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Original Research Article

In Vivo Evaluation of Anti-obesity and Anti-inflammatory Effects of Ethanol Leaf Extract of *Anredera cordifolia* in Rats

Rusdiana Rusdiana[1]*, Sry Suryani Widjaja[1], Tri Widyawati[2], Dina Keumala Sari[3], Rusmalawaty Rusmalawaty[4]

[1]*Department of Biochemistry, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia*
[2]*Department of Pharmacology, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia*
[3]*Department of Nutrition, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia*
[4]*Facutly of Public Health, Universitas Sumatera Utara, Medan, Indonesia*

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ABSTRACT

Excessive fat accumulation in obesity contributes to inflammation. Macrophages are inflammatory cells that are abundant in adipose tissue. This study aim to evaluate the anti-obesity and anti-inflammatory effects of *Anredera cordifolia* leaves. Powdered leaves of *Anredera cordifolia* were extracted with ethanol by maceration. The anti-obesity effect of the extract was assessed by measuring Extracellular Regulated Kinase (ERK) levels and the number of adipocytes in high fat diet-induced obesity rats. The anti-inflammatory effect was assessed by examining the number of macrophages in adipose tissue. Thirty-six male Wistar rats were divided into six groups: control groups (K1, K2, and K3); treatment groups (P1, P2, and P3). All groups except K1, were fed with high fat diet. K2 was not treated, K3 received orlistat (positive control). The treatment groups received *A. cordifolia* leaf extract as follows: P1 (50 mg/kg), P2 (100 mg/kg), and P3 (150 mg/kg). Following a 4-week treatment period, the body weight, abdominal circumference, ERK levels and macrophages in the adipose tissue of the rats were measured. There was a decrease in the body weights of rats treated with *A. cordifolia* leaf extract at all doses. There was a substantial reduction in abdominal circumference, and a decrease in ERK levels at 50 mg/kg and 100 mg/kg doses, and an increased amount of adipocytes in all the treatment groups. The extract also caused a significant decrease in the number of macrophages in adipose tissue. Therefore, *A. cordifolia* leaf extract has the potential to be used as anti-obesity and anti-inflammatory agent.

Keywords: *Anredera cordifolia*, Obesity, Extracellular Regulated Kinase, Adipocyte, Inflammation.

Introduction

Accumulation of immune cells associated with obesity leads to chronic inflammatory condition. Macrophage cells are prevalent inflammatory cells in adipose tissue and are the main mediators of meta-inflammation, insulin resistance, and decreased adipocyte function.[1] Numerous factors contribute to the processes that lead to obesity, such factors include genetics, a sedentary lifestyle or lack of physical activity, high calorie consumption, depression, and metabolic dysregulation.[2] In theory, obesity is a metabolic state produced by increase in adipose tissue, which results from a significant increase in mature adipocytes,[2,3] occurring through two main steps: a rise in the amount of adipocytes (hyperplasia) and a rise in size of adipocytes (hypertrophy) or both. Adipocyte progenitor cells (preadipocytes) undergo maturation into adipocyte cells.[4] Therefore, weight loss in obesity, besides limiting food consumption and raising energy expenditure,[5] can be achieved through effective inhibition of adipogenesis by inhibiting the proliferation as well as differentiation of adipocyte cells.[6]

*Corresponding author. Email: rusdiana@usu.ac.id
Tel: 081260463371

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One of the signalling pathways of adipogenseis is the insulin signalling pathway, the insulin receptor comprises two external α subunits and two intracellular β subunits on the cell membrane. The insulin signalling is mediated through high affinity binding to insulin receptors specifically in the tissues and cells, insulin signalling cascade activates insulin receptor substrate 1 (primary IRS1) and activates PI3K and AKT1 or AKT2 kinases.[7] This receptor contains a tyrosine kinase enzyme (located on the β subunit) that is stimulated when insulin binds to the receptor.[8] Research has shown that inhibition of lipid accumulation can be done by down-regulating the main transcription factors of adipogenesis, namely; PPARγ, C/EBPα and C/EBPβ, through regulation of extracellular signal-regulated kinase (ERK) pathway signalling in differentiated 3T3-L1 adipocyte cells.[9] Another study mentioned that early in differentiation, inhibiting the ERK pathway suppresses adipogenesis, indicating a positive role of the ERK pathway in adipogenesis.[10] It has also been mentioned that once mature adipocytes are formed, activating mitogen activated protein kinase (MAPK) will inhibit PPARγ expression through extracellular signal regulated kinase phosphorylation and inhibit the mechanism of adipogenesis.[11]

