



Decreased Blood Glucose and Histopathology Improvement in Hyperbaric Hyperoxic Conditions Alloxan-induced rats(Laboratory Experimental Study)

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Abstract : Background: Diabetes mellitus is still a problem in Indonesia, related to the number of organs involved. This disease has relatively high morbidity and mortality rates. Adjuvant therapy is needed considering the long-term therapy that must be consumed by patients. **Objective:** To explain the effect of hyperbaric oxygen in reducing blood glucose levels, and repairing histopathological damage to the pancreas and liver. **Method :** this study was an experimental laboratory study using whistar strain rats (*Rattus Norvegicus*) which were given a normal diet then induced alloxan to create a hyperglycemia condition. After that, 12 rats from the treatment group were given 3 x 30' hyperbaric oxygen exposure for 6 days. Blood glucose levels, histopathological changes in the pancreas and liver was measured between the treatment group and the control group were not given hyperbaric oxygen exposure. **Results:** There was a significant difference ($\alpha < 0,05$) in decreasing blood glucose and repairing histopathological damage in pancreatic and liver tissue between treated group and control group. **Conclusion:** hyperbaric oxygen treatment as much as 3 x 30'for days at 2.4 ATA O₂ 100% reduce blood glucose levels and repair histopathological damage to pancreatic tissue and liver of alloxan- induced white rats.

Key words : hyperbaric, alloxan, blood glucose, histopatology.

Background

Diabetes mellitus treatment requires a long time and expensive cost. Currently diabetes mellitus treatment uses insulin and Anti-Diabetes drugs that put patients at risk of complications due to side effects from medications such as diarrhea, nausea, abdominal pain, and vertigo. (Ahmad, 2008).

The International Diabetes Federation (IDF) predicts an increase in the number of people with diabetes in Indonesia from 9.1 million in 2014 to 14.1 million in 2035 (PERKENI, 2015).

As advances in science and technology, hyperbaric oxygen therapy (HBO) that uses 100% O₂ in high pressure rooms can be an adjuvant therapy for several diseases. (Angelica and Wiliyams, 2013).

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HBO has been used as a complementary therapy and is approved for use in various studies and trials, for healing diabetic ulcer wounds, infected wounds such as clostridial myonecrosis, necrotising soft tissue infections, fournier gangrene, traumatic wounds, crush injuries, compartment syndrome, graft and flap injuries, as well as thermal burns (Buthani, 2010).

HBO at a pressure of 2.4 ATA can increase tissue sensitivity to insulin and reduce blood sugar levels. Another theory states that hyperbaric oxygen therapy can increase the amount of dissolved oxygen in such a way that it is easier to enter tissue through direct diffusion. (Zaetun, Kerti and Srigele, 2015).

This study aims to look at the effect of hyperbaric oxygen therapy in alloxan-induced experimental animals, in reducing blood glucose levels and repairing histopathological damage that occurs in the pancreas and liver due to the alloxan induction.

Method

This study is an experimental study in white whistar rats (*Rattus norvegicus*). The study design was Post Test Only Group Design. Using 3 groups of *Rattus norvegicus*, the first group was given standard feed (negative control group), the second group was given standard food and induced alloxan (positive control group) and the third group was given standard food and alloxan induced, then given 2.4 ATA hyperbaric oxygen for 3 x 30 minutes O₂ 100% for 6 days (treatment group).

Rattus norvegicus whistar strain used in this study has a healthy condition, white, male, 8-12 weeks old, body weight 150-200 grams. Experimental animals were adjusted for 3 days before entering the treatment stage.

The sample size used for each group was 12 rats. So that the total sample used was 36 rats. This research was conducted at the Hyperbaric Laboratory of the Faculty of Medicine, Hang Tuah University and the Biochemistry Laboratory of the Faculty of Veterinary Medicine, Airlangga University, Surabaya.

A preliminary study was conducted to find out the dose of alloxan which creates a hyperglycemic condition. The dose given to experimental animals is 150 mg / kg BW, 160 mg / kg BW and 170 mg / kg BW. After alloxan induction, blood glucose was checked on the 3rd day post induction.

Hyperglycemic conditions are induced by injecting alloxan intraperitoneally parallel to the feet and then pushed through the abdominal wall into the intraperitoneal space (Prameswari, Okky; Widjanarko, 2014). The rat's tail is dipped in warm water so that blood flow increases. After the tip of the tail is cut, blood is dripped on a strip of gluco-test. Blood glucose was checked from the tail of the rat using a glucometer strip. Hyperglycemia if blood sugar > 135 mg / dL.

