Original Article

EPIGALLOCATECHIN GALLATE IMPROVES CAUDAL FIN REGENERATION IN THE STREPTOZOTOCIN-INDUCED DIABETIC ZEBRAFISH MODEL

Ajungla Jamir, Sentiyanger Longkumer, Pranay Punj Pankaj*

Department of Zoology, Fish Biology and Fisheries Laboratory, Nagaland University, Lumami - 798627, India.
*e-mail: pranaypunj@gmail.com

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Abstract

Impaired wound healing is the leading manifestation of diabetes mellitus (DM). It is mainly triggered by chronic inflammation, hyperglycemia, hypoxia, and delayed neuropeptide signaling. The rising number of DM patients exhibiting poor healing of DM wounds has, thus, given rise to the need for therapeutic intervention. Natural extracts are said to contain high anti-oxidant and anti-inflammatory properties that decrease the pathogenesis of DM. It was recorded that epigallocatechin gallate (EGCG) can improve the blood glucose level and caudal fin regeneration in the streptozotocin-induced zebrafish model. The animals were categorized into four groups. The hyperglycemic state was maintained throughout the experiment for 21 days in the test subject. After the treatment with EGCG, the test zebrafish showed a decrease in blood glucose level from 295.5±4.7 mg/dl to 99.6±4.7 mg/dl on day 21. Results showed better regeneration of amputated caudal fin in the group treated with EGCG. The percentage regeneration in the caudal fin was 60% in diabetic subjects treated with EGCG and 66% in control subjects treated with EGCG, whereas diabetic zebrafish showed only 41% regeneration on day 21. It can be concluded that EGCG has the potency to treat impaired wounds in the DM model of zebrafish.

KEYWORDS: Blood glucose level, diabetes mellitus, epigallocatechin gallate, fin regeneration, zebrafish

INTRODUCTION

Diabetes mellitus (DM) is a metabolic dysregulation characterized by elevated blood glucose levels or hyperglycemia caused by disruption of glucose homeostasis in the blood. Ultimately several complications are accompanied, such as retinopathy, cardiovascular disease, stroke, delayed wound healing, and neuropathy [1,2]. Impaired wound healing is considered one of the leading manifestations of DM, a long-term complication that significantly degrades the quality of life [3]. Impaired wound healing is mainly triggered by chronic inflammation, hyperglycemia, hypoxia, sensory neuropathy, and delayed neuropeptide signaling [4,5]. DM is said to cause delayed wound healing by affecting the biological mechanisms of the patients [6]. Secondary complications such as the increased risk of infections, inflammatory phase around the wound, decreased cell number and growth factor response resulting in decreased local angiogenesis, inhibition of blood flow in peripheral blood vessels to chronic injuries, and amputations are prone to occur [7]. The rising number of DM patients exhibiting poor healing of the DM wound has, thus, given the need for therapeutic intervention [8].

Zebrafish (*Danio rerio*) is a well-established animal model used to study developmental biology, human disease, and regeneration [9]. It is considered a valuable model system for studying regenerative biology as it can restore poorly restored organs in other mammals [10]. The caudal fin of the zebrafish is considered the most convenient tissue approach experimentally due to its accessibility, simple structure, and fast regeneration [11]. Studies showed that streptozocin (STZ) injection in adult zebrafish sustained hyperglycemia as the blood glucose level and biochemical properties were elevated [12]. Further, zebrafish consists of critical signaling pathways that play essential roles in regulatory pathways and are associated with wound healing, including Hedgehog (HH), bone Morphogenetic Protein (BMP), and Wnt/catenin signaling pathways [13] .

Although synthetic drug therapies are used to treat DM wounds to subdue chronic injury and restore tissue regeneration, side effects are always associated with it. Therefore, alternative therapies with minimal side effects showing positive results are a point of concern and a clinical challenge for researchers today. Natural extracts are said to contain high anti-oxidant and anti-inflammatory properties that decrease the pathogenesis of DM [14]. One such natural product is Epigallocatechin gallate (EGCG), an extract from green tea known for its high antioxidant and anti-inflammatory properties [15]. EGCG has been effectively used to treat cancer and cardiovascular diseases and has also emerged as a potent anti-diabetic agent [16,17]. In an experiment with mice induced with STZ, EGCG reportedly inhibited the high glucose-caused mesangial cell damage by activating the nuclear factor erythroid 2-related factor 2 (NRF-2), which plays a critical role in EGCG protection against the oxidative damage and inflammation in diabetic nephropathy [18].

Further, EGCG inhibits adipocyte differentiation and proliferation, thus increasing glucose reception in cells through AMP-activated protein kinase activation [19]. The use of EGCG as a dietary supplement in Goto-Kakizaki rats was also reported to prevent hyperglycemia-evoked inflammation in the adipose tissue of the animal [20]. Despite the extensive literature on DM-based animal models, there are few studies on using EGCG in regenerating the caudal fin of diabetic zebrafish models. Therefore, understanding the potency of EGCG in the hyperglycemic condition of zebrafish fin regeneration needs to be done. This study may provide new insights as a first step in understanding the safe use of EGCG for the application of wound healing and regeneration under the hyperglycemic conditions of zebrafish.

