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*Original Research Article*

# Hydrogen-Rich Water Ameliorates Pancreatic Cell Necrosis and Regulates Cytokine Levels in Streptozotocin-Induced Diabetic Rats

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ABSTRACT

Water plays an important role in the metabolic processes. An unhealthy lifestyle leads to an increase in free radicals, which causes type 2 diabetes mellitus (T2DM). Hydrogen-rich water (HW) is water with dissolved hydrogen gas and has been implicated to have antioxidant activity. This study was aimed at analyzing the effect of HW on pancreatic cell necrosis and cytokine level in streptozotocin (STZ)-induced diabetic rats. Male Wistar rats were fed a high-fat diet (HFD) for four weeks before being injected intraperitoneally with low dose STZ (35 mg/kg bw) to obtain DM rats. Blood glucose values of >200 mL/dL were considered diabetic rats. The experimental rats were randomly divided into five groups: healthy (normal rats without STZ injection), DM, DM + metformin (45 mg/kg bw), DM + HW, and DM + HW + metformin. The treatments were administered orally for fourteen days. After that, the rats were dissected using Ket-A-Xyl; serum and liver samples were obtained to determine the concentrations of IL-10 and IL-1$\beta$. The pancreas was collected and examined for cell damage, while histology of the Islet of Langerhans was analyzed descriptively. The results showed that HW reduced blood glucose levels, body weight, and IL-1$\beta$ concentration, increased IL-10 concentration, and improved necrotic cells of the pancreas in comparison to DM rats. HW could be one of the therapies for T2DM patients by lowering glucose levels, regulating cytokine levels, and repairing $\beta$-cells in the pancreas.

***Keywords:*** Diabetes mellitus, Hydrogen-rich water, IL-1$\beta$, IL-10, Necrosis.

## Introduction

Water is a vital component for the body, especially for maintaining a healthy body and preventing various diseases.[1] Lack of water intake may lead to dehydration and increase the risk of various diseases such as headaches, urolithiasis, obesity, and diabetes mellitus.[1,2] According to Goodman, drinking more water is linked to a reduction in body weight.[2] Adequate water consumption is better than a fiber diet in a 24-week weight loss program in overweight and obese women.[3,4] Consumption of 1 L of water daily for three months and 0.8 L of water daily for six months significantly reduced fasting blood glucose.[5] More water intake can treat obesity. Adequate water consumption could affect the removal of excess glucose in the kidney by creating more urine. Dehydration is associated with an increase in blood glucose level caused by the inability to excrete glucose in the urine.[1] Diabetes mellitus (DM) is a metabolic disease that disrupts the metabolism of carbohydrates, fats, and proteins characterized by an increase in the normal level of blood glucose.[6] The prevalence of diabetes has increased over the decades.[7] Diabetic patients accounted for 463 million (9.3% of the world's population) in 2019, with ages ranging from 20 to 79 years, and this figure is anticipated to rise to 700 million (10.9%) by 2045.[7,8] The increasing prevalence of people with diabetes is also higher in low and middle-income countries compared with high-income countries.[9]

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T2DM is the type of diabetes that mainly occurs within society.[10] It is caused by insulin resistance, which is closely linked to obesity due to an unhealthy lifestyle.[11] The impaired insulin production was frequently accompanied by chronic low-grade inflammation for a prolonged time which may lead to tissue damage, including $\beta$-cells of the pancreas.[12–14] An excessive proinflammatory cytokines production, mainly TNF-α, IL-1$\beta$, IL-6 were increased through NFκB signaling pathways during T2DM progression.[15] In contrast, interleukin-10 (IL-10) as an anti-inflammatory cytokine that is secreted by regulatory T cells (Tregs) is decreased during T2DM progression.[16] IL-10 has pivotal roles in suppressing cytokine production and immune cell activation. In high-fat diet (HFD) mice, IL-10 expression is drastically reduced, along with Treg function and density.[17,18] Several treatments for DM have been developed, but effective treatment is required to restore the cytokines balance and improve damaged pancreatic $\beta$-cells. Recent studies found that hydrogen-rich water (HW) has an antioxidant effect that prevents or treats various diseases related to oxidative stress, including diabetes, atherosclerosis, and heart failure.[19] HW contains high levels of dissolved hydrogen ($H_2$). These $H_2$ molecules have antioxidant activity as a scavenger of hydroxyl radicals (•OH).[20] Kamimura *et al.* (2011)[19] reported that oral administration of HW by gavage in obese rats reduced oxidative stress in the liver and improved hepatic steatosis. However, research on HW on the histological improvement of Langerhans islets in the DM animal model is still limited. Therefore, the aim of this study was to investigate the effects of hydrogen-rich water on blood glucose levels, pancreatic cell necrosis, and cytokine levels in diabetic rats.

