Research Journal of Cell Sciences

PRIME OPEN ACCESS

Volume 1, Issue 2

Research Article

Date of Submission: 07 Apl, 2025
Date of Acceptance: 30 June, 2025
Date of Publication: 17 July, 2025

Pharmacological Modulation of Adrenergic Pathways Influencing Melanophores in Fresh Water Teleost: Balantiocheilos Melanopterus

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Citation: Pathak, T., Johri, S., Bhat, J. L. (2025). Pharmacological Modulation of Adrenergic Pathways Influencing Melanophores in Fresh Water Teleost: Balantiocheilos Melanopterus. *Res J Cell Sci, 1*(2), 01-09.

Abstract

In the present study, the dorso-lateral trunk scales of Balantiocheilos melanopterus (Bleeker) were used as a model to investigate sympathetic–neural melanophore responses. Pharmacological modulation was examined using adrenergic agonists such as norepinephrine, salbutamol, terbutaline, and ephedrine, alongside adrenergic antagonists including yohimbine, propranolol, phenoxybenzamine, and metoprolol. The adrenergic agonists induced pigment dispersion in melanophores in a dose-dependent manner, indicating strong stimulation of pigment innervation. In contrast, adrenergic antagonists—particularly those blocking α2-adrenoceptors—effectively inhibited this action, suggesting a regulatory role of these receptors. The responses were quantified using the Melanophore Index (MI) in isolated scale preparations. The findings support that melanophores in B. melanopterus possess functionally active adrenoreceptors, including α2-subtypes, which mediate bidirectional pigment translocation through microtubule-associated mechanisms.

Keywords: α2-Adrenoceptors, Adrenoreceptors, Melanophore Dynamics, Pharmacological Modulation, Pigment Translocation, Teleost Fish

Introduction

Physiological color change in teleost fishes is a rapid and reversible process driven by the intracellular redistribution of pigment granules within specialized cells known as chromatophores. Among these, melanophores—responsible for black or brown coloration—play a central role in modulating body color in response to environmental cues, stress, camouflage needs, and social signaling. This pigmentory response is primarily under the control of the autonomic nervous system, although endocrine factors often act in synergy to fine-tune the reaction based on physiological and behavioral contexts.

In teleosts, melanophores are especially sensitive to sympathetic nervous stimulation. Numerous studies have demonstrated that pigment aggregation within these cells is largely mediated by post-ganglionic sympathetic fibers. This neuroregulation occurs predominantly through adrenergic neurotransmission, with norepinephrine (NE) acting as the principal effector molecule that promotes melanosome aggregation [1]. Historical investigations by Frisch (1911) and subsequent researchers have consistently shown that melanophores receive dense sympathetic innervation, highlighting a conserved neurophysiological mechanism across diverse fish taxa [2].

Adrenergic receptors on melanophores are broadly categorized into α and β subtypes. Accumulating evidence indicates that α_2-adrenoceptors are the primary mediators of pigment aggregation. Pharmacological studies have consistently shown that α_2-agonists elicit stronger and more rapid aggregation responses compared to α_1-agonists, and α_2-specific antagonists more effectively inhibit their effects [3,4]. These findings underscore the dominant regulatory role of α_2-

adrenoceptors in pigment cell function in teleosts [5,6].

In addition to catecholaminergic control, purinergic signaling also plays a modulatory role. ATP, co-released with norepinephrine at sympathetic nerve endings, is hydrolyzed extracellularly to adenosine, which binds to its receptors and promotes pigment dispersion—thus antagonizing the aggregating effect of NE [7]. This bidirectional pigment movement reflects the complex neurochemical environment governing melanophore physiology.

At the cellular level, a radial microtubule network extending from the perinuclear region to the cell periphery coordinates melanosome transport within melanophores. Motor proteins under the control of intracellular second messengers, particularly cyclic AMP (cAMP), which modulates kinesin and dynein activity regulate movement along these tracks [8]. Although Parker's dual-innervation model proposes contributions from both sympathetic (aggregating) and parasympathetic (dispersing) fibers, sympathetic regulation is generally regarded as the principal effector pathway in teleost pigment dynamics [9].

