RESEARCH ARTICLE

Granule Nanoparticle *Citrus sinensis* (L.) Osbeck Peel Lowers Blood Glucose Levels and HbA1C in Alloxan-induced Diabetes Rats

Maya Sari Mutia[1,*], Yelvreza Rastiani Sipayung[2], Dwi Elmiam[2], Widya Yanti Sihotang[3], Juliana Lina[4]

[1]Department of Histology, Faculty of Medicine, Dentistry, and Health Science, Universitas Prima Indonesia, Jl. Sampul No. 3, Medan, Indonesia
[2]Department of Medicine, Faculty of Medicine, Dentistry, and Health Science, Universitas Prima Indonesia, Jl. Sampul No. 3, Medan, Indonesia
[3]Department of Public Health, Faculty of Medicine, Dentistry, and Health Science, Universitas Prima Indonesia, Jl. Sampul No. 3, Medan, Indonesia
[4]Department of Anatomical Pathology, Faculty of Medicine, Dentistry, and Health Science, Universitas Prima Indonesia, Jl. Sampul No. 3, Medan, Indonesia

*Corresponding author. Email: mayasarimutia@unprimdn.ac.id

Received date: Sep 17, 2024; Revised date: Jan 16, 2025; Accepted date: Jan 28, 2025

Abstract

BACKGROUND: The peel of *Citrus sinensis* (L.) Osbeck (sunkist orange) peels, which are often seen as waste, actually contains valuable properties such as antioxidants, hypoglycemic, nephroprotective, and anti-inflammatories. The potential effects of *C. sinensis* peel on diabetes have been discussed but not clear yet. Therefore, this study was performed to evaluate the effects of Granule Nanoparticle Sunkist Peel (GNSP) extracts as an antidiabetic agent in alloxan-induced diabetic rats.

METHODS: The nanoparticle suspension was prepared by mixing a formulation of 0.2% chitosan and 0.1% sodium tripolyphosphate. The characteristics of nanoparticles were measured by flow time, tap index and angle of repose. Rats were induced with alloxan injection to create diabetes rat models. Rats were divided into five groups; normal control group, diabetic controls, and diabetic rats receiving either 50, 100, or 200 mg/kg/day GNSP. After 28 days of diabetes induction, rats were euthanized, and blood as well as tissue samples were collected. Blood glucose levels, HbA1c, and histopathology of the liver, kidneys, and pancreas were then assessed.

RESULTS: The particle size of the synthesized material was 92.3 nm, which confirmed the nature of nanoparticle. The characteristics of the granule nanoparticle were also in accordance with the standards for drugs suitable for consumption. The administration of GNSP in dose dependent manner significantly decrease blood glucose levels and HbA1C to normal levels compared to control group ($p<0.05$). Histopathological analysis indicated recovery in pancreas, liver, and kidney tissues following GNSP administration.

CONCLUSION: GNSP administration lowers blood glucose levels and HbA1C, as well as improved histopathological condition of pancreas, liver, and kidney in diabetic rats. These findings suggest the potential of utilizing GNSP as a potent antidiabetic agent.

KEYWORDS: *Citrus Sinensis* (L.) Osbeck, histopathology, hyperglycemia, nano medicine, and type 2 diabetes mellitus

Indones Biomed J. 2025; 17(1): 60-9

Introduction

Diabetes mellitus presents a rapidly escalating global health challenge, with an estimated 285 million affected individuals in 2010 and a projected rise to 430 million by 2045.(1) Preventing type 2 diabetes mellitus (T2DM) is a multifaceted challenge, but evidence indicates that it's achievable through lifestyle changes and identifying high-risk individuals.(2) While metformin, liraglutide, and sulfonylureas are effective in treating T2DM, they can cause gastrointestinal side effects.(3) These side effects

Copyright © 2025 The Prodia Education and Research Institute.
This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 International (CC-BY-NC) License.

can be particularly problematic for patients, leading to discomfort and potentially impacting treatment adherence. However, recent advances in transdermal drug delivery systems for metformin may help alleviate these side effects.(4) Additionally, it's important for physicians to be aware of rare side effects such as liver injury associated with liraglutide.(5) Therefore, research scientists are actively racing to develop more efficacious diabetes treatments with fewer side effects.

Medicinal plants, especially, offer a wealth of natural antioxidants and bioactive phytochemicals, making them a promising substitute for synthetic medications.(6) Natural therapies have proven effective to manage diabetes with several studies indicating the potential of certain herbs in this regard.(7) Around 400 species of plant products (including seed, peel, root, fruit, bulb, flower) have been recognized for their antidiabetic properties, such as *Aloe barbadensis*, *Cinnamomum zeylanicum*, *Scoparia dulcis*, *Vinca rosea*, etc.(8,9) The active compounds in these plants, including phenolic compounds, flavonoids, terpenoids, and coumarins, have been discovered to lower blood glucose levels.(10) One of the traditional plants known for its effects in preventing diabetes is *Citrus sinensis* (L.) Osbeck (sunkist orange) peel. Previous studies indicate that *C. sinensis* peel extract exhibits several pharmacological effects.(11,12) *C. sinensis* peel contains secondary metabolites include flavonoids, saponins, citronella, and steroids.(13) Futhermore, extracts from various citrus fruit peels, such as orange, lime, and sour orange, also have shown antihyperglycemic effects in animal models.(14)

