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Oral Administration of Ethanol Extract of Moringa Leaves (*Moringa oleifera*) Reduces F2-Isoprostane and Monocyte Chemoattractant Protein (MCP-1) Levels in Wistar Rats (*Rattus norvegicus*) as an Obesity Model

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ABSTRACT

Background: Obesity is a chronic condition causes oxidative stress and low-grade chronic inflammation characterized by increased oxidative stress biomarkers F2-Isoprostane and proinflammatory biomarkers Monocyte Chemoattractant Protein (MCP-1). Oxidative stress and inflammation accelerate aging process. Moringa leaves (*Moringa oleifera*) are known as one of the antioxidant-rich plants containing polyphenols, flavonoids, saponin and tannin which have strong antioxidant and anti-inflammatory effects. This study was conducted to prove oral administration of ethanol extract of Moringa leaves in reducing F2-Isoprostane and MCP-1 levels in Wistar rats (*Rattus norvegicus*) as an obesity model.

Methods: This study was a pure experimental study using a Randomized Pretest-Posttest Control Group Design. Thirty Wistar rats, male, aged 3.5-4.5 months used in this study. Twenty rats were induced obesity with high-fat-diet until reached Lee's Index ≥ 0.30 g/cm, then 30 rats were divided into three groups, each consisting of 10 rats, P0 group was given normal diet, P1 group was given high-fat-diet and aquadest 1cc/day/rat per sonde and P2 group was given high-fat-diet and moringa leaf extract doses 300mg/kgBW/rat/day per sonde. Obesity induction was carried out for 32 days and treatment was given for 32 days. F2-Isoprostane and MCP-1 levels were measured before and after treatment.

Results: Serum F2-Isoprostane and MCP-1 levels showed a significant decrease in obese rats fed with high-fat-diet and moringa leaf extract for 32 days ($p<0.001$). Moringa leaf extract also prevent weight gain in obese rats.

Conclusion: Moringa leaf inhibit oxidative stress and inflammatory process related to obesity and comorbidities thus expected to inhibit the aging process.

Keywords: moringa leaf; F2-Isoprostane; MCP-1; obesity; aging

INTRODUCTION

Obesity is a world health problem that can occur in children, adolescents and adults. The number continues to increase every year and based on a national survey conducted in Indonesia, the prevalence of obesity was 23.1% and central obesity was 28%.[1] Obesity causes oxidative stress and inflammation which accelerate the aging process. In aging, inflammation occurs which is known as "inflammaging" and any disruption of metabolic pathways can cause inflammation is called "metaflammation".[2,3] Obesity is a chronic condition that causes low-level chronic inflammatory process associated with elevated

inflammatory biomarkers MCP-1. Monocyte Chemoattractant Protein (MCP-1) is the main chemoattractant for monocytes, T lymphocytes and basophils which plays an important role in the recruitment of leukocytes from the circulating blood to injured tissues. Therefore, MCP-1 is considered as one of the main markers involved in the pathogenesis of several conditions associated with monocyte cell infiltration.[4]

Obesity also causes oxidative stress resulting in an increase in Reactive Oxygen Species (ROS), oxidative phosphorylation, and disruption of fat metabolism which will induce an increase in biomarker of oxidative stress F2-Isoprostane. F2-Isoprostane is a specific product of lipid peroxidation that is used as a gold standard biomarker of oxidative stress because it has the advantage of being chemically stable, not affected by lipid content in food, and is formed in vivo.[5,6] In addition, the biomarkers F2-Isoprostane and MCP-1 also have the advantage that an increase in these biomarkers are associated with an increased risk of atherogenesis where the process of atherogenesis is closely related to obesity.[4,5,7,8]

Pharmacological therapy for obesity is still limited considering many side effects it causes. A number of studies on medicinal plant extracts have been carried out to help improve the condition of obesity, diseases related obesity, and prevent the onset of chronic and degenerative diseases due to obesity, one of which is by using the extract of the Moringa oleifera plant or known in Indonesian as "Kelor".[9,10] Moringa oleifera (MO) is one of the most useful plants in the world because almost every part of the plant can be used for food, medicine and industrial purposes. Its availability in Indonesia is quite abundant, easy to cultivate in a tropical climate, rich in nutrients and phytochemicals, and affordable. The part of the plant that is often used is the part of the leaf which can be consumed as a vegetable or as an extract.

