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Original Research Article

Beneficial Effects of Self-nanoemulsifying Drug Delivery System Extract of *Curcuma longa* on Polycystic Ovary Syndrome Rats Model Through Insulin Sensitization Activity

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ABSTRACT

Insulin resistance contributes to the Polycystic Ovary Syndrome (PCOS) pathogenesis. Although *Curcuma longa* improves insulin sensitivity, it is limited by its low bioavailability. This study aims to demonstrate the application of the Self-Nanoemulsifying Drug Delivery System (SNEDDS) on *Curcuma longa* extract on the improvement of insulin resistance through measuring Glut-4 expression, fasting insulin levels, fasting blood glucose levels, and Homeostatic Model Assessment-Insulin Resistance (HOMA-IR) of PCOS model rat. This experimental research was conducted with a post-test-only control group design. This study employed 36 female Wistar rats aged three months divided into six groups: N: normal rats; PCOS: PCOS rats without treatment; PM: PCOS rats with metformin 20mg/kgBW/day; PSC25, PSC50, and PSC100: PCOS rats with SNEDDS extract of *Curcuma longa* 25, 50 and 100 mg/kgBW/day. Letrozole and a high-cholesterol, high-fructose diet was used to induce PCOS in all rats, except the control group, for 21 days before treatment began. Rats were sacrificed on the fifteenth day, and blood samples and gastrocnemius muscle were taken. A statistical test used the Anova and Kruskal-Wallis test with a p-value < 0.05 considered significant. Self-nanoemulsifying Drug Delivery System (SNEDDS) extract of *Curcuma longa* at doses of 50 and 100 mg/kgBW could significantly increase Glut-4 in muscle cells while decreasing FBG, insulin, and HOMA-IR score (P<0.05). Self-nanoemulsifying Drug Delivery System (SNEDDS) extract of *Curcuma longa* at 50 and 100 mg/kgBW improved expression of Glut-4, FBG, Insulin level, and HOMA-IR score in PCOS rats via insulin sensitizer activity.

***Keywords*:** SNEDDS *Curcuma longa* extract, PCOS, GLUT4, Insulin, HOMA-IR

Introduction

Polycystic ovary syndrome (PCOS) is a prevalent endocrine disorder that causes chronic anovulation and affects 8-13% of reproductive-aged women.[1–3] PCOS responsible for anovulatory infertility, menstrual irregularity, and abortions.[4,5] A number of recent studies have documented a rise in prevalence attributed to unhealthy lifestyles, including excessive caloric intake, carbohydrate and cholesterol consumption, fructose consumption, and rarely physical activity. This disorder is correlated with a higher prevalence of obesity, which exacerbates insulin resistance.[5–7]

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The cause of PCOS is unidentified, but genetics, carbohydrate and lipid metabolism imbalances, oxidative stress, insulin resistance, and oxidative stress play a role.[8–11] Insulin resistance is characterized by a diminished capacity of insulin to facilitate glycogenesis, the process by which glucose is absorbed, thus inhibiting lipolysis and further necessitating insulin to perform this metabolic function. Consequently, insulin circulation increases, but blood glucose levels continue to be elevated.[12]

In women with polycystic ovary syndrome, insulin resistance is linked to hormonal abnormalities, which include hyperandrogenic consequences.[12] Hyperandrogens influence insulin sensitivity by increasing lipase expression in visceral adipose tissue and decreasing PPAR- expression. It results in elevated levels of oxidative stress.[11,13,14] Oxidative stress in pancreatic beta cells causes mitochondrial dysfunction that affects the coupling mechanism of glucose metabolism. It increases the expression of Ins-1 and Ins-2 genes in pancreatic β cells, resulting in hyperinsulinemia, accompanied by impaired glucose absorption in tissues, further aggravating insulin resistance and hyperglycemia.[11,13,15]

The medication for PCOS often causes unpleasant side effects such as nausea, vomiting, and diarrhea that will increase non-adherence to taking medication. There is an urgent need to discover alternative therapeutic approaches for women with PCOS. Consequently, it's essential to formulate natural medicine with few adverse effects.[5]

