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ORIGINAL ARTICLE

Effect of sunkist orange peel nanoparticle granules on cardiac and aorta histopathology in diabetic rats

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Abstract

Sunkist oranges (*Citrus sinensis (L.) Osbeck*) is rich in anthocyanins (95% of which are represented by cyanidin-3-glucoside and cyanidin-3-6"-malonyl-glucoside), flavanones (hesperidin and narirutin), hydroxycinnamic acid, carotenoids, sugars, minerals, and fiber, which offer health benefits. This study aimed to evaluate the efficacy of sunkist orange peel nanoparticle granules on the histopathological appearance of the cardiac and aorta in alloxan-induced diabetic rats. This study used a post-test experimental design, with a control group. Twenty-five male Wistar rats were randomly divided into five groups: negative control (alloxan+distilled water), positive control (alloxan+metformin), treatment 1 (alloxan+granular nanoparticles 50 mg/kg BW), treatment 2 (alloxan+granular nanoparticles 70 mg/kg BW), and treatment 3 (alloxan+granular nanoparticles 100 mg/kg BW). The heart and aorta were prepared for observation using 10x ocular magnification and 40x objective lens magnification. Kruskal-Wallis statistical test results revealed a significant difference in the average cardiac histopathological score between the treatment groups ($p = 0.011$). The amount of aortic endothelial cell damage can be seen from the presence of foam cells in the K(-) treatment group, while the K(+), P1, P2, and P3 groups did not have foam cells in the aortic endothelium. The findings of this study indicate that sunkist orange peel extract nanoparticles in granular form improve cardiac and aortic histopathology, with a dose of 100 mg/kg BB being the best dose for improving cardiac and aortic histopathology.

Keywords: sunkist orange peel extract, granule, nanoparticle, cardiac, aorta, histology

Introduction

Citrus contains numerous bioactive compounds that are beneficial for human health. These compounds are rich in vitamin C, flavonoids, phenolics, carotenoids, sugars, minerals, fiber, and pectin.[1,2] Sunkist orange (*Citrus sinensis (L) Osbeck*) contains significant amounts of anthocyanins, 95% of which are cyanidin-3-glucoside and cyanidin-3-6″-malonyl-glucoside, flavanones such as hesperidin and narirutin, hydroxycinnamic acids (caffeic, coumaric, synaptic, and ferulic acids), carotenoids, sugars, and minerals. Furthermore, it is a highly dietary source with a high fibre content of various bioactive substances with beneficial effects on human health.[1,3] Flavonoids have various biological effects, such as antioxidant, anti-tumor, anti-heart and vascular diseases, and anti-inflammatory properties.[4] Sunkist orange peel has pharmacological effects that include hypoglycaemic and hypolipidaemic benefits, antioxidant properties, and improved myocardial energy metabolism and oxidative stress.[2,3,5–8] Consuming 100 mg/day of Sunkist orange reduced TNF-α levels and improved overall oxidative stress.[3]

Various natural antidiabetic chemicals and drugs have been transformed into effective nanoparticles for delivery.[9] Nanoparticles offer many advantages for drug delivery applications.[10] These include improved bioavailability and pharmacokinetics, reduction of harmful side effects, controlled release, and enhanced

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paracellular absorption of the drug.[10,11] The mechanisms through which the pathophysiology of diabetes induces structural, metabolic, and functional changes in the heart can lead to diabetic cardiomyopathy and heart failure in individuals with diabetes mellitus.[12,13] Type 2 diabetes mellitus is a pivotal factor in reducing arterial flexibility. The loss of elasticity in the aorta affects the diastolic function of the heart, elevates systolic blood pressure and oxygen consumption, and can lead to heart valve damage.[14] Multiple studies have investigated the pharmacological impacts of Sunkist orange peel on hypoglycemia and hypolipidemia while showcasing antioxidant properties and improving myocardial energy metabolism and oxidative stress.[2,3,5–8] However, no existing research has examined the impact of Sunkist orange peel nanoparticle granules on the histopathology of the heart and aorta. As a result, researchers are keen to investigate the effectiveness of sunkist orange peel nanoparticles in effervescent granule form on the histopathological presentation of the heart and aorta in rats with induced diabetes using alloxan.