Rats were placed in a 40 x 30 cm tub that had been given grass. Each tub contains 12 mice. The cage is placed in a room with sufficient air and light, not humid, far from noise, not exposed to direct sunlight.

Rats were divided into 3 groups. First group, consisting of 12 mice with standard feed, the second group, consisting of 12 mice with standard feed and alloxan induced. third group, consisting of 12 mice with standard feed, alloxan induced and hyperbaric oxygen therapy 2.4 ATA for 6 days 3 x 30 minutes O₂ 100%.

In each group, pancreatic and liver histopathology were observed. Surgery of the rat by cutting along the thorax to the pubis. Pancreatic and liver tissue are inserted into a 10% formaldehyde buffer neutral fixation solution. Then labeled the group name, type of treatment, type of specimen, date of collection, then placed on the tube on the outside.

Pancreatic and liver histopathology preparations. Fixation the tissue into a 10% formalin buffer solution for 2 times 24 hours. It aims to stop the process of autolysis in cells, which is caused by enzymes released when cells die. Fixation also serves to make the cell unchanged due to subsequent treatment.

Then ethanol is removed from the tissues with chloroform and replaced with a material that can bind paraffin, so that blocks can be made.

After the block is made, cut 4-5 μ thick using a microtome. Then stained using Hematoxylin Eosin and histopathological observation of pancreatic and liver tissue seen on a microscope with a magnification of 400 x.

Observation of tissue histopathology

The parameters used are general morphological changes, changes in the shape and structure of the Langerhans islands and changes in cells.

Observations made by percentage change in the tissue. For liver damage, the rates of lobular inflammation, intravascular inflammation, cell degeneration and necrosis are assessed, scoring as follows: 0: no histopathological changes; 1: histopathological changes 1-20%/field; 2: histopathological changes 21 -50% /field ; 3: histopathological changes 51-75% / field, 4: histopathological changes > 75%/field.

Scoring the degree of damage to the pancreas using the following parameters (Nordback, 1986): Score 0: cell necrosis did not occur in Langerhans island, Score 1: cell necrosis occurred in Langerhans island as much as 1% - 25%, Score 2: cell necrosis occurred in langerhans island 26% - 50%, Score 3: cell necrosis occurred in Langerhans island 51% - 75%, Score 4: cell necrosis occurred in Langerhans island 76% - 100%.

Blood Glucose Levels

Rat blood was examined by the GOD-PAP method, on the basis of glucose will be oxidized by oxygen by catalyzing the enzyme glucose oxidase (GOD) to form gluconic acid and hydrogen peroxide (H₂O₂). Hydrogen peroxide will react with 4-aminoantipyrin and phenol with a catalyst peroxidase (POD) to form quinoneimine and water. Quinoneimine is an indicator that shows glucose levels in the blood (Barham and Trinder, 1972).

Result

1. Blood Glucose level

The results of the examination in each treatment group can be presented as follows:

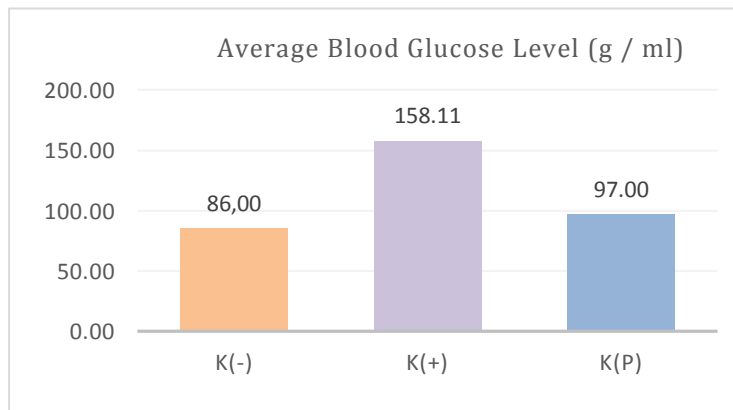


Figure 1. Average Glucose Level (g / ml)

The mean blood glucose in control negative group K (-) was 86.00 mg / dl, control positive group (K (+)) was 158.11 mg / dl, and treatment group (K (P)) was 97 mg / dl. These results indicate that the sample group induced by alloxan and given hyperbaric oxygen therapy had lower blood glucose levels than the control positive group.

