GENERAL PSYCHOPHARMACOLOGICAL ACTIVITY OF DOCOSAHEXANOIC ACID IN RODENTS

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ABSTRACT

Psychosis represents a class of severe psychiatric disorders marked by significant disruptions in thought processes, behavior, perception, and the ability to distinguish between reality and delusions. These conditions encompass a variety of mental health disturbances, including cognitive disorders, functional disorders, affective disorders, and neuroses. Psychopharmacology refers to the study of the impact of substances on behavior, particularly the way drugs influence cognitive and emotional states. One such compound, Docosahexaenoic Acid (DHA), an Omega-3 fatty acid from the broader group of polyunsaturated fatty acids, plays a crucial role in the prevention and management of various diseases. DHA is the predominant PUFA in the brain, serving as an essential structural component of neuronal membranes and supporting the development and functionality of the brain. DHA is characterized by its 22-carbon chain structure with six cis double bonds, the first of which is positioned on the third carbon from the omega end, denoted as 22:6, n-3. DHA exhibits a broad spectrum of biological activities, including cardiovascular effects, enhanced visual function, anti-inflammatory properties, and potential roles in managing conditions like arthritis, atherosclerosis, diabetes, hypertension, thrombosis, and certain cancers. Antipsychotic drugs are commonly utilized in the treatment of psychiatric disorders, though substances like alcohol and tobacco remain prevalent psychoactive agents in society. Benzodiazepines are typically prescribed for acute treatment of psychopharmacological disorders, offering intermittent or long-term relief in select patients, despite the limitations posed by their toxicity with prolonged use. This study aims to investigate the psychopharmacological effects of DHA through animal models. Preliminary data suggest that DHA may exert central nervous system depressant properties, but the existing scientific literature on its psychopharmacological effects remains sparse. Thus, this research seeks to explore DHA's efficacy using rodent models, employing the Rotarod and Open Field tests, and comparing its effects with the established anxiolytic, Diazepam (2 mg/kg), across varying doses of DHA (100, 200, and 400 mg/kg).

Keywords: Psychopharmacology, Psychosis, Docosahexanoic acid, rodents, Rotarod apparatus.

INTRODUCTION

The central nervous system (CNS) is of critical importance, governing all major physiological systems and ensuring homeostasis within the body. Disruptions in the intricate balance of neurochemical and psychological processes can lead to a wide array of CNS disorders, such as epilepsy, Alzheimer's etc. Collectively, these conditions contribute significantly to global morbidity and mortality rates (Thompson et al., 2000). Neurological disorders, like epilepsy, affect approximately 5% of the global population, with 30% of patients experiencing persistent seizures despite existing treatments. In addition, modern lifestyles have exacerbated the prevalence of behavioural disorders, including anxiety and depression.

Psychosis

Psychosis is a severe mental health condition that involves profound disturbances in cognitive processes, emotional regulation, and perceptual abilities. It is often characterized by delusions, hallucinations, and a fundamental inability to discern reality from illusion.

Acute and chronic organic brain syndromes (cognitive disorders)

This category encompasses conditions like delirium and dementia, often triggered by underlying toxic or pathological causes. Prominent symptoms include confusion, disorientation, memory deficits, and disorganized behavior.

Functional disorders

Functional disorders are those in which no specific pathological cause can be identified. While memory and orientation may be preserved, alterations in emotion, thought, reasoning, and behavior are profound. Schizophrenia, for instance, is marked by a disconnection between perception and reality, resulting in hallucinations and incoherent thought patterns.

Affective disorders

Affective disorders are characterized by significant disturbances in mood. These include:

- **Mania**: Elevated or irritable mood, decreased need for sleep, increased energy, racing thoughts, and potentially reckless behavior.
- **Depression**: Persistent sadness, loss of interest in activities, feelings of worthlessness, guilt, and physical or mental sluggishness.

Neurosis

Neurosis refers to less severe mental disorders where the individual retains an understanding of reality, though they may endure intense emotional distress. Neuroses encompass conditions such as anxiety disorders, phobias, OCD, reactive depression, PTSD, and hysterical states. Anxiety disorders, in particular, involve excessive worry or fear, which can manifest in physical symptoms like rapid heartbeat or trembling.

