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EFFECT OF GLUCOSE ON TAIL FIN REGENERATION IN *GAMBUSIA AFFINIS*

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ABSTRACT

Regeneration is a unique biological phenomenon wherein the organism can reform the lost parts of its body. A variety of growth and differentiation inducing molecules play crucial roles during these reformation events. Glucose is used by animals as a major source of energy for all biological processes. Even though glucose is inevitable for this reason, it should be present within its optimum levels only. When these levels are disturbed, it may lead physiological pathologies like either increased glucose i.e. hyperglycemia or hypoglycemia i.e. low glucose situations. In both the cases, animals suffer from various health problems. Since regeneration demands huge amount of energy and glucose serves as one of the major sources for energy, the present study aimed at elucidation of effects of altered levels of glucose on regeneration process. To study this, *Gambusia affinis* was selected as a model system for its tremendous regeneration capacity and its use as a favorite vertebrate model system for regeneration studies. In the present study an important role of glucose in the tail fin regeneration of *Gambusia affinis* is demonstrated.

KEYWORDS: *Gambusia affinis*, glucose, amputation, regeneration, tail fin

INTRODUCTION

Regeneration is a process of renewal, restoration and growth of damaged part of any living organism. Every animal species has regeneration capacity to various intensities depending on the organ that is lost. Regeneration can either be complete where the new tissue is the more or less same as the lost tissue or incomplete where the regenerating tissue undergoes necrosis leading to tissue fibrosis (Li *et al.,* 2012). Complete regeneration is essential to restore the integrity and function of the injured organ. During this process, immune cells play important roles for supporting the re-growth of the damaged tissue (Gilbert, 2006; Li *et al.,* 2012).

Gambusia affinis, commonly known as mosquito-fish, has been effectively used as a model organism for regeneration studies due to its regeneration ability. *Gambusia*, mostly found in freshwater, is benthic non-migratory fish that is mostly found in standing or slow flowing waters (McDowall, 2000; Pyke, 2005). *Gambusia affinis* has streamlined body that reaches maximum length of about 6-7 cm in case of female fish while males may reach around 4cm. Dorsal and caudal fins are rounded with almost invisible lateral line. Body is usually greenish olive to brown above, grey- blue on the sides and slivery-white from ventral side. Small black dots are present on the body and tail. The species is sexually dimorphic with adult males smaller than females. The maximum age reported for this species is 3 years (McDowall, 1990). In *Gambusia affinis,* epimorphic regeneration is observed. In epimorphic regeneration, the tissue near the lost or amputated region undergoes dedifferentiation to form undifferentiated cell mass that later becomes specified into the lost part (Gilbert, 2006).

Gambusia can very well regenerate its lost or damaged parts like brain, eye, kidney, heart and fins. After amputation stem cells accumulate at the injury site in a structure called the blastema. Fish mostly use glucose as a major source of energy. Glucose molecules are broken down within cells in order to produce ATP molecules; energy – rich molecules, which power numerous cellular processes. Elevated or reduced glucose levels lead to various physiological complications in animals (Olson *et al,* 2010; Fahmy *et al.,* 2013). Glucose is thought to increase oxidative stress which is likely to hamper the regeneration process as well. In the present study, the possible effects of glucose on regeneration process are tested using *Gambusia affinis* as a model system. Regenerating tail fins; after surgical amputation of tail fins; in presence or absence of various doses of glucose was monitored for different time periods. This study clearly demonstrates a crucial role of glucose, at its optimum levels, in the regeneration process.

MATERIALS AND METHODS

Procurement of animals

Gambusia affinis were collected from the local pond of the S.N Arts, D.J.M Commerce and B.N.S Science College, Sangamner, Ahmednagar, India and transported to laboratory in a plastic container. Then fish of same size and colour were selected for experiment and rest were released back in the pond.

Amputation of tail fin

Fish were anesthetized with an aquatic anesthetizer MS222 (0.2%; Fisher Scientific) and were taken on a glass slide one by one. Tail fin amputation was carried out using a sharp blade taking a single sharp cut leaving behind intact tail fin of 2mm. Fish with amputated tails were randomly distributed into control and treated groups. Filtered pond water was used for treatment of fish. Fish of equal body length, tail fin length, tail fin thickness were used as the experimental and control fish.

