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Research Article

Antianxiety Effect of Black Pepper on Indomethacin Induced Anxiety in Zebrafish

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ABSTRACT

Background: Anxiety disorders are the most common prevalent condition that affects both emotion and cognitive behavior. Annual prevalence of generalized anxiety disorder increased from 5.4% in 2020 to 6.6% in 2023, with a 3-year prevalence of 10.3% over the 2021–2023 period. The projected annual incidence ranged from 2.1% to 2.3%. Studies shown anxiolytic property due to the presence of chemical compounds like piperine, paprazine, cepharadione a, ciperolactam d, 10 – tricosanone and sylvamide. It also decreases motor deficits and improves memory function.

Materials and Methods: It is a preclinical study, done in animal house, DSMCH. The study was initiated after getting approval from IAEC. Sixty adult zebra fish are grouped in 6 groups as normal control, toxic control (indomethacin), standard drug (Diazepam), Test drug treatment (Piper Nigrum low, intermediate and high dose). Anxiety was induced in zebrafish by exposing to indomethacin 8 mg/l for 60 min and standard drug/ test drug for 30 min. Behavioral assessment was done using novel tank test.

Results: Indomethacin treated shows increased time spent in lower zone while treatment groups (group 3, 4, 5 & 6) showed more time spent on upper zone similar to control group. Number of transitions were more in toxic group and reduced in treatment and normal control groups. Abnormal swimming speed, erratic movements and thigmotaxis were found in all 10 fishes in indomethacin group while abnormal swimming speed, erratic movements and thigmotaxis were found in 3 fishes in group 3 & 5, 5 fishes in group 4, 2 fishes in group 6. Abnormal swimming pattern, Zig zag swimming, bottom dwelling was found in all 10 fishes in group 2 while swimming pattern and Zig zag swimming were found in 3 fishes in group 3 & 5, 5 fishes in group 4, 2 fishes in group 6. Bottom dwelling was seen 5 in group 3, 3 in group 4 and 2 in group 5 & 6. Freezing episodes were seen only in group 2

Conclusion: Piper nigrum (black pepper) treated group showed decreased in anxiety. Reduction levels are statistically significant with diazepam treated groups

Keywords: Piper Nigrum, Black Pepper, Indomethacin, Anxiety, Diazepam.

INTRODUCTION

Anxiety disorders are the most common prevalent condition that affects both emotion and cognitive behavior [1]. They are associated with substantial impairments in both productive and social roles. Adverse psychosocial factors, diseases, drugs, stress and environmental changes affect the neuronal system of an organism which leads to behavioral disturbances [2]. Anxiety is the most important component of psychiatric condition which ultimately decreases the quality of life. Annual prevalence of generalized anxiety disorder increased from 5.4% in 2020 to 6.6% in 2023, with a 3-year prevalence of 10.3% over the 2021–2023 period. The projected annual incidence ranged from 2.1% to 2.3%. [3]. Cognitive behavioral therapy is the gold standard for anxiety treatment. Drugs like escitalopram, venlafaxine and sertraline are the first line drugs. Benzodiazepines group of drugs like diazepam, alprazolam and lorazepam act by facilitating GABA transmission also used. Major disadvantages with above drugs are dependence, abuse liability and sedative property [4]. Black pepper (fruit of piper nigrum -L) is a food spice. Studies shown that black pepper has Antioxidant, antidiabetic, anti-

obesity, antihypertensive, Anti-inflammatory, anticancer, hepatoprotective, and Neuroprotective properties. It has been postulated, presence of anxiolytic property is due to the presence of chemical compounds like piperine, paprazine, cepharadione a, ciperolactam d, 10 – tricosanone and sylvamide. The postulated mechanism is it upregulates bdnf and creb expression and suppresses TNF – A, IL – 1B, AND IL – 6 expressions in frontal cortex and hippocampus, thereby promotes neurogenesis, decreases corticosterone and MDA levels, Increases catalayse and SOD activities in frontal cortex and hippocampus. It also decreases motor deficits and improves memory function [5,6]. Zebrafish (Danio rerio) have been utilized as animal models in biomedical research, particularly in CNS disorders. Because the zebrafish is a basic vertebrate animal that shares many physiological characteristics and neurotransmitters with humans. Their neuroendocrine system also generates strong physiological responses to stress. Zebrafish is also an excellent choice for scientific research since they are cheap, low-maintenance and have high numbers of progeny [7]. Indomethacin is the NSAID. Studies has been done in zebrafish and has been used to create zebra fish anxiety model [8]. This study evaluates whether black pepper oil reduces Indomethacin induced anxiety-like behaviours in adult zebrafish.

