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Original Article

The Effects of Leaves Extract of *Toddalia* aculeata on Body Weight, Body Mass Index, Body Fat Content and Resistive Index in Male Rats with High-Fat Diet

Prasetyo Sarwono Putro[1,2], Dono Indarto[1,3,4,*], Bambang Purwanto[1,5], Widyastuti Soewondo[2], Sulistyani Kusumaningrum[1,2], Khim Yatul Nguzum[4]

Prasetyo Sarwono Putro[1,2], Dono Indarto[1,3,4,*], Bambang Purwanto[1,5], Widyastuti Soewondo[2], Sulistyani Kusumaningrum[1,2], Khim Yatul Nguzum[4]

[1]Doctorate Program of Medical Sciences, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, INDONESIA.

[2]Department of Radiology, Dr. Moewardi General Hospital/Faculty of Medicine, Universitas Sebelas Maret, Surakarta, INDONESIA.

[3]Department of Physiology, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, INDONESIA.

[4]Biomedical Laboratory, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, INDONESIA.

[5]Department of Internal Medicine, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, INDONESIA.

Correspondence

Dono Indarto

Doctorate Program of Medical Sciences, Department of Physiology, Biomedical Laboratory, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, INDONESIA.

E-mail: dono@staff.uns.ac.id

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ABSTRACT

Introduction: *Dictamine* is found in the *Toddalia aculeata* plant and is able to interact with *Flavin-Containing Monooxygenase-3* (FMO3) *in silico*, which mediates endogenous atherosclerosis formation. Therefore, this study aimed to investigate the effects of *T. aculeata* extract (TAE) administration on Body Weight (BW), Body Mass Index (BMI), Body Fat Content (BFC), and Resistive Index (RI) in rats with a high-fat diet. **Methods:** Forty male Sprague-Dawley rats were randomly divided into the negative control (NC), positive control (PC) + 2.98 µg/kg BW *dictamine* and treatment (TAE1-3) + 200, 400, and 800 mg/kg BW TAE respectively. All rats were given a high-fat diet (HFD) for 28 days. The data were analyzed statistically using one-way ANOVA, repeated-measured ANOVA, and Friedman's tests with $p<0.05$. **Results:** The average of rats' BW in TAE2 (270±13.78 g) and TAE3 (276.62±40.31 g) was lower than that of the NC (304.12± 4.16 g) but only the TAE2 group was significantly different ($p=0.032$). Rats in the TAE 1-3 groups had the average BMI (26.03±5.61; 21.84±0.81; 20.78±2.17 g/cm^3) significantly lower than the NC (29.61±2.28 g/cm^3) and the PC (28.67±1.68 g/cm^3) for TAE2 and 3. The same pattern was also observed in BFC. All treatment groups had significantly lower RI compared to the NC group (0.62±0.07 cm). **Conclusion:** Administration of 400 and 800 mg/kg BW TAE for 28 days decreases BW, BMI, BFC, and RI in rats with a high-fat diet. *Dictamine* in the ethanol extract of *T. aculeata* leaves might contribute to the reduction of RI in rats with a high-fat diet.

Key words: Body Mass Index, Body Fat Content, Obesity, Resistive Index, *Toddalia Aculeata* Extract.

INTRODUCTION

Obesity is fat accumulation in the human body which results from an imbalance of nutrient intake and energy expenditure.[1,2] The prevalence of obesity has globally increased by which the World Health Organization (WHO) reported that obesity in children aged 5 to 19 years old for both sexes was 6.8% in 2016, increased by 3.9% in comparison to 2000.[3] In Indonesia, the prevalence of obesity has increased from 15.4% in 2013 to 21.8% in 2018, which females are more dominant than males.[4] In addition, obesity is the main risk factor for some non-communicable diseases such as cardiovascular diseases (CVDs), diabetes, stroke,[5] and atherosclerosis.[6] The CVDs are the number one cause of death globally and it is estimated that 17.9 million people died in 2016.[7] A reported data in Indonesia showed that 36.33% of total deaths are due to heart diseases, including hypertension (34.1%) and stroke (10.9 $^0/_{00}$).[4] Atherosclerosis is found in most CVDs patients, which begins from early life and develops for long time periods until the advanced stages with clinical manifestations.[8]

Atherosclerosis is a multiple process that is initiated by endothelial dysfunctions resulting from an accumulation of fats and fibrotic elements in the tunica intima-media of large arteries, including common carotid artery, inflammation, and calcification. These pathological processes have a bad impact on narrowing the artery due to plaque formation.[9] Resistive Index was initially used to assess the arterial lumen in some renal diseases[10] and right now widely used to assess the arterial lumen in heart diseases as well.[11] However, the RI used in obesity cases has not been established.

