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Neuropharmacological Assessment of Dual Herbal Combination Using Zebrafish Model

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Abstract: ***The goal of neuropharmacological screening is to evaluate new pharmacologically active drugs. The purpose of this study is to look at the neuropharmacological effects of 1:1 combination of flax seed oil and allspice oil in different zebrafish models. Flaxseed is a dietary supplement that can help with constipation, diabetes, high cholesterol, heart disease, cancer, and a variety of other problems. Lignans.antioxidants, fibre, protein, and polyunsaturated fatty acids like alpha-linolenic acid (ALA), or omega-3, are all found in flaxseed. Allspice oil has anticancer. Antifungal, antibacterial, nematicidal, anti-diabetic, anaesthetic, antiseptic, acaridical, and anti-oxidant properties. The zebrafish did not demonstrate behavioural changes or other symptoms of toxicity or mortality after consuming allspice and flax seed, indicating their safety profile. Because of the presence of aromatic oil content the product has significant anaesthetic characteristic in the zebrafish model of anaesthesia. It is crucial to investigate the effects of flaxseed essential oil and allspice oil in the zebrafish Y maze and dark/light paradigm, which showed memory-enhancing and anxiolytic advantages via cholinergic regulation and antioxidant capacities. Individual examination findings did not reveal the same impact in the case of CRC of ACH utilising the combination of dual herbal oil, indicating that they had cholinomimetic effects. Their antioxidant properties and suppression of acetylcholine esterase has shown its potential to be investigated further as a therapeutic drug for Alzheimers disease.***

Keywords: Zebrafish (daniorerio), Flax seed, Allspice, Anxiolytics, Anasthetics, Cognitive

1. Introduction

Flaxseed (Linumusitatissimum Linn), they're an oil seed that's high in lignans, lipids, proteins, fibre, carbohydrates, and minerals. Due to their well-established high nutritional profile, flaxseeds have earned a reputation as a dietary source of high-value functional components. Using a variety of bioprocessing techniques, these important constituents in flaxseeds can be made available for a range of uses, including nutraceuticals, cosmetics, and the food industry. According to epidemiological research, these substances have been associated to lower bioavailability of key nutrients and/or health concerns.

Figure 1: Flax seed and oil

Allspice, Pimentadioica (linn) Merr, and it belongs to the Myrtaceae[1]. Allspice leaves contain spices such as cinnamon, nutmeg, cloves, juniper, and pepper. Their structure is hermaphrodite, yet their function is dioecious. Eugenol, Methyl eugenol, caryophyllene, and Myrcene are found in allspice leaves. It has anticancer, antifungal, antimicrobial, nematicidal, antidiabetic, anaesthetic, antiseptic, acaridical, anti-oxidant activity. It's utilized as an anodine for rheumatism and neuralgia, as well as an aromatic stimulant in digestive issues, tonics, and cleanser[1].

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Figure 2: Allspice leaf and oil

Zebrafish**,** Daniorerio, originally known as Brachy Daniorerio, is a tiny tropical freshwater fish that originated in India's Ganges River and its tributaries[2].Zebrafish are frequently located near the bottom of the ocean in their natural habitat to avoid predators. Zebrafish are currently thought to be a good model for studying development, genetics, immunity, behavior, physiology, and nutrition.

