Smoke Exposure Effect Of Motor Vehicles Against Blood Sugar Levels And Pancreatic Histopathology Wistar Rats

Uly Astuti Siregar, Udadi Sadhana, Yan Wisnu Prajoko

Abstract: Diabetes is a disease caused by many factors such as lifestyle, environmental and genetic factors. The increasing incidence of type 2 diabetes is closely linked to obesity and insulin resistance. Environmental factors constitute a serious threat to health associated with the increasing air pollution. Analyzing blood sugar levels and pancreatic microscopic damage in Wistar rats by exposure to smoke in motor vehicles. This researchuse *posttest control group design*, Eighteen (18) tail white male rats were divided into three groups, namely by exposure to smoke motors for 100 seconds / day (X1), a group that is exposed to the smoke motors for 100 seconds / day and a diet high in fat (X2), and a control group. Fasting blood sugar levels (FBS) is checked on day 0 and day 30, while the degree of insulitis examined on the 30th day . Analysis of the data increase in FBS (final FBS levels reduced initial FBS levels) done by one way ANOVA test followed LSD Post Hoc Test, on the degree of insulitis do Kruskal Wallis test, followed by Mann Whitney test. The mean increase in fasting blood glucose level groups X1, X2, and C is 60.50; 98.33;6:00gr / dl, multivariate analysis showed no significant differences (P <0.05). Post Hoc Test LSD FBS levels show significant differences between treatment groups (X1 and X2) as compared with controls and compared with the group X1 X2. The degree of insulitis showed no significant differences between the treatment groups (X1 and X2) to controls. Exposure to smoke in motor vehicles can increase blood sugar levels and cause damage to the pancreas in Wistar rats.

Index Terms: Motor vehicle fumes, PM 2.5, Blood Sugar, damage to the pancreas.

1 INTRODUCTION

Diabetes mellitus (DM) describes a group of metabolic disorders characterized by an increase in blood sugar levels. People with diabetes are at risk of serious health problems that lead to high medical care costs and decreased quality of life. International Diabetes Federation (IDF) estimates that in 2017 there were 424.9 million people with diabetes worldwide, 279 million live in urban areas and 146 million in rural areas.(1) The prevalence of DM in Indonesia also showed an increase from year to year, the results of Health Research (Riskesdas) in 2013, showed the prevalence DM patients experienced an increase of 330.512 patients,(2)The increasing incidence of type 2 diabetes is closely linked to obesity and insulin resistance. Environmental factors constitute a serious threat to health associated with the increasing air pollution. Air pollution in Indonesia have ranks in eighth position which most deadly in the world with an average mortality rate of 50 thousand people in every year. The high levels of pollution, among others caused by the amount of emissions by motor vehicles .(3) Contributor to air pollution in Indonesia is motor vehicle emissions of around 85%. (13) The relationship positive between air pollution and type 2 diabetes was found in the study meta-analysis of 13 studies conducted in Europe and North America, but not reported on dose-response effects. (4) Meta-analysis of studies on the relationship between air pollution and type 2 diabetes, the majority stated that there is a correlation between an increased risk of type 2 diabetes with long-term exposure to air pollution.

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(4)(5)(6)(7) Experimental research on the effects of exposure to particle pollution (PM < 2.5) in mice also proved that air pollution is a risk factor increasing the incidence of insulin resistance and diabetes. (8)(9) Many studies have shown that exposure to smoke in motor vehicles the long term can lead to insulin resistance which ended up being type 2 diabetes, but there has been no experimental studies in vivo that relate to damage the pancreas, it is necessary to do research on the effects of exposure to smoke in motor vehicles on blood sugar levels and histopathology of the pancreas in *wistar* rats.

2 METHOD

2.1 Samples of Motor Vehicles

The smoke coming from the exhaust of motor vehicles matic pertalite 125 cc fuel output in 2017. Concentration of the amount and concentration of smoke particles with a diameter of ≤ 2.5 μm measured using the Model 3443 Kanomex.

