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Effect of Mango Leaf Shoot Extract (*Mangifera indica* L.) on Zebra Fish (*Danio rerio*) Cell Regeneration Induced by Hyperglycemia

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Abstrak. Regenerasi sel dalam proses penyembuhan luka dapat terganggu oleh kondisi hiperglikemia. Tanaman mangga memiliki manfaat sebagai tanaman obat karena memiliki metabolit sekunder yang dapat membantu menyembuhkan luka. Penelitian ini bertujuan untuk mengetahui pengaruh pemberian ekstrak pucuk daun mangga terhadap kadar glukosa darah ikan zebra (*Danio rerio*) hiperglikemia akibat induksi aloksan-glukosa dan peran ekstrak pucuk daun mangga terhadap regenerasi sirip ekor ikan zebra. Pada penelitian ini digunakan lima kelompok perlakuan: kontrol, hiperglikemia, metformin 0,75 b/v, ektrak 10 µg/ml dan ekstrak 20 µg/ml. Parameter yang diamati meliputi kadar glukosa darah, panjang sirip dan tingkat kelangsungan hidup. Hiperglikemia diukur dengan kadar glukosa darah, regenerasi sirip ekor dianalisis menggunakan panjang sirip, serta faktor lingkungan dan kelangsungan hidup diukur. Hasil penelitian menunjukkan bahwa ekstrak pucuk daun mangga 20 µg/ml efektif menurunkan kadar glukosa darah mencapai 68,31% dan mendekati perlakuan metformin 0,75 b/v dengan penurunan kadar glukosa darah mencapai 70,1%. Ekstrak pucuk daun mangga konsentrasi 20 µg/ml berbeda nyata ($p<0,05$) dari kelompok hiperglikemia dan ekstrak 10 µg/ml. Ekstrak 20 µg/ml efektif membantu proses regenerasi dengan pertambahan panjang sirip ekor mencapai 0,94 cm selama masa perlakuan.

Kata Kunci: *Hiperglikemia; Ikan Zebra; Mangga; Regenerasi*

Abstract. Cell regeneration in wound healing process could be interfere by hyperglycemia. Mango plants has benefits as medicinal plants because they have secondary metabolites that can help heal wounds. This study aims to determine the effect of mango leaf shoot extract on blood glucose levels of zebrafish (*Danio rerio*) hyperglycemia due to alloxan-glucose induction and role of mango leaf shoot extract on zebrafish caudal fin regeneration. Five group treatments are used in this study: control group, hyperglycemia, metformin 0,75 w/v, extract 10 µg/ml and extract 20 µg/ml. Parameters observed include blood glucose level, fin length and survival rate. Hyperglycemia was measured by blood glucose level, caudal fin regeneration was analayzed using fin length, also environmental factors and survival rate were measured. Result showed that mango leaf shoot extract 20 µg/ml was efective in reducing blood glucose levels, reaching 68,31% and aproaching metformin 0,75 w/v treatment with a decrease in blood glucose levels reaching 70,1%. Mango leaf shoot extract with concentration 20 µg/ml significantly different ($p < 0,05$) from hyperglycemia group and extract 10 µg/ml. Extract 20 µg/ml was effective in assisting the regeneration process with an increase caudal fin length reaching 0.94 cm during the treatment period.

Keywords: *Hyperglycemia; Mango; Regeneration; Zebrafish*

INTRODUCTION

Hyperglycemia is one of the main signs of diabetes (Mustofa, Yuniastuti, Ari, dan Marianti, 2012; American Diabetes Association, 2014). This condition is caused by abnormality of

insulin production and performance, abnormality of lipid and protein metabolism that involved in formation of free radicals (Alza, 2013; Permatasari, et al., 2018). If that condition is not treated properly, that conditions can cause several disorders such as damage to the eyes, kidneys, nerves and difficulty in wound healing (García, 2017; Hakim et al., 2010).

One of the ways to prevent diabetic wounds is generally used antiseptic. However, the continuous use of antiseptic can cause several effects. For generations, Indonesian people have used plants as alternative medicine. Advantages of using plants such as ingredients used are easy to obtain, have useful compounds for health problems and affordable price (Mustofa et al., 2012; Permata & Khoirunnisa, 2020).

Mango plant has benefits as a medicinal plant and has been widely used as traditional medicine (Luqyana & Husni, 2019). Mango plants have potential to help wound healing process of diabetics (Risa et al., 2018). In Indonesia, mango leaf shoot are consumed as salad. While in India, young leaves are used as a diabetic medicine (Prommajak et al., 2014).

Several studies have shown that mango leaf shoots has compounds that have benefit for pharmacology. Mangiferin is found in mango plants and has benefits as anticancer, antiaging, hepatoprotective, analgesic effect, immunomodulatory, antiobesity, antibacterial, antiviral, antidepressant, antioxidant, anti-infective, anti-inflammatory and antidiabetic effects (Dar et al., 2005; Wang et al., 2011; Matkowski et al., 2013; Pokorski, 2014; Luo et al., 2015; Tayana et al., 2019). Mangiferin compounds can be found in all parts of mango plant, such as in seeds, roots, peel, and leaves (Dinesh et al., 2011).

