Hematological parameters in streptozotocin-induced diabetic rats and the effect of Rosa damascena Mills. extract

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ABSTRACT

**Introduction:** Chronic inflammation is the primary factor contributing to the development of diabetes mellitus and its associated problems in affected patients. White blood cells are of significant importance in the degenerative mechanism of blood vessel walls in individuals with diabetes, ultimately leading to the development of atherosclerosis and initiating the rupture of unstable plaques, which subsequently causes thrombosis. Red roses contain anthocyanins, with cyanidin being the specific compound that demonstrates the greatest antioxidant action. The main aim of this study was to investigate the impact of various doses of ethanol extract derived from Rosa Damascena on hematological markers in rats with diabetes.

**Methods:** This study used an experimental design with a randomized post-test-only control group. A total of twenty male Wistar rats were allocated into five distinct groups for the purpose of this study. The groups were categorized as follows: Healthy Control Group (HCG), Diabetes Group (DG), and Extract Group, which was further separated into three subgroups denoted as P1 (250 mg/kgBW), P2 (500 mg/kgBW), and P3 (1,000 mg/kgBW). The experimental groups received a single injection of streptozotocin at a dosage of 50 mg/kgBW to induce diabetes. Additionally, they were administered oral treatment of Rosa Damascena ethanolic extract for a duration of 2 weeks.

**Results:** The group denoted as P2 has the lowest NLR, as seen by a mean value of 0.06. In contrast, the group administered with Metformin exhibited the lowest leukocyte value, with a mean of 7.98±6.0. Nevertheless, there are no substantial disparities in the remaining variables.

**Conclusion:** The results of this study indicate that the ethanol extract obtained from Rosa Damascena did not have a statistically significant effect on the hematological parameters of rats with streptozotocin-induced diabetes.

**Keywords:** Rosa Damascena, Hematology profile, Streptozotocin.


INTRODUCTION

Based on data provided by the World Health Organization (WHO), the prevalence of Diabetes Mellitus (DM) as a non-communicable illness is at 9%. Furthermore, it has been shown that DM is the cause of around 80% of fatalities in both developed and developing countries.¹ In the course of the last thirty years, there has been a consistent upward trend in the occurrence of diabetes across various income brackets, with rates increasing from 4.7% to 8.5%. The prevalence of diabetes mellitus (DM) among individuals aged 18 to 65 years is estimated to be 8.5% of the total population.²

Insulin problem is implicated in the development of insulin resistance, a condition widely regarded as the primary contributor to impaired glucose tolerance, diabetes, obesity, dyslipidemia, and hypertension.³ Multiple research studies have substantiated a correlation between insulin resistance and systemic inflammation, hence leading to immune system modifications in the pathophysiology of individuals afflicted with diabetes.³

In patients with diabetes mellitus, the most significant factor contributing to the development of this disease and its complications is chronic inflammation.⁴ White blood cells play a crucial role in the degenerative process of blood vessel walls in diabetic patients, leading to atherosclerosis and triggering the rupture of unstable plaques, resulting in thrombosis. Lately, examining leukocyte subtypes such as the neutrophil-lymphocyte ratio (NLR) has become a consideration as an affordable and convenient inflammation parameter in simple blood tests.⁵

Leukocytosis is commonly employed as an indicator of inflammation in routine clinical settings. Numerous studies have provided evidence of a correlation between the count of white blood cells and the prognosis and occurrence of coronary heart disease.⁶ Lymphocytes are known to play a crucial role in the modulation of the inflammatory response throughout the progression of atherosclerosis. Lymphocytes play a crucial role in both the immunological and inflammatory pathways, ultimately resulting in the induction of death in the lymphocytes themselves.⁷

According to data provided by the Indonesian Central Statistics Agency in 2017, the rose plant ranks second in terms of cultivation area and production among decorative plants, following...
chrysanthemums. East Java province is renowned for being the primary hub of rose cultivation in Indonesia, with the highest production volume in the country. A decade-long investigation spanning from 2009 to 2019 has yielded significant findings regarding the impacts of red rose extract. The research has revealed that red rose extract exhibits a wide range of beneficial properties, including antibacterial, antifungal, antioxidant, hypnotic, analgesic, anticonvulsant, antidepressant, anti-HIV, anti-inflammatory, and antidiabetic activities.

