



EVALUATION OF INVITRO ANTIOXIDANT AND INVIVO ANTI-ANXIETY ACTIVITIES OF ETHANOL EXTRACT OF ABELMOSCHUS MANIHOT (L.) IN ADULT ZEBRA FISH.

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ABSTRACT

A medicinal plant is a plant that is used in herbal medicine. In addition to being used for medicinal purposes, plants are also studied for their use. There is approximately one-third of adults who suffer from anxiety, making it among the most prevalent mental illnesses. A volume of 5L of this solution was prepared and poured into the tank. There were no abnormalities detected in the primary or secondary lamellae in the gills. Zebra fish exhibited symptoms of agitation and freezing when exposed to an ethanol solution. As a result of exposure to different concentrations of ethanolic extracts of *Abelmoschus pungen* (100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, and 6.25 mg/ml), there was no mortality or morbidity among the fish. Consequently, only botanically based medications were considered. Zebra fish were injected with ethanol-induced anxiety and reserpine-induced depression in order to test the effect of *Abelmoschus pungen* extract's powerful antioxidant properties.

Key words: *Abelmoschus Manihot*, Zebra Fish, Antioxidant, Anti-Anxiety.

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INTRODUCTION

Plants used in herbal medicine are sometimes called medicinal plants ("herbology" or "herbal medicine"). Plants are used for medicinal purposes and their use is also studied. In common usage, herb refers to any plant part, including fruit, seeds, stems, bark, flowers, leaves, stigmas, roots, or any nonwoody plant [1]. Previously, "herb" was a generic term only applicable to plants that are not woody,

such as those that grow in trees and bushes. As well as being used for food, medicine, and perfume, these medicinal plants have also been used for spiritual purposes. India is known among ancient civilisations for being a storehouse of medicinal herbs [2]. Many medicinal and aromatic plants grow in the forests of India, and these plants are mostly used as raw materials to produce pharmaceuticals and perfumery products [3]. AYUSH, India's traditional medicine system, contains a catalog of 8,000 herbal treatments. Folk medicine (tribal) and Ayurveda are four of the most important indigenous systems of medicine. Approximately 80 percent of people use herbal medicine as part of their primary healthcare, according to the World Health Organization [4].

Herbs with medicinal properties and their importance

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Black pepper, cinnamon, myrrh, aloe, sandalwood, ginseng, burdock, bayberry, and safflower are herbs used to heal wounds, sores, and boils. A kitchen garden is an excellent place to grow medicinal herbs such as basil, fennel, chives, cilantro, apple mint, thyme, golden oregano, variegated lemon balm, rosemary, and variegated sage.

Benefits of herbal medicine

HM refers to herbs, herbal materials, herbal preparations, and finished herbal medicines that contain active components from plants or from combinations of plants, primarily used for preventative or therapeutic purposes. Scientific evidence suggests that HMs contain complex chemical components associated with pharmacological effects that may provide beneficial effects on health or cause toxic effects.

Depression treatment with drugs

In the treatment of depression, there are a number of different types of drugs [5]:

- Antidepressants (SSRIs) that selectively block serotonin reuptake
- The 5-HT₂ antagonists (5-HT₂ blockers) modulate serotonin.
- A reuptake inhibitor of serotonin and norepinephrine
- Inhibitor of norepinephrine-dopamine reuptake
- Antidepressants heterocyclics
- A MAOI is an inhibitor of monoamine oxidase (MAO).
- Antidepressant melanocortinoid
- Drugs like ketamine

Corticosteroids, certain beta-blockers, interferon, and reserpine, among other medications, can cause depression. Some recreational substances (such as alcohol and amphetamines) can cause or exacerbate depression. Transient depressed symptoms might be caused by toxic effects or medication withdrawal. Some patients make use of medicinal plants. Although there is conflicting evidence, St. John's wort may be useful for moderate depression. Other antidepressants and medicines may interact with St. John's wort. Studies showing that taking eicosapentaenoic acid for 1 to 2 g once a day can help with depression have been done in placebo-controlled situations [6].