In recent years, some studies have reported the use of medicinal plants to treat obesity instead of the use of standard therapy. Green tea, *Rosmarinus officianalis* and *Nigella sativa* are commonly used in traditional and modern societies for the treatment of some human diseases including obesity.[12-14] However, the safety and efficacy of those plants over long time periods have not been determined. From *in silico* study, *dictamine* becomes a potential FMO3 inhibitor[15] which is able to interact with the FMO3 protein similar to the standard ligand, *methimazole*.[16] The FMO3 is an enzyme that plays a pivotal role in converting trimethylamine (TMA) to Trimethylamine-N-Oxide (TMAO)[17,18] for inhibition of cholesterol excretion.[19] Therefore, the inhibition of FMO3 activity is expected to reduce atherosclerosis through increasing cholesterol excretion, bile acid synthesis,[19] and reducing fat accumulation.[16]

Recently, the root bark of *Dictamnus dasycarpus Turcz* (a member of the Rutaceae family) has been identified to have some bioactive compounds such as *dictamine*, which belongs to the alkaloid compound.[21] Some studies have revealed that *dictamine* has therapeutic effects such as vasodilation,[22] neuroprotection,[23] anticancer,[24]

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antimicrobials,[25] and antivirus.[26] However, there is a limited study related to the administration of any part of the *T. aculeata* plant in obesity. A recent study has reported that a combination of leaves extracts of *T. aculeata* and star fruit significantly reduced BW (-69.8 g), BMI (-14.51 ± 0.65 g), and BFC (-13.95 ± 0.63 g/cm) compared to the control group in rats with obesity.[27] Therefore, the objective of this study was to investigate the effects of TAE administration on BW, BMI, BFC, and RI in rats with HFD.

MATERIALS AND METHODS

Extraction of T. *aculeata* leaves

Fresh *T. aculeata* leaves were obtained from the Tawangmangu forest, Central Java, and were authenticated by the Center for Research and Development of Medicinal Plants and Traditional Medicines (B2P2TOOT) Tawangmangu with the number KM.04.01/2/1168/2022. The powder of *T. aculeata* leaves was extracted using an existing method by which was soaked in 70% volume/volume (v/v) ethanol (Merck, Germany) at 28^{0}C for 2 days and then filtered to obtain the filtrate.[28] The filtrate was concentrated using a rotary vacuum evaporator at 60^{0}C with 80 rpm speed and 175 bar pressure. The ethanol extract was stored in a glass bottle and kept in the refrigerator before further analysis.

Experimental study design

This study was a true experiment with a randomized pre-posttests control group design. This study used male Sprague Dawley rats (*Rattus novergicus*), aged 3 months, and approximately weighed 250g. The research samples were calculated using Federer's formula: (T-1) (N-1) > 15 with an additional 20% of total rats, T= group number, N= rat number and we got at least 6 rats/group.[29] Selected male rats were randomly divided into 5 groups: negative control (NC), positive control (PC), and 3 treatment groups (TAE1-3). A total of 40 rats were adapted in 10 different cages with 12 hours of light and 12 hours of darkness at 25^{0}C with a standard feeding (Broiler-2 feed) and drinking ad-libitum for 3 days. The protocol of this research study was approved by the Health Research Ethics Committee of Dr. Moewardi General Hospital Surakarta with Number: 405/III/HREC/2021.