Curcumin (diferuloylmethane) is the most active polyphenol in turmeric. Turmeric derived from the rhizome of *Curcuma longa L*. has been recognized as anticancer, anti-inflammatory, antioxidant, antimicrobial, and antirheumatic for its bioactive effects.[16] Previous studies reported that *Curcuma longa* can reduce female reproductive health issues through its anti-inflammatory and antioxidant effects.[17,18] Based on the pharmacokinetic profile of curcumin, curcumin has a low bioavailability that results in poor absorption, low water solubility, rapid metabolism, and swift systemic elimination. Thus, the clinical application of curcumin has been limited until now.[19] Self-Nanoemulsifying Drug Delivery Systems (SNEDDS) are isotropic homogeneous mixtures of active compounds such as lipids, surfactants, and cosurfactants. SNEDDS was created to enhance the solubility and therapeutic effectiveness (bioavailability) of drugs with low water solubility. SNEDDS enhance the drug's surface area, mucosal permeability, lipoprotein incorporation, and lymphatic secretion, decreasing the liver's metabolic rate. SNEDDS provides several benefits, including reduced drug doses, minimal adverse effects, and controlled drug release.[20,21]

This study created a PCOS-IR animal model by combining letrozole with a diet high in fructose and cholesterol. SNEDDS were utilized to give *Curcuma longa* extract to increase its bioavailability. The impact of curcumin nanoparticles on PCOS-IR animal models has been investigated;[22,23] however, there is a lack of studies utilizing SNEED *Curcuma longa* extract. This study investigated the impact of SNEDDS of *Curcuma longa* extract on improvements in insulin resistance in PCOS model rats by insulin sensitization activity. These results might offer a brand-new clinical strategy for PCOS treatment.

Materials and Methods

Preparation of SNEDDS Extract of Curcuma longa

Curcumin derived from the *Curcuma longa* plant contained 73.95% curcumin and 90.35% curcuminoids, which were utilized in SNEDDS. Extract of *Curcuma longa* in powdered was acquired from PT Soho Pharmaceutical Industry, located in Jakarta, Indonesia. In addition, SNEDDS preparations were developed from the extract at the Faculty of Pharmacy, Indonesian Islamic University, Yogyakarta. Next, a co-powder comprising 250 mg was added to the solution; this co-powder must be completely dissolved in Myritol to produce SNEDDS curcumin. After that, 1 mL of propylene glycol was added as a co-surfactant to the mixture, and it was agitated for 15 minutes at 500 rpm using a magnetic stirrer. Then, 6 m of Cremophor RH and Tween 20, a surfactant, were added. The Particle Size Analyzer and the Zeta Potential Analyzer were used to calculate the average particle size, polydisperse index, and zeta potential. In this formulation, 31.25 mg of *Curcuma longa* extract were present as nanoparticles in 1 mL of SNEDDS. The dosage of nanocurcumin was determined by adjusting the previously established protocol[23] and preliminary study.

Animal

In this study, the post-test-only control group design method was utilized. The research was carried out at the Pharmacology and Toxicology Laboratory, Faculty of Pharmacy in Universitas Gadjah Mada, Indonesia. Regarding samples, 36 females *Rattus norvegicus* strain Wistar rats aged 3 months and weighing between 200 and 250 grams were examined. Rats were confined in a specialized animal laboratory with the same temperature (25-28°C), humidity (40-60%), and light (normal) conditions. Standard beverages and food were provided. After a week of acclimation, the rodents' estrus cycles were examined.[24] Thirty rats in estrus were selected at random to produce a PCOS model.

PCOS-IR Model Creation in Rats

The PCOS model is generated from a previous investigation that combined letrozole with a diet heavy in fat. In this study, a PCOS-inducing regimen of letrozole and a high-fructose, high-cholesterol diet was implemented. Preliminary research demonstrated that inducing insulin resistance and PCOS-model rats was possible with the intragastric administration of 1 mg/kg/day letrozole dissolved in 0.5 percent carboxymethylcellulose (CMC) over the course of 21 days in combined with a high-cholesterol and high-fructose diet.[25] The control group (n=6) rats had a daily conventional diet and beverages. Meanwhile, 30 rats comprised the PCOS model group. Rats in the PCOS model group were given daily letrozole (1 mg/kgBW/day, TCI/L0248, Tokyo, Japan) intragastrical along with a high-fat, high-fructose diet of about 20 g/day/rat (Comfeed PAR-s 60 percent, Flour 27.8%, cholesterol 2%, cholic acid 0.2 percent, and lard 10%; fructose 2 mg/kg BW/day; Center for Nutrition Studies, Gajah Mada University, Yogyakarta, Indonesia) and free water. Using a vaginal swab stained with Giemsa, the rats' oestrous cycle was monitored daily. PCOS develops when the oestrous cycle endures a series of alterations leading to persistent vaginal cornification.

