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Comparative Analysis of Hematological Profiles in Diabetic Mice Treated with D-Allulose and Neoagaro-Oligosaccharides

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Abstract: *Diabetes mellitus is characterized by chronic hyperglycemia that induces oxidative stress and inflammation, contributing to hematological dysfunction and increased systemic risk. This study comparatively evaluated the effects of D-allulose and neoagaro-oligosaccharides (NAOS) on hematological parameters in BALB/c mice with type 2 diabetes induced by streptozotocin. Experimental groups consisted of control, negative control, positive control (metformin), and treatment groups receiving D-allulose (0.05, 0.1, 0.2 g/kg BW) or NAOS (100, 200, 400 mg/kg BW) orally for 28 days. Hematological assessments included red blood cell (RBC) count, erythrocyte indices (hemoglobin, hematocrit, mean corpuscular hemoglobin), leukocyte count, and lymphocyte levels. Negative controls displayed decreased RBC parameters and lymphocytes, with elevated leukocytes, indicating hematological impairment associated with hyperglycemia. D-allulose improved RBC count, hemoglobin, and MCH, while reducing leukocyte count and elevating lymphocytes, especially at lower doses. Neoagaro-oligosaccharides primarily enhanced erythrocyte quality by improving hemoglobin, hematocrit, and MCH, while also modulating leukocyte and lymphocyte profiles. Overall, D-allulose was more effective in restoring erythrocyte quantity, whereas NAOS contributed more prominently to erythrocyte index improvement. Both agents show potential in reducing hematological disorders associated with inflammation in diabetes cases.*

Keywords: *BALB/c mice; Diabetes mellitus; D-allulose; Hematological profile; Neoagaro-oligosaccharides*

1. INTRODUCTION

Diabetes mellitus (DM) is one of the largest global epidemics of the 21st century, with an increasing number of cases exceeding 422 million people worldwide. The World Health Organization projects that the prevalence of diabetes will rise to approximately 592 million individuals by 2035, highlighting a growing global health burden (Milosevic & Panin, 2019). Clinically, DM is a chronic disorder characterized by elevated blood glucose levels resulting from inadequate insulin production or impaired insulin utilization by the body (Ebrahim et al., 2022). Diabetes is classified into several categories, with type 2 diabetes mellitus (T2DM) being the most prevalent, accounting for nearly 90% of all

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diagnosed cases (Bambo et al., 2024). T2DM is marked by persistent hyperglycemia accompanied by disturbances in carbohydrate, protein, and lipid metabolism (Yang & Kang, 2018). Sustained hyperglycemia is strongly associated with increased oxidative stress and inflammatory responses, both of which impair metabolic signaling pathways and contribute to organ dysfunction in T2DM (Sibony et al., 2024).

Long-term exposure to elevated blood glucose levels also affects the hematopoietic system, which is reflected in alterations in the structural and metabolic characteristics of erythrocytes (RBCs) and leukocytes (WBCs). These abnormalities can result in anemia, indicated by declines in hemoglobin (Hb), red blood cell count, and hematocrit (HCT), as well as disturbances in coagulation and immune function (Al-Dewachi et al., 2024). In addition, hematological alterations have been linked to metabolic features associated with diabetes, including hyperglycemia, hyperinsulinemia, and insulin resistance (Biro et al., 2021), indicating that hematological markers may serve as prognostic indicators for the progression of diabetic complications (Cardoso et al., 2021). Beyond conventional pharmacological interventions, interest has grown in the development of bioactive antidiabetic agents that may improve metabolic function and modulate hematological parameters. This study therefore highlights two bioactive compounds, D-allulose and neoagaro-oligosaccharides (NAOS), which were administered independently as treatment groups.