Advancements in pharmacy science and technology have led to develop nanoparticle as a system to delivery the drug, which are considered the most promising due to their adjustable size and adaptable basic properties such as solubility, diffusivity, and absorption. Nanoparticles have shown significant potential in drug delivery systems, as evidenced by their applications in various fields, including education and daily life.(15) Nanoparticles present a promising drug delivery system as they can be tailored in size and properties, providing benefits such as enhanced solubility, reduced drug doses, and improved absorption.(15) They are applicable across various routes of administration and for a wide range of treatments, including cancer, diabetes, and infections.(16) Controlled drug delivery using nanoparticles can also enhance therapeutic control and patient compliance.(17) Although the previous studies have shown that *C. sinensis* peel has antidiabetic potential, however the mechanism and pharmaceutical dosage forms in the treatment of diabetes are still unclear. The potential of nanoparticles in drug delivery is further underscored by their capacity to improve solubility, absorption, and targetability.(18) Our previous study found the potential effect of the methanol extract of *C. sinensis* peel on diabetic rats.(12,19) The current study used different materials and examined different parameters (methanol extract vs. nanoparticle granule), which focused on the right dosage form to be used as an antidiabetic. This is crucial since the dosage form may have a huge influence on how the body absorbs the drug so that it can work optimally in the body. The quality of a drug's design and formulation directly affects how well the body absorbs it. Hence, this study was carried out to experimentally validate the antihyperglycemic, antihyperlipidemic, and antioxidant properties of Granule Nanoparticle Sunkist Peel (GNSP). This study might give suggestions regarding the appropriate pharmaceutical dosage form of GNSP for diabetes.

Methods

Preparation of Determination of Formula for GNSP

C. sinensis (L.) Osbeck (sunkist oranges), which was originally from Berastagi, was purchased from a market in Medan, North Sumatra, and were carefully selected for their freshness and quality. The *C. sinensis* peels were dried in a shaded room with natural air circulation and then crushed. Subsequently, 250 g of *C. sinensis* peel powder was soaked in 5 L of ethanol (EMSURE® ACS Merck, Darmstadt, Germany) for 72 hours. Then, the extract was filtered with filter paper and stored at 4°C.(20) Fifty mL of *C. sinensis* peel extract was dissolved in 0.5 mL DMSO (Cat No. 102.952; Sigma-Aldrich, St. Louis, MO, USA) and then mixed with 0.1% natrium triphosphate (Cat No. 7758-29-4; Henan Honghai Chemical, Henan, China). This mixture was slowly added to a 0.2% chitosan solution containing 0.5 mL Tween 80 (Cat No. 9005-65-6; Sigma-Aldrich), using a magnetic stirrer until all the sodium tripolyphosphate solution was used and a nanoparticle suspension was formed. The nanoparticle suspension was frozen in a freezer for 24 hours, then mixed with Avicel PH 101 (Cat. 9004-34-6; DuPont Nutrition, Wilmington, DE, USA) and homogenized to form granules. Lactose (Cat. No. 63-42-3; Sigma-Aldrich) was added and homogenized. In another container, sodium and *C. sinensis* peel nanoparticles with a concentration of 4.8% were dissolved in 50 mL distilled water. The mixture was sifted using a 14-mesh sieve, and the granules were dried in an oven at 50°C for 14 hours. Afterward, sifting was performed again using a 16-mesh

sieve, and the granules were weighed and their physical characteristics evaluated.(21) The detailed composition of the formulation could be seen in Table 1.

Table 1. The formula of GNSP.

Material	Formula (%)			
	I	II	III	IV
C. sinensis peels extract	2	4	6	8
Pvp 5%	1	1	1	1
Amylum	10	10	10	10
Lactose	77	84	83	81
Distilled water	10	0	0	0

Evaluation of GNSP

The particle size of granule nanoparticle was measured with Particle Size Analyzer (PSA) ANALYSETTE 22 NanoTec (FRITSCH GmbH, Idar-Oberstein, Germany). During stability assessments, GNSP underwent tests for organoleptic properties, flow time, mass density, angle of repose, and determination. Organoleptic evaluation involved assessing the appearance, color, smell, and taste of the granules to ensure consistency in shape and color among the samples produced.(22) To ensure high-quality granules, the procedure involves weighing 100 grams of material and placing it in a closed funnel of the flow time test apparatus. The time was recorded when the cover was opened until the granules start flowing. This process was repeated three times. A good granule flow was indicated by no more than 10 seconds required for 100 grams to flow.(23,24)

The process for obtaining high-quality granules involves several steps. First was the flow time test as mentioned earlier. Next step was the calculation of repose angle by passing 25 grams of granules through a flow properties test funnel and measuring the angle formed between the powder pile and the horizontal plane. Additionally, the flow properties were measured by tapping a certain amount of granules using a volumeter. The determination of the index was conducted by placing 50 grams of granules in a measuring cup and recording the initial volume. The measuring cup was then tapped manually 100 times until the granule volume stabilizes. Granules with a firming index of less than 20% exhibit good flow properties.(23)

Experimental Induction of Diabetes

The induction of diabetes was performed in rats by administering a single intra-peritoneal injection of 125 mg/kg alloxan (Sigma Chemical Company, St. Louis, MO, USA).(24) Alloxan has been widely used to induce experimental diabetes in animals, primarily through its ability to selectively destroy pancreatic β-cells with dosage injections 100-150mg/kg.(25) In this study, alloxan was chosen since it has consistent ability to induce diabetes in experimental animals and offers a lower risk of animal death.(26) Diabetes was confirmed by analyzing blood from the rats' tails using the glucose oxidase method with a glucometer, 72 hours after alloxan injection. Glucose levels ≥200 mg/dL indicated hyperglycemia in rats that received alloxan.