## Materials and Methods

*Ethical approval*

Ethical approval for this study was obtained from the Ethics Committee of Politeknik Kesehatan Malang with approval number EC00974 2020.

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*Animal model and experimental design*

This research used thirty male Wistar rats (weight 200 ± 20 g, 10 ± 2 weeks old) which were placed in standard cages. The rats were acclimatized for a week, with free access to feed and water. After acclimatization, the rats were divided into two groups, namely normal feed (n = 5) and high-fat diet (HFD; n = 25). The normal diet contained 60% carbohydrates, 16% protein, 3% fat, and 21% vitamins as well as minerals. HFD contained 8.84% carbohydrates, 8.50% protein, 34.20% fat, and fiber, vitamins, as well as minerals.[21] For 30 days, the rats were administered with normal and HFD,[22] and the fasting blood glucose of the rats that were fed with HFD was measured. Then, the HFD-fed rats were injected with low dose of streptozotocin (at 35 mg/kg bw dissolved in 0.1 M citrate buffer, pH 4.5) intraperitoneally.[22] The blood glucose levels were measured 3 days after injection and DM rats were characterized by fasting blood glucose levels >200 mg/dL.[23] The experimental animals were randomly divided into 5 groups: normal (normal diet), DM, DM + metformin (45 mg/kg bw), DM + HW + metformin (45 mg/kg bw)[24] and DM + HW (Table 1.)

*Preparation and administration of hydrogen-rich water*

Hydrogen-rich water was prepared from a hydrogen-water electric generator (DR + Water Electric Generator, PT. Altra Multi Sukses). Freshly made hydrogen-rich water contains 1 ppm of hydrogen. The rats were orally administered with 3 mL of fresh hydrogen-rich water by gavage and *ad libitum* of hydrogen-rich water for 14 days.[25] At the end of the treatment, the rats were sacrificed using ketamine and xylazine (Ket-A-Xyl) as anesthetic solutions after which the pancreas was collected and stored in 10% formalin.

*Histological analysis of experimental rats' pancreas*

The pancreas of the experimental rats was fixed in 10% formalin, hydrated, and embedded in paraffin. It was then cut into 5 µm size, stained using hematoxylin-eosin (HE).[26] Slides of the Langerhans islet were examined with a microscope (Olympus, BX51) at a 1000x magnification. The pancreatic histology were analyzed descriptively to determine changes in cell architecture, including fibrosis, necrosis, and vacuolization in the Langerhan islet.[27]

*Measurement of IL-10 and IL-1β concentrations in serum and liver tissues*

Blood samples were collected in a centrifuged tube from the heart after the rats were fasted overnight and allowed to stand for one hour at room temperature. The blood was then centrifuged at 3000 rpm for 20 min to separate the serum, the supernatant was then collected and stored at -20$^{o}$C before use. The liver was removed and washed three times in phosphate buffer saline (PBS) pH 7.4, dried with filter paper, and weighed. After that, the liver was chopped into small pieces, about 0.5 g, and homogenized in PBS (pH 7.4). The homogenate was then centrifuged at 3000 rpm for 20 min, and the supernatant was stored at -20$^{o}$C before use.[21] ELISA commercial kits (Bioenzy, BZ-08188010-EB, and BZ-08189110-EB) were used to quantify the concentrations of IL-10 and IL-1, respectively in serum and liver tissues according to the manufacturer's protocol.

*Statistical data analysis*

Data related to blood glucose levels, body weight, organ weight, IL-10, and IL-1$\beta$ concentrations were analyzed using one-way analysis of. variance (ANOVA). Duncan Multiple Range Test (DMRT) was used as a post hoc test with $p$-value < 0.05 considered significant.

## Results and Discussion

*Hydrogen-rich water affected glucose level, body and organ weights in diabetic rats*

In the present study, when DM rats were compared to normal groups, they had significantly ($p$<0.05) higher levels of glucose and body weight (Table 2). HW administration reduced the glucose levels and DM rats' body weight significantly ($p$<0.05). Interestingly, the results of the current study revealed that HW alone has a stronger effect on decreasing glucose levels and body weight than the combination of HW with metformin. Also, there was no significant difference in organ weight between the DM rats and treatment groups.