MATERIALS AND METHOD

Drugs and chemicals

EGCG (≥95%), Streptozotocin, Tricaine MS-222 (Ethyl 3-aminobenzoate methanesulfonate salt), Sodium chloride, Accu Check glucometer, and blood glucose test strips were obtained from Sigma-Aldrich and Accu-Chek Active, Mumbai, India, respectively.

Acclimatization of Zebrafish

Adult Zebrafish (300-500 mg) were procured from Aqua fish & pets, Jorhat Assam, India. Zebrafish was cultured in a zebrafish housing system (Model-NT-ZB-11; Make-Narshi Technologies) to ensure constant temperature (28±2°C), persistent chemical, biological, and mechanical water filtration and aeration (7.20 mg O_2/L). Polycarbonate fish tanks were maintained under a 14h/10h: day/night photoperiod cycle. Adult zebrafish were fed three times daily with commercially available feed containing protein 28%, crude fat 3%, fiber 4%, and moisture 10%. The experimental subjects were divided into the following four experimental groups (n= 18). Group I - control zebrafish, Group II- STZ-induced, Group III- STZ + EGCG, Group IV- Control + EGCG

Induction of hyperglycemia

Induction of hyperglycemia in the zebrafish was initiated by injecting an STZ dose of 0.35mg/g of body weight into the peritoneal cavity of the subjects with the aid of a ½ cc syringe equipped with a 27-gauge needle. 2-Phenoxyethanol with a dilution ratio of 1:1000 was used for anesthetizing the subjects. In contrast, normal fish tank water was used as the recovery medium. Anesthetized fish was placed briefly on a paper towel to absorb any excess water, after which the weight of the fish was measured. Zebrafish were placed on a firm surface for

injection. After the injection, the fish were transferred to a recovery water tank and observed for signs of irregular swimming activity, which were absent. This was accomplished by transferring the fish to a standard living tank maintained at a decreased temperature of 22 - 24°C. The zebrafish were injected with 6 STZ injections for prolonged hyperglycemia over four weeks. The six doses were administered in the following way: 3 injections in the first week, followed by booster doses, one each in weeks 2, 3, and 4, on days 12, 19, and 21, respectively [21,22].

Blood glucose levels determination

Blood glucose levels were determined in the test animals (n=6), fasted for 12 hours, and placed for at least 15 minutes in a test fish tank without glucose to avoid contamination of the glucometer strip. Test animals were anesthetized in 0.04% Tricaine MS-222 (Tricaine methanesulfonate). Zebrafish were removed from the solution, patted dry with lens cleaning wipes, and placed on a glass surface. The zebrafish blood was obtained by decapitating using a sharp blade behind the eyes, where the heart is located. After this, the blood was collected on a strip directly from the punctured heart, and the blood glucose level was read by placing a glucometer test strip (One-Touch Ultra, Accu Check).

Treatment with EGCG

The effective dose was calculated using different concentrations of the compound EGCG. Further, the concentration of 6mg was culled for this study. EGCG powder (6mg) was dissolved in distilled water before being mixed into a 1-liter water tank container. The treatment continued for 21 days. The body weight of each group was weighed on day 1 and day 21 of the experiment.

Caudal fin regeneration analysis

Zebrafish (n=6) were anesthetized by immersion in an ice water bath for 1–2 minutes**.** The fish were laid in a transverse position in a petri dish, the caudal fin of the zebrafish was carefully stretched using a brush, and fin amputation was performed. A sterile scalpel blade size ten was used to amputate a straight line proximal to the first lepidotrichia branching point while viewing the fin through a dissecting microscope. The zebrafish were then returned to recover in the respective treatment and control fish water tank for the regenerative growth phase of the assay. The fins were imaged on day 0 (before amputation), day 1 (initial cut), day 7, day 14, and day 21 to examine the regenerative growth. The length of the amputation site along the dorsal-ventral axis was measured and compared with treated subjects. An Olympus Stereo Zoom microscope (Model SZX10) attached with Sony digital camera model E31SPM20000KPA (USB 2.0), and Image J software installed computer desktop (Intel Quad Core 2.8 GHz desktop computer with 64 GB RAM under Windows 7) was used for imaging the caudal fin growth of the amputated zebrafish. The regeneration percentage was calculated by using the formula described by Sun et al., 2020 [23]. Wound healing rate (%) = (IWA-UWA)/IWA × 100, where IWA stands for the initial wound, UWA stands for the wound made on the first day.