Animal Treatments

This study involved adult male Wistar rats, each weighing between 250-300 grams. The rats were housed in polycarbonate cages. They were provided with appropriate living conditions and had unlimited to access daily food or water. Body weights were monitored weekly using digital scales. The rats were allocated into 5 groups, with each containing 5 rats. Group 1: Normal control group; Group 2: Diabetic rats received no treatment as diabetic control group; Group 3: Diabetic rats treated with 50 mg/kg BW/day GNSP; Group 4: Diabetic rats treated with 100 mg/kg BW/day GNSP, Group 5: Diabetic rats treated with 200 mg/kg BW/day GNSP. All the treatments groups were administered with GNSP for 28 days.

At the end of the study, the rats were anesthetized with chloroform and the blood samples were collected, before being euthanized. Several organs were collected and 10% formalin was used to preserve liver, kidney, and pancreas tissues. This study protocol was approved by the Health Research Ethics Commission of Universitas Prima Indonesia (No. 067/KEPK/UNPRI/XI/2023).

Measurement of Blood Glucose Levels

Blood samples were collected from all groups following an overnight fast using the tail prick method. A small drop of blood was collected from the tail tip using a sterile lancet and immediately applied to a glucose test strip. Blood glucose levels were measured using a glucometer (Ascensia Diabetes Care, Basel, Switzerland) according to the manufacturer's instructions. The glucometer utilizes a glucose oxidase-based electrochemical sensor to measure the glucose concentration in the blood sample. The results were displayed in milligrams per deciliter (mg/dL). Measurements were taken on day-0, -7, -14, and -28 to assess baseline glucose levels and monitor changes over time.

Serum HbA1c Examination

Serum samples were obtained by centrifuging blood samples at 4000 rpm for 10 minutes. HbA1c levels were measured using a Rat HbA1c Assay Kit (Cat. No. 80300; Crystal Chem, Elk Grove Village, IL, USA) with assay range 3.5-13% according to the manufacturer's instructions. The assay utilized a colorimetric method to quantify glycated hemoglobin, a marker of long-term glycemic control. The absorbance of the samples was measured at 450 nm using a microplate reader. HbA1c levels were calculated based on a standard curve generated using a HbA1c standard provided in the kit.

The Histological Examination

The organs were promptly removed, rinsed with cold normal saline, dried, and then weighed. Preserved tissue underwent dehydration, cleaning, and embedding in paraffin wax. Seven µm tissue sections were cut from the paraffin blocks and stained H&E dyes. The slides were captured at 400x magnification of a light microscope for histopathological analysis.

Results

Particle Size of GNSP

The Polydispersity Index (PI) suggested a broad distribution of particle sizes in the sample, characterized by multiple peaks and varying particle sizes (heterogeneous). The result of the particle size obtained from the third quartile (%) was 9.23 x 0.01 µm. Therefore, the nanoparticle size obtained from the PSA calculation was 92.3 nm (Figure 1), which strongly affirming the definite nano-scale of the synthesized material, which typically falls within the range of 1 nm to 100 nm.

The flow time test of granulated GNSP revealed that the water flow times at F1, F2, F3, and F4 were 5.11±9.194, 4.71±9.088, 4.71±9.432, and 3.9±8.656 seconds, respectively. However, the results of the flow time test met the requirements after three repetitions, where good granule flow was achieved if the time required to flow 100 grams is no more than 10 seconds. Additionally, the mean±SD values indicate that F1>F4, with values of 5.11±9.194 being greater than 3.9±8.656 (Table 2).

The angle of repose test was considered to flow well if they had an angle of repose between 25-45 degrees. F1 angle was 13.2±25.15º, F2 angle was 13.16±25.22º, F3 angle was 13.37±26.06º, and F4 angle was 12.16±24.96º (Table 2), all falling within the required range. Additionally, the average height (h)±diameter (d) for F3 is greater than that for F4, with values of 3.1±13.2 and 2.83±12.16, respectively. In the tap index test, nanoparticles were in granule form. From these results, the tap index was easy to flow, the granules properties were very good. Based on the data, formula F2 has the lowest tap index (6.89%). A lower tap index indicated that the granule flows more easily, which means formula F2 had the best flow properties among the four formulas. Therefore, formula F2 was used in this study.

GNSP Decreased Blood Glucose Level in Dose-dependent Manner after Diabetic Induction

The diabetic rats with no treatment (Group 2) experienced diabetes which was characterized by the increasing of blood glucose level to 401.7±2.20 mg/dL. However, compared to the first day of treatment, blood glucose level decreased to 220±1.49 mg/dL after 28 days of treatment with GNSP 200 mg/kg BW of rats (Group 5). A significant decrease in glucose levels after GNSP treatment was observed on day-7, followed by day-14 and -28. This indicated the hypoglycemic (blood sugar lowering) effect of GNSP. The

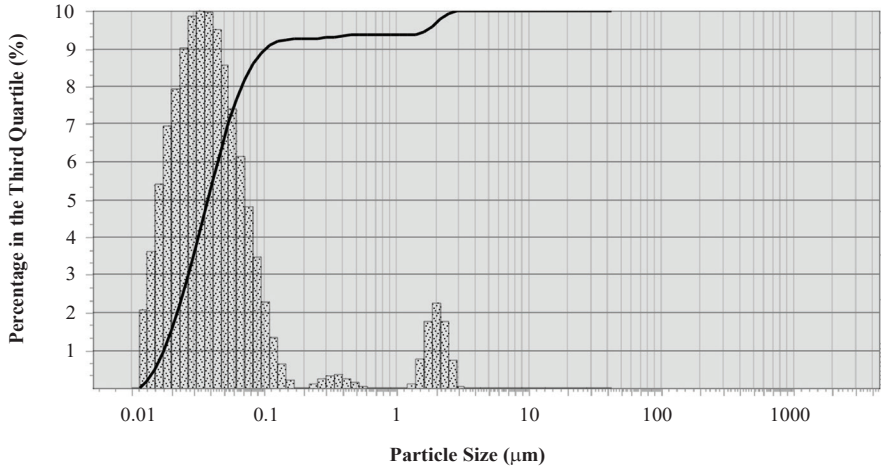

Figure 1. Particle size of GNSP.