TAE intervention

A high-fat diet (HFD) in this study was adopted from the Sundari *et al.* (2022) research study[27] with slight modification. Each kg of HFD consisted of 400 mg Broiler-2 feed (Japfa Comfeed, Indonesia), 6 duck egg yolks, 6 dried organs of chicken liver, and 100 ml lard.[30] The NC group was given HFD and 1ml of 1% (v/v) Carboxymethyl Cellulose Sodium solution and the PC group was given HFD and 2.98 µg/kg BW *dictamine* (PhytoLab, Germany Cat.80583). Meanwhile, the TAE1-3 groups were given HFD and 200, 400, and 800 mg/kg BW TAE solution respectively for 28 days.

Measurements of BW, BMI, and BFC

Rats' BW and length were measured using a standard scale and ruler and monitored weekly. BMI was measured using the Rõhrer index = {Body weight (g)/Naso-anal length (cm)3} ×10^3.[31] The BFC was calculated using a formula: 0.581×TM index-22.03[32] and the TM index was determined using a formula: Body weight (g)/Naso-anal length (cm)$^{2.823}$ x 10^3.[33]

Measurement of RI common carotid artery

Measurements of RI in the common carotid artery were carried out before treatment (day 0), during (day 14), and after treatment (day 28) using an Ultrasonography (USG) device from General Electric USA, type Logiq E with 2d linear probe composition with button L4-12t RS frequency 13 Mhz, sample volume length 1mm, color flow angle and doppler 0 degrees. The RI measurement was performed three times by a radiologist, according to the manufacturer's guidelines.

Statistical analysis

All collected data were analyzed using SPSS (Statistical Program for Social Science) version 26 and presented as mean ± Standard Deviation (SD). The averages of BW, BMI, BFC, and RI among groups were analyzed using the one-way ANOVA, followed by the LSD test. Duration of TAE intervention within groups was assessed using the repeated-measured ANOVA for BW, BMI, BFC, and RI except for abnormal data using the Friedman's test. The significant value was set up as $p<0.05$.

RESULTS

TAE administration increased BW in rats with HFD

Figure 1 indicated that TAE administration increased the average of rats' BW except the TAE2 group. In the NC group, the average of rats' BW increased significantly to reach 304.12±24.16 g (p=0.032) at the end of treatment. The same pattern was observed in the PC and TAE3 groups but the final average of rats' BW (290.25±35.66 g and 276.62±40.31 g) was lower than the NC group. However, the increased average of rats' BW in the TAE3 group did not reach a statistical difference (p=0.095). For the TAE1 and 2 groups, the average of rats' BW increased 299.12±31.92 and 270 ±13.78 g respectively. From Figure 1b, the decreased rats' BW in the TAE2 group significantly differed from the NC group (p=0.032) but the other treatment groups did not significantly differ.

TAE administration reduced BMI in rats with HFD

Table 2a showed that TAE administration significantly reduced rats' BMI at the end of treatment except in the TAE1 group (26.03±5.61 g/cm^3). Before treatment, all rat groups had an average BMI lower than 30 g/cm^3. On day 28, the average BMI increased significantly in the NC (29.61 ± 2.28 g/cm^3; p=0.004) and PC (28.67±1,68 g/cm^3; p=0.029) groups but did not significantly increase in the TAE1 group (26.03±5.61 g/cm^3 p=0.607), compared to their counterparts before treatment. In contrast, the average BMI reduced significantly in the TAE2 (21.84±0.81 g/cm^3; p=0.002) and 3 (20.78±2.17 g/cm^3; p=0.006) groups. From Figure 2b, the decreased rats' BMI in the TAE groups significantly differed from the NC group (p<0.001) in the day 28 treatment. In addition, decreased rats' BMI in TAE2 and 3 groups significantly differ from the TAE1 (p=0.008 and p=0.001) and the PC groups (p<0.001).

