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Pharmacological Modulation of Adrenoreceptors Regulating Melanophore Dynamics in the Freshwater Teleost Fish, Balantiocheilos melanopterus

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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:
Melanophore dynamics, Adrenoreceptors, Pharmacological modulation, Teleost fish, α2-adrenoceptors, Pigment translocation

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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 pigmentary 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 (David, 2007; Bear et al., 2007). 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.

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 their effects are more effectively inhibited by α_2-specific antagonists (Fujii, 2000; Karlsson et al., 1987). These findings underscore the dominant regulatory role of α_2-adrenoceptors in pigment cell function in teleosts (Burton & Vokey, 2000; Acharya & Ovais, 2007).

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 (Rather & Jain, 2010; Agarwal, 2016; Yadav, 2016). This bidirectional pigment movement reflects the complex neurochemical environment governing melanophore physiology.

At the cellular level, melanosome transport within melanophores is coordinated by a radial microtubule network extending from the perinuclear region to the cell periphery. Movement along these tracks is regulated by motor proteins under the control of intracellular second messengers, particularly cyclic AMP (cAMP), which modulates kinesin and dynein activity (Nascimento et al., 2003). 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 (Singh & Jain, 2017; Yadav, 2021).

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* (Dubey et al., 1980), 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 (as per Rather and Jain, 2012; Singh and Jain, 2017).

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). 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

Effects of Adrenergic Agonists on Melanophore Pigment Dynamics
A range of adrenergic agonists including norepinephrine, ephedrine, salbutamol, and terbutaline were evaluated to assess their effects on the bidirectional movement of pigment granules (melanosomes) in the melanophores of B. melanopterus.

Effect of Norepinephrine
Norepinephrine (NE), a catecholamine released from the adrenal medulla, demonstrated potent aggregation of melanosomes. Isolated scale melanophores in physiological saline solution (PSS) equilibrated for 15 minutes showed full

dispersion (Melanophore Index, M.I. = 5). Upon exposure to NE at concentrations ranging from 10^{-8} to 10^{-5} M, the optimal response was observed at 10^{-6} M, with complete aggregation (M.I. = 1) achieved within 10 minutes. Subsequent perfusion with PSS resulted in gradual dispersion, returning to M.I. = 5 over 60 minutes, indicating the reversible nature of NE's effect (Fig. 1).

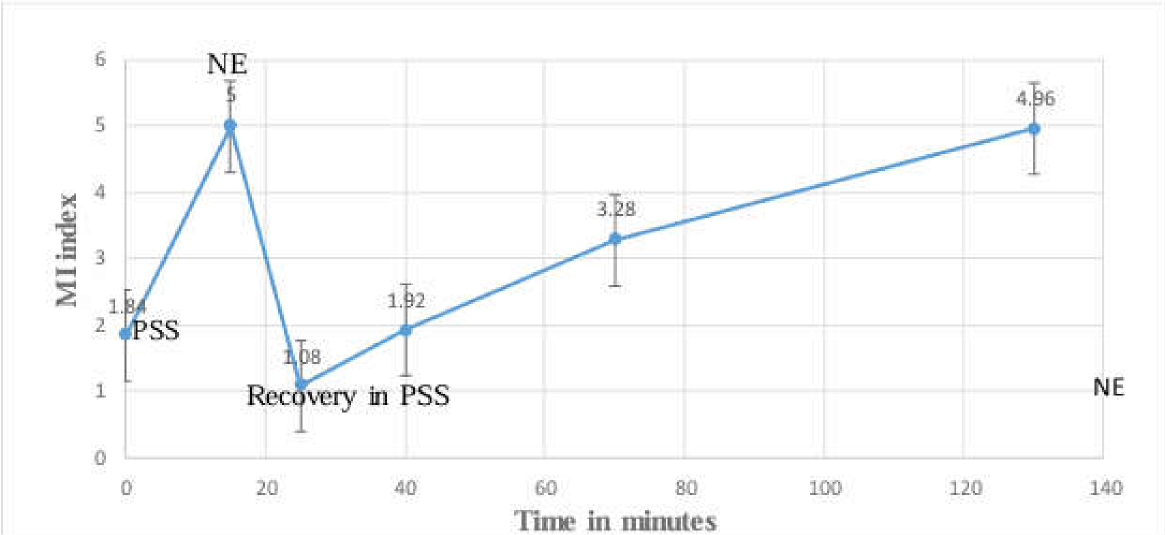

Figure 1: Melanosome aggregation in melanophores was observed following treatment with 10^{-6} M norepinephrine (NE), followed by recovery in physiological saline solution (PSS). The results are presented as mean ± SEM, based on five measurements taken from scale slips of five individual Bala shark (B. melanopterus) specimens.