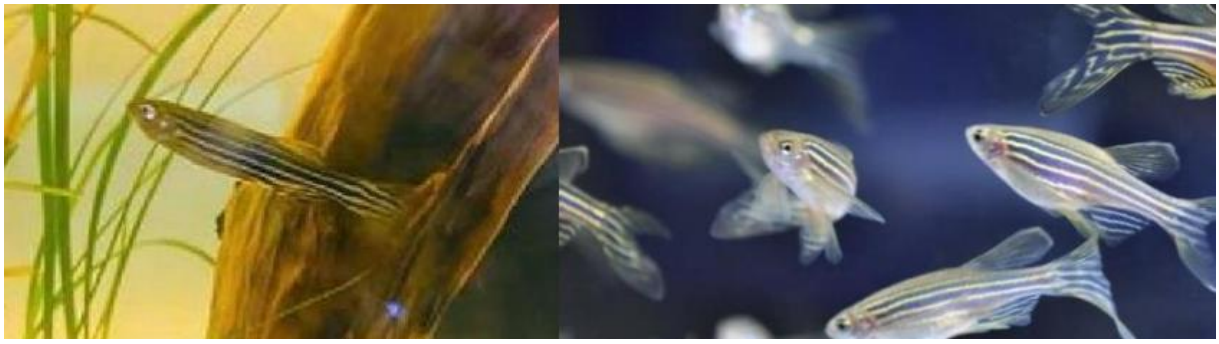

Figure 3: Adult male and female zebrafish

2. Material and Methods

Procurement of herbal oil

Flax seed oil and Allspice oil were procured from local market (fssailic no:1131301001157and11312007000136) of Kerala.

Animals

Thirty adult male zebrafish (Daniorerio) with body length 3–5 cm and weight 500 mg were purchased from Whizbang bioresearch Pvt Ltd, Chennai and cultured in an acrylic tank with an aerator, keeping the water in the tank for 24 hours before use. The density was set at 5 fish per 7.5 liters. Water temperature and pH were maintained at 28 ± 2°C and 6.5–7.5, respectively, with 12- hour light to dark cycle. The fish were fed with Brine shrimp twice a day (morning-evening).

Approval of Protocol

All the experimental procedures and protocols used in this study were reviewed and approved by the Institutional Animal Ethical Committee (IAEC) of JamiaSalafiya Pharmacy College constituted under Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India 1686/JSPC (Reg. No. 1686/JSPC). Ethical guidelines were strictly followed during all the experiments.

Chemicals

i. Ethanol – New material Industries, Changshu ii. Acetyl choline- Ultra Pure Lab Chem Industries LLP, Thane, Mumbai-401102, Maharashtra, India

Acute Toxicity Studies Acute Toxicity Study. An acute toxicity study was determined using 30 fish of both sexes divided into 4 groups as follows: a control group and ASO+FSO (10, 25, and 50 µ L/L) groups. The fish were monitored for two weeks for any symptoms of mortality and toxicity.

Apparatus of Behavioral Tests - Y maze

Animals were tested in a Y-Maze transparent plastic aquarium with three arms (25 cm long, 8 cm wide and 15 cm high) starter arm, other two arms with red and green colored at the ends. The apparatus is filledwithnormal water.

Fig 4: Y-maze for zebrafish

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3. Methods

Y-Mazetask

The Y-Maze task consisted of two trials. During the first trial (training, 5 min), fish were allowed to explore only two arms (start and another arm (red)), with the third arm (green arm) closed. For the second trial fish allowed to explore on start arm and third arm (green), with the second arm closed (red). During the trials, in the second arm (red) we disturb the fish and in the third arm (green) we provided the fish with food. Training and test sections were recorded. The time spent in each arm was determined. Then the time spent in each arm by the control, and drug treated were determined and compared[4,5,6].

Cholinomimetic activity of 1:1 combination of flax seed oil and allspice oil using isolated chicken ileum

Fresh intestine of chicken was collected and transfer to a beaker containing modified Tyrode solution maintained at 37°C and aeration was provided. Ileo-caecal junction was identified and cut into 2-3 Cm length. Loops were carefully made at both ends without closing the lumen. Preparation was mounded in an organ bath containing modified Tyrode solution and provide aeration.

Combination of allspice oil and flaxseedoil (1:1)

Concentration of 0.2, 0.4, 0.08 mLof Acetylcholine was added and record the contraction of tissue. Then add the combination of the drugs at a concentration 0.2, 0.4, 0.8, 1.6 mL and record the response

Individual response of drugs

Concentration of 0.2, 0.4, 0.8 mL of Acetylcholine was added and record the contraction of tissue. Concentrations of acetyl choline was added along with a fixed dose of drugs individually and record the response individually[6,7].