In addition to nutrition, Moringa leaves contain phytochemicals such as polyphenolic compounds, flavonoids, glycosides, saponins and tannins which have benefits for health.[11] The content of flavonoids (quercetin) and isothiocyanates in Moringa is known to have strong antioxidant and anti-inflammatory effects, anti-diabetic, hypocholesterolemic, and anti-obesity.[12] Several studies have been conducted to prove this effect. Research by Oathman et al in 2019 using Moringa leaf extract 300 mg/kg for 6 weeks in rats fed with high-fat diet stated that Moringa leaf extract help prevent weight gain, improve glucose resistance, lipid profiles, reduce levels of oxidative stress such as malondialdehyde and nitric oxide and enhance antioxidant activity.[10] Another study states that moringa leaf extract has the effect of reducing VEGF, TNFα, IL-2, IL-1β, IL-6 which play a role in the inflammatory process.[13] Based on the theory, this study was conducted to prove that oral administration of ethanol extract of Moringa leaves can reduce levels of F2-Isoprostane and MCP-1 in obese wistar rats (Rattus norvegicus).

MATERIALS & METHODS

Preparation of Moringa oleifera leaf extract

Moringa oleifera leaf extract was obtained from a Moringa leaf plantation in Lokapaksa Village, Buleleng, Bali and was subjected to phytochemical tests at the Department of Agriculture, Udayana University, Bali. The leaves are cleaned, dried and blended. Moringa leaf powder (300 g) was macerated in ethanol (96%) 1:20 (w/v) with stirring for 24 hours. The extract was filtered and evaporated with a vacuum rotary evaporator at 50^{o}C for 24 hours to obtain a crude extract. The dose used in this experiment was 300 mg/kg and dissolved in distilled water for oral administration.

Animal groups

This study was a pure experimental study using a Ranzomized Pretest-Posttest Control Group Design. In this study, 30 male Wistar white rats (*Rattus norvegicus*), aged 3.5-4.5 months, 170-190 g were used. The animals were placed in plastic cages under normal laboratory conditions and exposed to 12-h light/dark cycles. All rats were acclimated for 7 days, obesity induction was carried out for 32 days and treatment was given for 32 days. The experimental protocol was performed in accordance with the guidelines of the Animal Ethics Committees Faculty of Veterinary Medicine, Udayana University, Bali (Ethical Clearence Publication No:B/198/UN14.2.9/PT.01.04/2022)

In P0 group (control group), 10 rats received normal diet. Another 20 rats were induced obesity with a high-fat diet until they reached Lee's Index ≥ 0.30 g/cm, then divided into two groups, each consisting of 10 rats, namely group P1 (HFD-aquadest group) was given a high-fat diet and aquadest 1cc/day/rat per sonde and P2 group (HFD-moringa group) was given a high-fat diet and ethanol extract of Moringa leaves at a dose of 300mg/kgBW/rat/day given per sonde.

Body weight, F2-Isoprostane and MCP-1 levels were measured at day 39 and day 71. Blood samples were collected from medial canthus vein of the orbital sinus. Rats were anaesthetized using Ketamine and Xylazine mixture before the procedure.

Biochemical analysis

F2-Isoprostane and MCP-1 levels were measured using the sandwich ELISA (Enzyme-linked immunosorbent assay) method following the manufacturer's instructions of ELISA Kit from Bioassay Technology Laboratory, China.

Statistical Analysis

Statistical analysis was performed using IBM SPSS software for Windows version 26.0. All data variable were tested for normality using Shapiro-Wilk, homogeneity test was performed with Levene's Test. Comparison test were tested using One-Way Analysis of Variance (ANOVA) and treatment effect analysis test was performed with Paired T-test. P value < 0.05 was considered statistically significant.

RESULT

Obesity induced HFD rats (P1 and P2) showed an increase in body weight compared to normal diet rats (P0). Oral administration of Moringa leaf ethanol extract to HFD rats (P2) for 32 days prevented the percentage of weight gain compared to HFD rats given aquadest (P1) (Table.1).

Table 1. Effect of Moringa leaf extract (P2) on body weight gain in rats fed with high-fat diet (HFD) for 32 days. Moringa leaf extract prevents weight gain in obese rats. Values mean ± SD (n = 10).

	P0	P1	P2
Day 39	204.8 ± 12.06	240.8 ± 21.56	266.3 ± 21.85
Day 71	230.7 ± 20.42	271.9 ± 29.93	281.2 ± 24.82
% increase in body weight	12.65	12.92	5.6

Obese rats induced by HFD (P1 pre and P2 pre) for 32 days showed a significant increase in F2-Isoprsotane and MCP-1 levels compared with control rats fed with normal diet (P0 pre) (Fig.1). Oral administration of moringa leaf extract to HFD rats for 32 days (P2 post) showed a significant decrease in F2-Isoprostane and MCP-1 levels and showed values close to the normal diet control group (Fig.1).

