Effect of Dahlia Tuber Inulin Creamer on Urea and Creatinine Levels In Rat: A Study on Kidney Function and Hyperlipidemia Prevention

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Abstract. The administration of a high-fat creamer will cause hyperlipidemia, an atherosclerosis risk factor. If atherosclerosis occurs in the renal arteries, it can cause impaired kidney function. Inulin, a prebiotic, can be used as a creamer ingredient to produce a low-fat creamer. This study aims to determine the effectiveness of dahlia tuber inulin creamer on urea and creatinine. This study involved 20 male rats (aged 2-3 months, 150–200 grams), divided into four groups: Group I received standard food only; Group II received inulin creamer; Group III received creamer without inulin; and Group IV received commercial creamer. Serum urea and creatinine levels were measured using the colorimetric method.The results of the analysis using ANOVA ($p < 0.05$) showed that there was a significant difference in creatinine levels, followed by the LSD test, and a significant difference was obtained between groups III and IV with group I. Meanwhile, the results of the Kruskal-Wallis analysis did not show significant differences in urea levels between the groups. This study concluded that inulin was effective in preventing an increase in creatinine levels in rats given creamer.

Keywords: creamer, creatinine, dahlia tuber, inulin, urea

1 Introduction

ver the last few years, cases of obesity and atherosclerosis have increased throughout the world. According to World Health (WHO) data, obesity worldwide has tripled since 1975 [23]. One of the factors that has influenced this increase is the habit of drinking coffee with a sweet taste, with added sugar and milk or creamer. Most of these creamers are considered unhealthy because they contain a lot of saturated fat and/or hydrogenated oil [10]. Research shows an increase in the concentration of triglycerides, LDL cholesterol, VLDL cholesterol, and the LDL/HDL ratio in mice given creamer that does not contain milk.

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This is because creamer contains hydrogenated palm oil with many short-chain saturated fatty acids. An increase in LDL levels and the LDL/HDL ratio indicates an increase in cholesterol synthesis compared to phospholipids, while DLcholesterol contains more phospholipids than cholesterol [8]. Atherosclerosis is an inflammatory process in the arteries that occurs slowly and progressively. This process is characterized by fat deposits, accumulation of leukocytes, formation of foam cells, migration and proliferation of smooth muscle cells, and extracellular matrix deposits, making blood vessels thicker and stiffer [28].

In the renal arteries, atherosclerosis will cause reduced blood flow to the kidneys, resulting in impaired kidney function, characterized by increased urea and creatinine serum levels. Ureum is the result of amino acid metabolism, and creatinine is the result of creatinine phosphate [24].

One effort can be made to replace sugar or milk with creamer from inulin, which is low in fat and contains prebiotics, which are suitable for the body and digestive health. Inulin is a low-calorie ingredient that can improve a food's rheological characteristics and nutritional properties [17]. The prebiotic ability contained in inulin can support the growth of flora in the digestive system and provide health effects. Consuming inulin can improve lipid metabolism and influence blood fats to normal levels. The function of inulin as a food additive is that it can stimulate probiotic microorganisms such as Bifidobacterium, L. casei, and L. plantarum and can inhibit the growth of pathogenic bacteria, such as E. coli and Clostridia, without changing the taste of food [18].

The inulin used in this research came from dahlia tubers. Dahlia tubers are known to contain compounds that have high bioactivity [21]. Until now, no research has discussed the effect of Dahlia tuber inulin creamer on kidney function. Based on this, the researchers intended to analyze the impact of Dahlia tuber inulin creamer on rat kidney function by measuring serum urea and creatinine levels.

2 Methods

This research is an experimental laboratory study with a control group post-test only that was designed in vivo and administered with a creamer for 6 weeks. The research was conducted at the Animal House and Biochemistry Laboratory, Faculty of Medicine, Riau University. This research has received a letter of ethical suitability from the Medical and Health Research Ethics Unit, Faculty of Medicine, Riau University, with number B/070/UN19.5.1.1.8/UEPKK/2024.

