

## ***The Effect of Ethanol Ants Nest Extract on Profil Lipid Mice Model Obesity with Type 2 Diabetes***

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### **ABSTRACT**

*The Ants Nest plant (Myrmecodia pendans) is known to have the potential to reduce blood glucose cholesterol, LDL (Low-Density Lipoprotein), and HDL (High-Density Lipoprotein) in obese patients with Type 2 Diabetes (T2D) because it contains high flavonoid and tannin content that can prevent oxidative stress and inhibit the work of pancreatic  $\beta$  cells,  $\alpha$ -glucosidase work, Glut-2, and lipase enzymes. This study aims to study the effect of the management of Ethanol Ants Nest Extract (EANE) on changes in the cholesterol, LDL, and HDL levels of obese rats with T2D. The extraction of the ants nest was carried out using the maceration method with ethanol solvent. Cholesterol, LDL, and HDL values will be obtained through the CHOD PAP. The rats were divided into 6 groups, namely: KNo (standard control), KN (T2D obesity), KP (T2D obesity given metformin), P1, P2, and P3 (T2D obesity given ethanol extract of ant nest 150mg/KgBW/day, 300mg/KgBW/day, and 600mg/KgBW/day for 14 days by gastric sonde). Induction of DM model using streptozocin and nicotinamide. Data were analyzed using Paired T-test and continued by using One-way ANOVA. There was a decrease in cholesterol, LDL, and HDL after 14 days of intervention, which was highest in the P3 group with cholesterol, LDL, and HDL of  $64,06 \pm 1,97$  mg/dl ( $p < 0.0001$ ),  $44,56 \pm 2,22$  mg/dl ( $p < 0.0001$ ), and  $45,33 \pm 2,56$  mg/dl ( $p < 0.0001$ ). The treatment of ethanol extract from ants nest has an effect in reducing cholesterol, LDL, and HDL.*

**Keywords:** Cholesterol, Ethanol Ant Nest Extract, Diabetes, LDL, HDL

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### **INTRODUCTION**

Ant's Nest (*Myrmecodia pendans*) from Papua is a typical Indonesian plant considered a traditional medicine with various benefits but has not been clinically tested. The anthill plant is also said to be antiobesity and antidiabetic. This is because the anthill plant contains high

antioxidants, namely flavonoids and tannins with an IC<sub>50</sub> value of 8.18 ppm and in the Ethanol Ant Nest Extract (EANE) obtained an IC<sub>50</sub> value of 32.48 ppm (very strong)<sup>1</sup>. The content cannot only ward off free radicals but also antimicrobial and anti-inflammatory<sup>2</sup>. Flavonoids preserve pancreatic  $\beta$ -cells, restore cellular insulin receptor sensitivity, and

maximize insulin sensitivity. Flavonoids keep pancreatic  $\beta$ -cells, restore cellular insulin receptor sensitivity, maximize insulin sensitivity, inhibit pancreatic lipase enzymes that lower cholesterol, TG, and BW levels, and reduce cholesterol synthesis by inhibiting acyl-CoA cholesterol acyl transferase (ACAT) enzyme activity<sup>3</sup>.

Diabetes Mellitus is a chronic metabolic disorder described by high blood sugar levels associated with impaired insulin resistance and secretion caused by the main factor, one of which is obesity<sup>4</sup>. According to the International Diabetes Federation (IDF) in 2021, there were 537 million people or 10.5% of the total number of adults in the world suffering from Diabetes, it is estimated that the number of Diabetes sufferers will increase to 643 million people, in 2030 and 783 million people, in 2045 in adults aged 20-79 years<sup>5</sup>. According to WHO, the largest increase in Diabetes sufferers occurred in Southeast Asia, including Indonesia, which ranked 5th at 19.5%<sup>6</sup>. *The World Health Organization (WHO)* estimates that in 2030, diabetes will become the 7th cause of death worldwide. According to Suwinawati et al. (2020), 90 - 95% of the disease burden that causes 70% of deaths from diabetes cases worldwide is T2D due to an unhealthy lifestyle. The number of patients is increasing due to the lack of knowledge of T2D management<sup>7</sup>. Infodatin data, 2020 noted that the prevalence of DM in the 2018 Riskesdas in the Papua region was 1.1%, while compared to the West Java, Central Java, and East Java regions, it was 1.7%, 2%, and 2.5<sup>5</sup>.