TAE administration reduced BFC in rats with HFD

We calculated the Rats' BFC using Lee's formula [41] in order to figure out the fat distribution after TAE treatment (Figure 3a & b). In the beginning, the average of rats' BFC in the TAE2 group (125.76±18.452 g/cm) was higher than other rats' groups but it was not significantly different (p=0.345). During TAE treatment, the average of rats' BFC in the TAE2 (98.54±2.53 g/cm) and 3 groups (94.65±11.55 g/cm) decreased significantly, compared to the before treatment (p=0.004 and p=0.016 respectively). Meanwhile, the average of rats' BFC increased significantly in the NC (134.25±9.48 g/cm; p=0.002) and PC groups (128.63±5.42 g/cm; p=0.012), compared to the same groups before treatment. In addition, a slight reduction of BFC was observed in the TAE1 group (118.75±24.69 g/cm). Decreased rats' BFC in TAE2 and 3 groups significantly differed from the TAE1 (p=0.004 and p=0.001) and the PC groups (p<0.001).

TAE administration reduced RI in rats with HFD

In order to figure out fat accumulation in the inner layer of the common carotid artery, we evaluated RI among rat groups with and without TAE treatment (Figure 4). Before TAE treatment, the TAE1 and 2

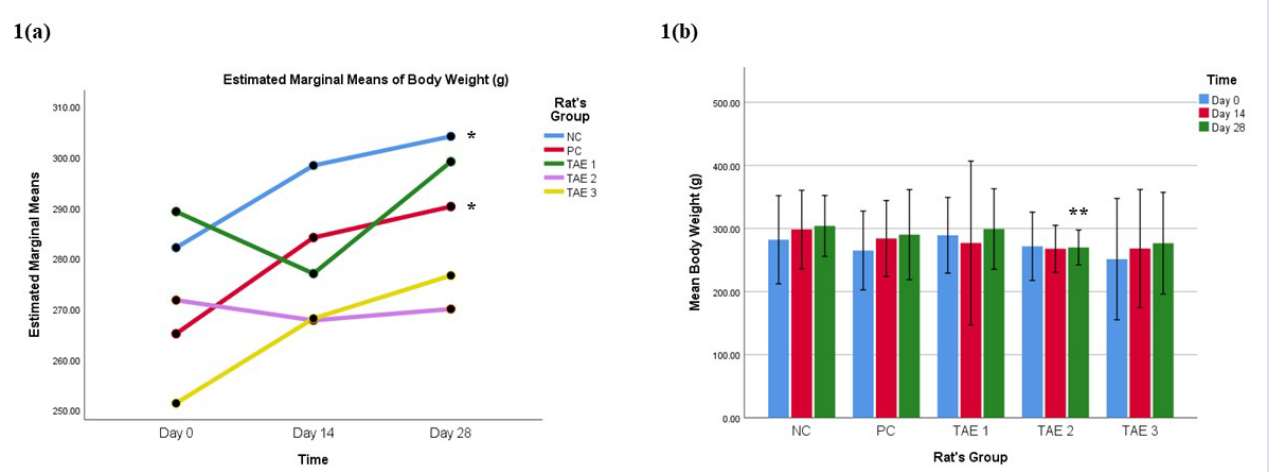

Figure 1: BW changes in 5 rats' groups with or without TAE administrations. (a) Comparative effect of TAE administration among male rats with HFD on day 0, 14, and 28 interventions. (b) Comparative effect of TAE administration within male rats' groups on BW from day 0, 14 to 28 interventions. *was designated a significant difference, compared to the NC group and **was significantly different among serial times within the group.

