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The Effect of *Cordyline terminalis's* Leaf Extract on Lipid Profile, Obesity and Liver Function in Obese Induced Rats

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ABSTRACT
A high diet in saturated fat and lack of physical activity can increase blood plasma fat content and can cause obesity, which is a risk factor for coronary heart disease, type 2 diabetes mellitus, hyperlipidemic, atherosclerosis, liver function dan hypertension. This study aimed to observe effect of andong leave extract (*Cordyline terminalis*) on the profile of lipid, obesity and function of liver onrats. The study was conducted using 24 wistar rats, which was divided into 4 groups: six rats were as control with a standard diet (group I) and 18 rats were obese induced by high-fat diet for 3 groups: Group II obesity with high-fat diet (HFD), Group III and IV (obesity plus extract 100 mg / kgbw and 200 mg / kgbw). Rats received andong leaf extract orally after induction of obesity. After 30 days of research the rat's blood was drawn for testing of lipid profile, obesity, and liver function. The results showed that a decrease in body weight, Lee Obesity Index (LOI), abdominal circumference (AC),visceral fat weights, organs weight, daily diet intake, and serum total cholesterol (TC), LDL cholesterol, triglycerides (TG), alanine aminotransferase (ALT) and aspartate aminotransferase (AST), and an increase in serum HDL cholesterol, daily fecal weight, total cholesterol and fat in feces by significant ($p < 0.05$) in the treatment extract of andong leave than HFD group. According to the research results it can be concluded that the andong leaf extract are proven to have hypolipidemic effects, antiobesity and improve liver function in rats

Keywords: Obesity, *Cordyline terminalis*'s leaf, lever function, hypolipidemic, lipid profile.

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INTRODUCTION
Obesity is a disorder of the metabolic syndrome and increase the development risk factor of diseases of degenerative such as coronary heart, IItype diabetes, hypertension continues to increase, dyslipidemia, nonalcoholic fatty liver, some cancers, atherosclerosis and arthritis. Dyslipidemia is a disease that arises because of the impact of obesity and can be independent risk factors or together can increase oxidative stress that accelerates the formation of atherosclerosis[1–6].The profile of dyslipidemia in obese patients are characterized by increased cholesterol of LDL and TG levels and decrease levels of HDL cholesterol[7–9]. In a state of obesity can trigger oxidative stress due to imbalance of prooxidants and antioxidants in the body. In overweight excessive lipogenesis occurs and inhibition of lipolysis. The deposition process of fat which includes the synthesis fatty acidsand triglycerides that occurs in the liver in the cytoplasmic and mitochondrial regions and adipose tissue is called lipogenesis. Excess Energy in the body from fats, carbohydrates, and dietary protein will be stored in fat tissue. Fat in the form of TG can be obtained from HFD intake or the synthesis of fat in the liver[7]. Excess fat in the body also become first cause of metabolic disorders including glucose and lipid metabolism. That gives to effect the functions of organs involving cardiac, gastrointestinal, liver and reproductive functions [3,10].Hight-fat diet consumption is one of the factors causing obesity. Furthermore, lead to increases of the weight of body, the mass of visceral fat, and the weight of organs[3,11-13].

The liver is one of the most important organs for the body. The physiological function of the liver in the body is as a place for metabolism (carbohydrate, protein, and fat), detoxification of toxins, a place for the formation of red blood cells and blood filter. If the liver is disrupted, various bodily functions will be disrupted so that it greatly impacts the health of the body. Obesity is proven to cause fatty liver that can cause impaired liver function [1]. The parameters of liver damage can be known from changes in the activity of levels of enzymes in the blood by observing substances in the blood formed by liver cells. Alanine aminotransferase (ALT) and Aspartate aminotranferase (AST) are some of the enzymes used as indicators of liver damage[14,15]

The one of remedies of natural that can be used to prevent obesity and decrease cholesterol in the body is to consume foods that contain antioxidant compounds.Antioxidants are substances that can inhibit, delay, and prevent the reaction of free radical in lipid oxidation[16]. Natural plant antioxidant compounds are generally compoundsof phenolic or polyphenolic which may be flavonoids, cinnamic acid derivatives, coumarin, tocopherol, and

polyfunctional organic acids. This polyphenolic compound is multifunctional and can react as a reducing, free radical capture, metal chelating and dampening of the singlet oxygen [16].