Preparation of Rat Samples in Both Groups

The prepared PCOS model rats were randomly divided into the following groups (n = 6): normal, PCOS, PCOS + metformin 20 mg/kgBW/day, PCOS + SNEDDS curcumin 25 mg/kg/day, PCOS + SNEDDS curcumin 50 mg/kgBW/day, and PCOS + SNEDDS curcumin 100 mg/kgBW/day. As a positive control in this study, metformin was used as a standard therapy of PCOS on rats with a dosage of 20 mg/kgBW/day.[26] Meanwhile, determining the dosage of SNEEDS extract of *Curcuma longa* in this study was the modification of the nano curcumin dosage that was used in the previous study.[23] Thus, in this study, Metformin and SNEEDS curcumin were taken orally for 14 days. Furthermore, the rats were subjected to a 12-hour fast, light ether anesthesia and blood collection from the retro-orbital venous plexus of the eye using a heparinized capillary tube on day 15. Blood samples were drawn into tubes and centrifuged for ten minutes to separate the serum. The insulin levels were assessed after the serum had been separated, kept at -20 °C, and prepared for ELISA analysis. Before making paraffin blocks, the right gastrocnemius muscle was removed and fixed for 48 hours in 10% formalin buffer. Histological preparations have been made, and then immunohistochemical staining of Glut-4 was carried out. An optical microscope with 400x magnification was used to examine the histological images.

The Health Research Ethics Committee of the Faculty of Medicine, Universitas Muhammadiyah Yogyakarta, Indonesia, approved this study with letter number 048/EC-KEPK/FKIK UMY/VI/2021.

Results and Discussion

Insulin resistance, as well as hyperandrogenism in women with PCOS, have been implicated in dysfunction of the hypothalamic-pituitary-ovarian axis, leading to hormonal imbalance in this axis. It began with hypersensitivity of the pituitary to GnRH caused by an increase in the frequency and amplitude of GnRH. In response, the pituitary gland secretes a greater quantity of LH than FSH. Luteinizing hormone (LH) induces an overproduction of androgens by ovarian theca cells.[8,15]

Hyperandrogens are responsible for the accumulation of visceral fat. PPAR-γ activation is additionally impaired under these conditions, leading to a reduction in adiponectin, an increase in lipolysis, and the release of substantial quantities of free fatty acids (FFA), all of which contribute to heightened oxidative stress.[11] High levels of oxidative stress in PCOS inhibit IKkB and stimulate NFkB, increasing TNFα, IL 6 and CRP. These three inflammatory mediators are important in the induction of hyperandrogenism and insulin resistance in PCOS.[10,27,28]

Tumor necrosis factor-alpha (TNFα) also reduces insulin sensitivity by increasing IRS-1 serine phosphorylation. IL-6 plays a role in regulating IR by increasing serine phosphorylation of IRS-1. In addition, TNFα inhibits the phosphorylation of the substrate Akt 160, which causes reduced expression of glucose transporter type 4 (Glut-4) so that glucose transport into cells will be hampered.[27] In addition, with increased oxidative stress, pancreatic beta cells experience mitochondrial dysfunction followed by increased expression of Ins-1 and Ins-2 genes, which further causes hyperinsulinemia. Hyperinsulinemia, accompanied by impaired glucose absorption in tissues, causes insulin resistance and hyperglycemia. Hyperinsulinemia strengthens the LH mechanism in regulating androgen biosynthesis.[10,15]

Free Fatty Acid (FFA) also causes functional and structural changes in hepatocytes and myocytes by the accumulation of metabolites of long-chain FFA, including Acyl-CoA and diacylglycerol. This molecule will