Effect of Ephedrine

Ephedrine, a non-catechol α- and β-adrenergic agonist, induced concentration-dependent aggregation of melanosomes. Following equilibration in PSS (M.I. = 5), treatment with ephedrine (10^{-8} to 10^{-5} M) produced gradual aggregation, with full aggregation (M.I. = 1) at 10^{-6} M. Restoration of dispersion upon PSS perfusion was slow, achieving M.I. = 5 within 60 minutes, confirming ephedrine's adrenomimetic activity (Fig. 2).

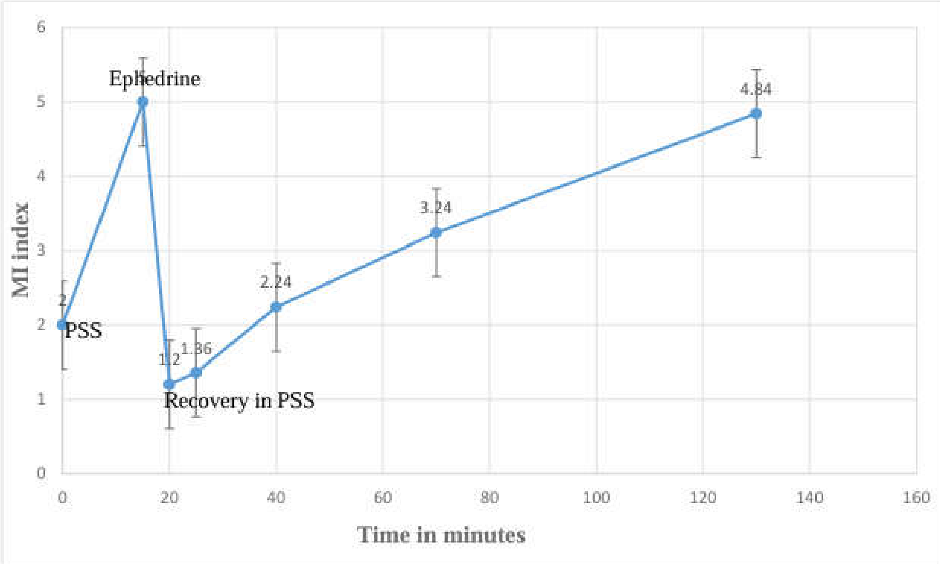

Figure 2: Melanosome aggregation in melanophores was induced by treatment with 10^{-6} M ephedrine, followed by recovery in physiological saline solution (PSS). Data are expressed as mean ± SEM from five measurements on scale slips taken from five different individuals of the fish species B. melanopterus.

Effect of Salbutamol

Salbutamol, a selective β_2-adrenoceptor agonist, was tested on melanophores pre-aggregated with epinephrine (10^{-6} M). Treatment with salbutamol (10^{-4} M) reversed aggregation, restoring full dispersion within 15 minutes. Pretreatment with propranolol (10^{-5} M), a non-selective β-antagonist, blocked this effect completely. Even after subsequent treatments with salbutamol or PSS, dispersion was not re-established, confirming the β-adrenoceptor-mediated action of salbutamol and its inhibition by propranolol (Fig. 3)

