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THE EFFECT OF VITAMIN A TREATMENT ON RENAL GLOMERULAR AREA OF RATS MODEL DIABETES MELLITUS TYPE 2

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Abstract

Background: Diabetes mellitus (DM) type 2 is a metabolic disorder due to defects in insulin secretion. A microangiopathy complication of DM in the kidney can lead to renal enlargement. This augmentation in renal size can be attributed to several factors, including glomerular hypertrophy, tubular hypertrophy, and interstitial expansion. Retinoic acid, a vitamin A derivative, serves numerous cellular roles, including the induction of cell differentiation, the regulation of apoptosis, and exhibiting anti-inflammatory and antifibrotic properties.
Objective: This study aims to evaluate the effect of vitamin A administration on the renal glomerular area in a rat model of type 2 diabetes mellitus.
Methods: This study was experimental with five groups, including a negative control group, a positive diabetes mellitus group, and three groups of vitamin A treatment. The primary variable assessed was the glomerulus area across the different groups.
Results: No significant decrease in glomerular area was observed in the vitamin A treatment group within the positive diabetes (ANOVA, p = 0.052).
Conclusions: The findings of this study suggest that the administration of vitamin A does not significantly affect the reduction of renal glomerular area in rats with diabetes mellitus.

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INTRODUCTION

Diabetes mellitus (DM) represents a complex metabolic disorder marked by elevated blood glucose levels, which arise from anomalies in insulin secretion, insulin action, or a combination of both. Type 2 diabetes mellitus constitutes the predominant variant of the condition. The underlying causes exhibit considerable variability, from a preponderance of insulin resistance accompanied by a relative deficiency of insulin to a predominance of defects in insulin secretion coupled with insulin resistance (1). According to data from RISKESDAS 2018, the national prevalence of DM is 8,5%, equating to approximately 20.4 million individuals within Indonesia who have been diagnosed with this condition. The International Diabetes Federation (IDF) indicates a growth in diabetes patients, escalating from 10.7 million to 13.7 million individuals in 2030 (1).

The absence of glycemic control in diabetes mellitus may precipitate acute metabolic disturbances and chronic vascular complications, manifesting as either microangiopathy or macroangiopathy, ultimately resulting in a spectrum of persistent complications affecting the ocular system, renal function, peripheral nerves, and vascular integrity. The renal manifestation characterized by microangiopathy is referred to as diabetic nephropathy. It is estimated that 20-30% of individuals diagnosed with type 2 diabetes will experience diabetic nephropathy, which has the potential to progress to renal failure (2).

Diabetic nephropathy (DN) represents a consequential complication associated with diabetes mellitus, distinguished by persistent albuminuria, which is quantitatively defined as urinary albumin excretion surpassing 300 mg within 24 hours. The primary abnormality that occurs in diabetic nephropathy is glomerular alteration. Glomerular area initially peaks in mild diabetes, indicating structural changes. In diabetic nephropathy, the glomerular area is affected by structural changes, including increased mesangial volume and occlusion of glomeruli (3).

Renal enlargement is a significant early change in diabetes mellitus, primarily due to hypertrophy and hyperfunction of renal cells. This enlargement is attributed to various factors, including glomerular and tubular hypertrophy, a compensatory response to hyperglycemia. Increased size of glomeruli is observed, leading to elevated glomerular filtration rates (GFR) in early diabetes (4). Recent research has shown that the increased size of the kidney occurs in the first month, and the condition will worsen at the end of the fourth month (4).

Another abnormality that occurs in the kidneys, especially in the glomerulus of people with diabetes mellitus, is the presence of microalbuminuria. Microalbuminuria is defined as excessive albumin excretion and is considered essential to develop uncontrolled diabetic nephropathy. It will grow into proteinuria clinically, continue with the decrease of glomerular filtration rate function, and end in renal failure. The prevalence of microalbuminuria among individuals diagnosed with Type II diabetes mellitus was found to be 30.4% in the conducted study, signifying a noteworthy prevalence of this initial indicator of diabetic nephropathy within the clinical demographic assessed (5).