Table 2. Flow rate of GNSP.

Sample	Flow Rate (s)	Angle of Repose (°)	Index Tab
	Mean±SD		%
Formula I	5.11±9.19	13.20±25.15	8.47
Formula II	4.71±9.09	13.16±25.22	6.89
Formula III	4.71±9.43	13.37±26.06	8.55
Formula IV	3.90±8.66	12.16 ± 24.96	8.67
Standard	< 10 s	20° < Ø < 40°	< 20%

decrease of blood glucose was in dose-dependent manner, and the most significant decrease could be seen in Group 5 (Figure 2).

GNSP Increased Body Weight after Diabetic Induction

The current study's findings revealed variations in weight among the experimental groups. Based on one way ANOVA analysis, there was a significant difference in body weight of the rats after being administered GNSP (p=0.023). Notably, the diabetic control group (Group 2) exhibited significant weight loss compared to Group 3, Group 4, and Group 5 (p=0.023). From this study, rats in Group 2 had a weight loss from 255.2±2.23 to 232.33±2.01 grams. However, this substantial weight loss was mitigated in diabetic rats treated with GNSP extract. The increase in the body weight of the rats after the GNSP treatment was observed on day-7 and continued to increase on day-14, -21, and -28. The rats gained of weight from 254.20±1.92 grams in initial week and increased to 271.99±2.84 grams after administered with 200 mg/kg BW GNSP (p<0.05) (Figure 3).

GNSP Decreased HbA1c in Dose-dependent Manner after Diabetic Induction

Based on the results from one-way ANOVA analysis, there was statistically significant difference in HbA1c levels between the groups after 28-day GNSP treatment (p<0.001). Group 2 experienced diabetes which was characterized by an increase in the percentage of HbA1c levels reaching 7.3%Hb. However, GNSP administration showed a significant decrease in dose-dependent manner. The most significant decrease was seen in the Group 5 group, with the decreased of HbA1c as much as 4.2 %Hb (Figure 4).

Effect of GNSP on Organ Weight

Based on the results presented in Figure 5, one-way ANOVA revealed significant differences in the mean weights of the pancreas (p<0.001), kidney (p<0.001), and liver (p<0.001) after 28 days of GNSP treatment. These results illustrated the weights changes of the pancreas, kidneys, and liver in four experimental groups. The weights of organs were higher in treatment group with GNSP (Group 3, Group 4, and Group 5) compared to Group 2 (p<0.05). Rats treated with GNSP showed increased organ weights, with pancreatic, kidney, and liver weight.

Histological Results of Pancreas, Kidney, and Liver

Microscopic analysis of kidneys from the diabetic group (Group 2) showed marked abnormalities in renal parenchymal architecture. The glomerulus showed Bowman's space dilation, degenerated cells and inflammation. In contrast, GNSP-treated diabetic rats (Group 3, Group 4, and Group 5) showed restoration of renal structure to a better appearance on histologic examination. Bowman's space dilation and degenerated cells were still found in each group. However,

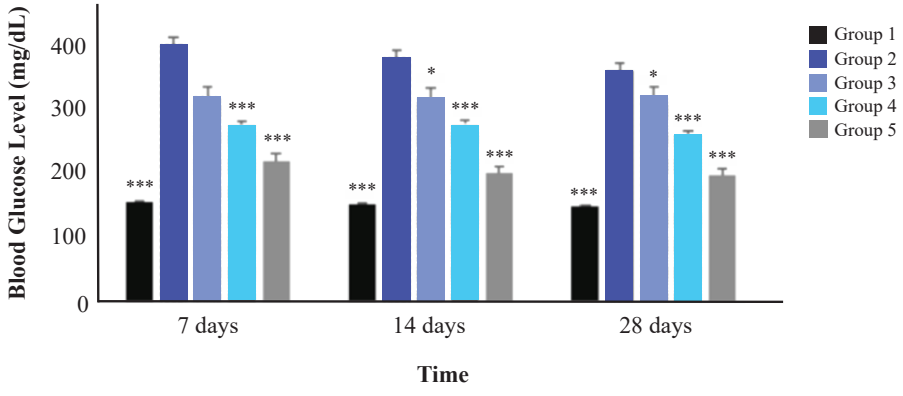

Figure 2. Blood glucose levels in the treatment groups. Values are presented as mean±SEM and analyzed with one-way analysis of variance (ANOVA) to compare mean values between the experimental groups, followed by Tukey post-hoc test for detailed group analysis. *p<0.05 compared with Group 2; **p<0.01 compared with Group 2; ***p<0.001 compared with Group 2.