activate a serine/threonine kinase underlying insulin resistance by increasing serine phosphorylation of IRS-1.[29,30] In adipocytes, testosterone also appears to induce serine phosphorylation of IRS-1. FFA induces RI in muscle at the level of glucose transport by impairing the insulin signaling pathway.[31] Thus, increased oxidative stress and accumulation of lipid peroxidation metabolites in the body, pancreatic beta cell dysfunction, mitochondrial dysfunction, and decreased FA β oxidation are other factors that can lead to insulin resistance.[32] Several insulins signaling pathways have been identified. The phosphoinositide 3-kinase (PI3K)/Akt pathway plays a role in translocating glucose transporter 4 (Glut-4) from intracellular vesicles into cells, thereby increasing glucose uptake into skeletal muscle and ultimately reducing blood glucose levels. Other pathways are the mitogen-activated protein kinase (MAPK), adenosine monophosphate-activated protein kinase (AMPK) pathway, and the c-Jun-N-terminal kinase (JNK) pathway.[33]
Insulin binding to insulin receptors on cells triggers autophosphorylation of intracellular receptor substrates 1 and 2 (IRS-1/IRS-2). Disruption of the signaling pathway is caused by serine phosphorylation of the insulin receptor and IRS-1 secondary to intracellular serine kinase. RI is characterized by a decrease in receptor concentration and kinase activity, PI3K activity, concentration and phosphorylation of IRS-1 and IRS-2, and glucose transporter translocation.[13,34,35]
Curcumin is a natural ingredient that contains lipophilic polyphenols with low water solubility. The curcumin content is around 80% of the total curcuminoids in *Curcuma longa* extract.[16,19] Many previous studies have proven that curcumin has the activity of increasing insulin sensitivity, a powerful antioxidant, anti-inflammation, and others.[23] Several formulations, including nanoparticles, have been prepared to increase bioavailability, permeability, and resistance to metabolic processes. One form of nanoparticle whose manufacturing method is easy and simple is auto emulsification, known as SNEDDS.[20,36]

Administration of SNEDDS Curcuma longa Extract on Glut-4 Expression in Gastrocnemius Skeletal Muscle Cells in PCOS Model Rats

Glut-4 expression was observed in histopathological preparations of gastrocnemius muscle tissue using IHC staining and appeared brown in the cytoplasm of skeletal muscle cells. Glut-4 expression was observed with a microscope with 400x magnification. The data for each sample was assessed semi-quantitatively according to the modified Remmele method, where the Remmele Scale Index (Immuno Reactive Score/IRS) is the result of multiplying the percentage score of immunoreactive cells by the color intensity score of immunoreactive cells. A microscopic view of Glut-4 expression in skeletal muscle cells in each group is shown in Figure 1.
In Figure 2 and Table 1 Analysis using the Kruskall Wallis test followed by a post hoc test with Mann Whitney showed that there were significant differences in all groups ($p < 0.05$). Glut-4 expression in the PCOS group was significantly lower than in the normal and PCOS groups treated with metformin and SNEED Curcuma 50 mg and 100 mg ($p<, 0.05$), but like one of the PCOS group that treated with SNEEDS curcumin 25 mg ($p> 0.05$). On the other hand, there was no significant difference ($p>0.05$) observed between the normal, PCOS groups treated with curcumin and medium and large dosages of SNEEDS curcumin. It has been demonstrated that giving SNEDDS of *Curcuma longa* extract at doses of 50 mg/kgBW and 100 mg/kgBW increases Glut-4 expression in the gastrocnemius muscle of PCOS model rats, getting close to levels in normal rats which are already comparable to metformin treatment.
The glucose uptake mechanism in tissues and cells requires a glucose transporter (GLUT), one of which is Glut-4. Glut-4 is a major contributor to glucose homeostasis. Glucose Transporter-4 (Glut-4) is expressed in tissues associated with insulin-mediated glucose uptake, such as skeletal muscle and adipose tissue. As Glut-4 depends on insulin for glucose uptake, its damage can lead to insulin resistance.[37] Glut-3 expression is affected by oxidative stress. High levels of oxidative stress in PCOS inhibit IKkB and stimulate NFkB, which in turn increases TNFα, IL 6 and CRP.[28,38] This pro-inflammatory agent plays a role in increasing serine phosphorylation of IRS-1 and decreasing Glut-4. The next step is reducing post-receptor insulin sensitivity.[5,27,38,39] In PCOS, Glut-4 levels will decrease due to increased expression or exposure of the androgen receptor.[37,40]
Research on PCOS model mice showed that administration of pure curcumin at 100 and 200 mg/kgBW doses for 30 days increased Glut-4 expression.[41] Curcumin administration increased Rac1 Expression in insulin-dependent Glut-4 translocation and maintained normal glucose tolerance and insulin sensitivity in bone tissue.[42]