Vitamin A is an active vitamin that, when dissolved in fat, is stored by the body in the liver. Scientifically, there are two forms of vitamin A: pre-form vitamin A and provitamin A. There are four types of pre-form vitamin A: retinol, retinoic acid, retinal, and retinyl ester. Retinoic acid, a compound derived from vitamin A, plays various roles at the cellular level, such as promoting cell differentiation, regulating apoptosis, and suppressing

inflammation and cell proliferation. Retinoic acid is necessary for kidney development and cell differentiation in damaged podocyte cells of glomeruli (6). The mechanism of retinoic acid's beneficial effects, especially on the kidney, is multifactorial, ranging from its anti-inflammatory and anti-fibrotic effects to the upregulation of podocyte differentiation markers in renal podocyte cells (7).

Supplementation with vitamin A as antioxidants has been linked to improved renal function profiles in diabetic models, suggesting a protective effect against oxidative stress. In experimental studies, vitamin A supplementation has been associated with reduced glomerular damage and improved kidney morphology in diabetic rats, indicating potential benefits for glomerular area preservation (8).

Based on the description above, vitamin A has benefits in repairing renal glomerular cell damage, and further investigation is needed about the use of vitamin A as an inhibitor of the development of advanced kidney damage in type 2 diabetes mellitus and from this research, expected that vitamin A can later be used as an alternative treatment for kidney disease mainly caused by type 2 diabetes mellitus.

MATERIALS AND METHODS

The research method was conducted using an experimental design (actual experimental design) in the laboratory, using an in vivo study with a randomized post-test control only. The research subjects used were male Wistar strain white rats (Rattus norvegicus) maintained in the Biochemistry Biomolecular Laboratory of the Faculty of Medicine, Brawijaya University.

A total of 30 male Wistar rats (6-8 weeks old, 150-200 grams) were acclimatized for 7 days with a 25 mg regular diet before the experiment. The sample was chosen by random sampling and divided into 5 (five) treatment groups as follows:

1. Negative control group (KN): Normal diet,
2. Positive control group (KP): Provision of high-fat diet, then rats were injected with Streptozotocin (STZ) but not given vitamin A,
3. Group treatment I (VAP1): Provision of high-fat diet, then the rats were injected with STZ and given 50 mg/kg b.w of vitamin A,
4. Group II treatment (VAP2): Provision of high-fat diet, then the rats were injected with STZ and given 100 mg/kg b.w of vitamin A,
5. Group III treatment (VAP3): Provision of high-fat diet, then the rats were injected with STZ and given 150 mg/kg b.w of vitamin A.

In the second week, a high-fat diet comprising 25 grams was initiated for the positive control group (KP) and the treatment groups (VAP1, VAP2, and VAP3). This high-fat diet was formulated utilizing a composition that included 221.75 grams of BR1, 123.25 grams of wheat flour, 0.098 grams of cholic acid, 7.105 grams of cholesterol, and 184.25 grams of lard (9). A high-fat diet contains more calories from fat (45-60% of calories). A high-fat diet in rats leads to increased body weight and fat pad weights, decreased glucose tolerance, and elevated fasting insulin levels (10).

In the seventh week, an intraperitoneal administration of streptozotocin (STZ) at a dosage of 30 mg/kg body weight (BW) was executed (11). Streptozotocin (Calbiochem, Catalog No. 572201) was prepared by dissolving 100 grams in 3 mL of citrate buffer at pH

4.5, followed by vortexing until a homogeneous solution was achieved, resulting in a stock STZ solution that was subsequently stored at a temperature of 4 °C.

After the STZ injection, blood glucose levels were evaluated in the eighth week to ascertain the presence of type 2 diabetes mellitus (DM). Rats were classified as diabetic if their fasting glucose parameters exceeded 125 mg/dl (12) and exhibited clinical signs of polyuria, polyphagia, polydipsia, and weight reduction (13). Blood glucose levels were determined via a sample obtained from the tail tip of the rat (lateral vein) utilizing a glucose strip (Easy Touch).

