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Effectiveness of *Moringa oleifera* Nanoparticles (*Self Nano Emulsifying Drug Delivery System)* on Insulin Resistance in the Prediabetes *Rattus norvegicus* Model

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ABSTRACT

The present study aimed to investigate the effect of *Moringa oleifera* nanoparticles (MoNP) supplementation based on the Self Nano Emulsifying Drug Delivery System on insulin resistance in prediabetes models. Twenty-five *Rattus norvegicus* were split into five groups, containing five rats each: normal control group (given a standard diet), as well as a prediabetes control group and three intervention groups fed a high-fat diet for four weeks. Those with fasting blood serum (FBS) ranged betwen 100-130 mg/dL were deemed prediabetic. The intervention groups were then given MoNP at dosages of 75, 125, and 225 mg/kg bw, respectively, whereas the normal and prediabetic groups received a regular diet. FBS, fasting insulin, TNF-*α*, IL-6, triglycerides, and HOMA-IR were all examined after treatment. MoNP significantly reduced TNF-*α*, IL-6, triglycerides, and HOMA-IR levels ($p<0.05$). It also generated a considerable rise in insulin levels in contrast to the prediabetic control group ($p<0.05$). Supplementation of MoNP at the lowest dose of 75 mg/kg body weight was able to lower FBS to normal levels from 130.04 mg/dL to 97.34 mg/dL and reduce HOMA-IR from 4.20 to 3.71 ($p < 0.05$). The administration of MoNP can reduce inflammatory cytokines and insulin resistance in the prediabetes model.

***Keywords*:** HOMA-IR, inflammatory cytokines, insulin resistance, *Moringa oleifera*, nanoparticles, prediabetes.

Introduction

Glucose metabolism abnormalities and dyslipidemia are substantial predictors for certain diseases e.g., cardiovascular problems, type 2 diabetes (T2DM), stroke, and all-cause mortality.[1,2] Prediabetes is defined as impaired glucose metabolism that does not meet the diagnostic criteria for type 2 diabetes.[3] The risk of developing diabetes for non-diabetes people was 14.0%, while the risk of developing diabetes was 21.9% for patients with a history of impaired fasting glucose (IFG).[4]

Evidence suggests that obesity considerably increases the risk factor for the progression of prediabetes to T2DM.[1] Individuals who possess a body mass index (BMI) higher than 23 Kg/m2 are more prone to diabetes by 43% for men, and 41% for women.[5] Obesity is associated with the presence of chronic low-grade inflammation and oxidative stress-inducing insulin resistance.[6,7] Obesity, insulin resistance, as well as decreased insulin secretion are the initial stage of the main indicators of prediabetes.[1] Malecular mediators from adipocytes and macrophages contribute to the pathogenesis of insulin resistance as shown by increased levels of proinflammatory cytokines TNF-*α*, IL-6 in prediabetes.[8]

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Preventive therapy is required due to the progressive nature of prediabetes and the development of early macrovascular, as well as microvascular complications, including nephropathy, retinopathy, and autonomic neuropathy. Lifestyle changes, as well as improved adherence to a plant-based diet[9], and flavonoid diet[10] play an pivotal role in controlling glucose metabolism. Flavonoids possess antiobesity and antidiabetic properties.[11] As a result, they have an influence on blood glucose levels, glucose tolerance, and insulin sensitivity.[12]

Moringa oleifera (*M. oleifera*) is a herbal remedy that contains a high concentration of flavonoids. The main flavonoids in *M. oleifera* are quercetin, kaempferol, apigenin, luteolin, and myricetin glycosides. *M. oleifera* mostly contains quercetin (43.75%), with the total concentration of phenolic content in its methanol extract ranges from 71.08 ± 12.05 to 76.63 ± 10.63 mg GAE/g, which is 22% greater than spinach. *M. oleifera* is therefore a superior source of phytochemicals.[13] Various treatments have been carried out at this point to examine the efficacy of *M. oleifera* in glycemic control. Related studies on animals suggest that *M. oleifera* can improve glycemic control by modulating hyperinsulinemia, Peroxisome proliferator-activated receptor γ (PPARγ), and inflammatory cytokines.[14] However, studies on human reveal contradicting results, suggesting *M. oleifera* has insignificant effect on glycemic control.[15,16] Quercetin is a hydrophobic compound with high permeability but poor solubility. Quercetin is water insoluble (4.5 µg/ml), while quercetin and kaemferol are the main bioactive components as hypoglycaemic and hypolipidemic agents in *M.oleifera*.[17] Evidence suggests that quercetin has a low bioavailability of <17% in rats, and <2% in humans.[18] Wanjiru et al. also reported that polyphenols have low plasma membrane permeability, limiting their absorption into target cells.[19] It is also known that polyphenols in *M. oleifera* are destroyed by digestive enzymes and gastric secretions when taken orally.[20] However, drug delivery methods in nanoparticle formulations have the potential to improve therapeutic value by lowering toxicity, boosting

