The Role of Interleukin 1β (IL-1 β) and Interleukin 6 (IL- 6) on Diabetes Mellitus and its Correlation with Cardian Rythm in Wistar Rats (Rattus norvegicus)

by Anis Irmawati

Submission date: 02-Apr-2023 11:47AM (UTC+0800)
Submission ID: 2053195285
File name: Jurnal_2.pdf (194.77K)
Word count: 5218
Character count: 26472

ORIGINAL ARTICLE

The Role of Interleukin 1β (*IL-1* β) and Interleukin 6 (IL-6) on Diabetes Mellitus and its Correlation with Cardian Rythm in Wistar Rats (*Rattus norvegicus*)

*Retno Indrawati[1], Sidarningsih Sidarningsih[1], Anis Irmawati[1], Alya Sahilla Fahri[2], Hasina Tasya[2] and Fatahilah Abdul Razak[3]

[1] Department of Oral Biology, Faculty of Dental Medicine, Universitas Airlangga, 60115 Surabaya, Indonesia
[2] Undergraduate Program, Faculty of Dental Medicine, Universitas Airlangga, 60115 Surabaya, Indonesia
[3] Departement of Oral and Craniofacial Sciences, Faculty of Dental Medicine, Universiti Malaya, 50603 Kuala Lumpur, Malaysia

ABSTRACT

Introduction: IL-1 and IL-6 are proinflammatory cytokines produced due to the binding of Advanced Glycation End-products (AGEs). in diabetics, molecularly, diabetes mellitus can be caused by disturbances in the circadian rhythm system. This study aims to determine the role of IL-1β and IL-6 in type 2 diabetes mellitus and their relationship to circadian rhythm. **Materials and Methods:** This study was conducted on male wistar rats treated with circadian rhythm and increased dietary glucose levels, then measured blood sugar and blood draw from the heart to measure levels of IL-1β and IL-6 using ELISA. Data analysis used One-way ANOVA, repeated measure ANOVA, and Pearson Correlation Test. **Results:** The dark group showed a significant increase in blood glucose at week 7 ($p = 0.039$) and 9 ($p = 0.002$). There was a significant increase in blood glucose at week 9 ($p = 0.000$) in the light group. There were significant differences between normal, dark, and light groups ($p < 0.05$) at the 9th-week blood glucose level ($p = 0.000$). In the correlation test, there was a significant relationship with the positive correlation of Interleukin 1 β and blood glucose levels in rats ($r = 0.599$; $p = 0.009$), there was a significant relationship between Interleukin 6 and blood glucose levels with positive correlation ($p = 0.003$; $r = 0.652$). **Conclusion:** Circadian rhythm affects the role of Interleukin 1β (IL-1β) and Interleukin 6 (IL-6) in wistar rat with diabetes mellitus.

Keywords: Circadian rhythm, Diabetes mellitus, Interleukin 1β, Interleukin 6, Wistar Rats (*Rattus norvegicus*)

Corresponding Author:
Retno Indrawati, DDS, MDS
Email: retnoindrawati@fkg.unair.ac.id
Tel: +62-315030255

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder that affects human body in terms of physical, psychological, and social health which was the leading cause of death in 2016 (1, 2). The world health organisation, the World Health Organization (WHO) predicts diabetes mellitus will afflict more than 21 million Indonesians in 2030. The threat of diabetes mellitus is undoubtedly one of the leading health problems that need significant attention in Indonesia (3, 4). Demographic transition combined with urbanization and industrialization has resulted in drastic changes in lifestyles globally and associated with an increased risk of related disease patterns like diabetes, obesity, cardiovascular disease, and cancer (5, 6).

The circadian rhythm system is an internal regulator in the human body that coordinates physiological and behavioral activities in a 24-hour cycle (7, 8). In mammals, circadian rhythms are regulated by the suprachiasmatic nucleus (SCN) which is located in the anterior hypothalamus and other are managed by "laborer" vibration separate in brain sector or body tissues. It's responsible for receiving information about the light-dark cycle through particular neural pathways, namely the retinohypothalamic fibers that pass to the SCN that drives the daily regulation (24h) of physiological, biochemical, and endocrine rhythms (9, 10). Efferent nerve-fibers from the SCN initiate signals that act on the circadian rhythm so that they can affect the secretion of ACTH, growth hormone (GH), melatonin, Thyroid-Stimulating Hormone (TSH), cortisol, ghrelin, and leptin (11, 12). Some of the causes of a person experiencing circadian rhythm disorders are eating at night and sleeping during the day, increasing the risk of metabolic syndrome (13).