Vitamin A (IPI 6.000 IU) was obtained in pill form, ground in a blender, and divided by dosage, 50 mg/kgBW, 100 mg/kgBW, and 150 mg/kgBW. Administered to rats using tubes, the vitamin A was diluted with two cc of aquades, dissolved, inserted into the syringe, and administered per os/sonde to the rats. Vitamin A was given for 4 weeks in the 8th to 11th week. Chronic toxicity is a consequence of the prolonged consumption of vitamin A over an extended duration of months or years. Daily consumption levels of 25,000 IU for six years and 100,000 IU for six months are considered toxic (14).

At the beginning of the 12th week, kidney tissue was taken and stained using Hematoxylin and Eosin (HE). Histopathologic preparations were scanned and observed using the Master Scan Dot Slide application with 400x magnification to calculate glomerular area by counting 5-10 representative glomeruli on each slide or kidney preparation (15).

This research analyzed all the data using SPSS for Windows Version 16.0 software. Data analysis in this study includes a normality test with the Shapiro-Wilk test to analyze normality and distribution of data, a homogeneity test with Levene's test to analyze the homogeneity of data between groups, and a comparative analysis with one-way ANOVA. Post-hoc test to determine the differences that occurred between groups. Correlation and regression tests are used to determine the relationship between variables.

RESULTS AND DISCUSSIONS

Fasting Blood Glucose Level in Rats

Based on data on average blood glucose measurements of rats before injection of STZ, all groups had normal blood glucose levels. After STZ injection, the KN group had a lower or normal blood glucose level than the other groups. Normal fasting blood glucose levels of rats are <100 mg/dL (17). The data show that the different groups, except the KN group, have blood glucose levels above normal or hyperglycemia.

Table 1. Average of fasting blood glucose

Group	Average of Fasting Blood Glucose (mg/dL)	
	Before	After
KN	71,20	88,67
KP	91.30	246,33
VAP1	85,00	179
VAP2	76,40	221
VAP3	71,80	158,67

STZ is a glucosamine-nitrosourea compound that induces diabetes primarily by damaging pancreatic β-cells by generating free radicals. This process leads to significant cellular damage, including DNA strand breaks and other genotoxic effects, ultimately impairing insulin production. The destruction of β-cells leads to an initial surge in insulin followed by a deficiency, causing hyperglycemia (16,17). The combination of high-fat feeding and STZ injections further supports the occurrence of hyperglycaemia in rats. Experimental animals with a high-fat diet caused obesity, hyperinsulinemia, and insulin resistance in rats (12). Diabetes mellitus can be induced by combining high-fat feeding that results in insulin resistance and STZ administration that causes pancreatic beta cell dysfunction and leads to hyperglycemia (18).

Glomerular Surface Area of Kidney in Rats

The measurement of the glomerulus surface area performed at the end of the study showed varying results for each group. The result of the average surface area of the glomerulus is shown in the following graph.