2.1 Animal treatment

This research used male rats aged 2-3 months weighing 150-200 grams. The 16 rats were divided into four groups: Group I namely the group that was only given standard feed; Group II, the group that was given inulin creamer; Group III, the group that was given creamer without inulin; and Group IV, the group that was given commercial creamer. All groups received standard feed and water

ad libitum during this experiment. The human equivalent dose equation (HED) was used to determine the doses of liquid and powder creamer by multiplying the animal dose by the ratio of animal weight to human weight, multiplied by 0.33. The recommended daily intake of creamer liquid is 30 mL per cup for a 70 kg individual, totaling 90 mL for three cups. The inulin creamer dose was converted to animal dose as 1.33 mL/130 g, with 342 mg dissolved in 1.33 ml of water and administered to the rat. The control group was given the same amount of water to balance the stress. The dose was calculated by considering the weight of the rat each week as the average weight (g) of all groups [2]. After 6 weeks, all rats were euthanized following standard protocols, and blood samples were taken by cardiac puncture.

Inulin extraction

Dahlia tubers are harvested from newly grown dahlia plants in Bukittinggi, West Sumatra Province. Dahlia tuber extraction was carried out to obtain inulin. The process starts with warming up 2500 grams of recently harvested dahlia tubers, cleaned and thinly cut, in 5000 milliliters of distilled water at 80-90°C for half an hour. Cool down to room temperature, strain, and collect the filtered liquid and solid. Ethanol was mixed with the filtrate in a 1:1 (v/v) ratio and then chilled at four °C for 18 hours. Following this, the filtrate underwent centrifugation at a speed of 9000 rpm for a duration of 10 minutes, forming a white inulin precipitate. The white precipitate was dried at 60°C until it was scorched, obtaining yellowish-white dry inulin [11], [19].

Creamer preparation

The creamer emulsion was made by mixing 8% w/w hydrogenated palm kernel oil and 0.5% w/w soy lecithin (emulsifier) in a 100 mL beaker, then covering it with aluminum foil to prepare the dispersed phase. The blend was warmed to 80°C and stirred at 100 rpm for 20 minutes in a temperature-controlled water bath. The liquid phase was created by gradually mixing sodium caseinate (2.5% w/w), silicon dioxide (used as an anti-caking agent, 1.0% w/w), and di-potassium hydrogen phosphate (as a stabilizer, 2.5% w/w) into 100 mL of hot distilled water (80 ± 5 °C), then adding skimmed milk powder (7% w/w) and solid corn syrup (15% w/w). The mixture was agitated using a magnetic stirrer at 100 rpm for 5 minutes until fully dissolved and smooth.

Then, maltodextrin at a concentration of 25% and inulin at 7.5% w/w were slowly introduced into the water phase to create various continuous phases. The mixture was stirred at 100 rpm for 5 minutes at 60 ± 1°C. Finally, the dispersed phase was slowly added to the continuous phase. Next, the blend was agitated for 10 minutes to create a rough creame emulsion. Ultimately, the rough emulsion underwent homogenization using a high-pressure homogenizer at 200 and 180 MPa before drying. Afterward, the homogenized creamer mixture is dehydrated using a spray dryer to produce standard creamer and instant cream [10]. For comparison, commercial inulin and no inulin (0% inulin) were used as controls.

2.2 Blood sampling

At the specified time, blood is drawn from the heart. Previously, the rats were anesthetized by placing them in a vessel saturated with ether vapor. Blood is collected in a tube. The blood is then centrifuged at a speed of around 1000 g at a temperature of 2-8 ° C for 15 minutes to obtain serum, which is then stored in a refrigerator at -40 ° C until the measurement time.

2.3 Examination of Serum Urea and Creatinine Levels

The blood samples obtained were centrifuged for 15 minutes at 3000 rpm to obtain serum for use in examining urea and creatinine levels. The examination was carried out using the colorimetric method using a micro AB 300 LX spectrophotometer with special reagents. Serum urea was determined by DiaSys Urea CT FS (DiaSys Diagnostic Systems, Holzheim, Germany). Serum creatinine levels were determined by enzymatic colorimetric assay with the DiaSys Creatinie PAP FS kit DiaSys Diagnostic Systems, Holzheim, Germany).

2.4 Statistical analysis

Data processing was done manually, with calculators and computers, while data analysis was done using the Statistical Package for Social Science (SPSS) program. Statistical tests were used to compare serum urea and creatinine levels between groups. The ANOVA test was used to compare serum creatinine levels and continued with the LSD test. Meanwhile, creatinine levels are not normally distributed, so the Kruskal-Wallis test is used. Differences are statistically significant if ($p < 0.05$).

3 Results

3.1 Effect of giving inulin creamer from dahlia tubers on serum urea levels in Wistar rats

The meaning of serum urea levels in the control and treatment groups that were given creamer can be seen in Figure 1 below.