Based on research by Permatasari et al. (2018), mango leaf extract of Cengkir cultivar can reduce blood glucose levels in fructose-induced mice, with an optimal dose 105 mg/kg/BW. Risa et al. (2018) also proved that there was a significant effect on given mango leaf extract of Manalagi cultivar on wound healing in mice with optimal concentration 20%. This is also supported by Ayuningtyas (2020) that states a mixture of honey and dried mango leaf powder can assist wound healing.

In this study, we used mango leaf shoot of Arumanis cultivar which were expected to has compounds that could help in wound healing process. According to Medina Ramírez et al. (2016), the highest mangiferin in mango plants is found in young mango leaves or mango leaf shoots. It is supported by Cahyanto et al. (2020) which stated that the highest mangiferin was found in mango leaf shoot extract of Arumanis cultivar with percentage 20.83%. The high of mangiferin can describe antioxidants in mango leaf shoot extract which has a role in increasing cell regeneration in wound healing process.

Zebrafish is animal test we used. It has 70% genetic similarity with humans. Zebrafish that induced by diabetogenic have difficulty in regenerating their caudal fins. It’s same with diabetic

humans that have difficulty in wounds healing (Utami, 2018). Therefore, this study was conducted to determine effect of mango leaf shoot extract on reducing blood glucose levels and regeneration caudal fin of hyperglycemic zebrafish. It also as information for further utilization of mango leaf shoot and as alternative treatment in assisting diabetic wound healing process.

MATERIALS AND METHODS

Tools and materials

This study used aerators, aquariums, surgical instruments, stirring rods, blender, bunsen, funnel, erlenmeyer (250 ml and 500 ml), fishnets, beaker glass (100 ml, 500 ml and 1000 ml), measuring cup (100 ml), glucometer (EasyTouch®GCU), hot plate, wire gauze, fluorescent lamp, UV lamp, mortar and pestle, loop, magnetic stirrer, stereo microscope (Nikon SMZ 18), millimeter block, digital ohaus, pH meter, dropper, drip plate, test tube rack, rotary evaporator, filter, spatula, test tube, thermometer and timer.

The materials we used are UV water, distilled water, 70% alcohol, alloxan monohydrate, aluminum foil, ice cubes, 1% $FeCl_3$, glucose, 37% HCl, 2N HCl, zebrafish (*Danio rerio*), filter paper, 70% methanol, metformin, fish feed (TertaBits), Dragendoff's reagent, Mayer's reagent, Wagner's reagent, mango leaf shoot sample (*Mangifera indica* L.) Arumanis cultivar, Mg powder and glucose test strip (EasyTouch®).

Method

Sampling and Mango Leaf Shoot Extract Making

Mango leaf shoot sample is taken were light green colors, light green brown, pink, purplish and reddish brown. Samples were cleaned, then air-dried for 5 to 7 days. Dry sample was mashed with a blender, then filtered. The method used to make mango leaf shoot extract is maseration method by immersing sample in methanol solvent. The advantage of using this method is that it doesn't use heating which may damage thermolabile compounds. Resulting filtrate is then evaporated to produce a thicker extract.

Identification of Secondary Metabolic Compounds

Identification of secondary metabolites using a simple descriptive method. Identified secondary metabolites are alkaloids, flavonoids, saponins and tannins which are thought to play a role in wound healing process (Hakim et al., 2010). Alkaloid test was performed by dissolving extract and HCl and then filtered. Filtrate obtained was divided into 3 different test tubes, then added by Mayer, Wagner and Dragendoff reagents. Flavonoid test was performed by mixing extract and Mg powder, then dripping with HCl. Saponin test was performed by homogenizing extract and hot water, then adding HCl. While tannin test was performed by adding extract into distilled water and then filtered. Filtrate obtained added by $FeCl_3$ (Alasa et al, 2017).

Hyperglycemia Preliminary Test

Preliminary test was performed by comparing two treatment groups. They are control group by immersing fish in UV water and hyperglycemic group with modification based on Hayati et al. (2017) by immersion fish in solution of 400 mg of alloxan in 1 liter of distilled water for 30 minutes then immersion it in solution of 2 grams of glucose in 1 liter of distilled water for 24 hours. Alloxan and glucose inducted for three days. On the fourth day, blood glucose level of fish from each treatment was measured.

Treatment Hyperglycemia and Amputation

Fish group with weights ranging from 0.1-0.4 grams made hyperglycemia by induction of alloxan and glucose for 3 days. On the fourth day, the fish's blood glucose level was measured. After reaching a hyperglycemic condition, the fish were anesthetized by immersion in water with a temperature lowered from 17-12°C. Then, caudal fin was amputated vertically with a length 1/2 of fin or 0.3 cm from the outer tip of caudal fin. Then, allowed fish to regenerate in normal temperatures (Poss et al., 2000).

Treatment of Metformin and Mango Leaf Shoot Extract

After amputation, the fish were divided into 4 different treatment groups, they are hyperglycemia group, metformin 0.75 w/v, mango leaf shoot extract 10 µg/ml and 20 µg/ml. This treatment was performed for 5 days.