Rats have a 24-hour circadian rhythm with a 12-hour

dark and 12-hour light cycle. Rats are nocturnal animals that are active for exploration, eating, and drinking at night (14). Bright light at night in nocturnal animals will improve sleep quality and decrease food intake (15). Meanwhile, constant-dark conditions will fight the physiology and behaviour of rats, resulting in disturbed circadian rhythm (16). Molecular disturbances in the circadian rhythm system can trigger metabolic disorders, diseases, and tumours (17). Normal blood glucose levels in rats are 50-135 mg/dL (18).

IL-1β and IL-6 are proinflammatory cytokines produced as a result of Advanced Glycation End-products (AGEs) binding to their receptors (RAGE) and can be found in saliva (19, 20). In normal healthy adults, the serum concentration of IL-1β is about 0.5 – 12 pg/mL, and IL-6 is about 0.5 to 5 pg/mL. In normal healthy Wistar rats, the serum concentration of IL-1β is around 300-700 pg/mL and IL-6 is about 4.627 ng/L (21).

Currently, many companies are implementing a shift work system, but the current work schedule prevent people from getting enough sleep (22). Based on some of the findings above, if these conditions are left unattended and do not receive attention, it will have a systematic effect on workers' health. Therefore, serious attention is needed from company leaders and support from all related parties in solving these problems. Present study among the Railway employees according to the scale about 12.05% employees had Pre-Diabetes and 8.49% were suffering from Diabetes. Altered blood sugar level may lead to various disease, so diabetes control and management programs are needed for its prevention (23). Based on existing theory or data, the researchers conducted a study to determine the concentration of IL-1β and IL-6 in diabetes mellitus due to circadian rhythm disturbances in experimental Wistar rats. IL-1β and IL-6 concentrations were measured using ELISA (Enzyme-linked Immunosorbent Assay) because this tool has high sensitivity and specificity, and the examination process is relatively fast and straightforward.

METHODS

Samples

This research has been approved with ethical clearance from the Committee of Dental Medicine, Universitas Airlangga, Indonesia (No. 320/HRECC.FODM/VII/2020). This type of research is an experimental laboratory study with a pre-post-test control group design using 18 male Wistar rats (*Rattus norvegicus*), eight weeks old, weighing 140-200 grams, healthy characterised by active movement, no defects in all parts of the body, there is no tooth decay and has been adapted for one week. There were three groups in this study (dark, normal, light) which each group consisted of 6 samples.

Preparation of the light exposure to animal model

The normal group is the sample group treated with 12 hours of light and 12 hours of darkness treated with circadian rhythms in the form of a photoperiod. The dark group is the sample group treated with 24 hours of dark and 0 hours of light (did not receive any light). The light group is the sample group treated with 24 hours of light (did not receive any dark period). The length of treatment for Wistar sacrifice is eight weeks with high carbohydrate feed and who gave drinking water ad libitum with a feed composition of 65-70% carbohydrates, 20-25% protein, and 5-12% fat, and the total calories are 2900 kcal/kg while 10-15% fructose solution in drinking water (24). Photoperiod treatment with fluorescent lamps and a time switch to adjust the duration of the light in each group. The distance between the floor of the lighting room and the light source is ±75 cm. The amount of light received by rats ranges from 100-110 lux. Rat body weight measurements were carried out every seven days with an analytical balance at an accuracy of 0.1 grams.

Measurement of Interleukin 1β and Interleukin 6 levels

The rat blood serum was taken from the tail end of the tested animal (Wistar rats) every two weeks to measure the blood sugar levels. Blood is dripped on a glucometer strip to read at the blood glucose levels on a glucometer monitor (25). To measure Interleukin 1β and Interleukin 6 levels, the rat blood serum was taken through the heart surgically. Then the needle was inserted directly into the heart (intracardiac) so that the rats needed to be sacrificed (26). At least 3 mL of blood was taken and stored in a vacutainer, then centrifuged at 3000 rpm for 10 minutes. After centrifugation, the blood will be separated into two parts: the serum on the top and the red blood cells on the bottom. The serum formed was taken using a micropipette, then placed in a vial tube and stored at -80 C. According to the manufacturer's regulations, the blood serum is used to measure Interleukin 1β and Interleukin 6 levels, tested using a sandwich rat ELISA kit.

Statistical analysis

After the Wistar rats were sacrificed, it was continued with the measurement results from the first week until the ninth week were summed and calculated for one preparation. The results were compared between the control group and the treatment group. The data obtained were analysed using the Kolmogorov-Smirnov Test, Shapiro Wilk test, Levene test, one way ANOVA, Repeated Measure ANOVA and Pearson Correlation tests.

RESULTS

The examinations carried out on the subjects of this study were the analysis of Interleukin 1β and Interleukin 6 using ELISA taken from rat blood serum and random blood sugar test. The results obtained mean and standard deviation (SD) in each group from all the data obtained.