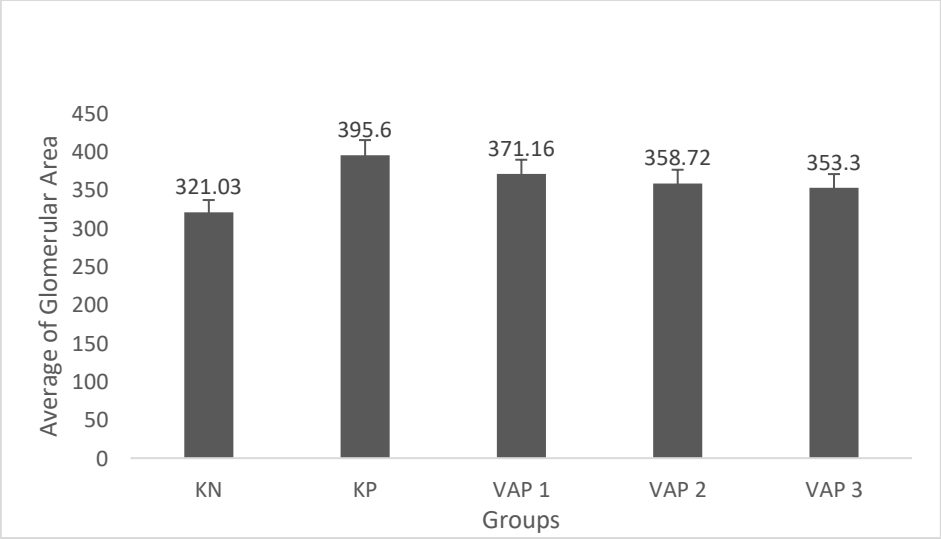

Figure 1. Average of Surface Area Results of Glomerulus

The average of the glomerulus area in the KN group was smaller than that of the KP group and the three other treatment groups. The KP group has a larger average area value than the other groups. The VAP1 group has a larger glomerular area than VAP2 and VAP3, but smaller than the KP group. The VAP2 group has a smaller glomerular area than VAP1 but larger than VAP3. Based on the comparison of each group, the smallest average area of glomerulus was found in the KN group {321,03}, and the largest average area was found in the KP group {395,6}.

