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Evaluation of Anti-Angiogenic Effect of Naturally Occurring Compound from Ficus.Bengalensis on Regeneration & Development of Zebra-Fish Fin

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Abstract

Angiogenesis is the process that leads to the formation of new blood vessels or neovascularisation. Angiogenesis inhibitors are designed to prevent the formation of new blood vessels. The main aim of this study is to evaluate Anthocyanin obtained from medicinal plant Ficus.bengalensis for Anti-angiogenesis to standardize a method for the study of angiogenesis. A variety of animal models have been described to provide more quantitative analysis of in vivo angiogenesis and to characterize pro- and anti Angiogenic molecules.

Index terms— anthocyanin, regenerative angiogenesis, vegf (vascular endothelial growth factor), zebrafish embryos (larvae).

1 Introduction

ngiogenesis, the process that leads to the formation of new blood vessels or neovascularisation, which is highly important during development but is largely not observed in the adult, except physiological exceptions in which angiogenesis occurs under tight regulation found in the female reproductive system and during wound healing. (Malin **??**ollinger et al.) In pathological situations, however, angiogenesis may be turned on, which contribute to the onset and progression of most severe human pathologies characterized by high mortality, including cancer, diabetes, obesity and retinopathies. Thus, angiogenesis is one of the largest and fastest evolving areas of research today, the knowledge of the molecular mechanisms that regulate neovascularisation continues to emerge, and there is increasing hope for the new discoveries that will lead to newer therapies targeting angiogenesis. (Uday P Kundap et al 2013) Angiogenesis is the physiological process through which new blood vessels develop from pre-existing vessels. This is distinct from vasculogenesis, which is the de novo formation of endothelial cells from mesoderm cell precursors. Anti-angiogenesis is a form of targeted therapy that uses drugs or other substances to stop tumors from making new blood vessels. Without a blood supply, tumors can't grow. Anti-angiogenesis research began more than 35 years ago with the work of the late Judah Folkman, MD. (Bagchi. D et al 2004, Han-Chung Wul et al) Anthocyanin are the flavonoid compounds that produce plant colours ranging from orange and red to various shades of blue and purple. Anthocyanin are members of the flavonoid group of phytochemicals, which is a group predominant in teas, honey, wines, fruits, vegetables, nuts, olive oil, cocoa and cereals. The flavonoids are thought to be perhaps the most important single group of phenolic in food. **??**Gael McGill et al.) Adult zebrafish have a remarkable regenerative capability. Many tissues which may not be regenerated in mammals are quickly regenerated in zebrafish. Among these are the heart, retina, maxillary barbell and fins importantly, as they regenerate, new blood and lymph vessels grow into the regenerating tissue -which enables studies on regenerative angiogenesis. One commonly used assay in the adult zebrafish, based on this principle is the regenerating tail fin. After amputation, the tail fin will re-grow and after approximately 1 month, the fin is back to its original size.

The zebrafish embryo has become an important vertebrate model for assessing drug effects. It is well suited for studies in genetics, embryology, development, and cell biology. (L. D. Jensen1, et al) Zebrafish embryos exhibit unique characteristics, including ease of maintenance and drug administration, short reproductive cycle, and transparency that permits visual assessment of developing cells and organs. Because of these advantages, zebrafish bioassays are cheaper and faster than mouse assays, and are suitable for largescale drug screening. The main aim of this study is to evaluate Anthocyanin obtained from medicinal plant Ficus.bengalensis for Anti-angiogenesis & to standardize a method for the study of angiogenesis. A variety of animal models have been described to provide more quantitative analysis of in vivo angiogenesis and to characterize pro-and anti Angiogenic molecules. However, it is still necessary to establish a quantitative, reproducible and specific.

2 a) Collection of plant

The dried stem bark of Plant Ficus.bengalensis Linn. Was collected from Uran region of Navi-Mumbai Maharashtra India & were authenticated from Agarkar Research Institute, G. G. Agarkar Road, Pune, Sample deposited on 13/9/2012 & voucher number allotted is S/B-110.