D-allulose (D-psicose) is a rare monosaccharide and a C-3 epimer of D-fructose that became more widely available following advancements in enzymatic production technologies during the 1990s (Yuma et al., 2023). It possesses sweetness comparable to sucrose but has a very low caloric value (approximately 0.2 kcal/g), low absorption in the intestinal tract, and minimal contribution to energy storage, making it a promising low-calorie sweetener for individuals with diabetes (Chan et al., 2012; Zhang et al., 2016; Jiang et al., 2020). Previous studies report that D-allulose can reduce postprandial blood glucose, improve insulin sensitivity, and influence lipid metabolism and oxidative status in diabetic animal models (Ochiai et al., 2014; Hossain et al., 2015; Chen et al., 2017). Clinical studies in patients with type 2 diabetes further demonstrate improvements in glycemic control, including reductions in postprandial glucose, fasting glucose, and glycated hemoglobin (HbA1c), following D-allulose intake (Tanaka et al., 2019; Tak et al., 2023).

Neoagaro-oligosaccharides (NAOS) are oligosaccharides generated from the enzymatic hydrolysis of agarose using β-agarase (Cheong et al., 2018). Neoagaro-oligosaccharides is known to possess several biological activities, including antioxidant, antidiabetic, anti-inflammatory, anti-obesity, and prebiotic effects (Hong et al., 2017; Higashimura et al., 2014). Mechanistically, NAOS inhibits α-glucosidase and α-amylase activity in the digestive tract, reducing glucose absorption and aiding in blood glucose regulation (Hong et al., 2017). They also enhance cellular antioxidant capacity, protect pancreatic β-cells against oxidative damage, and modulate glucose metabolism by increasing the expression of glucose transporter type 2 (GLUT2) and adenosine triphosphate (ATP) production (Canfora et al., 2015; Hajiaghaalipour et al., 2015). Furthermore, NAOS upregulate the expression of peroxisome proliferator-activated receptor gamma (PPARγ), which plays an important role in insulin sensitivity and lipid metabolism (Lin et al., 2019).

Although both D-allulose and NAOS have demonstrated antidiabetic, antioxidant, and anti-inflammatory effects, most prior research has focused primarily on metabolic parameters such as blood glucose levels, insulin sensitivity, and lipid profiles. Systematic comparative evidence regarding their influence on hematological parameters, particularly in diabetic animal models, is still limited. Given that hematological markers reflect systemic inflammation, oxidative stress, and cardiovascular risk in diabetes, this study aims to comparatively evaluate hematological changes in diabetic mice treated with D-allulose and NAOS, providing deeper insight into their potential to ameliorate hematological dysfunction associated with diabetes.

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2. RESEARCH METHOD

Animals

Male BALB/c mice, four weeks age and weighing approximately 18-22 g, were obtained from the Faculty of Pharmacy, Universitas Gadjah Mada. The animals were housed under controlled environmental conditions consisting of a 12 h light/12 h dark cycle, a temperature of 25°C, and relative humidity of 50-60%. Standard laboratory feed (RatBio) and water were provided *ad libitum*. All experimental procedures were reviewed and approved by the Ethics Committee of the Faculty of Veterinary Medicine, Universitas Gadjah Mada (Ethical approval No: 79/EC-FKH/int./2025).

Preparation of D-allulose

D-allulose used in this study was obtained from a commercial product, Amalose® (PT. Amalose TeDCo Indonesia, BRIN). Amalose is a water-based honey solution enzymatically processed using D-psicose 3-epimerase (DPEase), which converts a portion of the honey's fructose into D-allulose. According to product documentation and compositional data, Amalose contains 13 g of D-allulose per 100 mL of solution (equivalent to 0.13 g/mL). The treatment doses (0.056, 0.1, and 0.2 g/kg BW) represent equivalent doses of pure D-allulose, calculated through interspecies dose conversion based on Km factors following Sari (2023) and Jacob et al. (2022). The volume of Amalose® required to achieve each equivalent dose was determined based on the actual D-allulose content (13 g/100 mL), then diluted with water for injection (WFI) to obtain a standardized administration volume of 0.5 mL per mouse. The caloric content of Amalose is minimal because D-allulose has an energy value of approximately 0.4 kcal/g based on FDA reference data, resulting in an estimated caloric value of 0.052 kcal per mL of Amalose. Prepared solutions were stored at 4°C and then equilibrated to room temperature before oral administration.