Antidiabetic drugs such as glinide, metformin, etc. Non-pharmacological therapy means planning nutritional therapy, namely 3J (schedule, type, and amount)<sup>7</sup>. Consumption of several diabetes drugs with a long duration has adverse side effects such as increased body weight (BW), allergy to insulin, gastrointestinal disorders, flatus, genital and urinary tract infections, and can trigger ketoacidosis<sup>8</sup>. People today prefer alternative medicine to traditional medicine, one of which is the Ant Nest plant. People today prefer alternative treatment to conventional medicine, one of which is the Ant Nest plant. The use of extracts from ant nest simplisia is thought to have a hypoglycemic mechanism and be able to reduce cholesterol levels, as well as affect LDL and HDL levels, which involve the inactivation of peroxide free

radicals that can damage pancreatic  $\beta$  pancreas<sup>3</sup>.

This study aims to determine the effect of anthill ethanol extract in reducing LDL and cholesterol levels and increasing HDL levels in obese conditions with T2DM. The results of this study are expected to be used as a reference for further research and may be applied to humans.

## METHOD

This study uses a true-experimental method to observe the impact of the administration of Ethanol Ant Nest Extract (EANE) on cholesterol, LDL, and HDL levels. The research design used was pre-posttest with a control group. The research was conducted from May to June 2022 at the Laboratory of the Center for Food and Nutrition Studies (PSPG), Gadjah Mada University, Yogyakarta. This research has received approval from the Research Ethics Committee of the Faculty of Medicine, Sebelas Maret University, with number 108/Un27.06.11/KEP/EC/2023.

Wistar rats used in this study were treated and raised in the Laboratory of the Center for Food and Nutrition Studies (PSPG), Gadjah Mada University, Yogyakarta. The sampling technique was purposive sampling with 30 samples, 8 weeks old, 150-200gram weight. Rats were adapted within 7 days with drinking water ad libitum and comfeed feed. After the rats were acclimated for 7 days, the rats were made obese, induced with HFHF diet for 14 days given 2 times (morning and evening) ad libitum<sup>9</sup>. In addition to being given HFD, obesity-induced rats are also given 10% Fructose by dissolving 20 ml of High Fructose Syrup 55% in 100 ml of distilled water until homogeneous<sup>10</sup>. After 14 days, the lee index was measured. Mice were classified as obese if the lee index exceeded 300. After reaching obesity, mice were induced with streptozotocin (STZ) as much as 45 mg/kgBW/day and nicotinamide (NA) as much as 110 mg/kg BW/day for 72 hours. STZ + NA induction is done through injection into the abdominal cavity because the abdominal cavity has many blood vessels that cause hyperglycemia. Rats are said to be hyperglycemia if their blood glucose levels exceed 250 mg/dl<sup>11</sup>. Experimental animals were divided into 6 groups, namely: KNo (standard control), KN (T2D obesity), KP (T2D obesity given metformin), P1, P2, and P3 (T2 D obesity given

ethanol extract of ant nest 150mg/KgBW/day, 300mg/KgBW/day, and 600mg/KgBW/day).

To prepare the Ethanol Extract of the Ant Nest, the first step is to peel the ant nest that has met the inclusion and exclusion criteria set. Then, the peeled ants' nests were thoroughly dried and not wet so that they became thin like crackers. After that, the anthill pieces were blended to make a coarse powder. The next step uses a maceration method that Ahmad & Lestari (2011) modified. A total of 300 g of finished anthill coarse powder was added with 70% ethanol in a ratio of 1:7. This mixture was placed in a tightly closed bottle container and then allowed to stand for 2 days with stirring once. After that, the resulting macerate will go through a distillation or evaporation process using soxhlet at a maximum temperature of 78oC. This is done so that the extract is not damaged or degraded<sup>12</sup>.