Anredera cordifolia has weight reducing effect, and has also been shown to have anti-inflammatory activity. Phytochemical analysis of the ethanol extract of *A. cordifolia* has revealed the presence of alkaloids, flavonoids, saponins, tannins, triterpenoids/steroids, and phenolic compounds. LC-MS analysis identified compounds such as quercetin,[12] and vitexin,[13,14] and these compounds contribute to the anti-obesity effect of *Anredera cordifolia* extract.[15] Thus, this study investigated the anti-obesity as well as an anti-inflammatory effects of *A. cordifolia* leaf extract by measuring ERK protein levels and the amount of adipocytes and macrophage cells in high fat diet-induced obesity rat model.

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Materials and Methods

Collection and identification of plant material
Dried leaves of binahong (*A. cordifolia*) were collected from the highland area of Susuk village, Tiganderket sub-district, Tanah Karo district, Indonesia in July, 2022. The plant material was identified at the Herbarium Bogoriense, Biology Research Centre-LIPI Bogor, Indonesia, with voucher number LHU57697.

Preparation of extract
The powdered leaves of *A. cordifolia* were macerated in 70% ethanol at room temperature. The extract was concentrated in a rotary evaporator at reduced pressure. The concentrated extract was stored in an air-tight container and kept in the refrigerator until ready for use.

Phytochemical screening
The ethanol extract of *A. cordifolia* leaves were analysed for the presence or absence of secondary metabolites including alkaloids, flavonoids, saponins, tannins, and steroids/triterpenoids according to standard methods.

Experimental animals
Thirty-six (36) male Wistar rats were used for the experiment. The rats were acclimatized to the laboratory condition, they were fed with rodent pellets and allowed access to drinking water *ad libitum*. Ethical approval was obtained from the health ethics committee of the Universitas Sumatra Utara USU) with approval number 705/KEPK/USU/2022.

Induction of obesity and administration of extract
The rats were divided into six groups of 6 animals each, three groups were used as the controls; K1 (rats fed normal diet without extract treatment), K2 (rats fed high fat diet without extract treatment) and K3 (rats fed high fat diet with orlistat treatment), and the remaining three groups P1, P2, and P3 were rats fed with high fat diet and treated with *A. cordifolia* leaf extract at doses of 50 mg/kg bw,100 mg/kg bw, and 150 mg/kg bw, respectively. Treatment was administered orally, once daily for 4 weeks. Rats were considered obese on weight gain of greater than 20% after high fat diet. Anthropometric measurements including body weight (BW) and abdominal circumference (AC) were taken pre and post high fat diet and treatment.

Measurement of ERK and macrophage levels
Analysis of ERK levels was performed using Enzyme Linked Immunosorbent Assay (ELISA). Rats were euthanized, fats were removed from the abdominal cavity, and placed in a cylindrical microbottle. The fat was frozen in liquid nitrogen, fixed in 10% neutral buffered formalin solution, and then followed by Hematoxylin and Eosin (H&E) staining for the examination of adipose tissue, and enumeration of adipocytes and macrophages.

Statistical analysis
Data were analysed using SPSS software version 20.0. Differences between means were analysed using One way analysis of variance (ANOVA) followed by least significant difference (LSD) post hoc test. Significant difference was considered at $p < 0.05$.

Results and Discussion

Effect of Anredera cordifolia leaf extract on body weight of obese rats
There was a greater than 20% increase in body weight of rats in all groups after being fed with high fat diet (Table 1). After treatment with *A. cordifolia* extract, there was a significant ($p < 0.05$) reduction in the body weight of rats in the treatment groups at all doses tested compared to the controls (K1 and K2). The average weight loss at a dose of 50 mg/kg bw (P1) was 6.03 g (2.87%), at 100 mg/kg bw (P2), there was 6.16 g (2.90%) weight reduction, while at a dose of 150 mg/kg bw (P3), the average weight loss was 1.00 g (1.00%). This observation has shown that the ethanol extract of *A. cordifolia* leaves has anti-obesity effect. Similarly, in the group treated with orlistat (K3), there was a significant ($p < 0.05$) weight loss of 20.67 g (10.18%) compared to the control groups (K1 and K2) (Table 2).