2. The degree of pancreatic histopathological damage

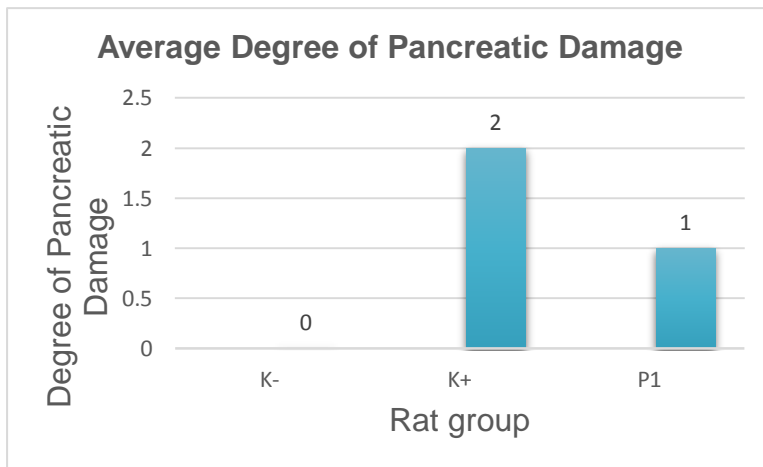


Figure 2 Average Degrees of Pancreatic Damage

The degree of pancreatic damage is seen from the percentage of cell damage on the island of langerhans. Based on the analysis of the average data of the degree of pancreatic damage, the degree of cell necrosis in the Langerhans island region through histopathological features of control negative (K-) group is degree 0, control positive (K+) group is degree 2, and treatment (P1) group is grade 1.

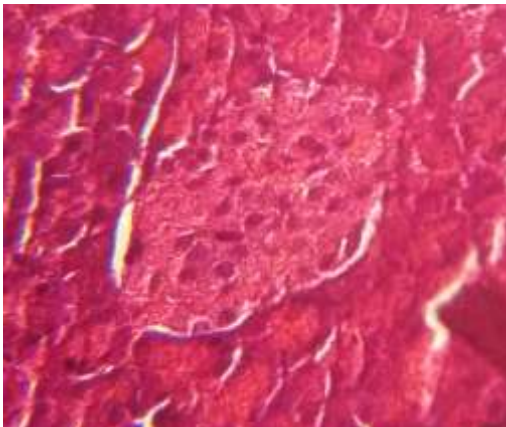


Figure 3. Histopathology of the pancreas with 400x magnification in the control negative group

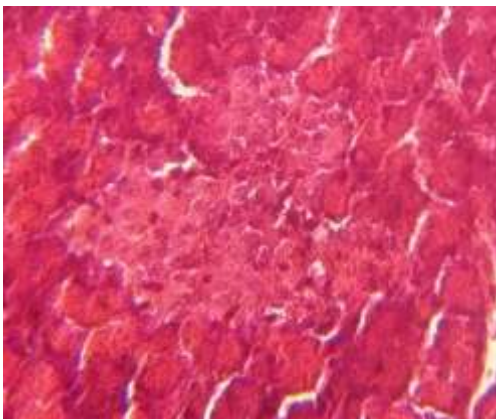


Figure 4. Histopathological picture of the pancreas with 400x magnification in the positive control group.

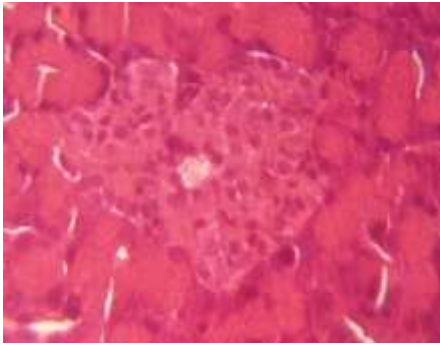


Figure 5 Histopathological picture of the pancreas with 400 x magnification in the treatment group.

3. The degree of pancreatic histopathological damage

For scoring liver damage, evaluated rates of lobular inflammation, intravascular inflammation, cell degeneration and necrosis.

