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Ameliorative Effects of Methanolic extract of *Erythrina variegate* in Scopolamine-Induced Amnesic in Adult Zebrafish (*Daniorerio*) Model

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ABSTRACT

Erythrina variegate (EV) possesses a wide range of biological activities, including-anticancer,-antidiabetic, hepatoprotective, -antirheumatic, antibacterial, and antiviral properties. Methanolic extract of *Erythrina variegate* (MetEEV) has not been studied for its neuroprotective properties. The purpose of this study was to look into the effect of MetEEV-on-scopolamine (Sco)-induced anxiety, amnesia, and brain oxidative stress in zebrafish, in addition to figuring out the underlying mechanism. The zebrafish were given MetEEV (260, 520, and 780g/ L) for seven days in a row before being given Sco (100µM) for 30 minutes before the behavioral tests (locomotor activity and cognitive function). The *in-vivo* antioxidant status and Acetylcholinesterase- (AChE) -activity were also -evaluated for the further assessment of possible mechanisms of MetEEV in enhancing memory capacities in zebrafish. MetEEV-could-improve-memory-and-reduce-brain oxidative stress in sco-induced zebrafish, as well as regulating cholinergic function by inhibiting AChE activity. Our findings suggest that MetEEV could be a promising candidate compound for treating amnesia by restoring cholinergic activity and alleviating brain oxidative stress.

INTRODUCTION

Alzheimer's disease (AD) is a neurological disorder characterized by declines in memory, thinking, behavior, and cognitive function **(Katsouri *et al.*, 2020)**. Most AD patients are older than 65. Nearly 10 million new instances of dementia are recorded each year, affecting approximately 50 million individuals worldwide **(Gustavsson *et al.*, 2022)**. More than 5 million Americans suffer from AD **(Jadenur *et al.*, 2022)**, and the number of people over 65 with the condition doubles every 5 years **(Peeters *et al.*, 2022)**.

AD is a neurological disease that worsens over time. Two histopathological features of AD include neurofibrillary tangles (NFTs) composed of hyperphosphorylated microtubule associated proteins, and extracellular amyloid plaques (Aβ plaques) **(Otero-Garcia *et al.*, 2022)**. Microglia, also referred to as neurotoxic microglia, is stimulated by the development of

amyloid plaques to signal via toll-like receptors (TLRs) and receptors for advanced glycation end- products (RAGE). Since the main neurons are involved in learning and memory, this directly affects cholinergic neurons by inducing transcription factors like NF-B and AP-1 that produce ROS and stimulate the release of inflammatory mediators such as cytokines (TNF- and IL-1) **(Thal & Tome, 2022)**.

A defective APP protein-controlledgene can cause an excessive amount of Aβ to be produced. Two gene products called APPa and APPb, which are homologues of the human APP gene, are expressed in ZF **(Kiper & Freeman, 2002)**. Hyperphosphorylated Tau can aggregate and lead to destabilized microtubules, which can impair neuron function. Both MAPTa and MAPTb mRNAs were expressed in the developing CNS, representing two zebrafish paralogues of MAPT. Acetylcholinesterase (AChE), an enzyme that hydrolyzes acetylcholine, the neurotransmitter of cholinergic neurons, contributes to the formation of plaques and NFTs. Morover, the memory is aided by acetylcholine (ACh) **(Takata *et al.*, 2022)**.

Acetylcholinesterase inhibitors like rivastigmine, galantamine, and donepezil are currently approved to treat mild to moderate AD, whereas N-methyl D-aspartate receptor antagonists (NMDA) like memantine are approved to treat moderate to severe Alzheimer's disease **(Świetlik *et al.*, 2022)**. Treatment can improve the quality of life for Alzheimer's patients and momentarily slow the emergence of dementia symptoms. Currently, the majority of medications used to delay disease development are sourced from natural products **(Yang *et al.*, 2022)**. The anti-inflammatory, anti-microbial, and antioxidant effects of plant extracts and phytochemicals are widely established and have significant therapeutic applications.