In freshwater fishes, particularly those within the family Cyprinidae, body color changes are typically manifested along a grayscale spectrum from black to white. Melanophores are the predominant chromatophore type responsible for these changes. However, the pharmacological control of melanophores in lesser-known cyprinid species remains poorly characterized. Previous studies from the Chambal river region have focused primarily on common genera such as Labeo, Catla, Cirrhinus, Garra, Rasbora, and Puntius, with little attention given to other taxa.

The present study aims to elucidate the pharmacological regulation of melanophore pigment movement in the freshwater cyprinid Balantiocheilos melanopterus, an understudied species in this regard. By employing a range of adrenergic agonists and antagonists, we investigated the receptor-specific modulation of pigment aggregation and dispersion, with particular emphasis on the functional role of α_2-adrenoceptors. This work contributes to a deeper understanding of neuropharmacological mechanisms underlying physiological color change in teleost fishes.

Materials and Methods

Fish Used

The freshwater teleost fish Balantiocheilos melanopterus (Bleeker), commonly known as Bala shark, silver shark, tricolor shark, or shark minnow, was selected for the present study. Specimens of both sexes, with average weight and length, were utilized for experimentation.

Care and Maintenance

The selected fish were treated with fresh aerated water containing potassium permanganate ($KMnO_4$) to eliminate microbial and other infections. They were maintained in transparent glass aquaria (30 × 30 × 60 cm) for a period of 10 days at a temperature range of 22–30 °C under natural photoperiodic conditions.

The aquaria were filled with aerated water maintained at a pH of 6.9–7.8. Natural photoperiod was simulated using an overhead 10 W CFL light positioned 30 cm above the water surface. Fish were fed once daily with a commercial diet (3% of the total body weight). Aquarium tanks were cleaned regularly using a drain-off method to remove fecal matter and uneaten food.

Preparation of Isolated Scale Slips

Isolated scale slips were collected from the dorsal surface of the fish (anterior to the dorsal fin) using fine forceps. These slips were immediately immersed in physiological saline solution and replaced with selected agonist or antagonist drug solutions as required.

Bidirectional movement in melanophores was observed in the area of skin attached to the isolated scale using a light microscope. The perfusion chamber was cleaned between treatments using a suction pump with an outlet pipette, Pasteur pipette, or filter paper, and subsequently refilled with the desired drug solution via an inflow pipette [10].

Assessment of Drug Effects on Melanophores

To evaluate drug responses, five preparations were made from five adjacent melanophores per fish. Each experiment thus included at least 25 melanophores from five isolated scales obtained from five individual fish. The observations were recorded using the Melanophore Index (M.I.), originally developed by Hogben and Slome (1931) [11]. This index categorizes melanophore states from Stage I (maximal pigment aggregation) to Stage V (maximal pigment dispersion).

Preparation and Administration of Drug Doses

An isotonic physiological saline solution (PSS) was prepared and used for the experiments. A K^+ -rich variant of this solution, in which equimolar NaCl was replaced with KCl, was also utilized. Stock solutions of all drugs were prepared using either PSS or distilled water. Epinephrine injections were diluted with PSS prior to use.

Drugs Used

The following agonists and antagonists were used in the study:

- **Epinephrine/Adrenaline Tartrate** (M.I. Pharmaceutical Works Pvt. Ltd., Kolkata): α- and β-agonist
- **Norepinephrine/Noradrenaline Bitartrate** (Samarth Life Sciences Pvt. Ltd., Mumbai): α- and β-agonist
- **Ephedrine Hydrochloride** (US Pharmacopeia): α- and β-agonist
- **Salbutamol** (Cipla Ltd., Mumbai): Selective β_2-agonist
- **Terbutaline Sulphate API** (A.B. Enterprises, Mumbai): β_2-agonist
- **Yohimbine** (Poul Neeuoundrof, Germany): α_2-antagonist
- **Propranolol** (Ranbaxy Laboratories Ltd., India): Non-selective β-antagonist
- **Metoprolol** (Ranbaxy Laboratories Ltd., India): β_1-selective antagonist
- **Phenoxybenzamine** (RBI, U.S.A.): Non-selective α-antagonist

Ethical Statement:

All experimental procedures involving animals were conducted in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and were approved by the Institutional Animal Ethics Committee (IAEC) of ITM University.
Approval Reference Number: IAEC/ITMU/SOP/2022-01/02.

