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Antiobesity Effect of NADES (Natural Deep Eutectic Solvent) Extract of Purslane Herb (*Portulaca oleracea* L.) on Male Rats (*Rattus norvegicus*)

Rizqi Nur Azizah*, Irma Santi, Muhammad Noer Awali, Bayu Putra, Faradiba

Faculty of Pharmacy, Universitas Muslim Indonesia, Makassar, South Sulawesi, Indonesia

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Corresponding author e-mail:
rizqi.azizah@umi.ac.id

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ABSTRACT

Introduction*: Obesity is a metabolic disorder characterized by excessive fat accumulation, leading to various health complications. One potential approach to managing obesity is through natural products, such as medicinal plants.* ***Aims:*** *This study evaluates the anti-obesity activity of purslane (Portulaca oleracea L.) extract obtained using the green extraction method in male rats (Rattus norvegicus).* ***Method:*** *A total of 18 male rats were divided into six groups: normal control, negative control (Na-CMC + NADES), positive control (orlistat), and three treatment groups receiving NADES Purslane Herb Extract (NPHE) at doses of 50, 100, and 200 mg/kg BW. The treatment groups were induced with a high-fat diet for 30 days, followed by 14 days of therapy.* ***Result:*** *Statistical analysis using One-Way ANOVA and Bonferroni post-hoc test showed that NADES purslane herb extract exhibited significant anti-obesity effects, with the most effective dose being 200 mg/kg BW, which reduced body weight by 13.004% and BMI by 17.935% in male rats.* ***Conclusion:*** *These findings suggest that purslane extract has potential as a natural anti-obesity agent. Further research is needed to explore its bioactive compounds and mechanisms of action, supporting its potential development in pharmaceutical and nutraceutical applications.*

KEYWORDS*: Anti-obesity, green extraction, NADES, orlistat, Portuluca oleracea L.*

INTRODUCTION

Obesity is a condition in which an individual experiences excessive body weight due to uncontrolled fat accumulation. The primary cause of this condition is an imbalance between energy intake and energy expenditure, where high-calorie food consumption is not balanced with adequate physical activity. Obesity is highly detrimental to human health as it is associated with other health issues such as cardiovascular disorders, diabetes mellitus, and certain types of cancer, making its management crucial (Toar et al., 2023; Septiyanti & Seniwati, 2020).

One of the medicinal plants known for its therapeutic properties is purslane (*Portulaca oleracea* L.). This plant has been traditionally used for various conditions

such as burns, headaches, and coughs (Jung et al., 2023). Several studies have been conducted on purslane. According to research by Jung et al. (2021), rats fed a high-fat diet supplemented with 10% purslane extract exhibited a significant 34% reduction in weight gain compared to those receiving only a high-fat diet. Another study conducted by Azizah et al. (2022) demonstrated that a 96% ethanol extract of purslane herb at a dose of 200 mg/kg BW effectively reduced body weight in obese rats, with a weight loss percentage of 21.868%.

The extraction of bioactive compounds from plants is commonly performed using maceration techniques with organic solvents such as methanol, ethanol, acetone, and ethyl acetate. However, these methods have limitations, including prolonged extraction times and the use of large solvent volumes, which may leave harmful residues. To overcome these drawbacks, a novel green extraction method utilizing Natural Deep Eutectic Solvents (NADES) has been developed, which is more environmentally friendly (Puspita et al., 2023).

In terms of physicochemical properties, NADES provide several advantages over conventional solvents, such as high stability at elevated temperatures, low volatility, non-toxicity, and environmental safety. NADES also exhibit high efficiency in extracting both polar and non-polar compounds, as well as other secondary metabolites. Furthermore, these solvents can dissolve macromolecules, enhancing extraction efficiency (Ahmad et al., 2020; Puspita et al., 2023). A study by Liu et al. (2019) demonstrated that extraction using citric acid-glucose-based NADES yielded higher curcuminoid content than organic solvents like ethanol and methanol, reinforcing the superiority of NADES as a green solvent for bioactive compound extraction.