Andong is one of the local plants in Bali that contains multifunctional antioxidant compounds. Red andong is type of plant that has a red color and many benefits, especially in Bali as a religious ceremony.Phytochemical testing of andong leafextract containing saponins, polyphenols, flavonoids, alkaloids, steroids and tannins[9]. Saponins of andong leaf have been shown to have anti-cholesterol and anti-obesity activities [17,18]. Leaf extract of andongalso has been shown to have antidiabetic activity[9]. The activity of andong leaf extract may be caused by the secondary metabolites it contains.

Based on the above background the researchers intend to reveal the extract effect of andong leaf on profile of lipid, obesity and the function of liver in wistar rats have not done so much so expected with andong leaf extract level P100 and P200, can cause loss of weight, Lee obesity index, visceral fat weight, fats of blood; ALT, AST and increased total cholesterol and fat of fecal to prevent degenerative diseases.

MATERIALS AND METHODS

Materials

The sample used is andong leaf(*Cordylineterminalis* Kunth) which grow in Gianyar regency of Bali, lard, duck egg yolk, Alanine_aminotransferase (ALT) and Aspartate_aminotranferase (AST), cholesterol (Sigma diagnostic), lipid propile kit (Sigma Diagnostic Ltd) for cholesterol-total, Triglycerides (TG), and high_density_lipoprotein (HDL), Bile acid kit (Sigma Dianostic)

Collection and determination of plants

Andong leaves are collected from the Tampaksiring area, Gianyar, Bali, in January. Determined by the Head of the Plant Conservation Center of the Botanical Garden 'Eka Karya' Bali-LIPI. The leaves obtained are then cleaned of dust and other dirt, and cut into small pieces then dried at room temperature in an open room to a moisture content of ±9%, then dried leaves are ground in a blender flask and filtered to 100 mesh fineness. Powder material is extracted by maceration [9].

Andong leaf extraction

The fine powder of 0.5 kg was put into a 2.5-liter beaker, 2 liters of methanol were added to extract all secondary metabolites and allowed to stand for 24 hours. The mixture is filtered and collected, combined and evaporated [19]. This Andong leaves extract was used for the in vivo assay.

Diet of high fat

Diet of high fat was prepared by mixing a standard dietCP 550 (60%) and 20% pork fat and 20% duck egg yolks. Diet is prepared in form of pellet and given for 60 days [9].

Animals of experiment

White female wistar rats as a research protocol were taken from the laboratory of the Study Center of Animal Diseases of the Faculty of Veterinary Medicine of Udayana University

11-12 weeks old and 100-150 g of weight were divided into 4 groups, one control group (6 normal rats with standard diet Cp 550), 3 treatment groups (18 rats were obese to the Lee obesity index> 0,3) by a calculation of Campos et al., 2008 (1) [20] with a diet high in fat 4 weeks according to Figure. 1. All the experimental works with the animal were carried out after obtaining approval from the organization of Animal Ethics Committees (Nomor: 26/UN14.2.9/PT.01.04/2020).

$$\text{Lee's Obesity Index} = \sqrt{\frac{weight(gram)}{nasoanal(mm)}} x10 \quad (1)$$

Serum lipid analysis

Total cholesterol (TC) was analyzed using CHOD-PAP method according to E. Merck with the span cholesterol diagnostic kit. Serum TG and HDL-C were done by using the span TGs diagnostic kit [21] . Low_density_lipoprotein (LDL) cholesterol was calculated using formula: LDL = total cholesterol-HDL-TGs/5

VLDL was calculated using the formula:

VLDL = TGs/5

Atherogenic index (AI) was calculated using the following formula:

AI = LDL cholesterol/HDL cholesterol [21,22].

Analysis of total cholesterol, total Fat and bile acid of fecal

Three days end of the study period. The number of fecal total cholesterol was measured by spectrophotometry IKM 30, and fecal fat of total was measured by graphimetry [22]. Bile acid was also measured by spectrophotometer with method of Bogoriani et al., 2015 [17].