Table 1: Baseline Body weight (BW) of experimental animals before and after feeding with a high-fat diet (before administration of *A. cordifolia* leaf extract**)**

Group	BW before induction (g)	BW after induction (g)	Percentage increase in BW (%)	P-value
K1	106.67 ± 8.04	178.33 ± 3.72	67.2	0.027*
K2	110.83 ± 12.69	219.67 ± 21.18	98.2	0.043*
K3	118.67 ± 16.90	223.67 ± 17.95	88.5	0.043*
P1	112.00 ± 11.98	215.83 ± 18.87	92.7	0.043*
P2	114.67 ± 18.48	218.33 ± 22.39	90.4	0.028*
P3	110.67 ± 15.54	208.17 ± 17.29	88.1	0.028*

Values are mean ± SD, n = 6. * = significant ($p < 0.05$).

Table 2: Body Weight (BW) of obese rats (High fat diet induced) before and after treatment with *Anredera cordifolia* ethanol leaf extract

Group	BW obesity before treatment (g)	BW after treatment (g)	P-value
K1	178.33 ± 3.72	212.00 ± 2.61	0.027*
K2	219.67 ± 21.18	252.00 ± 32.33	0.043*
K3	223.67 ± 17.95	203.00 ± 18.64	0.068
P1	215.83 ± 18.87	209.80 ± 13.81	0.686
P2	218.33 ± 22.39	212.17 ± 21.27	0.075
P3	208.17 ± 17.29	207.17 ± 12.42	0.528

Values are mean ± SD, n = 6. * = significant ($p < 0.05$).

Orlistat is a well-known anti-obesity drug that works by reversibly inhibiting the lipase enzyme in the stomach and pancreas, so that the inactivation of this enzyme prevents the breakdown of triglycerides into free fatty acids. The use of orlistat is most effective when combined with diet and exercise.[16] In this study, the average weight loss obtained at extract doses of 50 mg/kg bw and 100 mg/kg bw were similar, but at a dose of 150 mg/kg bw, the weight loss was small with only about 1 g reduction in weight after 4 weeks of treatment. This is in agreement with previous research which reported that 100 mg/kg dose of *Anredera cordifolia* leaf extract is effective in losing weight.[17]

Effect of Anredera cordifolia leaf extract on abdominal circumference and abdominal visceral fat

In the standard diet (K1) and high fat diet fed rats (K2) without extract treatment, there was a notable rise in abdominal circumference (AC) ($p < 0.05$). The administration of *Anredera cordifolia* ethanol extract caused a considerable decrease in the abdominal circumference of the rats. The reduction in abdominal circumference was significant in the groups treated with 50 mg/kg (P1) and 100 mg/kg (P2) of *A. cordifolia* extract ($p < 0.05$) compared to the control groups (K1 and K2). However, in the 150 mg/kg of *A. cordifolia* extract treated group (P3), and the group treated with orlistat (K3), the decrease in abdominal circumference was not statistically significant ($p > 0.05$) (Table 3). Table 4 shows the mean abdominal visceral fat weight in the control and the treatment groups. Administration of a high fat diet (K2) resulted in a significant increase in the weight of visceral fat compared to the normal diet fed rats (K1) ($P < 0.05$). Treatment with *Anredera cordifolia* leaf extract at doses of 50, 100, and 150 mg/kg bw (P1 – P3), as well as orlistat treatment (K3) resulted in a significant decrease in the mean weight of abdominal visceral fat compared to the high fat diet control group (K2) ($P < 0.05$). Based on these observations, one might suggest that the use of *Anredera cordifolia* leaf extract in addition to causing weight loss can also reduce abdominal circumference. This shows that anthropometric measurements of abdominal circumference are strongly correlated with fat content.[18] In the same vein, it can also be said that the use of ethanol extract of *Anredera cordifolia* leaves in addition to losing weight can also reduce abdominal fat. Obesity is an excessive accumulation of fat that can pose a significant health risk. This accumulation results from an abundance of visceral and subcutaneous fats, which is due to disparity in energy expenditure as a result of dietary intake and reduced of physical activity.[19]

Table 3: Abdominal circumference (AC) after high fat diet induced-obesity and treatment with *Anredera cordifolia* ethanol leaf extract

Group	AC before treatment (cm)	AC after treatment (cm)	P-value
K1	12.670 ± 0.106	13.617 ± 0.133	0.026*
K2	13.917 ± 0.585	16.260 ± 0.750	0.041*
K3	14.083 ± 0.937	13.680 ± 0.438	0.063
P1	14.050 ± 0.362	13.800 ± 0.4472	0.041*
P2	13.900 ± 0.573	13.350 ± 0.1378	0.043*
P3	13.700 ± 0.623	13.417 ± 0.3251	0.345

Values are mean ± SD, n = 6. * = significant ($p < 0.05$).