Stress

Anxiety and stress can arise in response to life events, such as financial concerns or chronic illness. Social pressures, body image concerns, and evaluation anxiety are common stressors among adolescents and young adults. Anxiety is also prevalent among older adults, particularly those experiencing dementia.

Depression

Depression is characterized by a persistent low mood and disinterest in previously enjoyable activities. Affected individuals may feel hopeless, worthless, guilty, irritable, or restless, significantly impacting their thoughts, behaviours, and overall well-being.

Epilepsy

Epilepsy is a neurological disorder marked by recurrent, spontaneous seizures. These seizures can vary in severity, ranging from brief, subtle episodes to prolonged, violent convulsions, often resulting in physical injuries such as fractures**.[1]**

PSYCHOPHARMACOLOGY

Psychopharmacology explores how pharmacological substances influence behavior. Psychoactive or psychotropic drugs, which alter perceptions, emotions, or cognitive functions, are used to treat psychiatric conditions. These substances exert their effects by interacting with the brain and nervous system, altering mental states and influencing behavior.**[2]**

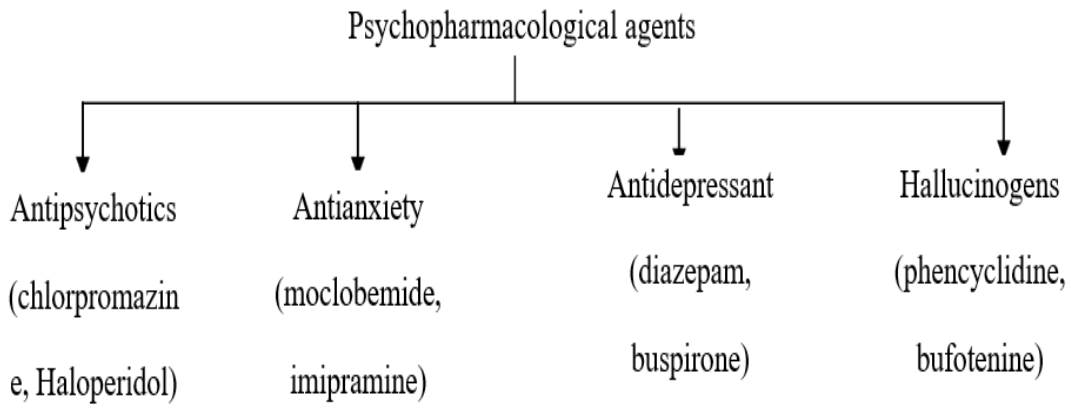

Figure 1. Psychopharmacological agents.

DOCOSAHEXANOIC ACID

DHA, essential Omega-3 fatty acid, a key component of neuronal membranes. It constitutes a significant portion of the phospholipids in the brain's grey matter and in retinal photoreceptor cells, where it supports cellular processes such as membrane fluidity, permeability, and viscosity. DHA also plays a pivotal role in neurotransmission, gene expression, and the modulation of enzymes, receptors, and ion channels. DHA is critical for fetal brain development, the enhancement of motor skills and visual acuity in infants, cognitive support

in older adults, and lipid metabolism throughout life. In addition to its neuroprotective effects, DHA is also beneficial in managing certain types of cancer. DHA's influence extends beyond brain health, as it supports synaptic function by maintaining plasma membrane integrity and regulating the ionic permeability of membranes. DHA's role in synaptic plasticity and cognitive function is linked to the activation of critical metabolic pathways that impact neurotrophins such as brain-derived neurotrophic factor and insulin-like growth factor 1, both of which are vital for maintaining cognitive abilities and facilitating synaptic communication. **[3]**

Drug profile

Chemical Names: Docosahexaenoic acid; Doconexent; Cervonic acid; Doconexento; Doconexentum; Doxonexent

Molecular Formula: $C_{22}H_{32}O_2$.

IUPAC Name: (4Z,7Z,10Z,13Z,16Z,19Z)docosa4,7,10,13,16,19hexaenoic acid.

Molecular Weight: 328.48828 g/mol.