Results and Discussion

This study investigated the pharmacological modulation of melanophore pigment dynamics in Balantiocheilos melanopterus through adrenergic agonists and antagonists, highlighting the role of specific adrenoceptor subtypes.

A range of adrenergic agonists—norepinephrine, ephedrine, salbutamol, and terbutaline—were tested for their effects on bidirectional pigment granule movement (melanosomes) in isolated scale melanophores. These cells exhibited a fully dispersed state (Melanophore Index, M.I. = 5) after equilibration in physiological saline solution (PSS) for 15 minutes.

Norepinephrine (NE), a catecholamine and sympathetic neurotransmitter, triggered potent aggregation of melanosomes in a concentration-dependent manner. The optimal aggregation (M.I. = 1) occurred at 10^{-6} M within 10 minutes. Upon removal and perfusion with PSS, dispersion gradually resumed to M.I. = 5 over 60 minutes, indicating reversibility of NE's action.

Ephedrine, a non-catechol α- and β-adrenergic agonist, also induced melanosome aggregation in a dose-dependent manner. Full aggregation (M.I. = 1) was noted at 10^{-6} M, and subsequent PSS perfusion restored dispersion over 60 minutes, confirming its adrenomimetic activity.

Salbutamol, a selective β_2-agonist, was applied to melanophores pre-aggregated with epinephrine (10^{-6} M). At 10^{-4} M, salbutamol induced complete pigment dispersion within 15 minutes. This response was entirely blocked by propranolol (10^{-5} M), a non-selective β-antagonist, confirming the involvement of β-adrenoceptors in the dispersion mechanism.

Similarly, terbutaline, another β-agonist, reversed epinephrine-induced aggregation. At 10^{-4} M, re-dispersion initiated within 5 minutes and was complete within 30 minutes, further validating the role of β-receptors in pigment dispersion.

To examine receptor subtype contributions, adrenergic antagonists were employed. Yohimbine, a selective α_2-adrenoceptor antagonist, maintained melanophores in a dispersed state (M.I. = 5) even after epinephrine treatment, highlighting effective α_2-receptor blockade.

Propranolol (10^{-5} M) alone did not affect baseline dispersion. However, after epinephrine administration, aggregation ensued (M.I. = 1), and only partial dispersion (M.I. = 4.8) occurred upon PSS treatment, indicating transient β-receptor blockade.

Phenoxybenzamine, a non-selective irreversible α-adrenoceptor antagonist, failed to allow phenylephrine-induced aggregation, even after 60 minutes. This suggests stable blockade of α-receptors, with α_2-receptors likely playing the predominant role.

Finally, metoprolol, a β_1-selective antagonist, was tested following sequential treatments. After epinephrine-induced aggregation and salbutamol-induced dispersion, pretreatment with metoprolol inhibited salbutamol's effect, preventing re-dispersion. This confirmed selective β_1-receptor involvement in regulating β_2-agonist responses. These experimental outcomes align with established literature describing dynamic melanophore responses in teleosts during stress, background adaptation, and social interaction. Color change in fishes is orchestrated via sympathetic nervous control, mediated by pigment redistribution through cytoskeletal elements such as microtubules and actin filaments [12].

The current findings underscore a dominant role for α_2-adrenoceptors in melanosome aggregation and β-adrenoceptors in dispersion. Yohimbine's blockade of NE-induced aggregation supports the presence of functional α_2-receptors, while the limited efficacy of phenoxybenzamine suggests a secondary role for α_1-receptors. This selective receptor profile is

consistent with reports in other cyprinids [9,10].

Additionally, the effects of β-agonists and their inhibition by propranolol and metoprolol affirm the participation of β-adrenoceptors in pigment dispersion. These receptors may also contribute to the "excitement darkening" effect or socially modulated pigmentation, contrasting with α-receptor-mediated background-induced paling [13].

In Summary, Balantiocheilos melanopterus melanophores are under dual adrenergic control, with α_2-adrenoceptors primarily mediating pigment aggregation and β-adrenoceptors responsible for dispersion. These insights reinforce the species' utility as a model for studying adrenergic regulation of chromatophore physiology. Future research focusing on receptor localization and intracellular signaling cascades could further elucidate the underlying molecular mechanisms.