Observation of Caudal Fin Regeneration

Observation of caudal fin regeneration was performed on sixth day after immersion using metformin 0.75 w/v, extract 10 µg/ml and extract 20 µg/ml. Observations were made from base of fin to the tip of outer caudal fin.

Data analysis

The results of data collection on blood glucose levels and length of caudal fin were processed using IBM SPSS Statistics version 26 (Prayoto dan Nugroho, 2021). Data from observations of decreasing blood glucose levels and increasing length of caudal fin were processed usinf ANOVA to determine whether there was an average difference between treatment groups. If data doesn’t meet assumptions of parametric test, then data is processed with a non-parametric alternative test Kruskal Wallis.

RESULT AND DISCUSSION

Mango leaf shoots (*Mangifera indica*L.) Arumanis cultivar obtained from Kuningan, West Java with temperature of around 28-33°C and average pH of about 6.7. These environmental conditions are included in general conditions of growing mango plants.

In this study, extraction is performed by maceration method. This method was chosen because it's often used and is fairly simple method (Susanty & Bachmid, 2016). The principle of maceration method is secondary metabolites in cytoplasm can dissolve in solvent because cell walls and cell membranes are broken. It happened because pressure differences from inside and outside cell (Firdaus et al., 2015).

Filtrate as an extraction result were evaporated using a rotary vacuum evaporator until obtaining a thicker extract. Futhermore, identification of secondary metabolites was performed to determine compounds in extract which could be use as parameters related to pharmacological effect. The result of identification secondary metabolites in mango leaf shoot extract can be seen in table 1.

Table 1. Results of Identification of Secondary Metabolic Compounds Extract of Mango Leaf Shoot (*Mangifera indica* L.) Arumanis Cultivars

Sample	Compound Component Test			
	Alkaloids	Flavonoids	Saponins	Tannins
Mango leaf shoot extract of Arumanis cultivar	+	+	+	+

Based on identification results, it was found that alkaloid compound in mango leaf shoot extract sample was evidenced by formation of white or yellowish precipitate after addition of Mayer reagent, dark red color was formed after addition of Wagner's reagent and orange precipitate was formed after addition of Dragendoff's reagent. Positive results were shown in flavonoid test as evidenced by formation of orange color when adding HCl to a mixture of extracts and Mg powder. Other compounds that showed positive results were saponin compounds as evidenced by formation of stable foam for more than 5 minutes. Identification of tannin compounds also showed positive results as evidenced by formation of dark blue or dark black color in solution after addition of 1% $FeCl_3$ reagent.

Hyperglycemia Preliminary Test

Preliminary test to determine appropriate method for animal test to achieve hyperglycemic conditions. This test consisted of two groups, they are control group and hyperglycemic group with alloxan and glucose induction. Induction of hyperglycemia was performed for 3 days. Glucose level of fish was measured from each treatment in fourth day. The results of measuring fish blood glucose levels after 3 days of alloxan and glucose induction can be seen in Figure 1.

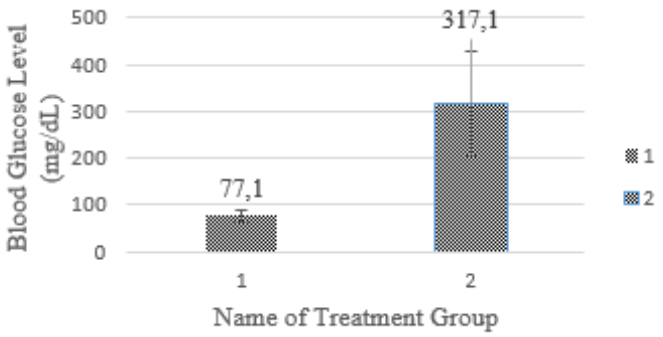

Figure 1. Blood Glucose Level in Preliminary Test

Description : 1: Control; 2: Hyperglycemia

Based on figure 1, it shows that there are differences blood glucose levels between control treatment and hyperglycemia treatment. The average blood glucose level in control treatment was 77.1 mg/dL, meanwhile the average blood glucose level in hyperglycemia treatment was 317.1 mg/dL. These results indicate a significant difference in blood glucose levels between both. According to Heckler and Kroll (2017), blood glucose level of zebrafish in hyperglycemic conditions can reach 310 mg/dL. It's mean induction of alloxan and glucose for 3 days can significantly increase blood glucose levels.

Alloxan is an unstable hydrophilic compound that acts as a diabetogenic. Its unstable can be causes alloxan to easily redox reactions. Alloxan reduction will produce dialuric acid while dialuric acid reoxidation will become alloxan (Hayati et al., 2017). In this process, alloxan radicals are released which will produce superoxide radicals which will dismutation into hydrogen peroxide (H_2O_2) which has potential to become hydroxyl radicals (Szkudelski, 2001;Ighodaro et al., 2017).

The Effect of Inducing Mango Leaf Shoot (*Mangifera indica* L.) Extract Arumanis cultivar on Blood Glucose Levels

Alloxan and glucose induction were performed for three days, then blood glucose level of fish was measured. The results of measuring fish blood glucose levels after three days induction can be seen in Figure 2.