bioavailability, and improving drug stability against enzymatic degradation.[21] *Self Nano-Emulsifying Drug Delivery System* (SNEDDS) is a nano-sized drug delivery technology that can improve medication solubility and bioavailability [22]. SNEDDS can be used as a solution in the oral administration of lipophilic compounds.[21] While most studies on *M. oleifera* focus on the comsumption of fresh or dried leaves[23], investigations on prediabetes remain uncommon. Thus, in order to obtain further data regarding the efficacy of *M. oleifera* in treating insulin resistance, investigations on the role of *M. oleifera* in decreasing insulin resistance by engaging attempts to boost bioavailability are needed.

Materials and Methods

Preparation of the M. oleifera leaves' ethanol extract

Both young and old *M. oleifera* leaves, were collected in Klaten, Central Java, Indonesia (-7.613431,110.689477) in July 2022. It was identified in the laboratory of biology, Applied Science and Technology Faculty, Universitas Ahmad Dahlan, Yogyakarta, Indonesia, with voucher number of 382/Lab.Bio/B/VIII/2023. The samples were then sorted, rinsed, and drained before dried in a 50^{0}C oven for two days. Subsequently, they were crushed and sifted through mesh 40. A total of 341.3 grams of dried *M. oleifera* leaf powder was macerated using 96% ethanol solvent in a 1:5 ratio for three days, with daily stirring for $\pm$10 minutes. After the third day, the maceration results were filtered and the dregs were re-macerated in a 1:3 ratio for three days. The acquired product was processed using a rotary evaporator to produce a thick extract, which was then evaporated again to improve the thickness and avoid leaking. It produced a 41.9 g thick extract with a 12.2% yield.

Preparation of Self Nano Emulsifying Drug Delivery System (SNEDDS)

Oleic acid, Tween 80 and PEG 400 were mixed in the ratio of 1:8:1. After homogenizing the mixture with a vortex for 5 minutes, 125 mg of *M. oleifera leaf* extract was added in SNEDDS base solution (125 mg/3 ml). The misture was then homogenized for 30 minutes at 40^{0}C using a hotplate magnetic stirrer, followed by sonication for 15 minutes of at 40^{0}C. The resulting SNEDDS formula of *M. oleifera* leaf extract is called *M. oleifera* Nanoparticles (MoNP).

The formulation was evaluated by measuring the percentage of transmittance at 650 nm with a UV-Vis spectrophotometer (Genesys, USA). Emulsification time was calculated by estimating the time necessary for full suspension of SNEDDS in distilled water using an 80-rpm magnetic stirrer (IKA C-MAG HS7, Malaysia). Particle size, polydipersibility (Pdi), and potential zeta were determined by gently mixing 1 mL of SNEDDS with 100 mL of distilled water using magnetic stirrers. The Particle Size Analyzer (Malvern, United Kingdom) was used to measure these parameters.

Making a high-fat diet

The composition of a high-fat diet including 60% Comfeed PAR-S, 27.8% flour, 10% *pork* fat, 2% cholesterol, and 0.2% cholic acid, and in 100 grams of feed. The mixture was also crushed and shaped into pellets, which were oven-dried for 2 hours at 180^{0}C. The high-fat diet formulation was adapted from a prior research with minor modifications.[24]

Protocol approval

The Diponegoro University Health Research Ethics Committee approved the experimental protocol titled "Effectiveness of *Moringa oleifera* nanoparticles (*Self Nano Emulsifying Drug Delivery System)* on insulin resistance in the prediabetes *Rattus norvegicus* model" with reference number 25/EC/H/FK-Undip/IV/2022.

Animals used in the study

This study included 25 8-week-old male *Rattus norvegicus* weighing between 173 and 188 g, obtained from the Laboratory of Nutrition, Center for Food and Nutrition Studies, Gadjah Mada University, Indonesia. The rats were randomly separated into five groups. Each group of five rats was kept in a cage with unrestricted acces to Comfeed Par-S (Jabfa Comfeed Indonesia) and water.. In addition, the temperature in the cage was kept constant at 22±2^{0}C, with a 12-hour light-dark cycle.