Conclusions

The present study demonstrates that melanophore responses in Balantiocheilos melanopterus are predominantly regulated by adrenergic mechanisms involving post-ganglionic sympathetic pigment-aggregating fibers. The aggregation of melanosomes within these chromatophores is primarily mediated via α2-adrenoceptors located on the cell membranes, as evidenced by the strong responses to α2-agonists and their effective inhibition by α2-antagonists. The efficacy of both adrenomimetic and adrenolytic agents highlights the pharmacological sensitivity and regulatory complexity of the pigmentory system in this species.

Furthermore, the results suggest the probable co-existence of both α1 and α2-adrenoceptor subtypes on the melanophores of B. melanopterus, consistent with earlier findings in other teleosts such as Puntius spp. Labeo rohita, Clarias sp. and Rasbora elanga [7,14-16]. These findings collectively support the presence of a conserved yet nuanced adrenoceptor-based regulatory mechanism in teleost melanophores. The study provides valuable groundwork for further exploration into the adrenergic control of chromatophores and their role in physiological color change among freshwater teleosts [17-36].

Acknowledgments

The authors express their sincere gratitude to the Dean, School of Sciences, ITM University, Gwalior, India, for providing the necessary research facilities and institutional support that enabled the successful completion of this study.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this research. All experiments were conducted independently, without any financial or commercial relationships that could be construed as a potential conflict of interest.

S. No.	Ingredients/Chemicals	Quantity in /100ml.
1	Sodium Chloride (NaCl)	12.8
2	Potassium chloride (KCl)	2.68
3	Calcium Chloride	1.8
4	Glucose	5.6
5	Sodium hydroxide buffer(Hepes)	5.0
6	Ph. value	7.4

Table 1. Composition of Physiological Saline Solution used in present study

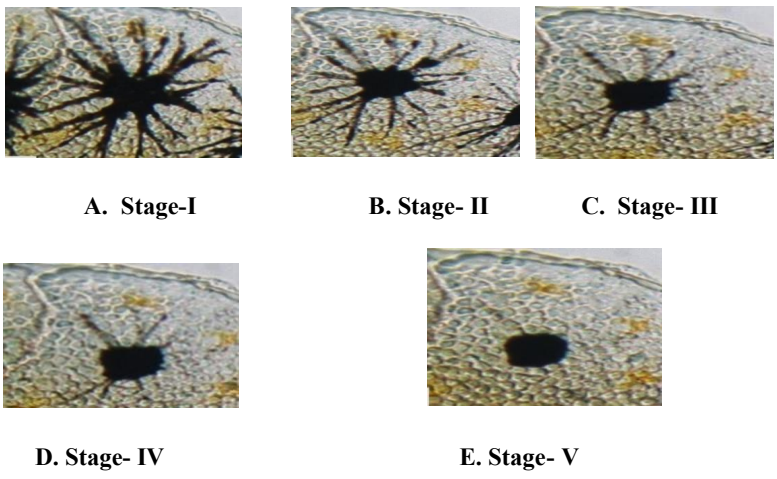

Figure 1: Sequential Microscopic photographs of a typical single melenophore showing Aggregation of melanosome on Melenophore Index (MI). As utilized for assessment of effect of drug induced (in vitro) in relation to Bidirectional movement in Melenophore of Selected fish species

(A) MI= 5: Fully Dispersed State
(B) MI= 4
(C) MI= 3 } **MI 4, 3 and 2 showing intermediate state of melanosome**
(D) MI= 2 }
(E) MI= 1: Fully Aggregate State

