.63± 2.51^{a}	37.7± 1.54^{a}	40.2± 4.16^{c}	61.61± 2.06^{c}	52.78± 3.04^{b}	41.04± 0.21^{a}
Neutrophil (10^3/µL)	10	12.27± 0.22^{a}	17.8± 4.71^{a}	14.3± 2.29^{a}	16.57± 5.05^{a}	11.68± 0.61^{a}	11.42± 0.51^{a}
	20	11.46± 0.58^{a}	12.89± 0.5^{a}	12.1± 0.31^{a}	17.81± 0.25^{a}	12.45± 0.29^{a}	12.13± 0.58^{a}
MXD (10^3/µL)	10	20.1± 1.13^{a}	25.91± 3.8^{a}	20±2.74^{a}	31.61± 6.33^{a}	19.02± 1.43^{a}	19.38± 0.69^{a}
	20	21.11± 0.99^{a}	20.5± 0.58^{a}	19.2± 0.14^{a}	19.81± 0.69^{a}	19.84± 0.68^{a}	20.73± 0.44^{a}
Lymfosit%	10	29.53± 5.09^{b}	16.4± 1.21^{a}	16.70± 1.3^{b}	31.84± 5.76^{b}	21.68± 1.78^{ab}	23.8± 3.25^{ab}
	20	21.62± 0.54^{a}	20.5± 0.58^{a}	22.1± 1.01^{b}	33.27± 0.55^{c}	28.11± 1.02^{b}	31.57± 0.38^{c}
Neutrophil%	10	59.54± 5.33^{b}	23.8± 8.64^{a}	67.8± 7.41^{b}	54.07± 8.35^{b}	68.29± 1.54^{b}	65.47± 3.01^{b}
	20	67.05± 0.83^{c}	67.6± 0.56^{a}	69.4± 0.51^{c}	55.19± 1.26^{a}	60.24± 2.27^{b}	67.33± 0.56^{c}
MXD%	10	10.92± 0.75^{a}	13.3± 3.97^{a}	8.26± 0.53^{a}	17.08± 1.83^{a}	10.95± 0.47^{a}	10.63± 0.34^{a}
	20	12.10± 0.75^{a}	10.55± 0.24^{a}	10.6± 0.17^{a}	11.01± 0.50^{a}	10.77± 0.28^{a}	11.09± 0.20^{a}
Erytrosit (10^6/µL)	10	3.80± 0.84^{bc}	1.52± 0.52^{a}	2.97± 0.32^{abc}	2.11± 0.54^{ab}	6.13± 0.76^{d}	4.63± 0.94^{cd}
	20	5.66± 0.12^{c}	4.84± 0.38^{bc}	5.71± 0.26^{c}	2.91± 0.47^{a}	4.17± 1.20^{b}	5.01± 0.81^{bc}

Parameters	Days	Groups					
		KN	K-	K+	P1	P2	P3
Hematocrit%	10	15.96± 3.50[a]	50.0± 2.92[a]	18.8± 3.11[a]	52.13± 4.97[a]	26.10± 1.75[a]	19.95± 3.11[a]
	20	23.57± 0.71[c]	23.4± 2.99[b]	23.87± 1.1[c]	12.52± 0.92[a]	16.65± 1.72[ab]	19.29± 1.9[bc]
MCV (fL)	10	41.9± 1.28[a]	40.7± 1.82[a]	34.4± 17.2[a]	36.83± 0.85[a]	43± 2.21[a]	42.76± 1.42[a]
	20	39.83± 0.29[a]	42.9± 0.33[b]	39.9± 0.49[a]	39± 0.17[a]	42.13± 0.24[b]	39.06± 0.18[a]
Platelets (10^3/µL)	10	121.3± 34.2[a]	497± 7.67[a]	965± 45.2[a]	333.6± 25.4[a]	123.7± 10.2[a]	452.7± 33.3[a]
	20	349.67± 18.4[a]	92± 5.50[a]	270.7± 2,2[a]	269.7± 7.01[a]	164.3± 18.4[a]	187.3± 3.92[a]

Observation group: (KN) normal control without treatment; (K-) negative control with alloxan treatment; (K+) as a positive control with alloxan and glibenclamide treatment; P1 treated with alloxan and Combretum indicum leaf extract at a dose of 100 mg/kgBW; P2 with treatment with alloxan and Combretum indicum leaf extract at a dose of 200 mg/kgBW; P3 with alloxan treatment and Combretum indicum leaf extract at a dose of 200 mg/kgBW.

Values are presented as Mean±SE of 3 mice (n=3) followed by different superscript letters (a,b,c,d) in the same row indicates a significant difference in groups with significant ($p \leq 0.05$).

The number of lymphocytes, MXD, and neutrophils on day 10 did not differ. However, on day 20, the mean number of lymphocytes differed. Lymphocytes in the P1 group differed from the other groups on the 10th and 20th days of data. On day 20, the KN group's average percentage decreased, while the other groups increased. Each group had the same average percent of MXD levels, while the P3 group had a higher average percent of neutrophils.