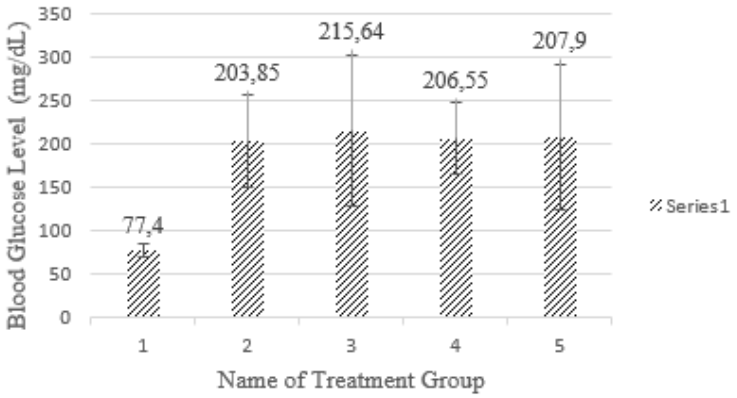

Figure 2. Blood Glucose Level After Induction by Alloxan and Glucose

Description : 1: Control; 2: Hyperglycemia; 3; metformin 0.75 w/v;
4: Extract 10 µg/ml; 5: Extract 20 µg/ml

Figure 2 showed that there was a difference in average blood glucose level between treatment groups. The average blood glucose level in control group was 77.4 mg/dL, hyperglycemia group was 203.85 mg/dL, metformin 0.75 w/v group was 215.64 mg/dL, extract group 10 µg/ml was 206.55 mg/dL and extract group 20 µg/ml was 207.9 mg/dL. According to Hayati et al. (2017), stated that blood glucose level in zebrafish under normal conditions was 50-75 mg/dL. It means that induced fish by alloxan and glucose were declared to have hyperglycemia with blood glucose levels more than 200 mg/dL.

Hyperglycemic group then was anesthetized and their caudal fin was amputated. After that, the fish were treated by metformin 0.75 w/v, extract 10 µg/ml and extract 20 µg/ml for 5 days. On day 6, blood glucose level was measured. The results of measuring fish blood glucose levels after 5 days of exposure to mango leaf extract can be seen in Figure 3.

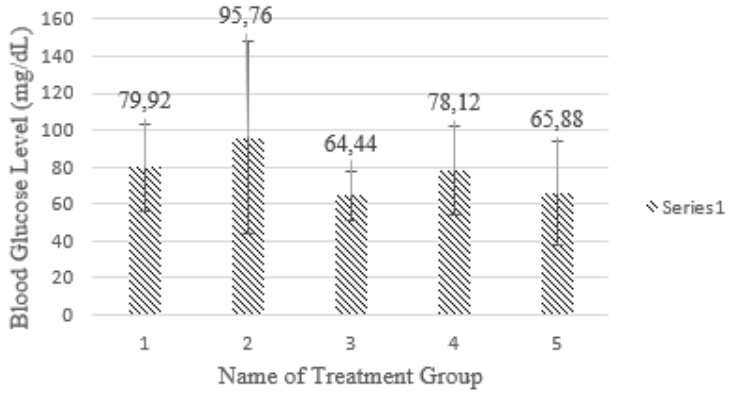

Figure 3. Blood Glucose Level After Induction of Mango Leaf Extract

Description : 1: Control; 2: Hyperglycemia; 3: Metformin 0.75 w/v;
4: Extract 10 µg/ml; 5: Extract 20 µg/ml

Based on figure 3, blood glucose level in metformin 0.75 w/v group was 64.44 mg/dL, extract 20 µg/ml group was 65.88 mg/dL, extract 10 µg/ml group was 78.12 mg/dL and control group was 95.76 mg/dL. The highest decrease blood glucose level was metformin 0.75 w/v with percentage of 70.1% and extract 20 µg/ml with percentage of 68.31%.

Metformin is an oral antihyperglycemic or antidiabetic medicine that has main function of lowering blood glucose levels (Gumantara & Oktarlina, 2017). Metformin works by repairing and increasing insulin receptor sensitivity and inhibiting gluconeogenesis in liver. Giving extract can reduce blood glucose levels in animal test because compounds contained in mango leaf shoot extract have potential as antidiabetic.

The compounds in extract include alkaloids, flavonoids, saponins and tannins which are thought to have hypoglycemic activity or can reduce high blood glucose levels. These compounds play a role in repair of pancreatic cells, stimulate insulin release, restore insulin receptor sensitivity, increase glucose absorption into cells so that glucose in blood decreases, inhibit disaccharide activation, activate glycogen synthesis, inhibit gluconeogenesis, inhibit glucosidase activity and prey on free radicals (Miten & Setiasih, 2014; Syaputri, 2014; Elza et al, 2016; Amiraragab et al, 2017; Haryoto & Devi, 2018; Herlina et al, 2020; Indrayani & Mustarichie, 2020;).