Analysis of alanine _aminotransferase (ALT) and aspartate _aminotransferase (AST).

Rat blood samples were collected with tubes, then centrifuged and serum taken. 0.1 ml of rat serum was added with 1 ml of a mixture of four parts of one reagent (R1) (100 mmol / l TRIS buffer pH 7.0; 0.500 mmol / l L-alanine, 1200 U / l LDH) and part 2 (R2) (0.18 mmol / l NADH and 15 mmol / l 2-oxoglutarate). The mixture was shaken and incubated in a water bath at 37^0C for 1 minute, then measured with a spectrophotometer at 340 nm wavelength.

The analysis of statistic

The research data were analyzed with statistics. Values were expressed as mean ± SD. The results were analyzed by one-way ANOVAwith least-significant-difference test (LSD) as the post hoc test and evaluated the difference among the treatments. The differences of significant is determined with $p<0.05$

RESULTS

Effect of Andong Leaf Extract (*Cordyline terminalis*) on Rats Body Weight

The development of body weight of the the study rats was determined weekly. Growth of rats body weight for 4 weeks is presented in Figure 2.The results of the research in Figure 2 shows that the development of rats body weight decreased by administering methanol extract of andong leaves at a dose of P100 (mg/kgbw) and P200 (mg/kgbw) compared with the group of high_fat diet. The body weight of the methanol extract group at a dose of P100 decreased by 7. 38% and with P200 decreased by 8.86% so that the difference was significantly ($p <0.05$) compared to the group of high fat_diet which experienced an increase of 6.82%.

Based on Figure 1 it can be seen that the weight of the control group was significantly different at the start of the study ($p <0.05$) compared to the other three groups. The

average body weight of the control group was 190.94 ± 8.95 g, the treatment group with high_fat was 290.93 ± 9.13 g, the treatment group with 100 mg andong leaf extract was 289.09 ± 8.70 g, and the treatment group with 200 mg / kg andong leaf extract was 288.99 ± 0.70 g.Weight gain for 4 weeks occurred in the control group and high_fat group. Significant weight gain occurred every week of the study ($p < 0.05$) in the groups of control and high fat diet.

The extract effect of andong leaf on serum lipid profile, liver function and fecal

The Table 1. Shows the effects of andong leaf extract on profile lipid of serum, total cholesterol, total fat, bile acid of fecal and liver function.

Effect of andong leaf extract on organs weight

The effect of Cordyline terminalis leaf extract on organ weight can be shown in the Table 2.

It shows that the andong leaf extract at a dose of P100 and P200 reduced organs weight: heart, liver, kidney, spleen significantly different ($p < 0.05$) than the HFD group.

Effect of andong leaf extract to visceral fat weight

The effect of andong leaf extract to visceral fat weight can be seen in the Table 3.

It shows that andong leaf extract at a dose of P100 and P 200 reduced mass of visceral fat: retroperotonial, perirenal, perianal were significantly different ($p < 0.05$) than the group of HFD.

Effect of andong leaf extract to physiological rats

The effect of andong leaf extract on AC, LOI, Fecal Weight, and food intake can be seen the Table 4. It shows that extract of andong leaf at a dose of P100 and P200 influence physiological of rats : AC, LOI, Fecal Weight, Food Intake were significantly different ($p < 0.05$) than the HFD group.

DISCUSSION

The figure 2 shows that after 4 weeks of research, the effect of andong leaf extracts, there was a tendency to lose with the treatment of P100 and P200 of andong leaf extract respectively at 7.38% and 8.86% compared control and HFD. In the HFD group there was a gain of weight of 6.82% resulting in a significant difference $p < 0.05$. This proves that a high-fat diet can accelerate weight gain. This research is also supported by the results of research which states that high-fat diets and lack of physical activity cause obesity [3,8,22,23]. High-fat diets derived from animals such as egg yolks and pork oil have been shown to be able to increase body weight with significant differences [24,25].According to research result indicate that the andong leaf extract both at P100 and P200 reduced weight of rats and Lee obesity index below 0.3 with a significant difference.

