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Original Research Article

Evaluation of the Combined Effect of Turmeric (*Curcuma domestica*) Rhizome and Pegagan (*Centella asiatica*) Leaf Ethanol Extracts in the Prevention of Endothelial Dysfunction in Obese Rat Model

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ABSTRACT

Obesity is recognized as a significant global health issue, and has been declared a global epidemic by the WHO. This study aimed to investigate the effects of combined turmeric rhizome and pegagan leaf extracts on endothelial dysfunction in obese rats, as well as to determine the effective dose of the combined extracts that would significantly influence this condition. Wistar rats were divided into eight groups: Normal control, induction, orlistat, curcumin, turmeric, pegagan, turmeric/pegagan (100/200 mg/kg), and turmeric/pegagan (200/100 mg/kg) groups. Animals in all the groups except the normal control group were fed with a high-fat and carbohydrate diet for 60 days to induce obesity. The rats were thereafter treated with the extracts and standards according to their respective groups for 60 days. The anti-obesity effect of the treatments was assessed by measuring body weight, fat index, and organ weight index. The ability of the treatments to prevent obesity-induced endothelial dysfunction was assessed by measuring heart rate, pulse wave velocity, and nitric oxide levels in the rats after 60 days of treatment. Results showed that high-fat and high-carbohydrates diet effectively induced obesity in rats. The administration of curcumin, turmeric rhizome and pegagan leaf extracts, and the combined extract of turmeric rhizome and pegagan leaf demonstrated anti-obesity effect, and a positive effect on endothelial dysfunction. The most effective dose of the combined extract was 200 mg/kg turmeric extract and 100 mg/kg pegagan extract. These findings revealed the potential therapeutic effect of turmeric and pegagan in the management of obesity-induced endothelial dysfunction.

Keywords: Obesity, Endothelial dysfunction, Pegagan, Turmeric.

Introduction

Endothelial dysfunction is a condition that results from the abnormal functioning of the endothelium (the layer of cells lining the blood vessels).[1–4] This condition has a substantial effect on cardiovascular health, because the endothelium regulates blood pressure, plays a role blood coagulation, and maintain vascular-tissue homeostasis.[5] Obesity is one of the established risk factors for endothelial dysfunction, a disorder that is becoming increasingly prevalent around the world.[6] Obesity is a global health issue that is closely linked to heart disease, stroke, and type 2 diabetes.[7] Obesity is also known to cause changes in the endothelium, including a decrease in the synthesis of nitric oxide (NO), a molecular signal that is vital in maintaining vascular tone.[8] As a result, developing natural therapies or supplements to combat endothelial dysfunction in obese condition is crucial. Although, several pharmaceutical therapies have been developed for the treatment of endothelial dysfunction, their use is frequently limited due to undesirable side effects and non-availability.[9]

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As a result, there is an increasing interest in the search for alternative natural remedies for the treatment of endothelial dysfunction. Natural compounds have established pharmacological potentials, such as anti-inflammatory, antioxidant, and vasodilatory properties that can aid in the repair of endothelial dysfunction.[10,11] Turmeric (*Curcuma domestica*) and pegagan (*Centella asiatica*) are two plants that have long history of use in traditional medicine due to their numerous health benefits. Turmeric contains curcumin, a polyphenol compound with demonstrated anti-inflammatory and antioxidant properties.[12,13] Pegagan on the other hand, contains triterpenoids and other substances that have beneficial effects on blood, blood vessels, and the cardiovascular system.[14,15] Previous research suggests that substances capable of reducing endothelial dysfunction can be identified using carrier molecules as part of drug modeling approach. This method predicts how drugs interact with molecular targets that regulate endothelial function.[16] Furthermore, assessing the biological activity of plant extracts might provide valuable insights into possible substances that can be used as therapy or supplements to combat endothelial dysfunction in obesity. In this context, the present study aimed to uncover effective natural inhibitors of endothelial dysfunction in obese rat model, with focus on turmeric and pegagan extracts, two plants known for their anti-inflammatory and antioxidant properties which may be beneficial in maintaining endothelial function. It is envisaged that by combining the two plant extracts, there would be greater and substantial outcome in combatting endothelial dysfunction in obesity. The findings from the study are likely to make significant contribution to the development of natural supplements for improving vascular health in obese patients.

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Materials and Methods

Collection and identification of plant materials

The rhizomes of turmeric (*Curcuma domestica* Val.) and leaves of pegagan (*Centella asiatica* (L.) Urban) were collected in February 2024 from the Spice and Medicinal Plants Research Center (BALITTRO), Bogor City, Indonesia (6°34'44"S 106°46'44"E). The plant materials were identified and authenticated at the Plant Taxonomy Laboratory, Department of Biology, FMIPA, Padjajaran University, Indonesia, with voucher numbers 61/HB/05/2024 and 62/HB/05/2024 for *Centella asiatica* and *Curcuma domestica*, respectively.

Determination of total ash content

The powdered plant samples (2 g each) were placed in clean dry porcelain crucibles. The samples were ignited in the furnace at 600ºC until they were charred. The crucibles were removed from the furnace, allowed to cool, after which the charred samples were weighed. The total ash content was calculated using the formula below (Equation 1).[20]

$$\text{Total ash } (\%) = \frac{Wb}{Wa} x\ 100 \quad \text{----------} \quad \text{(Eq.1)}$$

Where; Wa is the weight of powdered sample, and Wb is the weight of ash.