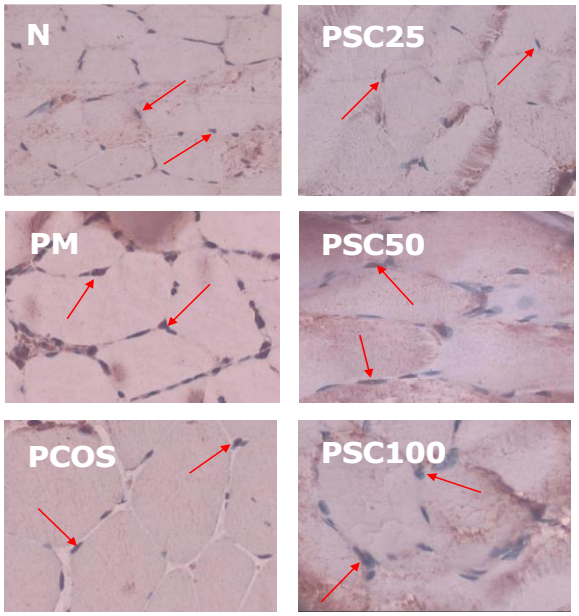

Figure 1: Glut-4 skeletal muscle cells gastrocnemius expression score overview
Note: Glut-4 expression of skeletal muscle cells. Glut4 expression appears as a brown color on the cytoplasm of skeletal muscular cells (mark"→"); (IHC painting, 400x zoom). N: Normal control group with no therapy; PM: PCOS rats group with metformin 20mg/kgBW per oral; PCOS: negative control group, PCOS rats without treatment; PSC25: PCOS rats group with SNEDDS *Curcuma longa* extract 25 mg/kgBW/day per oral; PSC50: PCOS rats group with SNEDDS *Curcuma longa* extract 50 mg/kgBW/day per oral; PSC100: PCOS rats group with SNEDDS *Curcuma longa* extract 100 mg/kgBW/day per oral.

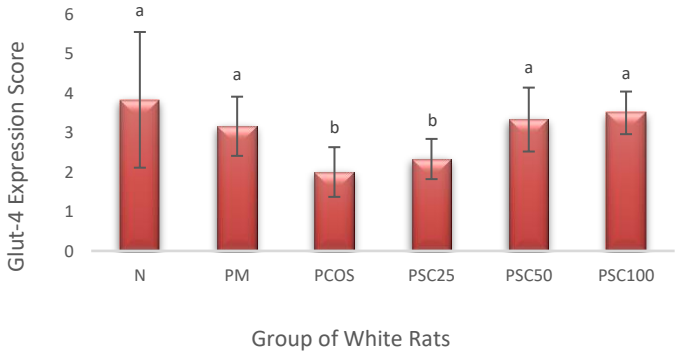

Figure 2: Histological score of Glut-4 expression in the Gastrocnemius in each group
Note: N: Normal control group and not receiving treatment; PM: PCOS rats group receiving metformin at 20 mg/kgBW/day per oral dose; PCOS: Negative control group, PCOS rats without treatment; PSC25: PCOS rats group receiving curcumin in SNEDDS at 25 mg/kgBW/day per oral use; PSC50: PCOS rats group receiving curcumin in SNEDDS at 50 mg/kgBW/day per oral use; PSC: PCOS rats group receiving curcumin in SNEDDS at 100 mg /kgBW/day per oral use. *a: significantly different from the PCOS group; b: significantly different from the control group (N).

Administration of SNEDDS Curcuma longa Extract on HOMA-IR Scores in PCOS Model Rats

Homeostatic Measurement Assessment – Insulin Resistance (HOMA-IR) is a score to assess insulin resistance and is obtained by entering fasting blood glucose and insulin levels in a special formula.[43]

Table 1: The result of Kruskal Walls and Mann Whitney on Glut-4 Expression

Group	Median (Min-Max)	P
N	3 (3-8)	
PM	3 (2-4)	
PCOS	2 (1-3)	0.001*
PSC25	2 (2-3)	
PSC50	3.5 (2-4)	
PSC100	3.5 (3-4)	

Note: N: Normal control group; PM: PCOS rats group with metformin 20mg/kgBW per oral; PCOS: negative control group, PCOS rats without treatment; PS 25: PCOS rats group with SNEDDS *Curcuma longa* extract 25 mg/kgBW/day per oral; PSC50: PCOS rats group with SNEDDS *Curcuma longa* extract 50 mg/kgBW/day per oral; PSC100: PCOS rats group with SNEDDS *Curcuma longa* extract 100 mg/kgBW/day per oral.