Fig 1.1: Drugs inhibition of depression

ANXIETY

One-third of the adult population suffers from anxiety, making it among the most prevalent disorders in society. There are various types of anxiety disorders, including panic attack, post-traumatic stress disorder, generalized anxiety disorder, and specific phobias. Anxiety disorders are characterized by an acute and excessive sense of concern in response to potential problems, which adversely affects the quality of life and job performance of the individuals suffering from them. The first line of defense

against anxiety disorders consists of SSRIs and cognitive behavioral therapy [7].

PLANT PROFILE

Abelmoschus pungens

Habitat

A. pungens var. *mizoramensis* occurs at an altitude between 150 to 800 m, in association with *Solenanthera heterophylla* Lour., *Trichosanthes* sp., *Thladiantha cordifolia* Cogn. (Cucurbitaceae), *Solanum violaceum* Ortega (Solanaceae), *Musabalisiana Colla* (Musaceae), etc. Generally observed on the edges of secondary forests, roadsides, grasslands and forest slopes. Common in Lungle and Serchhip, and occasional in Kolasib, Aizawl and Lunglei districts [8].

Global Distribution

Asia: Bhutan, China, India, Indonesia, Malaysia, Myanmar, Nepal, Pakistan, Philippines, Taiwan, Thailand.

Salient features of zebra fish as a model organism

The characteristics of the *Danio rerio* make it ideal as a model organism, a fact that is intrinsic to its popularity among scientists. As the embryo develops outside the mother while being opaque and optically transparent, it is readily observable and studied. It is approximately 18 hours old when the embryo is transparent, and the blastula stage lasts for just three hours. Gastrulation takes five hours. It is possible for the embryo to be translucent and to see very well-formed ears, eyes, segmenting muscles, and brain. All the major organ systems have been established by the end of the day. Approximately 72 hours after emergence, the embryo begins to seek nourishment. Four days after being injected into a uterus, an embryo becomes a miniature version of an adult. As a result of the rapid development, discoveries in development and genetics are made easier. Zebra fish reach sexual maturity within a period of about 10 weeks, and this little fish has a high reproductive rate. According to, zebra fish will lay about 200 eggs per week when kept under ideal conditions [9]. In addition to being a difficult fish to grow, zebra fish are also easy to keep. Zebra fish are useful and require very little space as well as very little maintenance. This fish has many of the traits necessary to be an intriguing model organism for studies on developmental, toxicological, and transgenic mechanisms. We will discuss recent developments in zebrafish research, including evolutionary genetics, toxicity, transgenic experiments, human illness, medication development, cancer, etc.

Advantages as model for anxiety and depression

While fish behaviour was often thought to be mostly rudimentary and subconsciously motivated, recent research has shown the dynamics of zebra fish behaviour and its relevance to modelling fear- and anxiety-like emotions. Increasing evidence reveals that zebra fish's

anxiety-like behaviour is triggered by environmental cues similar to those experienced by rats, and that the zebra fish's anxiety-like behaviour is governed by evolutionarily conserved circuitry that mediates unpleasant learning and emotionality [10]. Furthermore, strong anxiogenic endocrine and genomic responses in zebra fish have been documented, which are similar to those seen in people and rats002E.

MATERIALS AND METHODS

PLANT PROCESSING

In December, the entire plant of *Abelmoschus pugnens* was obtained from a local farm in Chennai, India [11].

ANIMALS STUDIES

Acute toxicity studies

The adult zebra fish was used in the acute toxicity trials. Whinzbang bioresearch Pvt. Ltd. in Chennai provided both sexes of zebra fish.

Extract dosing

The extract was weighed and dissolved in distilled water to obtain the concentration of 1g/L. This was further diluted with distilled water to obtain different variable concentrations of 100 mg/L, 50 mg/L, 25 mg/L, 12.5 mg/L and 6.25 mg/L. This solution was prepared to a volume of 5L and poured in the tank.

Histopathological examination

The fish were sacrificed at the end of the experiment by euthanizing them with hypothermic shock. Fish parts such as the liver, kidneys, and other organs were meticulously extracted, and the resulting tissue was kept in formalin solution and prepared for section cutting.