Preparation of turmeric and pegagan extracts

Fresh turmeric rhizomes and pegagan leaves were sorted, washed clean with water, thinly sliced, and dried in an oven at 40°C for 3 days. The dried samples were pulverized, and the powdered sample (1 kg each) were extracted separately by maceration in 96% ethanol (10 L) at room temperature for 72 h. The extracts obtained were then filtered and the filtrates were concentrated using a rotary evaporator (QR 2005-V, Shimadzu, Japan) at 50-60°C under reduced pressure.

Determination of water soluble extractive value

Five grams of the powdered plant sample were weighed and placed into a stoppered flask, then 100 mL of chloroform-saturated water was added. The mixture was shaken repeatedly for the first 6 hours and then left to stand for 18 hours. The extract was filtered, and 20 mL of the filtrate were evaporated to dryness in a flat-bottomed shallow dish that had been preheated to 105°C and tared. The remaining filtrate was also heated at 105°C until a constant weight was achieved. The content was calculated as a percentage of the water-soluble extract.[20]

Determination of alcohol soluble extractive value

The powdered plant sample (5 g each) was dissolved in 96% ethanol and made up to 100 mL. The mixture was shaken every 30 minutes for 6 hours, then 25 mL of the sample was pipetted into a porcelain dish, and evaporated at 78°C for 3 h. After cooling for 1 h, the extract was weighed, and the process of heating and cooling was repeated until a constant weight was achieved.[20]

Phytochemical analysis

Turmeric rhizome and pegagan leaf extracts were subjected to qualitative phytochemical screening for the presence of alkaloids, flavonoids, saponins, tannins, and steroids/triterpenoids according to standard procedures.[22]

Preparation of drug/chemicals

Preparation of positive control drugs

Orlistat and curcumin were used as the positive controls. Orlistat was suspended in a 1% sodium carboxymethyl cellulose (Na-CMC) solution. The orlistat dose used was extrapolated from human dose of 120 mg/70 kg BW to the dose for test animals weighing 200 g. Curcumin was prepared by dissolving the drug in 0.5% of sodium carboxymethyl cellulose (Na-CMC) to make a suspension of 20 mg/kg.

Preparation of high-fat and carbohydrate feed

The ingredients used for the preparation of high-fat and carbohydrate feed included; corn flour, fish flour, green bean flour, wheat flour, rice flour, vegetable oil, and beef fat (Table 1). All the ingredients were weighed, mixed, then blended into a homogeneous paste, which was then baked in an oven until evenly cooked.

Animals grouping and induction of obesity

Thirty-six healthy male Wistar rats weighing between 170 – 230 g were obtained from the animal facility of the Pharmacology Laboratory, Faculty of Pharmacy, Bhakti Kencana University, Bandung, Indonesia. The rats were kept in well-ventilated cages, and acclimatized to the laboratory conditions for one week. Obesity was induced in the rats by feeding them with a high fat and carbohydrate diet for 60 days, followed by 30% fructose. The rats were divided into eight groups of 4 animals per group and treated as shown in Table 2.

Table 1: Composition of normal and obesity-induction feed

No	Composition	Normal Feed (%)	High fat and Carbohydrates feed (%)
1	Cornstarch	251.1	204
2	Fish meal	185.5	130
3	Green bean flour	166	114
4	Wheat flour	377	172
5	Rice flour	-	189
6	Vegetable oil	20	20
7	Beef fat	-	120

Table 2: Animal grouping and treatments

Group	Treatment
Negative Control (-)	Normal feed
Positive Control (+)	Induction feed + 30% fructose
Comparator 1 (Orlistat)	Induction feed + 30% fructose + orlistat 120 mg/kg BW
Comparator 2 (Curcumin)	Induction feed + 30% fructose + curcumin 20 mg/kg BW
Test Group 1	Induction feed + 30% fructose + ethanol extract of turmeric rhizome (200 mg/kg BW)

Test Group 2	Induction feed + 30% fructose + ethanol extract of pegagan leaves (200 mg/kg BW)
Test Group 3	Induction feed + 30% fructose + ethanol extracts of turmeric rhizome (200 mg/kgBW) and pegagan leaves (100 mg/kgBW)
Test Group 4	Induction feed + 30% fructose + ethanol extracts of turmeric rhizome (100 mg/kgBW) and pegagan leaves (200 mg/kgBW)

Ethical approval
Ethical approval with reference number 706/UN6.KEP/EC/2024 was obtained from the Padjadjaran University Research Ethics Commission.

Evaluation of anti-obesity activity
The anti-obesity activity of the turmeric and pegagan extracts and their combination was evaluated by measuring various anthropometric parameters following extracts treatments. The parameters measured were body weight, fat index, and organ weight index.