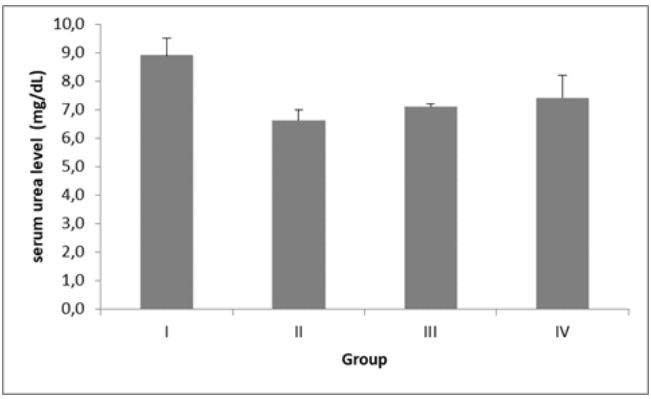

Fig. 1. Serum Ureum Levels

Note: The data shows the mean ± SE of rat urea levels after the study. The data was tested using Kruskal-Wallis, and no significant differences were found

$$(p > 0.05)$$

The results of statistical tests on the serum urea levels of Wistar rats fed the inulin creamer diet showed a decrease, but it was not significant in group II compared to group I ($p > 0.05$) Groups II and IV had higher urea levels than group II, although this was not statistically significant.

Effect of giving inulin creamer from dahlia tubers on serum creatinine levels in Wistar rats

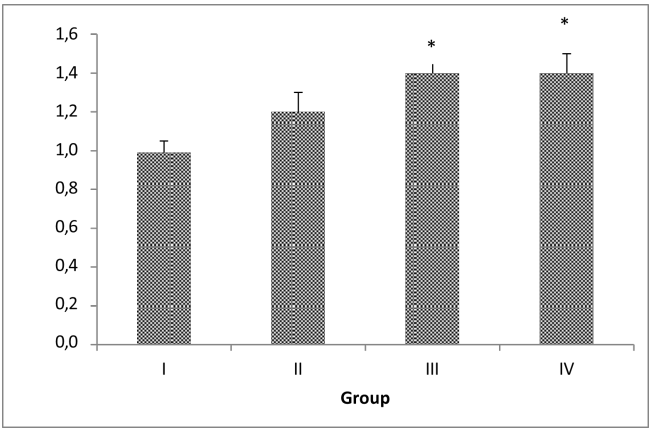

Fig. 2. Serum creatinine levels

Note: Data shows the mean ± SE of rat creatinine levels after the study. Data were tested using ANOVA and continued with the LSD test, which was significant to the Group I.There was an increase in serum creatinine levels in group II, but it was not statistically significant, whereas, in groups III and IV, there was an increase in creatinine compared to group I, and it was statistically significant ($p < 0.05$).

4 Discussion

The lowest serum urea levels were found in treatment group II, rats given inulin creamer. Giving inulin creamer to dahlia tubers reduced urea levels not significantly (figure 1). Meanwhile, the highest serum creatinine levels were found in groups II and IV and the lowest in group I. The increase in serum creatinine was statistically significant in the group given creamer without inulin and commercial creamer compared to the control group. Meanwhile, in group II, namely the group given dahlia tuber inulin creamer, although there was an increase, it was not statistically significant (figure 2). This shows that administering cream containing inulin can prevent an increase in creatinine. Novianti et al. [16] also found similar research results, namely that giving a high-fat and high-fructose diet for 10 weeks only increased serum creatinine levels but did not increase urea levels. Research by Dissard et al. [6] showed that giving a high-fat and high-fructose diet for 8 weeks only caused minimal injury to the kidneys [16]. Research by Dissard et al. [6] showed that giving a high-fat and high-fructose diet for 8 weeks only caused minimal injury to the kidneys [6]. A high-fat diet will increase the lipolysis process in fat tissue, thereby increasing the formation of LDL and reducing the formation of HDL, resulting in hyperlipidemia [3].

Hyperlipidemia can trigger endothelial dysfunction, resulting in a decrease in nitric oxide (NO) and an increase in reactive oxygen species (ROS). ROS will bind to low-density lipoprotein (LDL) to form oxidized LDL and cause activation of macrophages [4]. Macrophages will phagocytose oxidized LDL to form foam cells, which then trigger atheroma plaque formation [9]. The presence of atherosclerosis in the renal arteries can cause a decrease in blood flow to the kidneys. It can disrupt kidney function, reducing the glomerular filtration rate (GFR), followed by increased serum urea and creatinine levels [27]. Besides that, excessive production of ROS will affect kidney structure and function. This oxidative stress causes inflammation, fibrosis, and endothelial dysfunction and subsequently causes terminal-stage kidney disease [4]. Hyperlipidemia can also cause edema in the glomerulus due to mesangial expansion, which can reduce the surface area of the glomerular capillaries, which function for filtration. As a result, there will be a decrease in the eGFR (Estimated Glomerular Filtration Rate), which will also cause kidney function to decrease. Decreased kidney function will cause decreased secretory function, which is characterized by increased levels of serum urea and creatinine [6].