The Effect of Extract of Mango Leaf Shoot (*Mangifera indica* L.) Arumanis cultivar on Caudal Fin Regeneration of Zebra Fish Hyperglycemia

The regeneration process in zebrafish is characterized by an increase in length of caudal fin. Metformin 0.75 w/v, mango leaf shoot extract 10 µg/ml and 20 µg/ml were induced for the first five days after amputation. The average increase in the length of zebrafish caudal fin in all treatments can be seen in Figure 4.

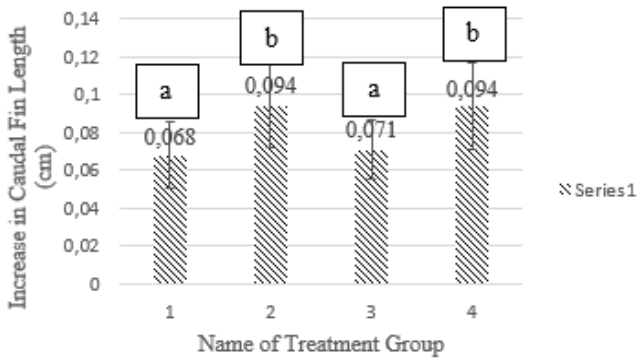

Figure 4. Increase in the length of zebrafish caudal fin hyperglycemia after amputation and induction of mango leaf shoot extract

Description: 1: Hyperglycemia; 2: Metformin 0.75 w/v; 3: Extract 10 µg/ml; 4: Extract 20 µg/ml

Based on figure 4, it was found that there was no significant difference between hyperglycemia group and extract 10 µg/ml group. Meanwhile, the metformin 0.75 w/v group did not different significantly from the extract 20 µg/ml group. However, significant results were shown by hyperglycemia control and extract 10 µg/ml group with metformin 0.75 w/v and extract 20 µg/ml group.

The difference is thought because of effect from treatment with immersion animal tests in metformin and extract solution. According to Spampinato et al. (2020), antidiabetic medicine such as metformin has a role in anti-inflammatory and cell proliferation that play a role in wound healing, increase the proliferation of keratinocytes and fibroblasts, angiogenesis and increase the formation of granulation tissue. While the compounds in extract such as alkaloids, saponins, tannins and flavonoids act as antioxidants (Hakim et al., 2010; Laut et al., 2019).

Antioxidants efficiently stopping free radical reactions, are able to reduce superoxide and prevent cell damage, chelate iron ions and slow down oxidation, prevent ROS regeneration and can increase the activity of cellular antioxidant enzymes, as superoxide scavangers, peroxyl radicals and peroxynitrite by transferring H^+ atoms, prevents the formation of ROS by chelating Fe^{2+} and Cu^{2+} metals thereby preventing redox reactions that generate free radicals (Syarif et al., 2008; Juniarti, 2011; Hardiningtyas et al, 2014; Fithriani et al., 2015).

The keys of regeneration process is formation of blastema or accumulation of undifferentiated proliferative cells that contain most of cells needed to regenerate structures lost after initial wound closure. Blastema proliferation in zebrafish is regulated by *Wnt signaling/β-catenin pathway* and Notch pathway which have a role in the regulation of wound area, blastema proliferation, osteoblast maturation, maintaining blastema cells in a plastic and undifferentiated state. Hyperglycemic conditions have a direct impact on regenerative capacity of zebrafish because it can reduce proliferative ability of regenerating limbs (Olsen et al., 2011).

Cells that help in wound healing will produce ROS (*Reactive Oxygen Species*). In addition, the balance between production and disposal of ROS must be homeostasis. If there is an imbalance, it will cause oxidative stress that can interfere with the wound healing process and can reduce the activity of antioxidant enzymes which causes a decrease in non-enzymatic antioxidants. ROS can be converted rapidly to H_2O_2 by SOD or superoxide dismutase. H_2O_2 is then detoxified by catalase enzymes or antioxidant enzymes into H_2O and O_2. However, if more ROS accumulates then H_2O_2 will be reduced by Fe^{2+} or Cu^{2+} to hydroxyl radicals such as OH^- and OH* which are highly reactive (Djuanda et al., 2012; Paredes et al., 2019).

pH and Water Temperature

The environment parameters measured in this study were pH and water temperature in the treatment aquarium. This measurement was performed to determine changes in temperature and

pH caused by room temperature and the results of fish metabolism. The data on measurement of external factors in the rearing aquarium can be seen in table 2.

Table 2. Environment Factors of pH and Water Temperature in Aquarium Maintenance

Parameter	Treatment			
	Hyperglycemia	Metformin 0.75 w/v	Extract 10 µg/ml	Extract 20 µg/ml
Temperature (°C)	25-26	25-26	25-26	25-26
pH	7	7.04	7.02	7.02

Based on table 2, water temperature of each treatment aquarium ranged from 25-26°C. according to Muchdar et al. (2020), stated that zebrafish live in a temperature range of 24-30°C with the optimal temperature for activity which is around 27°C. Water temperature is maintained at general environmental conditions for zebrafish. according to Darniwa et al. (2020), stated that the higher of water temperature, the stress on zebrafish can have an impact on fish health. While pH of water from each treatment aquarium showed a value of 7. According to Arip Rahman et al. (2012), stated that zebrafish are distributed in fresh waters of the tropics which have pH values ranging from 7-8

Survival Rate

Survival rate is the ratio of the fish number that live at the beginning to the end of study. Survival refers to the survival rate of a population in a certain period of time. Survival data during the observation can be seen in table 3.

