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Short Communication

Biochemical And Triglyceride-Glucose Index (Tyg) Profile In High Doses Streptozotocin-Nicotinamide Produce Diabetes Mellitus In Rats Model

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ABSTRACT

Preclinical test is a stage in evaluating potential drug candidates for diabetes mellitus (DM). However, developing an animal model that accurately replicates the various pathophysiological and etiological aspects of DM as seen in humans presents significant challenges. To induce diabetes, streptozotocin (STZ) in single doses or combination with nicotinamide (NIC) is often used. Treating diabetes conditions has a low success rate, compounded by the complexity of numerous affected biochemical profiles, presenting a significant research challenge. Therefore, this research aimed to determine the biochemical and triglyceride-glucose index (TyG) profile in high doses of STZ-NIC-induced diabetes in the rat model. The population consisted of 18 rates divided into three groups, each containing six. Group I (the normal group) consisted of healthy rats who were given standard feed and drink. In groups II and III, diabetes was induced intraperitoneally with 50 and 65 mg/kg of STZ in addition to 230 mg/kg of NIC. Observations were made for 6 weeks after the rats were diagnosed with diabetes having blood glucose levels of ≥ 250 mg/dL 72 hours after STZ-NIC induction. The data were evaluated statistically by one-way analysis of variance (ANOVA), followed by the least significant difference (LSD) test ($p \leq 0.05$). The result showed significant differences in blood biochemistry, specifically in the parameters of blood glucose, SGPT, and SGOT with increasing doses of STZ-NIC ($p<0.05$), but not in total cholesterol, triglycerides, albumin, total protein, and TyG ($p<0.05$). The high doses of STZ-NIC administration can produce DM models with different blood biochemical profiles but not in TyG.

***Keywords*:** Diabetes Mellitus; Blood Biochemical; Triglyceride-Glucose Index; Marker

Introduction

Diabetes mellitus (DM) is a metabolic disease characterized by increased blood glucose levels and followed by changes in other biochemical profiles.[1] The increasing incidence of DM and the side effects of oral antidiabetic drugs enable worldwide research to develop natural anti-diabetic therapies.[2] Preclinical screening and investigation of new anti-diabetic drugs for type 2 diabetes mellitus (T2DM) require chemically generated rat models. However, developing an animal model that includes many pathophysiological aspects of T2DM and diabetes sequelae with a comparable etiology of clinical presentation presents significant challenges.[3] The in vivo test was used as a protocol because the pharmacodynamic and pharmacokinetic profiles were similar to those in humans.[4] Previous research have developed various models on experimental animals, such as chemical induction, high glucose, and high-fat diets.[5] Diabetogenic chemical ingredients, such as streptozotocin (STZ) and alloxan, are often used for testing in single doses or combination.[6]

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Previous research reported the effect of variations in the doses of STZ and NIC on the profile of kidney function due to complications of diabetes. These results showed that STZ at a dose of 65 mg/kg with a combination of 230 mg/kg NIC produced a pre-nephropathy model.[7] Furthermore, STZ induces DM from 35 mg/kg to 65 mg/kg, depending on the research objectives. Recent research has shown that modeling DM using STZ induction combined with nicotinamide (NIC) provided a model of T2DM.[8] Nicotinamide reduced experimental animal mortality by decreasing baroreflex and parasympathetic sensitivity modulation.[9] Despite numerous reports about the development of STZ-NIC-induced rat models, the results exhibited variability. This was due to several factors, such as the type of rat, age, diet, how long it was fed, and the amount of STZ.[3] Due to the varied results on the biochemical profiles, the research screened most of the parameters of organ function. This screening incurs significant fees in addition to the prepared technical components. Consequently, a reference was needed for any biochemical profiles that do not require testing when developing T2DM. Based on this background, it is very important to develop animal models with T2DM that can be easily replicated and modified by existing procedures when examining glycemic status, body weight profile, lipid profile, and atherogenic risk. Therefore, this research aimed to optimize biochemical markers in the Wistar rat model induced by high doses of STZ-NIC to build a pre-clinical model of diabetes.

Methodology

Materials

The chemicals used in this experiment, which included streptozotocin (STZ) and nicotinamide (NIC), were of analytical grade and purchased from Sigma-Aldrich in Germany. Some reagents were purchased from

Biosystem in Germany, including blood glucose, total cholesterol, triglycerides, serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), total protein, and albumin kits.

Ethical clearance
The present method was approved by the ethics committee of the Integrated Research and Testing Laboratory (LPPT) at Gadjah Mada University (No: 00034/04/LPPT/VIII/2021).