Measurement of body weight
The body weight of the mice was measured every day for 60 days using a digital scale in grams. The percentage change in body weight was calculated using the formula below (Equation 2).[23]

$$\% \text{ change in body weight} = \frac{W final - W initial}{W initial} \times 100 \quad \text{(Eq. 2)}$$

Measurement of fat index
On the 61st day, the rats were sacrificed, the abdominal cavity was surgically opened, and the subcutaneous fat was removed and weighed. The fat index was calculated using the formula below (Equation 3).[24]

$$\text{Fat index (\%)} = \frac{weight\ of\ fat}{Body\ weight} \times 100 \quad \text{(Eq. 3)}$$

Measurement of organ weight index
Following the 60 days treatment period, all the rats were sacrificed on the 61st day, and vital organs, including the heart, aorta, lungs, liver, kidneys, spleen, and testes were surgically removed. The organs were weighed, and the organ weight index was calculated using the following formula (Equation 4).[25]

$$\text{Organ weight index} = \frac{Organ\ weight}{Body\ weight} \times 100 \quad \text{(Eq. 4)}$$

Evaluation of endothelial dysfunction
Electrocardiography (Heart rate and Pulse wave velocity measurement)
After the treatment period, the rats were placed in an ECG chamber connected to a laptop with a Serial Plot application. The rats were anesthetized using carbon dioxide (CO_2) gas at high pressure for 30 seconds. After applying the ECG gel, the electrodes were placed on the rats' four limbs, and the tail was placed on the photoplethysmography (PPG) sensor. The ECG and PPG signals were viewed using the Serial Plot application. The pulse arrival time (PAT) was determined from the ECG and PPG waveforms. PAT is the duration of time from the first reference time point (R wave) to the second reference time point (PPG foot). A short duration is required between the time of ventricular contraction and ejection of blood from the heart valves. This short duration is called the pre-ejection period (PEP). Impedance cardiography (ICG) can be used to measure the time of blood ejection from the heart (B-Point). The PEP value in rats is about 15 ms and is relatively unchanged with changes in blood vessel stiffness.[26,27]

Determination of nitric oxide level
Preparation of Griess reagent
Solution I was prepared by dissolving 0.5 g sulfanilic acid in 150 mL of 30% v/v acetic acid. Solution II was prepared by heating 0.1 gram of naphthylethylenediamine in 20 mL of distilled water until dissolved, then poured while still hot into 150 mL of glacial acetic acid. Solutions I and II were mixed in a 1:1 ratio and stored in an amber coloured bottle.[28]

Preparation of sodium nitrite calibration curve
Sodium nitrite ($NaNO_2$) (100 mg) was dissolved in distilled water and made up to 100 mL to obtain a concentration of 1000 ppm. From the 1000 ppm solution, 10 mL was taken and diluted to 100 mL with distilled water to make a concentration of 100 ppm. From the 100 ppm solution, 1 mL was taken and diluted to 10 mL with distilled water to make a concentration of 10 ppm. Further series of concentrations were prepared from the 10 ppm solution by pipetting 1.0, 1.4, 1.8, 2.2, 2.6, and 3.0 mL from this solution, and diluting to 10 mL with distilled water to obtain 1.0, 1.4, 1.8, 2.2, 2.6, and 3.0 ppm solutions, respectively. Exactly 2 mL of Griess reagent was added into each of the solutions and incubated at room temperature for 30 minutes, after which an absorbance spectrum was read from 400 to 800 nm. The wavelength that gave maximum absorbance (λmax) was recorded.

Collection of blood samples
After the treatment period, the rats were anesthetized with CO_2 gas, blood samples (2 mL) were collected from the retro-orbital vein with the aid of a hematocrit capillary tube. The collected blood was placed in an Eppendorf tube and stored in the refrigerator until used for the analysis.

Measurement of nitric oxide level
The blood sample was centrifuged at 10,000 rpm for 10 minutes to obtain serum. The serum sample (400 µL) was deproteinated with 80 µL of 6% zinc sulphate ($ZnSO_4$) followed by centrifugation at 4000 rpm for 30 min. The supernatant was reacted with 80 µL of 6% cadmium for 15 minutes. A total of 380 µL of this solution was added to 1.5 mL of Griess reagent, then incubated at room temperature for 1 hour. The absorbance of the resulting solution was measured at the predetermined λmax of 535 nm using a UV-Vis spectrophotometry (UV-1280, Shimadzu, Japan).[29]

Histological examination of the aorta
The hearts of the rats which were previously harvested following hyperbaric CO_2 anaesthesia, were cleaned with normal saline (0.9% NaCl). Thereafter, the aorta was removed, and fixed with 10% neutral buffered formalin for 24 h, after which they were dehydrated with graded concentrations of ethanol (80%, 95%, and 100% for 3 h each), cleaned, and then embedded with paraffin in a tissue processor. The paraffin-embedded aortic tissues were cut into 3 - 4 µm sections using a microtome. The tissue sections were deparaffinized with xylene, followed by rehydration with alcohol, and then stained with hematoxylin-eosin (HE). The stained tissue sections were mounted on a microscope slide, and then examined under a microscope (XSZ-107BN, Microscope Binocular 107BN, China) at 100x magnification.[30]

Statistical analysis
Numerical data were presented as mean ± standard deviation (SD). The data were subjected to one way analysis of variance (ANOVA). Statistically significant differences between mean values were established at P-value < 0.05.

Results and Discussion

Phytochemical constituents of turmeric and pegagan extracts
The phytochemical constituents of turmeric rhizome and pegagan leaf ethanol extracts are presented in Table 3. Turmeric rhizomes tested

positive for alkaloids, flavonoids, tannins and steroids, while saponins were absent. These results are in agreement with the findings from the work of Arista *et al*. (2020) where turmeric rhizome extract was shown to contain alkaloids, flavonoids, tannins and steroids.[31] On the other hand, pegagan leaf extract showed the presence of flavonoids, tannins, saponins, and steroids, while alkaloids were absent.