Induction of prediabetes

Following one week of acclimatization, 25 rats were placed into two groups: normal control group (n=5) and prediabetes group (n=20). Those in the normal control group (NC) (n=5) were fed with a regular diet, whereas the others were given a high-fat diet (HFD; n=20) for four weeks to induce prediabetes. The state of prediabetes was confirmed when fasting blood serum (FBS) levels ranged between 100 and 160 mg/dl.[8] The results of FBS measurements in all groups fed a high-fat diet for 4 weeks yielded an average FBS value of 131.68 mg/dL ± SD 3,30 (126.04 - 140 mg/dL), which matched the prediabetes parameters.

Subsequently, rats in this weight range were split and divided into four groups, i,e., the prediabetes control group, the MoNP dose of 75, 150, and 225 mg/kg bw. Following that, each group received the following interventions:

Group I: Prediabetes rats administered with 75 mg/kg bw *M. oleifera* nanoparticles (MoNP 75).

Group II: Prediabetes rats administered with 150 mg/kg bw *M. oleifera* nanoparticles (MoNP 150).

Group III: Prediabetes rats administered with 225 mg/kg bw *M. oleifera* nanoparticles (MoNP 225).

Group IV: Normal control rats were given standard food (NC).

Group V: Prediabetes control rats were given standard food (PreDM-C).

The interventions lasted for four weeks. Furthermore, blood samples of the rats were taken at the end of of the fourth week through retro-orbital to examine fasting glucose level, fasting insulin, TNF-*α*, IL-6, TG, and HOMA-IR.

Blood sampling and biochemical assays

The rats were starved overnight. Blood samples were then drawn from each rat via the retro-orbital vein before being euthanized with chloroform. Furthermore, the blood samples from all groups were centrifuged at 3000 rpm for 10 minutes in a vacutainer. From each sample, 1 cc of serum was extracted and kept at –20^0 C.

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Glucose and lipid profile

Serum TG level was analyzed using a Triglyceride Kit Diasys (Germany). Assessment of fasting blood glucose levels was done using glucose kits for Rats from Diasys (Germany) and examined with spectrophotometers from Shimatzu (Japan). Fasting plasma insulin was assessed with a kit for Rats Fine Test (China) as well as ELISA examination devices from Zenix (New England). Furthermore, insulin sensitivity was examined using the HOMA-IR index, with the following formula HOMA-IR = (fasting glucose (mg/dL) x fasting insulin (μU/mL)/405.

Inflammatory profile

TNF-*α* and IL-6 levels were measured using RAT IL-6 Immunoassay Kit from Finetest (China) and assessed with an ELISA device from Zenix (New England).

Statistical analyses

The data obtained were processed using SPSS version 2.2 software. All quantitative variables were then written by reporting mean ± standar deviation. Normality test was carried out using Shapiro-Wilk (α = 0.05), while homogeneity testing was performed with Levene Test (α = 0.05). Since the data were deemed normal, as well as having the same variance, the analysis of variance (ANOVA) was done. The ANOVA results showed p <0.05. Thus, a post hoc least significant difference (LSD) test was also performed. All the analyses carried out in this study were done with 95% confidence.

Results and Discussion

The effects of *M. oleifera* leaf extract nanoparticles in self nano emulsifiying delivery drug (SNEDDS) preparation on blood glucose levels, fasting insulin levels, TNF-*α* levels, IL-6 levels, triglycerides,

and HOMA-IR in prediabetic rat models for 4 weeks were detected in this study. Different from the diabetic control group, the MoNP groups had lower fasting serum glucose levels, increased fasting insulin levels, lower levels of TNF-*α*, IL-6, triglycerides, as well as HOMA-IR.

Characterization of SNEDDS Moringa oleifera

The SNEDDS formulation in this investigation contains M. oleifera 125 mg/3mL in basic formula. The percentage of transmittance from SNEDDS *M. oleifera* was 98.073 with an emulsification time of 5.9 seconds. Physical observations after 24 hours at 5^0C, revealed that the formulation was clear, with no sediment or phase separation. As shown in Figure 1, MoNP have an average particle size of 86.48 nm, a polydispersity index of 0.2 (Figure 1a), and a zeta potential of -32.6 mV (Figure 1b). Nanoparticles with a diameters ranging from 10 nm to 200 nm have demonstrate satisfactory drug delivery properties.[25] Drug absorption is influenced by droplet size; the smaller the droplet size, the larger the interface surface for absoprtion.[26] Furthermore, the polydispersity (Pdi) of the SNEDDS *M. oleifera* was 0.2. The droplet dispersion and homogenity are reflected in Pdi. Pdi ≤0.25 indicates that the particle size distribution is homogenous and stable. For oral preparations, the Pdi value is less than 0.5.[27] In addition, zeta potential also monitors the charge on the surface of nanoemulsion droplets.[28]According to the current investigation, the zeta potential is -32.6 mV. Zeta potential values greater than >+30mV or less than ‗30 mV are regarded to have a high degree of stability in nano-drug delivery systems.[25]