Table 4: Weight of abdominal visceral fat in obese rats

Group	Weight of visceral fat (g)	P-value	Post Hoc LSD				
			K2	K3	P1	P2	P3
K1	2.52 ± 0.44		0.002				
K2	6.04 ± 3.36			0.036	0.012	0.017	0.013
K3	3.65 ± 2.60	0.045					
P1	3.12 ± 0.88						
P2	3.40 ± 0.44						
P3	3.27 ± 0.69						

Values are mean ± SD, n = 6.

Effect of Anredera cordifolia leaf extract on adipocytes and macrophages in obese rats

A greater number of adipocytes with smaller sizes were observed in the extract treated groups (P1, P2, P3) compared to the number and size of adipocytes in the control groups (K1 and K2) (Table 5). On the other hand, the number of macrophage cells in the orlistat treated group (K3) was significantly higher compared to the control groups K1, and K2 as well as the extract treated groups (P – P3). For the extract treated groups, there was a decrease in the number of macrophages in all the treatment groups (P1, P2, and P3). However, only the 150 mg/kg (P3) group showed a significant decrease in the number of macrophage cells compared to the K2 control group ($P < 0.05$) (Table 6). The process of adipogenesis is the ability of preadipocytes to proliferate and undergo differentiation into fully developed adipocytes. Obesity develops due to a chronic positive energy balance, leading to an elevated adipose tissue volume. There are two primary processes involved in this phenomenon: (i) adipocyte hypertrophy, which is an elevation in the size of fat cells, and (ii) hyperplasia, which involves the rise in the amount of fat cells.[20] In this study, it was found that in all the extract treated groups (P1, P2, and P3), there was an elevated amount of fat cells with a decrease in the size of these fat cells. Hench, the anti-obesity effect of *A. cordifolia* leaf extract could generally be linked to a reduction in the size of adipocytes. It is interesting to know that the histological analysis of the adipose tissue indicated an increase in the number of adipocytes with a decreased size in all the treatment groups (P1, P2, and P3) compared to the control groups (K1 and K2) (Figure 1). These observations corroborated earlier studies which found that adipocytes size decreased following weight loss,[21] and increased adipocyte size led to an increase in the proportion of infiltrating macrophages.[22] In another study, it was found that the enlargement of fat cells (adipocyte hypertrophy) is also linked to a higher ratio of M1/M2 macrophages.[23]

Effect of Anredera cordifolia leaf extract on ERK levels

An examination of ERK markers derived from the abdominal fat cells did not reveal substantial differences among the control groups ($p > 0.05$). The average ERK levels in the group given standard diet (K1) was 9.012 ± 2.38 ng/mL, the average ERK levels in the control group without treatment (K2) was 10.17 ± 2.98 ng/mL, while in the orlistat group (K3), the average ERK levels was 11.11 ± 1.40 ng/mL. In the extract treated groups (P1, P2, and P3), the average ERK levels were 8.62 ± 1.28 ng/mL, 8.97 ± 1.29 ng/mL, and 10.22 ± 2.30 ng/mL, respectively. It was observed that the average ERK levels in the treatment groups (P1 and P2) decreased in comparison to the average ERK levels in the control groups without extract treatment (K1, K2, K3) (Table 7). Elevated ERK levels in high fat diet fed rats has been reported in a previous study where it was found that ERK activation led to adipose tissue hypertrophy.[24] In this study, it was observed that treatment with *A. cordifolia* leaf extract at doses of 50 and 100 mg/kg caused a decrease in ERK levels while at a dose of 150 mg/kg, there was a slight elevation in ERK levels compared to the ERK levels in the K2 group. It is well known that adipogenesis occurs through activation of the ERK signaling pathway which result in an increased levels of ERK.[25,26]

Table 5: Number of adipocytes in abdominal adipose tissue

Group	Adipocyte	P-value
K1	52.00 ± 17.37	
K2	62.60 ± 13.18	0.07
K3	107.80 ± 63.76	
P1	78.40 ± 36.36	
P2	91.17 ± 23.17	
P3	65.50 ± 5.57	

Values are mean ± SD, n = 6.