Table 3: Phytochemical constituents of turmeric rhizome and pegagan leaves

Phytochemical	Inference	
	Turmeric	Pegagan
Alkaloid	+	-
Flavonoid	+	+
Tannin	+	+
Saponins	-	+
Steroid	+	+

Key: (+): Detected, (-): Not detected

Extraction yields

Table 4 shows the yields for turmeric rhizome and pegagan leaf extracts. The results indicate an efficient extraction with ethanol for both plants samples, producing percentage yields of 30.73% and 20.47% for turmeric rhizome and pegagan leaf extracts, respectively.

Table 4: Extraction yield

Extract	Extract Weight (g)	Sample Weight (g)	Yield (%)
Turmeric rhizome	461.0	1500	30.73
Pegagan leaves	307.1	1500	20.47

Proximate parameters of turmeric rhizomes and pegagan leaves

Quantitative proximate analysis was done with the aim of ensuring that the crude plant materials meet the required quality standards. The parameters investigated were total ash content, acid insoluble ash content, ethanol and water-soluble extractive values. The results as presented in Table 5 showed that the total ash content, ethanol-soluble extractive value, and water-soluble extractive value for turmeric rhizomes were 6.6%, 22.3%, and 15.2%, respectively. For pegagan leaves, the values were 9.0%, 27.0%, and 23.5% for total ash content, ethanol-soluble, and water-soluble extractive values, respectively (Table 6). The values obtained for both plants materials met the FHI standard.

Table 5: Proximate parameters of turmeric extract

Parameter	Value (%)	FHI Standard (%)
Total ash content	6.6	≤ 8.3
Ethanol soluble extract	22.3	≥ 11.4
Water soluble extract	15.2	≥ 11.5

Table 6: Proximate parameters of pegagan extract

Parameter	Value (%)	FHI Standard (%)
Total ash content	9.0	≤ 11.6
Ethanol soluble extract	27.0	≥ 4.4
Water soluble extract	23.5	≥ 15.4

Effect of turmeric rhizome and pegagan leaf extracts on body weight of obese rats

Obesity in experimental animals can be induced by high fat diet, and confirmed by measurement of body weight. Obesity is indicated by an increase in body weight up to 20% and above. The purpose of body weight measurement was to assess whether the test extracts have the ability to reduce or maintain the body weight of mice after induction of obesity. The rats' body weights were measured every 10 days.

Administration of high-fat and carbohydrate feed resulted in 35.39% increase in body weight of the rats, whereas rats in the normal control group experienced an increase in body weight of 16.26% (Table 7). This observation indicated that the administration of high-fat and carbohydrate feed successfully induced obesity in the rats. The results of this study are in line with a previous study where the administration of high-fat and carbohydrate feed was shown to lead to more than 20% increase in body weight and cause fat accumulation in the skin.[32]

It was observed that the administration of turmeric rhizome, and pegagan leaf extracts, curcumin, as well as the combination of turmeric and pegagan extracts significantly decreased body weight compared to the body weights of rats in the induction group, which means that turmeric rhizome extract, pegagan leaf extract, and curcumin are thought to be able to maintain body weight and have anti-obesity effect. The active compound, curcumin contained in turmeric has been shown to reduce body weight and the percentage of body fat in overweight and obese individuals,[33] and induced inhibition of adipogenesis and lipogenesis or activation of lipolysis, this indicates that turmeric can prevent obesity and excess fat accumulation through the action of curcumin.[34]

Effect of turmeric rhizome and pegagan leaf extracts on fat and organ weight indices

The turmeric rhizome ethanol extract significantly reduced the fat index in obese rats compared with the induction group (Table 8). However, there were no significant differences in the organ weight index across all groups, indicating that the administration of high-fat and carbohydrate feed did not affect organ weight indices (Table 9). High-fat diet induction may not necessarily translate to observable changes in organ weight. For instance, an agent might improve endothelial integrity or reduce oxidative stress without causing direct organ weight alteration. It is possible that the administered doses of the controls (Orlistat and Curcumin) as well as the extracts (turmeric rhizome, pegagan leaf, and their combination), and the duration of treatment were effective in addressing endothelial dysfunction but were not sufficient to induce significant changes in macroscopic organ structure or overall fat reduction. Different doses or a longer treatment period might be required to cause such macroscopic changes.

Table 7: Average weight of rats before and after treatment with turmeric and pegagan extracts

Average body weight (g)			
Group	D0	D60	Change in body weight (%)
Normal	194.25 ± 4.35	225.75 ± 1.89*	16.26 ± 2.73* $^{\beta}$
Induction	220.50 ± 22.78	296.75 ± 24.15 $^{\#}$	35.39 ± 14.34 $^{\#\beta}$
Orlistat 120 mg	200.75 ± 1.50	167.25 ± 12.34 $^{\#}$*	-16.69 ± 6.07 $^{\#}$*
Curcumin 20 mg	176.50 ± 6.61	161.50 ± 4.43 $^{\#}$*	-8.36 ± 5.25 $^{\#}$*
ERK 200 mg	195.25 ± 19.19	178.25 ± 18.84*	-8.62 ± 5.35 $^{\#}$*
EDP 200 mg	192.75 ± 16.64	186.00 ± 13.86 $^{\#}$*	-3.12 ± 8.70 $^{\#}$* $^{\beta}$