Experimental set up

Each experimental unit consisted of glass bowls containing 5 fish per bowl. The pond water was renewed after every 24 hours. Control animals were kept in plain filtered pond water while the treated groups received either 10mM, 20mM, 50mM or 100mM of glucose solution. In one set of experiment, the glucose treatment was given to fish only on the first day of regeneration i.e. 24 hrs post-amputation. This treatment is known as chronic treatment while in the other set of the treatment, solution of the same glucose concentration was replaced daily for 8 days respectively (acute treatment). The tail fin of each fish from control and treated groups were measured daily for 8 days post-amputation. The experiment was repeated at least 4 times with 20 fish as the sample size for control and treated groups.

RESULTS

Response of regenerating tail fin of Gambusia affinis to chronic treatment:

Fish did not exhibit any regeneration in terms of length enhancement for the first 3 days post-amputation. Blastema formation and actual regeneration began from 4th day onwards. Complete regeneration was observed within 8 days in natural conditions. However in 10 mM glucose treated fish, regeneration appeared to begin on 3rd day itself indicating promoting effect of glucose on regeneration. Throughout the observation period, 10 mM glucose solution accelerated regeneration process in tail fins as compared to controls and other glucose treated fish as seen in table no 1 and figure 1. At the remaining tried doses i.e. 20 mM, 50 mM and 100 mM, glucose accelerated regeneration as compared to control, however the acceleration was not statistically significant.

Table1: Effect of glucose on regenerating Gumbusia tail fin (Chronic treatment)

Treatment	**Tail Length (mm)**				
	Day 4	**Day 5**	**Day 6**	**Day 7**	**Day 8**
Control	0.5±0	0.69±0.25	1±0	1.61±0.29	1.96±0.13
10 mM glucose	0.66±0.24	1±0	1.54±0.14	2.08±0.28	2.81±0.40
20 mM glucose	0.65±0.24	1±0	1.25±0.33	1.68±0.46	2.18±0.51
50 mM glucose	0.65±0.24	1±0	1.2±0.25	1.7±0.45	1.75±0.45
100 mM glucose	0.64±0.23	1±0	1.4±0.20	1.9±0.30	1.9±0.30

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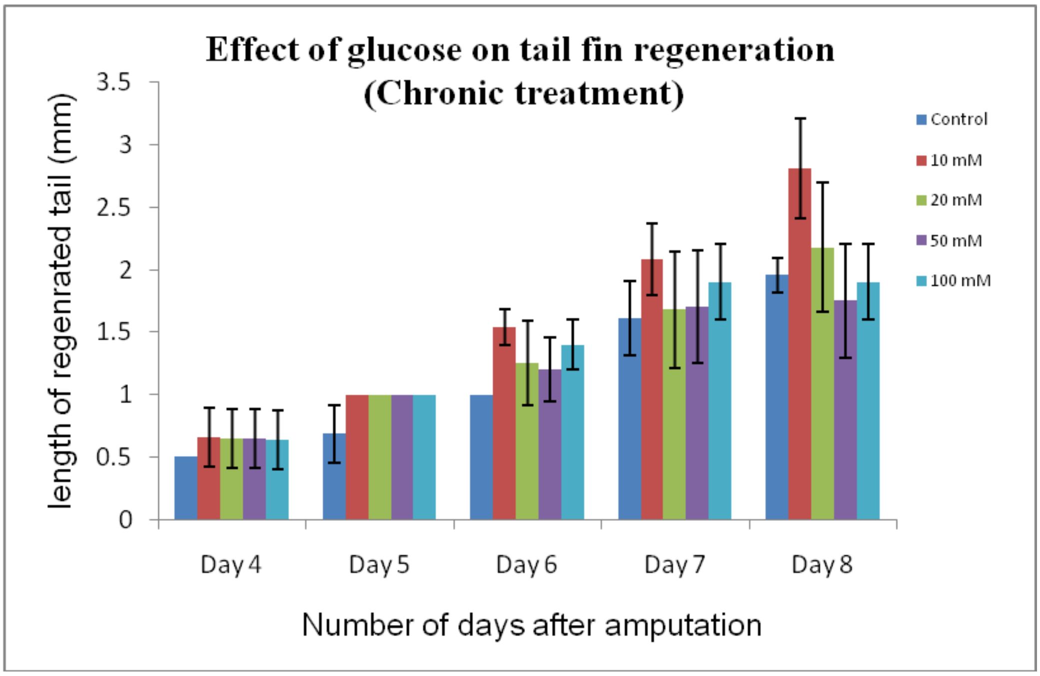