According to research results in Table 1 anincrease in the levels of cholesterol of total, LDL, TG and a decrease in HDL levels, will cause the risk of atherosclerosis, because the increase in total cholesterol of the serum is above the maximum threshold (130 mg / dl). Likewise, the increase in triglycerides was already above the maximum threshold (145 mg / dl), while the levels of HDL decreased significant different ($p < 0.05$) in the rats fed of HFD [26–29].

The levels of LDL cholesterol, total cholesterol, TG significantly decreased ($p < 0.05$) in the treatment of P100 andong leaf extract and P 200 compared with HFD group. VLDL levels, ratio of total cholesterol to HDL and ratio of AI also showed a significant decrease ($p < 0.05$) in the P100 and P200 treatments compared to HFD group. The treatment with P200 showed the highest decrease ($p < 0.05$). It shows that the andong leaf extract has hypolipidemic actvity. Garg and Singh, 2015 have reported that decrease in LDL and VLDL level and the HDL cholesterol level increased are beneficial in hyperlipidemic conditions. An increase in HDL is the effect of an antiatherogenic by counteracting LDL and VLDL oxidation and facilitating the translication of cholesterol from peripheral tissue like arterial walls to liver for catabolism. Thus, combined reduction of TC, TG, LDL and VLDL reduces the fat mass of obese rats [8,30].

HDL cholesterol levels significantly increased ($p < 0.05$) in the blood serum of rats given P100 and P200 extract compared to HFDgroup and lower than controls. Foods high in fat cause obesity. The impact of obesity causes impaired liver function. Impaired liver function occurs due to fatty liver. Fatty liver is a condition where there is a buildup of fat in the liver which makes the liver function deteriorate slowly eventually developing into liver damage. It is proven that rats with high-fat diet treatment produced the highest ALT and AST compared with control and treatment rats at P100 and P200, so that the potential for liver damage. ALT and AST are enzymes used to determine the degree of liver damage [15,31].

Intake of andong leaf extract at P100 and P200 in experimental rats can prevent an increase in average total cholesterol (39.71%; 39.05 %); LDL (80.23%; 82.25%); TG (43.90%; 51.74%); VLDL (44.29%; 51.75%); the ratio of total cholesterol / HDL (48.75%; 53.44%) and reduction of HDL cholesterol (21.10%; 33.25%) compared to the HFD group. The increase in HDL cholesterol levels in P100 was lower than in controls and higher than in the HFD group with a significant difference ($P < 0.05$). Decrease in lipid profile of rat blood serum is caused by the content of phenolic and saponins compounds in leaves of Andong which can inhibit the absorption of cholesterol in the intestine, so that serum lipid decreases[9,17,18].

The results of these studies indicate that the P100 and P200 andong leaf extract is suspected to have biological activity as anticholesterolemia and antiobesity which can prevent atherosclerosis, which is is one of the triggers of heart disease [28,29]. Andong leaf extract intake is thought to not only bind cholesterol from the food consumed, but also bind cholesterol from the liver which is secreted to the intestine along with bile acid.

The Table 1 shows that the average total cholesterol and total bile acid (folic acid) feces in the lowest high-fat group and the highest high-fat + P200 treatment group ($p < 0.05$) compared to the control group. The results of the study with HFD + P100 and P200 treatment show that the average total feces fat of rats (Table 1) gave a significant difference ($p < 0.05$) compared to the control group and higt-fat diet group. Thus, the intake of andong leaves extract can increase the excretion of total bile acids (cholic acid) and total cholesterol of fecal in rats with a significant increase ($p < 0.05$), compared to other groups. Andong leaf extract intake can also bind fat, resulting in an increase in total fat excretion in the feces of wistar rats.