Based on the aforementioned discussion, this study aims to evaluate the anti-obesity effects of purslane extract using a green extraction method in male rats (*Rattus norvegicus*). The findings will provide scientific evidence supporting the potential use of purslane extract as a reliable anti-obesity agent.

MATERIAL AND METHODS

Materials

The materials used in this study include, distilled water, citric acid, and glucose (CV. Sentana Sempurna, Makassar), purslane herb, sodium carboxymethyl cellulose (PT. Sumber Rejeki, Makassar), orlistat (Novell), high-fat diet feed, standard feed, and picrate.

Sample Collection and Processing

The purslane plant was collected from Makassar, South Sulawesi, Indonesia and

has been determined by the Plant Determination Unit of the UMI Makassar Pharmacognosy-Phytochemistry Laboratory. The plant was sorted by removing impurities and then washed with running water. The purslane herb was drained and chopped into pieces of 1–2 cm in length. The chopped herb was placed in a drying cabinet. After drying, the sample was sorted again and powdered to obtain the simplicia powder.

NADES Preparation

Citric acid and glucose are weighed 5 g and 1 g respectively. The two components were melted at a specific temperature using a magnetic stirrer at 50°C. After melting, distilled water was gradually added in an amount equal to 30% of the final desired NADES volume, followed by homogenization. The solution was then cooled to room temperature and stored in a tightly sealed bottle until use (Ahmad et al., 2020).

Extract Preparation

A total of 3 g of purslane herb simplicia powder was extracted using ultrasound-assisted extraction (UAE) with NADES as the solvent, at a solvent-to-sample ratio of 1:15 g/mL. The ultrasonic extraction was performed for 15 minutes at 40°C. The resulting solution was filtered using a Buchner funnel, and the filtrate was discarded. The liquid extract obtained was then placed in a dehydrator to produce a thick extract (Ahmad et al., 2020 ; Afandi et al., 2021).

High-Fat Diet (HFD) Preparation

A total of 1 kg of high-fat diet feed was prepared using the following composition: 20% standard feed (Confeed PARS CP594), 20% wheat flour, 20% butter, 30% chicken eggs, and 10% milk powder. The standard feed was ground using a blender and sieved through an 80-mesh sieve, then mixed thoroughly with wheat flour and milk powder (Component A). Butter and eggs (both yolk and white) were mixed separately, and Component A was gradually added to this mixture while continuously stirring until a uniform dough was formed. The dough was weighed and shaped using a pelleter, then baked in an oven at 160°C for 60 minutes. The pellets were then removed from the oven and cooled (Rosnah et al., 2022).

Orlistat Preparation

Ten capsules of 120 mg orlistat were weighed, and their average weight was calculated. A total of 27.754 mg of orlistat powder was weighed and suspended in 1% w/v sodium carboxymethyl cellulose (Na-CMC) until homogeneous. The suspension was then transferred to a volumetric flask and diluted to a final volume of 12 mL (Saputri et al., 2023).

Test Formulation Preparation

Suspensions of NADES-extracted purslane herb were prepared in three dose variations: 50 mg/kgBW, 100 mg/kgBW, and 200 mg/kgBW. First, 100 mg, 200 mg, and 400 mg of NADES purslane herb extract were weighed, respectively. Each dose variation was then dissolved in 12 mL of 1% Na-CMC until a homogeneous suspension was obtained (Azizah et al., 2022).

Animal Treatment

Anti-obesity activity assay has received approval from the ethics committee (Number: UMI012503137). A total of 18 test animals were used and divided into six groups. Before the experiment, the animals underwent a two-week acclimatization period and were provided with a standard diet. The initial body weight and naso-anal body length were measured. The test animals, except for the normal control group, were induced with a high-fat diet (HFD) at 30 g ad libitum for 30 days. The animals were divided into six treatment groups: Group I (normal control, no treatment), Group II (negative control, Na-CMC + NADES), Group III (positive control, orlistat), Group IV (NADES purslane herb extract at 50 mg/kgBW), Group V (NADES purslane herb extract at 100 mg/kgBW), and Group VI (NADES purslane herb extract at 200 mg/kgBW). The test formulations were administered orally for 14 days. Body weight and naso-anal body length were measured on day 7 and day 14 of treatment (Azizah et al., 2022).