Preparation of Neoagaro-oligosaccharides (NAOS)

Neoagaro-oligosaccharides (NAOS) used in this study were obtained from the National Research and Innovation Agency (BRIN), Indonesia. Agar was dissolved in Tris-HCl buffer to a final concentration of 0.3% (w/v) and heated in a water bath for 10 min. After cooling, crude β-agarase was added to the agar solution, followed by incubation at 45°C for 24 h with shaking at 120 rpm. Insoluble components were removed by centrifugation at 12,000 × g for 30 min. The supernatant was subsequently subjected to membrane processing, including ultrafiltration to remove proteins and nanofiltration to eliminate low–molecular weight compounds such as salts, in order to isolate and purify NAOS. Further purification was carried out using gel filtration chromatography. The purified NAOS were analyzed using HPLC-ELSD, and their structural and molecular mass characteristics were confirmed by ESI-TOF-MS and NMR, according to Lin et al. (2019).

Experimental Design

Prior to treatment, all mice were acclimated for 7 days. After acclimation, the mice were randomly assigned to two groups: a standard diet group (n = 5) and a high-fat diet (HFD) group (n = 25). The HFD formulation consisted of 40% corn oil, 30% beef fat, 15% butter, 12% coconut oil, and 3% quail egg yolk, as described by Ifada et al. (2020).

Table 1. Experimental Groups for D-allulose and NAOS Treatment

Group	Description
C	Normal mice fed standard food
DC	Diabetic mice
PC	Diabetic mice + Metformin 50 mg/kg BW
D-allulose 0.05	Diabetic mice + Allulose 0.056 g/kg BW
D-allulose 0.1	Diabetic mice + Allulose 0.1 g/kg BW
D-allulose 0.2	Diabetic mice + Allulose 0.2 g/kg BW
NAOS 100	Diabetic mice + NAOS 100 mg/kg BW
NAOS 200	Diabetic mice + NAOS 200 mg/kg BW
NAOS 400	Diabetic mice + NAOS 400 mg/kg BW

After 14 days of HFD feeding, mice in the HFD group were administered a single intraperitoneal injection of streptozotocin (STZ) at a dose of 55 mg/kg BW, dissolved in citrate

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buffer at pH 4.5. Following STZ administration, all animals were allocated into six experimental groups (n = 5 per group). Treatment group assignments are presented in Table 1 for D-allulose and NAOS. Metformin, D-allulose, and NAOS treatments were administered by oral gavage once daily for 28 days, with a constant administration volume of 0.5 mL per mouse.

Hematological Profile Assessment

After 28 days of treatment, all mice were fasted for 8–10 hours prior to anesthesia using ketamine: xylazine (50 mg/kg BW: 5 mg/kg BW, intramuscular) (Struck et al., 2011). Blood samples were collected from the retro orbital sinus using a microhematocrit capillary tube (± 0.5 mL) and transferred into EDTA tubes. Following blood collection, euthanasia was performed by administering an intramuscular overdose of ketamine: xylazine, followed by cervical dislocation to ensure death (Struck et al., 2011). Hematological parameters were analyzed using an automated hematology analyzer, including red blood cell (RBC) and white blood cell (WBC) counts, hemoglobin concentration, hematocrit, mean corpuscular hemoglobin (MCH), and lymphocyte count.

Statistical analysis

Data are presented as mean ± standard deviation (SD). Statistical analyses were performed using GraphPad Prism 10 (GraphPad Software, Inc., San Diego, CA, USA). Hematological parameters were analyzed using one-way ANOVA with a significance level of 5% ($p < 0.05$). When significant differences were detected, post hoc analysis was conducted using the Duncan Multiple Range Test.

3. RESULTS AND DISCUSSION

Diabetes mellitus (DM) is a chronic metabolic disease characterized by hyperglycemia resulting from impaired insulin production or action, leading to widespread disturbances across multiple physiological systems, including the hematological system. Hematological profile assessment plays an essential role in evaluating physiological status and detecting systemic alterations (Alekseeva et al., 2024). The parameters examined in this study include red blood cell (RBC) and white blood cell (WBC) counts, hemoglobin concentration, hematocrit, mean corpuscular hemoglobin (MCH), and lymphocyte count.