Experimental animals
Male Wistar rats weighing around 180–200 grams were obtained from the Laboratory of Animal Life Science in Karanganyar, Indonesia. The animals lived in a controlled environment with 12-hour cycles of light and dark in the integrated laboratory at Universitas Sebelas Maret. Before commencing the research, the animals were allowed to adapt for seven days under regulated food and water conditions.

Streptozotocin-nicotinamide treatment
The research used 18 male rats, divided into 3 groups, with 6 in each, as shown in Figure 1. Group I (normal group) contained healthy rats given standard feed and drink for 6 weeks of the experiment. Group II was induced intraperitoneally with 50 mg/kg STZ + 230 mg/kg NIC. Meanwhile, group III was induced intraperitoneally with 65 mg/kg STZ + 230 mg/kg NIC). STZ was dissolved in citrate buffer (0.1 M, pH 4.5) before injection into rats, 15 min after administration of NIC in saline solution. The rats with blood glucose levels of more than 250 mg/dL were used as a diabetes model.[10]

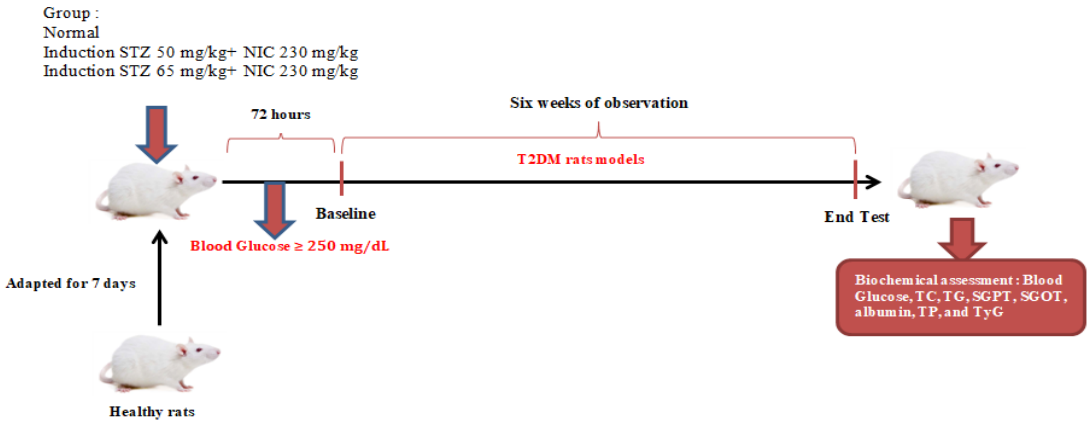

Figure 1:The study design of diabetes mellitus models in rats

Biochemical parameters assessment
At the baseline day (the diagnosis of diabetes) and the end of the research, rats fasted, and the blood was taken through the eye vein. The blood was then centrifuged (Thermo Scientific Fresco™ 17 Microcentrifuge, Germany) at 3000 rpm for 10 minutes to collect the serum. Biochemical profiles were measured with commercial kits, namely blood glucose, total cholesterol, triglycerides, serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), and total protein, albumin using a spectrophotometer (Biosystem BTS-350, Germany).

The triglyceride- glucose index (TyG) calculation
The formula used to evaluate TyG index [11] was equationly :

$$TyG = \text{Ln}\left[\frac{\text{triglycerides (mg/dL) x glucose (mg/dL)}}{2}\right] \quad \text{.....(1)}$$

Statistical analysis
The data were evaluated statistically using IBM SPSS version 27 and presented as the mean ± standard error mean. One-way analysis of variance (ANOVA) was carried out followed by post hoc analysis using the least significant difference (LSD) test for statistical comparisons at a p-value of less than 0.05.

Result And Discussion

The use of streptozotocin (STZ) and nicotinamide (NIC) as chemicals to produce a type 2 diabetes mellitus (T2DM) model was developed by Masiello et al.[12] The results were then used as a reference for the development of new traditional medicines, through in vivo testing. However, modeling results showed different biochemical profiles, and preliminary research on STZ-NIC doses was suggested. Table 1 shows the biochemical profile for 6 weeks of observation. After 72 hours, STZ induction at 50 mg/kg and 65 mg/kg in combination with NIC was successful in increasing rats' blood glucose levels. After 6 weeks of observation, blood glucose levels were stable and tended to increase. A dose of 65 mg/kg STZ produced a rat model of DM with significantly different blood glucose levels when compared to 50 mg/kg ($p<0.05$).