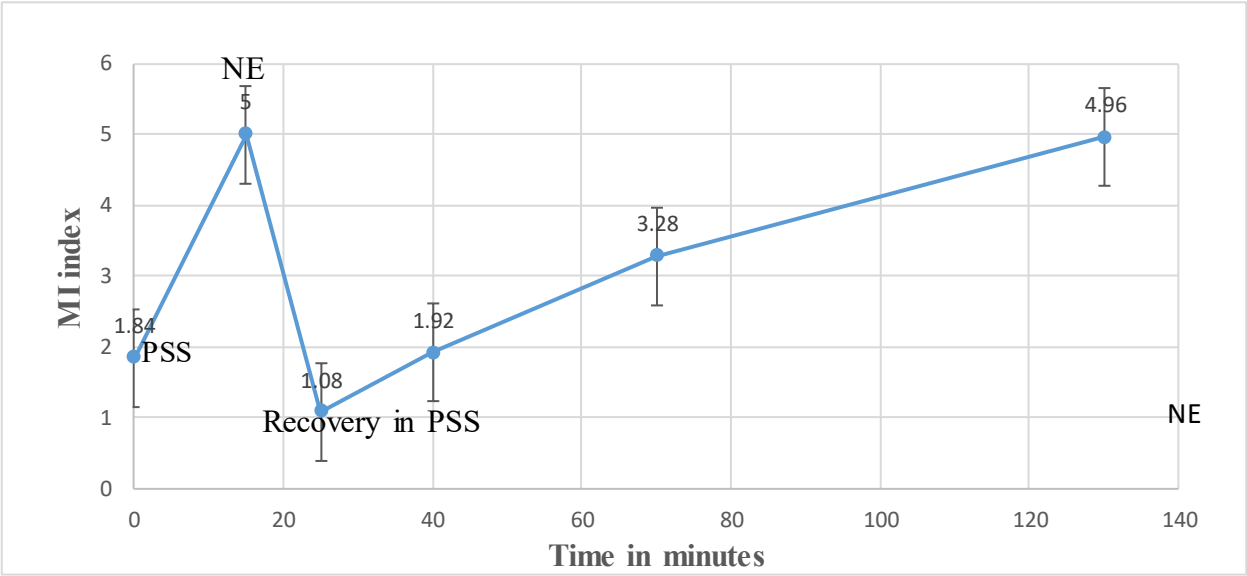

Figure 2: Melanosome Aggregation in melenophore when treated with NE (Norepinephrine) with 10-6M and then recovery in PSS. The data are shown as mean± SEM via five measurements on scale slips of five different individuals of selected fish B. melanopterus.

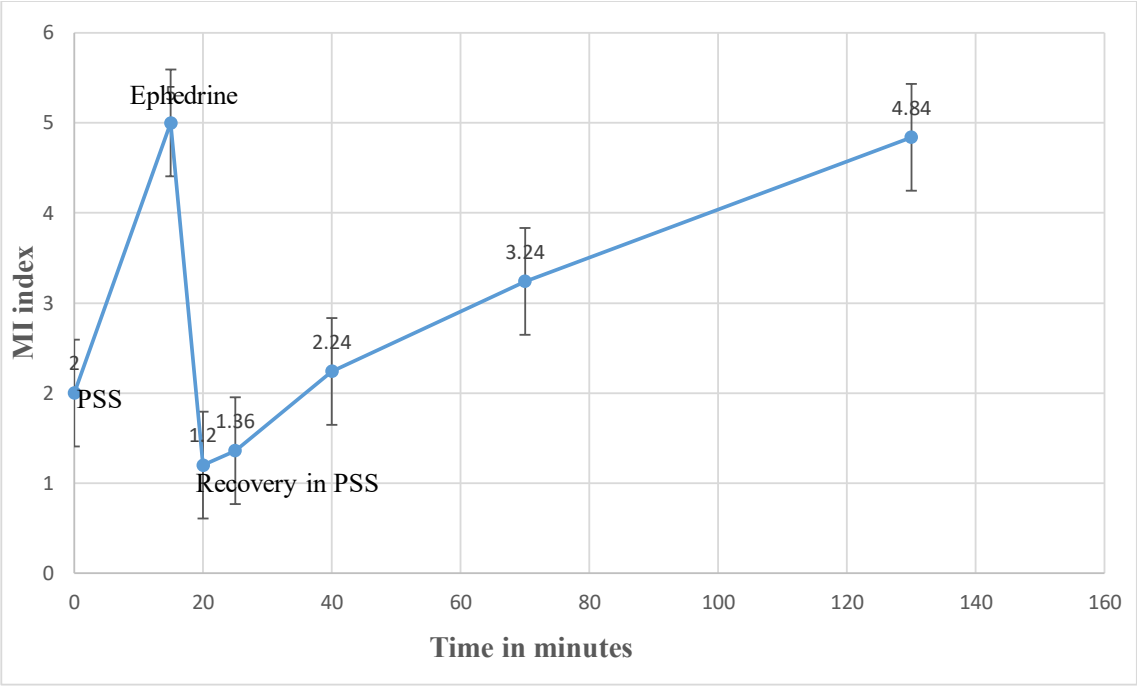

Figure 3: Melanosome Aggregation in melenophore when treated with Ephedrine with 10-6M and then recovery in PSS. The data are shown as mean± SEM via five measurements on scale slips of five different individuals of selected fish B. melanopterus.