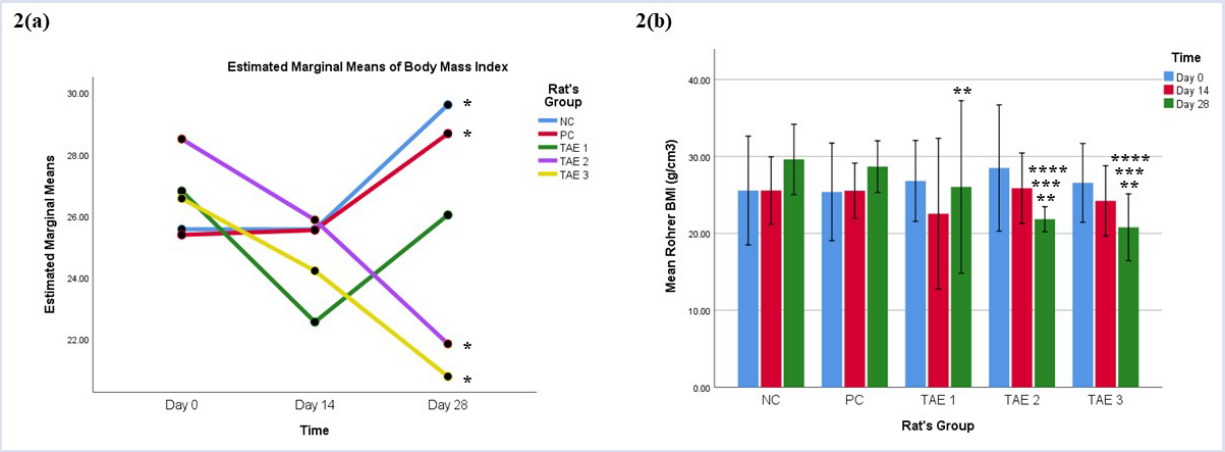

Figure 2: BMI changes in 5 rats' groups with or without TAE administrations. (a) Comparative effect of TAE administration among male rats with HFD on day 0, 14, and 28 interventions. (b) Comparative effect of TAE administration within male rats' groups on BW from day 0, 14 to 28 interventions. *was designated a significant difference, compared to the NC group. **was significantly different among serial times within the group. ***was significant difference, compared to the PC group. ****was significant difference among TAE groups.

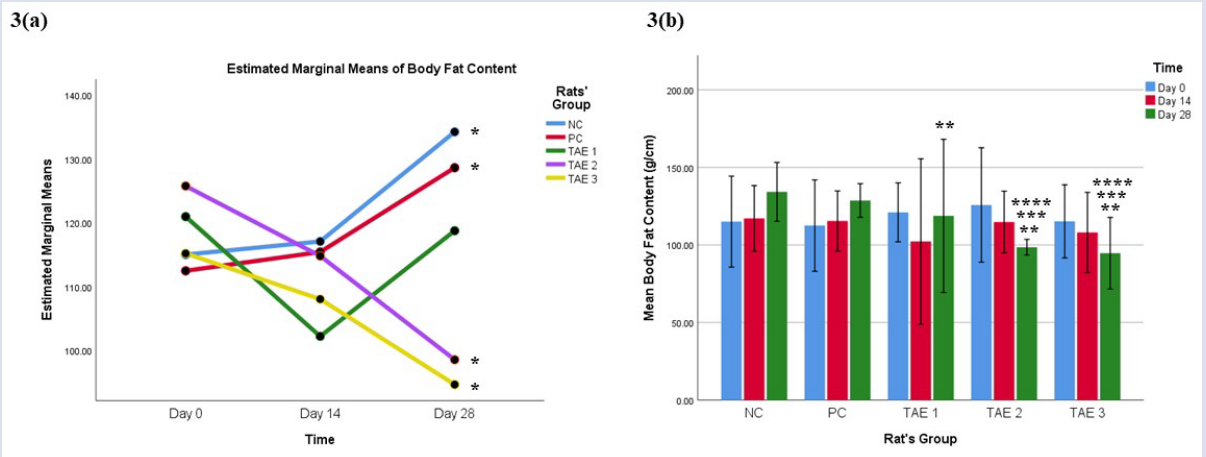

Figure 3: BFC changes in 5 rats' groups with or without TAE administrations. (a) Comparative effect of TAE administration among male rats with HFD on day 0, 14, and 28 interventions. (b) Comparative effect of TAE administration within male rats' groups on BW from day 0, 14 to 28 interventions. *was designated a significant difference, compared to the NC group. **was significantly different among serial times within the group. ***was significant difference, compared to the PC group. ****was significant difference among TAE groups.