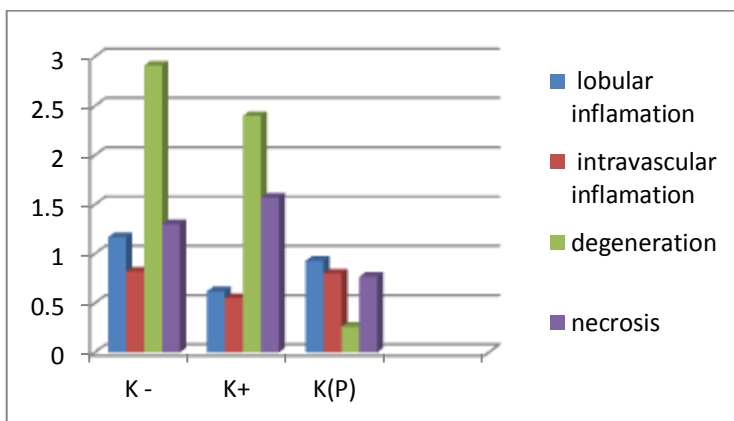


Figure 6. Histopathological damage score of liver tissue (with various types of damage) in all groups (K-: negative control, K +: positive control, KP: treatment group)



Figure 7. Histopathological changes in liver of mice (HE staining, 200x). Hepar without Alloxan-induced and without hyperbaric (K-) treatment, Alloxan-induced liver without hyperbaric (K +) treatment, Alloxan-induced liver and hyperbaric (KP) treatment. Blue arrows indicate infiltration of inflammatory cells, green arrows indicate hepatocyte degeneration (balloning), and yellow arrows indicate hepatocyte necrosis. VC = central venous vein.

There were a significant differences found in the all groups ($\alpha < 0.05$), that is lobular inflammation ($\alpha = 0.021$), degeneration ($\alpha = 0.01$) and necrosis ($\alpha = 0.06$).

However, histopathological changes in the form of vascular inflammation were not significant between the all treatment groups (with $\alpha = 0.063$)

It can be concluded that hyperbaric oxygen has a significant effect on liver histopathological improvement, where the treatment group had less histopathological damage than the positive control group.

Discussion

Analysis of decreased blood glucose after exposure to hyperbaric oxygen

Based on the results of the study, the average glucose level in the negative control group was 85.3 mg / dl, the average glucose level in the positive control group was 158.11 mg / dl, and the average glucose level in the treatment group was 97.0 mg / dl.

The mean difference was analyzed by the ANOVA test showing a significant difference in the average glucose level in the three study groups ($p < 0.05$). This shows that hyperbaric oxygen therapy can reduce glucose levels to become normal.

These results are similar to Inggried's (2017) study which showed that hyperbaric oxygen therapy significantly reduced blood sugar levels. This decrease in blood sugar levels through increased aerobic metabolism and decreased HbA1c levels (Irawan, Semadi and Widiana, 2018).

Other studies also explain that hyperbaric oxygen increase the amount of oxygen in blood plasma. In the air (20% Oxygen) with normal pressure (1 ATA), the amount of hemoglobin is 20.1% and plasma oxygen is 0.32%. If given 100% oxygen and normal pressure (1 ATA), hemoglobin oxygen remains 20.1% and plasma oxygen becomes 2.14%. When the oxygen pressure is 100% raised to 3 atmospheres, the amount of oxygen in the blood plasma is 3 times (6.42%). Increased pressure and volume of oxygen cause oxygenation in hypoxic tissue, the formation of new blood vessels, cell proliferation and the ability of white blood cells (Susan, 2013).

Other studies on *Rattus Norvegicus* with diabetes mellitus who were given hyperbaric oxygen at 2.4 ATA and 100% oxygen for 10 sessions were able to increase Akt expression and reduce glucose levels, so the role of hyperbaric oxygen therapy in lowering glucose levels through the insulin-Akt pathway cannot be ignored (Rusdiana, 2014).

Analysis of pancreatic histopathological improvement after administration of hyperbaric oxygen

This research shows that alloxan can increase the degree of pancreatic histopathological damage. Alloxan can cause diabetic conditions (hyperglycemia) in experimental animals in a short time, cause insulin-dependent diabetes mellitus (type 1). Alloxan is selectively toxic to pancreatic beta cells that produce insulin through glucose transporters, GLUT 2 (Yudita, 2009).

In a preliminary study to determine the dose of alloxan, a dose of 150 mg / kg BW increase blood glucose in experimental animals on the third day (397.33 mg / dl).