Objectives

1. To assess the effect of black pepper oil (three doses) on amphetamine-induced hyperactivity in adult zebrafish.
2. To compare the efficacy with the standard anxiolytic diazepam.
3. To assess behavioural pattern and histopathological examination

Materials and Methods

Study Design: Pre clinical study (Experimental animal study).
Animals: Adult zebrafish (Danio rerio).
Age: 3-6 months
Gender: Male
Sample Size: 60 (20 in each group)
Study Duration: 3 months (Includes acclimatization, exposure, treatment, testing)
Study Setting: Central Animal house, Dhanalakshmi Srinivasan Medical College and Hospital, Siruvachur, Tamil Nadu.

Groups

- Group I (N=10): Normal control (no Indomethacin, vehicle only)
- Group II (N=10): Toxic control — Indomethacin (8 mg/L)
- Group III (N=10): Standard — Diazepam (5 mg/L) + Indomethacin (8 mg/L)
- Group IV (N=10): Piper nigrum PN (1.25 µg/mL) + Indomethacin (8 mg/L)
- Group V (N=10): Piper nigrum PN (5.00 µg/mL) + Indomethacin (8 mg/L)
- Group VI (N=10): Piper nigrum PN (10.00 µg/mL) + Indomethacin (8 mg/L)

Dose Preparation

Black pepper was shade dried and grinded. 3.5g of powder is mixed with 35 ml hot water (100º c). Then, the final filtrate is collected and then dilute into tank water to achieve final concentrations (1.25, 5.0, 10.0 µg/mL).

Experimental Procedure

The study was initiated after getting approval from IAEC. Adult Zebrafishes (Danio rerio) were collected from TRI-BIOTECH animal facility, Trichy (2295/PO/RcBiBt/S/2024/CCSEA). The animals were maintained in the department animal unit in groups of 25, in 15 liters aquarium tanks for 15 days prior to the setup of the experiment. The animals were provided with a clean environment in a properly functioning aquarium under controlled condition of alkalinity, pH (6.8-7.5), temperature (26- 28.5 °C), light condition 12:12 (light: dark) hardness (75–200 mg/L CaCO3), ammonia, dissolve oxygen (7.8 mg/L at 28.0 °C), salinity (0.25–0.75 ppt) and conductivity was monitored on regular basis to ensure good water quality for housing zebrafish [9]. Animals were fed 1-2 times daily of fish food. On the day of experiment animals were divided into 6 groups. Baseline locomotor activity was recorded. After 24 hrs, animals were exposed to Indomethacin (8mg/l) for 60 min to induce anxiety. Behavioral test was done to confirm anxiety model. Then the animals are correlated with standard drug/test drug for 30 min. Behavioral assessment was done at the end of experiment for 3 minutes

Behavioural Tests [10,11]

Novel Tank Diving Test

Parameters assessed - Time spent in upper, middle and bottom zone, number of transitions between zone, freezing episodes, swimming speed, thigmotaxis, Erratic movement, Zig zag swimming and bottom dwelling.

Safety / Tolerability

- Mortality, gross appearance, abnormal swimming outside expected model effects and feeding behaviour.
- Acute sedation was also recorded

Statistical Analysis

- Data reported as Mean ± SD
- For mean values, One-way ANOVA across groups for each endpoint, followed by Tukey's post hoc for pairwise comparisons and Repeated measures ANOVA was done for within the group analysis.
- $p < 0.05$ was considered significant.

RESULTS

Table-1 shows the zebra fish time spent in lower, middle and upper zone; it was noted that group 2 shows increased time spent in lower zone while treatment groups (group 3, 4, 5 & 6) showed more time spent on upper zone similar to control group. Number of transitions were more in toxic group and reduced in treatment and normal control groups. (Table-2) shows the locomotor activity parameters like swimming speed, erratic movements and thigmotaxis. Abnormal swimming speed, erratic movements and thigmotaxis were found in all 10 fishes in group 2 while abnormal swimming speed, erratic movements and thigmotaxis were found in 3 fishes in group 3 & 5, 5 fishes in group 4, 2 fishes in group 6 (Table-3). shows swimming pattern parameters like swimming pattern, Zig zag swimming, bottom dwelling and freezing number. Abnormal swimming pattern, Zig zag swimming, bottom dwelling was found in all 10 fishes in group 2 while swimming pattern and Zig zag swimming were found in 3 fishes in group 3 & 5, 5 fishes in group 4, 2 fishes in group 6. Bottom dwelling was seen 5 in group 3, 3 in group 4 and 2 in group 5 & 6. Freezing episodes were seen only in group 2. By summarizing the data, indomethacin 8mg/l has induced anxiety and groups treated with diazepam and piper nigrum produces anti-anxiety effect similar to control.