In addition, the Table 2, 3 show that occur increases of organs weight in high-fat diet rat group and also increases of mass of visceral fat signifacantly ($p < 0.05$) compared with control rats group, P100 and P200 andong leaves extract doses. On the Table 2 shows that occur enlargement of liver and spleen ($p<0.05$). Organs of liver and spleen were known as marker for condition of pathologic on the body of animal [3,32]. Moreover, the accumulation of fat on the region of visceral adipose can influence health level and stimulate released of

adipocytokinine from adipose tissue, so that give effect on the dysregulation of metabolim of lipid and glucose. Dysregulation of metabolim of lipid and glucose resulting lipotoxicity. Lipotoxicity effect is the change of hormonal modulation, dysregulation of immune-system, dysfunction of mitochondrial, and oxidative stress [3,6]. Conversely, groups treated with andong leaf extract with dose P100 and P200 didn't effective to decrease liver weight caused by fat accumulation. Futhermore, andong leaves extract at the dose of P100 and P200 showed a significant decrease in spleen weight compared to high-fat diet group. As shown in Table 2 andong leaves extract at the dose of P200 shows a significantly lower spleen weight and heart compared to high-fat diet group ($p<0.05$).

In addition, Table 4 shows that the treatment group of rats with high-fat diets increased including body weight (obesty Lee index), abdominal circumference, the amount of diet consumed and decreased stool weight compared to P100 and P200 with significant differences ($p <0.05$). that means calorie intake is influenced by appetite factors. weight gain occurs if the appetite increases and consumes an excessive high-fat diet causing weight gain and visceral fat mass to increase.

CONCLUSION

It can be concluded that *Cordyline terminalis's* leaf extract intake have had effect as an antiobesity and hypolipidemic as well as prevent liver damage by reducing profile lipid, AI, body weight, Lee Obesity Index, abdominal circumference, visceral fat weight, organs weight, daily diet intake, daily fecal weight, ALT, AST and increase serum HDL cholesterol, total cholesterol, fecal total fat and bile acids in obese wistar rats

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CONFLICT OF INTERESTS

The authors declare and no conflict of interests for this study

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TABLE AND FIGURE

Table 1. Mean levels of blood serum lipid profile, bile acid, liver function and rat feces

Group	Control	High_Fat Diet	P100	P200
Serum Tot Chol. mg/dl	116.67±1.63$^{b, c, d}$	211.37±7.79$^{a, c, d}$	91.00± 2. 37$^{a, b, d}$	83.83±1.17$^{a, b, c}$
LDL mg/dl	58.62±2.02$^{b, c}$	138.53±8.25$^{a, c, d}$	27.90±2.56$^{a, b, d}$	14.32±0.98$^{a, b, c}$
HDL mg/dl	46.85±1.38$^{b, d}$	14.37±0.64$^{a, c, d}$	46.10±1.85$^{b, d}$	55.88±1.45$^{a, b, c}$
TG mg/dl	56.83±1.94$^{b, c, d}$	324.83±3.31$^{a, c, d}$	84.17±2.48$^{a, b, d}$	68.17±1.17$^{a, b, c}$
VLDL mg/dl	11.37±0.39$^{b, c,}$	64.87±0.73$^{a, c, d}$	16.73±0.60$^{a, b, c}$	13.63±0.23$^{b, c}$
Tot Chol/HDL	2.49±0.12$^{b, c, d}$	14.71±0.25$^{a, c, d}$	1.97±0.11$^{a, b}$	1.50±0.10$^{a, b}$
LDL/HDL	1.25±0.07^{b,}	9.61±1.00$^{a, c, d}$	0.60±0,07$^{a, b}$	0.26±0.02$^{a, b}$
Bile Acid(µMol/L)	46.75±0.65$^{c, d}$	47.39±1.43^{cd}	26.52±0.97$^{a, b}$	23.67±0.87$^{a, b}$
ALT (U/l)	28.38±1.15$^{b, c, d}$	86.87±1.97$^{a, c, d}$	28.60± 0.80 $^{a, b}$	25.03±0.59$^{a, b}$
AST (U/l)	78.20±1.80$^{b, c}$	213.32±1.26$^{a, c, d}$	78.60±0.89$^{a, b, d}$	68.73±0.84$^{b, c}$
Fecal Fat (g/100g)	3.52±0.45$^{b, c, d}$	7.01±0.17$^{a, c, d}$	8.58±0.47$^{a, b, d}$	9.20±0.31$^{a, b, c}$
Tot Chol (mg/kg)	580.84±8.10$^{b, c, d}$	216.28±1.83$^{a, c, d}$	1354.38±46.96$^{a, b, d}$	1442.70±26.73$^{a, b, c}$
Bile acid (µmol/d/100g bw)	18.60±0.65$^{b, c, d}$	3.83±0.46$^{a, c, d}$	34.10±0.64$^{a, b}$	35.93±0.56$^{a, b}$