On day 20, the average number of MXD increased in groups P2 and P3, while decreasing in groups K-, KN, K+, and P1. On day 20, the number of MXD in group P1 decreased the most. On day 20, the average number of lymphocytes decreased in groups K-, KN, and P1, whereas it increased in the other groups. Groups K- and KN exhibited a decrease, while group P2 increased. The average number of neutrophils increased on day 20 in groups P1, P2, and P3. The other groups experienced a decrease in same-day data (day 20).

Each group had the same average percent of MXD levels, while the P3 group had a higher average percent of neutrophils. Group K- had a higher percentage of neutrophils on day 20. On the day of erythrocyte observation, the average number of erythrocytes remained low. Then, on day 20, the P2 group had less erythrocytes than the other groups, which had more. On average MCV, data collected on days 10 and 20 revealed a little rise in the average number in groups K-, K+, and P1 on day 20.

On day 20, the number of MCV became in each of these groups, whereas the average number of MCV in the KN, P2, and P3 groups decreased slightly. The average platelet count showed no difference between days 10 and 20, and the K-, K+, P1, and P3 groups showed a drop in the average number on day 20, however the KN and P2 groups increased.

Insulin insufficiency in mice can result in excessive blood glucose levels and lead to blood cell abnormalities and indices (Islami *et al*., 2021). But overall, lymphocytes, MXD, and neutrophils in group P1 performed well by boosting neutrophils. The increase in neutrophils is caused by inflammation, which causes neutrophils to migrate to the area of inflammation, causing phagocytes that clean the remaining neutrophils that have undergone apoptosis to be unable to thoroughly clean the remaining neutrophils, resulting in inflammation again (Raharjo *et al*., 2020). Differences in the ratio of neutrophils to lymphocytes can be utilized as an early indicator of prediabetes and diabetes (Mertoglu & Gunay, 2017).

On day 10, the average number of erythrocytes remained low. Hyperglicemia affects the characteristics of the erythrocyte membrane, making it more fragile, causing erythrocytes to shatter and lyse prematurely (Sharma *et al*., 2017). On the 20th day, the average number of erythrocytes increased, but the K-, P1, and P2 groups remained below the usual limit of 5,0-10,10 X 10^6/µL (Susanti *et al*., 2021). This is consistent with prior research Handayanti *et al*., (2020) and Kumar *et al*., (2017), which found that diabetics hematological profiles are more susceptible to anemia. According to the author, bleeding, a lack of oxygen, and certain disorders can all cause the development of erythrocytes. These events can cause the loss of a large number of erythrocytes, prompting the body to stimulate erythrocyte synthesis again. On the tenth day, the mice were given blood through the orbital sinus, causing them to experience a scarcity of blood, causing the body to naturally increase erythrocyte production on the twentyth day (Sirait *et al*., 2022).

On day 10, the average hematocrit count showed no difference, but on day 20, there was. However, a hematocrit of 32-52.7 percent in the blood is still considered normal (Arifin *et al*., 2019). Other groups also demonstrate that hematocrit levels remain below average. The average number of MCV was different in group P2. However, the obtained findings are still normal, ranging from 40,1 to 49,4 fL (Siregar *et al*., 2020). On

day 10, the average number of MCV in the K + and P1 groups was still lower than normal, indicating that the group was anemic, as were the P1, K +, and KN groups on day 20. In mice, platelet counts typically range from 325 to 888x10^3/µL (Santos *et al*., 2016). The elevated platelet count in the K+ group on day 10 may be due to acute inflammation in the body. Erythrocytes in the K+ group indicate that the body's erythrocyte count is below normal. This can have an impact on the quantity of platelets since acute infections and acute inflammation in the body cause platelet counts to rise. The decrease (platelet deficit) in groups K, P1, and P2 can be caused by a variety of factors, including anemia, viral or protozoal infections, platelet production failure, or aberrant platelet distribution (Wijayanti *et al*., 2013).

Studies on hematology induced with *Combretum indicum* extract and aloxane showed that if the extract alters the hematological profile, there is a recognized alteration in the amount of neutrophils and lymphocytes present, and then there is an increase in the quantity of both neutrophils and lymphocytes.

CONCLUSION

Based on the observations made, the results of the lipid profile and hematological profile in this study show that the methanol extract of *Combretum indicum* leaves, which is known to contain flavonoids, saponins, tannins, and other antioxidant compounds, has an influence by helping to reduce total cholesterol and LDL while increasing HDL during 20 days of treatment. Furthermore, it can considerably enhance the percentage of lymphocytes and neutrophils, as well as the amount of lymphocytes, neutrophils in alloxan-induced mice. So, in aloxan-induced mice, this extract can alleviate the symptoms of diabetes with optimum dose of extract is 200 mg/kgBW.

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