Anxiolytic activity

Animals were divided in to 3 groups (control, test, and standard) each containing 2 adult zebra fish[8]. This test is conducted in a tank consist of half black and white portion that is separated by using a plastic barrier or a sliding door[8]. Each zebra fish is initially placed in the light area, and the number of entries and time spent in the light area are assessed[9]. Drug solution was prepared with distilled water. The fish was immersed into the beaker containing drug solution for about 1 hour and after their exposure immediately place them in to the center portion or intersection compartment which was removed after 5 minuets of their acclimation period[10]. Then fish were allowed to explore for 10 minutes. The parameters were analyzed the total time that the animal remained in the dark and light compartment, and the no: of crossing. The time in dark indicate anxious behavior. Thelatency is a measure of impulsivity when they exploring in new environments, and the number of crossings is the measurement of motor activity and which was recorded by using video camera[8]. The control group of fish was immersed individually in to the beaker without any drugs, and standard group of animals was treated with ethanol.

Anesthetic activity in Zebrafish

Zebrafish were immediately placed in the anaesthetic water bath after the anaesthetic solution was homogenised, and a time count was started to quantify loss of equilibrium, time for induction, and loss of sensitivity to gentle and painful stimuli, as well as monitor anaesthetic parameters. The time of anaesthetic induction was measured from the beginning of immersion until the fish stopped moving and remained in a total and permanent dorsal decubitus at the bottom of the beaker. The respiration rate was recorded after the loss of balance and the absence of responsiveness to a light touch. The time took the fish to start moving and re-establish equilibrium in a ventral posture was then recorded. The activity of zebrafish was tested five and twenty-four hours after complete recovery. Fish activity was determined by the number of times a longitudinal line in the tank crossed each minute (crossings/min).Before their activity was assessed, the animals in the control group were split into small tanks and placed in repose for 5 hours to allow them to acquire acclimated to their environment. After 48 hours of recovery, the animals were taken individually and transported to a larger tank that had been set up to accommodate all of the fish who had already undergone the anaesthetic protocols[11].

Statical Analysis

All of the data is presented as a mean ± (SEM) (standard error mean). The data was analysed using one-way ANOVA and Dunnett's test, with p 0.001 being judged statistically significant using Graph Pad instat.

4. Result and Discussion

Acute Toxicity Study After the administration of ASO+FSO (10, 25, and 50 µ L/L), the zebrafish did not show behavioral changes or other signs of toxicity and mortality, indicating the safety profile of the ASO+FSO doses.

Behavioral effects One-way ANOVA demonstrated overall changes of the colour arm preferences ($p < 0.01$), on the time spent in green arm 173.20±8.52 sec in cotrol, $p < 0.0001$ and in test conc 1 (50 µ L)211.60±11.52 sec, $p < 0.0001$. The red arm entrance times for control and test conc 1 are 74±8.37 sec and 40.60±7.56 seconds respectively.

Table 1: Mean time in sec of zebrafish in discrimination sessions.
Values are expressed as mean ±SEM, n = 5

Control	Green Arm preference	173.20± 8.52 sec
	Red Arm preference	74 ± 8.37 sec
Test Cone 1 (50 μL)	Green Arm preference	211.60 ± 11.52 sec
	Red Arm preference	40.60 ± 7.56 sec