Table 1: General characteristics of the group at baseline and after 4 weeks of intervention

Variables	Measurement period	Prediabetic rats MoNP (mg/kg bw)			Control group	
		75 (n=5)	150 (n=5)	225 (n=5)	Prediabetes rats (n=5)	Normal (n=5)
BW (g)	Before	231 ± 3.08	232.4 ± 2.7	232.8 ± 4.7	238.8 ± 3.11	212 ± 3.16
	After	254 ± 2.73*	258.2 ± 4.14*	257.2 ± 5.89*	295.4 ± 3.43*	235.4 ± 2.70*
Indeks Lee	Before	323.5 ± 4.04	331.2 ± 7.15	324.8 ± 3.14	330.09 ± 5.97	296.9 ± 0.80
	After	297.08 ± 2.61*	297.3 ± 2.05*	295.8 ± 2.16*	350.8 ± 3.48*	297.7 ± 1.18*
FBS (mg/dL)	Before	130.04 ± 1.68	132.23 ± 3.94	131.85 ± 0.78	133.81 ± 4.89	71.17 ± 1.21
	After	97.34 ± 1.81*	93.43 ± 2.55*	88.78 ± 2.34*	136.53 ± 4.01*	71.88 ± 1.28*
Insulin (μIU/L)	Before	13.07 ± 0.077	13.33 ± 0.18	13.48 ± 0.10	13.26 ± 0.18	16.85 ± 0.09
	After	15.44 ± 0.09*	15.80 ± 0.12*	16.29 ± 0.11*	13.03 ± 0.19*	16.69 ± 0.07*
HOMA-IR	Before	4.20 ± 0.07	4.35 ± 0.16	4.39 ± 0.01	4.38 ± 0.19	2.96 ± 0.05
	After	3.71 ± 0.06*	3.64 ± 0.09*	3.57 ± 0.11*	4.39 ± 0.16	2.96 ± 0.11

The results are described as means ± Standard Deviation (SD)
P value obtained from Paired t-test
* P-value < 0.05 before and after treatment for 4 weeks
Bw: Body weight
FBS: Fasting Blood Serum
HOMA-IR: Homeostatic Model Assessment of Insulin Resistance

Table 2: Effects of 4 weeks intervention MoNP on TNF-α, IL-6, FBS, fasting insulin, trygliserida, body weight, and HOMA-IR in the interventions group compared control group

Variables	Prediabetic rats MoNP (mg/kg bw)			Control group	
	75 (n=5)	150 (n=5)	225 (n=5)	Prediabetes rats (n=5)	Normal (n=5)
TNF-α (Pg/mL)	9.83 ± 0.67*,**	7.9 ± 0.31*,**	7.0 ± 0.47*,**	17.9 ± 0.43	5.1 ± 0.37
IL-6 (Pg/mL)	18.14 ± 1.32*,**	14.07 ± 0.67*,**	12.26 ± 0.84*,**	36.74 ± 0.65	8.56 ± 0.46
FBS (mg/dL)	97.3 ± 1.81*,**	93.4 ± 2.55*,**	88.7 ± 2.34*,**	136.5 ± 4.0	71.8 ± 1.28
Insulin (pg/mL)	15.44 ± 0.09*,**	15.80 ± 0.12*,**	16.29 ± 0.11*,**	13.03 ± 0.19	16.69 ± 0.07
Trygliserida (mg/dL)	91.3 ± 3.51*,**	83.4 ± 1.69*,**	80.8 ± 2.87*,**	136.5 ± 3.84	73.7 ± 2.11
BW (g)	254 ± 2.73*,**	258.2 ± 4.14*,**	257.2 ± 5.89*,**	295.4 ± 3.43	235.4 ± 2.70
HOMA-IR	3.71 ± 0.06*,**	3.64 ± 0.09*,**	3.57 ± 0.11*,**	4.39 ± 0.16	2.96 ± 0.11

The results are described as means ± Standard Deviation (SD)
P value obtained from one-way ANOVA followed by Tukey's test for post hoc analysis
* P-value < 0.05 between the MoNP group and the prediabetes group
**P-value < 0.05 between the MoNP group and the normal group
TNF-α : Tumor Necrosis Factor-α
IL-6 : Interleukin-6

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Basic characterization of research subjects

There were no study fatalities or adverse effects in any of the trial animals treated with MoNP during the course of the 9-week investigation. As indicated in Table 1, there was a substantial drop in the Lee index and FBS in all intervention groups, whereas an increase was noted in the controls. The average body weight of the groups given MoNP 75, 150, and 225 mg/kgbw was respectively 254±2.73, 258±4.14, 257±5.89 g. These numbers were lower than the average body weight of prediabetic control group 295,4±3.43 g. These findings support prior research suggesting that daily treatment of *M. oleifera* extract radically reduced body weight and liver hyperplasia in animals fed a fat/carbohydrate diet. Treatment with M. oleifera reduced de novo lipogenesis by upregulating lipogenic genes such as sterol regulatory element-binding transcription factors-1c (SREBF-1c) and fatty acid synthase (FASN).[29] However, only the groups treated with MoNP had a significant rise in insulin levels, while the others' remained unchanged.