Blood Sugar Levels Data Results

Based on Table I, the light group's mean blood sugar levels in the first week were the greatest compared to the normal and dark groups. But at the third and fifth week, the normal group's mean blood sugar levels were the greatest compared to the dark and tight groups. Then in the seventh and ninth week, the dark group's mean blood sugar level was the greatest compared to the normal and light groups. So, it was found that the blood sugar levels in the dark group experienced a significant increase compared to the light and normal groups.

Blood sugar levels above were tested for normality using the Kolmogorov Smirnov and Shapiro-Wilk test to see if the data were normally distributed, then followed by a different test using measured ANOVA in each group to see if there was an increase in blood glucose levels from the first week until the ninth week in each group. The normality test results show that all data variables were normally distributed (p>0.05). The repeated measure ANOVA test shows that the increase in glucose levels from the first week until the ninth week could be performed.

Data Results of Interleukin 1β

Based on Table II, the average level of Interleukin 1β and Interleukin 6 of Wistar rats in the dark group was the greatest compared to the normal and light groups. So, it was found that Interleukin 1β in the dark group was higher than the normal and light group.

Repeated measure ANOVA test of blood glucose levels in the normal group, it can be interpreted that there is no significant difference between the first week until the ninth week with the significance value (p>0.05) (Table III). Repeated measure ANOVA test of blood glucose levels in the dark group, it can be interpreted that initially, there was an increase in blood glucose levels at the third week but not significant (p>0.05). Blood sugar levels began to decrease in the fifth week but were not significant (p>0.05). There was a substantial increase in blood sugar levels (p<0,05) (Table IV). Based on Table V, repeated measure ANOVA test of blood glucose levels in the light group, it can be interpreted that there was an increase in the blood glucose levels from the first week until the seventh week but not significant (p>0.05). Then in the ninth week of observation, there was a substantial increase in the blood glucose levels (p>0.05).

Blood glucose levels at the ninth week show that Interleukin 1β and Interleukin 6 have a normal data distribution. So that a different test was carried out using one way ANOVA, but before the other test was carried out, the data was tested for homogeneity with the Levene

Table II: The mean and standard deviation of Interleukin 1β and Interleukin 6 of the three groups of Wistar rats

Data Type	Sample Group	N	Mean (pg/mL)	SD
Interleukin 1β	Normal	6	701.137	132.851
	Gelap	6	899.585	71.860
	Terang	6	798.924	113.088
Interleukin 6	Normal	6	4.702	0.940
	Gelap	6	5.482	0.525
	Terang	6	5.015	0.572

Table I: The mean and standard deviation of the three groups of wistar rats in 8 weeks to measure the blood sugar levels

Data Type	Weeks	Normal			Dark			Light		
		N	Mean	SD	N	Mean	SD	N	Mean	SD
Blood Glucose (mg/dL)	1st	6	108.167	8,750	6	108.833	8.495	6	111.500	6.411
	3rd	6	126.667	16,318	6	112.833	5.811	6	115.833	7.250
	5th	6	116.500	7.765	6	106.167	7.305	6	113.833	6.555
	7th	6	122.167	7.414	6	124.833	7.055	6	118.000	6.603
	9th	6	120.000	5.727	6	158.500	5.128	6	134.167	7.935

Description:

a. 1st Week: 9 October 2020
b. 3rd Week: 23 October 2020
c. 5th Week: 6 November 2020
d. 7th Week: 20 November 2020
e. 9th Week: 2 December 2020

Table III: The results of measure anova blood glucose levels test in the normal group

Group	Time		Mean ± SD	Increase(+)/ Decraese(-) (units in grams)	Value (p)
Normal	1st Week (9 October)	1st Week (9 October)	108.17 ± 8.75		
		3rd Week (23 October)	126.67 ± 16.32	+ 18.50	0.589
		5th Week (6 November)	116.50 ± 7.77	+ 8.33	0.890
		7th Week (20 November)	122.17 ± 7.41	+ 14	0.221
		9th Week (2 December)	120.00 ± 5.73	+ 11.83	0.268

*there is a significant increase/decrease in α = 0.05.

test first. The homogeneity test results of the three groups of Wistar rats using the Levene test is p>0.05. It shows that the data is homogeneous or has the same variance. Results of the different tests using one way ANOVA in the normal-dark-light group obtained p=0.000 or p<0.05, which means a significant difference in blood glucose levels in the normal-dark-light group. The result of blood glucose levels data at the ninth week was tested for correlation with Interleukin 1β and Interleukin 6, but a normality test was carried out first. Blood glucose levels and Interleukin 1β were tested for normality using the Shapiro-Wilk test, and it was found that both variables were normally distributed because all the p-value were more than 0.05 (p>0.05). After all of the tests, such as the different and normality tests, continue with the correlation test using the Person Correlation.