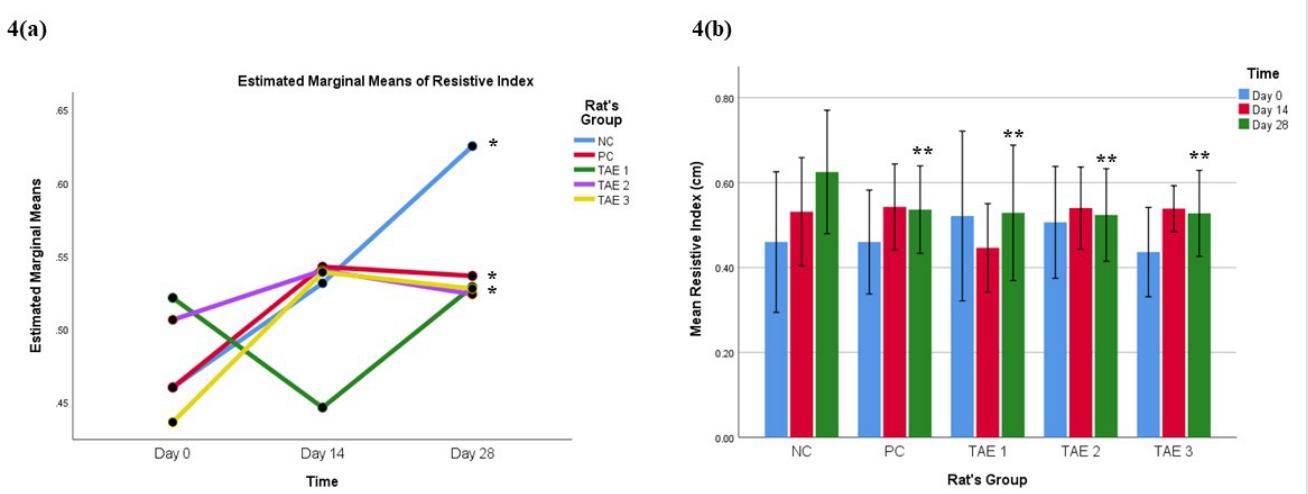

Figure 4: RI changes in 5 rats' groups with or without TAE administrations. (a) Comparative effect of TAE administration among male rats with HFD on day 0, 14, and 28 interventions. (b) Comparative effect of TAE administration within male rats' groups on BW from day 0, 14 to 28 interventions. *was designated a significant difference, compared to the NC group and **was significantly different among serial times within the group.

groups had a similar RI (0.52±0.01 and 0.51±0.07 cm) and were higher than the other groups but it was not significant (p=0.105). Figure 4a showed that the mean RI in the NC group significantly increased on the day 28 treatment (0.62±0.07 cm; p=0.009). The same pattern was also observed in the PC and TAE3 groups (0.54±0.05 cm; p=0.001 and 0.53±0.05 cm; p=0.017 respectively) while the mean RI of TAE1 and 2 groups slightly increased (0.53±0.08 cm; p=0.195 and 0.52±0.05 cm; p=0.544 respectively). At the end of the treatment (Figure 4b), the PC and TAE groups had lower mean RI than the NC group and it reached significance (p=0.008, p=0.004, p=0.003, and p=0.004). The highest reduction of mean RI was found in the TAE3 group and followed by the TAE2 group but it was not significantly different from the PC group.

DISCUSSION

Herein, we have demonstrated that the administration of 400 mg/kg BW TAE seemed to keep the average BW remained stable and significantly lower than the average BW in the NC group. In addition, administration of 400, and 800 mg/kg BW TAE significantly reduced rats' BMI, BFC, and RI of the common carotid artery, compared to the NC group. These findings suggest that TAE administration potentially becomes an alternative therapy to reduce BW for obesity management. Our study is different from a previous study conducted by Sundari *et al.* (2022) that TAE administration is used for obesity treatment instead of obesity prevention. They combined 100 mg TAE with 750 mg star fruit extract and which significantly reduced BW from 220.60±9.27 g to 150.80±4.70 g. Although TAE contains 20±0.5 µM *dictamine* and has low cytotoxicity with 245.22 g/mL IC_{50} value, the action mechanism underlying the effect of TAE administration on BW reduction remains unknown.[27] In contrast, another research study reported that administration of 200 and 400 mg/kg BW leaves extracts from *T. aculeata* significantly reduced serum levels of total cholesterol, triglyceride (TG) and low-density lipoprotein-cholesterol (LDL-C) in rats with hyperlipidemia but the researchers did not identify their active compounds and reported BW reduction.[34]