Statistical analysis

Data analysis was performed for blood glucose level, body weight, and caudal fin regeneration. Values were represented as Mean ± SEM. The glucose level in the blood and fin growth percentage was performed using GraphPad Prism 8 software (Trial version). One-way analysis of variance (ANOVA) and a post hoc Tukey Honest Significant Difference test was done to determine the significant differences between the treatment groups ($p<0.05$). All experiments were done in triplicate.

Ethics statement

All experimental animal procedures were conducted by the guidelines of the Declaration of Helsinki and approved by the Institutional Animal Ethics Committee (IEAC), Nagaland University, Lumami, with IAEC approval no. NU/ZOO/IAEC/Meeting No 1/2020, Protocol No. 03.

RESULTS

Effect of EGCG on blood glucose level and body weight

The experimental zebrafish model was made diabetic by administering the diabetogenic agent STZ. A hyperglycemic state was maintained throughout the experiment for 21 days. The result showed that the hyperglycemic group (group-II) had the highest blood glucose level during the investigation (day 1; 297.5±4.0, day 21; 301.6±4.3) mg/dl. Fasting blood glucose levels (FBGL) of adult zebrafish were recorded as 62.6±3.5 mg/dl on day 1 and 71.5±3.4 mg/dl on day 21 (Figure 1). After the exposure to EGCG, the zebrafish showed improvement in their blood glucose level. In group III, the blood glucose level dropped from (day 1: 295.5±4.7 to day 21: 99.6±4.7) mg/dl. Further, in group IV, where the control group was treated with EGCG, the blood glucose level was recorded (day 1: 63.8±5.5 to day 21: 65.1±5.1) mg/dl. The result of the body weight indicates that before the induction of DM into the test animal, there was no significant change in the weight. After induction of DM, the animals in group II were seen to experience weight loss during the 21 days of exposure, from 623±11 on day 1 to 547± 13 on day 21 of the experiment. In group–III, the zebrafish were seen to exhibit lesser significant weight loss as compared to group-II. There was no significant difference in weight between group-I and IV during this study (Figure 2).

Figure 1: Blood glucose level (mg/dl) on day 1 and day 21 for group- I, group - II, group - III and group - IV

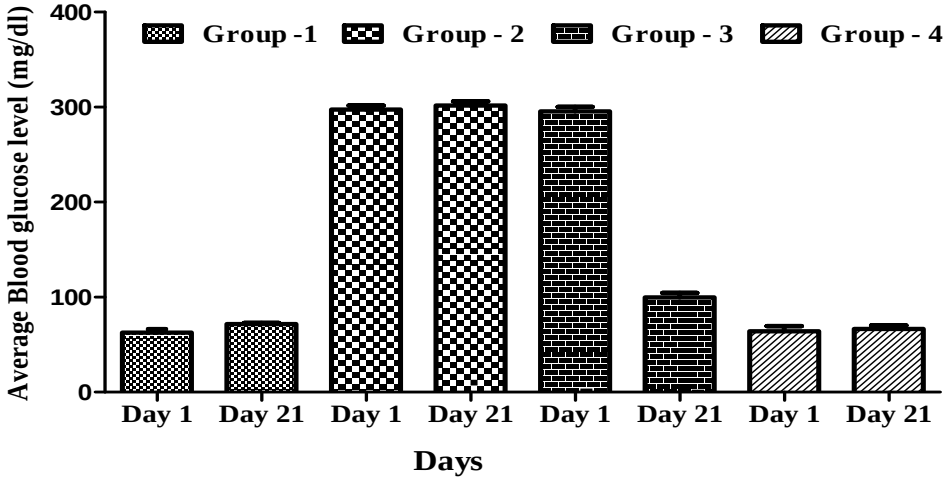
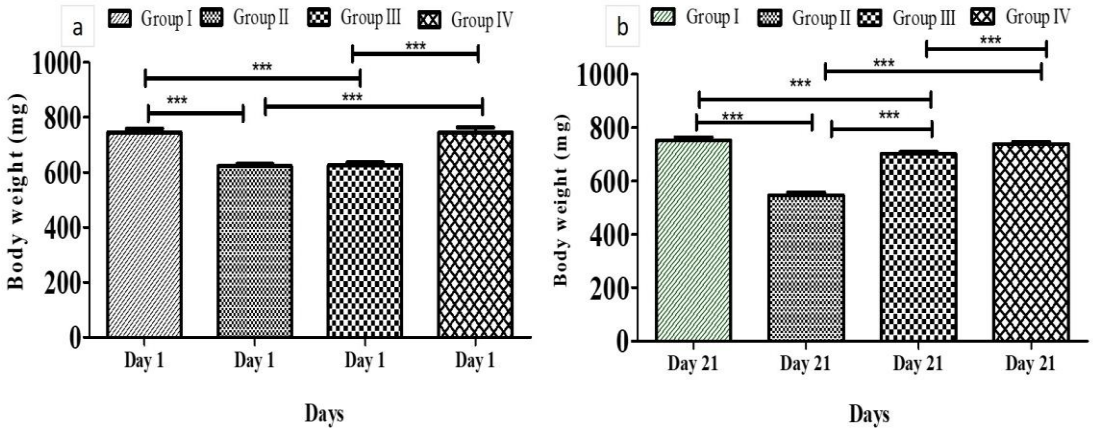