Figure 1: Effect of glucose on tail fin regeneration on *Gambusia* in chronic treatment

Regeneration of tail fin of Gambusia affinis under acute treatment: As observed in controls kept in chronic treatment experiments, controls of acute treatment also showed the regeneration from 4th day onwards. In treated groups, initially there was no significant difference in regeneration rate as compared to controls however by the last day of experiment i.e. by 8th day, there was significant reduction in the length of the regenerating tail fin in fish treated with 10 mM, 20 mM and 100 mM glucose doses (please refer to table no. 2 and figure no 2).

Table 2: Effect of glucose on regenerating *Gambusia* fins (Acute treatment).

Treatment	Tail Length (mm)				
	Day 4	**Day 5**	**Day 6**	**Day 7**	**Day 8**
Control	0.45±0.14	0.91±0.28	1.5±0	2.12±0.23	2.87±0.23
10 mM glucose	0.5±0	0.77±0.26	1.1±0.51	1.6±0.51	2±0
20 mM glucose	0±0	0.5±0	1±0	1.38±0.22	1.38±0.22
50 mM glucose	0.33±0.24	0.82±0.24	1.32±0.24	1.85±0.30	2.61±0.48
100 mM glucose	0±0	0.5±0	1±0	1.31±0.25	1.71±0.26

Figure 2- Effect of glucose on tail fin regeneration in Gambusia in Acute treatment

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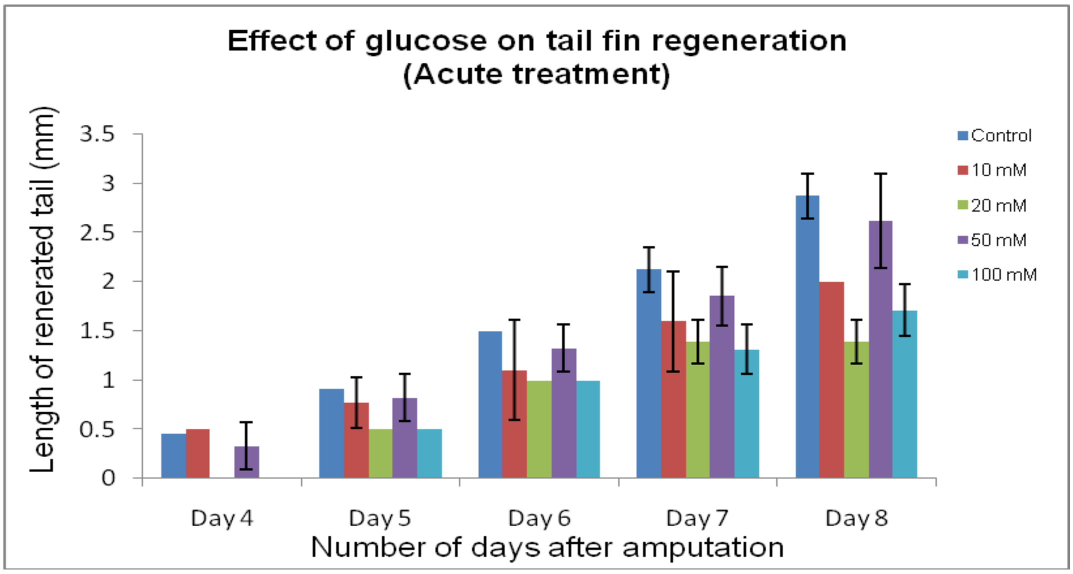