Blood sampling was carried out two times after the rats were induced with STZ-NA for 3 days, which was to measure the rats' blood sugar levels as a sign of diabetes after induction. At both times of blood sampling, rats were fasting for at least 8 hours. Blood samples were taken by first preparing the rats to be taken blood. The retro-orbital plexus method takes blood from the orbital sinus (eye vein). Second, prepare supporting equipment for taking blood samples (1mL syringe and Eppendorf). Third, the rat was held by the nape of the neck and massaged with fingers. Fourth, microhematocrit was scraped on the medial canthus. Blood was drawn as much as 1mL and collected in Eppendorf<sup>13</sup>.

Examination of total cholesterol, LDL and HDL using by enzymatic colorimetry CHOD-PAP using blood from orbital vein of rats<sup>14</sup>. Total Cholesterol Level Examination, Homogenize 1000 µl standard cholesterol reagent + 10 µl serum 10 minutes at room temperature. Then measured the absorbance with a 546nm spectrophotometer and then compared with the standard. LDL Level Examination, using the Fridewald and Fredicson formula, total cholesterol - HDL - triglycerides: 5. HDL Level Examination, working reagent was made by mixing between 1000 µl distilled water and 4 parts of 4000 µl cholesterol reagent placed in a bottle. The next step is to make a supernatant by homogenizing the sample which is added with 200 µl serum left for 10 minutes at 20-25 Degrees Celcius then centrifuged at 4000 rpm, 10 minutes. The

last step is to homogenize working reagent 1000 µl + serum 10 µl 10 minutes at room temperature. Then measured the absorbance with a 546nm spectrophotometer and then compared with the standard<sup>15</sup>. Statistical data analysis using SPSS 26, where the normality test uses paired t-test parametric test, and homogeneity test using Levene. The test used to determine group differences is continued using ANOVA (Analysis of Variance). The significance value used is = 0,05<sup>16</sup>.

## RESULTS

**Table 1. Cholesterol Levels in Mice.**

Group	Mean ± SD			P <sup>b</sup>
	Initial Cholesterol (mg/dl)	Final Cholesterol (mg/dl)	Δ Cholesterol (Difference) (mg/dl)	
KNo	82,64 ± 1,40	92,33 ± 1,92	9,69 ± 2,79	0,001
KN	168,06 ± 3,46	229,10 ± 4,26	61,05 ± 6,77	0,000
KP	162,93 ± 2,67	108,24 ± 2,90	54,69 ± 3,95	0,000
P1	167,91 ± 4,13	142,08 ± 47	25,83 ± 4,63	0,000
P2	169,96 ± 4,01	130,18 ± 2,18	39,78 ± 4,62	0,000
P3	165,42 ± 2,22	101,36 ± 1,49	64,06 ± 1,97	0,000
P <sup>a</sup>	0,00	0,00	0,00	

Description:

*Kno*: Group of normal/healthy experimental animals

*KN*: Type 2 Diabetic obesity trial group

*KP*: Type 2 Diabetic obesity trial group + metformin 9mg/200gBW

*P1*: Type 2 Diabetic obesity trial group + Ethanol Ants Nest Extract 150mg/KgBW

*P2*: Type 2 Diabetic obesity trial group + Ethanol Ants Nest Extract 300mg/KgBW

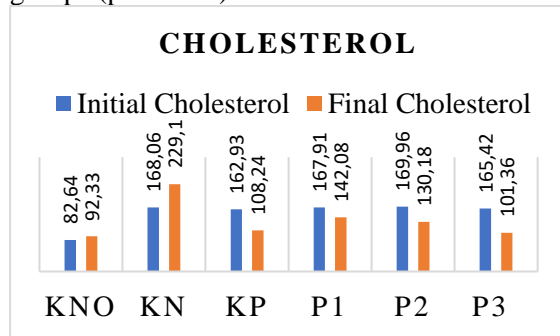
*P3*: Type 2 Diabetic obesity trial group + Ethanol Ants Nest Extract 600mg/KgBW

*P<sup>a</sup>*: (p<0,05) Uji One Way Anova

*P<sup>b</sup>*: (p<0,05) Uji Paired T-test

Table 1 shows that there is a decrease in cholesterol in the P1 group by 25.83 ± 4.63 (p < 0.0001), P2 by 39.78 ± 4.62 (p < 0.0001), and in the P3 group by 64.06 ± 1.97 (p < 0.0001). In the KNo group, where the rats are in average condition (p = 0.001), there is no significant decrease in cholesterol levels in contrast to KN and KP rats in the negative control group (p < 0.0001), which shows there is a substantial decrease in cholesterol levels. Based on the ANOVA test, it was found that there was a significant difference between the treatment

groups ( $p < 0.0001$ ).