India is home to the medium-sized deciduous Erythrina variegate (EV) (Fabaceae) tree, which has thorny branches, leaves with triangular leaflets, and enormous coral-red blooms **(Tang *et al.*, 2022)**. Astringent, febrifuge, anti-bilious, and anthelmintic properties are all applied to the plant's bark. Additionally, it helps with skin conditions and ophthalmia **(Tang *et al.*, 2022a)**. The flavonoids, alkaloids, and terpenoids that make up EV's phytochemicals have been employed as nerve tonics, sedatives, febrifuges, and neuroprotective **(Tang *et al.*, 2022b)**. In order to avoid learning deficits caused by scopolamine in zebrafish, they are used as models for human neurological disorders due to their similar basic nervous system **(Tang *et al.*, 2022c)**. This study investigated the pharmacological properties of EV leaf extract. To assess the potential of EV extract as a dietary supplement or medication for the amelioration of the beginning of Alzheimer's disease, its effects on scopolamine-induced memory defects in zebrafish (ZF) were examined **(Singsai *et al.*, 2021)**.

MATERIALS AND METHODS

1. Animal husbandry

In this investigation of neurocognitive function, male adult Daniorerio's aged 8 months served as the apprentices. Each zebrafish (ZF) was housed in a 20L housing tank. The tank was maintained using an aerator at a temperature of 25°C, and photoperiodic cycles of light and dark (11– 12h). Prior to the animal testing in the more recent testing chamber, ZF specimens were acclimated for two weeks. To prevent aberrant neurobehavioral patterns and neuroendocrine-associated neurobiological interactions, all behavioral observations were made between the hours of 9:00 AM and 1:00 PM.

2. Animal experimentation procedure

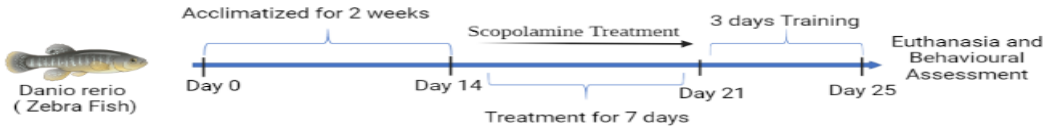

Fig. 1. The experimental design of the study

The ZF samples, after the acclimatization phase, were separated into six groups, each containing eight fish,. Every 24 hours, water was changed in the separate aerated tanks where each group was kept. The test groups were exposed to methanolic extract of Erythrina variegate (MetEEV) at concentrations of 260, 540, and 780ug/ L, which were chosen based on prior acute toxicity studies. The trial lasted for a duration of 7 days. For seven days, the control group was kept under the identical circumstances but without MetEEV. Moreover, the fish were individually placed in Sco (100μM) solutions for 1 hour prior to each behavioural test in order to experimentally induce an animal model of cognitive impairment. Scopolamine was given 30 minutes prior to each behavioral test. After day 7, the behavioral indicators were examined to determine how well the memory functioned. Animals were put to death after the behavioral evaluation, and brain samples were taken for biochemical analysis (Fig. 1 &Table 1).

Table 1. Grouping and treatment of the study

SI.NO.	Grouping	Treatment
1	Normal	Vehicle
2	Scopolamine (100 μM)	Scopolamine induced AD
3	Standard (Donepezil)	Scopolamine +Donepezil
4	MetEEV (260 ug/L)	Scopolamine + MetEEV (260ug/L)
5	MetEEV (520 ug/L)	Scopolamine + MetEEV (520ug/L)
6	MetEEV (780ug/L)	Scopolamine + MetEEV (780ug/L)

3. Behavioral assessments

3.1 Locomotor activity

To assess the changes in locomotor activity, average speed (meter/sec), mobility rate (%), explorative rate (%), total distance moved (M), and total freezing (s) of fish individuals were measured.