Figure 2. Effect of glucose on tail fin regeneration in *Gambusia* in Acute treatment

DISCUSSION

Regeneration is a fascinating biological phenomenon which helps the animal to restore important organs after losing them accidently. By the time the animal can regenerate the lost part, it can survive only if the temporary loss of that organ can be tolerated by the animal. As compared to highly evolved animals, less evolved exhibit tremendous regeneration capacity. Some animals such as sponges, sea anemones, corals, turbellarians, annelids and starfish have such high powers of regeneration that fission and regeneration serves as a mode of asexual reproduction in natural conditions. Starfish (arms), crabs, insect and spiders (legs) can regenerate frequently. In vertebrates, amphibians have an amazing ability to regenerate limbs, tail, eye, lens, jaws, external gills etc. The regeneration of internal organs such as liver and bone occurs to some extent in most vertebrates including humans (Verma and Agrawal, 1997). It has been proved that regeneration is guided by specific hormones and the chemicals released by the injured or damaged organ that act as a stimulus for regeneration (Verma and Agrawal, 1997; Muller, 2008).

Glucose is a monosaccharide and has six carbon atoms. In animals, glucose is biosynthesized in the liver or kidneys from non-carbohydrate intermediates, such as pyruvate and glycerol, by the process gluconeogenesis. In animals going through stressful conditions, glucose metabolism pathway is one of the primary metabolic pathways which aid in various cellular processes like growth, proliferation and survival (Ha *et al.* 2015). When the optimum levels of glucose are increased, it may lead to several physiological complications such as hyperglycemia, also known as *Diabetes mellitus* in humans; while decreased levels of glucose are present in hypoglycemia or hyperinsulinism. Elevated or reduced glucose levels may hamper other important biological phenomenon like regeneration in addition to *Diabetes* or hyperinsulinism (Olson *et al*; 2010, Fahmy *et al*; 2013). Glucose is thought to increase the oxidative stress in animals, which may be a result of disturbed antioxidant levels (Meng *et al*; 2015). Antioxidants may delay, prevent or remove oxidative damage to a target molecule. Every metabolic process in fish or any other animal can create oxidative stress. For this reason, certain essential nutrients have an important antioxidants function in regeneration (Kolluru *et al.*, 2006; Petrulea *et al*; 2012).

Glucose accelerated regeneration process in chronic treatment. 10mM glucose solution accelerated the regeneration process as compared to other doses in chronic treatment. These results are in agreement with the unpublished work of Cao and Tran (The effects of glucose on totipotent cell formation and Differentiation; California State Science Fair, 2014 project summary, project number 34793) who have shown regeneration accelerating effect of glucose at lower doses as compared to higher doses in Planaria. In acute treatment, 20mM glucose solution delayed the regeneration of tail fin in *Gambusia affinis.* In another study, effect of insulin; a hormone which regulates blood glucose levels, has been studied on regeneration of tail fins of *Gambusia affinis. W*hen the fish were treated with 0.05, 0.1 unit/ml insulin solution acceleration of tail fin length was remarkable in 0.1 unit/ml insulin solution. As the dose of insulin was increased to 0.3 unit/ml, retarded regeneration speed was seen (Miyagi, 1962). One of the mechanisms by which insulin acts is by reducing blood glucose levels. So the accelerative effect at low doses and retardation effect at higher doses of insulin may be co-related with down-regulation of glucose at different levels. Experiments on tail fin regeneration in zebra fish have revealed that glucose may interfere with the regeneration process. One week exposure to high glucose resulted in statistically different rates of fin regeneration between control and hyperglycemic zebra fish for various time points like 24 hours, 48 hours and 72 hours. After 2 weeks of hyperglycemia, zebra fish exhibited a significant reduction at all-time durations of hyperglycemia (Olsen *et al*; 2010). So our study with *Gumbusia affinis* matches with the earlier reports on zebra fish. Our study thus clearly demonstrates an important role of glucose, at its optimum levels in the regeneration process. Elucidation of mechanism of glucose in regeneration is under progress.

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