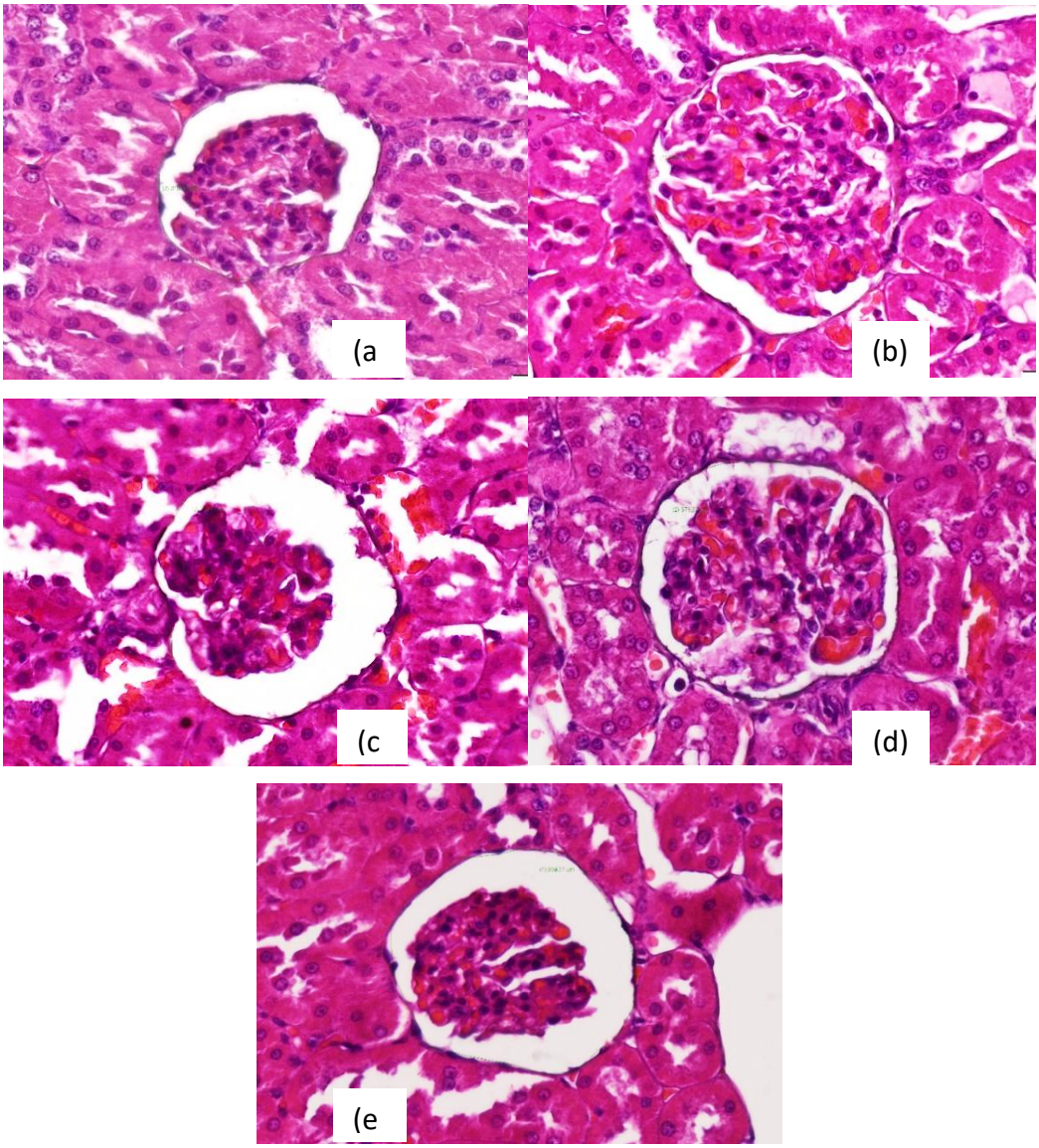

Figure 2. Glomerulus of Kidney**,** (a) Negative Control (KN), (b) Positive Control (KP), (c) Group treatment 1 (VAP1), (d) Group treatment 2 (VAP2), (e) Group treatment 3 (VAP3), Hematoxylin Eosin, 400x

The results of the normality test using the Shapiro-Wilk test showed that the significance value for glomerular area data was 0.339 (p>0.05), and the homogeneity test of variances showed that the value of the glomerular area was 0,066 (p>0,05). The data determined that the glomerular area data were normally distributed and homogeneous.

Based on the results of statistical tests using One Way ANOVA, the significance value for glomerular area was 0,052 (P>0,05). It can be concluded that there is no significant difference in the average area of the white rat glomeruli in each group. The method of analysis to determine the difference in measurements of the five groups can be identified using Post Hoc Multiple Comparison with the Turkey HSD test. In the Post Hoc Turkey HSD test, the results showed that there was a significant difference in the glomerular area of the negative control group (KN) with the positive control group (KP), which was 0,031 (P<0,05).

Diabetes mellitus is a metabolic disease with characteristics of hyperglycemia and is associated with an increase in free radicals (19,25). The prolonged condition of hyperglycemia in diabetes mellitus can induce the occurrence of oxidative stress (20). This increase of oxidative stress can lead to various reactions that can affect protein kinase C, the response of plasma non-enzymatic glycation and glomerular basement membranes protein, and trigger the rise of proinflammatory cytokines such as transforming growth factor β (TGF-β) and vascular endothelial growth factor. The accumulation of proinflammatory cytokines such as TGF-β and vascular endothelial growth factor may induce inflammatory processes as well as increased synthesis of extracellular matrix, which will ultimately lead to increased production of collagen, thickening of glomerulus basement membranes, and tubulointerstitial fibrosis (21,22). All of these reactions, supporting the enlargement of the glomerular area in diabetes mellitus, showed a significant difference in the average glomerular area between the negative control group (KN) and the positive control group (KP).

The result showed a decrease in glomerular area in treatment groups (VAP1, VAP2, VAP3) compared with KP, but there was no significant difference (p>0.05). Pearson correlation test showed that the significance was 0,054 (p>0.05), so there was no significant difference between vitamin A dosage and renal glomerular area. In addition, the correlation value was also obtained at -0.569. The correlation value indicates a strong relationship between a higher dosage and the reduction of the renal glomerulus area. The higher the vitamin A dosage, the smaller the renal glomerular area, but not significantly.

Vitamin A has derivatives, one of which is retinoic acid (RA), an anti-inflammatory effect proven in experimental animals that experienced damage to the glomerulus. The beneficial effects of RA have also been demonstrated in other tissues. In the in vivo and in vitro models of diabetic nephropathy, RA treatment improved podocyte damage. Notably, treatment of podocyte cultures with AR may reduce the activation of the inflammatory pathway by inhibiting the synthesis of monocyte-chemotactic-protein-1 (MCP-1), which MCP-1 may aggravate the diabetes condition (7,21).