According to previous research, STZ was a nitrosourea molecule, such as glucose.[13] As a glucose analog, it was characterized as STZ entering β cells through the glucose transporter-2 (GLUT-2) on the cell membrane due to the structural similarity to glucose.[14] The nitrosoamide moiety of STZ attacked DNA and caused alkylation in β cells, resulting in genotoxicity and cytotoxicity.[15] High blood glucose levels were often used as a reference to produce models of chronic diabetes complications.[16] Furthermore, the use of NIC reduced β-cell damage and prevented high mortality during testing.[7] Although the NIC dose also affects blood glucose levels, the 230 mg dose in this research has been widely used as a reference.[16] Increasing the STZ dose significantly affected the resulting blood glucose levels.[17] The results of this research were similar to the theory, where an increase in the STZ dose from 50 mg to 65 mg increased blood glucose levels during 6 weeks of observation.

The lipid profile of total cholesterol and triglycerides showed a significant improvement after 6 months of diabetes ($p<0.05$). Increasing the STZ dose from 50 mg to 65 mg has no significant difference in lipid profile, but the largest showed an insignificant increasing trend ($p>0.05$). A successful T2DM model should include some dyslipidemia, which would be valuable for testing novel antidiabetic drugs.[3] Table 1 also shows that after 72 hours of STZ induction, there was a significant increase in SGPT and SGOT in rats when compared to normal groups. This result showed that induction of STZ can increase the SGPT and SGOT enzymes, which are indicators of liver organ damage.

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Table 1: Changes in the biochemical profiles of rats from the baseline to the end of the research period.

Parameters	Baseline			End-Test			Difference		
	Normal	STZ 50+NIC	STZ 65+NIC	Normal	STZ 50+NIC	STZ 65+NIC	Normal	STZ 50+NIC	STZ 65+NIC
Blood glucose (mg/dL)	103.6 ± 8.1	444.0 ± 47.9*	445.0 ± 36.7*	99.4 ± 7.7	487.0 ± 20.7*	528.00 ± 40.1*#	-4.2 ± 0.4	43.0 ± 27.2*	83.0 ±3.4*#
Total cholesterol (mg/dL)	85.2 ± 5.7	82.8 ± 3.6	90.6 ± 1.7	86.0 ± 4.8	102.4 ± 4.2*	113.80 ± 8.6*	0.8 ± 0.7	19.6 ± 0.6*	23.2 ± 6.9*
Triglycerides (mg/dL)	141.4 ± 11.8	154.4 ± 10.9	155.4 ± 11.1	147.2 ± 14.2	175.2 ± 7.4*	176.60 ± 18.8*	5.8 ± 2.4	20.8 ± 3.5*	21.2 ± 7.7*
SGPT (U/I)	79.4 ± 3.3	141.8 ± 10.4*	113.8 ± 13.9*	81.6 ± 2.6	142.6 ± 9.1*	178.00 ± 26.9*#	2.2 ± 0.7	0.8 ± 1.3	64.2 ± 13.0*#
SGOT (U/I)	152.0 ± 13.9	285.8 ± 60.6*	321.2 ± 42.8*	260.0 ± 16.8	331.8 ± 70.4*	368.60 ± 28.3*#	108.0 ± 2.9	46.0 ± 9.8*	47.4 ± 14.5*
Albumin (mg/dL)	34.8 ± 1.3	32.9 ± 2.9	34.5 ± 3.6	35.7 ± 1.1	28.2 ± 2.5	25.93 ± 1.4*	0.9 ± 0.2	-4.8 ± 0.5	-8.6 ± 2.2*
Total protein (mg/dL)	81.3 ± 3.1	79.1 ± 3.4	81.8 ± 3.3	83.4 ±2.6	64.5 ± 2.3*	68.63 ± 4.6*	2.1 ± 0.5	-14.5 ± 1.1*	13.1± 1.3*

Note: Data are expressed as mean ± SEM (n=6). *, Significantly different compared to the normal group (p<0.05). #, Significantly different STZ 50+NIC compared to STZ 65+NIC (p<0.05). STZ, streptozotocin; NIC, nicotinamide.