Table 2: Kruskal Wallis and Mann Whitney Test Results on Fasting Blood Glucose Levels

Group	Median (Min-Max)	P
N	88.5 (84 – 106)	
PM	85.5 (74 – 96)	
PCOS	149 (138 – 196)	0.000*
PSC25	117.5 (95 – 143)	
PSC50	85.5 (69 – 101)	
PSC100	73 (67 – 85)	

Note: N: Normal control group; PM: PCOS rats group with metformin 20mg/kgBW per oral; PCOS: negative control group, PCOS rats without treatment; PS 25: PCOS rats group with SNEDDS *Curcuma longa* extract 25 mg/kgBW/day per oral; PSC50: PCOS rats group with SNEDDS *Curcuma longa* extract 50 mg/kgBW/day per oral; PSC100: PCOS rats group with SNEDDS *Curcuma longa* extract 100 mg/kgBW/day per oral

Table 3: The Result of The ANOVA test on Insulin Level

Group	Mean ± SD	P
N	4.69 ± 0.54^{a}	
PM	4.37 ± 0.83^{b}	
PCOS	6.45 ± 0.43^{a}	0.000*
PSC25	4.94 ± 0.89^{a}	
PSC50	4.35 ± 0.47^{a}	
PSC100	3.94 ± 0.5^{a}	

Note: N: Normal control group; PM: PCOS rats group with metformin 20mg/kgBW per oral; PCOS: negative control group, PCOS rats without treatment; PS 25: PCOS rats group with SNEDDS *Curcuma longa* extract 25 mg/kgBW/day per oral; PSC50: PCOS rats group with SNEDDS *Curcuma longa* extract 50 mg/kgBW/day per oral; PSC100: PCOS rats group with SNEDDS *Curcuma longa* extract 100 mg/kgBW/day per oral

Fasting Blood Glucose Level

In Table 2 and Figure 3, PCOS rats without medication had the highest fasting blood glucose levels, while PCOS rats treated with SNEDDS 100 mg *Curcuma longa* extract have the lowest levels. The Kruskal Wallis and Mann Whitney tests (Table 2) indicated that there were statistically significant differences ($p<0.05$) in the fasting blood glucose levels in serum across all sample groups. Serum fasting blood glucose levels were considerably higher in the group of PCOS rats who did not get medication compared to all other groups ($p < 0.05$). PCOS elevated fasting blood glucose levels, which decreased significantly when SNEDDS *Curcuma longa* extract at doses of 25, 50, and 100 mg/kgBW were administered, which is equivalent to standard PCOS therapy. Although the reduction in fasting blood glucose levels was more pronounced at dosages of 50 and 100 mg/kgBW, there was no statistically significant distinction between the two doses.

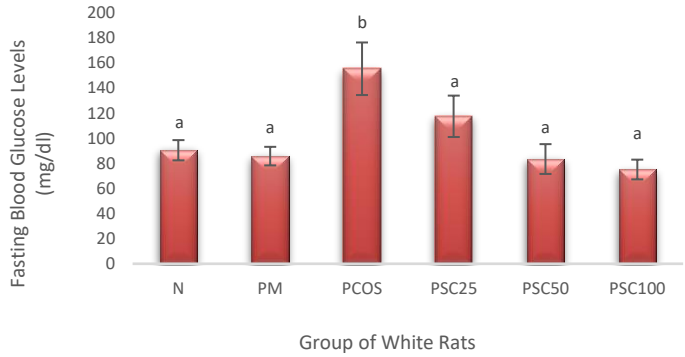

Figure 3: Graph of average Fasting Blood Glucose levels in each group

Note: N: Normal control group and not receiving treatment; PM: PCOS rats group receiving metformin at 20 mg/kgBW/day per oral dose; PCOS: Negative control group, PCOS rats without treatment; PSC25: PCOS rats group receiving curcumin in SNEDDS at 25 mg/kgBW/day per oral use; PSC50: PCOS rats group receiving curcumin in SNEDDS at 50 mg/kgBW/day per oral use; PSC: PCOS rats group receiving curcumin in SNEDDS at 100 mg /kgBW/day per oral use. a: significantly different from the PCOS rats group without therapy (PCOS); b: significantly different from the normal group (N)