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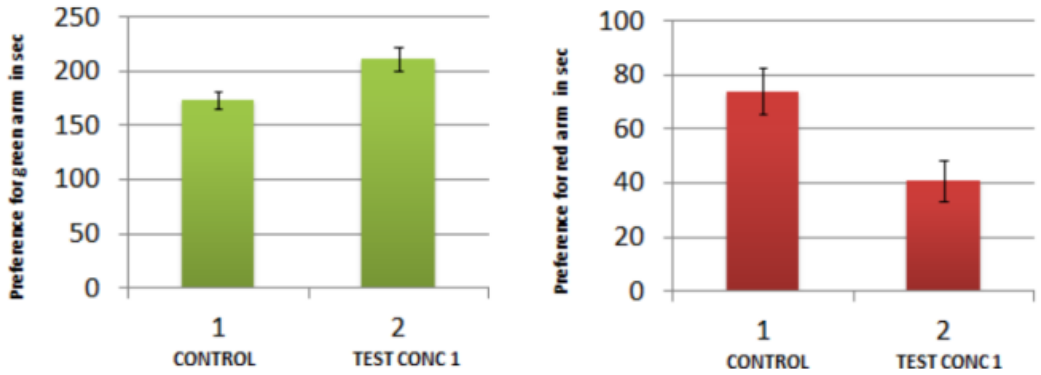

Figure 5: Effect of flaxseed oil+ Allspice oil on green/ red arm preference

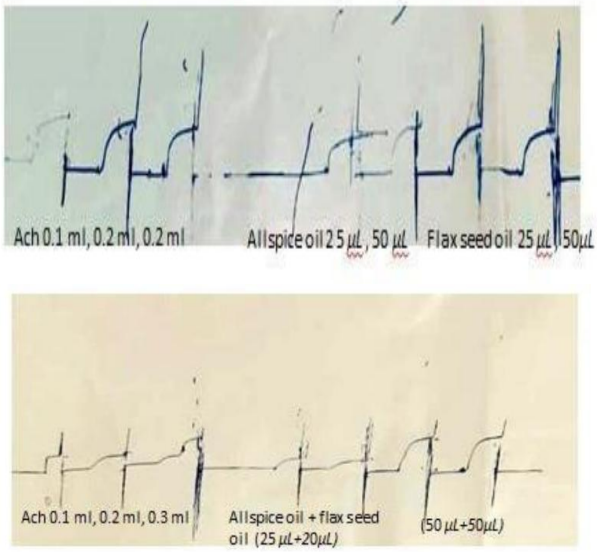

Figure 6: Effect of flaxseed oil+ Allspice oil on CRC of ACh using isolated chick ileum

The above-mentioned result (fig.9) indicated that the mix of two oils had cholinomimetic effects, while the individual examination results did not show the same impact. The existence of cholinergic impairments doesn't explain the neuropathology of the brain seen in Alzheimer's disease. The clear evidence of cholinergic neuron degradation, on the other hand, suggests a new therapy option for Alzheimer's disease symptoms. The levels of ACh in the cortex and hippocampus area of Alzheimer's patients were shown to be lower.

The use of cholinesterase inhibitors, which block AChE and cause ACh to be hydrolyzed at the cholinergic synapse, is the current strategy that has been found to offer symptomatic relief. The findings suggest that the antioxidant action and inhibition of acetylcholine esterase might lead to their being considered as an effective, novel therapeutic agent for Alzheimer's disease.

Validation and evaluation of anxiolytic drugs in light/dark model The light/dark task has long been used in zebrafish as an anxiety test. Anxiolytics have been reported to enhance time spent in the bright zone. Because zebrafish prefer gloomy settings, this characteristic was used to study the effects of anxiolytics50-53 . The results of time spent in light have been presented in Fig 9. The time spent in light by the untreated control fish was 50.66 ± 3.09 whereas in the test dose group (50 µ L) administered 94.5 ± 10.25. The time spent in light for ethanol 0.5 % was 124 ±10.17.