Bond Structure and 2D structure of DHA

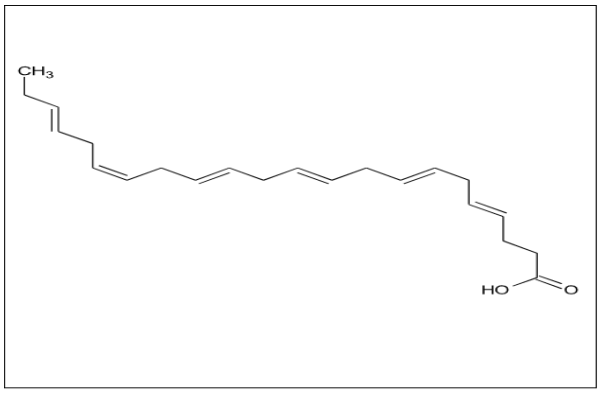

Figure 2. Bond structure of Docosahexanoic Acid

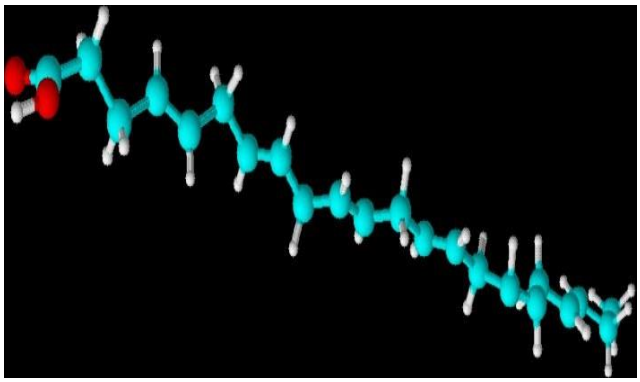

Figure 3. 2D structure of DHA

Mechanism of action

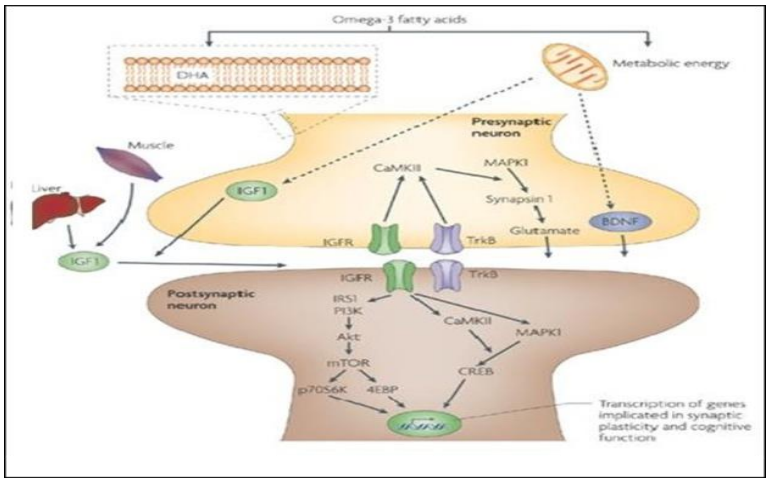

Figure 4. Brain foods: the effects of nutrients on brain function [4].

Researches involving psychopharmacological activity of Docosahexaenoic acid (DHA)

Table 1. Psychopharmacological activity of DHA

Compound/Derivative	Activity	References
Omega-3 DHA	Treatment of mood disorders	**[5]**
DHA	Antidepressant activity	**[6]**
Omega-3 DHA	Anxiety treatment	**[7]**
Omega-3 fatty acid DHA	Alzheimer's disease	**[8]**

MATERIAL AND METHODS

EVALUATION OF ANTIPSYCHOPHARMACOLOGICAL ACTIVITY

Grouping of Animals

Adult albino mice of 20-25 gm, selected, divided in 5 groups of 3 animals.

- Group I- Control group (animals only received vehicle).
- Group II treated with Standard drug Diazepam (2mg/kg).
- Group III treated with Docosahexanoic acid solution (100mg/kg).
- Group IV treated with Docosahexanoic acid solution (200mg/kg).
- Group V treated with Docosahexanoic acid solution (400mg/kg).
- Oral route of administration selected for treatment of animals.