ERK signalling is indispensable in the early stage of adipocyte differentiation, when the lack of ERK will lead to disruption of formation of adipocytes or disturbance in the process of preadipocytes growth into adipocytes. High fat diet administration has been shown to slow down the process of adipogenesis.[27]

According to an *in vitro* study, it has been demonstrated that persistent activation of ERK will decrease adipogenesis because persistent activation of ERK suppresses the expression of PPARγ which is mediated by MAPK phosphorylation.[28] With the reduction in ERK levels in the P1 and P2 groups, it can be suggested that the ethanol extract of *A. cordifolia* leaves exerts its weight reduction or anti-obesity effect by blocking the adipogenic process. The weight reducing effect of *A. cordifolia* extract could also be associated with a decrease in abdominal adipose mass. However, with orlistat, ERK levels were higher compared to the control (K2) group, which shows that the anti-obesity effect of orlistat is not mediated through ERK reduction but through ERK activation-induced hypertrophy of adipocytes.[29,30] In addition, this study also found a decrease in triglyceride levels resulting from the treatment with 50 and 100 mg/kg of *A. cordifolia* leaf extract, whereas, at a dose of 150 mg/kg of the extract, the triglyceride levels increased in comparison to the triglyceride levels in the control groups (K1, K2, and K3). Previous study showed that inhibition of ERK signaling results in decreased ERK levels which in turn causes a decrease in triglyceride levels.[31]

Conclusion

The findings from the present study have shown that the ethanol extract of *Anredera cordifolia* leaves has anti-obesity effect, which was confirmed by its ability to reduce body weight and abdominal circumference in high fat diet-induced obesity in rats. The extract is thought to exert its anti-obesity effect by inhibiting lipogenesis, reduction in the size of adipocytes, and decreasing the level of ERK in adipocytes. *Anredera cordifolia* leaves could also possess anti-inflammatory effect due to its ability to reduce the number of macrophages.

Table 6: Number of macrophage cells in abdominal adipose tissue

Group	macrophage cell	P-value	Post Hoc				
			K2	K3	P1	P2	P3
	Median (min-max)						
K1	4.5(1-7)		0.537	0.429	0.126	0.132	0.026**
K2	3 (2-13)			0.841	0.151	0.052	0.030**
K3	8 (1-14)	0.048*			0.151	0.082	0.082
P1	2 (0-5)					0.931	0.662
P2	1 (1-7)						0.699
P3	1 (0-3)						

*Kruskal-Wallis test $p<0.05$, **Man-Whitney test, n = 6.

Table 7: ERK levels in abdominal adipocytes

Group	ERK levels in adipocytes (ng/mL)	P-value
K1	9.012 ± 2.38	
K2	10.17 ± 2.98	
K3	11.11 ± 1.40	0.349
P1	8.62 ± 1.28	
P2	8.97 ± 1.29	
P3	10.22 ± 2.30	

Values are mean ± SD, n = 6.

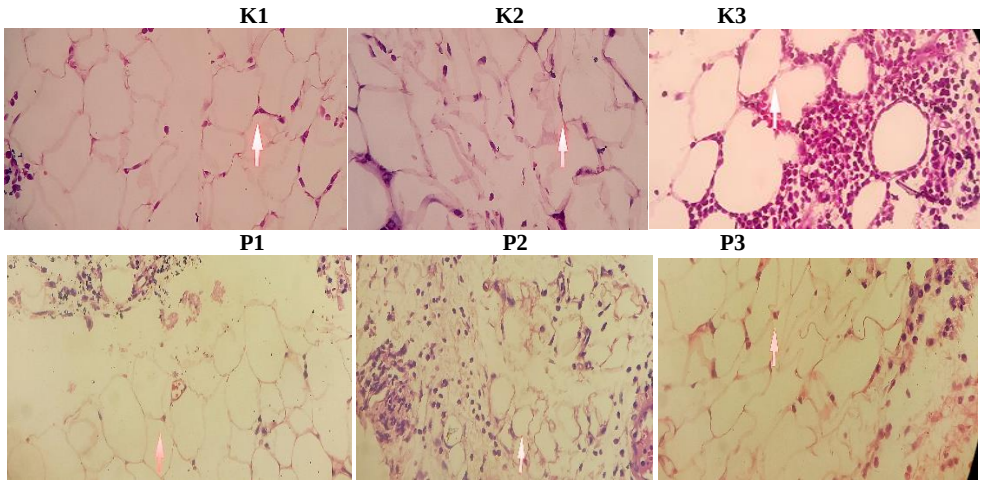

Figure 1: Adipocyte cell morphology
The size of fat cells decreased and the number of adipocytes (indicated by arrows) increased in all treatment groups (P1, P2, P3) compared to the number +and size of adipocytes in the control group (K1 and K2). 400x magnification.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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