Data Analysis

The obtained data consisted of body weight and body length measurements of the test animals. The data were statistically analyzed for normality, followed by One-Way ANOVA and Bonferroni post hoc test.

RESULTS AND DISCUSSION

In this study, 18 male rats (*Rattus norvegicus*) were used as test subjects. The test animals were divided into six groups: Group I served as the normal control (no treatment), Group II as the negative control (Na-CMC + NADES), Group III as the positive control (Orlistat), Group IV received NADES Purslane Herb Extract (NPHE) at a dose of 50 mg/kg BW, Group V received NPHE at 100 mg/kg BW, and Group VI received NPHE at 200 mg/kg BW. The test formulations were administered orally for 14 days. The induction agent used in this study was a High-Fat Diet (HFD), which was given to all test animals except for the normal control group. HFD was provided at 30 g ad libitum for 30 days. The test sample used in this study was NADES extract derived from the *Portulaca oleracea* L. herb simplicia. The extraction process employed a green extraction method, specifically Ultrasound-Assisted Extraction (UAE) using Natural Deep Eute-

Table 1. Data on weight loss of experimental animals

Treatment Group	Mean ± SD (gram)			
	Initial	Induction	Therapy	
			Day- 7	Day- 14
Normal Control	170,00 ± 18,02	195,66 ± 5,50	198,00 ± 3,00	198,00 ± 3,00
Negative Control (Na-CMC+NADES)	206,00 ± 16,62	295,66 ± 25,10	283,66 ± 11,31	302,66 ± 26,08
Positive Control (Orlistat)	220,00 ± 25,00	329,00 ± 14,73	312,66 ± 8,02	281,66 ± 10,40
NPHE 50mg/KgBB	287,66 ± 38,43	360,33 ± 49,69	344,66 ± 47,34	328,00 ± 47,63
NPHE 100mg/KgBB	269,00 ± 35,51	357,66 ± 20,52	347,00 ± 18,02	322,66 ± 20,52
NPHE 200mg/KgBB	279,66 ± 44,00	364,33 ± 38,47	345,00 ± 32,51	323,66 ± 39,70

ctic Solvent (NADES) composed of a combination of citric acid and glucose. This method was chosen to minimize the use of synthetic organic solvents, making it more environmentally friendly while preserving the bioactive compounds in the extract (Puspita et al., 2023) (Hikmawanti et al., 2021).

The study parameters included body weight and Body Mass Index (BMI) measurements in the test animals. The first parameter, body weight measurement, was conducted before HFD induction, after induction, and after therapy on day 7 and day 14.

Based on the data in Table 1, the test animals experienced a body weight increase of approximately 73 –109 grams from their initial weight. This data indicates that all groups showed a weight gain of more than 20%, except for the normal control group, which was only given a standard diet. These results are consistent with the study conducted by Rosnah et al. (2022), in which HFD was composed of butter, eggs, flour, powdered milk, and a mixture of standard feed. The study demonstrated that HFD administration led to a significant body weight increase in the test animals by the fourth week, with a weight gain of 10–20% compared to the standard feed group. High-fat diets can induce obesity and metabolic disorders in rodents, resembling metabolic syndrome in humans (Rosnah et al., 2022).

Based on the data in Table 2, the normal control group did not experience weight loss but instead showed a weight increase of 1.548% on day 7, which further increased to 3.581% on day 14. This was due to the fact that the normal control group was only given a standard diet without any treatment. The negative control group experienced a weight loss of 4.080% on day 7, however, by day 14, the body weight of the test animals increased again by 2.360% from the induction weight. This indicates that the observed weight loss was temporary and inconsistent, as evidenced by the weight regain after day 7. The negative control group was administered Na-CMC and NADES orally, with the purpose of ensuring

Table 2. Percentage data of weight loss of experimental animals

Treatment Group	% Decrease Percentage		P Value
	Day- 7	Day- 14	
Normal Control	-1,548	-3,581	0,000
Negative Control (Na-CMC+NADES)	4,080	-2,360	
Positive Control (Orlistat)	4,911	14,539	
NPHE 50mg/KgBB	4,342	4,435	
NPHE 100mg/KgBB	2,962	9,808	
NPHE 200mg/KgBB	5,160	13,004	

(-) sign: indicates no weight loss

that the anti-obesity effect observed in other groups was due to the purslane extract rather than NADES as the extraction solvent (Ulfa et al., 2024).