Rotarod model [9]

Animals categorized in 5 groups, each consisting of five albino mice, with body weights ranging from 20 to 30 grams. Prior to testing, all animals underwent a period of overnight fasting, although water was available ad libitum. Mice were first familiarized with testing apparatus through a pre-test session. The apparatus was set to rotate at a constant speed of 36 rotations per minute. A day before the actual experiment, the mice were acclimatized and trained to remain on the rotating rod for 3 minutes. Only those animals that were able to stay on the rotating rod for a minimum of 1 minute were selected for the test, while those with inadequate performance were excluded. The test substances were administered either intraperitoneally or orally. Following administration, the mice were placed on the rotating rod for 1 minute—30 minutes after intraperitoneal injection or 60 minutes after oral dosing. The number of mice that fell off the rod during this time interval was recorded.

Open field method [10]

The animals were assigned to five groups, each containing five albino mice, which were fasted overnight prior to the experiment, though water was provided freely. Prior to initiating the experiment, the animals were acclimatized in the behavioural room for at least 30 minutes, with the lights on and doors closed. If pharmacological intervention was involved, the route of administration and absorption time were considered before proceeding to the next phase. Video recording was started, and the animals were identified using a whiteboard displayed in front of the camera for approximately 3 seconds. Subsequently, each mouse was gently placed in the centre of the open field apparatus. Each trial lasted for 10 minutes. After completing each trial, the testing area was thoroughly cleaned using a 70% alcohol solution and dried with paper towels.

Inclined Screen Test [11]

Animals divided into 5 groups, each with 5 albino mice. These mice were fasted overnight, while water was provided ad libitum. Inclined plane test was designed to assess skeletal muscle relaxant activity. The test apparatus consisted of a transparent glass surface inclined at 30 degrees. Mice were placed at the top of the inclined surface and allowed to attempt to maintain their position on the plane without sliding off. Observations were made at 15-30 minute intervals after oral administration of DHA at doses of 100, 200, and 400 mg/kg. Mice were given 30 seconds to either hang on to the surface or fall off.

Pentylenetetrazol induced Convulsions [12]

Animals again divided into 5 groups, each containing 5 albino mice, fasted overnight with water available ad libitum. Pentylenetetrazol (PTZ)-induced seizures in rodents serve as a model for human absence epilepsy and myoclonic seizures. PTZ was administered subcutaneously to induce convulsions. DHA was administered orally at doses of 100, 200, and 400 mg/kg, 30 minutes prior to the injection of PTZ. Following treatment, the duration of convulsions was observed and recorded. Parameters such as the onset of convulsions, Straub tail, hind limb extension, and the animal's status at 30 minutes and 24 hours post-treatment were assessed. The percentage of protection against convulsions was also calculated.

STATISTICAL ANALYSIS

Outcomes expressed as the mean ± standard error of the mean. The significance of differences among the experimental groups was determined using one-way analysis of variance, followed by Dunnett's test. P-value less than 0.05 was statistically significant.

RESULTS AND DISCUSSION

PHARMACOLOGICAL ACTIVITY ASSESMENT

Muscle relaxant activity of DHA by Rotarod

The data collected at 0 and 30 minutes after treatment from the Rotarod test are summarized. DHA (100, 200, and 400 mg/kg) diminished time spent by mice on the rotating rod compared control group ($p<0.05$). Standard, diazepam (2 mg/kg), exhibited a significant effect when compared control. These results indicate that DHA significantly impaired motor coordination of tested animals.

Table 2. Effect of DHA on Muscle relaxant activity by Rotarod in mice

Group	Treatment	Dose	After 0 minutes (In sec.)	After 30 minutes (In sec.)
Control	DMSO	10 ml/kg	140.5+3.23	146.7+3.29
Standard	Diazepam	2 mg/kg	137+2.5	39.6+1.7
Treated 1	DHA-1	100 mg/kg	120+18.23	96.6+23.54
Treated 2	DHA-2	200 mg/kg	128.7+3.5	81.3+26.33
Treated3	DHA-3	400 mg/kg	130.1+1.2	72.2+16.2

Values obtained as Mean±SEM, n=5, $P<0.05$ when compared control statistical significance via ANOVA followed by Dunnett's t test.