Red roses are known to possess a range of significant polyphenolic compounds, including kaempferol, cyanidin 3.5-diglucoside, quercetin, and gallic acid. The content of anthocyanins in red roses can reach a maximum of 0.925% per 10g. Among the several types of anthocyanins present, cyanidin exhibits the highest level of antioxidant activity. It is widely hypothesized that anthocyanins possess several properties, including anti-hyperglycemic, anti-hyperlipidemic, and antioxidant actions. The primary objective of this study was to examine the effects of different doses of ethanol extract of Rosa Damascena on hematological parameters in diabetic rats.

MATERIAL AND METHODS

The animals utilized in this study consisted of 20 male white rats of the Wistar Strain, around 12 weeks old, with body weights ranging from 150 to 200 grams. All animals were in good health, exhibiting no physical impairments, as seen by their lustrous and clean hair, pink mucous membranes around the eyes, and overall agility. The exclusion criteria encompassed individuals who were sick or incapacitated. In the event that the experimental animals had perished throughout the course of the experiment, they would have met the requirements for dropout.

The animal samples were acquired from the Department of Pharmacology, Faculty of Medicine, Airlangga University. The subjects were maintained in laboratory conditions, adhering to conventional protocols, which included a 12-hour light/dark cycle and controlled temperature.

There were a total of five cages, each measuring 500 x 300 x 150 mm in terms of length, width, and height. Each enclosure is comprised of four rats. The subjects were provided with normal pellets and had unrestricted access to water.

The animal laboratories were administered a modest dosage of streptozotocin using a regimen of 50 mg per kilogram of body weight. The fasting blood glucose levels were assessed five days post-injection. If the detected glucose levels are above 200 mg/dl, the individual will be classified as having diabetes. The fasting glucose levels were measured using Easy Touch glucometers and Glucostrip. The animals were categorized into five distinct categories by the utilization of random sampling. The study consisted of many groups, namely the Healthy Control Group (HCG), Diabetes Group (DG), and Rosa Damascena Group. The Rosa Damascena Group was further divided into three subgroups, denoted as P1 (250 mg/KgBW), P2 (750 mg/KgBW), and P3 (1,000 mg/KgBW).

The experiment protocols conducted in this study received approval from the Ethics Committee Faculty of Dental Medicine Airlangga University, with reference number 456/HRECC.FODM/X/2020, located in Surabaya, Indonesia. These procedures were carried out in accordance with ethical standards, according strictly to the guidelines and objectives of animal research.

Extract of Rosa Damascena
A quantity of 100 grams of dried Rosa Damascena was immersed in a solution of 96% ethanol for a total of three consecutive periods of 24 hours each. Subsequently, the mixture was subjected to evaporation at a temperature of 50˚C, resulting in the formation of a thick and sticky extract. CMC-Na 0.1% was included in the mixture to enhance its viscosity and inhibit particle aggregation.

The extract will be administered in three distinct dosages, namely P1 at a dosage of 250 mg/kgBW, P2 at a dosage of 500 mg/kgBW, and P3 at a dosage of 1,000 mg/kgBW. The ethanol extract was solubilized in distilled water prior to oral administration to the rats.

A Biochemical Evaluation
Following a 14-day treatment period, the blood plasma samples were analyzed in order to assess the hematological profile of each individual unit. The procedure necessitated the administration of anesthesia and termination due to the utilization of the cardiac puncture technique. The blood sample was obtained by using a 5 ml syringe with a 23G needle and collected into a vacutainer plastic serum tube. The blood samples were transported to the laboratory at Surabaya Health Center for analysis via an automated analyzer.