Results of the correlation between the blood sugar levels and Interleukin 1β shows that there is a significant relationship (p>0.05), indicating that the relationship between the two is moderate, meaning that the higher the blood sugar is, the higher the Interleukin 1β, and vice versa. Results of the correlation between the blood sugar levels and Interleukin 6 also show a significant relationship (p>0.05), indicating that the relationship between the two is moderate, meaning that the higher the blood sugar is, the higher the Interleukin 6, and vice versa.

DISCUSSION

The habit of eating at night can increase the chance of Night Eating Syndrome (SMM) (27). This is evidenced by the continuous activity carried out by rats in the dark, resulting in an increase of cortisol which can cause an increase of hormone ghrelin which increases appetite (28). In the dark group, the amount of intake consumed by the Wistar rats was more or twice that of the normal group, which was 100 grams/day per rat. This is per the theory, which says that one of the risk factors supporting obesity is a disturbance in the performance of the circadian rhythm, which acts as a regulator of the body's biological functions and behaviour (17).

Overweight and obesity lead to serious health consequences based on the progressively increases of body mass index (BMI), such as cardiovascular disease, musculoskeletal disorders, and some cancers (29). Obesity can trigger inflammation and metabolic disorders that will result in increased oxidative stress and is a strong predictor of diabetes mellitus (30, 31). Obesity is a major risk factor for the development of type 2 diabetes (32). Obesity can also lead to hyperglycemia, which produces AGEs and forms inflammatory cytokines such as IL-1β and IL-6 (33). IL-1β contributes to cell failure, and also an increase in IL-6 levels can occur in patients with diabetes mellitus (34, 35).

AGEs can cause tissue damage in patients with diabetes mellitus because increased blood glucose levels can trigger an increase in glycation, so that the absorption of glycated proteins by receptors will activate Nf-kB transcription to then form proinflammatory molecules and cytokines (36). The accumulation of fat that cannot be metabolized by insulin will worsen insulin resistance and cause pancreatic beta cell death (37). This condition usually occurs in individuals who are overweight or obese. Decreased pancreatic beta cell function causes insulin resistance to increase and then chronic hyperglycemia occurs (38). Insulin resistance can also inhibit glucose uptake into weak muscles and cells so that blood glucose increases (39). When pancreatic

Table IV: The results of measure anova blood glucose levels test in the dark group

Group	Time		Mean ± SD	Increase(+)/ Decraese(-) (units in grams)	Value (p)
Dark	1st Week (9 October)	1st Week (9 October)	108.83 ± 8.50		
		3rd Week (23 October)	112.83 ± 5.81	+ 4.00	1.000
		5th Week (6 November)	106.17 ± 7.31	- 2.67	1.000
		7th Week (20 November)	124.83 ± 7.05	+ 16.00	0.039*
		9th Week (2 December)	158.50 ± 5.128	+ 49.67	0.002*

*there is a significant increase/decrease in α = 0.05.

Table V: The results of measure anova blood glucose levels test in the light group

Group	Time		Mean ± SD	Increase(+)/ Decraese(-) (units in grams)	Value (p)
Light	1st Week (9 October)	1st Week (9 October)	111.50 ± 6.41		
		3rd Week (23 October)	115.83 ± 7.25	+ 4.33	1.000
		5th Week (6 November)	113.83 ± 6.55	+ 2.33	1.000
		7th Week (20 November)	118.00 ± 6.60	+ 6.50	1.000
		9th Week (2 December)	134.17 ± 7.93	+ 22.67	0.000*

*there is a significant increase/decrease in α = 0.05.

beta cells can no longer produce insulin secretion high enough to compensate for insulin resistance, fasting hyperglycemia and diabetes will occur (40, 41).

Human beings are diurnal creatures, so they are active and eat during the day and sleep at night when dark (42). Rats are nocturnal rodents that make gene expression times 180° opposite to diurnal animals. So that the circadian rhythm of the mouse body also experiences the opposite 180° from that of human beings (43, 44). Rats have diabetes mellitus when blood glucose levels are more than 150 mg/dL (45).

Blood glucose levels were measured every two weeks. Based on table I, it was found that there was an increase and decrease in blood glucose levels in the first week until the seventh week, but when viewed from the seventh to the ninth week, it shows that there was an increase in blood glucose levels in the dark-normal-light groups. The results of this study are per the hypothesis, namely that the highest blood glucose levels in the ninth week is in the dark group, which the light group then followed, and the lowest was the normal group. Normally, rats experience a cycle of 12 hours dark, 12 hours light and have blood glucose levels of 50-135 mg/dL (18).