The effect of administering MoNP on the inflammatory profile

The current research studied the efficacy of MoNP in affecting TNF-*α* and IL-6 levels to determine its anti-inflammatory status. Its results showed a considerable reduction in inflammatory biomarkers TNF-*α*, and IL-6, in the intervention group, in comparison to the prediabetes control group ($p < 0.05$). Table 2 shows that the administration of MoNP with a measure of 75, 150, as well as 225 mg/kg substantially lower the average level of TNF-*α* and IL-6. Meanwhile, all of the samples in the control group had a rise in these proinflammatory markers. Figure 2 demonstrates that the average level of TNF-*α*, and IL-6 dropped as the dosage increased ($p <0.05$).

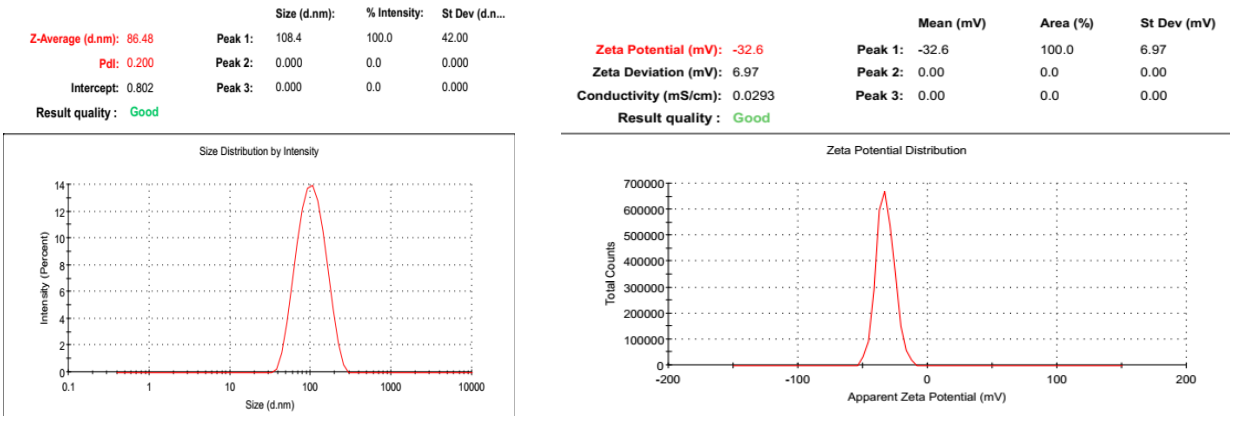
In vitro experiments revealed that *M. oleifera* leaf extract prepared by maceration with n-hexane, ethyl acetate, and ethanol demonstrated anti-inflammatory action, with IC50 values of 0.217 µg/mL, 0.257 µg/mL, and 1.029 µg/mL, respectively. The anti-inflammatory effect was strongest in the n-hexane axtract. However, the activity was still lower than diclofenac sodium as a positive control (IC50 = 0.110 µg/mL).[30] Phenolic compounds in *M. oleifera* have antioxidant and anti-inflammatory properties that can modify lipid peroxidation and prevent free radicals.[31] Fattah *et al*. also suggested that the treatment of *M. oleifera* extract at 200 mg/kg bw for 4 weeks significantly reduced TNF-*α* levels [32]. This is also in accordance with other studies examining the administration of *M. oleifera* 250 mg/kg bw in rat with diabetes induced by STZ 55 mg/kg bw which revealed a decrease in inflammatory cytokines in the kidneys (IL-1*α* (pg/ml), IL-12, IL-18) and liver (IL-1*α*, IL-12, IL-18) serum levels of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kb) and IL-18 in the kidneys, as well as IL-1*α*, IL-18 in the liver.[33] Kashyap et al. reported that *M. oleifera* has the activity of inhibiting IKBα phosphorylation, thus preventing the translocation and dimerization of IKBα and NFKB. This condition inhibits the formation of inflammatory proteins, e.g., TNF-*α*, cyclooxygenase-2 (COX-2), IL-6 and inducible nitric oxide synthase (iNos) thus reducing inflammation.[13]