DISCUSSION

Anxiety and Depression, both are neuropsychiatric syndrome characterized by behavioural, physiological as well as psychological symptoms which affects quality of life mainly in adult population [12]. Currently, available drugs are limited and have their own adverse effects. Hence need for herbal medicines is increasing. Several reports have shown that black pepper and its alkaloid piperine are potential antianxiety effect. Black pepper and its bioactive compound piperine are commonly used as traditional remedies for anxiety and depression. Maneenet et al. reported that black-pepper fruit extract given orally (100 or 500 mg/kg/b.w.) improved behavioural impairments [13]. Similarly, Maneenet et al. showed black-pepper fruit extract given orally with Kleeb Bua Daeng to UCMS-induced mice significantly improved their cognitive function. It also significantly decreased serum corticosterone and MDA levels but increased antioxidant activity like catalase and SOD activities in the frontal cortex and hippocampus [14]. Ghosh et al. subsequently reported that essential oil from the black-pepper fruit has antianxiety and antidepressant activity. Behavioral studies were done using FST, TST and the open-field test. study showed that black-pepper essential oil has potent activity at 5, 10, and 50 mg/kg/b.w. doses [15]. Methanolic black-pepper fruit extract (100, 200, and 400 mg/kg/b.w. doses) decreased immobility in mouse TST and FST [16]. Mao et al. reported that piperine treatment (10 mg/kg/b.w.) increased sucrose consumption and decreased immobility time in the FST and TST in mice [17]. Similarly, Mao et al. reported that piperine treatment (10 mg/kg/b.w.) significantly increased sucrose consumption and decreased immobility time in the FST rats [18].

CONCLUSION

The study concludes indomethacin can be used to produce anxiety model in zebra fish. Piper nigrum (black pepper) produces anti-anxiety effect similar to diazepam. No signs of toxicity have been noted. Our study highlights the use of herbal and natural products in treating anxiety.

Conflict of Interest: Nil

Funding: Self

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Table-1: Comparison of Mean Number of Transitions between Zones of Different Groups

Groups	**Time spent in lower zone of tank**		**Time spent in middle zone of tank**		**Time spent in upper zone of tank**		**Number of transitions between zone**		**Repeated measure ANOVA**
	Mean	S.D	Mean	S.D	Mean	S.D	Mean	S.D	

Group-1	53.6	4	38.6	3.6	27.8	2.5	98.5	9.2	p<0.001
Group-2	90.8	6.1	25.1	6.6	4.1	2.6	136.8	13.2	p<0.001
Group-3	42.4	7	43.1	6.3	34.5	9.6	81.3	7.8	p<0.001
Group-4	46.2	11.1	42.6	7.3	31.2	11.5	83.4	8.1	p<0.001
Group-5	27.2	5.5	33.8	4.2	59	5.2	75.4	6.9	p<0.001
Group-6	22.1	6.2	30.8	5.1	67.1	7	72.6	6.8	p<0.001
One way ANOVA	p<0.001		p<0.001		p<0.001		p<0.001		

(p<0.05 considered statistically significant)

Table-2: Comparison of Locomotor Activity between Groups

Scale	Grading	Group-1	Group 2	Group 3	Group 4	Group 5	Group 6
Swimming speed	Normal	10	0	7	5	7	8
	Abnormal	0	10	3	5	3	2
Erratic movement	Present	0	10	3	5	3	2
	Absent	10	0	7	5	7	8
Thigmotaxis	Present	0	10	7	5	3	2
	Absent	10	0	3	5	7	8

Table-3: Comparison of Swimming Pattern between Groups

Swimming pattern	Grading	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Swimming pattern	Normal	10	0	7	5	7	8
	Abnormal	0	10	3	5	3	2
Zig zag swimming	Present	0	10	3	5	3	2
	Absent	10	0	7	5	7	8
Bottom Dwelling	Present	0	10	5	3	2	2
	Absent	10	0	5	7	8	8
Freezing Number	Mean	0	2.8	0	0	0	0
	S.D	0	0.4	0	0	0	0

Image-1: Novel Tank Test