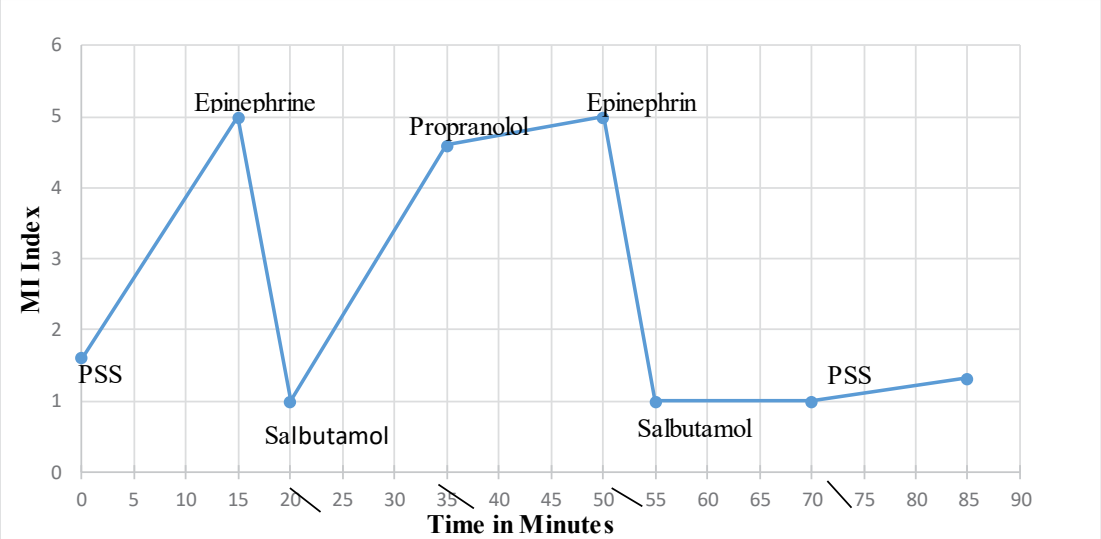

Figure 4: Effects of Salbutamol (10-4 M) on β- adrenoceptors blocked melanophores, induced by epinephrine (10-6M). The data are shown as Mean± SEM via five measurements on scale slips of five different individuals of selected fish B. melanopterus

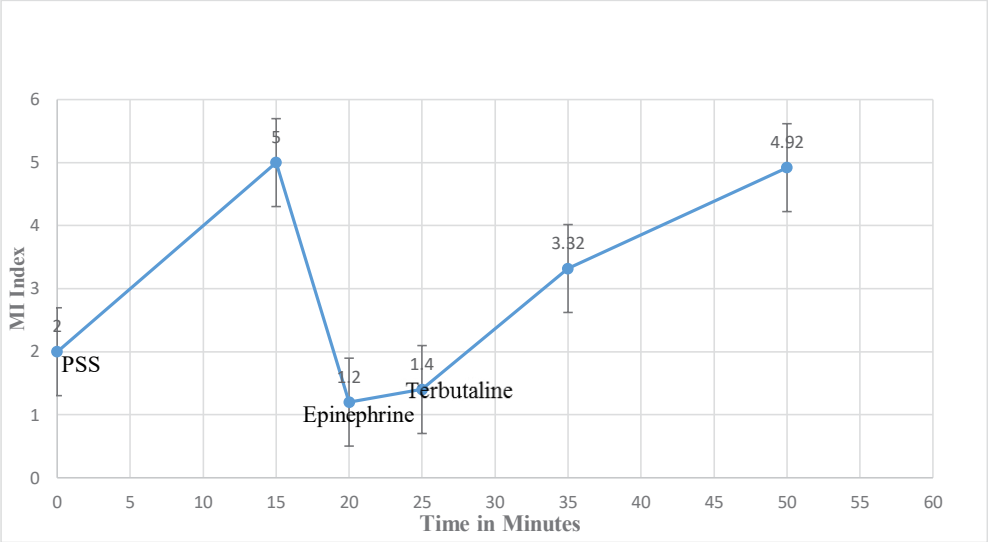

Figure 5: Effects of Terbutaline (10-4 M) on the Epinephrine (10-6 M) induced aggregation of melanophores in fish. The data are shown as Mean± SEM via five measurements on scale slips of five different individuals of selected fish B. melanopterus.