It was observed that HW supplementation reduced the body weight gain caused by HFD. Kamimura *et al*. (2011)[29] reported that HW administration increased the fibroblast growth factor 21 (FGF21) expression as a hepatic hormone with the function of maintaining energy homeostasis, especially with glucose and fat metabolism.[19,28] After treatment with HW, the final blood glucose levels were significantly decreased compared with DM rats, and the levels became close to that of normal rats (Table 2). A previous study demonstrated that drinking about 900 mL/day HW could normalize an impaired glucose tolerance as evidenced by the elevation of insulin level after glucose loading.[29]

*Hydrogen-rich water altered IL-10 and IL-1β concentrations in diabetic rats*

IL-10 levels decreased while IL-1$\beta$ levels increased in the DM rats as shown in Figure 1A-B. In contrast to the DM rats, HW administration enhanced the IL-10 levels and reduced the IL-1$\beta$ levels significantly ($p$<0.05). The results revealed that IL-10 levels in serum and liver followed a similar pattern (Figure 1A). Interestingly, in the DM rats, treatment with HW combined with metformin had a stronger effect on increasing IL-10 levels than treatment with HW alone. Furthermore, metformin had a better effect in lowering IL-1$\beta$ concentrations than HW or HW combined with metformin (Figure 1B). Surprisingly, there was no difference in IL-1$\beta$ concentrations in the liver of normal, DM, and treated rats.

HW altered the proinflammatory and anti-inflammatory cytokines in DM rats as highlighted in Figure 1A-B. A previous study reported that HW increased the IL-10 concentration and decreased the IL-1$\beta$ concentration in LPS-challenged mice.[30] Besides, HW at 0.5-0.6 ppm was reported to have shown anti-inflammatory activity, both in *in vitro* and *in vivo* studies.[31] The possible mechanism could be that $H_2$ inhibits the phosphorylation of IκB-α and interferes with NFκB signaling through declining oxygen free radicals. The NFκB signaling pathway blockade may ameliorate the inflammatory condition by suppressing IL-1$\beta$ expression.[32,33] The findings of this investigation showed that HW decreased IL-1 levels in DM rats after supplementation with HW, which was consistent with previous results. Furthermore, as the concentration of IL-1$\beta$ decreased, the concentration of IL-10 increased. IL-10 was critical in suppressing immune cell activation, leading to delayed inflammation.[34] Consistent with the results of this research, a previous study reported that HW enhanced IL-10 levels and restored regulatory T cells (Tregs) loss.[35]

*Hydrogen-rich water restored necrotic cells of the pancreas in diabetic rats*

The present study demonstrated that there was an increase in the number of necrotic cells in DM rats (Figure 2).

**Table 1:** Experimental design

| | **Acclimatization** | **Experimental Animal** | | **Sacrifice** |
|---|---|---|---|---|
| Male *Wistar* Rats (n = 30) | NHFD (n = 30) | NHFD (n = 6) | NHFD (n = 6) | Serum and Tissue Sampling |
| | | HFD (n = 24) | DM (n = 6) | |
| | | | DM+met (n = 6) | |
| | | | DM+met+HW (n = 6) | |
| | | | DM+HW (n = 6) | |
| Day | 0-7 | 8-38 | 39-52 → | 52 |