Method

This experimental study used a post-test-only control group design. This study was conducted from February to October 2023 at the Laboratory of Universitas Sumatera Utara. The equipment used included fan scales, ovens, analyzers, glucometer autocheck, glucose strips, mixers, petri dishes, sample bottles, microscopes, incubators, Pyrex beakers and glass jars, filter paper, gloves, masks, cages, and feeding and drinking stations for male white Wistar rats (*Rattus novergicus*). The materials used were sunkist orange peel extract, distilled water, 96% ethanol, alloxan, simvastatin,phosphate-buffered saline (PBS) solution, 70% alcohol, and rat food (pellets).

A total of 25 male white Wistar rats were acclimatized for seven days. Healthy rats chosen are agile, have fur that doesn't shed easily, are 2-3 months old, and weigh 150-200 grams.[15] Rats placed in plastic containers measuring 40 x 30 cm with 2-4 rats per container. Cages were placed in areas with adequate airflow and lighting to prevent condensation, free from distracting noises, and away from the direct path of the sun.[16] The experimental group was divided into five groups of five albino rats. The rats were acclimated to the environment for seven days.[17] Group I was used as a negative control and received a combination of alloxan and distilled water for induction, while group II was used as a positive control and was induced with 125 mg/kg BW alloxan and metformin. Group III received orange peel nanoparticles in granules at a dose of 50 mg/kg BW, whereas Group IV received the same nanoparticles in granules at a higher dose of 70 mg/kg BW. Finally, Group V received orange peel nanoparticles in granules at a dose of 100 mg/kg BW.

All rats were fasted on day one before induction and fasting blood glucose levels were measured. Alloxan was intraperitoneally injected to induce diabetes in Wistar rats. Twenty-four hours after alloxan injection, blood glucose levels of the rats were measured again. The criterion for effective induction was fasting glucose >125 mg/dL.[18] The nanoparticle granules of the sunkist orange peel extract were administered for 14 days. The rats were euthanized and necropsied to obtain cardiac and aortic organs, which were preserved in liquid formalin for 1 d. Subsequently, the heart organs were washed using physiological NaCl solution, weighed, and fixed in 10% Neutralized Buffered Formaldehyde (NBF) for histological staining. Tissue samples were dehydrated using a graded alcohol series and embedded in paraffin. Subsequently, the samples were placed on an object glass and treated with a Canadian balm before being covered with a cover glass. The preparations were incubated overnight at 37°C before staining. The stained preparations were then assessed using hematoxylin-eosin (H&E) staining and examined under a 400x light microscope. The assessment was performed in five fields of view as a means of evaluation.

The Shapiro-Wilk test was conducted to determine the statistical normality of the distribution ($p>0.05$) due to the limited sample size ($n \leq 50$). Collected data were analyzed using SPSS; the results showed an abnormal distribution ($p<0.05$), necessitating the use of the Kruskal-Wallis test.

Results

Histopathologic observation was performed on the cardiac and aorta of rats that were induced with alloxan after 14 days of administration of granular nanoparticles of sunkist orange peel extract. The cardiac

tissue was dissected and prepared, and then stained with the HE method for observation under 10x ocular magnification and 40x objective lens magnification.