3.2 Assessment of cognitive functions (Light and dark chamber test)

Light and dark chamber assessment was used for the assessment of spatial learning and memory functions, according to the method described by **Dubey *et al*. (2015)**.

4. Biochemical estimation

The homogenated brain samples were used for the estimation of catalase (CAT), reduced glutathione (GSH) malondialdehyde (MDA) levels, and acetylcholinesterase activity. Lowry's method was used to estimate the content of protein in the brain sample.

5. Statistical analysis

Results were expressed as Mean ±SEM. The data obtained from all behavior and biochemical tests were scrutinized by one-way analysis of variance (ANOVA). Further, Tukey'stest was considered for post-hoc analysis. The SPSS software version 20 was used for the statistical analysis of all the data.

RESULTS

1. Locomotory activity

Overall average speed resulted to be significantly ($P< 0.05$) decreased in scopolamine treated group when compared to the normal group. While, a concentration dependent significant ($P<0.05$) increase in average speed was observed (Table 2). Overall average acceleration resulted to be significantly ($P< 0.05$) decreased in scopolamine treated group, compared to the normal group. However, upon treatment with MetEEV, a concentration-dependent increase in average acceleration was observed, showing statistical significance ($P< 0.05$) (Table 2). The overall distance traveled was significantly ($P< 0.05$) decreased in scopolamine treated group when compared to the normal group. Though, upon treatment with MetEEV, a concentration-dependent increase in distance traveled was observed, showing statistical significance ($P< 0.05$) (Table 2). The mobility rate was significantly decreased ($P< 0.05$) in the scopolamine-treated group compared to the normal group. In contrast, upon treatment with MetEEV, a concentration-dependent, significant ($P< 0.05$) increase in the mobility rate was observed (Table 2).

The explorative rate was significantly ($P< 0.05$) decreased in scopolamine treated group compared to the normal group. However, upon treatment with MetEEV, a concentration dependent, significant ($P<0.05$) increase in explorative rate was observed (Table 2). Freezing

behavior of fish is a complete halting of the movement except the gills and eyes. Generally, a part of submissive behavior and a state of stress/anxiety are noted. The freezing time was significantly ($P< 0.05$) increased in scopolamine treated group compared to the normal group. While, upon treatment with MetEEV, a concentration-dependent, significant ($P< 0.05$) decrease in explorative rate was observed (Table 2).

Table 2. Effect of MetEEV on locomotory activity in adult ZF

Parameter	Average speed (m/ s)	Average acceleration (m/ s^2)	Total distance (m)	Mobility (%)	Exploratory (%)	Freezing (s)
Normal	103.5±4.01	54.61±3.65	61.53±6.21	68.25±2.58	39.75±1.19	58.87±2.60
Scopolamine (100µM)	20.60±1.70[a]	27.12±6.30[a]	29.01±1.01[a]	32.00±4.12[a]	11.25±2.31[a]	21.07± 9.00[a]
Standard (Donepezil)	81.80±6.21[b]	40.24±3.72[b]	22.97±2.47[b]	64.75±3.22[b]	23.75±2.72[b]	37.50±2.30[b]
MetEEV (260ug/ L)	36.10±1.14[b]	13.75±9.43[b]	82.11±1.00[b]	31.25±9.86[b]	14.25±1.54[b]	10.51±5.97[b]
MetEEV (520ug/ L)	47.97±1.30[b]	16.29±1.60[b]	83.12±7.02[b]	38.10±1.18[b]	18.50±1.73[b]	27.87±3.84[b]
MetEEV (780ug/ L)	61.10±5.08[b]	21.07±8.31[b]	100.0±7.58[b]	45.25±3.28[b]	27.87±3.84[b]	42.82±1.51[b]

Values are expressed as Mean± SEM. n= 8 ZF per group.

$P< 0.05$, a is significant when compared to the normal vs scopolamine group.