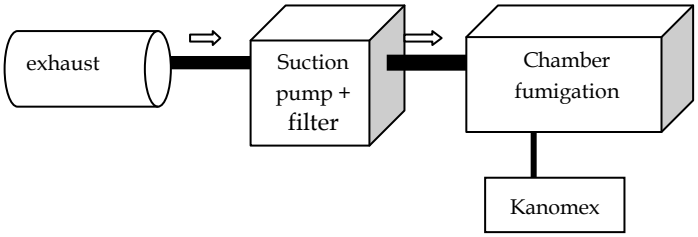
2.2 Animals

Management of animals trying to follow the "animal ethics". This study was submitted and approved by the Research Ethics Committee Medical / Health Faculty of Medicine, University Islam Sultan Agung Semarang, with certificate No. 418 / VII / 2019 / bioethics Commission on 26 July 2019. Eighteen (18) tail Animals used in this study were white rats (*Rattus norvegicus*) *Wistar* strain of 2-3 month old male weighing 100-200 gr were obtained of the Laboratory of Animal Physiology Faculty of Science and Technology of the State Islamic University (UIN) Malang. Animals acclimatized for 3 days in a curing chamber measuring 77 x 52 x 35 cm with room temperature ranging between 25-28^{0}C and room humidity levels ranged between 70-75%. Light reception arranged 12 hours of light and 12 hours dark. Mice given feed pellet-shaped type of AD II, high-fat feed and reverse osmosis drinking water provided ad libitum. Once acclimatized experimental animals were randomly divided into 3 groups: control group (C), the treatment group X1 by exposure to

smoke with a time of 100 seconds / day and the treatment group X2 by exposure to smoke with a time of 100 seconds / day and a diet high in fat, respectively each group contains 6 animals.

2.3 Exposure to Smoke in Motor Vehicles

Emissions of motor vehicles described by putting the test animals in a sealed enclosure that has a hole to the provision of smoke and holes for ventilation. Motor switched on for 80 seconds after smoke is produced, collected in a reservoir for later connected to the cage that has been vented (figure 1)



***Picture 1**. Smoke granting procedures*

2.4 Procedure Research Subjects

Animals acclimatized for 3 days, then on the fourth day to do the weighing and inspection of the initial fasting blood sugar levels. Weight weighed using digital scales Metler Toledo with a level of accuracy of 0.1 grams. The level of FBS is done by using a glucometer Nesco Multicheck N-01 with a blood sample taken from the tail vein of mice. Animals were divided into 3 groups randomly with the aid of a random number table. Each group included in individual cages, control group (C), X1 and X2 received standard feed to get high-fat feed. Exposure to exhaust fumes with a time of 100 seconds / day given to the X1 and X2, the smoke is given for 30 days in the treatment group. Anaesthetized mice by injection of ketamine 60 mg/kg body weight (i.m) before surgery. Pancreatic tissue obtained were fixed with a solution of 10% neutral buffered formalin, kept at room temperature for 48 hours. Next step is making preparations for histology and HE staining according to the procedure. Histological preparations that are ready to be read under a binocular light microscope 400x magnification.

2.5 Data Analysis

The data obtained is the data body weight, fasting blood glucose levels of data and the data on the degree of insulitis. The data is entered into a computer file with the aid of a computer program SPSS 25.0. Descriptive analysis displays average values and standard deviations body weight, FBS levels and the degree of insulitis of any existing group. The results are presented in tabular form. Body weight data normality and initial fasting blood glucose level checked using the Shapiro Wilk test. Descriptive analysis presented in the form of frequency tables and percentages, followed by one-way ANOVA tests the hypothesis when the data were normally distributed and kruscal wallis when the data are not normally distributed. The p-value was considered significant if $p < 0.05$ with 95% confidence interval.

3 RESULT

3.1 Parametersof Test Results

Here's a table that presents the results of sample testing of smoke parameter motorcycle used in this study.

***Table 1.** Parameters of test results*

Unit	Mean ± SD
$PM_{2,5}$ (mg / m^3)	0.628 ± 0.069
A (cm^2)	0.38
V (m / s)	0.77
Q (cm^3 / s)	29.77
T (s)	800

Motorcycles used is kind matic 125 cc. Dose PM 2.5 were exposed to mice per day per fumigation for 80 seconds with a time of 100 seconds for inhalation of 0.628 ± 0.069 mg / m^3. The flow rate of fluid (smoke motorcycles) of 29.77 cm 3 / s at a flow rate of 0.77 m / s. The temperature in the chamber at 26 ° C and humidity of 65%, with higher levels of CO gas (carbon monoxide) of 0 ppm (filtered).