RESULTS

The hemoglobin (Hb) levels observed in the diabetes group were found to be significantly higher compared to both the metformin group and the P1 and P2 groups. However, the Hb levels in the diabetes group were found to be nearly equivalent to those observed in the P3 group. The statistical analysis using the Kruskal-Wallis test did not yield any significant changes in the Hb (g/dl) levels between the treatment groups and the control group. The P2 group has a notable Neutrophil-to-Lymphocyte Ratio (NLR) with a mean value of 0.06, distinguishing it as the most favorable among the groups. In contrast, it was noted that among the groups that were administered Metformin, one group had the lowest leukocyte count, with a mean value of 7.98±6.0. Significantly, the other characteristics such as Thrombocyte, Hb, and Erythrocyte which were evaluated in this study exhibited no statistically significant variations among the different groups. This observation underscores the distinctive effect of P2 with respect to NLR, and the specific influence of Metformin on leukocyte counts, while simultaneously underscoring the general comparability in other measured variables across the groups (Table 1).

Regarding leukocyte outcomes, the metformin group exhibited the lowest mean value, whereas the RD extract groups administered at doses ranging from 250 mg/kg BW to 1,000 mg/kg BW demonstrated an elevation that did not display statistical significance when compared to the diabetes group. Nevertheless, the statistical analysis conducted using the difference test did not yield any statistically significant differences when comparing the experimental group...
to the control group.

The platelet values exhibited a decrease in the metformin group as compared to the diabetes control group. However, the administration of RD extract at a dosage of 1,000 mg/kg BW demonstrated a noteworthy rise in platelet values as compared to the control group.

The statistical analysis of the eosinophil differential count revealed a statistically significant disparity between the group administered with RD extract at a dosage of 1,000 mg/kg BW and the control group. Nevertheless, no substantial disparities were observed among the remaining groups. Significant changes were seen in the basophil difference test between the control group and the groups administered RD extract at dosages of 500 and 1,000 mg/kg BW.

The metformin group had the highest values for neutrophils, which were substantially different from the diabetes control group. However, the administration of RD extract did not yield any significant differences.

**DISCUSSION**

Diabetes mellitus is a prevalent and progressive metabolic condition defined by increased blood glucose levels resulting from either insulin resistance or decreased insulin production. Additionally, it induces oxidative stress that impacts several systems through diverse methods. Streptozotocin, a derivative of nitrosourea, is internalized by pancreatic β-cells, leading to DNA fragmentation by alkylation processes and resulting in rapid death of β-cells. A reduction in the rate of insulin production results in the development of stable hyperglycemia.

In a study conducted by Akpan and Ekaidem in 2015, it was shown that experimental rats exposed to streptozotocin exhibited hematological alterations. The hematological changes seen in this study are attributed to the presence of oxidative stress, which subsequently results in modifications in the amounts of biomolecules. Existing research suggests that individuals with diabetes mellitus (DM) exhibit changes in their hematological parameters, including decreased red blood cell (RBC) count, hemoglobin (HGB) level, and platelet...
The findings of this investigation suggest that the ethanol extract derived from *Rosa Damascena* does not exert a statistically significant impact on the hematological parameters of rats with diabetes caused by streptozotocin. The presence of various restrictions within this study may account for this phenomenon.

**CONCLUSION**

The findings of this investigation suggest that the ethanol extract derived from *Rosa Damascena* does not exert a statistically significant impact on the hematological parameters of rats with diabetes caused by streptozotocin. The presence of various restrictions within this study may account for this phenomenon.

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**AUTHOR CONTRIBUTIONS**

Conceptualization, L.D. and T.J.; methodology, L.D., T.J. and P.W.; software, T.J.; validation, T.J., and L.D.; formal analysis, T.J.; investigation, L.D. and T.J.; resources, T.J.; data curation, L.D., and T.J.; writing—original draft preparation, L.D., T.J., and P.W.; writing—review and editing, L.D. visualization, T.J, and P.W.; supervision, L.D.; project administration, P.W.; funding acquisition, L.D. and T.J. All authors have read and agreed to the published version of the manuscript.

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None.

**CONFLICT OF INTEREST**

None.

**ETHICAL PERMISSION**

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**REFERENCES**