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However, HW administration resulted in an improvement on the pancreas histology, indicating the reduction of necrotic cells and the increase in the number of cells in the Langerhans islet compared to the DM group without treatment (Figure 2). Oxidative stress agents, especially reactive oxygen species (ROS), play a role in the pathogenesis of diabetes.[36] Hyperglycemic conditions lead to high ROS production and decreased endogenous antioxidants.[37] In both *in vitro* and *in vivo* experiments, HW at 0.5-0.6 ppm showed antioxidant activity.[31] $H_2$ molecules are biologically active gases that can be used to improve various systemic pathological conditions. The molecules are involved in regulating gene expression, modulating signal transduction, protein phosphorylation cascades, and especially in metabolic pathways.[38,39] Excessive free radical production might lead to organ damage and failure. The results obtained from this study suggest that necrotic cells in DM rats were higher than the normal group and there was a decrease in the number of cells in Langerhans islet compared to the normal treatment group (Figure 2). Oxidative stress activates the cell death signaling pathway.[40] Smirnov *et al*. (2012)[41] reported that STZ-induced diabetic mice caused necrotic cells and reduced the number of *β*-cells in each Langerhans islet significantly. HW administration showed an improvement on the pancreas histology, suggesting the reduction of necrotic cells and the increase in the number of cells in the Langerhans islet compared to the DM group without treatment (Figure 2). In this study, it was found that HW supplementation restored glucose levels in DM rats to near-normal levels. Ming *et al*. (2020)[37] reported a decreased malondialdehyde (MDA) levels in the serum of T2DM mice model after treatment with HW. $H_2$ molecules increase the synthesis of hepatic glycogen, improve glucose intake in the liver, and lowers fasting blood glucose levels.[37] $H_2$ molecules improved blood glucose levels in STZ-induced diabetic mice by increasing the expression of Glucose Transport Type 4 (GLUT4) on the skeletal muscle membrane through the activation of phosphatidylinositol-3-OH kinase (PI3K).[20] Administration of HW resulted in decreased expression of Advanced Glycation-End product (AGE) and receptor of AGE (RAGE) in STZ-induced diabetic rats. AGE and RAGE as a non-enzymatic product of glycation process play a role in development of diabetic complication.[42]

**Table 2:** Effect of hydrogen-rich water on glucose levels, body weight, and organ weight of normal and diabetic rats

| Parameter | Treatment Groups | | | | |
|---|---|---|---|---|---|
| | N | DM | DM + Met | DM + HW | DM + MHW |
| **Glucose Levels** | | | | | |
| Initial | $85.60^{a} \pm 3.78$ | $415.67^{b} \pm 24.82$ | $384.33^{b} \pm 39.11$ | $406.67^{b} \pm 21.79$ | $395.33^{b} \pm 26.39$ |
| Final | $124.33^{a} \pm 8.26$ | $376.00^{c} \pm 10.58$ | $169.00^{b} \pm 17.08$ | $130.67^{a} \pm 11.50$ | $185.67^{b} \pm 7.51$ |
| **Body Weight** | | | | | |
| Initial | $165.67 \pm 16.01^{a}$ | $296.48 \pm 10.78^{b}$ | $321.67^{bc} \pm 14.57$ | $346.67^{c} \pm 12.74$ | $315.33^{b} \pm 17.09$ |
| Final | $184.33^{a} \pm 19.85$ | $374.00^{c} \pm 31.43$ | $265.67^{b} \pm 48.33$ | $185.67^{a} \pm 41.26$ | $252.67^{b} \pm 29.71$ |
| **Organ Weight** | | | | | |
| Pancreas | $7.38^{a} \pm 1.01$ | $11.50^{b} \pm 2.08$ | $10.71^{b} \pm 0.52$ | $11.18^{b} \pm 1.88$ | $11.23^{b} \pm 2.85$ |
| Liver | $27.21^{a} \pm 1.97$ | $47.57^{b} \pm 5.04$ | $42.85^{b} \pm 4.07$ | $55.61^{b} \pm 14.65$ | $49.15^{b} \pm 5.75$ |
| Kidney | $2.46^{a} \pm 0.69$ | $3.81^{ab} \pm 0.63$ | $3.58^{ab} \pm 0.85$ | $4.07^{b} \pm 0.82$ | $4.75^{b} \pm 1.05$ |

[a,b]:Different superscript within the same row indicates significant differences ($p < 0.05$); N: Normal rats; DM: DM rats without additional supplementation; DM + Met: DM rats supplemented with metformin; DM + HW: DM rats supplemented with hydrogen-rich water; DM + MHW: DM rats supplemented with metformin and HW combination.