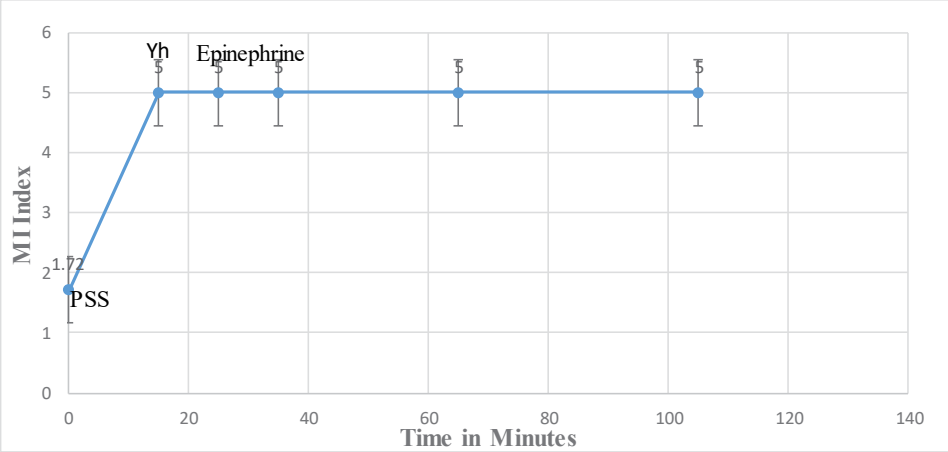

Figure 6: Complete blockade of epinephrine (10-6 M) induced melenophore aggregation via pretreatment of α2–adrenoceptor blocker, yohimbine (10-5 M). The data are shown as means ± SEM from five measurements on scale slips from five different individuals of selected fish B. melanopterus

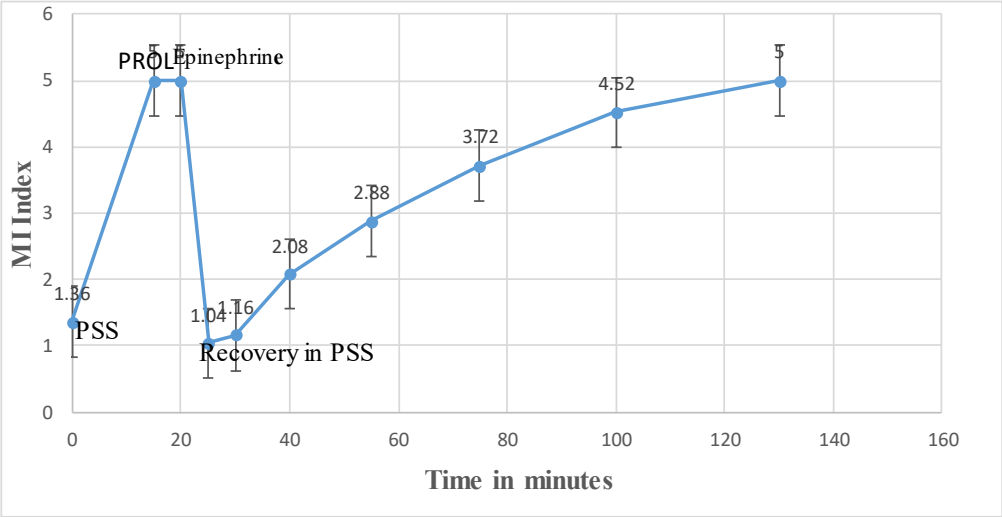

Figure 7: Effects of Propranolol (10-5 M) on the Epinephrine (10-6 M) induced aggregation of melanophores in fish scale. The data are shown as Mean± SEM via five measurements on scale slips of five different individuals of selected fish B. melanopterus