ERKDP 100 mg:200 mg	211.25 ± 9.91	194.25 ± 21.20*	-8.24 ± 5.72 #*
ERKDP 200 mg:100 mg	206.50 ± 11.36	180.00 ± 6.27 #*	-12.61 ± 6.23 #*

Values represent mean ± Standard deviation (SD). #: Significantly different when compared with the normal group (P < 0.05), *: Significantly different when compared with the induction group (P < 0.05), β: Significantly different when compared with the orlistat group (P < 0.05). D0: Day 0 before induction, D60: Day 60 after induction and treatment, ERK: Turmeric Rhizome Extract, EDP: Pegagan leaf extract, ERKDP: Turmeric rhizome and Pegagan leaf extract.

Table 8: Effect of turmeric and pegagan extracts on fat index

Group	Fat index					
	Lung	Liver	Heart	Kidney	Spleen	Testis
Normal	0.76 ± 0.16	0.76 ± 0.04	1.28 ± 0.19	4.05 ± 0.41	0.37 ± 0.07	0.37 ± 0.06
Induction	0.56 ± 0.11	0.77 ± 0.06	1.16 ± 0.19	3.73 ± 0.17#β	0.33 ± 0.02	0.36 ± 0.07β
Orlistat 120 mg	1.02 ± 0.23	0.93 ± 0.14*	1.37 ± 0.23	4.36 ± 0.50*	0.43 ± 0.07	0.41 ± 0.13*
Curcumin 20 mg	1.77 ± 0.71	0.91 ± 0.54*	0.73 ± 0.15	1.51 ± 0.19*	1.03 ± 0.49	2.46 ± 0.22
ERK 200 mg	0.82 ± 0.11	0.79 ± 0.05*	1.47 ± 0.12	4.18 ± 0.51*	0.40 ± 0.04	0.34 ± 0.08
EDP 200 mg	0.46 ± 0.50	0.42 ± 0.52	0.80± 0.95	2.35 ± 2.59*	0.22 ± 0.26	0.21 ± 0.19
ERKDP 200 mg:100 mg	0.79 ± 0.21	0.79± 0.12*	1.36 ± 0.19	4.16 ± 0.89*	0.39 ± 0.12	0.49 ± 0.11
ERKDP 100 mg:200 mg	1.88 ± 0.29	0.83 ± 0.05*	1.37 ± 0.12	4.28 ± 0.47*	0.47 ± 0.13	0.51 ± 0.14

Values represent mean ± Standard deviation (SD). #: Significantly different when compared with the normal group (P < 0.05), *: Significantly different when compared with the induction group (P < 0.05), β: Significantly different when compared with the orlistat group.
ERK: Turmeric rhizome extract, EDP: Pegagan leaf extract, ERKDP: Turmeric rhizome and Pegagan leaf extract.

Table 9: Effect of turmeric and pegagan extracts on organ weight index

Group	Organ weight index					
	Lung	Liver	Heart	Kidney	Spleen	Testes
Normal	1.67 ± 0.34	8.87 ± 0.81	0.81 ± 0.15	1.66 ± 0.07	0.81 ± 0.12	2,81 ± 0.38
Induction	1.64 ± 0.24	10.97 ± 0.84	0.98 ± 0.05	2.28 ± 0.34	1.06 ± 0.25	3.40 ± 0.45
Orlistat 120 mg	1.69 ± 0.30	7.27 ± 0.75	0.72 ± 0.09	1.55 ± 0.13	0.68 ± 0.22	2.29 ± 0.42
Curcumin 20 mg	1.77 ± 0.71	7.91 ± 0.54	0.73 ± 0.15	1.51 ± 0.19	1.03 ± 0.49	2.46 ± 0.22
ERK 200 mg	1.55 ± 0.39	7.45 ± 1.09	0.71 ± 0.04	1.45 ± 0.17	1.71 ± 0.31	2.37 ± 0.21
EDP 200 mg	1.67 ± 0.34	1.67 ± 0.34	1.67 ± 0.34	1.67 ± 0.34	1.67 ± 0.34	0.81 ± 0.12
ERKDP 200 mg:100 mg	1.53 ± 0.43	8.06± 0.43	0.75 ± 0.18	1.63 ± 0.17	0.95 ± 0.29	2.63 ± 0.25
ERKDP 100 mg:200 mg	1.57 ± 0.50	7.70 ± 0.63	0.85 ± 0.24	1.50 ± 0.09	0.92 ± 0.26	2.47 ± 0.29

Values represent mean ± Standard deviation (SD). ERK: Turmeric rhizome extract, EDP: Pegagan leaf extract, ERKDP: Turmeric rhizome and Pegagan leaf extract

Effect of turmeric rhizome and pegagan leaf extracts on electrocardiography parameters