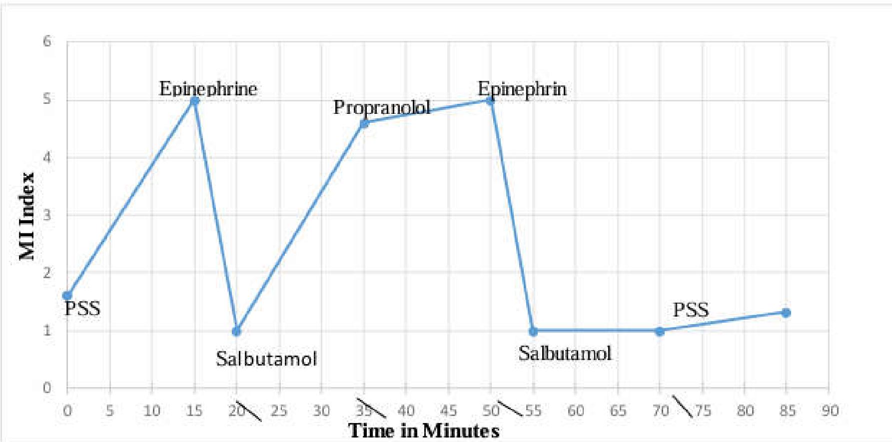

Figure 3: Effects of Salbutamol (10-4 M) on β- adrenoceptors blocked melanophores, induced by epinephrine (10-6M). The data are shown as Mean± SEM via five measurements on scale slips of five different individuals of selected fish B. melanopterus.

Effect of Terbutaline

Terbutaline, another β-agonist, was applied following epinephrine-induced aggregation (10^{-6} M). At a concentration of 10^{-4} M, terbutaline promoted re-dispersion of melanosomes, initiating recovery within 5 minutes and achieving complete dispersion (M.I. = 5) in 30 minutes (Fig. 4).

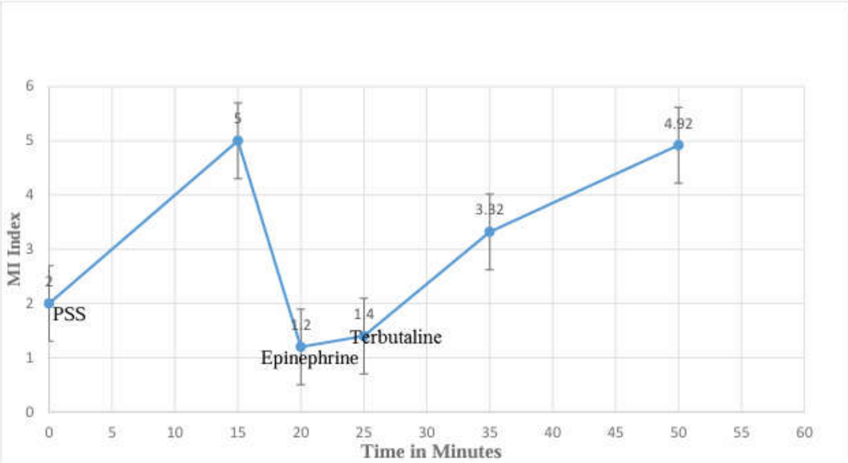

Figure 4: Effects of terbutaline (10^{-4} M) on epinephrine (10^{-6} M)-induced melanophore aggregation in fish were examined. The data are presented as mean ± SEM, based on five measurements from scale slips of five individual B. melanopterus specimens.

Effects of Adrenergic Antagonists on Melanophore Pigment Dynamics

Adrenergic antagonists including yohimbine, propranolol, phenoxybenzamine, and metoprolol were employed to investigate their role in modulating pigment movement via adrenoceptor blockade.

Effect of Yohimbine

Yohimbine, a selective α_2-adrenoceptor antagonist, maintained melanophores in a dispersed state (M.I. = 5) when applied at 10^{-5} M for 10 minutes. Subsequent treatment with epinephrine (10^{-6} M) failed to induce aggregation, and melanophores remained dispersed even after 50 minutes of incubation in PSS, demonstrating effective α_2-receptor blockade (Fig. 5).

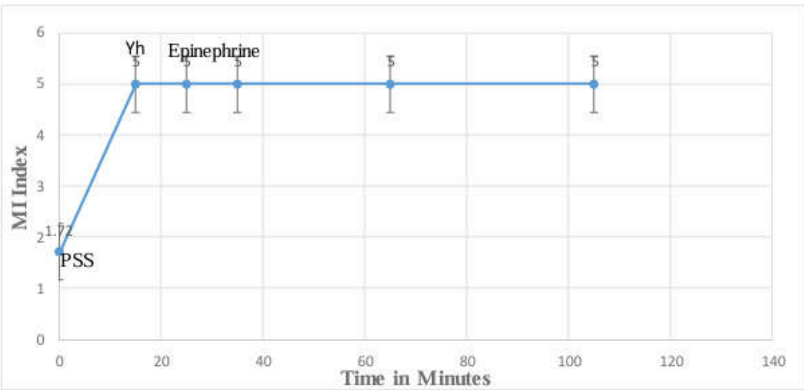

Figure 5: Complete inhibition of epinephrine (10^{-6} M)-induced melanophore aggregation was achieved by pretreatment with the α_2-adrenoceptor antagonist yohimbine (10^{-5} M). Data are expressed as means ± SEM from five measurements on scale slips obtained from five individual B. melanopterus specimens.