Alloxan doses vary from 90 - 200 mg / kg body weight (BW) and a dose of 150 mg / kg body weight is the most commonly dose. Alloxan provides two different pathological effects by inhibiting secretion insulin and the formation of reactive oxygen species (ROS) which results in beta cell necrosis in the pancreas. These two effects produce the pathophysiology of type 1 diabetes (Macdonald and Mohammed, 2018).

In some previous studies, it was found that OHB 2.4 ATA for 60 minutes / day on days 1, 3, 5, 7 and 10, was given at the same time in the morning. OHB reduce the amount of inflammatory cell infiltration. The diabetic rats that treated with OHB for 5 days showed a statistically significant increase in pancreatic regeneration as indicated by reduced the number of inflammatory cell infiltrations and there was no difference when compared to non-diabetic rats (Prabowo et al., 2014).

The results of this study, the positive control group and the treatment group were showed significant differences. That is because hyperbaric oxygen therapy produces antioxidants that can reduce the oxidants produced by alloxan.

Analysis of histopathological changes in liver tissue damage after hyperbaric oxygen

Giving alloxan to trigger of hyperglycemic condition gives histopathological changes in other tissues such as the liver (Adomorrow, Oyewole and Turay, 2011). In this study the histopathological changes observed were lobular inflammation, intravascular inflammation, degeneration process and necrosis.

Histopathological changes that can be observed in the liver of Alloxan-induced diabetic rats are caused by the toxic effects of the drug. Alloxan has a toxic effect on pancreatic beta cells, which causes type 1 Diabetes Mellitus, but this effect extends to the kidneys and liver of some species of experimental animals. The systemic toxic effects of Alloxan can generally be observed in the first 2 weeks after diabetes induction. However, Alloxan has a narrow safe line between diabetogenic doses and lethal doses. Death generally occurs due to diabetic ketoacidosis and / or kidney failure or liver failure. The facts require the importance of making experimental observations of liver tissue after the acute phase of administration of Alloxan (Adikut, Oyewole and Turay, 2011).

Changes in alloxan-induced hepatic rats are characterized by fatty vacuoles, sinusoid dilatation and progressive loss of organ structure. Inflammation is characterized by mononuclear infiltrates in the interlobular and periportal spaces in 40% of the liver tissue analyzed from animals made to diabetes with alloxan. Mild liver fibrosis around the portal vessels was also observed in 20% of the liver (Lucchesi, Cassettari and Spadella, 2015).

This research shows that alloxan cause significant liver damage, and hyperbaric oxygen can inhibit that damage. There was a significant differences between the positive control group and the treatment group.

Histopathological change of the liver in the positive control group showed fatty degeneration and necrosis. Degeneration results from hyperglycemia and inhibition of insulin production which is seen microscopically in the form of fat droplets in the liver lobules around the central vein and most often seen in the perilobular part. However, liver damage in the treatment group in the form of fatty degeneration and necrosis began to decrease. The liver cell structure of the treatment group appeared to have improved to approach the normal liver cell structure.

In this study hyperbaric oxygen was able to prevent liver cell damage due to alloxan induction. Significant damage can be prevented by hyperbaric oxygen (with $\alpha < 0.05$) includes lobular inflammation, degeneration and necrosis. While intravascular inflammation did not provide a significant difference between the treatment group and the positive control group.

In vitro studies also report hyperbaric oxygen being able to stimulate hepatocyte proliferation through normalizing Mrp-2 localization in the apical membrane and subsequently activating transporter function. Increased liver regeneration due to HBO is closely related to increased mitochondrial function. HBO also decreases MDA and increases antioxidant activity, including glutathione (GSH) and superoxide dismutase (SOD) activities that play a role in hepatocyte cell regeneration (Sun et al., 2018).

This study shows a significant difference between the positive control group and the treatment group. This is due to hyperbaric oxygen therapy producing antioxidants that can reduce oxidants caused by alloxan.

Conclusion

It can be concluded that administration of Hyperbaric Oxygen 2.4 ATA with 100% O₂ for 6 days in Rattus norvegicus-induced Alloxan, can reduce blood glucose levels, reduce the degree of histopathological damage to pancreatic and liver tissue. .