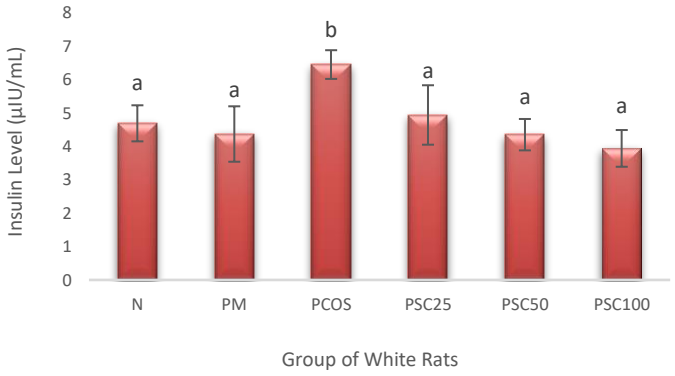

Figure 4: Graph of Mean Score (Mean ± SD) of Insulin Levels in Each Group

Note: N: Normal control group and not receiving treatment; PM: PCOS rats group receiving metformin at 20 mg/kgBW/day per oral dose; PCOS: Negative control group, PCOS rats without treatment; PSC25: PCOS rats g

roup receiving curcumin in SNEDDS at 25 mg/kgBW/day per oral use; PSC50: PCOS rats group receiving curcumin in SNEDDS at 50 mg/kgBW/day per oral use; PSC: PCOS rats group receiving curcumin in SNEDDS at 100 mg /kgBW/day per oral use. a: significantly different from the PCOS rats group with no therapy (PCOS); b: significantly different from the normal control group (N).

Insulin Level

Table 3 and Figure 4 show that insulin levels are highest in the group of PCOS rats without therapy (PCOS) and lowest in PCOS which is given SNEED Curcuma L 100 mg/kgBW. The ANOVA test revealed that the results of the mean difference test for serum insulin levels showed significant differences in all sample groups. (p<0,05)). On average, serum insulin levels in PCOS rats without therapy were significantly higher (p<0,05) than all other groups. However, there were no significant differences between treatment groups. These results showed that PCOS elevated insulin levels, which decreased in response to SNEDDS *Curcuma longa* extract at doses of 25, 50, and 100 mg/kgBW, which is equal to metformin administration. A substantial difference, however, was not observed between treatments.

HOMA-IR Score

Table 4 and Figure 5 show that HOMA-IR score are highest in the group of PCOS rats without therapy (PCOS) and lowest in PCOS which is given SNEED Curcuma L 100 mg/kgBW. The ANOVA test revealed that the results of the mean difference test for HOMA-IR score showed significant differences in all sample groups. (p<0,05). On average, HOMA-IR score in PCOS rats without therapy were significantly higher (p<0,05) than all other groups. However, there were no significant differences between treatment groups. These results showed that PCOS elevated HOMA-IR score, which decreased in response to SNEDDS *Curcuma longa* extract at doses of 50, and 100 mg/kgBW, which is equal to metformin administration. A substantial difference, however, was not observed between treatments.

Table 4: The result of the Kruskall Wallis test on HOMA-IR score

Group	Median (Min-Max)	P
N	1.05 (0.87 – 1.33)	0.000*
PM	0.89 (0.65 – 1.20)	
PCOS	2.42 (2.19 – 3.02)	
PSC25	1.33 (1.05 – 1.94)	
PSC50	0.80 (0.62 – 1.20)	
PSC100	0.78 (0.75 – 0.90)	

Note: N: Normal control group; PM: PCOS rats group with metformin 20 mg/kgBW per oral; PCOS: negative control group, PCOS rats without treatment; PS 25: PCOS rats group with SNEDDS *Curcuma longa* extract 25 mg/kgBW/day per oral; PSC50: PCOS rats group with SNEDDS *Curcuma longa* extract 50 mg/kgBW/day per oral; PSC100: PCOS rats group with SNEDDS *Curcuma longa* extract 100 mg/kgBW/day per oral

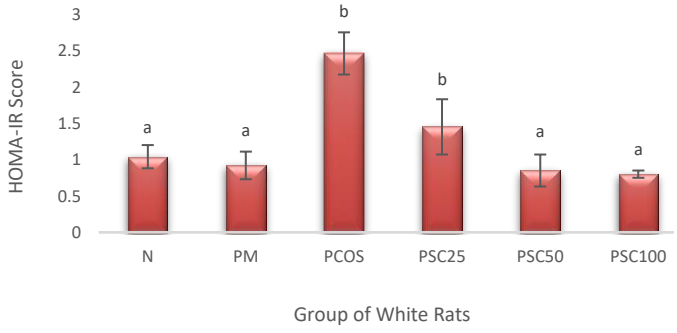

Figure 5: Graph of Mean Score of HOMA-IR Scores in Each Group

Note: N: Normal control group and not receiving treatment; PM: PCOS rats group receiving metformin at 20 mg/kgBW/day per oral dose; PCOS: Negative control group, PCOS rats without treatment; PSC25: PCOS rats group receiving curcumin in SNEDDS at 25 mg/kgBW/day per oral use; PSC50: PCOS rats group receiving curcumin in SNEDDS at 50 mg/kgBW/day per oral use; PSC: PCOS rats group receiving curcumin in SNEDDS at 100 mg /kgBW/day per oral use. a: significantly different from PCOS rats group with no therapy (PCOS); b: significantly different from normal control group (N)