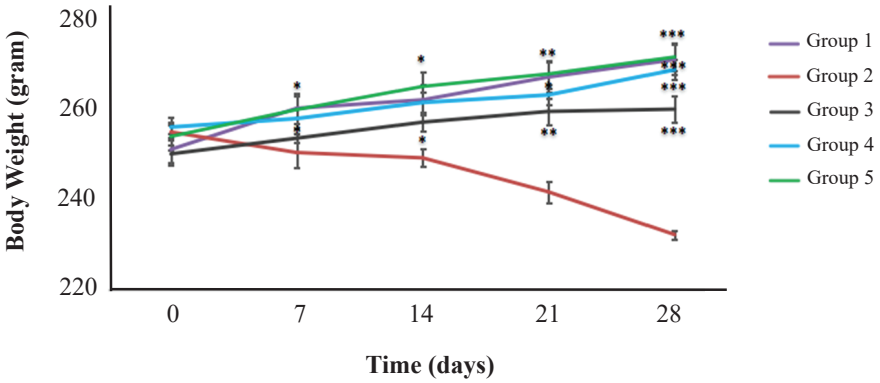

Figure 3. The GNSP effect on the percentage change in body weight. Values are presented as mean±SEM and analyzed with one-way analysis of variance (ANOVA) to compare mean values between the experimental groups, followed by Tukey post-hoc test for detailed group analysis. **p*<0.05 compared with Group 2; ***p*<0.01 compared with Group 2; ****p*<0.001 compared with Group 2.

it was clear that necrotic cells and degenerated cells decreased in all treatment groups after treatment with GNSP (Figure 6).

Discussion

Various medicinal plants contain natural compounds that can help in managing diabetes by regulating blood sugar levels.(27) These plants, such as bitter melon, fenugreek, cinnamon, and aloe vera, have shown antihyperglycemic effects by either increasing insulin production or reducing glucose absorption in the intestines. There is a growing interest in using these plant-based remedies for metabolic disorders, as they present potential benefits in diabetes management. Nonetheless, additional research is necessary to confirm their effectiveness and safety in treating diabetes. The peel of oranges, especially from the Sunkist variety,

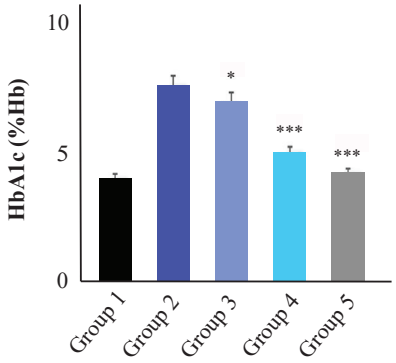

Figure 4. HbA1C levels in diabetic rats and after GNSP treatment. Values are presented as mean± SEM and analyzed with one-way analysis of variance (ANOVA) to compare mean values between the experimental groups, followed by Tukey post-hoc test for detailed group analysis. Values with distinct letters at the same treatment time indicate statistically there was a significant difference. **p*<0.05 compared with Group 2; ***p*<0.01 compared with Group 2; ****p*<0.001 compared with Group 2.

contains a range of pharmacological benefits, including antioxidant, anti-inflammatory, cholesterol-lowering, and blood sugar-regulating properties.(28)

In this study, granule formulation was chosen because it offers several advantages compared to other dosage forms. The chosen formulation might increase drug solubility.(29) The granulation process allows better control of particle size and flow properties so that it dissolves quickly and increases the body's absorption of the active substances of the drug.(30) Proper granule formulation and super disintegrant lead to superior drug products with improved shelf life, quicker onset of action, and increased therapeutic efficacy.(30,31) The flow rate of pharmaceutical preparations will affect the ability of drug delivery through tap index, flow rate, and angle of response. (29) The evaluation of GNSP showed a high tap index, a high flow rate, and a good angle of repose. The angle of response is a parameter to evaluate the flow properties of a powder or grain.(32) The smaller the response angle, the better the flow properties. Particles with a good index tab will be tightly packed, reducing the risk of segregation and volume changes during drug storage and transportation. In addition, the high index tab will also optimize the capsule or tablet filling process so that the right amount of dosage is obtained in each dosage unit.

The results of this study confirm that GNSP has a significant antidiabetic effect in diabetic rats. It decreases blood pressure and HbA1c levels. In diabetic rats, sustained high blood glucose levels lead to elevated HbA1c, which is a key biomarker in assessing long-term glycemic control. (33) High HbA1c is associated with several diabetes complications, including kidney damage.(34) GNSP showed a potential effect in preventing diabetes and its complications by lowering blood glucose and HbA1c levels. In terms of body weight, control rats continuously increased body weight, while diabetic rats decreased body weight from the first week after alloxan injection. This

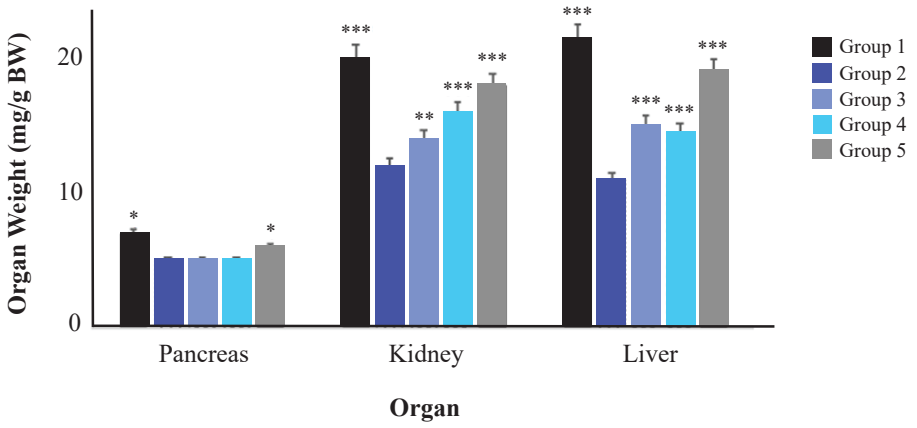

Figure 5. Effect of GNSP on Pancreas, Kidney, and Liver Weights. Values are presented as mean±SEM and analyzed with one-way analysis of variance (ANOVA) to compare mean values between the experimental groups, followed by Tukey post-hoc test for detailed group analysis. *p<0.05 compared with Group 2; **p<0.01 compared with Group 2; ***p<0.001 compared with Group 2.