Based on table I, the results also show that the dark group has an average blood glucose level exceeding the threshold for diabetes mellitus, so it can be concluded that the rats in the darkroom and behavioural, Physiology have diabetes mellitus (45). This is consistent with the results of other studies, which also state that if rats are in the dark for several weeks or experience long-term light deprivation, they will show different behavioural and anatomical characteristics and may be involved in the aetiology of depression that can exacerbate oxidative stress (16). Stress conditions of rats can cause disturbances in controlling blood glucose levels. The rat's body will produce the hormones epinephrine and cortisol, which can cause an increase in blood glucose levels automatically (46). In 24-hours light conditions, rats showed a continuous increase in blood glucose levels. However, the average blood glucose level was still within the normal threshold, so that it had not yet been classified as diabetes mellitus (47). Rats in this light group also experienced increased serotonin but not as much as the dark group. This is per the statement that light at night can cause a decrease in norepinephrine stimulation of the pineal gland, so that serotonin activity decreases (48).

Interleukin 1β and Interleukin 6 levels were examined by taking serum from rats, then measured using the Rat ELISA kit. As shown in table II, it is known that the mean levels of Interleukin 1β and Interleukin 6 were higher in the dark group compared to the normal and light groups. This is per the statement that increasing food intake can lead to obesity which can lead to hyperglycemia that produces AGEs. Ages bind to RAGE and produce Interleukin 1 and Interleukin 6, high levels of Interleukin 1β in the dark group indicate that hyperglycemia in rats with diabetes mellitus will activate monocytes and increase interleukin 1β (35). Meanwhile, the high levels of Interleukin 6 in the dark group indicate that these results are appropriate because Interleukin 6 can indeed cause insulin resistance by impaired phosphorylation of insulin receptors and insulin receptor substrate-1 (IRS1) late-night, so that Interleukin 6 is considered to be involved in the pathogenesis of diabetes mellitus (35). This explains therelationship between increased blood glucose levels and increased levels of Interleukin 1 and Interleukin 6, in humans, interleukin levels in patients with diabetes mellitus with retinopathy are higher than in patients with diabetes mellitus without retinopathy (43).

CONCLUSION

Circadian rhythm affects the role of Interleukin 1β (IL-1β) and Interleukin 6 (IL-6) in Wistar Rat with Diabetes Mellitus.

ACKNOWLEDGEMENTS

This research received PDUPT funding from the Indonesian government. The author would like to thank those who have helped me in completing this research.

REFERENCES

1. ADA. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2020. Diabetes care Am Diabetes Assoc. 2020;43(1):14–31. doi:10.2337/dc20-S002.
2. Kamtekar S, Keer V. Management of diabetes: A review. Res J Pharm Tech. 2014;7(9):1065-72.
3. Kemenkes RI. Hari Diabetes Sedunia Tahun 2018 Kementerian Kesehatan RI. 2018
4. Kemenkes RI. Riset Kesehatan Dasar (RISKESDAS) Kementerian Kesehatan Republik Indonesia. 2018
5. Panari H, Vegunarani M. Study on complications of diabetes mellitus among the diabetic patients. Asian J Nur Edu Res. 2016; 6(2):171-82. doi:10.5958/2349-2996.2016.00032.X.
6. Kelechi TN, Comfort CM, Frank O. Management of diabetes mellitus with combined therapy of reducdyn and metformin in streptozotocin-induced diabetic rats. Res J Pharm Tech. 2014;7(1):39-43.
7. Farhud D, Aryan Z. Circadian rhythm, lifestyle and health: A narrative review. Iran J Public Health. 2018;47(8):1068-76.
8. Dubey S. Circadian rhythm. Int J Nur Edu Res. 2019;7(1):112-4. doi:10.5958/2454-2660.2019.00022.X.
9. Kumaravel P, Prabhakaran AK, Melchias G, Edward A, Natarajan P, Sathishkum S. Monosodium glutamate exposure alters histopathological and