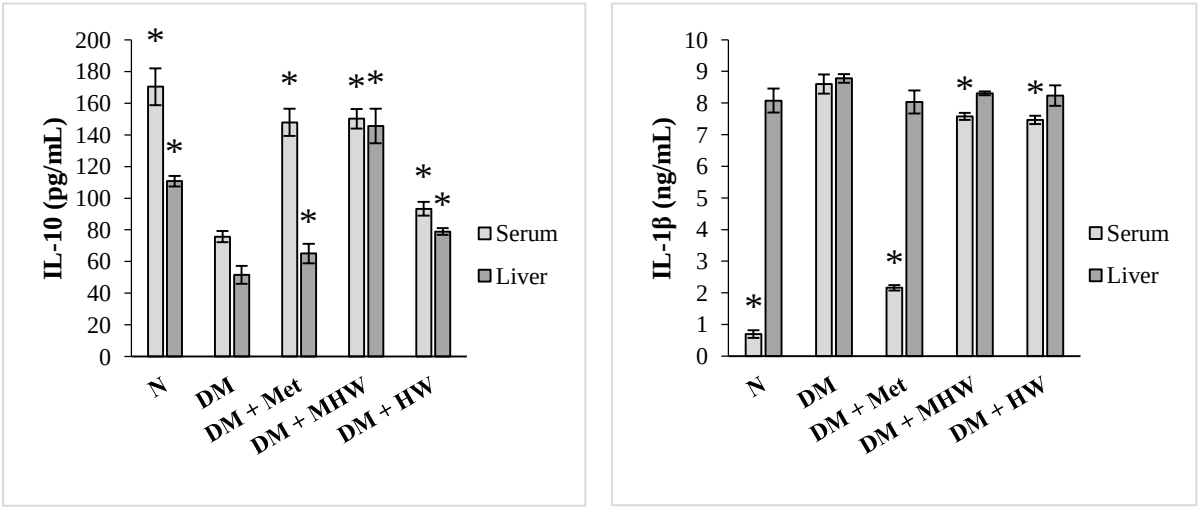


**Figure 1:** Effect of hydrogen-rich water on IL-10 and IL-1*β* concentrations in serum and liver of normal and diabetic mice.
DM: DM rats without additional supplementation; DM + Met: DM rats supplemented with metformin; DM + HW: DM rats supplemented with hydrogen-rich water; DM + MHW: DM rats supplemented with metformin and HW combination. *$p < 0.05$ significantly difference compared with DM group as control.

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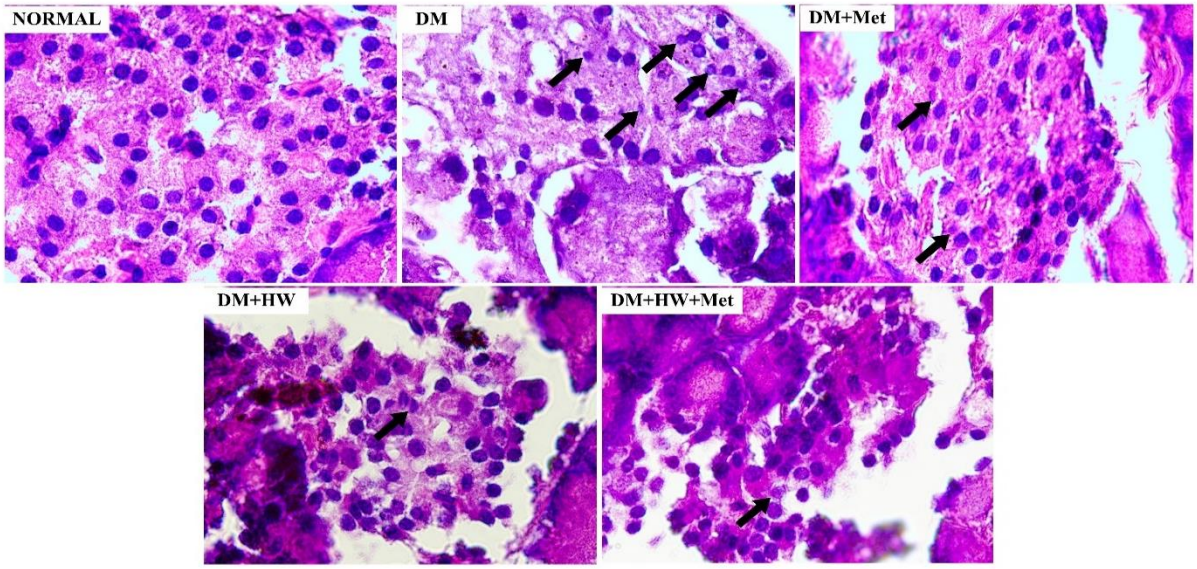


**Figure 2:** Histology of Langerhans islet of the different treatment groups.
The black arrow shows necrotic cells; DM: DM rats without additional supplementation; DM + Met: DM rats supplemented with metformin; DM + HW: DM rats supplemented with hydrogen-rich water; **D**M + MHW: DM rats supplemented with metformin and HW combination; H&E 1000x magnification.