Effect on heart rate

Heart rate is the number of heartbeats per unit of time, usually expressed in beats per minute (bpm) which is based on the number of ventricular contractions. Table 10 shows that the induction group has a mean heart rate of 238.46 bpm, which was significantly ($p < 0.05$) higher than that recorded in all the other groups. The high heart rate in the induction group is an indication that obesity causes fat accumulation in the arteries, leading to narrowing of the blood vessels, restriction of blood flow, and forces the heart to work harder, so that the heart rate increases.[35] It is worthy to note that the administration of turmeric rhizome extract, pegagan leaf extract, curcumin, and the combination of turmeric and pegagan extracts, all resulted in a significant ($P < 0.05$) reduction in heart rate compared to the induction group. It has been shown that pegagan leaves contain asiaticosides (triterpenoid glycosides) which play an important role in the healing of various diseases.[36] Meanwhile, turmeric contain many compounds that function as antioxidants, and regulate potassium, calcium and magnesium levels. Potassium is needed by the body to control heart rate and blood pressure.[27] Therefore, the combination of pegagan and turmeric has antihypertensive potential with a possible mechanism of increasing the elasticity of arterial vessels.[27]

Effect on pulse wave velocity (PWV)

It is known that arterial stiffness is caused by decreased endothelial function.[37] Previous studies have shown that the PWV approach can be used to estimate blood vessel stiffness in rats, and values have been found to range from 300 to 500 cm/s.[27]

As shown in Table 11, the induction group had a significantly higher PWV value (441.51 cm/s) compared to all the other groups ($p < 0.05$). This suggests that increase fat accumulation in obesity can lead to arterial stiffness, and stiff arterial walls have a negative impact on overall cardiovascular health.[27]

It is important to state that the administration of curcumin, turmeric rhizome and pegagan leaf extracts, as well as a combination of both extracts reversed the increased PWV caused by high-fat diet-induced obesity to near normal levels, with values of 308.93, 304.56, 298.18, 308.38, and 308.22 cm/s for curcumin, turmeric, pegagan, turmeric/pegagan (100/200), and turmeric/pegagan (200/100), respectively. Curcumin a major component of turmeric has been shown to increase vasodilation and reduce arterial stiffness,[37] while the flavonoid compounds contained in pegagan leaves has been shown to reduce blood pressure by inhibiting angiotensin converting enzyme (ACE).[38]

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Table 10: Average heart rate of rats after 60 days of treatment

Group	Heart rate (bpm)
Normal	184.73 ± 3.34 *
Induction	238.46 ± 8.92#βα
Orlistat 120 mg	185.45 ± 4.51*
Curcumin 20 mg	177.75 ± 4*
ERK 200 mg	175.96 ± 7.95*
EDP 200 mg	172.48 ± 4.71*
ERKDP 100 mg : 200 mg	183.90 ± 5.30*
ERKDP 200 mg : 100 mg	191.06 ± 3.92*

Values represent mean ± Standard deviation (SD). #: Significantly different when compared with the normal group ($P < 0.05$), *: Significantly different when compared with the induction group ($P < 0.05$), β: Significantly different when compared with the orlistat group, α: Significantly different when compared with the curcumin group ($P < 0.05$). ERK: Turmeric rhizome extract, EDP: Pegagan leaf extract, ERKDP: Turmeric rhizome and Pegagan leaf extract.

Table 11: Effect of turmeric and pegagan extracts on pulse wave velocity

Group	PWV (cm/s)
Normal	291.37 ± 4.29*
Induction	441.51 ± 20.52 #βα
Orlistat 120 mg	310.02 ± 9.19*
Curcumin 20 mg	308.93 ± 18.18*
ERK 200 mg	304.56 ± 10.19*
EDP 200 mg	298.18 ± 5.39*
ERKDP 100 mg : 200 mg	308.38 ± 5.84*
ERKDP 200 mg : 100 mg	308.22 ± 3.57*

Values represent mean ± Standard deviation (SD). #: Significantly different when compared with the normal group ($P < 0.05$), *: Significantly different when compared with the induction group ($P < 0.05$), β: Significantly different when compared with the orlistat group, α: Significantly different when compared with the curcumin group ($P < 0.05$). ERK: Turmeric rhizome extract, EDP: Pegagan leaf extract, ERKDP: Turmeric rhizome and Pegagan leaf extract.

Effect of turmeric rhizome and pegagan leaf extracts on nitric oxide (NO) level

Nitric oxide (NO) is a heterodiatomic free radical formed from the oxidation of L-arginine to L-citrulline due to the action of Nitric Oxide Synthase (NOS). NO plays an important role in the regulation of vascular tone, inhibition of platelet aggregation, and control of cytokine adhesion to the walls of blood vessels.[39]

From the results of the present study, high-fat and carbohydrate diet-induced obesity resulted in a decreased NO level from 65.25 µmol/L in the normal group to 14.38 µmol/L in the induction group (Table 12). This indicates that obesity contribute to endothelial dysfunction thereby inhibiting the production of NOS. Reduced expression of NOS causes decreased NO bioavailability causing blood vessels to become stiff and narrow leading to an increase in blood pressure.[40–42]

The results of this study have shown that curcumin, turmeric rhizome extract, pegagan leaf extract, and their combination normalized NO level in obese rats, with the highest level observed in the pegagan group with a value of 48.95 µmol/L, although this was still lower than that obtained in the orlistat group (73.51 µmol/L). Turmeric has been shown to reduce reactive oxygen species (ROS) production in endothelial cells, increase eNOS activation, and increase NO production. Pegagan on the other hand, due to its high asiatic acid content has been shown to contribute to the normalization of serum NO level and blood pressure due to increased eNOS protein expression, and improved vasodilation. In general, the beneficial effects of pegagan (*C. asiatica*) on endothelial function can be attributed to increased NO bioavailability, decreased cardiac ACE activity, and improved oxidative stress status. Asiatic acid a major component of pegagan has been shown to prevent cardiac and aortic remodeling including left ventricular hypertrophy, myocardial fibrosis, and aortic wall thickening in chronic NO deficiency.[43,44]