The effects of andong leaf extract showed significant differences p <0.05. ^{a} p < 0.05 represents as control; ^{b} p < 0.05 represents as High_Fat Diet; ^{c}p < 0.05represents as P100; ^{d} p < 0.05 represents as P200.

Table 2. Results of methanol extract of andong leaf on organ weight

Group	Control	High_Fat Diet	P100	P200
Kidney(g)	1.85±0.64$^{c, d}$	1.84±0.51$^{c, d}$	0.90± 0.05$^{a, b}$	0.93±0.05$^{a, b}$
Heart (g)	0.68±0.09$^{b, c}$	2.05±0.22$^{a, c, d}$	0.89±0.14$^{a, b, d}$	0.49±0.09$^{b, c}$
Spleen (g)	0.36±0.06$^{b, c, d}$	1.48±0.19$^{a, c, d}$	0.42±0.04$^{a, b}$	0.29±0.05$^{a, b}$
Liver (g)	4.64±0.04$^{b, c}$	7.94±0.03$^{a, c, d}$	5.46±0.17$^{a, b,}$	4.44±0.14$^{b, c}$

Effects of andong leaf on weight of organsshowed significant differences p <0.05. ^{a} p < 0.05 represents as control; ^{b} p < 0.05 represents as High_Fat Diet; ^{c} p < 0.05represents as P100; ^{d} p < 0.05 represents as P200.

Table 3. Results of methanol extract of andong leaf on visceral fat weight

Group	Control	High_Fat	P100	P200
Retroperitonial(g)	1.78±0.24$^{b, c, d}$	8.04±0.16$^{a, c, d}$	1.50± 0.40$^{b, d}$	0.95±0.14$^{a, b}$
Perirenal(g)	1.0±0.31$^{b, c}$	5.10±0.19$^{a, c, d}$	2.28±0.17$^{a, b, d}$	1.14±0.21$^{b, c}$
Perianal (g)	1.04±0.20$^{b, c, d}$	5.07±0.12$^{a, c, d}$	3.14±0.19$^{a, b}$	1.0±0.18$^{b, c}$

Effects of andong leaf extract on visceral fat weight showed significant differences p <0.05.^{a} p < 0.05 represents as control; ^{b} p < 0.05 represents as High_Fat Diet; ^{c} p < 0.05represents as P100; ^{d} p < 0.05 represents as. P200.

Table 4. The results of methanol extract on physiological of rats

Group	Control	High_Fat	P100	P200
AC (cm)	17,36±0,42^{b}	22,92±0,33$^{a, c, d}$	17,54±0,27$^{a, b}$	17,18±0,27$^{a, b}$
LOI	0,27±0,02^{b}	0,35±0,02$^{a, c, d}$	0,27±0,02^{b}	0,26±0,02^{b}
Fecal Weight (g)	3,46±0,33$^{b, c, d}$	0,38±0,05$^{a, c, d}$	4,66±0,04$^{a, b, d}$	5,75±0,19$^{a, b, c}$
Food Intake (g)	14.28±0,23$^{b, c, d}$	15,35±0,19$^{a, c, d}$	5,74±0,61$^{a, b, d}$	6,39±0,05$^{a, b, c}$

Effects of andong leaf extract on physiological showed significant differences with p <0.05. ^{a} p < 0.05 represents as control; ^{b} p < 0.05 represents as High_Fat Diet; ^{c} p < 0.05represents as P100; ^{d} p < 0.05 represents as P200.