ANTI-ANXIETY ACTIVITY-NOVEL TANK METHOD

To reach a concentration of 1g/L, 1g of extract was weighed and diluted in distilled water [12]. To obtain two solutions with concentrations of 50mg/L and 25mg/L, serial dilutions were performed.

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Water in the novel tank was changed for every 3 fishes and fishes were monitored for duration sent in upper and lower halves of the tank, time gap to enter into upper half, number of time fish entered into upper half, erratic movements and freezing patterns [13, 14].

ANTI-DEPRESSANT ACTIVITY

The principal drug tanks were constructed in the same way as the prior experiment. The medication solutions were made with the identical 50mg/L and 25mg/L concentrations. At a concentration of 10 mg/L, fluoxetine was chosen as the reference medication. The drug tanks were filled with the extract and medication solutions [15].

Statistical analysis

The values were considered as triplicate or 10 readings as per procedure and were represented as Mean and its standard deviation. The data was subjected to ANOVA one-way analysis to find the difference in the samples. The significant differences between groups were evaluated using dunnet's test and $P > 0.001$ were considered as significantly different from the comparing group.

RESULTS

5.1. ACUTE TOXICITY STUDIES

Zebra fish were randomly placed into six groups, each with ten fish. The extract was converted into solutions with concentrations of 100mg/L, 50mg/L, 25mg/L, 12.5mg/L, and 6.25mg/L. Zebra fish were exposed to the aforesaid solutions for four days, with readings obtained at the conclusion of each day to determine the extract's toxicity on the fish. Various factors such as changes in water pH, oxygen content, fish movement, and behaviour.

ANTI-ANXIETY ACTIVITY

Using a unique tank approach, the anti-anxiety effect of the ethanol extract of *Abelmoschus pugnens* was investigated against ethanol-induced anxiety. The outcomes are presented in the tables below.

ANTI-DEPRESSANT ACTIVITY

Groups II and III were extract-treated groups, with greater and lower doses of 50 and 25 mg/L, respectively. Fish in groups IV and V were given normal medication

treatments. The exposure of the fish to the reserpine medicine caused the fish to become depressed.

Table 1. Grouping and Dosing to Zebra fish.

Sl. no	Group	No. of fishes/ group	Concentration (mg/ml)
1	Group I	10	100
2	Group II	10	50
3	Group III	10	25
4	Group IV	10	12.5
5	Group V	10	6.25
6	Group VI (control)	10	No extract treatment, Fresh Water with 4pg/ml of 3,4- dichloroaniline

Table 2. Grouping and Dosing to Zebra fish to evaluate for anti-anxiety activity.

Sl. no	Group	Induction of anxiety	Treatment concentration of drugs		Number of fishes
1	Group I	0.5 %v/v ethanol in water	Normal water	--	10
2	Group II	0.5 %v/v ethanol in water	Extract-higher dose	50mg/L	10
3	Group III	0.5 %v/v ethanol in water	Extract-lower dose	25mg/L	10
4	Group IV	0.5 %v/v ethanol in water	Standard drug (Buspirone)	25mg/L	10
5	Group V	0.5 %v/v ethanol in water	Standard drug+Extract	25mg/L+25mg/L	10

Table 3. Grouping and Dosing to Zebra fish to evaluate for anti-depressant activity

Sl. no	Group	Induction of anxiety	Treatment concentration of drugs		Number of fishes
1	Group I	Reserpine	Normal water	--	10
2	Group II	Reserpine	Extract-higher dose	50mg/L	10
3	Group III	Reserpine	Extract-lower dose	25mg/L	10
4	Group IV	Reserpine	Standard drug (Fluoxetine)	25mg/L	10
5	Group V	Reserpine	Standard drug+Extract	25mg/L+25 mg/L	10

Table 4. Effect of extract on the movement and behavior of the zebra fish

Observation in days	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
12	Erratic swimming	Erratic swimming	No change	No change	No change	No change
24	Erratic swimming	No change	No change	No change	No change	No change
48	Occasional Erratic swimming	No change	No change	No change	No change	No change
72	No change	No change	No change	No change	No change	No change
96	No change	No change	No change	No change	No change	No change

Table 5. Effect on ethanol extract on the movement of fish in novel tank.