3 b) Extraction of Anthocyanin

This unit describes methods for extraction, isolation, and purification of anthocyanin pigments from plant tissues. The choice of extraction method should maximize pigment recovery with a minimal amount of adjuncts and minimal degradation or alteration of the natural state.

Basic Protocol 1 describes the extraction of Anthocyanin with acetone and their partition with chloroform. Basic Protocol 2 describes a simple, fast, and effective method for purification of Anthocyanin from polyphenol compounds, sugars, and organic acids using solid-phase adsorption. This produces a uniform composite sample with a high surface area, which allows for efficient pigment extraction. (Oszmianski and Lee, 1990, Sheikh Anis, et al. 2012) Basic Protocol -1

Acetone Extraction & Chloroform Partition of Anthocyanin: In this method, acetone extracts the Anthocyanin from the plant material and chloroforms partitioning further isolates and partially purifies the pigments. The addition of chloroform results in phase separation between the aqueous portion (which contains the anthocyanin, phenolics, sugars, organic acids, and other water-soluble compounds) and the bulk phase (which contains the immiscible organic solvents, lipids, carotenoids, chlorophyll pigments, and other nonpolar compounds). This method has the advantage of producing an extract with no lipophilic contaminants. The absence of a concentration step minimizes the risk of acid-dependent pigment degradation. (Oszmianski and Lee, 1990) Materials: Powdered plant material,Frozen Acetone 70% (v/v) aqueous acetone or aqueous acidified acetone: 70% aqueous acetone with 0.01% HCl, Chloroform, Acidified water: 0.01% (v/v) HCl in deionized, distilled water,Waring Blender with stainless steel container (Waring) or general-purpose homogenizer, Whatman no. 1 filter paper, Buchner funnel, Separatory funnel, 500-ml boiling flask Rotary evaporator with vacuum pump or water aspirator, 40°C Basic protocol -2 Anthocyanin Purification: Purification of anthocyanin-containing extracts is often necessary, as the solvent systems commonly used for extraction are not specific for anthocyanin. Considerable amounts of accompanying materials may be extracted and concentrated in the coloured extracts, which can influence the stability and/or analysis of these pigments (Jackman and Smith, 1996). Anthocyanin purification using solid-phase extraction (Figure) permits the removal of several interfering compounds present in the crude extracts. Mini-columns containing silica gel 60 chains bonded on silica retain hydrophobic organic compounds (e.g., anthocyanin, phenolics), while allowing matrix interferences such as sugars and acids to pass through to waste. Washing the retained pigments with ethyl acetate will further remove phenolic compounds other than anthocyanin. **??** Regenerated Zebrafish fin was observed under the microscope after 7-8 days of regeneration, fish from control group shows the normal & complete growth of blood vessels in fin. Vehicle control group also shows the complete & normal growth of blood capillary bloodvessels in regenerated fin. Unique observation was noticed in standard drug group, the growth of capillary blood vessels in fin was stunted, the growth of blood vessels was blocked in standard drug paclitaxal (0.5ppm conc). Fishes in the test drug shows the simillar features as standard drug group. The growth of the blood vessels was also blocked by the test compound. Adult zebrafish have a remarkable regenerative capability. Many tissues which may not be regenerated in mammals are quickly regenerated in zebrafish. Among these are the heart, retina, maxillary barbell and fins importantly, as they regenerate, new blood and lymph vessels grow into the regenerating tissue -which enables studies on regenerative angiogenesis. One commonly used assay in the adult zebrafish, based on this principle is the regenerating tail fin. After amputation, the tail fin will re-grow and after approximately 1 month, the fin is back to its original size. Angiogenesis process was activated in zebrafish by cutting there fin. Test compound (1mg/300ml of water) was added in the water.

4 Material & Methods

5 Discussion

standard drug group, the growth of capillary blood vessels in fin was stunted, and the growth of blood vessels was blocked in standard drug paclitaxel (0.5ppm conc). Fishes in the test drug (3.3ppm) shows the similar features

as standard drug group. The growth of the blood vessels was also blocked by the test compound which shows 78.21% inhibition of angiogenesis.