References

1. Adesokan, A., Oyewole, O. and Turay, B. (2011) 'Kidney and Liver Function Parameters in Alloxan-Induced Diabetic Rats Treated with Aloe Barbadosis Juice Extract', *Sierra Leone Journal of Biomedical Research*, 1(1), pp. 33–37. doi: 10.4314/sljbr.v1i1.52431.
2. American Diabetes Association (2018) 'STANDARDS OF MEDICAL CARE IN DIABETES',

- 41(January).
3. Angelica, I. and Wiliyams, V. (2013) 'Efektivitas terapi oksigen hiperbarik terhadap kadar gula darah pasien diabetes mellitus', 3(1), pp. 19–24.
 4. Azhar, N. (2015) 'Uji Efektivitas Terapi Oksigen Hiperbarik pada Pasien Diabetes Melitus di Rumah Sakit TNI ANgkatan Laut dr. Mintoharjo Jakarta Pusat'.
 5. Barrett, K. E. and Ganong, W. F. (2010) *Ganong's review of medical physiology, Ophthalmic surgery*.
 6. Birben, E. *et al.* (2012) 'Oxidative Stress and Antioxidant Defense', (January), pp. 9–19.
 7. Bocci, V. and Valacchi, G. (2015) 'Nrf2 activation as target to implement therapeutic treatments', *Frontiers in Chemistry*, 3(February), pp. 1–6. doi: 10.3389/fchem.2015.00004.
 8. Chougale, A. D. (2008) 'Optimization of Alloxan Dose is Essential to Induce Stable Diabetes for Prolonged Period'.
 9. Ganong, W. . (2008) *Fisiologi Kedokteran*. Jakarta: EGC.
 10. Girman, P. and Kriz, J. (2015) *Rat Experimental Transplantation Surgery*. doi: 10.1007/978-3-319-14559-4.
 11. Godman, C. A. *et al.* (2010) 'Hyperbaric oxygen treatment induces antioxidant gene expression', *Annals of the New York Academy of Sciences*, 1197, pp. 178–183. doi: 10.1111/j.1749-6632.2009.05393.x.
 12. Hall, J. E. and Guyton, A. C. (2011) *Guyton and Hall Textbook of Medical Physiology, Journal of Chemical Information and Modeling*. doi: 10.1017/CBO9781107415324.004.
 13. Halliwell, B. and Gutteridge, J. M. C. (2015) 'Free Radicals in Biology and Medicine', p. 906.
 14. iMedPub Journals (2015) 'The Inspiring Journey of Hyperbaric Oxygen Therapy , from the Controversy to the Acceptance by the Scientific Community Abstract The Historical Evolution of HBOT', pp. 1–8.
 15. Irawan, H., Semadi, I. N. and Widiana, I. G. R. (2018) 'A Pilot Study of Short-Duration Hyperbaric Oxygen Therapy to Improve HbA1c, Leukocyte, and Serum Creatinine in Patients with Diabetic Foot Ulcer Wagner 3-4', *Scientific World Journal*. Hindawi, 2018. doi: 10.1155/2018/6425857.
 16. Katzung, Bertram G; Masters, Susan B.; Trevor, A. J. T. (2013) *Farmakologi Dasar & Klinik*. 12th edn. Jakarta: ECG.
 17. Kibble, Jonathan D; Halsey, C. R. (2009) *Medical Physiology*.
 18. Komang, N. I. *et al.* (2015) 'Pengaruh Pemberian Ekstrak Bawang Merah (Allium Ascalonicum L) Terhadap Gambaran Histopatologi Hepar Tikus Putih (Rattus Norvegicus) yang Diinduksi Aloksan'.
 19. Kumar, V., A.K; Abbas, N. F. and J. and Aster (2018) *Robbins and Cotran's Pathological Basis of Disease*. USA: Elsevier.
 20. Lucchesi, A. N., Cassetari, L. L. and Spadella, C. T. (2015) 'Alloxan-induced diabetes causes morphological and ultrastructural changes in rat liver that resemble the natural history of chronic fatty liver disease in humans', *Journal of Diabetes Research*, 2015. doi: 10.1155/2015/494578.
 21. Macdonald, O. and Mohammed, A. (2018) 'ScienceDirect Alloxan-induced diabetes , a common model for evaluating the glycemic-control potential of therapeutic compounds and plants extracts in experimental studies', *Medicina*. The Lithuanian University of Health Sciences, pp. 1–10. doi: 10.1016/j.medic.2018.02.001.
 22. Mehta, Vandana; Balachandran, C. (2014) 'Hyperbaric Oxygen Therapy'.
 23. Mescher, A. L. (2010) *Junqueira's Basic Histology*. 12th edn. Singapore.
 24. Murray, R. K. (2014) *Biokimia Harper Edisi 27, Igarss 2014*. doi: 10.1007/s13398-014-0173-7.2.
 25. Naik, P. (2012) *Essentials of Biochemistry*. First edit. Jaypee Brothers Medical Publisher.
 26. Nordback, I. (1986) 'Clinical pathology of acute n-ecrotising pancreatitis', pp. 68–74.
 27. PERKENI (2015) *Pengelolaan dan Pencegahan Diabetes Melitus tipe 2 di Indonesia 2015*.
 28. Prabowo, S. *et al.* (2014) 'Hyperbaric Oxygen Treatment in a Diabetic Rat Model Is Associated with a Decrease in Blood Glucose , Regression of Organ Damage and Improvement in Wound Healing', (August), pp. 1950–1958.
 29. Prameswari, O. M. and Widjanarko, S. B. (2014) 'Uji Efek Ekstrak Air Daun Pandan Wangi Terhadap Penurunan Kadar Glukosa Darah dan Histopatologi tikus diabetes mellitus', 2(2), pp. 16–27.
 30. Putri, O. (2012) 'Pengaruh Pemberian Ekstrak Buah Labu Siam (Sechium Edule) Terhadap Penurunan Kadar Glukosa Darah Tikus Wistar yang Diinduksi Aloksan'.
 31. Putri, O. B. *et al.* (2012) 'Pengaruh Pemberian Ekstrak Buah Labu Siam (Sechium Edule) Terhadap Penurunan Kadar Glukosa Darah Tikus Wistar yang Diinduksi Aloksan'.
 32. Rigalli, A. and Loreto, V. E. di (2016) *experimental surgical models in the laboratory rat*. CRC Press.