A reduction in erythrocyte count was observed in the diabetic control group (DC) compared to the control group (C), accompanied by lower hemoglobin (Hb), mean corpuscular hemoglobin (MCH), and hematocrit (HCT). Although these differences were not statistically significant, they indicate early alterations in erythrocyte indices associated with hyperglycemic conditions. D-allulose treatment improved erythrocyte profiles, as evidenced by a highly significant increase in RBC count at 0.05 g/kg BW ($^{**}p < 0.01$) and a significant increase at 0.1 g/kg BW ($^{*}p < 0.05$) compared to diabetic control group (DC). The 0.2 g/kg BW dose and the positive control (PC; metformin) also showed higher RBC values, although these increases did not reach statistical significance (**Table 2**). In contrast, NAOS treatment did not result in significant changes in RBC counts; however, all NAOS doses demonstrated an increasing trend relative to diabetic control group (DC) (**Table 2**).

Consistent with RBC findings, D-allulose supplementation improved erythrocyte indices. Hb levels increased across all D-allulose-treated groups, with highly significant elevations at 0.05 and 0.1 g/kg BW ($^{**}p < 0.01$) and a significant increase at 0.2 g/kg BW ($^{*}p < 0.05$). A significant increase in MCH was observed at 0.1 g/kg BW ($^{*}p < 0.05$), whereas HCT values showed an increasing tendency without reaching statistical significance (**Table 2**). In the NAOS-treated groups, significant increases in Hb were observed in the the positive control (PC) and 200 mg/kg BW groups ($^{***}p < 0.001$ and $^{**}p < 0.01$, respectively). MCH increased significantly only at 100 mg/kg BW ($^{*}p < 0.05$), while higher doses exhibited non-significant upward trends. Notably, NAOS at 200 and 400 mg/kg BW resulted in significant increases in HCT ($^{**}p < 0.01$ and $^{*}p < 0.05$), indicating partial restoration of total erythrocyte volume in diabetic mice (**Table 2**).

In addition to alterations in erythrocyte parameters, diabetes also affected leukocyte (WBC) profiles. Leukocyte counts in the diabetic control group (DC) representing the diabetic condition were significantly elevated compared to the healthy control group (C) (*** $p < 0.001$), indicating a systemic inflammatory response

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associated with diabetes. Administration of D-allulose resulted in a significant reduction in leukocyte counts compared to diabetic control group (DC), particularly in the group receiving 0.2 g/kg BW (** $p < 0.01$). The positive control group (PC) also exhibited lower leukocyte levels than diabetic control group (DC), although the difference was not statistically significant (**Table 2**). Consistent with these findings, NAOS administration demonstrated modulatory effects on leukocyte counts. Neoagaro-oligosaccharides treatment showed a decreasing trend in leukocyte numbers, with the highest reduction observed at a dose of 400 mg/kg BW (** $p < 0.01$), suggesting that higher doses may suppress inflammatory responses associated with the diabetic state **(Table 2)**.

Table 2. Hematological parameters of mice following 28 days of D-allulose and NAOS treatment