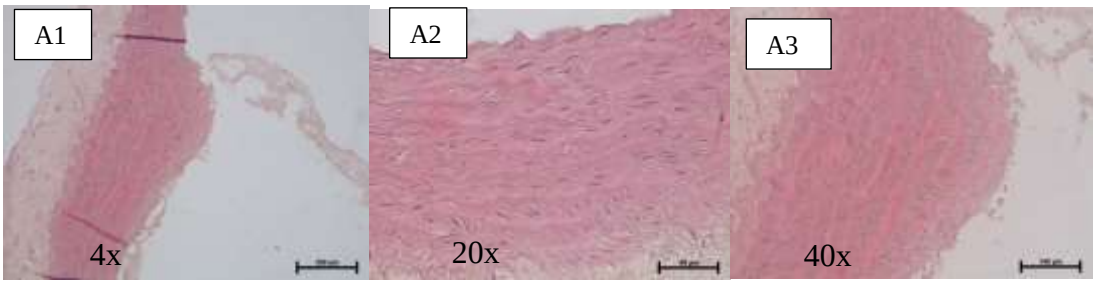
Table 12: Nitric Oxide levels in mice after 60 days of treatment

Group	Nitric oxide concentration (µmol/L)
Normal	65.25 ± 2.42 *
Induction	14.38 ± 1.58 #βα
Orlistat 120 mg	73.51 ± 8.52*
Curcumin 20 mg	44.40 ± 6.45*
ERK 200 mg	26.21 ± 6.45*
EDP 200 mg	48.95 ± 7.60*
ERK 100 mg : 200 mg	33.94 ± 3.61*
ERKDP 200 mg : 100 mg	37.57 ± 7.00*

Values represent mean ± Standard deviation (SD). #: Significantly different when compared with the normal group ($P < 0.05$), *: Significantly different when compared with the induction group ($P < 0.05$), β: Significantly different when compared with the orlistat group, α: Significantly different when compared with the curcumin group ($P < 0.05$). ERK: Turmeric rhizome extract, EDP: Pegagan leaf extract, ERKDP: Turmeric rhizome and Pegagan leaf extract.

Histological findings of the rats' aorta

In the normal group, the aortic lumen diameter and wall thickness were within physiological ranges, and only a minimal presence of foam cells was observed, indicating a healthy vascular architecture. The induction group showed a markedly enlarged aortic lumen and a thinner arterial wall, accompanied by an increased number of foam cells (Figure 1).



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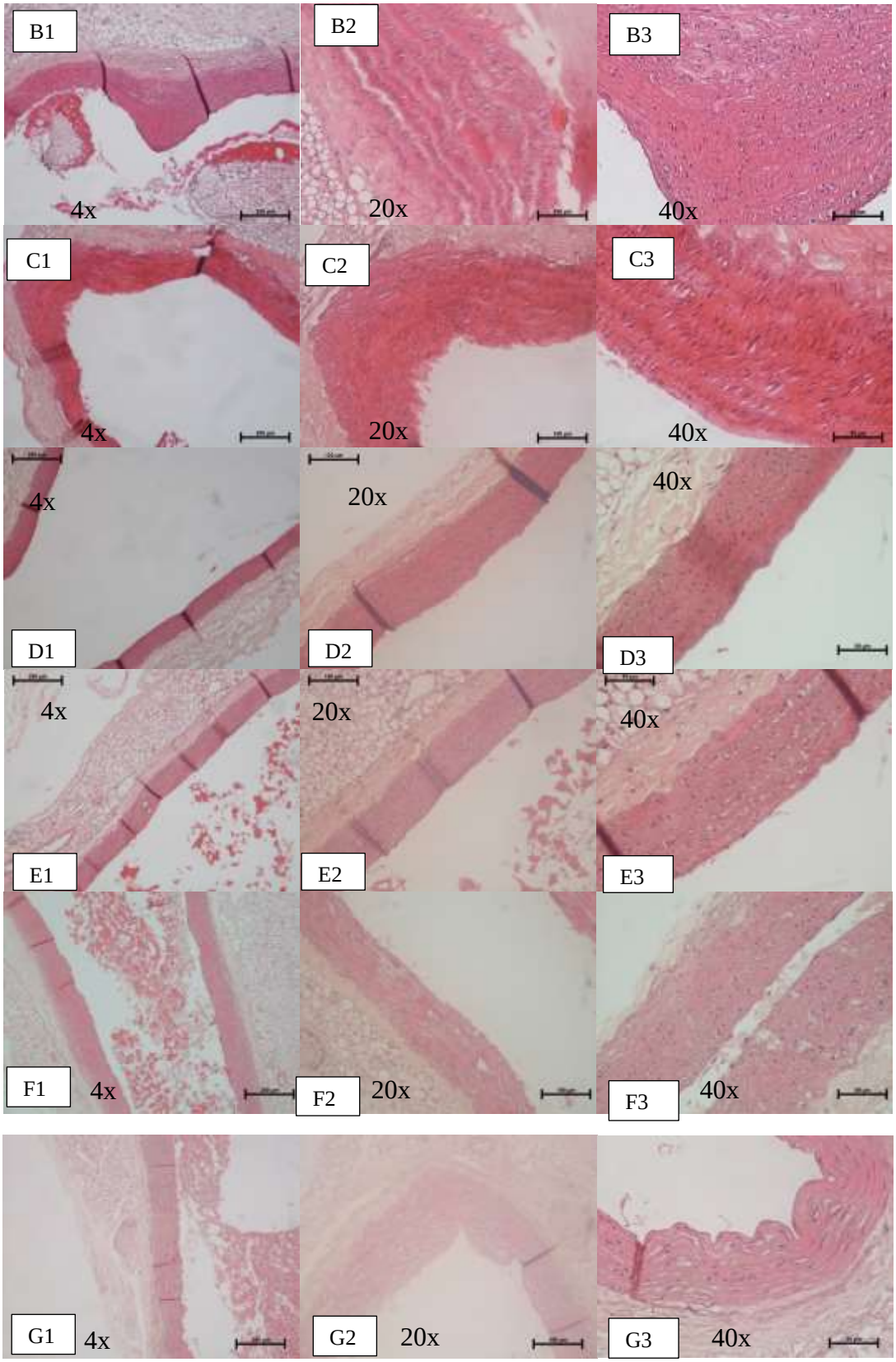