$P< 0.05$, b is significant whencompared to the scopolamine vs standard and treatmnet group.

2 Lights and dark test

Light and dark chamber assessment was used for the assessment of spatial learning and memory functions. The present study revealed that the exposure of scopolamine in the treated group significantly ($P< 0.05$) increased TSLC compared to the normal group. Whereas, upon treatment with MetEEV, a concentration-dependent, significant *($P<0.05$)* decrease in TSLC was observed (Table 3).

In addition, NEDC was significantly ($P< 0.05$) increased in the scopolamine treated group compared to the normal group. Whereas, upon treatment with MetEEV, a concentration-dependent, significant ($P< 0.05$) decrease in NEDC was observed (Table 3). These results agree with earlier studies, where in exposure of the adult zebrafish to cigarette smoke resulted in a significant increase of NEDC and a significant decrease in TSLC values compared to the normal control group.

Table 3. Effect of MetEEV on light and dark chamber test in adult ZF exposed for 7 days

Group	NEDC	TSLC
Normal	20.21±1.81	03.03±0.32
Scopolamine (100µM)	97.52±3.88[a]	15.00±2.39 [a]
Standard (Donepezil)	37.21±2.12[b]	06.71±1.71 [b]
MetEEV (260ug/ L)	94.60±1.07	14.05±1.62
MetEEV (520ug/ L)	75.15±2.43 [b]	08.62±0.71 [b]
MetEEV (780ug/ L)	63.80±1.11 [b]	07.54±1.05 [b]

Values are expressed as Mean± SEM. n= 8 ZF per group.

P< 0.05, a is significant compared to the normal vs scopolamine group.

P< 0.05, b is significant compared to the scopolamine vs standard and treatmnet group.

3 CAT and GSH specific activities

The CAT specific activity significantly (*P*< 0.05) decreased in scopolamine-exposed ZF compared to the normal group. While, a concentration-dependent, significant (*P*< 0.05) increase in CAT activity was observed (Table 4), supporting its antioxidant action.

Moreover, GSH specific activity significantly (*P*< 0.05) decreased in scopolamine-exposed ZF compared to the normal group. Meanwhile, a concentration-dependent, significant (*P*< 0.05) increase in GSH activity was denoted (Table 4), supporting its antioxidant potential. The results suggest that MetEEV exhibits neuroprotective effects against oxidative stress.

4 MDA level

The MDA level, an indicator of lipid peroxidation was significantly (*P*< 0.05) increased in the scopolamine treated fish compared to the normal group. While, a concentration-dependent, significant (*P*< 0.05*)* decrease in MDA level was observed (Table 4).

5 AChE activity

The AChE decreases acetylcholine level alleviate disease symptoms associated with the progressive loss of cholinergic functions in AD. Our results demonstrated that ZF group subjected to scopolamine treatment showed a significant (*P*< 0.05) increase in AChE activity in the brain compared to the normal group. While, a concentration-dependent, significant (*P*< 0.05) decrease in the AChE activity was obsereved (Table 4). Thus, MetEEV demonstrated an anti-AChE profile that corresponds to the improvments in memory parameters in ZF.

Table 4. Variation in the MDA, GSH, CAT and AchE level in the ZF brain tissue

Group	CAT (umol/ µg of protein)	GSH (umol/ mg of protein)	MDA (umol/ mg of protein)	AChE (umol/ mg of protein)
Normal	1.27±0.12	6.5±0.76	0.19±0.01	2.08±0.14
Scopolamine Control	0.65±0.03 [a]	0.67±0.61 [a]	0.31±0.01 [a]	3.68±0.09 [a]
Standard(Donepezil)	1.21±0.07 [b]	7.17±0.70 [b]	0.20±0.02 [b]	1.15±0.14 [b]
MetEEV (260µg/ L)	0.73±0.05	1.84±0.65	0.29±0.01	3.17±0.15
MetEEV (520µg/ L)	0.89±0.05	4.83±0.40 [b]	0.22±0.02 [b]	2.75±0.12 [b]
MetEEV (780µg/ L)	1.24±0.11 [b]	6.67±0.42 [b]	0.21±0.01 [b]	2.28±0.18 [b]

Values are expressed as Mean± SEM. n= 8 ZF per group.