In this study, NADES-extracted purslane herb extract at a dose of 200 mg/kg BW successfully reduced the body weight of test animals by 13.004% over two weeks of treatment. In contrast, a previous study conducted by Hussein (2010) demonstrated that administering 95% ethanol extract of purslane herb at a dose of 300 mg/kg BW for eight weeks inhibited the weight gain of test animals by 35.41%. These differences suggest that NADES-extracted purslane herb may provide a faster anti-obesity effect at a lower dose compared to ethanol-extracted purslane herb in Hussein's study. The advantage of NADES extraction lies in its ability to extract both polar and non-polar bioactive compounds. Additionally, NADES is food-grade, non-toxic, and easy to produce. The ultrasonic-assisted extraction (UAE) method used in this study further enhances NADES extraction efficiency by increasing the solubility of target compounds through heat generation and cavitation principles (Puspita et al., 2023) (Hikmawanti et al., 2021).

The percentage of weight loss was analyzed using One Way ANOVA, showing a significant difference among treatment groups ($P < 0.05$). The Bonferroni test revealed that the normal and negative control groups were not significantly different ($P > 0.05$), indicating that neither induced weight loss. In contrast, the positive control and NADES extract groups showed a significant difference compared to the normal and negative controls ($P < 0.05$), suggesting a weight loss effect. The NADES extract at a dose of 200 mg/KgBW was not significantly different from the positive control ($P > 0.05$), indicating similar effectiveness to the positive control and greater efficacy than the other doses.

The obesity status in rats can also be assessed using the Body Mass Index (BMI). Body fat and obesity in rats are better estimated using BMI than the Lee Index. Change in BMI are associated with dyslipi-

Table 3. Body mass index of experimental animals

Treatment Group	Mean ± SD (gram/cm^2)			
	Initial	Induction	Therapy	
			Day- 7	Day- 14
Normal Control	0,439 ± 0,02	0,506 ± 0,01	0,531 ± 0,03	0,530 ± 0,02
Negative Control (Na-CMC+Nades)	0,507 ± 0,02	0,703 ± 0,01	0,674 ± 0,00	0,720 ± 0,01
Positive Control (Orlistat)	0,498 ± 0,02	0,723 ± 0,01	0,609 ± 0,02	0,524 ± 0,01
NPHE 50mg/KgBB	0,559 ± 0,05	0,699 ± 0,01	0,660 ± 0,06	0,617 ± 0,04
NPHE 100mg/KgBB	0,564 ± 0,04	0,717 ± 0,02	0,676 ± 0,05	0,619 ± 0,04
NPHE 200mg/KgBB	0,543 ± 0,05	0,718 ± 0,02	0,661 ±0,02	0,589 ± 0,04

demia profiles, oxidative stress in serum, and obesity status in rats. The BMI of adult male Wistar rats ranges from 0.45 to 0.68 g/cm^2, therefore, rats are considered obese if their BMI is ≥ 0.68 g/cm^2 (Rosnah et al., 2022).

Based on Table 3, the induction data show an increase in BMI >0.68 g/cm^2 in all experimental groups except the normal control group. This is attributed to the administration of 30 grams of DTL feed for 30 days ad libitum, which led to weight gain and increased BMI in the test animals. In contrast, the normal control group, which received only standard feed, maintained a stable BMI throughout the induction period. The observed increase in BMI in the experimental groups suggests that the DTL feed was effective in inducing obesity-like conditions, providing a suitable model for evaluating the anti-obesity potential of the test extract. After 14 days of treatment, a noticeable reduction in BMI was observed in several groups, particularly those receiving the extract or the positive control treatment.