## Conclusion

The findings of this study reveal that hydrogen-rich water has the potential to be an alternative treatment option with beneficial effects such as lowering glucose levels, enhancing pancreatic histology, reducing proinflammatory cytokine, and increasing anti-inflammatory cytokine in DM rats. Improved glucose metabolism in the body, as well as an increase in anti-inflammatory cytokines and a decrease in the number of necrotic cells in Langerhans islets, may lead to improved pancreatic-cell function in DM patients. Therefore, hydrogen-rich water can be used as an effective therapy for DM, as it improves necrotic cells in the pancreas and lowers cytokine levels, resulting in reduced blood glucose levels. Hydrogen-rich water may have potential for use in diabetes mellitus prevention and management.

## 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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## References

1. El-Sharkawy AM, Sahota O, Lobo DN. Acute and chronic effects of hydration status on health. Nutr Rev. 2015; 73(2):97-109.
2. Goodman AB. Behaviors and Attitudes Associated with Low Drinking Water Intake Among US Adults, Food Attitudes and Behaviors Survey, 2007. Prev Chronic Dis. 2013; 10(4):120248-120259.
3. Madjd A, Taylor MA, Delavari A, Malekzadeh R, Macdonald IA, Farshchi HR. Beneficial effects of replacing diet beverages with water on type 2 diabetic obese women following a hypo-energetic diet: A randomized, 24-week clinical trial. Diabetes Obes Metab. 2017; 19(1):125-132.
4. Madjd A, Taylor MA, Delavari A, Malekzadeh R, Macdonald IA, Farshchi HR. Effects on weight loss in adults of replacing diet beverages with water during a hypoenergetic diet: a randomized, 24-wk clinical trial. Am J Clin Nutr. 2015; 102(6):1305-1312.
5. Naumann J, Biehler D, Lüty T, Sadaghiani C. Prevention and Therapy of Type 2 Diabetes—What Is the Potential of Daily Water Intake and Its Mineral Nutrients? Nutr. 2017; 9(8):914-931.
6. Elikee ER, Ezugwu CO, Ihekwereme CP, Nzekwe IT, Ajaghaku DL. Co-administration Effects of *Newbouldia laevis* (P. Beauv.) Leaves and Garcinia kola (Heckel) Seed Extracts on Animal Models of Hepatotoxicity and Diabetes. Trop J Nat Prod Res. 2021; 5(2):389-396.
7. Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, Colagiuri S, Guariguata L, Motala AA, Ogurtsova K, Shaw JE, Bright D, Williams R. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. Diabetes Res Clin Pract. 2019; 157 (2019):107843-107851.
8. International Diabetes Federation (IDF). IDF Diabetes Atlas: 9th Edition [Online]. 2019. Available from: https://www.idf.org/aboutdiabetes/what-is-diabetes/facts-figures.htmL
9. World Health Organization. Diabetes [Online]. 2020. Available from:https://www.who.int/news-room/fact-sheets/detail/diabetes
10. Khan MAB, Hashim MJ, King JK, Govender RD, Mustafa H, Al Kaabi J. Epidemiology of Type 2 Diabetes – Global Burden of Disease and Forecasted Trends. J Epidemiol Glob Health. 2020; 10(1):107-111.
11. Al-Goblan AS, Al-Alfi MA, Khan MZ. Mechanism linking diabetes mellitus and obesity. Diabetes Metab Syndr Obes Targets Ther. 2014; 7(2014):587-591.
12. Kahn SE, Cooper ME, Del Prato S. Pathophysiology and treatment of type 2 diabetes: perspectives on the past, present and future. Lancet. 2014; 383(9922):1068-1083.
13. Skyler JS, Bakris GL, Bonifacio E, Darsow T, Eckel RH, Groop L, Groop PH, Handelsman Y, Insel RA, Mathieu C, McElvaine AT, Palmer JP, Pugliese A, Scharz DA, Sosenko JM, Wilding