The effect of giving MoNP on insulin resistance, lipid profile, and body weight

Table 2 reveals that administering MoNP at dosages of 75, 150, and 225 mg/kg bw reduced FBS. On the other hand, it rised insulin to normal levels. However, these changes were only observed in the intervention group, as the characteristics remained constant in the controls. Insulin levels in prediabetes control rats were lower than in normal rats. In contrast to the prediabetes control group, the administration of the treatment at dosages of 75, 150, and 225 mg/kgbw dramatically reduces HOMA-IR index. Furthermore, there was a notable rise in HOMA-IR level in the prediabetes control group, compared tho the normal group.

The hypoglycemic effect of *M. oleifera* is mostly attributed to three types of phytochemicals: phenolic acids (chlorogenic acid), glavonoids (quercetin and kaempferol), and glucosinolates which contain antioxidant properties. Quercetin and terpenoids stimulate the activity of glucokinase enzymes and pancreatic β-cells, thereby reducing insulin resistance. While the presence of isothiocyanate suppresses gluconeogenesis and glycogenolysis in the liver, as well as glucose absorption into adipose tissue and muscle. Moreover, *M. oleifera* counteracts insulin resistance in muscle through the activation of glucose transporter type 4 (GLUT-4) through an increase in the Akt signaling pathway. *M. oleifera* also promotes fatty acid oxidation via the AMP-activated protein kinase (AMPK) and/or PPAR-*α* pathways, yet inhibits trisylglycerol and cholesterol production via the control of sterol regulatory element-binding protein-1 (SREBP-1).[34]

Table 2 shows that the intervention group had a significant reduction in triglyceride levels when compared to the prediabetes control group ($p<0.05$). Figure 3 demonstrates that there was no significant change in triglyceride levels between the groups administered MoNP at 150 mg/kg bw and MoNP at 225 mg/kg bw (p-value = 0.879). Tang et al. also found that in diabetic animal models, treatment of *M. oleifera* leaf extract at a concentration of 150 mg/kgbw for 5 weeks lowered triglyceride levels.[35] Consumption of *M. oleifera* leaves can lower total plasma cholesterol while rising high-density lipoprotein (HDL). Because phytosterols limit absorption of dietary cholesterol and enhance excretion in the stool, the presence of β-sitosterol concentration in *M. oleifera* leaves can lower plasma cholesterol.[36] This study, however, contradicts the research of Setyawati *et al*, who found that giving *M. oleifera* leaf extract for 14 days at 50 mg/kgbw, 100 mg/kgbw, and 200 mg/kgbw did not significantly decrease triglyceride levels in diabetic rat models induced with Streptozotocin (STZ) 50 mg/kgbw.[37]



a b

Figure 1 : (a) Particle size and polydispersity index SNEDDS extract leaf *Moringa oleifera* , (b) Zeta Potential SNEDDS extract leaf *M. oleifera*

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Previous studies on the administration of *M. oleifera* extracts revealed that the average dosage of extracts in rats was approximately 200-300 mg/kgbw, and no adverse effects with methanol extracts up to a dose of 3000 mg/kgbw were seen.[23] Other investigations have found that the most effective in vivo dosage of *M. oleifera* administered was 200 mg/kgbw, which is consistent with these findings.[38] In this study, the selection of the MoNP dose formula of 75 mg/kg bw, 150 mg/kg bw was lower than the standard dose (200 mg/kg bw) as previous studies have found that nanoherbal formulas can increase the solubility of active substances, reduce therapeutic doses, improve absorption, and bioavailability of drugs in the body.[39] In line with other studies, the absorption of nanoherbal in the human body is almost 100%, while the micron size is only 50%.[40] Our findings provide evidence that the administering MoNP at concentration of 75, 150, and 225 mg/kg bw improved the inflammatory response, reduced insulin resistance, and reduced triglycerides in prediabetic rats.