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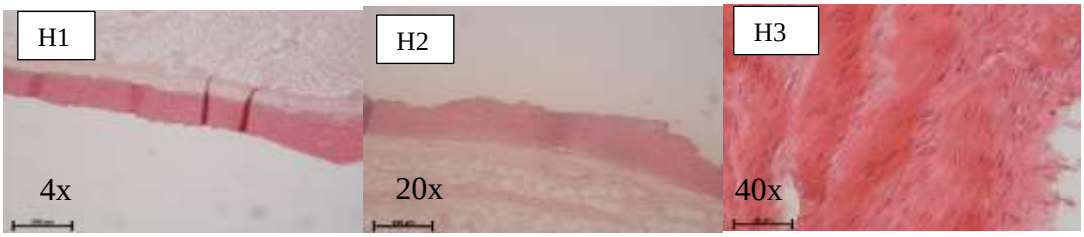

Figure 1: Photomicrograph of rat aorta (A) Normal group: lumen diameter 1,305,086 µm; Wall thickness 114,264 µm; foam cells present +; (B) Induction group: lumen diameter 1,679,978 µm; Wall thickness 82,847 µm; foam cells present ++; (C) Orlistat group: lumen diameter 1,267,437 µm; Wall thickness 75,993 µm; foam cells present ++; (D) Curcumin group: lumen diameter 1,108,834 µm; Wall thickness 116,726 µm; foam cells present +, (E) ERK group: lumen diameter 1,226,938 µm; Wall thickness 92,94 µm; foam cells present +++; (F) EDP group: lumen diameter 892,938 µm; Wall thickness 90,028 µm; foam cells present +; (G) ERKDP 200 mg:100 mg group: lumen diameter 1,240,544 µm; Wall thickness 93,235 µm; foam cells present ++; (H) ERKDP 100 mg:200 mg: lumen diameter 1,713,527 µm; Wall thickness 77,744 µm; foam cells present ++.
ERK: Turmeric rhizome extract, EDP: Pegagan leaf extract, ERKDP: Turmeric rhizome and Pegagan leaf extract.

These changes suggest endothelial dysfunction and early atherogenic remodelling as a consequence of the obese phenotype. Orlistat treatment partially normalized the aortic lumen diameter but did not substantially restore wall thickness. The persistence of foam cells indicates that while lipid absorption inhibition occurred, anti-inflammatory effects were limited. Curcumin treatment led to a reduced lumen diameter and increased wall thickness, together with a low presence of foam cells. These findings reflect its potent anti-inflammatory and antioxidant activities that protect the endothelium. The turmeric rhizome extract (ERK)-treated group exhibited moderate lumen size but a relatively thin wall and the highest foam-cells among all groups, suggesting that turmeric rhizome extract alone may not sufficiently counteract lipid deposition. On the other hand, pegagan leaf extract (EDP) produced the smallest lumen diameter and a low number of foam cells, indicating effective suppression of atherogenic expansion, although wall thickness remained slightly reduced. The combination of turmeric rhizome extract and pegagan leaf extract (100 mg:200 mg) formulation resulted in the largest lumen diameter and thin wall, with a high foam-cell count, suggesting a suboptimal synergistic effect and possible antagonism at this ratio. The synergy between turmeric (*Curcuma longa*) and pegagan (*Centella asiatica*) likely mediates endothelial protection through NF-κB inhibition, reduction of oxidative stress, and suppression of TGF-*β*-driven fibrosis. Varying ERK/EDP ratios may modify these pathways, emphasizing the importance of optimal dosing.

Conclusion

The administration of a combination of turmeric (*Curcuma domestica*) rhizome extract and pegagan (*Centella asiatica* (L.) Urban) leaf extract has been shown to influence endothelial dysfunction in an obese rat model, suggesting their potential therapeutic effect. The effective dose for this combination is 200 mg/kg BW of turmeric rhizome extract and 100 mg/kg BW of pegagan leaf extract. However, further research is necessary to elucidate the precise mechanism of action by which turmeric and pegagan exert their effects on endothelial dysfunction.

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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