Regarding the BMI reduction, the administration of 400 and 800 mg/kg BW TAE was better than the administration of 200 mg/kg BW TAE and 2.98 µg/kg BW pure *dictamine*. These findings are in accordance with the previous study conducted by Sundari *et al.* (2022) but the BMI reduction in our study (-6.65±3.3 g/cm^3) is lower than their result study (-14.51±0.65 g/cm^3). This discrepancy is probably caused by the role of *apigenin* and *epicatechin* compounds in the leaves extract of star fruits.[27] Aladaileh *et al.* (2019) stated that star fruit leaves contain *apigenin*, a derivative of flavones, which plays an important role in the inhibition of the pancreas lipase, resulting in TG low absorption in the small intestine. In addition, this plant leaves also contain *epicatechin* (a derivative of flavanols), which inhibits hydroxyl methylglutaryl coenzyme A reductase to synthesize endogenous cholesterol in the hepatocytes, leading to the reduction of plasma LDL-C and increase of cholesterol excretion.[34] However, we did not further analyze other phytochemicals in our extract instead of *dictamine*. To our knowledge, we do not find *dictamine* activity against pancreatic lipase. Some other phytochemicals in TAE extract might also be involved in fat and cholesterol metabolisms.

Since fat storage is distributed in all kinds of cells, especially in adipose tissues, we used the BFC parameter to evaluate the effect of TAE administration. In contrast to the BMI reduction, the BFC reduction in our study (-27.215±15.92 g/cm) is higher than the result of the previous study (-13.95±0.63 g/cm).[27] In addition, the average BFC in the PC group is significantly higher than the average BFC of rats treated with 400 and 800 mg/kg BW TAE. These data indicated that other phytochemicals in TAE appear to be involved in BFC reduction. The higher effect of BFC reduction after TAE administration probably increases the TG mobilization from storage cells. However, this explanation should be further explored as a limited study has reported the *dictamine* activity and other phytochemicals content in TAE, involved in fat metabolism.

The RI may be a good biomarker of vascular diseases[36] and potentially predict the incidence of CVDs, which is correlated to the thickness of the arterial tunica intima-media.[28,36] Our results indicated that the administration of pure dictamine and TAE decreased the RI in rats with obesity. However, the decreased RI in dictamine and TAE groups is similar. These findings are not parallel to the data on BMI and BFC in our study. Altogether, it indicates that *dictamine* decreased RI through a different mechanism. Lower RI in the PC and TAE1 groups is more likely to be related to the technical issue in the RI measurement using the portable USG. Therefore, this RI parameter may not be as good as BMI and BFC parameters to evaluate the effect of TAE administration.

There are several limitations in our present study. Although the administration of 400 and 800 mg/kg BW TAE is able to reduce BW, BMI, BFC, and RI, we are unable to confirm the role of *dictamine* in fat metabolism. In this study, we used the TAE to prevent atherosclerosis formation instead of treating atherosclerosis through the reduction of

fat absorption and cholesterol biosynthesis. Therefore, it is difficult to compare our data with other data in previous studies. Furthermore, we did not analyze other phytochemical contents in the TAE, which affect the fat and cholesterol metabolisms and have important roles in the reduction of BMI, and BFC. The other limitation is the availability of portable USG for animal experiments. In our study, we tried to use the portable USG for humans but the measurement data is less accurate than the expected data.