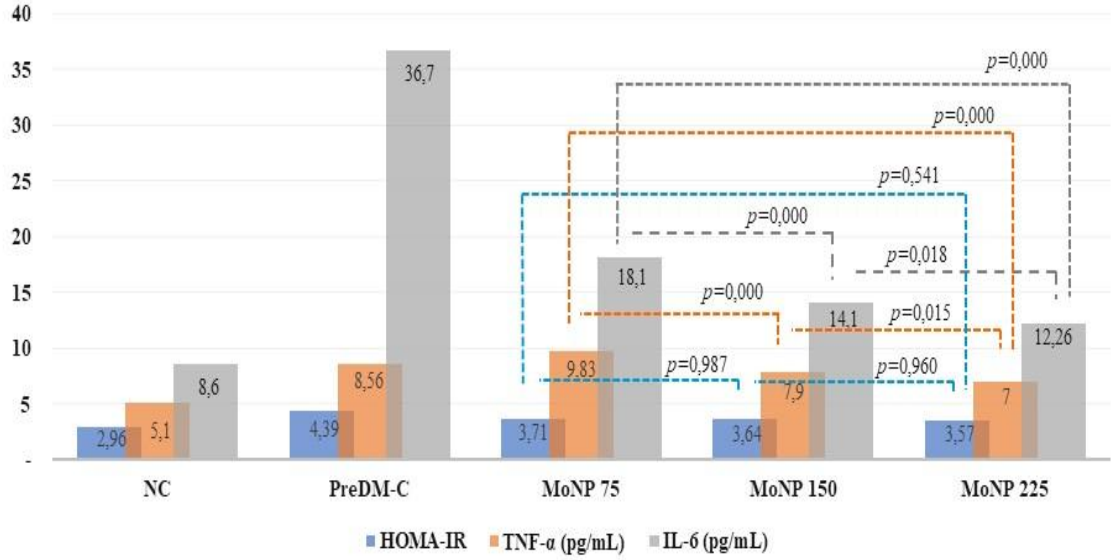

Figure 2: Differences in dosages of MoNP on levels of TNF-α, IL-6, and HOMA-IR. The results are described as means. P value obtained from one-way ANOVA followed by Tukey's test for post hoc analysis

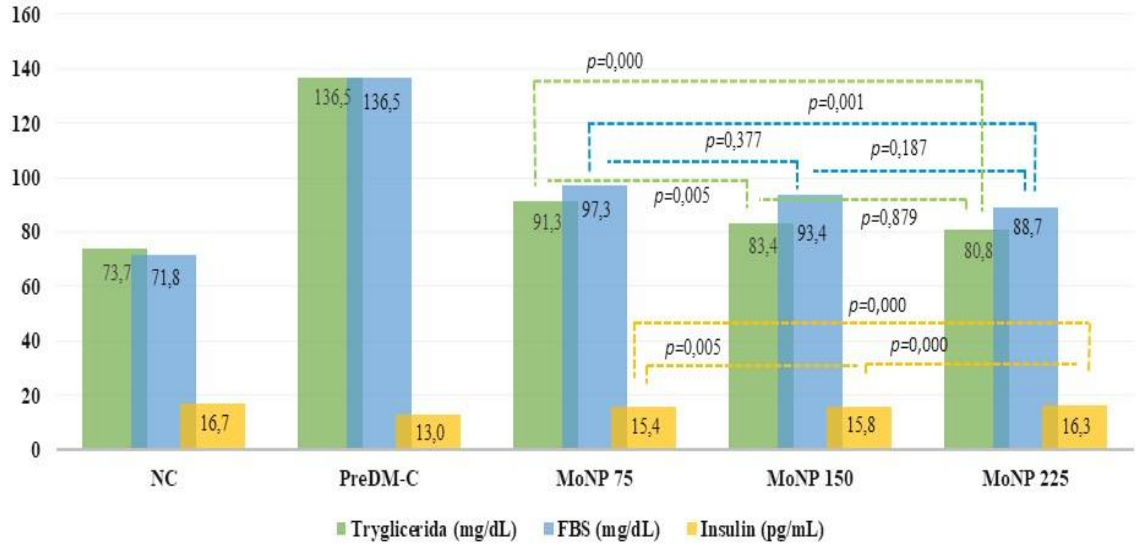

Figure 3: Differences in dosages of MoNP on levels of tryglicerida, FBS, and insulin. The results are described as means. P value obtained from one-way ANOVA followed by Tukey's test for post hoc analysis

Figure 3 shows the consumption of the intervention caused an increase in insulin levels and body weight, as well as a significant decrease in triglycerides between the dosage of administration. The reduction in FBS among rats given a dosage of 75 mg/kg bw did not statistically differ from the group given a concentration of 150 mg/kg bw. The highest decline in FBS was found in the group with 225 mg/kg bw MoNP. Furthermore, when the administration dosage was raised, FBS, triglyceride, and HOMA-IR levels reduced, whereas fasting insulin levels rose. The present study also reveals that there were no snoticeable variations in HOMA-IR between the dosing groups ($P > 0.05$).