biochemical variables in rats under constant light. Asian J Res Pharm Sci. 2014;4(1):01-04.
10. Tamilanban T, Mohamedmajeed A, Chitra V. Overview of chronopharmacology. Res J Pharm Tech. 2020;13(9):4457-63.
11. Barret KE, Barman SM, Boitano S, Brooks HL. Ganong's Review of Medical Physiology 24th Edition's Review of Medical Physiology. 2012.
12. Kim TW, Jeong JH, Hong SC. The impact of sleep and circadian disturbance on hormones and metabolism. Int J Endocrinol. 2015;2015:591729. doi:10.1155/2015/591729.
13. Manodpitipong A, Saetung S, Nimitphong H, Siwasaranond N, Wongphan T, Sornsiriwong C, et al. Night-shift work is associated with poorer glycaemic control in patients with type 2 diabetes. J Sleep Res. 2017;26(6):764-72. doi:10.1111/jsr.12554.
14. Steinlechner S. Biological Rhythms of the Mouse. In: The Laboratory Mouse. 2nd ed. San Francisco: Elsevier; 2012.
15. Stenvers DJ, van Dorp R, Foppen E, Mendoza J, Opperhuizen A-L, Fliers E, et al. Dim light at night disturbs the daily sleep-wake cycle in the rat. Sci Rep. 2016;6(1):35662. doi:10.1038/srep35662.
16. Gonz6lez MMC. Dim light at night and constant darkness: Two frequently used lighting conditions that jeopardize the health and well-being of laboratory rodents. Front Neurol. 2018;9:1–17. doi:10.3389/fneur.2018.00609.
17. Potter GDM, Skene DJ, Arendt J, Cade JE, Grant PJ, Hardie LJ. Circadian Rhythm and Sleep Disruption: Causes, Metabolic Consequences, and Countermeasures. Endocr Rev. 2016 Dec;37(6):584–608.
18. Wolfensohn S, Lloyd M. Handbook of Laboratory Animal Management and Welfare. 4th ed. Chichester: Wiley-Blackwell; 2013.
19. Brailo V, Vucicevic-Boras V, Lukac J, Biocina-Lukenda D, Alajbeg-Zilic I, Milenovic A, et al. Salivary and serum interleukin 1 beta, interleukin 6 and tumor necrosis factor alpha in patients with leukoplakia and oral cancer. Med Oral Patol Oral y Cir Bucal. 2012;e10–5. doi:10.4317/medoral.17323.
20. Mulyati S. Peranan advanced glycation end-products pada diabetes. J Cermin Dunia Kedokt. 2016;43(6):422–6. doi:10.55175/cdk.v43i6.69.
21. Setiawan D, Indra MR. Pengaruh leptin terhadap kadar interleukin 6 serum tikus yang diberi pakan tinggi lemak. Mutiara Med. 2014;14(1):85-92.
22. Thressia PA, Rajeev KN. Effect of sleep duration on obesity and the glycemic level in patients with type 2 diabetes. Asian J Nur Edu Research 2014:4(4):502-7.
23. Pareek S. Prevalence of pre-diabetes and diabetes mellitus among the northern railway employees: A retrospective study. Int J Adv Nur Manag. 2017;5(4):347-50. doi:10.5958/2454-2652.2017.00073.7.
24. Husna F, Suyatna FD, Arozal W, Purwaningsih EH. Model hewan coba pada penelitian diabetes. Pharm Sci Res. 2019;6(3):131-41.
25. Pitriya IA, Nurdin, Sabang SM. Efek ekstrak daun kelor (Moringa oleifera) menurunkan kadar gula darah pada mencit. J Akad Kim. 2017;6(1):35-42.
26. Nugroho RA. Mengenal Mencit Sebagai Hewan Laboratorium. In: Mengenal Mencit Sebagai Hewan Laboratorium. Samarinda: Mulawarman University Press; 2018.
27. Afriani AE, Margawati A, Dieny FF. Tingkat Stres, Durasi dan Kualitas Tidur, serta Sindrom Makan Malam Pada Mahasiswi Obesitas dan Non Obesitas Fakultas Kedokteran. Sport Nutr J. 2019 Dec;1(2):63–73.
28. Zarouna S, Wozniak G, Papachristou AI. Mood disorders: A potential link between ghrelin and leptin on human body? World J Exp Med. 2015;5(2):103-9. doi:10.5493/wjem.v5.i2.103.
29. Anilkumar BJ, Radhakrishnan G. Stress, obesity and selected health problems among professionals. Asian J Nur Edu Res. 2013;3(3):154-63.
30. Susantiningsih T. Obesitas dan stres oksidatif. J Kesehat Unila. 2015;5(9):89-93.
31. Liberty IA. Hubungan obesitas dengan kejadian prediabetes pada wanita usia produktif. J Kedokt dan Kesehat. 2016;3(2):108–13.
32. Ellulu MS, Patimah I, Khaza'ai H, Rahmat A, Abed Y. Obesity and inflammation: The linking mechanism and the complications. Arch Med Sci. 2017;13(4):851-63. doi:10.5114/aoms.2016.58928.
33. Murray RK, Davis JC. Molecular Physiology. In: Harper's Illustrated Biochemistry. 30th Ed. New York: Mcgraw-Hill Education; 2018.
34. Zhao G, Dharmadhikari G, Maedler K, Meyer-Hermann M. Possible role of interleukin-1β in type 2 diabetes onset and implications for anti-inflammatory therapy strategies. PLoS Comput Biol. 2014;10(8):e1003798. doi:10.1371/journal.pcbi.1003798.
35. Cutando A, Montero J, Gomez-de Diego R, Ferrera M, Lopez-Valverde A. Effect of topical application of melatonin on serum levels of C-reactive protein (CRP), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-a) in patients with type 1 or type 2 diabetes and periodontal disease. J Clin Exp Dent. 2015;7(5): e628-33. doi:10.4317/jced.52604.
36. Rodwell VW, Bender DA, Botham KM, Kennelly PJ, Weil AP. Harper's Illustrated Biochemistry. 13th ed. New York: McGraw-Hill Medical; 2015.
37. Skyler JS, Bakris GL, Bonifacio E, Darsow T, Eckel RH, Groop L, et al. Differentiation of diabetes by pathophysiology, natural history, and prognosis. Diabetes. 2017;66(2):241-55. doi:10.2337/db16-0806.
38. Decroli E. Diabetes mellitus tipe 2. Alexander A Eds. Padang: Pusat Penerbitan Bagian Ilmu