3.2 Characteristics of Experimental Animals

Here's a table that presents data in the form of the characteristics of the experimental animals weighing results of test animals each group at the beginning and end of the treatment.

***Table 2.** Results of experimental animals weighing*

Group	Weight (grams)	
	Early	End
X1	110.00 ± 7.375	150.67 ± 18.250
X2	104.83 ± 3.763	151.33 ± 14.278
C	107.00 ± 6.449	153.67 ± 11.219

* Body Weight Data show average ± SD

3.3 The Results of The Examination of Fasting Blood Sugar Levels (FBS)

Here's a table that presents the results of the fasting blood glucose level test animals each group at the beginning and end of the treatment.

Table 3. The results of the examination of fasting blood sugar levels

Group	Fasting plasma glucose (mg / dl)		
	Early	End	Δ FBS
X1	88.00 ± 12.116	148.50 ± 24.898	60.50 ± 18.415
X2	74.33 ± 7.005	172.67 ± 34.268	98.33 ± 37.745
C	78.33 ± 12.987	84.33 ± 17.351	6.00 ± 23.375

* FBS data shows the average ± SD

One way ANOVA test results showed there were no significant differences in fasting blood sugar levels ($p < 0.05$), then performed LSD Post Hoc Test to determine the groups that have significant differences.

Table 4. Post Hoc Test LSD fasting blood sugar levels at the end of treatment

Treatment	X1	X2	C
X1		0032*	0004*

X2		0000
C		

*Has a significant different

3.4 Insulitis degree assessment result at the end of treatment

Here's a table that presents the results of assessment of the degree insulitis all groups at the end of treatment

Table 5. Results of insulitis degree assessment at the end of treatment

Group	The degree of insulitis					
	Negative	Light	Moderate	Weight	End Stage Islet	Total
X1	0	1	4	0	1	6
X2	0	1	5	0	0	6
C	0	6	0	0	0	6

p: 0.006 (Kruskal Wallis test)

Results Kruskal Wallis test showed that there were significant differences in the degree of insulitis at the end of treatment ($p < 0.05$). To determine whether there is a significant difference between the treatment groups Mann Whitney test was used.

Table 6. Test insulitis degree difference between the treatment groups

Treatment	X1	X2	C
X1		0598	0.006*
X2			0.005*
C			

*Has a significant different

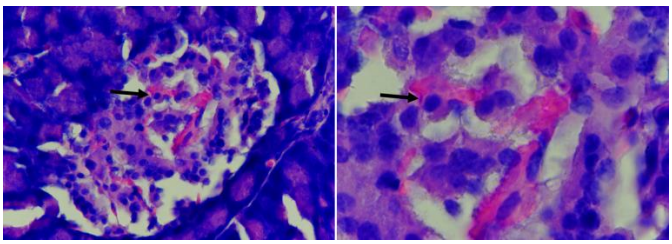


Figure 2. Result of parancreatic examination with HE staining (400x). (Black arrow : lymphocytes)

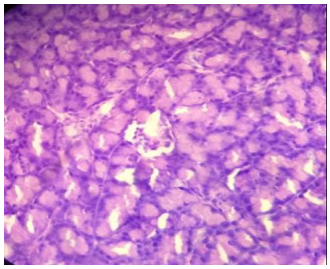


Figure 3*. End stage islet with HE staining (40X)*
(Looks atrophy islets of Langerhans)

4 DISCUSSION

4.1 Emissions of Motor Vehicles

Diabetes Mellitus type 2 (DM type 2) due to a combination of peripheral resistance to insulin action and insulin secretion compensatory response inadequate by the beta cells of the pancreas (relative insulin deficiency).(10) Many studies have revealed the relationship between dust particles (Particulate Matter / PM) in increasing the risk of diabetes.(11)Particle pollution can be either total suspended particulate / total suspended particles (TSP) with a diameter of up to 100 μm particles, particles less than 10 μm in diameter (PM 10), and a particle diameter of less than 2.5 μm (PM 2.5). Parameter particulate influential and sensitive given the criteria Air Quality Index (AQI) is PM 2.5.(12) Motor vehicles are the largest contributor to air pollution in urban areas and in the vehicle smoke emission smoke contains particles such as PM 2.5. The results of sample testing of emissions of motor vehicles fueled pertalite kind matic 125 cc in this research presented to the rat per day per fumigation for 80 seconds with a time of 100 seconds for the inhalation of 628 μg / m^3. The test results found to be higher than the threshold of PM 2.5 which is 65 μg / m^3 in line with the Government Regulation No. 41 of 1999 on Air Pollution Control.