condition may be due to decreased glucose metabolism and increased fat metabolism. GNSP showed a good increase in body weight of rats, especially when treated at a dose of 200 mg/kg body weight of rats. This may be due to *C. sinensis* peel extract has the ability in improving tissue antioxidant status, reducing lipid peroxidation, and preventing oxidative damage through various antioxidant compounds such as flavonoids and phenolics.(35) Antioxidants from medicinal plants are increasingly recognized for their ability to counteract oxidative stress and manage complications associated with diabetes.(36) The study revealed a strong blood sugar-lowering effect of the *C. sinensis* peel extracts with granule nanoparticle formulation on alloxan-induced diabetic rats, which was more pronounced than the effects of the extracts used individually.(37) The recovery was confirmed through histopathological assessment, revealing the restoration of pancreatic, liver, and kidney tissues. Pancreatic tissue repair, especially of insulin-producing cells, can increase insulin production and improve blood sugar control, while the liver is often damaged by fat accumulation and inflammation.(38) Liver tissue repair will improve liver function in regulating blood sugar levels and processing harmful substances.(39) Furthermore, the repair of kidney tissue leads to the prevention of complications such as diabetic nephropathy.(40) GNSP effectively shielded and rejuvenated these tissues, consistently demonstrating its antidiabetic effects throughout the study duration, encompassing the observation.

This study marks the first documentation of the antidiabetic of GNSP in an alloxan-induced diabetic rat model, suggesting encouraging results.(23) Further investigations combining extracts from various parts of the *C. sinensis* plant (such as leaves, seeds, roots, pods, fruit, and flowers) are recommended to identify the most potent hypoglycemic and antioxidant combination. These results suggest promising prospects for developing potent antidiabetic drugs from GNSP for diabetes treatment and management. However, there is limited research on the antidiabetic potential of *C. sinensis* peel in humans, with most studies focusing on alloxan or streptozotocin-induced diabetes models. Clinical trials on GNSP in human subjects are needed to confirm its protective effects and determine appropriate dosages for humans, potentially leading to the development of natural antidiabetic therapies. Another experimental study with bigger number of samples are also recommended to obtain more power for the statistical analysis.

Conclusion

The findings of this study indicate that GNSP administration effectively reduces blood glucose and HbA1c to normal levels compared to diabetic rats. The results also show that GNSP administration improve the histopathological condition of pancreas, liver, and kidney. Therefore, it is suggested that GNSP might be potential to be used as a traditional medicine in the treatment of diabetes and its complications.

Authors Contribution

MSM was involved in the concepting and planning of the research. YRS and DE performed the data acquisition/ collection. JL calculated the experimental data and performed the analysis. WYS drafted the manuscript and designed the figures. MSMs aided in interpreting the results. All authors took parts in giving critical revision of the manuscript.