Figure 2: Body weight in (mg) for group- I, group - II, group - III and group – IV. (a) Day 1 (b) Day 21



Effect of EGCG on Caudal fin regeneration

Amputation of the fins of test zebrafish was done to evaluate the impact of EGCG on hyperglycemic fin regeneration on the caudal fin of group-I, group- II, group-III, and group IV. Images of the regenerated fins were further taken on day 0, day 1, day 7, day 14, and day 21 (Figure 4). Our result showed improvement in the growth of the caudal fins with the treatment of EGCG in both group - III and group -IV. The development of blastema in the wound sight was recorded in group I, III, and IV of the experimental animals, while very little blastema growth was found in group II. On day 7th after amputation, the percentage growth of the caudal fin for group-II showed slower growth compared to group I, III and IV. The amputated fin was recorded for hyperglycemic zebrafish as 6% on day 7th, 26% on day 14th and 41% on day 21. On the 7th, 14th and 21st day post-amputation, the animal treated with EGCG showed improvement in the growth of the fin and increased the fin growth to 14%, 36% and 60% in group III and further in group IV the fin growth was recorded as 34%, 55% and 66% respectively. The control zebrafish fin growth was recorded as 39%, 45% and 65% on the 7th, 14th and 21st day after fin amputation. The data indicates that the hyperglycemic group II display impaired wound healing condition while treatment group III and IV show improvement in caudal fin regeneration compared to the diabetes group II (Figure 4 and 5).

Figure 3: Caudal fin regeneration in adult zebrafish on day 0 (pre amputation), day 1 (initial cut), day 7, day 14 and day 21. (a-e) Control caudal fin (f-j) Caudal fin of STZ-induced adult zebrafish, (k-o) Caudal fin of STZ-induced adult zebrafish exposed to EGCG, (p-t) Caudal fin of control zebrafish exposed to EGCG. (Image captured at 5X magnification)

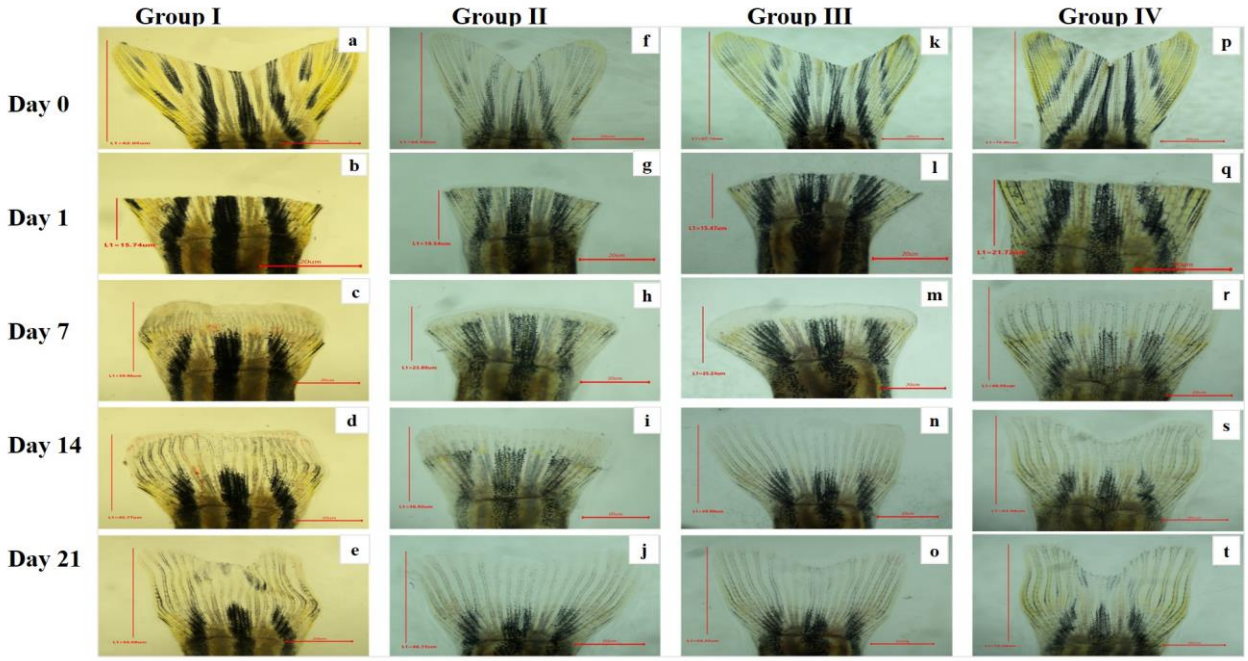


Figure 4: Average percentage growth of the caudal fin of adult zebrafish on days 7, 14 and 21 of group-I, group-II, group-III, group-IV.