CONCLUSION

Administration of 400 and 800 mg/kg BW TAE for 28 days reduces BW, BMI, BFC, and RI in rats with a high-fat diet. *Dictamine* is found in the ethanol extract of *T. aculeata* leaves, which might reduce RI in rats with a high-fat diet. Further studies are required to identify other phytochemicals in *T. aculeata* leaves using liquid chromatography-mass spectrometry, to measure total cholesterol, TG and LDL-C levels, and to examine the tunica intima of the common carotid artery using the *trichrome Masson staining* to identify foam cells, fat accumulation and arterial thickness in rats with a high-fat diet.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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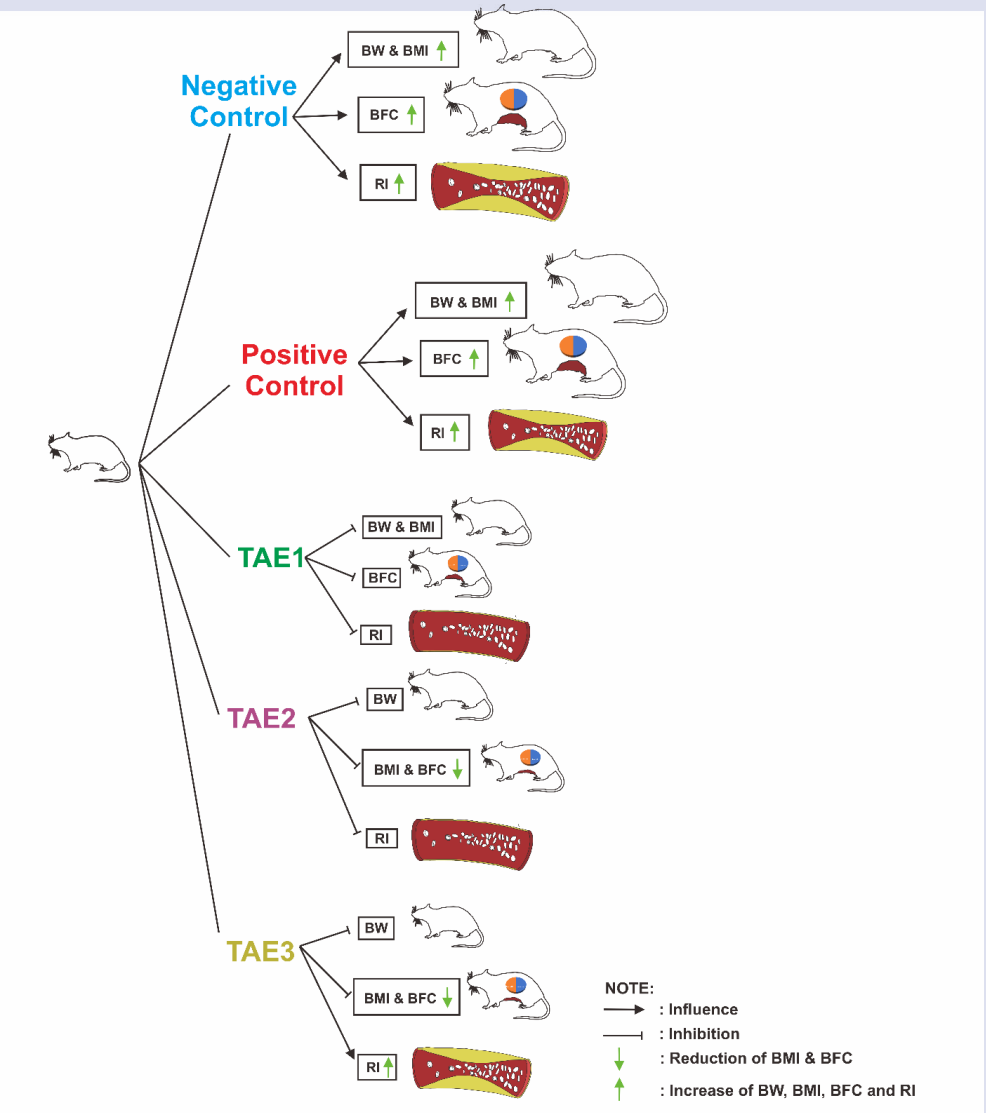
GRAPHICAL ABSTRACT



ABOUT AUTHORS

Prasetyo Sarwono Putro: Investigator, extraction of Toddalia aculeata leaves, assessment of resistive index, data curation, and writing the original draft.

Dono Indarto: Chief Investigator, conceptualization, methodology, writing, review, and editing.

Bambang Purwanto: Supervision, formal analysis, and validation.

Widiastuti Soewondo: Assessment of resistive index, supervision, methodology, and validation.

Sulistyani Kusumaningrum: Assessment of resistive index, methodology, and formal analysis.

Khim Yatul Nguzum: Investigator, data curation, formal analysis, and writing the original draft

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