P< 0.05, a is significant compared to the normal vs scopolamine group.

P< 0.05, b is significant compared to the scopolamine vs standard and treatment group.

4. DISCUSSION

The effect of methanolic extract of *Erythrina variegate* leaves (MetEEV) in preventing memory and cognitive impairment induced by scopolamine was studied. The postsynaptic muscarinic receptor's acetylcholine is where scopolamine binds, making it a highly effective muscarinic receptor antagonist. Generating amyloid beta causes neurodegeneration that may result in Alzheimer's disease **(Souza *et al*., 2022)**. Furthermore, it also raises the AChE activity in the cortex, levels of oxidative stress, and proinflammatory cytokines in the hippocampus, as well as elevated levels of APP and Tau. In ZF, the scopolamine can cause passive avoidance reactions and affect how long those reactions would last in response to learning **(Falsetti *et al*., 2022)**. To induce memory impairment in zebrafish, we utilized the toxin scopolamine in this study.

The rejuvenation of our body systems as well as illness prevention are given first priority in herbal therapy, which also lengthen life and foster health. Herbal medicines are a vital part of traditional medicine due to its wide availability, low cost, lack of unfavourable side effects, and long history of use. The current study aimed to ascertain whether MetEEV had any beneficial effects on cognitive function **(Tang *et al*., 2022)**. In this study, a male adult ZF that was eight months old was employed. Eight ZF were divided into six groups, each with its own division. The donepezil was adminstered to the normal group, control group, standard group, and three test groups, each of which got three different drug doses (260, 520, and 780ug/ L).

The ZF learning and memory are frequently tested using behavioral methods. The techniques include the light and dark chamber test and locomotor activity. By demonstrating a

drop in average speed, average acceleration, total distance, mobility, and exploratory rate, the scopolamine demonstrated a significant decrease in locomotory activity. Whereas, MetEEV increased locomotory activity by elevating the average speed, average acceleration, total distance, mobility, exploratory rate, and alleviating scopolamine-induced anxiety,.

The biochemical parameters, such as the lipid peroxidase, reduced glutathione, catalase, and acetylcholine esterase were estimated. Accumulating evidence suggests that the oxidative stress produced by the reactive oxygen species (ROS)/reactive nitrogen species (RNS) plays an essential role in the progression of AD in the ageing population. The ZF administered with scopolamine clearly showed suppressed antioxidant enzymes, such as CAT, GSH, and lipid peroxidation MDA.

Alternatively, when compared to the group treated with scopolamine, MetEEV administration reduced the levels of protein and lipid peroxidation and prevented scopolamine-induced oxidative stress in a dose-dependent way. The ACh, a vital cholinergic neurotransmitter, is degraded in large part by AChE. The ZF that had received scopolamine showed a significant rise in AChE activity than the normal group. In comparison to the scopolamine-treated group, the treatment of MetEEV dramatically decreased AChE activity in a dose-dependent way, and this was associated with an improvement in memory metrics.

The results of this study suggest that MetEEV has a strong memory-enhancing effect and may be useful in the treatment of dementia and other cognitive diseases. However, more research is needed to determine the precise mechanism of action in order to develop this medicine into a powerful memory enhancer. These all-natural memory-enhancing substances would aid in the development of novel medication candidates for the treatment of dementia.

CONCLUSION

MetEEV could effectively improve memory impairment in a scopolamine induced zebrafish model by improving cholinergic system function and through upstream antioxidant enzymes in the amnesic ZF model. The findings demonstrate the potential health benefits of MetEEV being investigated and point to its potential use in developing new medicines to treat dementia.

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