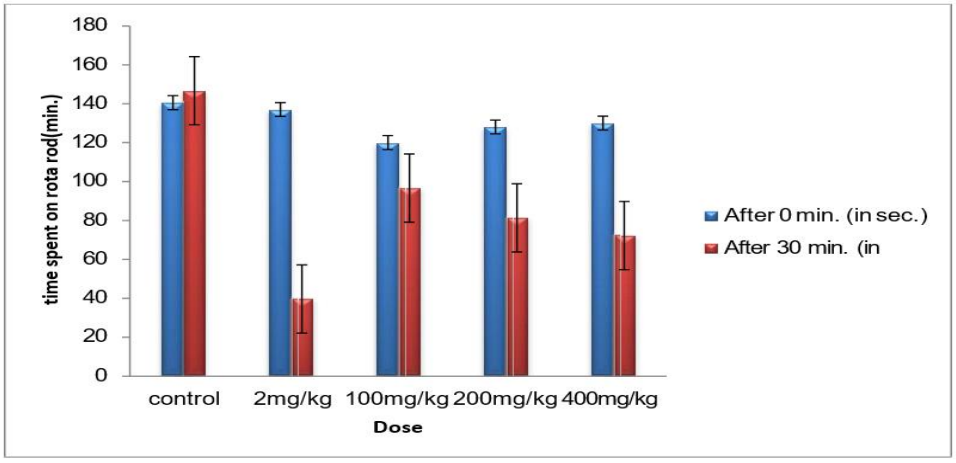

Figure 5. Graphical representation of Rotarod model

Muscle relaxant activity of DHA by Open Field Model

Results from the open field test, measured at 0 and 30 minutes post-treatment, are presented. DHA (100, 200, and 400 mg/kg) increased both distance moved and duration of movement compared to control ($p<0.05$). Standard, diazepam (2 mg/kg), depicted promising effect compared to the control. The data suggest that DHA reduced locomotor activity in the mice.

Table 3. Muscle relaxant activity of DHA by open field model

Group	Treatment	Dose	Rearing	Center field penetration
Control	DMSO	10 ml/kg	11.2±0.2	2
Standard	Diazepam	2 mg/kg	10.36±0.8	-
Treated 1	DHA-1	100 mg/kg	10.5±0.9	-
Treated 2	DHA-2	200 mg/kg	9.8±0.6	1
Treated3	DHA-3	400 mg/kg	8.3±0.7	-

Values obtained as Mean±SEM, n=5, P<0.05 when compared with control.