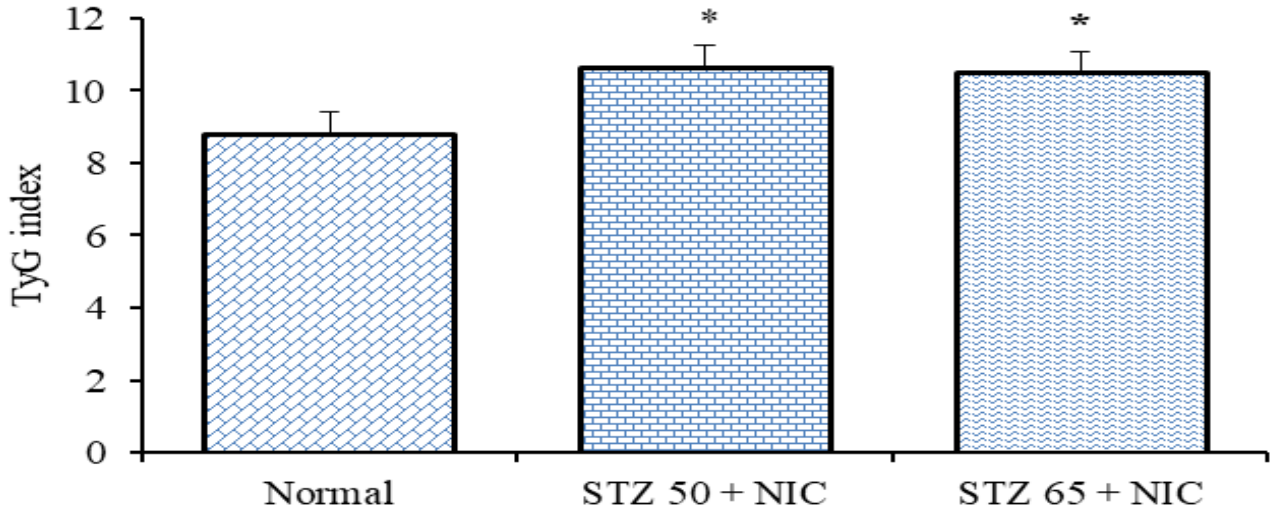

Figure 2: The TyG calculation after six weeks of experiments.
Note: Data are expressed as mean ± SEM (n=6). *, Significantly different compared to the normal group (p<0.05). STZ, streptozotocin at dose 50 and 65 mg/kg body weight; NIC, nicotinamide.

There was also a significant difference in the increase in SGPT levels with a dose of 65 mg when compared to 50 mg after 6 weeks of observation ($p<0.05$). According to previous research, STZ caused liver injury, which is marked by increased levels of SGPT and SGOT enzymes in the blood.[18,19] Other biochemical profiles, such as serum albumin and total protein, showed a significant decrease during the 6 weeks of observation. There was no significant difference in albumin and total protein levels between 50 mg and 65 mg of STZ induction combined with NIC. Figure 2 shows the TyG calculation after six weeks of experiments, which significantly increased at the 50 mg and 65 mg STZ doses when compared to the normal group ($p<0.05$).

STZ dosages ranging from 50 to 65 mg/kg have been found to cause hyperglycemia and partial β cell damage when combined with NIC-induced, as shown in T2DM.[20,21,22] High-dose STZ caused severe hyperglycemia and β cell destruction, although the STZ-NA combination reduced glucose tolerance and caused moderate hyperglycemia. The pancreas could still release insulin in the presence of glucose.[23] Furthermore, the TyG index generated from triglyceride and glucose levels was suggested as a marker of insulin resistance.[24] The index parameters were also crucial for evaluating novel drug candidates, since individual glycemic indicators may mislead.[3] For instance, a normoglycemic diabetic animal may have aberrant insulin sensitivity/resistance.[25] Based on this result, new antihyperglycemic drugs may be studied in a T2DM rat model using these indicators.[3] The TyG Index values in this research showed that the 50 mg and 65 mg STZ had no significant difference in combination with NIC. This result showed that the doses had no difference in insulin resistance during the 6 weeks of observation. The value of insulin resistance had no significant changes when STZ was given in high doses, but showed changes in low doses.[26,27] Further research is necessary to quantify insulin levels in the bloodstream, enabling the calculation of Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) and Quantitative Insulin Sensitivity Check Index (QUICKI) values. The resistance index could be used to determine the type of diabetes resulting from STZ-NIC induction.

Conclusion

In conclusion, the induction of high doses of STZ-NIC produced DM in the rat model, but the type of DM could not be determined. Increasing doses of STZ-NIC showed differences in blood biochemistry profile, specifically in the parameters of blood glucose, SGPT, and SGOT. However, differences were not found in total cholesterol, triglycerides, albumin, total protein, and TyG.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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