Design and development of small molecule therapeutics to inhibit angiogenesis has gained considerable importance in anti-angiogenesis research. We demonstrate that the zebrafish is a viable model for screening small molecules that can inhibit angiogenesis. The mechanism of Anthocyanin action is not yet known, but hypotheses include decreased levels of tumor necrosis factor alpha (TNF-a)-induced VEGF expression), inhibition of H2O2- and as well as through the inhibition of VEGF and VEGF receptor expressionThus, our study suggests that even mill molar concentration of test drug (Anthocyanin) could be an effective drug for in vivo inhibition of angiogenesis and thus might gain significance in future therapeutics. [1]

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Figure 1: Figure 1 :Figure 2 :

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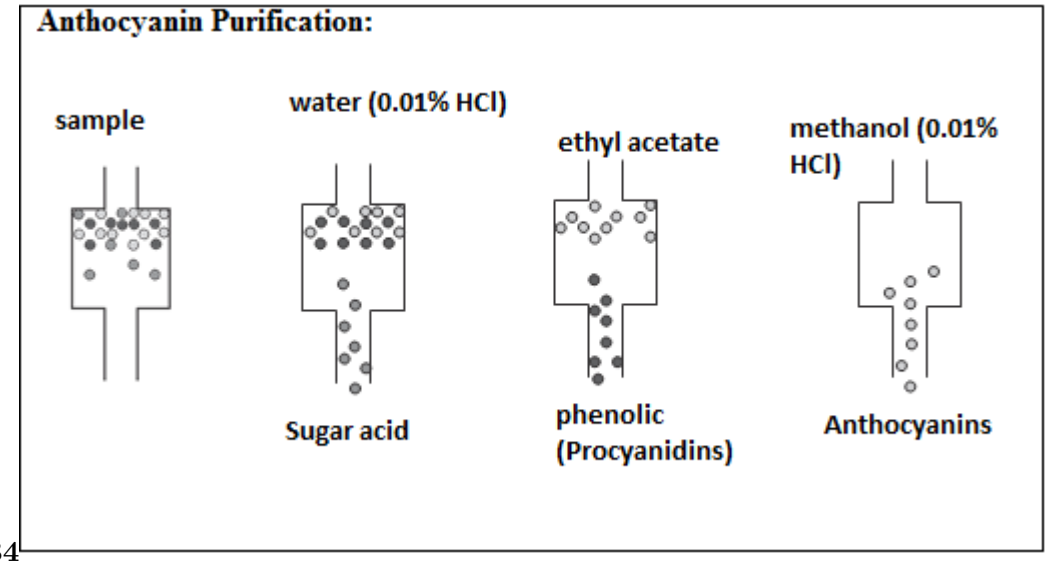
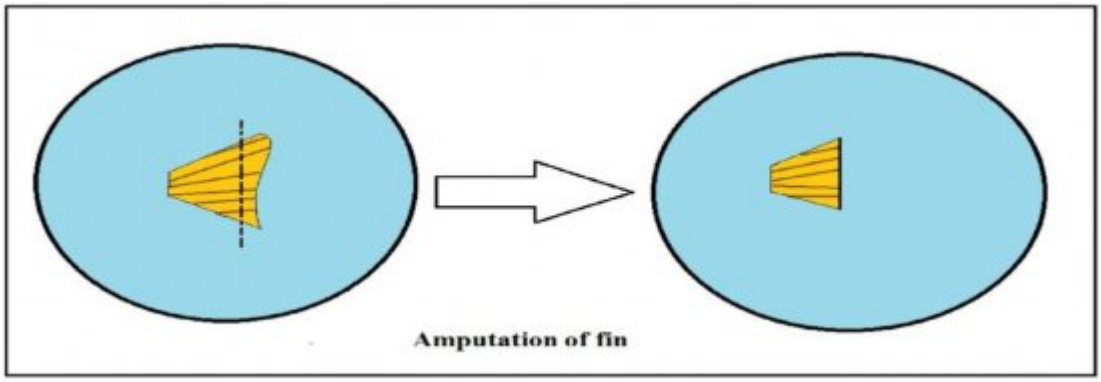