Penyakit Dalam Fakulltas Kedokteran Universitas Andalas 2019.
39. Masi G, Oroh, W. Hubungan obesitas dengan kejadian diabetes mellitus di wilayah kerja puskesmas Ranomut kota Manado. J Keperawatan. 2018;6910:1-6. doi:10.35790/jkp.v6i1.25183.
40. Cantley J, Ashcroft FM. Q&A: insulin secretion and type 2 diabetes: why do β-cells fail? BMC Biol. 2015;13:33. doi:10.1186/s12915-015-0140-6.
41. Wisudanti DD. Aplikasi terapeutik geranin dari ekstrak kulit rambutan (Nephelium lappaceum) sebagai antihiperglikemik melalui aktivasinya sebagai antioksidan pada diabetes mellitus tipe 2. NurseLine J. 2016;1(1):120-38.
42. Sole MJ, Martino TA. Diurnal physiology: Core principles with application to the pathogenesis, diagnosis, prevention, and treatment of myocardial hypertrophy and failure. J Appl Physiol. 2009;107(4):1318-27. doi:10.1152/japplphysiol.00426.2009.
43. Schweinfurth MK. The social life of Norway rats (*Rattus norvegicus*). Elife. 2020;9:e54020. doi:10.7554/eLife.54020.
44. Nunez AA, Yan L, Smale L. The cost of activity during the rest phase: animal models and theoretical perspectives. Front Endocrinol (Lausanne). 2018;9:72. doi:10.3389/fendo.2018.00072.
45. Nurmawati T. Study of physiological response and white rats (Rattus noorvegicus) blood glucose levels that streptozotocin exposed. J Ners Kebidanan (J Ners Midwifery). 2017;4(3):244-7.
46. Saputra NT, Suartha IN, Dharmayudha AAGO. Agen diabetagonik streptozotocin untuk membuat tikus putih jantan diabetes mellitus. Bul Vet Udayana. 2018;10(2):116. doi:10.24843/bulvet.2018.v10.i02.p02.
47. Emmer KM, Russart KLG, Walker WH, Nelson RJ, DeVries AC. Effects of light at night on laboratory animals and research outcomes. Behav Neurosci. 2018 Aug;132(4):302-14. doi:10.1037/bne0000252..
48. Yasa IWPS, Wande IN, Susila NKN, Budhiastra P, Pemayun CID, Herawati S. Kadar Il-6 Plasma Pasien Diabetes Melitus Dengan Dan Tanpa Pengidap Retinopati Diabetika (The Level of Interleukin-6 Plasma in Diabetes Mellitus Patients With and Without Diabetic Retinopathy). Indones J Clin Pathol Med Lab. 2018;21(1):1-4.

The Role of Interleukin 1β (IL-1 β) and Interleukin 6 (IL- 6) on Diabetes Mellitus and its Correlation with Cardian Rythm in Wistar Rats (Rattus norvegicus)

ORIGINALITY REPORT

20%	17%	11%	0%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	ijanm.com Internet Source	1%
2	www.researchgate.net Internet Source	1%
3	journal2.uad.ac.id Internet Source	1%
4	Shabrina Arwi Laily, Titiek Hidayati, Akrom Akrom, Vivi Sofia. "The Effect of Black Cumin Seed Oil on Active Smokers Based on Interleukin-1β Activity and Neutrophil", PHARMACY: Jurnal Farmasi Indonesia (Pharmaceutical Journal of Indonesia), 2021 Publication	1%
5	pinpdf.com Internet Source	1%
6	iabdm.org Internet Source	1%
	repository.unair.ac.id	