Parameters Measured	RBC ($\times 10^6/\mu L$)	WBC ($\times 10^3/\mu L$)	Hb (g/dL)	HCT (%)	MCH (pg)	LYM (%)
C	8.28 ± 0.48	4.33 ± 0.79***	12.40 ± 0.14	42.05 ± 1.10	15.43 ± 0.83	81.16 ± 5.6
DC	7.65 ± 0.23	7.92 ± 0.53	12.13 ± 0.08	40.68 ± 0.66	14.92 ± 0.58	64.89 ± 4.49
PC	8.19 ± 0.45	6.23 ± 0.50	13.05 ± 0.25	43.50 ± 2.13	15.55 ± 0.50	83.45 ± 4.01
D-allulosa 0.05	8.72 ± 0.44**	6.13 ± 0.29	13.43 ± 0.41**	43.93 ± 2.49	16.20 ± 0.47	86.90 ± 2.14*
D-allulosa 0.1	8.52 ± 0.44*	5.62 ± 0.66	13.62 ± 0.40**	41.70 ± 4.16	16.70 ± 0.37*	87.22 ± 2.05**
D-allulosa 0.2	8.46 ± 0.41	4.98 ± 0.29**	13.32 ± 1.05*	40.86 ± 3.36	15.74 ± 0.56	84.86 ± 2.77
NAOS 100	7.69 ± 0.03	6.35 ± 0.61	12.68 ± 0.09	42.30 ± 0.99	16.2 ± 0.23*	72.24 ± 5.43
NAOS 200	8.00 ± 0.01	5.57 ± 0.10	12.84 ± 0.15**	45.75 ± 1.52**	15.70 ± 0.42	77.30 ± 6.23
NAOS 400	8.13 ± 0.24	5.15 ± 0.18**	12.68 ± 0.10	44.77 ± 1.43*	15.55 ± 0.15	83.40 ± 1.39*

D-allulose supplementation increased lymphocyte percentages compared to diabetic control group (DC), with statistically significant improvements observed at doses of 0.05 and 0.1 g/kg BW (* $p < 0.05$ and ** $p < 0.01$, respectively), while the 0.2 g/kg BW dose showed a non-significant increase. The positive control group (PC) also showed higher lymphocyte percentages than diabetic control group (DC), albeit not significantly (**Table 2**). NAOS administration at 400 mg/kg BW significantly increased lymphocyte percentages compared to DC (* $p < 0.05$). Metformin treatment (PC) likewise resulted in a significant increase in lymphocyte percentages (** $p < 0.01$). Overall, these findings demonstrate that both D-allulose and NAOS have potential to ameliorate lymphocyte reduction associated with diabetes, indicating improvement in immune dysregulation under diabetic conditions.

Hyperglycemia, a hallmark of diabetes, plays a central role in inducing oxidative stress and significantly affects erythrocytes. Elevated glucose levels trigger the production of reactive oxygen species (ROS) within erythrocytes through multiple pathways, including the polyol pathway, formation of advanced glycation end products (AGEs), and increased mitochondrial oxidative stress. This heightened oxidative burden and the resultant structural alterations impair erythrocyte deformability, ultimately leading to increased erythrocyte hemolysis (Obeagu, 2024). Impaired erythrocyte deformability also significantly influences various erythrocyte indices such as hemoglobin (Hb), mean corpuscular hemoglobin (MCH), and hematocrit (HCT). Although the decreases in these parameters were not entirely statistically significant, the observed trends indicate alterations in erythrocyte indices associated with hyperglycemia. These patterns align with previous studies reporting disrupted glucose homeostasis and changes in erythrocyte indices in diabetic animal models (Bella et al., 2017; Rehman et al., 2023).

The increases in erythrocyte count and indices observed in the D-allulose treatment groups, particularly at doses of 0.05 and 0.1 g/kg BW, are hypothesized to be related to the ability of D-allulose to mitigate hyperglycemia-induced oxidative stress. D-allulose has been reported to activate the nuclear factor erythroid 2–related factor 2 (Nrf2) pathway, enhancing the expression of protective antioxidant genes such as heme

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oxygenase-1 (HO-1) and increasing the activity of key antioxidant enzymes, including superoxide dismutase (SOD) and catalase (Ahmed et al., 2017; Shintani et al., 2017; Molonia et al., 2024). This reduction in oxidative stress may contribute to improved erythrocyte membrane stability and reduced hemolysis, thereby supporting enhanced erythrocyte viability and lifespan in circulation (Delesderrier et al., 2020). On the other hand, improvements in erythrocyte count and indices in NAOS treatment groups are likely associated with the ability of NAOS to enhance antioxidant status through increased activities of SOD and glutathione peroxidase (GPx) (Lin et al., 2019a). Additionally, NAOS has been shown to activate the Nrf2 and mitogen-activated protein kinase (MAPK) pathways, contributing to the upregulation of antioxidant enzymes such as HO-1 and NAD(P)H quinone dehydrogenase 1 (NQO1). This mechanistic action helps attenuate oxidative damage and the formation of AGEs (Liu et al., 2017; Sampath et al., 2017; Ebrahimi et al., 2025).