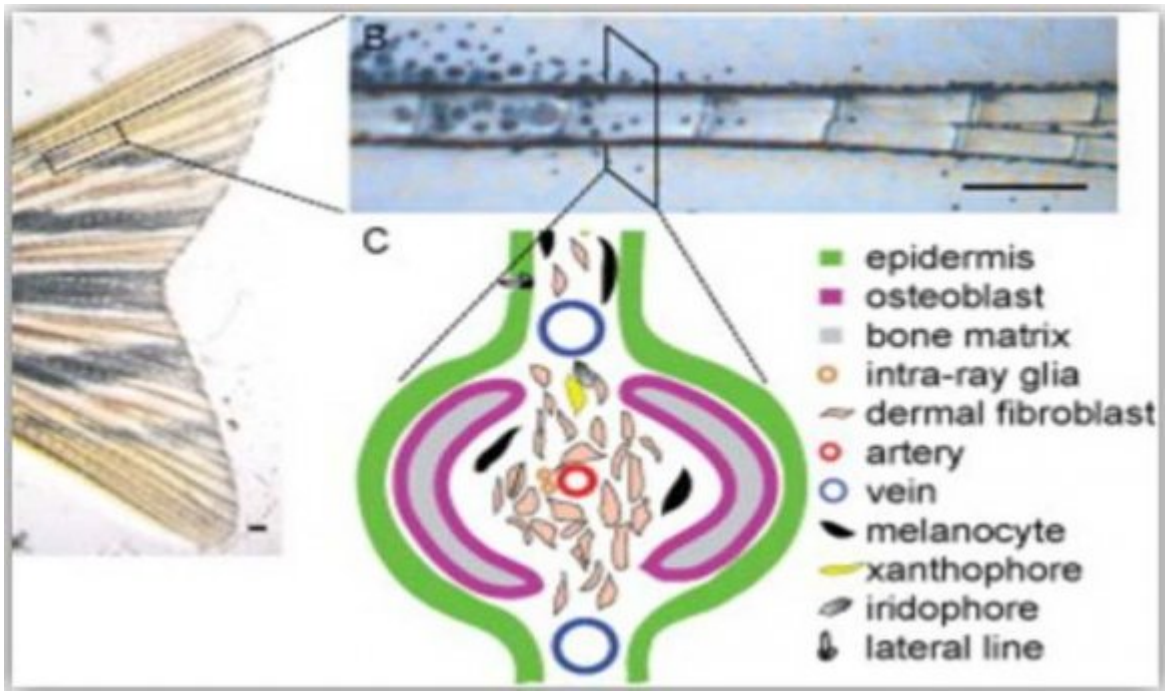
34

Figure 2: FigureFigure 3 Figure 4 :



56

Figure 3: Figure 5 :Figure 6 :