The study using the Kruskall Wallis test, and the Mann Whitney post hoc test indicated that there were statistically significant differences (p <0.05) among all groups. Although the HOMA-IR score of the PCOS group was similarly to that of the PCOS group that received SNEEDS curcumin 25 mg (p>0.05), it was considerably higher in the PCOS group than in the normal, PCOS groups treated with metformin and SNEED Curcuma 50 mg and 100 mg (p<0,05). On the other hand, there was no significant difference (p>0.05) observed between the PCOS groups receiving metformin, medium and large dosages of and curcumin SNEEDS therapy and the normal group. PCOS increased the HOMA-IR score, but treatment of SNEDDS *Curcuma longa* extract at doses of 50 and 100 mg/kgBW was associated with a decrease in the score, which was equal to the administration of Metformin.

The pathogenesis of insulin resistance in PCOS involves several factors, namely hyperandrogenism, high oxidative stress, and chronic inflammatory processes.[11] Hyperandrogens increase visceral fat lipolysis by reducing PPAR-γ expression in adipocytes. As a result, excessive release of FFA ultimately triggers an increase in oxidative stress. Oxidative stress directly impacts pancreatic beta cells' mitochondrial dysfunction, influencing glucose metabolism's coupling mechanism. There is an increase in the expression of the Ins-1 and Ins-2 genes in pancreatic β cells, resulting in hyperinsulinemia.[13,29] In addition, oxidative stress also increases cytokines such as TNF-α, IL-6 and IL-1β. Proinflammatory cytokines can induce insulin resistance by various mechanisms, such as activation of Ser/Thr kinase, decreased IRS-1, Glut-4 and decreased PPAR-γ and activation of SOCS-3.[44]

Various research has been conducted on the effects of pure curcumin. Curcumin has been proven to reduce the HOMA-IR score as an indicator of insulin resistance in PCOS model rats at several doses and durations of administration, namely doses of 100 and 300 mg/kgBW for 14 days[45], doses of 100 and 200 mg/kg BW for 30 days;[41,46] and doses of 50,100 and 200 mg/kgBW in the form of nano curcumin powder for 15 days.[23]

This research revealed that administering SNEDDS *Curcuma longa* extract at doses of 50 mg/kgBW and 100 mg/kgBW could increase Glut-4 expression in gastrocnemius skeletal cells and reduce fasting blood glucose levels, insulin levels, and HOMA-IR scores. In previous research, Curcumin isolate was needed at a dose of 100 – 300 mg/kgBW/day for 14-30 days to cause improvement in PCOS in various parameters.[45] Furthermore, this research showed that with SNEDDS *Curcuma longa* extract, a smaller dose was needed for a short time, namely 50 mg/kgBW/day for 14 days.The results showed that administration of SNEDDS *Curcuma longa* extract at doses of 25, 50 and 100 mg/kg BW significantly reduced Fasting Blood Glucose, insulin, and HOMA-IR scores in PCOS model mice. It indicated that SNEDDS *Curcuma longa* extract could improve insulin resistance in PCOS model rats.

Research limitations: (1) The PCOS model mice in this study were not reexamined for PCOS severity or HOMA-IR scores. The PCOS mouse model was established in strict accordance with preliminary research. This permits variations in study results; (2) PCOS was induced in rats by feeding them a diet high in sugar and cholesterol together with letrozole. The same standard food and drink were given to both PCOS models and normal rats during treatment. However, it was not established in this study whether switching from a high-fructose, high-cholesterol diet during PCOS induction to a standard diet during treatment had a positive impact on PCOS improvement.

Conclusion

Self-Nanoemulsifying Drug Delivery System (SNEDDS) *Curcuma longa* extract could increase the bioavailability of curcumin. Therefore, smaller doses could improve clinical and laboratory rats' models of PCOS through insulin sensitization activity . It was proven that the Self-Nanoemulsifying Drug Delivery System (SNEDDS) Extract of *Curcuma longa* at dose 50 and 100 mg/kgBW/days during 14 days,

increased skeletal muscle Glut-4 expression, decreased blood glucose levels, fasting insulin, and HOMA-IR scores in PCOS rat models.

Conflict of Interest

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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