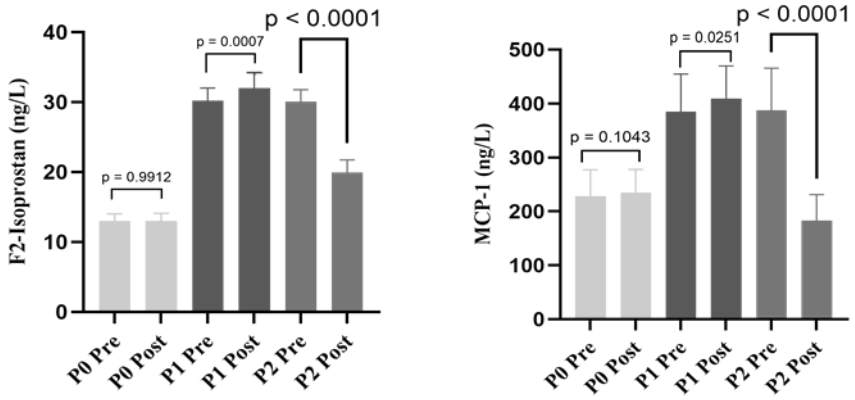

Figure 1. Effect of Moringa leaf extract (P2 post) on F2-Isoprostane and MCP-1 levels in rats fed with high-fat diet (HFD) for 32 days. Moringa leaf extract significantly reduced levels of F2-Isoprostane and MCP-1. Values mean ± SD (n=10).

DISCUSSION

High-fat diet (HFD) led to an increase in body weight and obesity in rats (Lee index ≥ 0.30 g/cm). Obesity induces systemic oxidative stress through various biochemical mechanisms. Biochemical processes that are affected by oxidative stress due to increased adipocytes are glyceraldehyde auto-oxidation, oxidative phosphorylation, protein kinase C (PKC) activation, increased production of superoxide radicals by Nicotinamide Adenine Dinucleotide Phospate (NADPH) oxidase (NOX) and hexosamine and polyol metabolic pathways.[2,14]

There was an increase in oxidative stress biomarkers F2-Isoprostane levels in the blood serum of obese rats fed with HFD. These results are in line with previous studies which state that F2-Isoprostane levels increase in obese conditions.[6,15] The results of this study showed that oral administration of ethanol extract of Moringa leaves containing polyphenolic compounds, flavonoids, saponins, and tannins significantly reduced F2-Isoprostane levels of obese rats that were given HFD (P2 post) when compared to the HFD-aquadest rat group (P1 post). These results confirm previous study which states that moringa leaf extract could reduce levels of malondialdehyde and nitric oxide.[10] The mechanism of Moringa leaves as an antioxidant is by increasing the Nuclear Factor Erythroid Related Factor-2 (Nrf2) pathway, modulating antioxidant enzymes such as superoxide dismutase (SOD), gluthathione (GSH), gluthathione peroxidase (GPx) and as a free radical scavenger which will inhibit oxidative stress processes (Fig.2).[10,14,16,17]

Obesity is also associated with low-grade chronic inflammation with increased activity of pro-inflammatory chemokines as indicated by increased MCP-1 in obese adipose tissue.[4,18] This theory is consistent with what was found in present study, that there was an increase in MCP-1 levels in blood serum of obese rats fed with HFD.

In this study, Moringa leaf extract prevented weight gain and significantly reduced MCP-1 levels in obese rats given HFD (P2 post) compared to the HFD-aquadest (P1 post) rat group. These results are also in line with other studies which state that moringa leaf extract reduced IL6 levels and body weight of HFD rats.[19] As an anti-inflammatory, Moringa leaf extract increases Nrf2 activity which will inhibit the inflammatory pathways of NF-κB (Nuclear factor kappa-light-chain-enhancer of activated B cells), MAPK (Mitogen-Activated Protein Kinase), and macrophage infiltration, also increase adiponectin which enhances anti-inflammatory factors such as IL-10 (Fig.2).[14,17,20–22] These mechanisms will inhibit the inflammatory process. Moringa leaf extract also increase AMPK

(Adenosine Monophosphate-Activated Protein Kinase) which increases catabolic, reduces adipogenesis and gluconeogenesis which provide anti-obesity effects (Fig.2).[14,23,24] These antioxidant, anti-inflammatory and anti-obesity effects inhibit inflammation, metaflammation, oxidative stress which are expected to inhibit the aging process.