Medicinal plants have low bioavailability and bioactivity. Nanostructured formulations of herbal extracts can potentiate their antidiabetic properties through pharmacokinetic regulation and bioavailability enhancement. Nano-sized droplets offer improved absorption and permeation of drugs through the gastrointestinal tract due to the large interface area.[41] Under hyperglycemic conditions, nanoformulations stimulate insulin release in INS 1 pancreatic beta cells and increased glucose uptake in the liver compared to extracts.[42]

Many studies have been reporting the antidiabetic effects of *M. oleifera* on animal models of steptozotocin-induced diabetes. Most of the previous studies used *M. oleifera* leaf extract preparations. The current investigation is the first study to evaluate the antidiabetic effect of MoNP in SNEDDS preparation in prediabetic animal models.

Previous research suggests that consumption of *M. oleifera* leaf extract at a dose of 500 mg/kg bw for 54 days, induced by a single treatment of STZ 45 mg/kg bw decreases blood glucose levels, fasting insulin levels, and weight loss, as well as and improves liver function in diabetic rats, however *M. oleifera* extract at a dose of 250 mg / kg bw only gave a partial antidiabetic effect.[43] Another study also demonstrated a decrease in serum glucose levels and an increase in insulin levels after consumption of *M. oleifera* leaf extract at a dose of 150 mg / kg body weight for 5 weeks in a diabetic animal model.[35] The results are in line with previous research, indicating the efficacy of nanoparticles preparations in glucose control. For instance, the administration of zinc oxide nanoparticles at a dosage of 7.5 mg/kg bw added to *M. oleifera* 250 mg/kg bw in diabetic animal samples provides a normoglycemic effect of 154.4 ± 4.5 mg/dL compared to the diabetic group (positive control) 315.7 ± 3 mg/dL, it also gives a normalizing effect on insulin secretion of 13.9 uiL/ mL in contrast to the positive control of 6.2 UiL/mL (p, 0.05).[44] In our study, the administration of *M. oleifera* leaf extract nanoparticles at a concentration of 75 mg/kg bw was able to normalize blood glucose levels (130.04 ± 1.68 mg/dL to 97.34 ± 1.81 mg/dL). The dosage employed in the current investigation was substantialy lower than in previous studies. The impact improves when the dosage is increased and is consistent with prior research findings.[43]

Moreover, insulin sensitivity in the present study was evaluated using the HOMA-IR index. A decrease in the HOMA-IR index indicates an increase in insulin sensitivity. The normal cut-off value of HOMA-IR is 1.85.[45] It is know from our study that the HOMA-IR index in the intervention group dropped considerably compared to the diabetic control group ($p < 0.05$), as presented in Table 2. The administration of MoNP in prediabetic rats with the smallest dose of 75 mg / kgbw has been able to reduce HOMA-IR in prediabetic rats from 4.40 ± 0.07 to 3.71 ± 0.06. Figure 2 shows that although there was a decrease in HOMA-IR, the HOMA-IR index did not differ significantly amongst the groups given dosages of 75, 150, and 225 mg/kg bw (p-value > 0.05).

According to earlier research, treatment with M. oleifera extract at concentrations of 100, 200, and 400 mg/kg bw in rat models is reported to reduce HOMA-IR levels.[14] Diabetic hyperglycemia induces significant oxidative stress and lowers the activity of the antioxidant defense system, increasing the development of free radicals. These free radicals promote insulin resistance by damaging pancreatic cells. Administering *M. oleifera* extract at a concentration of 200 mg/kg bw for 21 days in diabetic animal models can protect against oxidative damage, as demonstrated by a rise in the production of antioxidant enzymes, e.g., such as catalase (CAT), superoxide dismutase (SOD), glutathione-S-transferase (GST), as well as decreased lipid peroxidation (LPO).[46] Phenolic compounds posses important antioxidant properties due to, a large number of hydroxyl groups in their structure, which have a remarkable affect on scavenging free radicals. Polyphenols have the ability to aliminate free radicals by providing electrons or hydrogen atoms to different ROS. Nanoemulsions have been used to encapsulate various polyphenolic compounds due to their small size, large surface area, good stability and the ability to increase drug bioavailability.[47]

Conclusion

Administration of MoNP to prediabetic rats for 4 weeks caused a decrease in TNF-*α*, IL-6, FBS, TG, HOMA-IR and increased fasting insulin levels in all intervention groups. The smallest dose of MoNP (75 mg/kg bw) was able to reduce pro-inflammatory cytokines, TG, and HOMA-IR. This dose was much lower than the dose of *M. oleifera* administered in other studies. Therefore, it can be concluded that nanoparticle size can increase the bioavailability of *M. oleifera*, promising more beneficial effects in preventing the progression of prediabetes to the diabetic stage. The MoNP supplement can be regarded a good therapetic method for the prevention and treatment of prediabetes; nevertheless, due to limited scientific findings in this subject, further studies are required.

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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