**Figure 1. Comparison graph of Cholesterol levels before and after treatment.**

Figure 1 shows that the comparison graph of Cholesterol levels before and after treatment in all groups with Ethanol Ants Nest Extract and metformin shows there was an decrease. In the KN and KNO group, the Cholesterol value has increased significantly. This is different in the KP, P1, P2, and P3 groups, which experienced a decrease in Cholesterol levels after treatment. If you look back at the highest decline, it is P3, namely in the group of obese T2D animals given EANE 600mg / KgBW.

### Results of Statistical Test of *Low-Density Lipoprotein (LDL) Levels*

Contains exposure to the results of related analysis based on statistical tests to determine the effect of EANE administration in lowering LDL levels in Table 2. It can be seen that there is a decrease in LDL in group P1 by  $31.98 \pm 1.79$  ( $p < 0.0001$ ), P2 by  $38.56 \pm 3.38$  ( $p < 0.0001$ ), and group P3 by  $44.56 \pm 2.22$  ( $p < 0.0001$ ). In the KN group, the rat negative control group ( $p=0.009$ ) showed no significant decrease in GDP levels. Then, in the KNo group where the rats were in normal condition ( $p < 0.0001$ ), it was also found that there was a significant difference. Based on the ANOVA test, it was found that there was a substantial difference between the treatment groups ( $p < 0.0001$ ).

**Table 2. Low-Density Lipoprotein (LDL) Levels in Mice**

Group	Mean $\pm$ SD			$P^b$
	Initial LDL (mg/dl)	Final LDL (mg/dl)	$\Delta$ LDL (Difference) (mg/dl)	
KNo	$21,27 \pm 1,55$	$22,68 \pm 1,75$	$1,40 \pm 0,28$	0,001
KN	$71,91 \pm$	$74,50 \pm$	$2,59 \pm$	0,009

	1,91	1,66	1,22	
KP	$74,90 \pm 3,22$	$32,44 \pm 1,64$	$42,46 \pm 1,74$	0,000
P1	$76,11 \pm 1,80$	$44,12 \pm 1,78$	$31,98 \pm 1,79$	0,000
P2	$74,16 \pm 2,37$	$35,60 \pm 2,85$	$38,56 \pm 3,38$	0,000
P3	$71,91 \pm 27,35$	$27,35 \pm 1,32$	$44,56 \pm 2,22$	0,000
$P^a$	0,00	0,00	0,00	

Description:

KNo : Group of normal/healthy experimental animals

KN : Type 2 Diabetic obesity trial group

KP : Type 2 Diabetic obesity trial group + metformin 9mg/200gBW

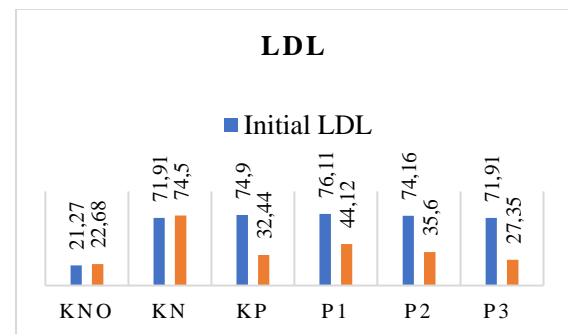
P1 : Type 2 Diabetic obesity trial group + Ethanol Ants Nest Extract 150mg/KgBW

P2 : Type 2 Diabetic obesity trial group + Ethanol Ants Nest Extract 300mg/KgBW

P3 : Type 2 Diabetic obesity trial group + Ethanol Ants Nest Extract 600mg/KgBW

$P^a$  : ( $p < 0,05$ ) Uji One Way Anova

$P^b$  : ( $p < 0,05$ ) Uji Paired T-test



**Figure 2. Comparison Graph of LDL levels before and after treatment.**

Figure 2 shows that the comparison graph of LDL levels before and after treatment in all groups with Ethanol Ants Nest Extract and metformin shows there was an decrease. In the KN and KNO group, the Cholesterol value has increased significantly. If you look back at the highest decline, it is P3, namely in the group of obese T2D animals given EANE 600mg / KgBW.