The data in Table 4 show that the highest percentage reduction in BMI was observed in the positive control group at 27.479%. The positive group (orlistat) exhibited the highest percentage of weight loss, reaching 14.539%. Orlistat is a commonly used anti-obesity drug that works by preventing the hydrolysis of dietary fat into absorbable free fatty acids and glycerol, thereby facilitating fat excretion from the body (Rajan et al., 2021).

Meanwhile, the normal control and negative control groups did not experience any reduction in BMI. This is because the normal group did not receive any treatment, while the negative control group was treated with Na-CMC + NADES administered orally.

Statistical analysis using One Way ANOVA revealed significant differences among treatment groups ($P < 0.05$), prompting further analysis with the Bonferroni test. The results showed no significant difference between the normal and negative control groups ($p > 0.05$),

indicating that neither influenced BMI reduction in test animals. The negative control group, compared to the positive control and extract groups, showed a significant difference ($P < 0.05$), suggesting that the positive control and extract groups contributed to BMI reduction. Further comparisons revealed that all extract doses significantly differed from the positive control ($P < 0.05$), except for the 200 mg/KgBW dose ($P > 0.05$), indicating that this dose had a similar effect to the positive control. Comparisons among the extract doses (50 mg/KgBW, 100 mg/KgBW, and 200 mg/KgBW) showed no significant differences ($P > 0.05$), suggesting that all extract doses had a comparable effect on BMI reduction in test animals.

These findings demonstrate that the NADES extract of *Portulaca oleracea* exhibited greater efficacy in reducing BMI compared to ethanol extract. According to Azizah et al. (2022), administration of a 96% ethanol extract of P. oleracea at a dose of 100 mg/kg BW resulted in a BMI reduction of 5.728%. In contrast, the NADES extract at a lower dose of 50 mg/kg BW produced a significantly greater reduction in BMI, reaching 11.691%. Furthermore, Sutjiatmo et al. (2021) reported that ethanol extract of P. oleracea at a dose of 110 mg/kg BW inhibited weight gain in obese rats by only 8.58% on day 21. This finding differs considerably from the NADES extract at a dose of 100 mg/kg BW, which reduced body weight by 9.8% on day 14. This difference may be attributed to the extraction method used. Green extraction using NADES solvents and the Ultrasound-Assisted Extraction (UAE) technique is known to be more environmentally friendly and more efficient in extracting bioactive compounds compared to conventional methods. This allows for the optimal acquisition of phytochemicals with anti-obesity effects. On the other hand, ethanol extract may extract different bioactive compounds, but its effectiveness in reducing BMI appears lower than that of NADES extract (Ahmad et al., 2020) (Puspita et al., 2023).

Portulaca oleracea L. is believed to exhibit anti-obesity effects due to its chemical constituents, including flavonoids, carotenoids, and saponins. A study by Lee et al. (2019) showed that homoisoflavonoids extracted from *Portulaca oleracea* could reduce lipid accumulation. Flavonoids act as antioxidants that counteract excessive free radicals from bile acid synthesis, thereby enhancing lipoprotein lipase activity, which hydrolyzes triglycerides into free fatty acids and glycerol for storage in adipose and muscle tissues. Carotenoids, particularly β-carotene, function as antioxidants that prevent lipid oxidation. Uncontrolled lipid oxidation can facilitated

cholesterol penetration into arterial walls leading to blockages. Carotenoids also exert anti-obesity effects through another mechanism by inhibiting the activity of HMG-CoA reductase, an enzyme involved in cholesterol synthesis via the mevalonate pathway. Meanwhile, saponins contribute to anti-obesity effects by inhibiting pancreatic lipase activity, which is responsible for triglyceride breakdown. Consequently, fat absorption in the intestine is reduced, and triglycerides are excreted in the feces (Lee et al., 2019) (Azizah et al., 2022) (Vajdi et al., 2023).

CONCLUSION

Based on the results of this study, the NADES extract of *Portulaca oleracea* exhibited anti-obesity effects in male *Rattus norvegicus*, with an optimal dose of 200 mg/kg BW, which effectively reduced body weight by up to 13.004% and Body Mass Index by 17.935%. Further research is recommended to explore the long-term effects, potential toxicity, and underlying molecular mechanisms of this extract, as well as its applicability in human models.

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