The observed increase in leukocyte count in the diabetic group (DC) indicates a chronic inflammatory response associated with hyperglycemia. This condition is closely associated with hyperglycemia-induced endothelial dysfunction and the activation of the Renin–Angiotensin–Aldosterone System (RAAS), leading to increased glomerular capillary pressure and oxidative stress (Li et al., 2023). In addition to endothelial-derived oxidative stress, hyperglycemia directly activates intracellular inflammatory pathways, particularly nuclear factor kappa B (NF-κB), which enhances the transcription of proinflammatory cytokines such as TNF-α and IL-6. These cytokines contribute to chronic inflammation and stimulate leukocyte production and recruitment (Verdile et al., 2015; Liu et al., 2024). Similar findings have been reported in previous studies showing elevated leukocyte levels in diabetic animal models compared to healthy controls (Ayodele et al., 2020).

Diabetes also affects leukocyte indices related to adaptive immunity, particularly lymphocytes. In the present study, the administration of D-allulose showed an improvement in lymphocyte profiles compared to the negative control group. This modulatory effect is likely associated with its capacity to suppress oxidative stress and inflammatory activation. D-allulose has been reported to modulate NF-κB signaling by inhibiting its activation and reducing the expression of proinflammatory cytokines, as demonstrated in adipocytes stimulated with palmitic acid (Molonia et al., 2024). Under normal physiological conditions, NF-κB remains in the cytoplasm bound to its inhibitor, IκB; however, upon inflammatory stimulation, NF-κB dissociates from IκB and translocates into the nucleus to initiate the transcription of proinflammatory genes (Kannan et al., 2025). Additional evidence shows that D-allulose supplementation reduces TNF-α, IL-6, and IL-1β expression in adipose tissue and serum, supporting its systemic anti-inflammatory activity in metabolic disorders (Zhao et al., 2024).

Similarly, treatment with NAOS also demonstrated improvement in lymphocyte profiles in diabetic mice. Neoagaro-oligosaccharides exhibits anti-inflammatory effects by inhibiting major pattern recognition receptor signaling pathways involved in innate immunity, particularly the NF-κB signaling axis. In vitro studies reported that NAOS, especially neoagarotetraose, suppresses NF-κB signaling in LPS-stimulated macrophages by reducing the phosphorylation of IκB kinase (IKK) and NF-κB p65, thereby preventing nuclear translocation and transcriptional activation (Wang et al., 2017). This inhibition results in decreased expression of inflammatory mediators including iNOS, TNF-α, IL-1β, and IL-6, which are crucial in amplifying inflammatory responses and leukocyte activation.

The systemic reduction of inflammation induced by both D-allulose and NAOS likely contributes to improved adaptive immune balance through the restoration of lymphocyte viability and regulation. Collectively, these findings indicate that D-allulose and NAOS hold potential as immunomodulatory agents capable of attenuating chronic inflammation associated with diabetes by targeting NF-κB-mediated pathways and oxidative stress.

4. CONCLUSION

The administration of D-allulose and neoagaro-oligosaccharides (NAOS) effectively improved the hematological profile of diabetic mice by suppressing oxidative stress and systemic

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inflammation. D-allulose demonstrated a more pronounced effect on increasing erythrocyte counts, whereas NAOS exerted a greater impact on enhancing erythrocyte quality, as reflected by improvements in erythrocyte indices, particularly hematocrit and mean corpuscular hemoglobin (MCH). Additionally, both compounds reduced leukocyte levels and enhanced lymphocyte counts, indicating an improvement in inflammatory status and the restoration of adaptive immune responses. These findings suggest that D-allulose and NAOS have the potential to ameliorate hematological disturbances associated with diabetes mellitus.

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