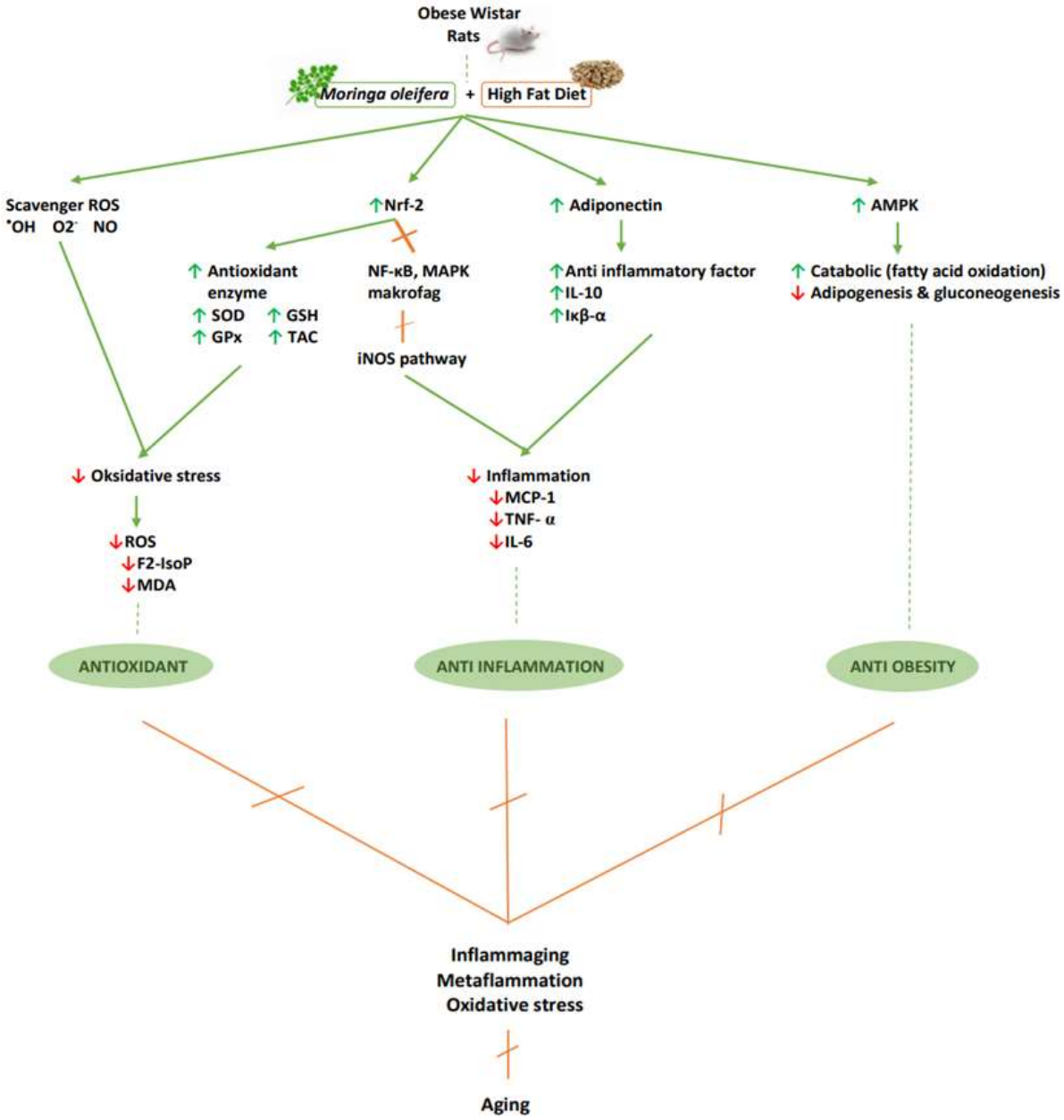

Figure 2. Mechanism of Moringa leaf extract (*Moringa oleifera*) as antioxidant, anti-inflammatory, and anti-obesity

CONCLUSION

From this study, it can be concluded that oral administration of ethanol extract of Moringa leaves (*Moringa oleifera*) is proven to significantly reduce F2-Isoprostane and MCP-1 levels, also preventing weight gain in obese rats given high-fat diet. The underlying mechanism is that Moringa leaf extract contains polyphenolic compounds, flavonoids, saponins, and tannins which have antioxidant, anti-inflammatory, and anti-obesity effects so that Moringa can be considered as a source of natural products that have the potential to be developed as medicinal plants for the prevention and treatment of obesity, thus expected to inhibit the aging process.

However, the weakness of this study is that no specific phytochemical test was carried out on quercetin and glycosides (glucocynolate and isothiocyanate) which are suspected as a strong antioxidant and anti-inflammatory. Furthermore, these results are expected to be used as a reference for subsequent research.

Declaration by Authors

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