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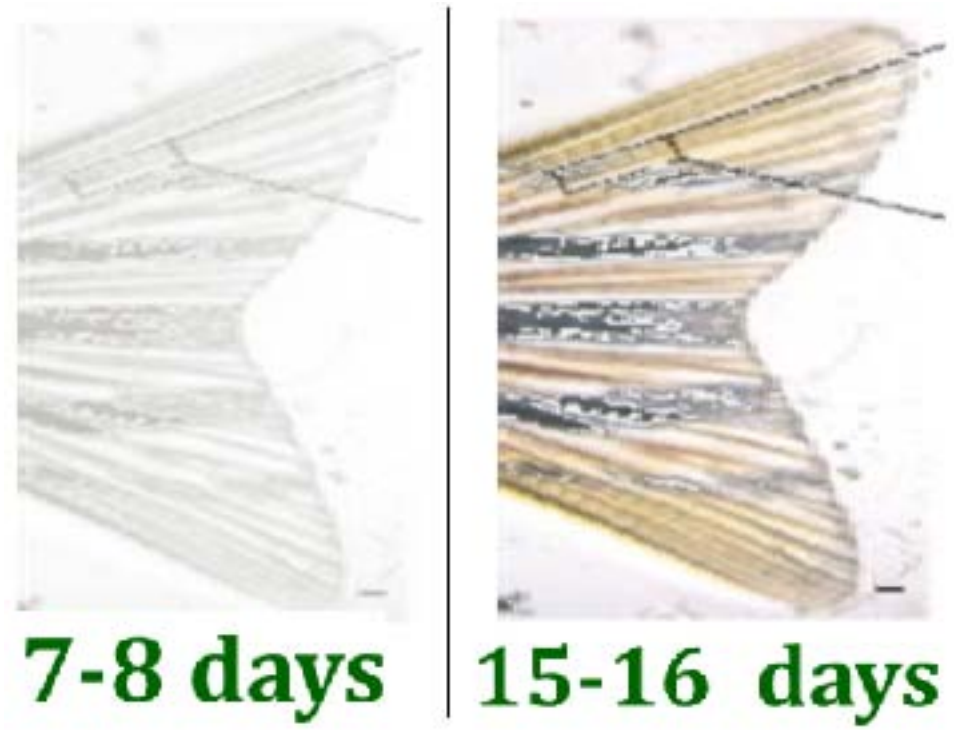

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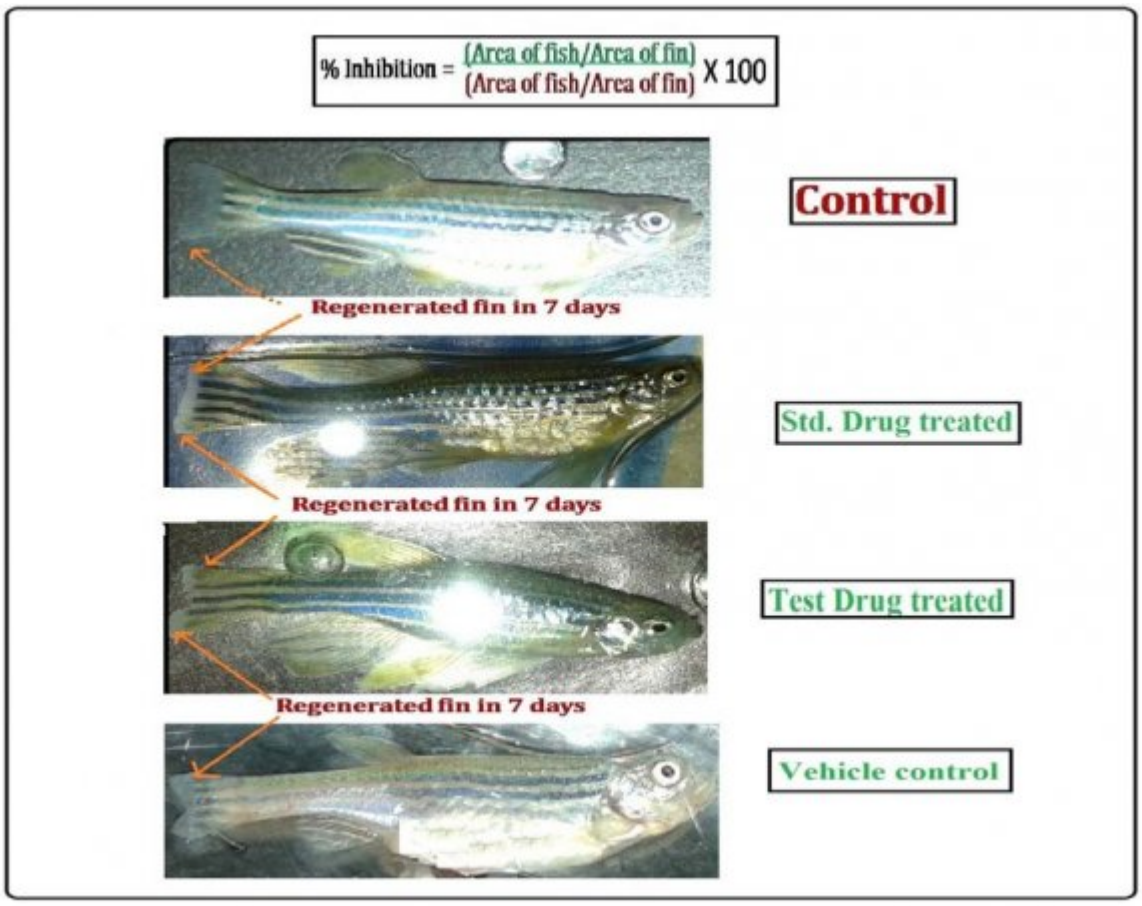
% Inhibition = (Area of fish/Area of fin) / (Area of fish/Area of fin) X 100
Control
Regenerated fin in 7 days
Std. Drug treated
Regenerated fin in 7 days
Test Drug treated
Regenerated fin in 7 days
Vehicle control