Table 3. Survival Rate of Animal Test During Observation

Parameter	Treatment			
	Hyperglycemia	Metformin 0.75 w/v	Extract 10 µg/ml	Extract 20 µg/ml
Survival	100%	80%	80%	90%

Based on table 3, the survival rate of animal test during the study period, ranging from 80% to 100%. The fish that did not survive were thought to be due to the lack of oxygen intake at the time of observation. Observation of the increase in caudal fin of zebrafish was performed on land with the head of fish given a little water so that the fish could breathe. According to Ismi (2017), stated that one of the main causes of sudden fish death is due to lack of oxygen.

CONCLUSION

Mango leaf shoot extract (*Mangifera indica* L.) Arumanis cultivar has an effect on reducing the blood glucose level of hyperglycemic zebrafish (*Danio rerio*) with a decrease in blood glucose level of 68.31% when given extract 20 µg/ml, and has an effect on regeneration of hyperglycemic zebrafish (*Danio rerio*) caudal fin with average length was 0.094 cm on the 20 µg/ml extract treatment.

REFERENCES

Alasa, A. N., Anam, S., & Jamaluddin, J. (2017). Analisis Kadar Total Metabolit Sekunder Ekstrak Etanol Daun Tamoenju (Hibiscus surattensis L.). *Kovalen*, *3*(3), 258–268.

Alza, Y. (2013). Hubungan Hiperglikemia Dengan Kadar Glyceraldehyde-3-Phosphate Dehydrogenase (Gapdh) Pada Penderita Diabetes Mellitus Tipe 2. *Photon: Jurnal Sain Dan Kesehatan*, *4*(1), 19–26.

American Diabetes Association. (2014). Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care*, *37*(1), 81–90.

Amiraragab, B., Hussein, S. A., Alm-Eldeen, A.-E., Hafe z, A., & Mohamed, T. (2017). Diabetes Management Saponins and Their Potential Role in Diabetes Mellitus. *Diabetes Manag*, *7*(1), 148–158.

Arip Rahman, Agus Arifin Sentosa, D. W. (2012). Sebaran Ukuran dan Kondisi Ikan Zebra Amatitlania nigrofasciata (Günther , 1867) di Danau Beratan , Bali in Lake Beratan , Bali. *Jurnal Iktiologi Indonesia*, *12*(2), 135–145.

Ayuningtyas, A. (2020). Penggunaan Madu Dan Mangga (Mangifera Indica) Pada Penyembuhan Luka. *Journal of Health Science and Physiotherapy*, *2*(1), 111–115.

Cahyanto, T., Fadillah, A., Ulfa, R. A., Hasby, R. M., & Kinasih, I. (2020). Kadar Mangiferin Pada Lima Kultivar Pucuk Daun Mangga (Mangifera indica L.). *Al-Kauniyah: Jurnal Biologi*, *13*(2), 242–249.

Dar, A., Faizi, S., Naqvi, S., Roome, T., Zikr-Ur-Rehman, S., Ali, M., … Moin, S. T. (2005). Analgesic and antioxidant activity of mangiferin and its derivatives: The structure activity relationship. *Biological and Pharmaceutical Bulletin*, *28*(4), 596–600.

Darniwa, A. V., Cahyanto, T., Kusumorini, A., Meidita, Z., Putri, E., Ulfa, R. A., … Hizqiyah, N. (2020). Uji Perilaku dan Preferensi Area pada Ikan Zebrafish (Danio rerio) yang Diinduksi Stres. *Biosfer*, *5*(2), 32–38.

Dinesh, M. R., Hemanth, K. N., Vasanthaiahm K. V., D. (2011). Wild Crop Relatives: Genomic and Breeding Resources. *Wild Crop Relatives: Genomic and Breeding Resources*, (June).

Djuanda, S. R. S., Novianto, E., Boediardja, S. A., & Jusman, S. W. A. (2012). Peran Stress Oksidatif pada Penuaan Kulit Secara Intrinsik. *Mdvi*, *39*(3), 127–133.

Elza Sundhani, Della Caya Nur Syarifah, Lita Ratriyana Zumrohani, N. A. N. (2016). Efektivitas Ekstrak Etanol Daun Adam Hawa (Rhoeo discolor) dan Daun Pucuk Merah (Syzygium campanulatum Korth.) Dalam Menurunkan Kadar Gula Darah Pada Tikus Putih Jantan Galur Wistar dengan Pembebanan Glukosa. *Pharmacy*, *13*(02), 137–149.

Firdaus, I., Retnowati, R., & Sutrisno. (2015). Fraksinasi Ekstrak metanol Daun Mangga Kasturi (Mangifera casturi Kosterm) Dengan Pelarut n-Butanol. *Kimia Student Journal*, *1*(1), 787–790.