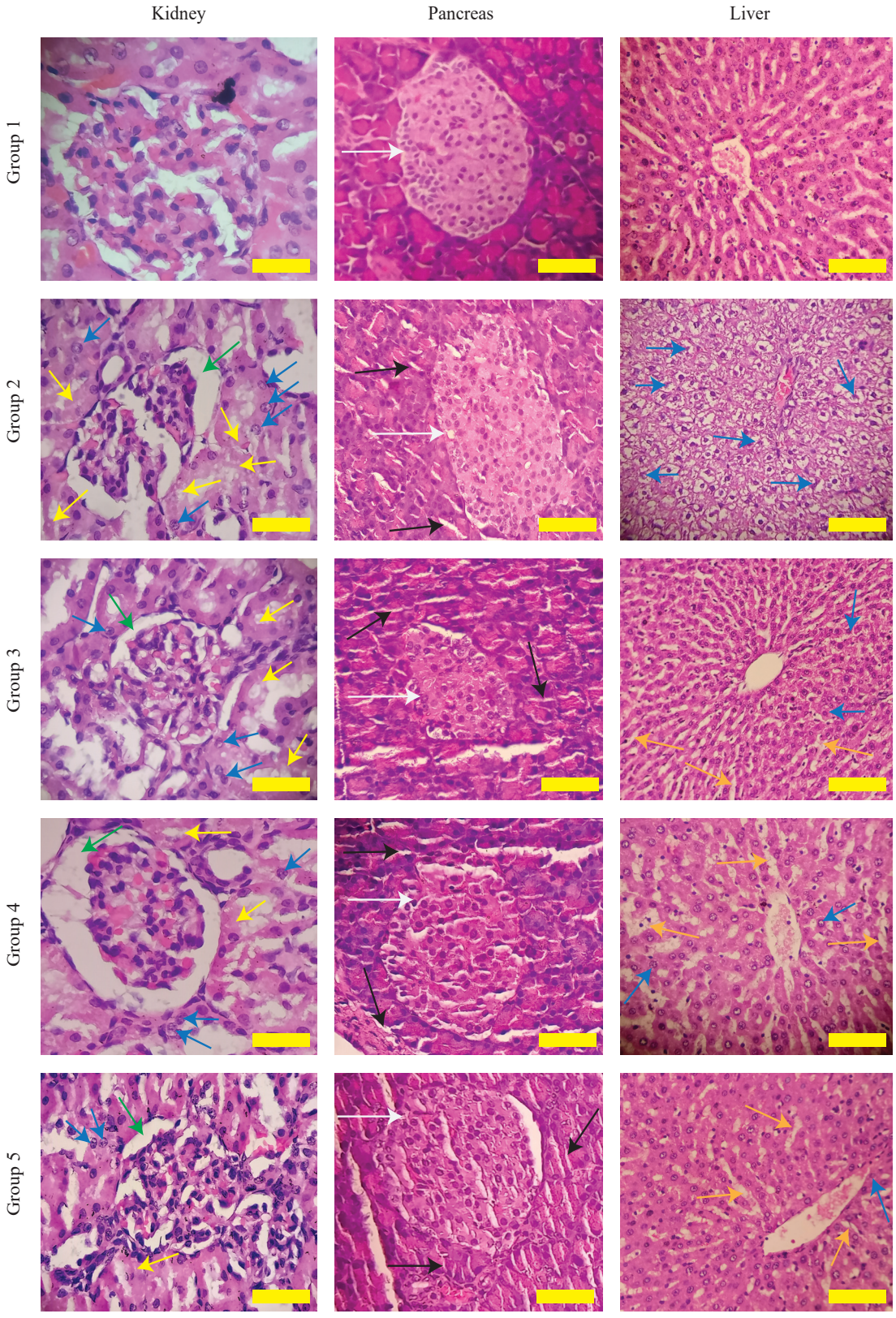

Figure 6. Histological of the kidney, pancreas and liver after 28 days of treatment with GNSP. Sections were stained with hematoxylin and eosin (H&E) and examined under a microscope at 400 × magnification. Yellow bar: 10 µm. Blue arrow: degenerative cell; Yellow arrow: necrosis; Green arrow: dilation of Bowman chamber; White arrow: Langerhans island; Black arrow: pancreatic acini; Orange arrow: dilation of liver sinusoids.