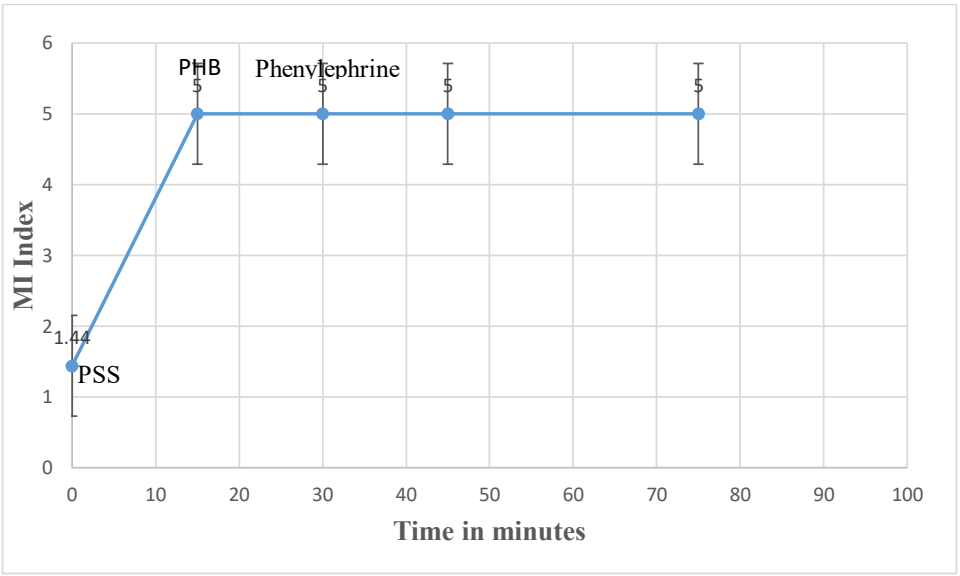

Figure 8: Complete blockade the effects of phenylephrine (10-6 M) aggregation inducing by the treatment with adrenoceptor blocker phenoxybenzamine (10-6 M). The values are expressed as mean ±SD from five different fishes

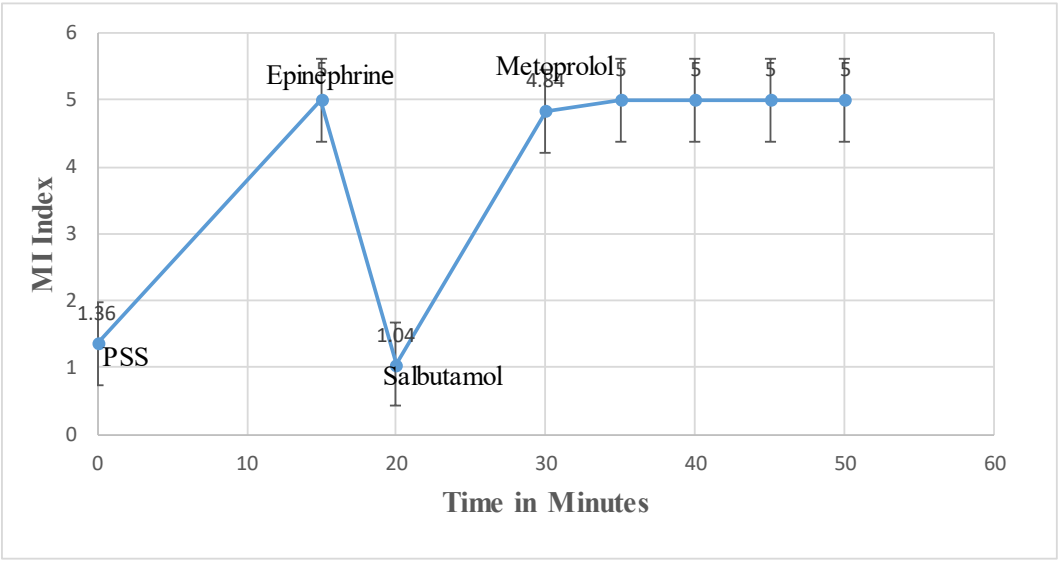

Figure 9: Effect of Metoprolol (10-4 M) on Epinephrine (10-6 M) induced aggregation of melanophores of fish. The data are shown as Mean± SEM via five measurements on scale slips of five different individuals of selected fish B. melanopterus

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