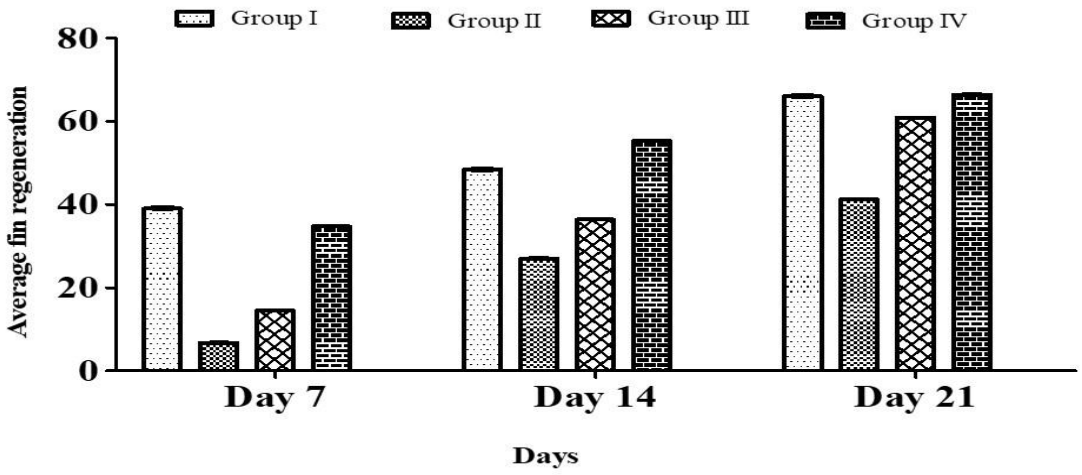
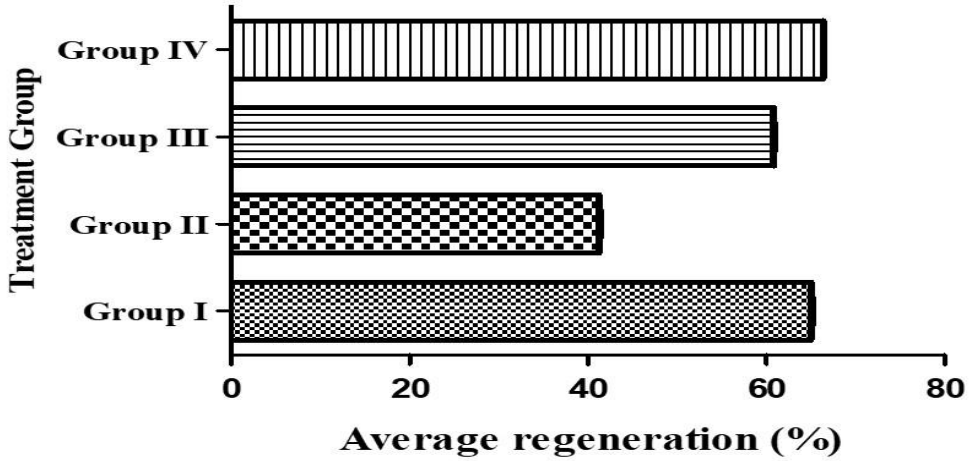


Figure 5: Average percentage growth of the caudal fin of adult zebrafish on day 21 for group - I, group - II, group - III and group - IV.



DISCUSSION

Delayed wound healing is considered the main reason behind lower limb amputation in a DM patient. Growth factors, namely insulin-like growth factor, platelet-derived growth factor, fibroblast growth factor, and vascular endothelial growth factor alter in DM patients [24]. Hyperglycemic condition is found to be associated with preventing circulating nutrients from reaching wounds, and dysfunction of endothelial cells, thus slowing the rate of healing of the wounds. Further, hyperglycemia also interferes with processes necessary for re-epithelialization, such as migration, protein synthesis, and proliferation of keratinocytes and fibroblasts [25]. Another way hyperglycemia impairs wound healing is through free radical damage caused by decreased activity of the antioxidant enzymes glutathione peroxidase and superoxide dismutase [26]. Hyperglycemia causes reactive oxygen species (ROS) to be produced via the polyol, hexosamine, protein kinase C, and advanced glycation end-product pathways [27]. Although ROS are known to be necessary for the initial stages of wound healing, an

imbalance in ROS production is harmful to later stages of wound healing. Thus, maintaining blood glucose levels in the normal range is the chief priority for any therapy.