Effect of Propranolol

Propranolol (10^{-5} M), a non-selective β-blocker, did not alter the dispersion state of melanophores (M.I. = 5). Following epinephrine administration (10^{-6} M), rapid aggregation (M.I. = 1) was observed within 5 minutes. Upon drug removal, partial dispersion resumed (M.I. = 4.8) within 20 minutes, confirming propranolol's transient blocking effect on β-receptor-mediated responses (Fig. 6).

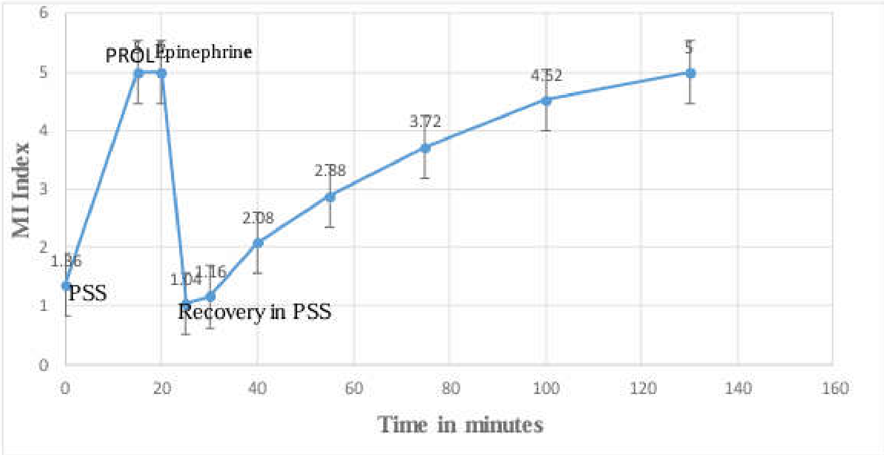

Figure 6: The effects of propranolol (10^{-5} M) on epinephrine (10^{-6} M)-induced melanophore aggregation in fish scales were investigated. Data are presented as mean ± SEM based on five measurements from scale slips of five different B. melanopterus individuals.

Effect of Phenoxybenzamine

Phenoxybenzamine, a non-selective irreversible α-adrenoceptor antagonist, was applied to disperse melanophores (10^{-6} M, 15 minutes). Melanophores retained full dispersion (M.I. = 5). Subsequent administration of phenylephrine (10^{-6} M), an α-agonist, did not alter pigmentation even after 60 minutes, confirming stable and irreversible α-receptor blockade (Fig. 7).

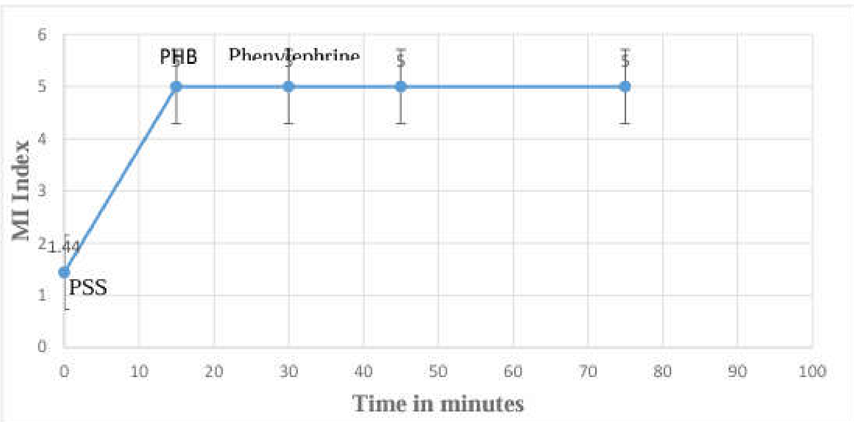

Figure 7: The phenylephrine (10^{-6} M)-induced aggregation was completely blocked by treatment with the adrenoceptor antagonist phenoxybenzamine (10^{-6} M). Values are expressed as mean ± SD from five different fish.