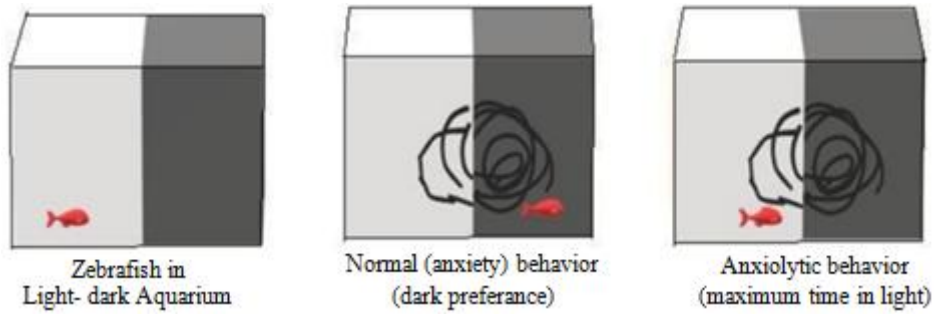

Figure 7: The anxiety like behaviour in the adult zebrafish using the light/dark box paradigm

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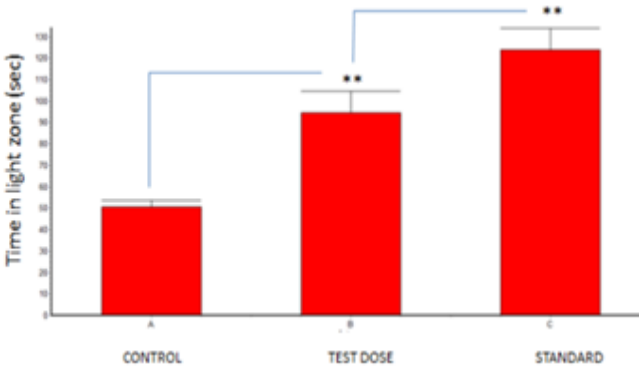

Figure 8: Effect of flaxseed oil+ Allspice oil on light/dark model

Our findings confirmed the zebrafish's light/dark preference and showed that a 1:1 mixture of allspice oil and flaxseed oil, as well as ethanol (control), was effective in increasing time spent in the light zone as compared to the usual control. The treatment with 1:1 mixture of flaxseed oil + allspice oil managed to anxious behavior in the fishes, without impairing their locomotion. This effect is most probably due to its natural biflavonoid composition53 .

Anesthetic activity

Figure 9: Effect of flaxseed oil+ Allspice oil on anaesthetic activity of zebrafish

Table2: Effect of flaxseed oil+ Allspice oil on zebrafish anesthetic model Values are expressed as mean ±SEM, n = 6

Treatment	Anaesthetic Induction Time (Sec)
Standard	55 ± 9.40
Test Cone 1 (5%)	534 ±13.13
Test Cone 2 (10%)	761.1± 610.37
Test Cone 3 (15%)	68.66± 5.74

In comparison to the treatments with a 1:1 combination of allspice oil and flaxseed oil($p<0.0001$),standard (clove oil 100 mg/l) ($p<0.0001$)triggered the loss of balance and onset of anesthesia more rapidly than test concentration however, by increasing test concentration 3 (15 ml/L).they were able to produce anaesthetia in less than 50 seconds.

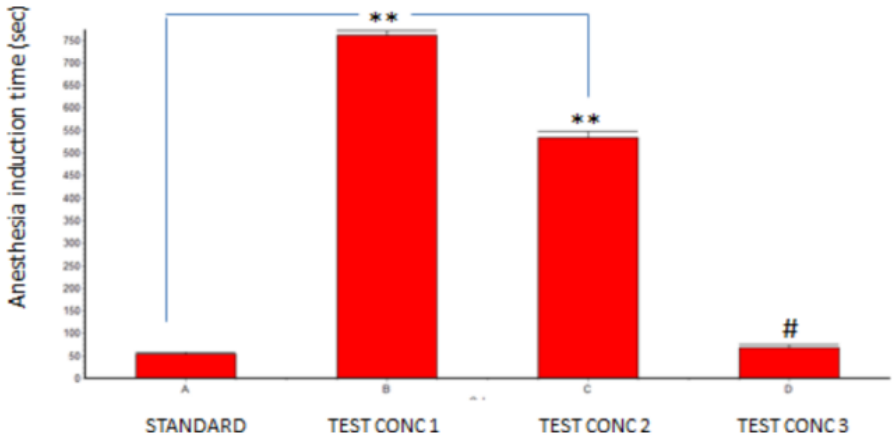

Figure 10: Effect of flaxseed oil+ Allspice oil on anaesthetic activity in zebrafish