Fithriani, D., Amini, S., Melanie, S., & Susilowati, R. (2015). Uji Fitokimia, Kandungan Total Fenol dan Aktivitas Antioksidan Mikroalga Spirulina sp., Chlorella sp., dan Nannochloropsis sp. *Jurnal Pascapanen Dan Bioteknologi Kelautan Dan Perikanan*, *10*(2), 101–109.

García, A. B. (2017). Brief Update on Diabetes for General Practitioners. *Rev Esp Sanid Penit*, (19), 57–65.

Gumantara, M. P. B., & Oktarlina, R. Z. (2017). Perbandingan Monoterapi dan Kombinasi Terapi Sulfonilurea-Metformin terhadap Pasien Diabetes Melitus Tipe 2. *Majority*, *6*(1), 55–59.

Hakim, I. R., Lestari, F., & Priani, S. E. (2010). Kajian Pustaka Tanaman yang Berpotensi dalam Penyembuhan Luka Bakar. *Prosiding Farmasi*, 14–20.

Hardiningtyas, S. D., Purwaningsih, S.-, & Handharyani, E.-. (2014). Aktivitas Antioksidan Dan Efek Hepatoprotektif Daun Bakau Api-Api Putih. *Jurnal Pengolahan Hasil Perikanan Indonesia*, *17*(1), 80–91.

Haryoto, H., & Devi, E. S. (2018). Efek Pemberian Ekstrak Etanol Daun Dan Batang Ubi Jalar Ungu (Ipomoea batatas L.) Terhadap Penurunan Kadar Glukosa Darah Pada Tikus Jantan Galur Wistar Yang Diinduksi Aloksan. *Talenta Conference Series: Tropical Medicine (TM)*, *1*(3), 139–143.

Hayati, Farida, Putri, Cyntia, A., Awaluddin, R., dkk. (2017). Pengembangan Metode Uji Antihiperglikemia Dengan Zebrafish. *Prosiding Seminar Nasional*, (7), 476–486.

Heckler, K., & Kroll, J. (2017). Zebrafish as a model for the study of microvascular complications of diabetes and their mechanisms. *International Journal of Molecular Sciences*, *18*(9), 1–9.

Herlina, Nina., Mulyati dan Ananda, P. (2020). Efektivitas Hipoglikemik Fraksi Etil Asetat Ampas Tahu Menggunakan Model Hewan Ikan Zebra (Danio rerio). *Jurnal Jamu Indonesia*, *5*(1), 16–21.

Ighodaro, O. M., Adeosun, A. M., & Akinloye, O. A. (2017). Alloxan-induced diabetes, a common model for evaluating the glycemic-control potential of therapeutic compounds and plants extracts in experimental studies. *Medicina (Lithuania)*, *53*(6), 365–374.

Indrayani, S., & Mustarichie, R. (2020). Aktivitas Antidiabetes Beberapa Tanaman Di Indonesia. *Farmaka*, *18*(1), 58–65.

Ismi, S. (2017). Pengaruh Penggantian Oksigen Pada Transportasi Benih Kerapu Dengan Sistem Tertutup. *Jurnal Ilmu Dan Teknologi Kelautan Tropis*, *9*(1), 385–392.

Juniarti, Y. dan. (2011). Analisis Senyawa Metabolit Sekunder Dari Ekstrak Metanol Daun Surian Yang Berpotensi Sebagai Antioksidan. *MAKARA of Science Series*, *15*(1), 48–52.

Laut, M., Ndaong, N., Utami, T., Junersi, M., Bria Seran, Y., Farmakologi dan Toksikologi FKH Undana Jl Adisucipto, B., … Kedokteran Hewan Jl Adisucipto -Penfui, F. (2019). Efektivitas Pemberian Salep Ekstrak Etanol Daun Anting-anting (Acalypha indica Linn.) Terhadap Kesembuhan Luka Insisi pada Mencit (Mus musculus). *Jurnal Kajian Veteriner*, *7*(1), 1–11.

Luo, Y., Fu, C., Wang, Z., Zhang, Z., Wang, H., & Liu, Y. (2015). Mangiferin attenuates contusive spinal cord injury in rats through the regulation of oxidative stress, inflammation and the Bcl-2 and Bax pathway. *Molecular Medicine Reports*, *12*(5), 7132–7138.

Luqyana Z. T. M, L. dan, & Husni, P. (2019). Aktivitas Farmakologi Tanaman Mangga (Mangifera indica L.): Review. *Jurnal Farmaka*, *17*(2), 187–194.

Matkowski, A., Kus, P., Goralska, E., & Wozniak, D. (2013). Mangiferin – a Bioactive Xanthonoid, not only from Mango and not just Antioxidant. *Mini-Reviews in Medicinal Chemistry*, *13*(3), 439–455.

Medina Ramírez, N., Monteiro Farias, L., Apolonio Santana, F., Viana Leite, J., De Souza Dantas, M., Lopes Toledo, R., … Machado Rocha Ribeiro, S. (2016). Extraction of Mangiferin and Chemical Characterization and Sensorial Analysis of Teas from Mangifera indica L. Leaves of the Ubá Variety. *Beverages*, *2*(4), 33.