Inulin is a polysaccharide composed of fructose connected by β (2-1)D-fructofuranoside bonds. This linkage hinders digestive enzymes' breakdown of

inulin, causing a delay in stomach emptying and a decrease in glucose absorption [1], [22]. Inulin has been known to have antidiabetic, anti-inflammatory, and antioxidant effects.

The prebiotic ability contained in inulin can support the growth of flora in the digestive system and provide health effects. Consuming inulin can improve lipid metabolism and influence blood sugar and blood fat to normal levels. The function of inulin as a food additive is that it can stimulate probiotic microorganisms such as Bifidobacterium, L. casei, and L. plantarum and can inhibit the growth of pathogenic bacteria, such as E. coli and Clostridia, without changing the taste of food [18].

The inulin used in this research came from dahlia tubers. Dahlia tubers are known to contain compounds that have high bioactivity [21]. Dahlia tubers are currently not optimal in society and are considered agricultural waste. Inulin can reduce blood sugar levels by increasing the circulation of the intestinal anorexia hormone GLP-1 [3]. Ingesting chicory root extract with inulin as the primary

bioactive component for 4 weeks can lead to a notable decrease in HbA1c levels, a standard indicator of blood sugar regulation [15]. Administering inulin extract from dahlia tuber at 0.5 g/kgBW, 1 g/kgBW, and 1.5 g/kgBW to rats with diabetes mellitus (DM) can effectively decrease levels of glucose in the blood. Nonetheless, only inulin extract at 1 g/kgBW and 1.5 g/kgBW in DM rats decreased damage significantly and the Homeostasis Assessment of Insulin Resistance (HOMA-IR) index in DM rats. On the other hand, administering 0.5 g/kgBB of inulin extract only decreases the severity of insulitis and does not impact HOMA-IR. Nevertheless, the administration of inulin extract does not affect insulin expression in pancreatic serum to counteract insulin resistance during the decompensation phase or in pancreatic tissue [12].

Inulin can be an antioxidant by effectively scavenging reactive oxygen species (ROS) [25]. As the body ages or undergoes stress, excess free radicals can harm cells, tissues, and organs, resulting in various diseases like inflammation, cancer, aging, and radiation damage [14]. ROS are generated during metabolic processes by free radicals like superoxide anion radicals, hydroxyl radicals, and their reactive derivatives [25]. Inulin with a lower polymerization degree exhibits more antioxidant activity than inulin with a higher polymerization degree [29]. New dahlia tubers typically contain inulin with a lower level of polymerization, which is more prevalent in tubers stored for 4 weeks than chicory inulin [11].

According to [13], gastric acid production can lead to a higher oxidation rate in lipids and other food components. Research conducted by Kanner and Lapidot [13], suggests that dietary antioxidants, such as inulin, may help prevent lipid peroxidation in the stomach. Adding inulin or oligofructose to your diet can help protect against oxidative stress and avoid inflammation linked to oxidative stress [5], [26]. Ghavidel and colleagues' study indicates that giving inulin and resveratrol can decrease IL-6, TNF-α, and IL-1β [7]. Inulin's potential as an antioxidant, anti-inflammatory, and anti-diabetic is thought to prevent kidney atherosclerosis and improve GFR to avoid increases in urea and creatinine levels in the serum.

Conclusion

This study concluded that inulin was effective in preventing an increase in creatinine levels in rats given creamer.