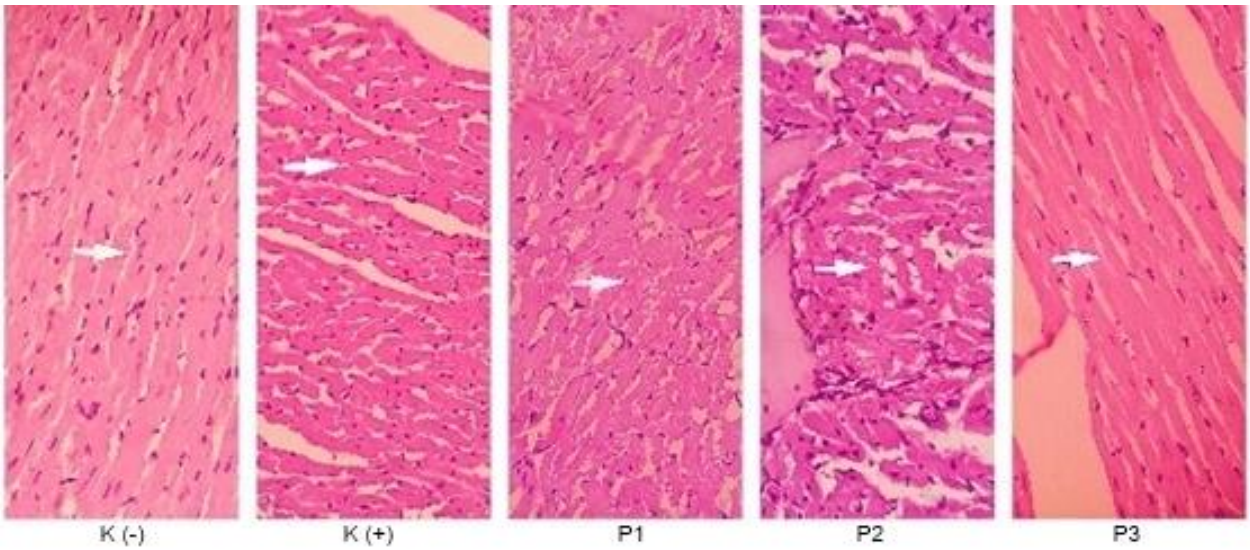

Figure 1. Cardiac histopathology by group
(K(-)=Group I; K(+)=Group II; P1=Group III; P2=Group IV; P3=Group V)

Table 1 shows that in the negative control group, myocyte necrosis was in 16-25% of all observed myocytes, where myocyte cell necrosis occurred in one fascicle (cluster). The positive control group exhibited no damage, although the damage was not entirely normal. In Groups III and IV, myocyte necrosis was observed in 6-15% of all myocytes. Group V was not completely normal, but no damage to the cardiac histopathology was observed. This can be explained by the changes in cardiac histopathology in group II given metformin and in group V given sunkist orange peel extract nanoparticle granules. The histopathological picture of the cardiac tissue was not completely normal and no damage was observed. The Kruskal-Wallis statistical test revealed a significant difference in the average score of heart histopathology between the treatment groups (p=0.001). The Mann-Whitney post hoc test results revealed significant differences (p<0.05) between all extract treatments and the myocyte necrosis score, except for group I compared to group III, and group II compared to group IV (p>0.05).

Table 1. Cardiac histopathological score

Group	Mean ± SD	p
I	2,33 ± 2,88	0,011
II	0,33 ± 2,88	
III	2,33 ± 2,88	
IV	1,16 ± 2,88	
V	0,50 ± 0,00	

Table 2. Foam cell count

Group	Count
I	1
II	0
III	0
IV	0
V	0

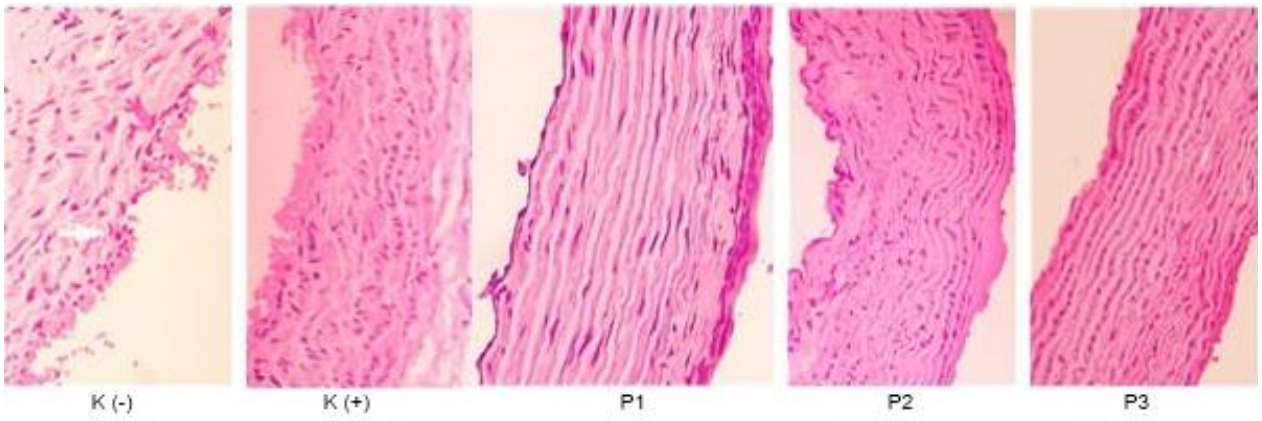

Figure 2. Aorta histopathology by group

In the table of the results of the number of endothelial cell damage, it can be seen that Group I has foam cells. In groups II, III, IV, and V, there were no foam cells in the aortic endothelium.