Another anti-inflammatory effect is proteinuria reduction and the inhibition of inflammation seen in diabetic rats treated with RA. RA treatment may reduce the occurrence of albuminuria, repair lesions in the glomerulus, and inhibit the expression of cytokines and chemokines in the kidneys. The administration of RA reduced proteinuria and improved other markers of kidney injury, suggesting a comprehensive renoprotective effect. RA

suppresses the transcription of some pro-inflammatory cytokines and chemokines that induce macrophages (23,24). This mechanism causes the administration of vitamin A in the VAP1, VAP2, and VAP3 groups to show a decrease in average glomerular area compared to the diabetic positive control group (KP).

Despite the decrease in glomerular area, the statistical difference between the average glomerular area is insignificant. Possibly, this is because the effects of vitamin A as an anti-inflammatory are only dominant in the damaged glomerular podocyte cells. In contrast, those affecting glomerular enlargement are the presence of mesangial hypertrophy, increased collagen production, and thickening of the glomerular basement membrane as a result of increased production of extracellular matrix.

Al-Hindi et al. found that administering vitamin A treatment for 16 weeks in streptozotocin-induced diabetic mice resulted in significant improvements in body weight, fat mass, lipid profile, and antioxidant enzyme levels, suggesting this duration may be optimal for therapeutic effects (23). In a study involving WNIN/GR-Ob obese rats, a diet enriched with 129 mg/kg of Vitamin A for 14 weeks significantly improved hyperglycemia, glucose clearance, and increased insulin and glycogen levels in the liver and muscle (26). Our study showed that vitamin A does not significantly affect the glomerular area. This suggests that the timing of vitamin A supplementation may be suboptimal for reducing glomerular area in diabetic rat models.

CLINICAL IMPLICATION

Given the role of vitamin A in regulating oxidative stress, cellular differentiation, and maintaining epithelial integrity, this study provides preliminary evidence supporting its utility as a nutritional adjunct in mitigating early glomerular damage associated with type 2 diabetes mellitus. These findings warrant further investigation through long-term studies and clinical trials to assess the therapeutic potential of vitamin A in diabetic renal complications.

LIMITATIONS

The relatively brief duration of vitamin A treatment is inadequate to assess the long-term administration on the advancement of renal impairment. This investigation did not assess biochemical indicators pertinent to renal function, such as serum creatinine concentrations or glomerular filtration rate (GFR), thereby constraining the interpretation of overall renal performance to morphometric data alone. The singular dosage of vitamin A administered has not been juxtaposed with alternative dosages to ascertain its optimal efficacy and potential toxicity.

CONCLUSIONS

Based on the results of a study on the comparative effects of vitamin A on the renal glomerular area of rats with type 2 diabetes mellitus, it can be concluded that there is a significant increase in the average glomerulus area in the positive control group compared with the negative control group. However, there was no significant effect of vitamin A in the 50mg/kg b.w, 100mg/kg b.w, and 150mg/kg b.w on the decreased rat renal glomerular area with diabetes mellitus.

CONFLICT OF INTEREST

The authors declare no conflict of interest related to this research.

AUTOR CONTRIBUTIONS

First Author engaged in the conceptualization, design of the study, interpretation of data, statistical analysis, drafting of the manuscript, and critical revision. The second author was involved in executing experimental procedures, encompassing STZ induction and administration of vitamin A, as well as the preparation and supervision of histological samples. Third Author responsible for interpreting morphometric data and contributing to the discussion section and histological imaging.

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The administered dosage of vitamin A tretamnet was not optimal in mitigating the hypertrophy of the renal glomeruli in diabetic rat models. Due to the absence of measurements regarding the thickness of the Bowman's capsule, it remains undetermined whether a correlation exists between glomerular enlargement and the thickness of the Bowman's capsule in the context of diabetes mellitus. The duration of vitamin A administration was insufficient to adequately enhance its efficacy in the reparative processes of the glomerulus.

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