4.2 Blood Sugar Levels

Increased blood sugar levels caused by exposure to particulate matter (PM) occurs due to inflammation in the gastrointestinal mucosa through the synthesis of cytokines and chemokines.(13)The diagnosis of diabetes can be enforced by the fasting blood sugar (FBS) or blood sugar 2 hour post prandial or HbA1C. In prediabetes fasting blood glucose level between 100 and 125 mg / dl, whereas the FBS diabetes ≥ 126 mg / dl. Factors that can increase the risk of type 2 diabetes include aging, obesity and lack of physical activity.(14) In this study, all animals try to meet the inclusion criteria which the initial fasting blood glucose level <110 mg / dl with weight ranges between 100-200 grams. The average increase in fasting blood sugar levels are 60.50 g / dl in the treatment group X1, 98.33 g/dl in the treatment group X2 and 6.00 gr / dl in the control group. Multivariate analysis showed significant differences ($p < 0.05$) between the group and the LSD post hoc test found significant differences (p, 0.005) between treatment groups X1 and X2 compared with controls, as well as the treatment group X1 compared with the Group X2. Elevated blood sugar levels due to exposure to PM 2.5 is consistent with research conducted Liu et al in which the experimental animals were given exposure to PM 2.5 and the high-fat diet can significantly increase the FBS. (8) The role of free fatty acids (ALB) is excessive and inflammation will trigger peripheral insulin resistance and beta cell dysfunction. ALB overload in beta-cell macrophages and can bind inflammasome, a multiprotein complex that causes cytoplasmic cytokine secretion of interleukin IL-113. Furthermore, IL-113 mediates secretion of other proinflammatory cytokines from macrophages, Langerhans islets, and other cells, which are then released into the circulation and work on the main place of insulin to increase insulin resistance.(15)The nutritional status in patients with type 2 diabetes mellitus are overweight or obese, where excess weight can lead to insulin resistance. Patients with type 2 diabetes who are not obese or overweight by measuring the Body Mass Index (BMI), body fat distribution possibilities dominant in the abdominal area.(16)

4.3 Histopathology of The Pancreas

Endocrine pancreas consisting of islets of Langerhans consists of four main types of beta cells, alpha, delta and PP (pancreatic polypeptide), which can be distinguished by the

morphologic, ultrastructural characteristics granules, and through hormones.(10) Histopathologic Changes in the islets of Langerhans in diabetic patients may occur both quantitatively, such as reducing the number or size, as well as qualitatively, such as necrosis, degeneration, and ameloidosis.(17) In patients with type 2 diabetes mellitus are ameloid deposits, fibrosis and cell death in the islets of Langerhans, a process that occurs mediated by the release of cytokines and chemokines through signal *interleukin* (IL) -1β as a result of metabolic stress.(18) Infiltration mononukleus cells, including macrophages, lymphocytes, and plasma cells can arise as ongoing chronic inflammatory response, including as a result of prolonged exposure to toxic agents.(10)

Rate the degree of insulitis in the pancreas of experimental animals, was found in 16.7% mild insulitis X1 and X2 group, 100% in the control group. Insulitis was at 66.7% in Group X1 and 83.3% of the Group X2. End stage islet was found in 16.7% of group X1. Multivariate analysis showed a significant difference ($p < 0.05$) in the degree of insulitis between groups, where the bivariate test by Mann Whitney test found significant differences in the degree of insulitis X1 and X2 treatment group compared with the control group. The results support the research conducted Xu et al, which states that chronic exposure to PM <2.5 µm triggers an inflammatory response in tissues, in this case lung and visceral fat and a systemic inflammatory response characterized by an increase in cytokines including *monocyte chemoattractant protein* 1 (MCP-1) and *monocyte / macrophage attractant* in the blood. (19)

5 CONCULSION

Giving smoke exposure motors for 100 seconds per day can increase blood sugar levels and cause damage to the pancreas significantly in both treatment groups with a normal diet or a high fat diet compared to the control group.

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