JPH, and Ratner RE. Differentiation of Diabetes by Pathophysiology, Natural History, and Prognosis. Diabetes. 2017; 66(2):241-255.
14. Gofur A, Witjoro A, Ningtiyas EW, Setyowati E, Mukharromah SA, Athoillah MF, Lestari SR. The Evaluation of Dietary Black Soybean and Purple Sweet Potato on Insulin Sensitivity in Streptozotocin - Induced Diabetic Rats. Pharmacogn J. 2019; 11(4):639-646.
15. Tsalamandris S, Antonopoulos AS, Oikonomou E, Papamikroulis G-A, Vogiatzi G, Papaioannou S, Deftereos S, and Tousoulis D. The Role of Inflammation in Diabetes: Current Concepts and Future Perspectives. Eur Cardiol Rev. 2019; 14(1):50-59.
16. Kartika R, Purnamasari D, Pradipta S, Larasati RA, Wibowo H. Impact of Low Interferon-γ and IL-10 Levels on TNF-α and IL-6 Production by PHA-Induced PBMCs in Type 2 Diabetes Mellitus. J Inflamm Res. 2020; 13(2020):187-193.
17. Atho'illah MF, Widyarti S, Rifa'i M. Elicited soybean (Glycine max L.) extract improves regulatory T cell activity in high fat-fructose diet mice. In Malang, Indonesia: AIP Conference Proceedings; 2017. 1-6 p. [cited 2020 May 9]. Available from: http://aip.scitation.org/doi/abs/10.1063/1.4983415
18. Lestari SR, Atho'illah MF, Christina YI, Rifa'i M. Single garlic oil modulates T cells activation and proinflammatory cytokine in mice with high fat diet. J Ayurv Integr Med. 2020; 11(4):414-420.
19. Kamimura N, Nishimaki K, Ohsawa I, Ohta S. Molecular Hydrogen Improves Obesity and Diabetes by Inducing Hepatic FGF21 and Stimulating Energy Metabolism in db/db Mice. Obes. 2011; 19(7):1396-1403.
20. Amitani H, Asakawa A, Cheng K, Amitani M, Kaimoto K, Nakano M, *et al*. Hydrogen Improves Glycemic Control in Type1 Diabetic Animal Model by Promoting Glucose Uptake into Skeletal Muscle. PLOS ONE. 2013; 8(1):53913-53926.
21. Arifah SN, Atho'illah MF, Lukiati B, Lestari SR. Herbal Medicine from Single Clove Garlic Oil Extract Ameliorates Hepatic Steatosis and Oxidative Status in High Fat Diet Mice. Malays J Med Sci. 2020; 27(1):46-56.
22. Gofur A, Witjoro A, Arifah SN, Atho'illah MF, Annisa Y, Lestari SR. Black soybean tempeh and purple sweet potato improve sperm quality in streptozotocin-induced diabetic rats. Vet World. 2020; 13(7):112534-2540.
23. Gofur A, Witjoro A, Widya Ningtiyas E, Setyowati E, Aminatul Mukharromah S, Suhartini S, Atho'illah MF, Lestari SR. The ameliorative effect of black soybean and purple sweet potato to improve sperm quality through suppressing reactive oxygen species (ROS) in type 2 diabetes mellitus rat (*Rattus novergicus*). Sci Asia. 2018; 44(5):303-310.
24. Syamsul ES, Nugroho AE, Pramono S. The Antidiabetics of Combination Metformin and Purified Extract of *Andrographis paniculata* (Burn).F.Ness in High Fructose-Fat Fed Rats. Maj Obat Tradis. 2015; 16(3):124-132.
25. Masuda H, Sato A, Miyata K, Shizuno T, Oyamada A, Ishiwata K, Nakagawa Y, Asahara T. Drinking Molecular Hydrogen Water Is Beneficial to Cardiovascular Function in Diet-Induced Obesity Mice. Biol. 2021; 10(5):364-381.
26. Arifah SN, Lukiati B, Lestari SR. Potency of single garlic oil from Indonesian tropical plant in high fat diet-induced hyperlipidemia mice. In Solo, Indonesia; 2018. 1-5 p. [cited 2020, Aug 12]. Available from: http://aip.scitation.org/doi/abs/10.1063/1.5054514
27. Shah R, Subhan F, Sultan SM, Ali G, Ullah I, Ullah S. Comparative evaluation of pancreatic histopathology of rats treated with olanzapine, risperidone and streptozotocin. Braz J Pharm Sci. 2018; 54(03):1-5.
28. Matikainen N. Chapter 39 - Fibroblast Growth Factor 21 is a Regulator of Energy Metabolism in the Liver and Adipose Tissue. In: Watson RR, editor. Nutrition in the Prevention and Treatment of Abdominal Obesity. San Diego: Academic Press; 2014. 461-472 p.