Our findings showed that the blood glucose level of the DM-induced zebrafish was improved with the treatment of 6mg/L EGCG, reducing the glucose level to 100 mg/dl, the hyperglycemic blood glucose level being considered as (200-300 mg/dl). Induction of diabetes with STZ was reported to be associated with high blood glucose levels and weight loss [28]. Further, the control group treated with EGCG showed similar blood glucose levels as that of fasting blood glucose levels. Similar results were noted where STZ-induced mice, when injected with different grades of EGCG (50,100, 200) mg for 17 weeks, significantly decreased their blood glucose level [29]. A study with male Sprague Dawley rats fed with a high-sucrose high-fat diet combined with tail vein injection of STZ for T2DM induction when treated with EGCG (25, 50, or 100 mg/kg/d) showed a significant decrease in blood glucose level compared to the untreated DM mice [30]. A further study on STZ diabetic mice treated with EGCG showed improvement in serum glucose as well as the body weight of the animal [31].

EGCG is essential in decreasing inflammatory response on the wound site [32]. Studies in wound tissue of diabetic mice treated with EGCG showed better wound re-epithelialization [33]. The phases of caudal fin regeneration of adult zebrafish involve a series of stereotypic successive steps and take approximately three to four weeks to completely regenerate the amputated fin [34]. To examine whether EGCG could improve diabetic wounds in adult zebrafish, we established the method of exposure in a manner where the fish fin was immersed in EGCG for 21 days. The wound recovery time was expressed directly by measuring the length of the regenerative fin. During the wound healing period, the blastema of the fish fin and the granulation tissue of the human are similar as both repair the damaged tissue by providing proliferative blastema cells [35]. Our result showed that the amputated fin gave positive growth with exposure to EGCG in both groups III and IV. The series in which a diabetic wound is healed and tissues are repaired is vital, and the underlying action of EGCG on the diabetic regenerative model of zebrafish is still unclear. DM is said to delay the wound healing process as it disturbs the different stages of wound repair, namely homeostasis, inflammation, proliferation, and remodeling [36].

Our study showed that the fin of the hyperglycemic adult zebrafish was severely impaired. It was observed that on day 7^{th}, 14^{th} and 21^{st}, the fin growth was significantly decreased compared to the group I, III and IV animals (Figure 3). Similarly, when studying the fin regeneration in STZ-induced adult zebrafish, it was recorded that the fin did not show significant growth during 72 hours following amputation. Still, later reductions in the development of the amputated fin were observed in the second and third weeks [37]. Our result also showed that when the hyperglycemic animals were treated with 6mg/L of EGCG, the hyperglycemic adult zebrafish expressed significant improvement in the regeneration of the fin length (Figure 3). Works with animal models have described the therapeutic insights of EGCG [38]. A study with STZ-induced diabetic mice reported that EGCG could improve wound healing before or after the inflammation phase by targeting the Notch signaling pathway [39].

In this study, we examined the effect of EGCG on blood glucose levels, body weight, and caudal fin re-growth in the hyperglycemic zebrafish model. Results clearly indicate that the hyperglycemic zebrafish model exhibits delayed wound healing. The blood glucose level of group II remained high for 21 days following injection with booster doses. EGCG, on the other hand, alleviates the blood glucose level and helps correct impaired fin regeneration. To the best of the author's knowledge, this study is the first to provide evidence of the potential effect of EGCG in fin regeneration. Further, the need to study and understand the mechanism of EGCG in improving caudal fin regeneration in DM-induced zebrafish may have broader applications. However, more attention needs to be paid to understanding the therapeutic approach of EGCG in the fin re-growth of zebrafish. Therefore, the study presented here may suggest using EGCG as a potent compound in treating impaired wound healing of DM models of zebrafish.

CONCLUSION

Treatment with EGCG, a green tea extract in STZ-induced zebrafish, significantly facilitates the healing of the amputated fin. EGCG treatment attenuates increased blood glucose levels and improves the body weight of diabetic adult zebrafish. Therefore, EGCG may be a promising agent for future studies on wound healing, particularly for the diabetic zebrafish model.

REFERENCES

1. Artime, E., Romera, I., Díaz-Cerezo, S., & Delgado, E. Epidemiology and economic burden of cardiovascular disease in patients with type 2 diabetes mellitus in Spain: a systematic review. Diabetes Ther. 2021. 12(6), 1631-1659.

2. Dubey, R., Prabhakar, P. K., & Gupta, J. Epigenetics: Key to improve delayed wound healing in type 2 diabetes. Mol. Cell. Biohem. 2022. 477(2), 371-383.

3. Romanowski, K. S., & Sen, S. Wound healing in older adults with severe burns: Clinical treatment considerations and challenges. Burns Open. 2022. doi: 10.1016/j.burnso.2022.01.002.

4. Baltzis, D., Eleftheriadou, I., & Veves, A. Pathogenesis and treatment of impaired wound healing in diabetes mellitus: new insights. Adv Ther. 2014. 31, 817-836.