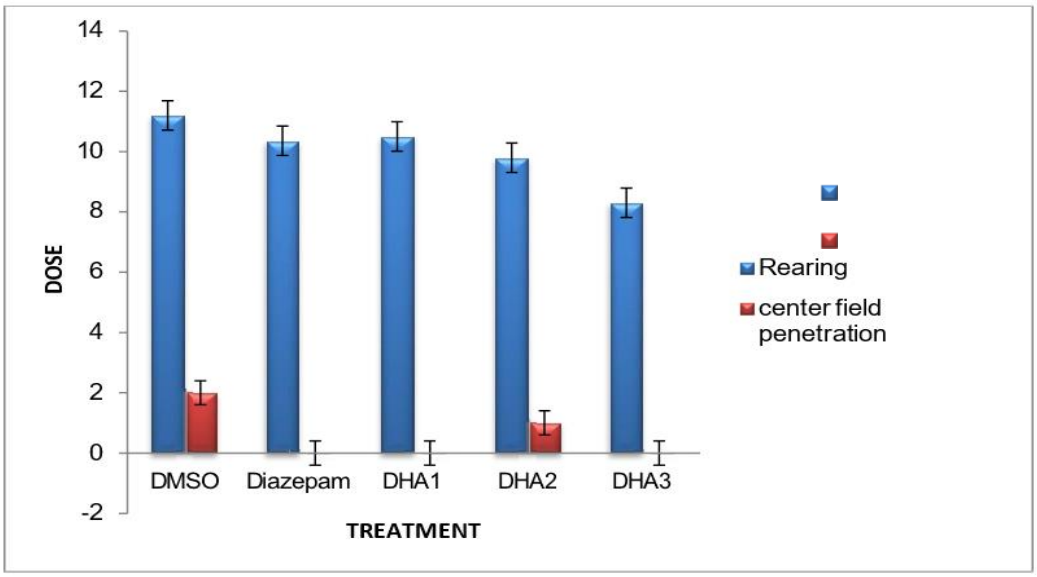

Figure 6. Graphical representation of open field test

Muscle Relaxant Activity of DHA by Inclined Plane test

The inclined plane test results showed that animals treated with diazepam (2 mg/kg, $p<0.05$) and DHA (400 mg/kg, $p<0.01$) exhibited reduction in their sliding time. Both 200 and 400 mg/kg doses of DHA, as well as diazepam, decreased the time spent sliding compared to the control group. 400 mg/kg dose of DHA demonstrated stronger muscle relaxant effects compared to 200 mg/kg dose.

Table 4. Muscle Relaxant Activity of DHA by Inclined Plane test

Group	Treatment	Dose	Sliding Time	
			After 0 min.(in sec.)	After 30 min. (in sec.)
Control	DMSO	10 ml/kg	6.23±0.07	7.89± 0.02
Standard	Diazepam	2 mg/kg	24.10±0.25	26.72 ±0.21
Treated 1	DHA-1	100 mg/kg	12.23± 0.06	11.36 ±0.92
Treated 2	DHA-2	200 mg/kg	16.43±0.21	17.02± 0.03
Treated 3	DHA-3	400 mg/kg	18.41±0.01	23.65± 2.43

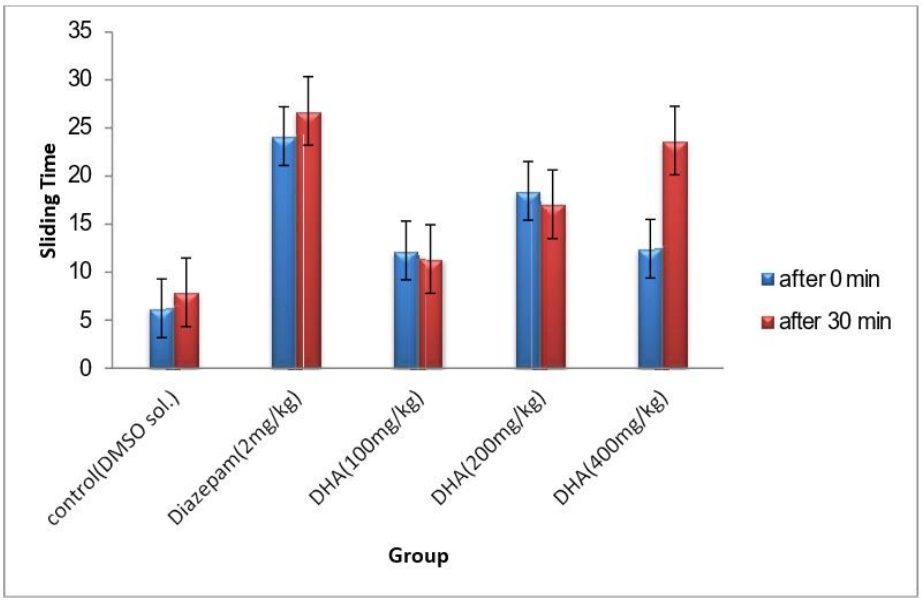

Figure 7. Graphical representation of inclined plane test

Pentylenetetrazol induced convulsion in mice

In the PTZ-induced seizure model, administration of DHA at doses of 100, 200, and 400 mg/kg, one hour before PTZ injection, delayed onset of convulsions and tonic seizures. DHA treatment prevented death in the animals and improved their overall protection against PTZ-induced seizures. Specifically, DHA doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg provided 20%, 60%, and 80% protection against seizures, respectively, when compared control group.