### Results of Statistical Tests of *High-Density Lipoprotein (HDL) Levels*

Based on the results of statistical tests to determine the effect of EANE administration in reducing HDL levels in Table 3. It can be seen that there was a decrease in HDL in group P1 by  $20.83 \pm 3.71$  ( $p < 0.0001$ ), P2 by  $33.07 \pm 1.38$  ( $p < 0.0001$ ), and group P3 by  $45.33 \pm 2.56$  ( $p < 0.0001$ ), in the KNo group, where the rats were in normal condition ( $p=0.004$ ) and KN rats negative control group ( $p=0.002$ ) showed no significant decrease in HDL levels. Based

on the ANOVA test, it was found that there was a substantial difference between the treatment groups ( $p < 0.0001$ ).

**Table 3. High-Density Lipoprotein (HDL) Levels in Mice.**

Group	Mean $\pm$ SD			$P^b$
	Initial HDL (mg/dl)	Final HDL (mg/dl)	$\Delta$ HDL (Difference) (mg/dl)	
KNo	85,10 $\pm$ 1,53	84,23 $\pm$ 1,57	0,87 $\pm$ 0,33	0,004
KN	32,19 $\pm$ 0,90	29,13 $\pm$ 1,56	3,06 $\pm$ 0,96	0,002
KP	33,62 $\pm$ 1,30	71,55 $\pm$ 2,35	37,92 $\pm$ 2,19	0,000
P1	31,55 $\pm$ 1,92	51,62 $\pm$ 2,53	20,83 $\pm$ 3,71	0,000
P2	34,10 $\pm$ 2,84	67,17 $\pm$ 2,77	33,07 $\pm$ 1,38	0,000
P3	33,47 $\pm$ 1,26	78,79 $\pm$ 2,42	45,33 $\pm$ 2,56	0,000
$P^a$	0,00	0,00	0,00	

Description:

KNo : Group of normal/healthy experimental animals

KN : Type 2 Diabetic obesity trial group

KP : Type 2 Diabetic obesity trial group + metformin 9mg/200gBW

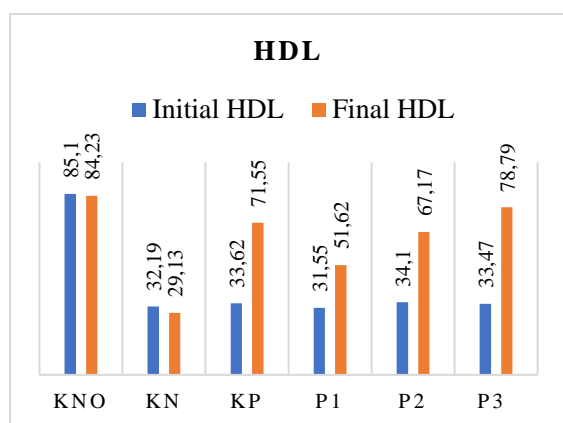
P1 : Type 2 Diabetic obesity trial group + Ethanol Ants Nest Extract 150mg/KgBW

P2 : Type 2 Diabetic obesity trial group + Ethanol Ants Nest Extract 300mg/KgBW

P3 : Type 2 Diabetic obesity trial group + Ethanol Ants Nest Extract 600mg/KgBW

$P^a$  : ( $p < 0,05$ ) Uji One Way Anova

$P^b$  : ( $p < 0,05$ ) Uji Paired T-test



**Figure 3. Comparison Graph of HDL levels before and after treatment.**

Figure 3 show that the comparison graph of HDL levels before and after treatment in all groups shows there was an increase. However, that there is slight change in KNO.

Meanwhile in the KN group there was a decrease. Looking back, the highest LDL levels is P3, namely in the group of obese T2D animals given EANE 600 mg/KgBW.