5. Partoazar, A., Kianvash, N., & Goudarzi, R. New concepts in wound targeting through liposome-based nanocarriers (LBNs). J Drug Deliv. Sci. Technol. 2022. 103878.

6. Tombulturk, F. K., Todurga-Seven, Z. G., Huseyinbas, O., Ozyazgan, S., Ulutin, T., & Kanigur-Sultuybek, G. Topical application of metformin accelerates cutaneous wound healing in streptozotocin-induced diabetic rats. Mol. Biol. Rep. 2022. 1-11.

7. Wibowo, I., Utami, N., Anggraeni, T., Barlian, A., Putra, R. E., Indriani, A. D., et al. Propolis can improve caudal fin regeneration in zebrafish (*Danio rerio*) induced by the combined administration of Alloxan and glucose. Zebrafish, 2021. 18(4), 274-281.

8. Kong, M., Xie, K., Lv, M., Li, J., Yao, J., Yan, K., et al. Anti-inflammatory phytochemicals for the treatment of diabetes and its complications: Lessons learned and future promise. Biomed. Pharmacother. 2021. 133, doi: 10.1016/j.biopha.110975.

9. Fontana, B. D., Norton, W. H., & Parker, M. O. Modelling ADHD-like phenotypes in Zebrafish. Curr. Top. Behav. Neurosci. 2022. 395-414.

10. Fan, C., Ouyang, Y., Yuan, X., & Wang, J. An enhancer trap zebrafish line for lateral line development and regulation of six2b expression. Gene Expr. Patterns. 2022. 43, doi: 10.1016/j.gep.2022.119231.

11. Ouyang, T., Yin, H., Yang, J., Liu, Y., & Ma, S. Tissue regeneration effect of betulin via inhibition of ROS/MAPKs/NF-κB axis using zebrafish model. Biomed. Pharmacother. 2022. 153, 113420.

12. Verma, B., Singh, C., & Singh, A. Effect of hydro-alcoholic extract of Centella asiatica on streptozotocin induced memory dysfunction in adult zebrafish. Curr opin. Behav. Sci., 2021. doi.org/10.1016/j.crbeha.2021.100059.

13. Longkumer, S., Jamir, A., & Pankaj, P. P. Maintenance and Breeding of Zebrafish under Laboratory Conditions for Animal Research. Agricultural Science Digest. 2022. DOI: 10.18805/ag.D-5599.

14. Shahwan, M., Alhumaydhi, F., Ashraf, G. M., Hasan, P. M., & Shamsi, A. Role of polyphenols in combating Type 2 Diabetes and insulin resistance. Int. J. Biol. Macromol. 2022. doi: 10.1016/j.ijbiomac.2022.03.004.

15. Longkumer, S., Jamir, A., & Pankaj, P. P. Evaluation of lipid profile in streptozotocin induced diabetic zebrafish, treated with C-phycocyanin and Epigallocatechin gallate. Biochem. Cell. Arch. 2022. 22(1), 1441-1446.

16. Alam, M., Ali, S., Ashraf, G. M., Bilgrami, A. L., Yadav, D. K., & Hassan, M. I. Epigallocatechin 3-gallate: From green tea to cancer therapeutics. Food Chem. 2022 doi: 10.1016/j.foodchem.2022.132135.

17. Beyaz, S., Özlem, G. Ö. K., & Aslan, A. The therapeutic effects and antioxidant properties of epigallocatechin-3 gallate: A new review. Int. J. Mol. Sci. 2022. 9(2), 125-136.

18. Sun, W., Liu, X., Zhang, H., Song, Y., Li, T., Liu, X., et al. Epigallocatechin gallate upregulates NRF2 to prevent diabetic nephropathy via disabling KEAP1. Free Radic. Biol. Med. 2017. 108, 840-857.

19. Asbaghi, O., Fouladvand, F., Gonzalez, M. J., Ashtary-Larky, D., Choghakhori, R., & Abbasnezhad, A. Effect of green tea on glycemic control in patients with type 2 diabetes mellitus: A systematic review and meta-analysis. Diabetes Metab Syndr. 2021 Clinical Research & Reviews, 15(1), 23-31.

20. Uchiyama, Y., Suzuki, T., Mochizuki, K., & Goda, T. Dietary supplementation with (−)-epigallocatechin-3-gallate reduces inflammatory response in adipose tissue of non-obese type 2 diabetic Goto-Kakizaki (GK) rats. J Agric. Food Chem. 2013. 61(47), 11410-11417.

21. Intine, R. V., Olsen, A. S., & Sarras Jr, M. P. A zebrafish model of diabetes mellitus and metabolic memory. J Vis Exp. 2013 (72), doi: 10.3791/50232.

22. Longkumer, S., Jamir, A., & Pankaj, P. P. Development and appraisal studies of chemically induced zebrafish hyperglycemia model. J. Exp. Zool. 2020. India, 23(2), 1305-1310.