Discussion

In Wistar rat cardiacs, histological scoring in Group I (negative control) revealed necrosis in 16-25% of all observed myocardial cells. Necrosis of myocardial cells occurred within the fascicle (cluster). Within Group III, myocardial necrosis ranging from 6-15% of the observed myocardial cells. Such necrosis could potentially arise due to the formation of free radicals, which subsequently damage the lipid membrane and nucleus of cardiac cells, leading to cell degeneration.[19] Individuals with diabetes mellitus who irregularly consume diabetes medication are at a 1.7 times higher risk of experiencing coronary heart disease compared to those who regularly adhere to diabetes medication.[20] In Group II (alloxan 125 mg/kgBW + metformin 4.5 mg/kgBW), albeit not entirely normal, no histological damage was observed in the heart. Metformin, a biguanide-class diabetes medication commonly used in diabetes therapy, exhibits an efficacy rate of up to 60%.[21] Furthermore, metformin has demonstrated effectiveness, safety, affordability, and potential in reducing the risk of heart disease and mortality.[22] Meanwhile, within Group V, the histopathological portrayal of the heart was not entirely normal but devoid of damage. Previous research has evidenced the influence of Sunkist orange peel extract on reducing blood glucose levels and lipid profiles in diabetic rats.[2,6] Flavonoids, terpenoids, steroids, and tannins have been noted to impact the levels of cardiac enzyme lactate dehydrogenase (LDH).[23]

The research findings revealed variations in the histopathological presentation of Wistar rat aorta induced by alloxan, as well as the influence of sunkist orange peel extract on endothelial cell damage. In Group I, endothelial cells were deemed abnormal if they did not adhere or detach from the vessel wall. Conversely, endothelial cells are considered normal when they exhibit a flattened shape and adhere to the vessel wall.[24] The production of foam cells signifies the initial marker within a series of structural changes occurring in the arterial vessel wall. This condition can eventually lead to vessel narrowing or complete occlusion, resulting in tissue hypoxia and necrosis.[25] Oxidative stress may hinder nitric oxide production and cause endothelial dysfunction in blood vessels. Consequently, the equilibrium between prostacyclin and thromboxane is disrupted: thromboxane levels increase, while prostacyclin levels decrease. This leads to blood vessel constriction and platelet aggregation within its lumen.[19]

In groups II (positive control), III, IV, and V, there was no observable presence of foam cells or damage to the endothelial cells. The decrease in the number of abnormal endothelial cells could potentially be attributed to the presence of isoflavones, which have been proven to act as antioxidants capable of combating free radicals generated by an increase in LDL levels in the bloodstream. This concept is substantiated by the explanation that antioxidants play a role in interrupting the chain reaction of free radicals within the body, thereby shielding the body's cells from damage caused by free radicals.[24] Antioxidant components such as flavonoids can prevent substrate oxidation within chain reactions, providing protection to cardiac muscle cells against free radicals by transferring electrons to free radical molecules. This results in the stabilization of free radicals and the cessation of chain reactions.[23] Flavonoids function as reducing agents and hydrogen donors, with the ability to chelate metals. Moreover, flavonoids possess antioxidant properties through redox mechanisms, acting as scavengers of hydroxyl free radicals. This aids in addressing vascular issues associated with diabetes and oxidative stress.[5]

The administration of nanoparticle doses of sunkist orange peel extract to aloxan-induced rats at doses of 50 mg/kg BW and 70 mg/kg BW revealed a histopathological depiction characterized by the presence of myocardial cell necrosis, which tended to remain high. However, at a dose of 100 mg/kg BW, the nanoparticle extract of sunkist orange peel induced changes in the quantity of myocardial cell necrosis and lowered blood sugar levels. Previously, the administration of a methanol extract of sunkist orange peel at a dose of 600 mg/kg BW has been shown to reduce diabetes.[5] From this study and previous research, it can be inferred that variations in dosage, coupled with smaller particle sizes, lead to an increased drug surface area, thereby accelerating drug release. Nevertheless, smaller particle sizes entail a higher risk of particle aggregation during storage, rendering the formulation unstable.[26]

Conclusion

The administration of sunkist orange peel nanoparticle extract in granule form has been shown to reduce blood glucose levels in diabetic rats. The effective dose found to act as an antidiabetic agent in this study was the nanoparticle extract of sunkist orange peel in granule form at a dosage of 100 mg/kg BW, displaying the lowest blood sugar levels observed after a 14-day treatment period. Nanoparticle extract of sunkist orange peel in granule form exhibited effects on the histopathological appearance of the cardiac and aorta, as evident from the level of myocyte necrosis and endothelial cell damage in rats induced with aloxan. The dosage of 100 mg/kg BW proved to be the most capable of eliciting reparative effects on the histopathological appearance of the cardiac tissue and aorta.

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