Miten, S. V., & Setiasih, L. N. E. S. dan S. K. W. (2014). Pemberian Ekstrak Etanol Kulit Batang Kelor Glukosa Darah Tikus Hiperglikemia. *Indonesia Medicus Veterinus*, *3*(4), 292–299.

Muchdar, F., Juharni, J., & Andriani, R. (2020). Utilization of Different Probiotics on Growth and Survival Rate of Blacktail Zebra fish (*Dascyllus melanurus*). *Agrikan: Jurnal Agribisnis Perikanan*, *13*(2), 222–231.

Mustofa, Yuniastuti, Ari, dan Marianti, A. (2012). Efek Pemberian Jus Lidah Buaya Terhadap Kadar Glukosa Darah Tikus Putih. *Unnes Journal of Life Science*, *1*(1), 35–40.

Olsen, Ansgar., Sarras, Michael and Intine, R. (2011). Limb Regeneration is Impaired in an Adult Zebrafish Model of Diabetes Mellitus. *NIPH Public Access*, *23*(1), 1–7.

Paredes, L. C., Camara, N. O. S., & Braga, T. T. (2019). Understanding The Metabolic Profile of Macrophages During The Regenerative Process in Zebrafish. *Frontiers in Physiology*, *10*, 1–19.

Permata, E. I., & Khoirunnisa, Y. (2020). Efek Mangiferin dalam Mengatasi Masalah Kesehatan.

Jurnal Penelitian Perawat Profesional, *2*(1), 31–38.

Permatasari, Sarah, Cahyanto, Tri, Adawiyah, Ayuni dan Ulfa, R. A. (2018). Pucuk Daun Mangga (Mangifera indica L.) Kultivar Cngkir Sebagai Penurun Kadar Glukosa Darah. *Bioma : Jurnal Biologi Dan Pembelajaran Biologi*, *3*(2), 102–112.

Pokorski, M. (2014). Mangiferin - A Nutraceutical with Clinical Implications. *Vitamins & Minerals*, *03*(01), 1–2.

Poss, K. D., Shen, J., Nechiporuk, A., McMahon, G., Thisse, B., Thisse, C., & Keating, M. T. (2000). Roles for Fgf signaling during zebrafish fin regeneration. *Developmental Biology*, *222*(2), 347–358.

Prayoto, D. B. N. S. (2021). Analisis Rasio-Rasio Keuangan yang Berpengaruh Terhadap Return On Asset Perusahaan Infrastruktur. *Sociaperti*, *1*(2).

Prommajak, T., Kim, S. M., Pan, C. H., Kim, S. M., Surawang, S., & Rattanapanone, N. (2014). Identification of antioxidants in young mango leaves by LC-ABTS and LC-MS. *Chiang Mai University Journal of Natural Sciences*, *13*(3), 317–330.

Risa, A. M., Pantiwati, Y., Mahmudati, N., Husamah, H., & Miharja, F. J. (2018). Daun Mangga (Mangifera indica L): Potensi Baru Penyembuh Luka Sayat. *Biota: Biologi Dan Pendidikan Biologi*, *11*(2), 96–106.

Spampinato, S. F., Caruso, G. I., De Pasquale, R., Sortino, M. A., & Merlo, S. (2020). The Treatment of Impaired Wound Healing in Diabetes: Looking Among Old Drugs. *Pharmaceuticals*, *13*(4), 1–17.

Susanty, S., & Bachmid, F. (2016). Perbandingan Metode Ekstraksi Maserasi dan Refluks Terhadap Kadar Fenolik dari Ekstrak Tongkul Jagung (Zea mays L.). *Jurnal Konversi*, *5*(2), 87–93.

Syaputri, R. R. (2014). Uji Efek Ekstrak Etanol 70% Kulit Buah Asam Jawa. *Universitas Muhammadyah Surakarta*.

Syarif, R. A., Muhajir, Ahmad, A. R., & Malik, A. (2008). Identifikasi Golongan Senyawa Antioksidan dengan menggunakan Metode Perendaman Radikal DPPH Ekstrak Etanol Daun Cordia myxa L. *Jurnal Fitofarmaka Indonesia*, *2*(1), 83–89.

Szkudelski, T. (2001). The Mechanism of Alloxan and Streptozotocin Action in B Cells of The Rat Pancreas. *Physiological Research*, *50*(6), 537–546.

Tayana, N., Inthakusol, W., Duangdee, N., Chewchinda, S., Pandith, H., & Kongkiatpaiboon, S. (2019). Mangiferin content in different parts of mango tree (Mangifera indica L.) in Thailand. *Songklanakarin Journal of Science and Technology*, *41*(3), 522–528.

Utami, N. (2018). Zebrafish (Danio rerio) Sebagai Hewan Model Diabetes Mellitus. *BioTrends*, *9*(1), 15–19.

Wang, R. R., Gao, Y. D., Ma, C. H., Zhang, X. J., Huang, C. G., Huang, J. F., & Zheng, Y. T. (2011). Mangiferin, an anti-HIV-1 agent targeting protease and effective against resistant strains. *Molecules*, *16*(5), 4264–4277.