23. Sun, M., Xie, Q., Cai, X., Liu, Z., Wang, Y., Dong, X., & Xu, Y. Preparation and characterization of epigallocatechin gallate, ascorbic acid, gelatin, chitosan nanoparticles and their beneficial effect on wound healing of diabetic mice. Int. J. Biol. Macromol. 2020. 148, 777-784.

24. Dinh, T., Elder, S., & Veves, A. Delayed wound healing in diabetes: Considering future treatments. Diabetes Management, 2011. 1(5), 509, DOI:10.2217/DMT.11.44.

25. Hu, S. C. S., & Lan, C. C. E. High-glucose environment disturbs the physiologic functions of keratinocytes: focusing on diabetic wound healing. J Dermatol. Sci. 2016. 84(2), 121-127.

26. Kant, V., Sharma, M., Jangir, B. L., & Kumar, V. Acceleration of wound healing by quercetin in diabetic rats requires mitigation of oxidative stress and stimulation of the proliferative phase. Biotech. Histochem. 2022. 97(6), 461-472.

27. Ighodaro, O. M. Molecular pathways associated with oxidative stress in diabetes mellitus. Biomed. Pharmacother. 2018. 108, 656-662.

28. Rad, M. G., Sharifi, M., Meamar, R., & Soltani, N. The role of pancreas to improve hyperglycemia in STZ-induced diabetic rats by thiamine disulfide. Nutr. Diabetes, 2022. 12(1), 32.

29. Yoon, S. P., Maeng, Y. H., Hong, R., Lee, B. R., Kim, C. G., Kim, H. L., et al. Protective effects of epigallocatechin gallate (EGCG) on streptozotocin-induced diabetic nephropathy in mice. Acta Histochem. 2014. 116(8), 1210-1215.

30. Zhu, T., Li, M., Zhu, M., Liu, X., Huang, K., Li, W., et al. Epigallocatechin-3-gallate alleviates type 2 diabetes mellitus via β-cell function improvement and insulin resistance reduction. Iran. J. Basic. Med. Sci. 2022. 25(4), 483, doi: 10.22038/IJBMS.2022.58591.13016.

31. Roghani, M., & Baluchnejadmojarad, T. Hypoglycemic and hypolipidemic effect and antioxidant activity of chronic epigallocatechin-gallate in streptozotocin-diabetic rats. Pathophysiology, 2010. 17(1), 55-59.

32. Li, M., Xu, J., Shi, T., Yu, H., Bi, J., & Chen, G. Epigallocatechin-3-gallate augments therapeutic effects of mesenchymal stem cells in skin wound healing. Clin. Exp. Pharmacol. Physiol. 2016. 43(11), 1115-1124.

33. Carvalho, M. T., Araújo-Filho, H. G., Barreto, A. S., Quintans-Júnior, L. J., Quintans, J. S., & Barreto, R. S. (2021). Wound healing properties of flavonoids: A systematic review highlighting the mechanisms of action. Phy Med. 2021. doi: 10.1016/j.phymed.2021.153636.

34. Dietrich, K., Fiedler, I. A., Kurzyukova, A., López-Delgado, A. C., McGowan, L. M., Geurtzen, K., et al. Skeletal biology and disease modeling in zebrafish. J. Bone. Miner. Res. 2021. 36(3), 436-458.

35. Pang, S., Gao, Y., Wang, F., Wang, Y., Cao, M., Zhang, W., et al. Toxicity of silver nanoparticles on wound healing: A case study of zebrafish fin regeneration model. Sci. Total. Environ. 2020. doi: 10.1016/j.scitotenv.2020.137178.

36. Patel, S., Srivastava, S., Singh, M. R., & Singh, D. Mechanistic insight into diabetic wounds: Pathogenesis, molecular targets and treatment strategies to pace wound healing. Biomed. Pharmacother. 2019. 112, doi: 10.1016/j.biopha.2019.108615.

37. Olsen, A. S., Sarras Jr, M. P., & Intine, R. V. Limb regeneration is impaired in an adult zebrafish model of diabetes mellitus. Wound Repair. Regen. 2010. 18(5), 532-542.

38. Xu, F. W., Lv, Y. L., Zhong, Y. F., Xue, Y. N., Wang, Y., Zhang, L. Y., et al. Beneficial effects of green tea EGCG on skin wound healing: A comprehensive review. Molecules, 2021. 26(20), 6123. doi: 10.3390/molecules26206123.

39. Huang, Y. W., Zhu, Q. Q., Yang, X. Y., Xu, H. H., Sun, B., Wang, X. J., & Sheng, J. Wound healing can be improved by (—) -epigallocatechin gallate through targeting Notch in streptozotocin-induced diabetic mice. The FASEB J.2019. 33(1), 953-964.