Table 5. Anticonvulsant activity of DHA by PTZ induced convulsion method

Group	Dose mg/kg	onset of convulsion (min)	Onset of tonic convul-sion (min)	Duration of convul-sion (min)	Status of animal alive after 1 hr	Status of anima l alive after 24 hrs	% protection
Control (N.S+PTZ)	10ml+60mg	5.34±0.6	7.4±0.4	19.3±0.8	5	0	0
Diazepam +PTZ	4mg+60mg	2.3±0.3	2.4±0.5	13.3±0.8	5	5	100
DHA1+PTZ	100mg+60mg	1.6±0.1	1.2±0.9	24.2±0.9	5	1	20
DHA2+PTZ	200mg+60mg	2.7±0.4	2.6±0.3	16.5±0.5	5	3	60
DHA3+PTZ	400mg+60mg	2.4±0.3	2.9±0.7	23±0.5	5	4	80

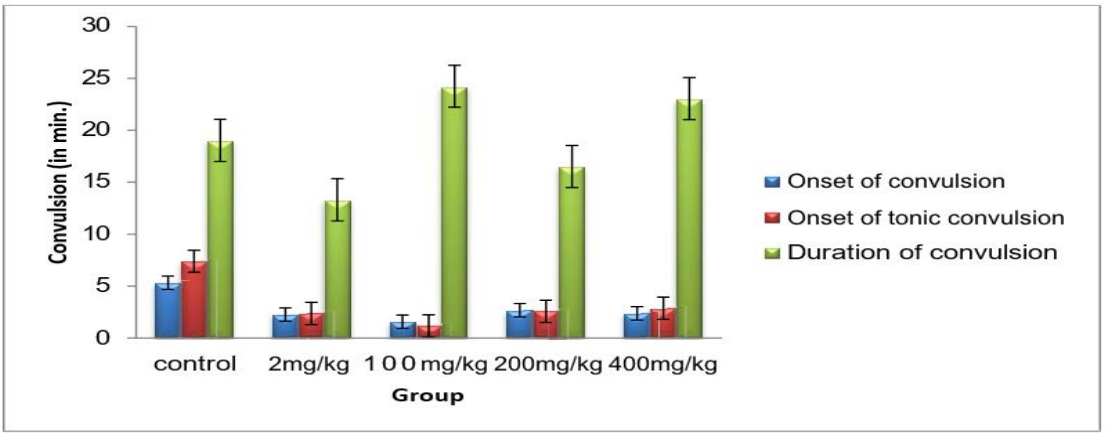

Figure 8. Oral administration effects of different doses of DHA on onset of convulsion, onset of tonic convulsion and duration of convulsion (min) induced by pentylenetetrazol 60 mg/kg. (n = 5)

CONCLUSION

Psychosis is a multifaceted condition that affects numerous species, both acutely and chronically. It is often associated with disorders such as depression, anxiety, stress, and epilepsy. Epilepsy, a chronic disorder characterized by recurrent, unpredictable seizures, is one of the more prevalent conditions related to psychosis. Psychopharmacological agents, including antipsychotics, anxiolytics, antidepressants, and hallucinogens, are employed to treat psychiatric illnesses. DHA, an omega-3 fatty acid and a significant PUFA in the brain, plays a vital role in the prevention and treatment of various diseases. DHA is particularly crucial for the structural integrity of neuronal membranes and the growth and development of the brain. Antipsychotic drugs are commonly used to treat psychopharmacological disorders, with alcohol and tobacco being the most widely used psychoactive substances in society. In this study, the psychopharmacological effects of DHA were evaluated using several animal models. The results from the muscle relaxant activity tests, including the Rotarod, Open Field, and Inclined Plane models, demonstrated that DHA (100, 200, and 400 mg/kg) significantly reduced motor coordination and locomotor activity in the animals. The PTZ-induced convulsion model further confirmed the anticonvulsant properties of DHA. The 400 mg/kg dose of DHA proved to be more effective than the 200 mg/kg dose, showing significant activity in both muscle relaxant and ant psychopharmacological models.

In conclusion, DHA demonstrates promising psychopharmacological activity, particularly as a muscle relaxant and anticonvulsant agent. However, further research is needed to elucidate the underlying mechanisms of DHA’s effects on psychopharmacological disorders.

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Conflict of Interest
Authors declare no conflict of interest.

Ethical approval
Not applicable.

Informed consent
Not applicable

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