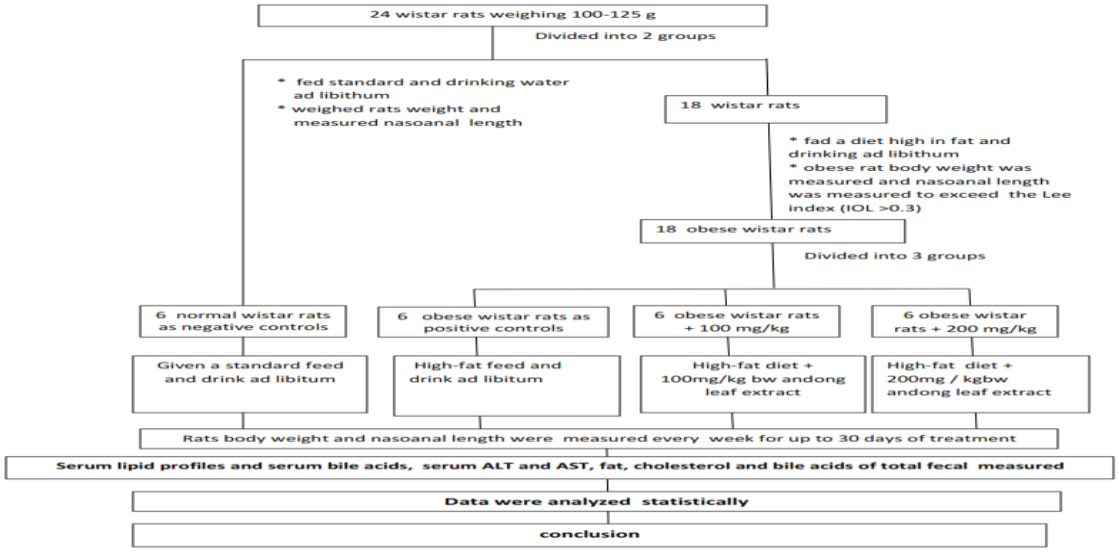

Figure. 1 schematic of research

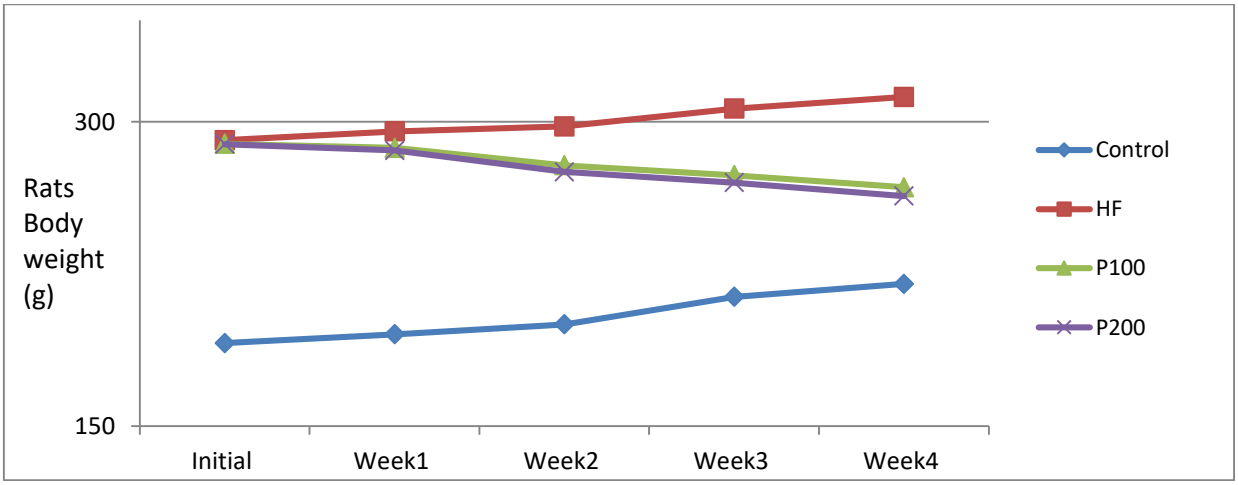

Figure. 2. Average body weight gain wistar ratsindicate significant differences p<0.05; HF (High_Fat), P100 (andong leaf extract with dose 100 mg/kg); P200 (andong leaf extract with dose 200 mg/kg)