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References

1. W. Ahmed and S. Rashid, "Functional and therapeutic potential of inulin: A comprehensive review," *Critical Reviews in Food Science and Nutrition*, vol. 59, no. 1, pp. 1–13, 2019.
2. A. Ali, S. Iqbal, M. Sohaib, A. U. Khan, R. M. W. Younis, and S. B. Junaid, "Effects of commercial non-dairy tea whitener consumption in comparison to milk on lipid profile, histopathology, and liver enzymes in animal model," *International Food Research Journal*, vol. 29, no. 5, pp. 1043–1052, 2022.
3. J. Anastasovska *et al.*, "Fermentable carbohydrate alters hypothalamic neuronal activity and protects against the obesogenic environment," *Obesity*, vol. 20, no. 5, pp. 1016–1023, 2012.
4. D. Burtenshaw *et al.*, "Reactive oxygen species (ROS), intimal thickening, and subclinical atherosclerotic disease," *Frontiers in Cardiovascular Medicine*, vol. 6, pp. 1–18, Aug. 2019.
5. J. Busserolles *et al.*, "Oligofructose protects against the hypertriglyceridemic and pro-oxidative effects of a high fructose diet in rats," *The Journal of Nutrition*, vol. 133, no. 6, pp. 1903–1908, 2003.
6. R. Dissard *et al.*, "Long term metabolic syndrome induced by a high fat high fructose diet leads to minimal renal injury in C57BL/6 mice," *PloS One*, vol. 8, no. 10, e76703, 2013.
7. F. Ghavidel *et al.*, "The combinational effect of inulin and resveratrol on the oxidative stress and inflammation level in a rat model of diabetic nephropathy," *Current Developments in Nutrition*, vol. 8, no. 1, p. 102059, 2024.
8. L. S. Hanna and H. A. A. Elmonem, "Evaluation of cardiac biomarkers in albino rats consumed instant coffee and non-dairy creamer," *The Journal of American Science*, vol. 10, no. 5, pp. 96–102, 2014.
9. J. Hartman and W. H. Frishman, "Inflammation and atherosclerosis: a review of the role of interleukin-6 in the development of atherosclerosis and the potential for targeted drug therapy," *Cardiology in Review*, vol. 22, no. 3, pp. 147–151, 2014.
10. S. Hedayatnia *et al.*, "Effect of different fat replacers and drying methods on thermal behaviour, morphology and sensory attributes of reduced-fat coffee creamer," *LWT-Food Science and Technology*, vol. 72, pp. 330–342, 2016.
11. H. Horiza, M. Azhar, and J. Efendi, "Ekstraksi dan karakterisasi inulin dari umbi dahlia (Dahlia Sp. L) segar dan disimpan," *Eksakta: Berkala Ilmiah Bidang MIPA*, vol. 18, no. 1, pp. 31–39, 2017.
12. I. Ismawati *et al.*, "Effect of inulin from Dahlia tubers (Dahlia variabilis) extract on insulitis severity and insulin expression in diabetic rats," *BioMedicine*, vol. 14, no. 3, p. 4, 2024.

13. J. Kanner and T. Lapidot, "The stomach as a bioreactor: dietary lipid peroxidation in the gastric fluid and the effects of plant-derived antioxidants," *Free Radical Biology and Medicine*, vol. 31, no. 11, pp. 1388–1395, 2001.
14. H. N. Liu *et al.*, "Effects of dietary supplementation of quercetin on performance, egg quality, cecal microflora populations, and antioxidant status in laying hens," *Poultry Science*, vol. 93, no. 2, pp. 347–353, 2014.
15. M. Nishimura *et al.*, "Effects of the extract from roasted chicory (Cichorium intybus L.) root containing inulin-type fructans on blood glucose, lipid metabolism, and fecal properties," *Journal of Traditional and Complementary Medicine*, vol. 5, no. 3, pp. 161–167, 2015.
16. V. D. Putri, S. Yanti, and F. Dyna, "Analisis inulin dari umbi dahlia (Dahlia variabilis) sebagai prebiotik antidiabetik," *Jurnal Katalisator*, vol. 7, no. 2, pp. 311–321, 2022.
17. N. Rachdaoui, "Insulin: the friend and the foe in the development of type 2 diabetes mellitus," *International Journal of Molecular Sciences*, vol. 21, no. 5, p. 1770, 2020.
18. X. Wan *et al.*, "The physiological functions and pharmaceutical applications of inulin: A review," *Carbohydrate Polymers*, vol. 246, p. 116589, 2020.
19. C. F. M. Welters *et al.*, "Effect of dietary inulin supplementation on inflammation of pouch mucosa in patients with an ileal pouch-anal anastomosis," *Diseases of the Colon & Rectum*, vol. 45, no. 5, pp. 621–627, 2002.
20. M. P. Wolf and P. Hunziker, "Atherosclerosis: insights into vascular pathobiology and outlook to novel treatments," *Journal of Cardiovascular Translational Research*, vol. 13, no. 5, pp. 744–757, 2020.