References

1. Agarwal C, Jahan T, Parveen N, Yousuf Y. Diabetes: The silent killer. 2018; 5(8): 444–6.
2. Bhatti JS, Sehrawat A, Mishra J, Sidhu IS, Navik U, Khullar N, *et al.* Oxidative stress in the pathophysiology of type 2 diabetes and related complications: Current therapeutics strategies and future perspectives. Free Radic Biol Med. 2022; 184: 114–34.
3. Haq FU, Siraj A, Ameer MA, Hamid T, Rahman M, Khan S, *et al.* Comparative review of drugs used in diabetes mellitus—New and Old. J Diabetes Mellit. 2021; 11(4): 115–31.
4. Wang J, Zhang Y, Li B, Zhao Z, Huang C, Zhang X, *et al.* Asthma and allergic rhinitis among young parents in China in relation to outdoor air pollution, climate and home environment. Sci Total Environ. 2021; 751: 141734. doi: 10.1016/j.scitotenv.2020.141734.
5. Salehi S, Mehrpour S, Koohmanaee S, Maleknejad S, Esfandiari MA, Maroufizadeh S, *et al.* Is there any need for the early detection of non-alcoholic fatty liver disease in children with type 1 diabetes mellitus? Hepat Mon. 2023; 23(1): e135941. doi: 10.5812/hepatmon-135941.
6. Lautié E, Russo O, Ducrot P, Boutin JA. Unraveling plant natural chemical diversity for drug discovery purposes. Front Pharmacol. 2020; 11: 1–37.
7. Dey P, Sowmya A, Jain AM, Sreenath A, Saji GS. Advances and prospects of medicinal herbs and their natural bioactive components for effective control of diabetics. Res J Chem Environ. 2022; 26(6): 142–9.
8. Rosenzweig T, Sampson SR. Activation of insulin signaling by botanical products. Int J Mol Sci. 2021; 22(8): 4193. doi: 10.3390/ijms22084193.
9. Patel D, Prasad S, Kumar R, Hemalatha S. An overview on antidiabetic medicinal plants having insulin mimetic property. Asian Pac J Trop Biomed. 2012; 2(4): 320–30.
10. Sun C, Zhao C, Guven EC, Paoli P, Simal-Gandara J, Ramkumar KM, *et al.* Dietary polyphenols as antidiabetic agents: Advances and opportunities. Food Front. 2020; 1(1): 18–44.
11. Mutia MS, Damayanti EWH, Suyono T, Sihotang WY. The antiacne activity of sunkist peels methanol extract in Propionibacterium acne-induced acne vulgaris. AVERROUS J Kedokt dan Kesehat Malikussaleh. 2023; 9(2): 10–22.
12. Sihotang WY, Putri IJD, Henny H, Suandy S, Mutia MS. Effect of sunkist orange peel nanoparticle granules on cardiac and aorta histopathology in diabetic rats. J Prima Med Sains. 2023; 5(2): 94–9.
13. Mutia MS. Ekstrak Kulit Jeruk Sunkist: Kajian Antioksidan Bagi Kesehatan Hepar. Medan: UNPRI Press; 2021.
14. Pallavi M, Ramesh CK, Siddesha JM, Krishna V, Kavitha GC, Nethravathi AM, *et al.* Anti-hyperlipidemic effects of citrus fruit peel extracts against high fat diet-induced hyperlipidemia in rats. Int J Res Pharm Sci. 2021; 12(3): 2226–32.
15. Ghaffari M, Dolatabadi JEN. Nanotechnology for pharmaceuticals. In: Industrial Applications of Nanomaterials. Amsterdam: Elsevier; 2019. p. 475–502.
16. Kadian R. Nanoparticles: A promising drug deliverry approach. Asian J Pharm Clin Res. 2018; 11(1): 30–5.
17. Adepu S, Ramakrishna S. Controlled drug delivery systems: Current status and future directions. Molecules. 2021; 26(19): 5905. doi: 10.3390/molecules26195905.
18. Beaven E, Kumar R, An JM, Mendoza H, Sutradhar SC, Choi W, *et al.* Potentials of ionic liquids to overcome physical and biological barriers. Adv Drug Deliv Rev. 2024; 204: 115157. doi: 10.1016/j.addr.2023.115157.
19. Sari M, Ginting CN, Yulizal O. Sunkist peel ethanol extract ameliorates diabetic nephropathy in streptozotocin-induced diabetic wistar rats. Open Access Maced J Med Sci. 2021; 9: 1086–91.
20. Alghazeer R, El-Saltani H, Saleh N, Al-Najjar A, Hebail F. Antioxidant and antimicrobial properties of five medicinal Libyan plants extracts. Nat Sci. 2012; 4(5): 324–35.
21. Souto EB, Souto SB, Campos JR, Severino P, Pashirova TN, Zakharova LY, *et al*. Nanoparticle delivery systems in the treatment of diabetes complications. Molecules. 2019; 24(23): 4209. doi: 10.3390/molecules24234209.
22. Liu Y, Yang G, Jin S, Xu L, Zhao CX. Development of high-drug-loading nanoparticles. Chempluschem. 2020; 85(9): 2143–57.
23. Nagaraja S, Ahmed SS, D R B, Goudanavar P, M RK, Fattepur S, *et al.* Green synthesis and characterization of silver nanoparticles of Psidium guajava leaf extract and evaluation for its antidiabetic activity. Molecules. 2022; 27(14): 4336. doi: 10.3390/molecules27144336.
24. Mutia MS, Harahap S, Nurhady FZ, Gunawan R, Dewani Y, Chairul M, *et al.* Effect of sunkist orange peel extract nanoparticle granules on the lipid profile levels in diabetic wistar rat. J Prima Med Sains. 2023; 5(2): 135–8.
25. Beheshtipour J. Comment on "Anion gap toxicity in alloxan induced type 2 diabetic rats treated with antidiabetic noncytotoxic bioactive compounds of ethanolic extract of Moringa oleifera." J Toxicol. 2019; 2019: 3930587. doi: 10.1155/2019/3930587.
26. Rohilla A, Ali S. Alloxan induced diabetes: Mechanisms and effects. Int J Res Pharm Biomed Sci. 2012; 3(2): 819–23.
27. Shehadeh MB, Suaifan GARY, Abu-Odeh AM. Plants secondary metabolites as blood glucose-lowering molecules. Molecules. 2021; 26(14): 4333. doi: 10.3390/molecules26144333.
28. Mojo T, Sutrisno, Marfuah S. Chemical content and pharmacology of sweet orange (Citrus sinensis) fruit peel: A review. E3S Web Conf. 2024; 481: 06002. doi: 10.1051/e3sconf/202448106002.
29. Grajang IB, Wahyuningsih I. Formulation of Sechium edule extract effervescent granule with the variation of citric acid, tartrate acid and sodium bicarbonate. In: Proceedings of the 1st Muhammadiyah International Conference on Health and Pharmaceutical Development. Yogyakarta: SCITEPRESS - Science and Technology Publications; 2018. p. 54–60.
30. Maysarah H, Sari I, Faradilla M, Kwok K. Formulation of effervescent granule from robusta green coffee bean ethanolic extract (Coffea canephora). J Pharm Bioallied Sci. 2020; 12(Suppl 2): S743–6.
31. Da R. Potential granule effervescent from a mixture of beetroot extract (Beta vulgaris L), Malang apple (Malus sylvestris L.) and Carrot (Daucus carota L.) as preparation for lowering blood cholesterol Levels. Int J Innov Sci Res Technol. 2022; 7(2): 934–8.
32. Orefice L, Remmelgas J, Neveu A, Francqui F, Khinast JG. A novel methodology for data analysis of dynamic angle of repose tests and powder flow classification. Powder Technol. 2024; 435: 119425. doi: 10.1016/j.powtec.2024.119425.
33. Bereda G. Brief overview of diabetes mellitus. Diabetes Manag. 2021; S1: 21–7.
34. Miller BR, Nguyen H, Hu CJH, Lin C, Nguyen QT. New and emerging drugs and targets for type 2 diabetes: Reviewing the evidence. Am Heal Drug Benefits. 2014; 7(8): 452–61.
35. Bai L, Li X, He L, Zheng Y, Lu H, Li J, *et al*. Antidiabetic potential of flavonoids from traditional Chinese medicine: A review. Am J Chin Med. 2019; 47(5): 933–57.
36. Widowati W, Wargasetia TL, Afifah E, Mozef T, Kusuma HSW, Nufus H, *et al.* Antioxidant and antidiabetic potential of Curcuma

longa and its compounds. Asian J Agri Biol. 2018; 6(2): 149–61.

37. Deshmukh D. A review: On herbal medicine used in the treatment of diabetes mellitus. J Emerg Technol Innov Res. 2021; 8(8): 312–22.
38. González P, Lozano P, Ros G, Solano F. Hyperglycemia and oxidative stress: An integral, updated and critical overview of their metabolic interconnections. Int J Mol Sci. 2023; 24(11): 9352. doi: 10.3390/ijms24119352.
39. Alotaibi MR, Fatani AJ, Almnaizel AT, Ahmed MM, Abuohashish HM, Al-Rejaie SS. In vivo assessment of combined effects of glibenclamide and losartan in diabetic rats. Med Princ Pract. 2019; 28(2): 178–85.
40. Anjajo EA, Workie SB, Tema ZG, Woldegeorgis BZ, Bogino EA. Determinants of hypertension among diabetic patients in southern Ethiopia: a case-control study. BMC Cardiovasc Disord. 2023; 23(1): 233. doi: 10.1186/s12872-023-03245-4.