7	Internet Source	1 %
8	media.neliti.com Internet Source	1 %
9	"The Diabetes Textbook", Springer Science and Business Media LLC, 2019 Publication	1 %
10	biotropika.ub.ac.id Internet Source	1 %
11	jpet.aspetjournals.org Internet Source	1 %
12	baadalsg.inflibnet.ac.in Internet Source	1 %
13	sci-hub.se Internet Source	1 %
14	edocs.maseno.ac.ke Internet Source	<1 %
15	bsdwebstorage.blob.core.windows.net Internet Source	<1 %
16	www.damanhour.edu.eg Internet Source	<1 %
17	www.ncbi.nlm.nih.gov Internet Source	<1 %
18	www.sciencegate.app Internet Source	

		<1 %
19	backend.orbit.dtu.dk Internet Source	<1 %
20	Submitted to Universitas Airlangga Student Paper	<1 %
21	M. J. Sole. "Diurnal physiology: core principles with application to the pathogenesis, diagnosis, prevention, and treatment of myocardial hypertrophy and failure", Journal of Applied Physiology, 10/01/2009 Publication	<1 %
22	archive.org Internet Source	<1 %
23	rjptonline.org Internet Source	<1 %
24	H.-Z. Pan. "The change of oxidative stress products in diabetes mellitus and diabetic retinopathy", British Journal of Ophthalmology, 4/1/2008 Publication	<1 %
25	ejournal.undiksha.ac.id Internet Source	<1 %
26	123dok.com Internet Source	<1 %

27	1library.net Internet Source	<1%
28	Merve Yilmaz, Aslı Ulubaş Hamurcu. "Relationships between socio-demographic structure and spatio-temporal distribution patterns of COVID-19 cases in Istanbul, Turkey", International Journal of Urban Sciences, 2022 Publication	<1%
29	ijshr.com Internet Source	<1%
30	trialsjournal.biomedcentral.com Internet Source	<1%
31	www.eamonryan.ie Internet Source	<1%
32	www.mdpi.com Internet Source	<1%
33	www.oncotarget.com Internet Source	<1%
34	Carlos Hernandez-Castillo, John Termini, Sarah Shuck. "DNA Adducts as Biomarkers To Predict, Prevent, and Diagnose Disease—Application of Analytical Chemistry to Clinical Investigations", Chemical Research in Toxicology, 2019 Publication	<1%

35	J. Dorrestijn, F. J. van Bussel, J. A. Maasen, D. S. Gomes de Mesquita. "Early Steps in Insulin Action", Archives of Physiology and Biochemistry, 2008 Publication	<1 %
36	basic.ub.ac.id Internet Source	<1 %
37	pubmed.ncbi.nlm.nih.gov Internet Source	<1 %
38	www.emerald.com Internet Source	<1 %
39	www.frontiersin.org Internet Source	<1 %
40	www.health.com Internet Source	<1 %
41	www.i-scholar.in Internet Source	<1 %
42	www.researchsquare.com Internet Source	<1 %
43	www.wjgnet.com Internet Source	<1 %
44	Murphy, H.M.. "Vasopressin deficiency and blood glucose interactions on passive avoidance behavior", Peptides, 1994 Publication	<1 %

45	bmccancer.biomedcentral.com Internet Source	<1 %
46	garuda.ristekdikti.go.id Internet Source	<1 %
47	link.springer.com Internet Source	<1 %
48	ppjp.ulm.ac.id Internet Source	<1 %
49	tumj.tums.ac.ir Internet Source	<1 %
50	www.jmchemsci.com Internet Source	<1 %
51	www.thieme-connect.com Internet Source	<1 %
52	Fajar Husen, Hernayanti Hernayanti, Nuraeni Ekowati, Dalia Sukmawati, Nuniek Ina Ratnaningtyas. "Antidiabetic Effects and Antioxidant Properties of the Saggy Ink Cap Medicinal Mushroom, Coprinus comatus (Agaricomycetes), in Streptozotocin-Induced Hyperglycemic Rats", International Journal of Medicinal Mushrooms, 2021 Publication	<1 %
53	Inflammatory Pathways in Diabetes, 2015. Publication	<1 %

Exclude quotes	Off	Exclude matches	Off
Exclude bibliography	On		

The Role of Interleukin 1β (IL-1 β) and Interleukin 6 (IL- 6) on Diabetes Mellitus and its Correlation with Cardian Rythm in Wistar Rats (Rattus norvegicus)

GRADEMARK REPORT

FINAL GRADE

/0

GENERAL COMMENTS

Instructor

PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6

PAGE 7