Effect of Metoprolol

Metoprolol, a β_1-selective antagonist, demonstrated its blocking action following a sequential treatment approach. Initially, epinephrine (10^{-6} M) induced full aggregation, which was reversed by salbutamol (10^{-4} M), leading to full dispersion. Upon metoprolol pretreatment, salbutamol failed to elicit dispersion in aggregated melanophores, confirming selective β_1-receptor inhibition and its role in preventing β_2-agonist action (Fig. 8).

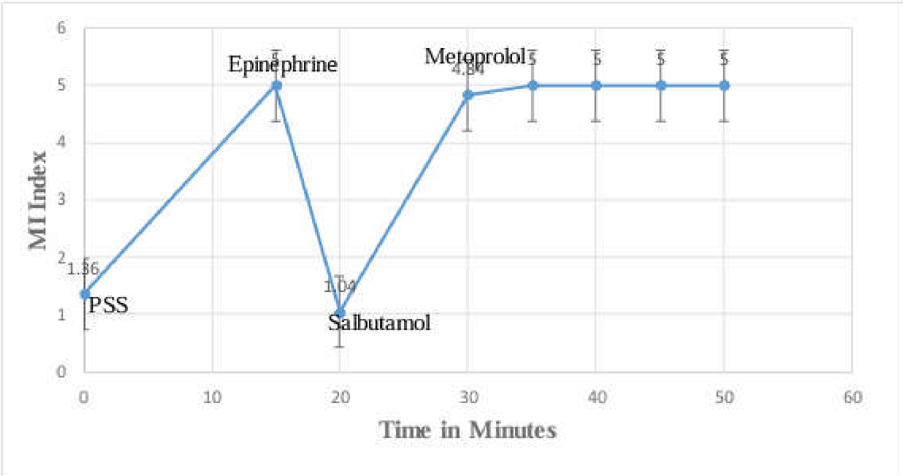

Figure 8: The effect of metoprolol (10^{-4} M) on epinephrine (10^{-6} M)-induced melanophore aggregation in fish was evaluated. Data are presented as mean ± SEM from five measurements on scale slips of five individual B. melanopterus specimens.

DISCUSSION

Teleost fish's exhibit dynamic color changes in response to environmental stimuli, often becoming darker against a dark background and paler in light surroundings. These changes are mediated by complex interactions between the neural and endocrine systems, frequently triggered by stress, background adaptation, or social stimuli. Among the cellular components involved, melanophores play a central role, with pigment redistribution being facilitated by intracellular molecular motors and cytoskeletal elements, particularly microtubules and actin filaments (Aspengren et al., 2006).

In many freshwater teleosts, melanophores are highly responsive to chemical and hormonal cues. These cells have therefore been employed in numerous studies as bioindicators for assessing the physiological impact of various exogenous agents, including neurotransmitters, environmental toxins, and pharmaceuticals (Chaplen et al., 2002; Dierksen et al., 2004; Mojovic et al., 2004; Sharma et al., 2005; Dukovcic et al., 2010; Munakata & Kobayashi, 2012). The present study, employing melanophore responses from the isolated scales of *Balantiocheilos melanopterus*, aimed to investigate the adrenoceptor subtypes involved in mediating pigment aggregation and dispersion. Melanophore activity was assessed using a modified Melanophore Index (MI) based on the classical Hogben and Slome method, which quantified pigment states on a scale from 5 (fully dispersed) to 1 (fully aggregated) (Fig. 9).