33. Rijadi MR, Sadewantoro, Guritno, A. M. (2009) 'Hiperbarik', in *Ilmu Kesehatan Penyelaman dan Hiperbarik*. Surabaya: LAKESLA, pp. 88–101.
34. Rohilla, A. and Ali, S. (2012) 'Alloxan Induced Diabetes : Mechanisms and Effects', 3(2), pp. 819–823.
35. Sayuti, Kesuma; Yenrina, R. (2015) *Antioksidan Alami dan Sintetik*.
36. Silveira, M. R. G. da *et al.* (2014) 'Effects of hyperbaric oxygen therapy on the liver after injury caused by the hepatic ischemia-reperfusion process', *Acta Cirurgica Brasileira*, 29(suppl 1), pp. 29–33. doi: 10.1590/s0102-86502014001300006.
37. Sun, Y. *et al.* (2018) 'Hyperbaric oxygen therapy in liver diseases', *International Journal of Medical Sciences*, 15(8), pp. 782–787. doi: 10.7150/ijms.24755.
38. Tejada, S. *et al.* (2016) 'Antioxidant Response of Chronic Wounds to Hyperbaric Oxygen Therapy', pp. 1–14. doi: 10.1371/journal.pone.0163371.
39. Ther, P. D. *et al.* (2013) 'Pancreatic Disorders & Therapy Hyperbaric Oxygen Therapy for Pancreatic and Gastrointestinal Disease'. doi: 10.4172/2165-7092.S4-00.
40. Uthbertson, C. H. M. C. and Hristophi, C. H. C. (2006) 'Review Article Potential Effects Of Hyperbaric Oxygen Therapy In Acute Pancreatitis', pp. 625–630. doi: 10.1111/j.1445-2197.2006.03793.x.
41. Uysal, B. *et al.* (2010) 'Efficacy of Hyperbaric Oxygen Therapy and Medical Ozone Therapy in Experimental Acute', 39(1), pp. 9–15.
42. Yudita, A. (2009) 'Efek Aloksan Terhadap Kadar Glukosa Darah Tikus Wistar'.
43. Zaetun, S., Kerti, L. B. and Srigede, L. (2015) 'pengaruh terapi oksigen hiperbarik terhadap kadar gula darah pasien diabetes mellitus di instalasi kesehatan penyelaman dan hiperbarik kantor kesehatan pelabuhan kelas II mataram', 9(1978).