Figure 6: (

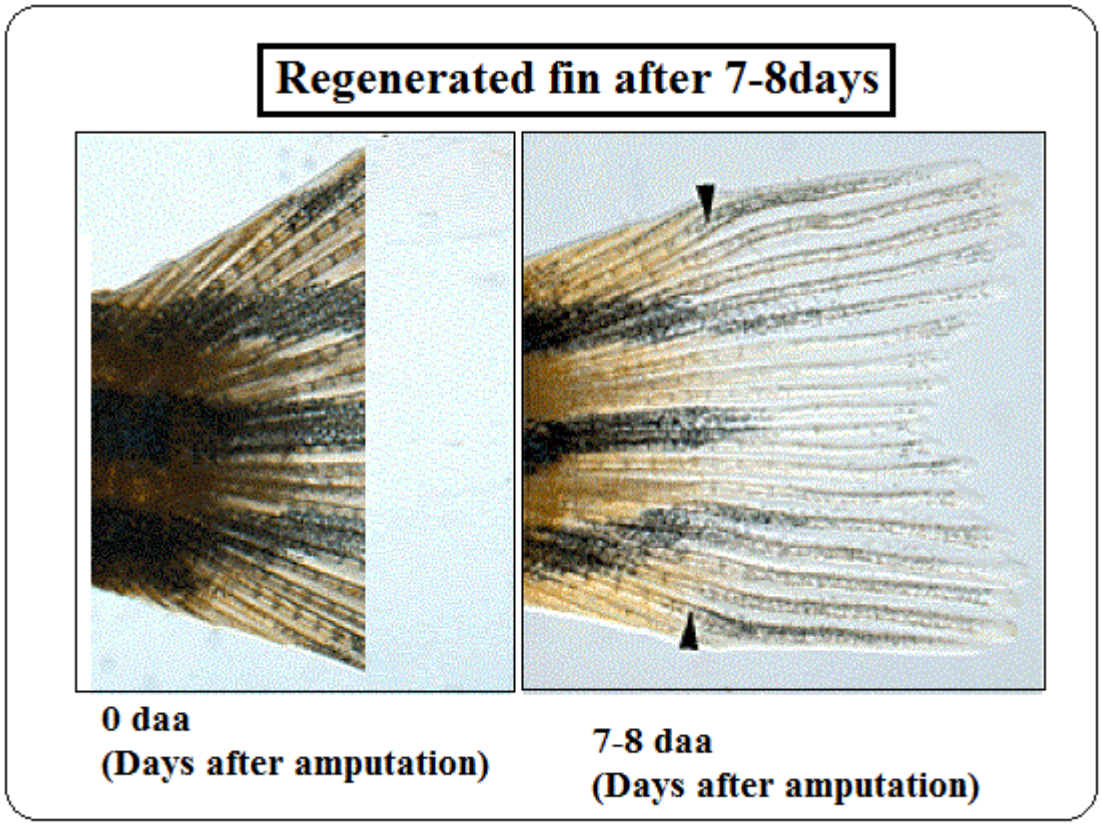
Regenerated fin after 7-8days
0 daa
(Days after amputation)
7-8 daa
(Days after amputation)

Figure 7:

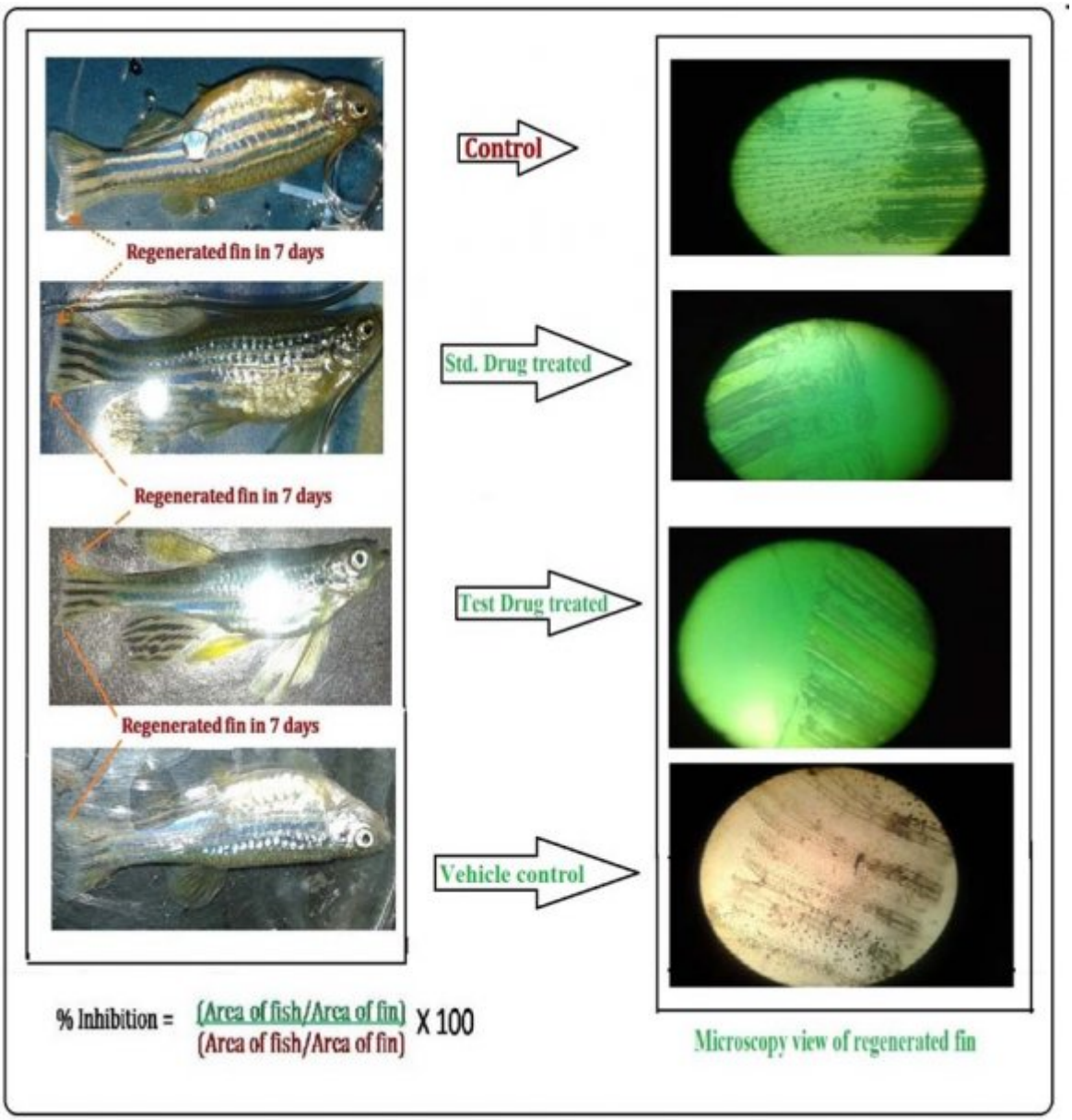

Figure 8:

2

Mean+ S. E : *= P<0.05; **= P<0.01; ***=P<0.0001

Figure 9: Table 2 :

.1 Acknowledgement

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