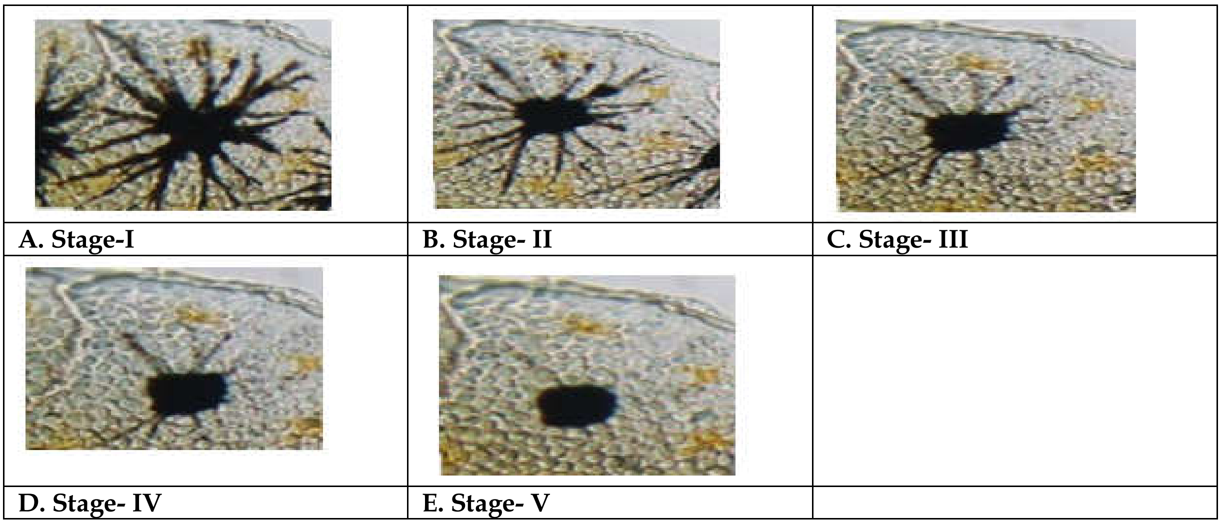

Figure 9: Sequential Microscopic photographs of a typical single melenophore showing Aggregation of melanosome on Melenophore Index (MI). As utilized for assessment of effect of drug induced (in vitro) in relation to Bidirectional movement in Melenophore of Selected fish species. (A) MI= 5: Fully Dispersed 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

Our findings demonstrate that melanophores in *B. melanopterus* are predominantly regulated by sympathetic postganglionic fibers, consistent with adrenergic modulation reported in other teleost species (Rather & Jain, 2012; Yadav, 2021). Norepinephrine, a key sympathetic neurotransmitter, exhibited significant pigment-aggregating effects at a concentration of 10^{-6} M. This aggregation response supports the hypothesis that melanosome centripetal transport is mediated by α-adrenoceptors, likely through α2-subtype receptors. The strong aggregation response to norepinephrine and ephedrine, both of which are sympathomimetic amines, reinforces the presence of adrenergic regulation in this species.

Application of α-adrenergic antagonists further elucidated the receptor subtype involvement. Yohimbine, a selective α2-adrenoceptor antagonist, effectively inhibited pigment aggregation induced by norepinephrine, suggesting a predominant role for α2-adrenoceptors in melanophore control. In contrast, phenoxybenzamine, a non-selective α1-antagonist, demonstrated limited efficacy, implying a minor or secondary role of α1-adrenoceptors in this process. These results indicate that α2-adrenoceptors are the principal mediators of norepinephrine-induced aggregation in *B. melanopterus*, although the existence of a mixed population of α1 and α2 receptors cannot be entirely excluded.

In addition to α-receptors, β-adrenoceptors were also examined. The dispersion of pigment granules observed upon β-agonist application, and the significant inhibition of this effect by β-blockers such as propranolol and metoprolol, indicate the functional presence of β-adrenoceptors on melanophores. These findings are consistent with previous reports suggesting that β-adrenoceptors mediate pigment dispersion in teleosts and play a role in background adaptation and stress-induced coloration (Yadav & Jain, 2017).

Notably, β-receptors are often associated with the "excitement darkening" phenomenon and socially driven pigmentation changes in fish, while α-receptors are linked to rapid aggregation and background-induced paling (Viamonte et al., 1991; Meitzen et al., 2011; Irion & Volhard, 2022). This study supports such a distinction in receptor function, with α2-receptors primarily involved in aggregation and β-receptors in dispersion mechanisms.

In Summary, the results of this study reveal that melanophore responses in *Balantiocheilos melanopterus* are under strong adrenergic control, primarily mediated by α2-adrenoceptors for pigment aggregation and β-adrenoceptors for dispersion. These findings contribute valuable insights into the neuropharmacology of pigment cells in cyprinid fishes and establish *B. melanopterus* as a promising model for further studies on adrenergic regulation of chromatophore physiology. Future work investigating receptor expression patterns and downstream signaling pathways could further elucidate the molecular mechanisms underlying these responses.

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. (Agarwal, 2016), *Labeo rohita* (Jain & Patil, 1992), *Clarias* sp. (Singh, 2015), and *Rasbora elanga* (Yadav, 2017). 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.

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Author Contributions
Trapti Pathak conducted the research and experimental work on the selected freshwater fish species. The study was carried out under the guidance and supervision of co-authors Dr. Sonia Johri and Dr. J.L. Bhat, who provided conceptual support, critical revisions, and oversight throughout the research process.

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