29. Kajiyama S, Hasegawa G, Asano M, Hosoda H, Fukui M, Nakamura N, Kitawaki J, Imai S, Nakan K, Ohta M, Adachi T, Obayashi H, Yoshikawa T. Supplementation of hydrogen-rich water improves lipid and glucose metabolism in patients with type 2 diabetes or impaired glucose tolerance. Nutr Res. 2008; 28(3):137-143.
30. Xie K, Yu Y, Huang Y, Zheng L, Li J, Chen H, Han H, Hou L, Gong G, Wang G. Molecular Hydrogen Ameliorates Lipopolysaccharide-induced Acute Lung Injury in Mice through Reducing Inflammation and Apoptosis: Shock. 2012; 37(5):548-555.
31. Lin C-P, Chuang W-C, Lu F-J, Chen C-Y. Anti-oxidant and anti-inflammatory effects of hydrogen-rich water alleviate ethanol-induced fatty liver in mice. World J Gastroenterol. 2017; 23(27): 4920-4934.
32. Shao A, Wu H, Hong Y, Tu S, Sun X, Wu Q, Zhao Q, Zhang J, Sheng J. Hydrogen-Rich Saline Attenuated Subarachnoid Hemorrhage-Induced Early Brain Injury in Rats by Suppressing Inflammatory Response: Possible Involvement of NF-κB Pathway and NLRP3 Inflammasome. Mol Neurobiol. 2016; 53(5):3462-3476.
33. Atho'illah MF, Safitri YD, Nur'aini FD, Widyarti S, Tsuboi H, Rifa'i M. Elicited soybean extract attenuates proinflammatory cytokines expression by modulating TLR3/TLR4 activation in high−fat, high−fructose diet mice. J Ayurv Integr Med. 2021; 12(1):43-51.
34. Lestari SR and Rifa'i M. Regulatory T cells and anti-inflammatory cytokine profile of mice fed a high-fat diet after single-bulb garlic (*Allium sativum* L.) oil treatment. Trop J Pharm Res. 2019; 17(11):2157.
35. Chen L, Ma C, Bian Y, Li J, Wang T, Su L, Lu J. Hydrogen Treatment Protects Mice Against Chronic Pancreatitis by Restoring Regulatory T Cells Loss. Cell Physiol Biochem. 2017; 44(5):2005-2016.
36. Barman AK, Ahmed T, Das H, Biswas B, Ali MS, Acharyya RN, Sarkar K, Dev S. Evaluation of Antidiabetic Potential of Extract of Sonneratia caseolaris (L.) Engl. Leaves against Alloxan-Induced Diabetes in Mice. Trop J Nat Prod Res. 2021; 5(1):77-83.
37. Ming Y, Ma Q-H, Han X-L, Li H-Y. Molecular hydrogen improves type 2 diabetes through inhibiting oxidative stress. Exp Ther Med. 2020; 20(1):359-366.
38. LeBaron TW, Singh RB, Fatima G, Kartikey K, Sharma JP, Ostojic SM, Gyozdjakova A, Kura B, Noda M, Mojto V, Niaz MA, and Slezak J. The Effects of 24-Week, High-Concentration Hydrogen-Rich Water on Body Composition, Blood Lipid Profiles and Inflammation Biomarkers in Men and Women with Metabolic Syndrome: A Randomized Controlled Trial. Diabetes Metab Syndr Obes Targets Ther. 2020; 13(2020):889-896.
39. Sobue S, Inoue C, Hori F, Qiao S, Murate T, Ichihara M. Molecular hydrogen modulates gene expression via histone modification and induces the mitochondrial unfolded protein response. Biochem Biophys Res Commun. 2017; 493(1):318-324.
40. Rojas J, Bermudez V, Palmar J, Martínez MS, Olivar LC, Nava M, Tomey D, Rojas M, Salazar J, Garicano C, Velasco M. Pancreatic Beta Cell Death: Novel Potential Mechanisms in Diabetes Therapy. J Diabetes Res. 2018; 2018(2):9601801-9601820.
41. Smirnov AV, Snigur GL, Voronkova MP. Pancreatic Islet Beta-Cell Apoptosis in Experimental Diabetes Mellitus (Chapter 9). Apoptosis Med. IntechOpen 2012. Available from: https://www.intechopen.com/books/apoptosis-and-medicine/pancreatic-islet-beta-cell-apoptosis-in-experimental-diabetes-mellitus
42. Retnaningtyas E, Susatia B, Indrajani O, Setyabudhi V, Santoso H. Hydrogen-rich water supplementation declines advanced glycation-end products (AGE) and receptor for AGE (RAGE) in streptozotocin-induced diabetic rats. In Malang, Indonesia; 2021. 1-6 p. [cited 2021 Jun 29]. Available from: http://aip.scitation.org/doi/abs/10.1063/5.0052797