During and after anaesthesia, all animals in all treatment groups lost all of their reflexes, and there was no mortality. Except for ketamine alone, which caused fish to undergo phases of excitationwith erratic, accelerated, and intense movements in almost all directions, with a predominance of circular movements, the anaesthetic protocols tested did not induce distressful behaviours in fish during induction or recovery. This effect might be due to the presence of eugenol abundantly in leaf and seed of allspice plant.

5. Conclusion

The current study was a satisfactory attempt to assess the neuropharmacological screening of an herbal combination

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(flaxseed oil and allspice oil in a 1:1 ratio) in several zebrafish models. Finally, our findings suggest that clarifying the effects of flaxseed essential oil and allspice oil (1:1) in the zebrafish Y maze and dark/light paradigm, displaying memory-enhancing and anxiolytic benefits via cholinergic modulation and antioxidant capabilities, is necessary. Our blend's anaesthetic effects were compared to clove oil's results. In a zebrafish model of anaesthesia, it possesses a significant anaesthetic property. The interaction between the exposure circumstances (target chemical concentration and speciation, as well as exposure duration) and the timeframe mechanisms involved in really implicated in anaesthetic property. Prior to recommending anaesthetic usage on a broad scale, behavioural and physiological variationsin the reaction of each fish species of interest to anaesthetic exposure must be investigated. In addition, additional research is needed to evaluate other immunological markers in fish anaesthetized with natural anaesthetics. The long-term consequences of clove oil exposure on fish species following surgical transmitter implantation have yet to be fully understood. For a prospective use of each essential oil (flaxseed and allspice), more research is needed toexplore its precise pharmacological characteristics.

Conflict of Interest

The authors have no conflicts of interest regarding this investigation.

References

[1] Priya S Rao et al an important spice, Pimentadioica (Linn.) Merill, International Current Pharmaceutical Journal 2012, 1(8): 221-225.
[2] Tavares, B. and S. Santos Lopes. The importance of zebrafish in biomedical research. Acta Med. Port. 2013. 26(5):583–592.
[3] Giana de P. Cognatoetal, Y-Maze memory task in zebrafish (Daniorerio): The role of glutamatergic and cholinergic systems on the acquisition and consolidation periods,
[4] Elsivier,Neurobiology of Learning and Memory, Volume 98,Issue 4, November2012, Pages 321-328.
[5] Aoki, R., et al., Y-maze avoidance: An automated and rapid associative learning paradigminzebrafish. Neurosci. Res. (2014)
[6] Kulkarni S.K. Handbook of experimental pharmacology, revised 3rd edition. VallabhPrakasan; Delhi.
[7] Kale S.R, kale R.R. Practical pharmacology, 3rd edition, VallabhPrakasan, Delhi.
[8] Magno LD, et al. Pharmacological study of the light/dark preference test in zebrafish (Daniorerio): Waterborne administration. PharmacolBiochemBehav. 2015 Aug; 135:169-76.
[9] Rishitha, N, et al. Therapeutic Evaluation of Solid Lipid Nanoparticle of Quercetin in Pentylenetetrazole Induced Cognitive Impairment of Zebrafish. 2018. Life Sci. 199, 80–87.
[10] Pushkarkulkarni, Zebrafish Models for Neuroscience Drug Discovery, Birla institute of technology and science, Pilani. 2018, 72-81.
[11] ValentimAM, et AL. A New Anaesthetic Protocol for Adult Zebrafish (Daniorerio): Propofol Combined with Lidocaine. PLoS One. 2016 Jan 25;11(1): e0147747.

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