## DISCUSSION

### Differences in Changes in Cholesterol Levels in Rats

The flavonoid component contained in the Ant's Nest plant (*Myrmecodia pendans* Merr. & Perry) shows an antihyperlipidemia effect because it can reduce the amount of overall cholesterol, Low-Density Lipoprotein (LDL) and prevent the oxidation process of LDL<sup>17</sup>. Lowering cholesterol levels is thought to be related to inhibiting enzyme activity by the ethanol extract of an ant nest. In the flavonoid group, the effect of ant nest is believed to be strongly associated with the content of other active compounds such as tannins, tocopherols, multimineral (Ca, Na, K, P, Zn, Fe, Mg), and polysaccharides. Flavonoids are contained in ant nests by inhibiting cholesterol synthesis through inhibitors of hydroxymethylglutaryl coenzyme-A (HMG CoA) reductase, which is an essential enzyme for cholesterol production in the body. Saponins can form insoluble complex bonds in cholesterol so that cholesterol cannot be absorbed by the intestine and tannin chemicals work by inhibiting fat absorption<sup>3</sup>.

### Differences in Changes in Low-Density Lipoprotein (LDL) Levels in Rats

Flavonoids have antioxidant properties that protect cells from oxidative damage caused by free radicals. In obese mice with DM, excessive oxidation will lead to increased inflammation and impaired lipid metabolism. Flavonoids have protective properties against pancreatic  $\beta$ -cells and restore the sensitivity of insulin receptors on cells and can maximize insulin sensitivity<sup>18</sup>. Flavonoids can boost several enzymes involved in the breakdown of LDL in the liver. Ethanol Extract has a high flavonoid content and plays a role in preventing LDL oxidation. LDL oxidation is one of the early developments of atherosclerosis disease and increases the level of obesity in obese mice models with T2D. Prevention of LDL oxidation will help reduce further disease complications.

The effectiveness of ethanol extract of anthill stem (*Hydnophytum formicarum*) on LDL levels (diabetic rats (*Rattus norvegicus*) induced with alloxan has the effect of being able

to reduce LDL significantly with a dose of 0.54 gr/kgBW equivalent to the administration of glibenclamide 0.5 mg/KgBW. In the treatment group with ESS water extract at a dose of 200 mg/kg and 400 mg/kg, there was a significant decrease in plasma LDL levels. This shows that there is an effect of treatment with ESS has a substantial impact in reducing LDL levels in the blood circulation of rats. This is because dietary cholesterol can suppress cholesterol biosynthesis. However, dietary cholesterol can increase plasma concentrations slightly, which helps reduce LDL levels<sup>19</sup>.

### **Differences in Changes in High-Density Lipoprotein (HDL) Levels in Rats**

The condition of rats before being given EANE decreased HDL due to the presence of cholesterol that accumulates. Free radical activity follows this buildup, which causes oxidative damage to several tissues<sup>20</sup>. Ant nest plant extracts *Myrmecodia pendens* Merr. & Perry) has the potential for anhiperkolesterolemia (total cholesterol and HDL cholesterol) in white rats (*Rattus norvegicus*) male Wistar strain at a dose of 800 mg/KgBW<sup>17</sup>. This is also in line with other studies which state that there is an effect after the administration of the first dose of ant nest plant 100mg/KgBW body weight of rats, which shows an increase in HDL concentration balanced with a decrease in LDL cholesterol<sup>3</sup>.

The limitations of this research are the parameters measured in this study are limited to cholesterol, LDL and HDL only so that other parameters such as HbA1c, GLUT-2, GLUT-4 or MDA levels need to be studied to further determine the antidiabetic activity of the ethanol ant nest extract. Then, the phytochemical test on ethanol ant nest extract was not conducted. In addition, there is limited literature related to ethanol ant nest extract or those associated with obesity with Diabetes Mellitus.

### **CONCLUSION**

Ethanol Ants Nest Extract can reduce LDL and cholesterol Nest levels and increase HDL levels. Dose 3 is the most effective dose using ethanol extract at 600mg/KgBW/day. Dose 3 can reduce LDL and cholesterol levels and increase HDL levels compared to the positive group (metformin). The results showed that the use of Ethanol Ants Nest Extract can be

considered as antidiabetic therapy and has an effect on cholesterol, LDL and HDL values but still needs further research such as acute cytotoxicity tests, chronic cytotoxicity tests. Ethanol Ants Nest Extract is still not recommended if used in humans because it still needs further research.

### **CONFLICTS OF INTEREST**

The authors declare no conflict of interest.

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