Groups	Time spent In Upper Half (sec)	% time in upper tank	Time spent In lower Half (sec)	% time in lower tank	No. of Entries to Upper half	Latency to enter upper half (sec)
Group I	51.2±4.6	16.58	250.1±4.5	83.42	1.6±0.5	45.9±5.2
Group II	55.9±6.9	18.75	243.5±6.5	81.25	1.6±0.6	88.9±14.7
Group III	55.2±4.8	18.21	246.1±4.5	81.79	1.6±0.5	58.5±16.8
Group IV	178.5±10.5	59.27*	123.5±10.5	40.73*	13.9±5.7*	19.5±12.5*
Group V	151.6±12.9	49.85*	150.2±12.7	50.15*	15.9±6.6*	25.9±9.6*

Table 6. Effect of plant extract on the erratic movement and freezing bouts in zebra fish.

Sl. no.	Groups	No. of erratic movements	No. of freezing bouts
1	Group I	1.5±0.8	0.6±0.4
2	Group II	1.5±1.4	0.6±0.5
3	Group III	0.4±0.4	1.2±0.6
4	Group IV	0	0.3±0.4*
5	Group V	0	0.3±0.3*

Fig 1.1: Drugs inhibition of depression

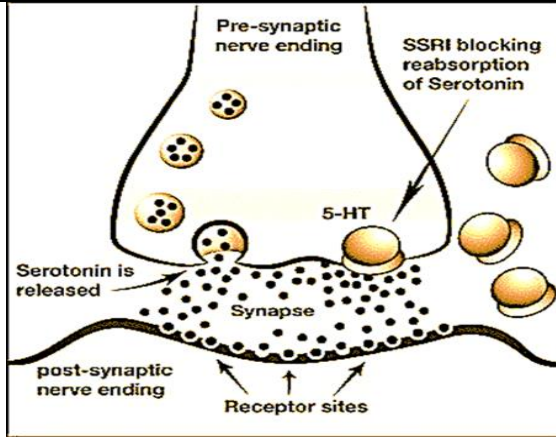


Fig 1.2: Drugs inhibition of depression

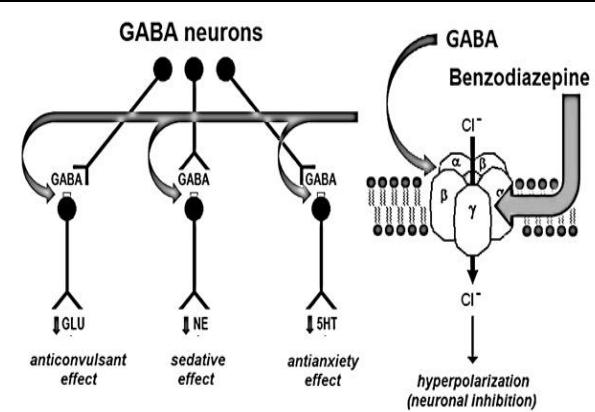


Fig 1.3: Plant of Abelmoschus pungens



Fig 1.4 Zebra fish in tank



Fig 1.5. Effect of Extract of Abelmoschus pungens on the pH of the tank

Fig 1.6. Effect of Extract of Abelmoschus pungens on the Dissolved oxygen

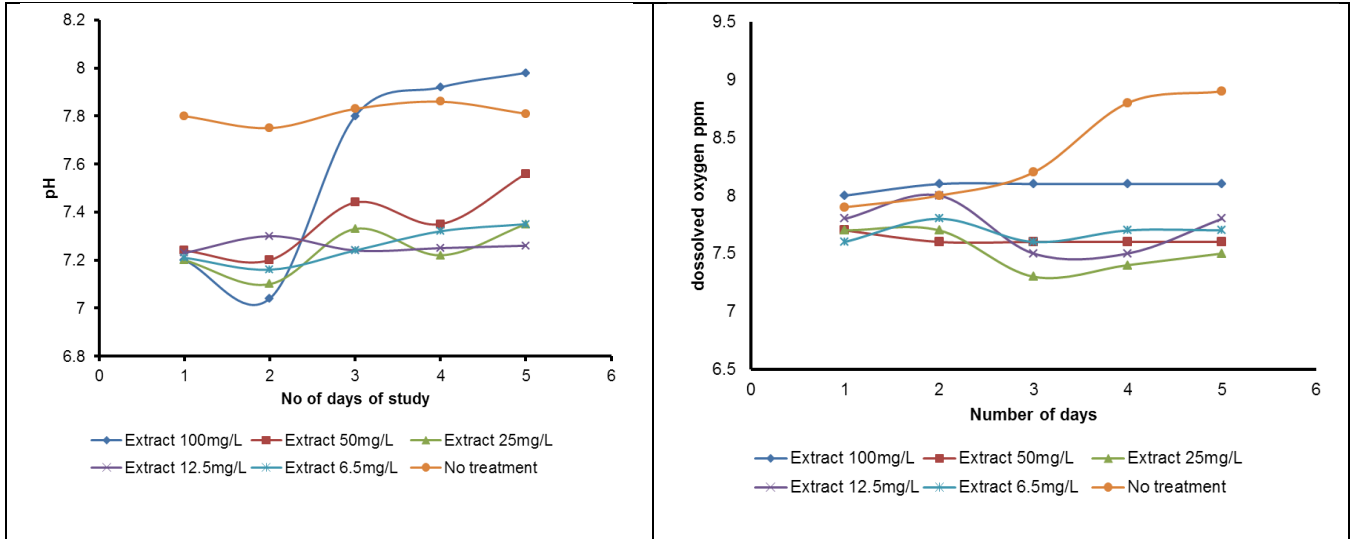


Fig 1.7. Acute toxicity studies histopathology.

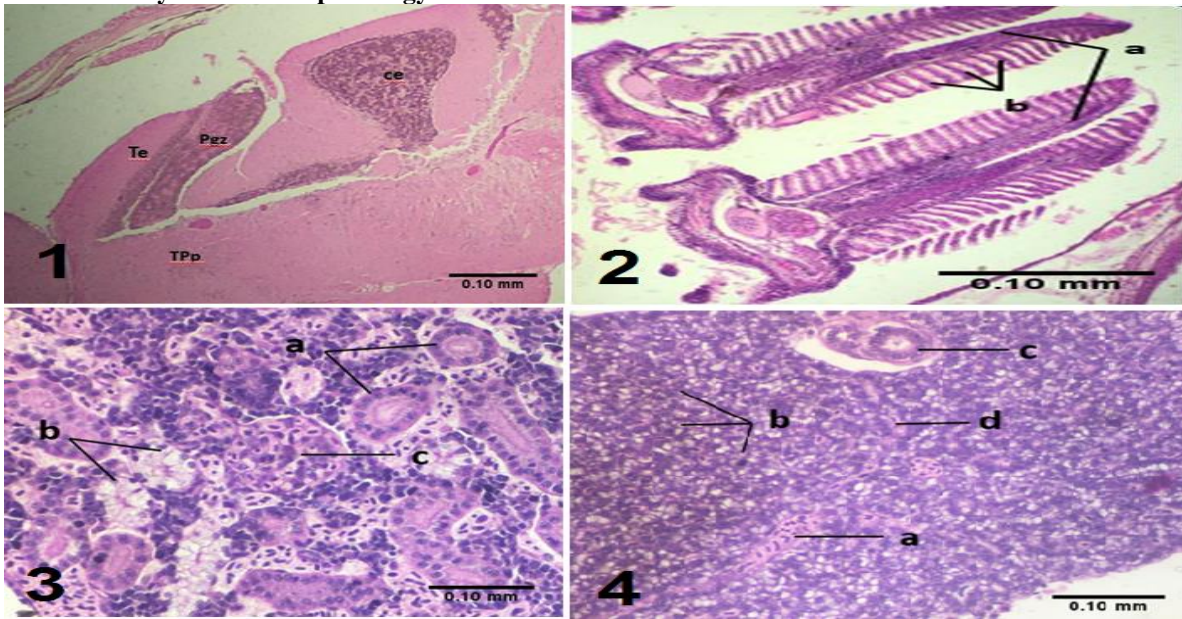
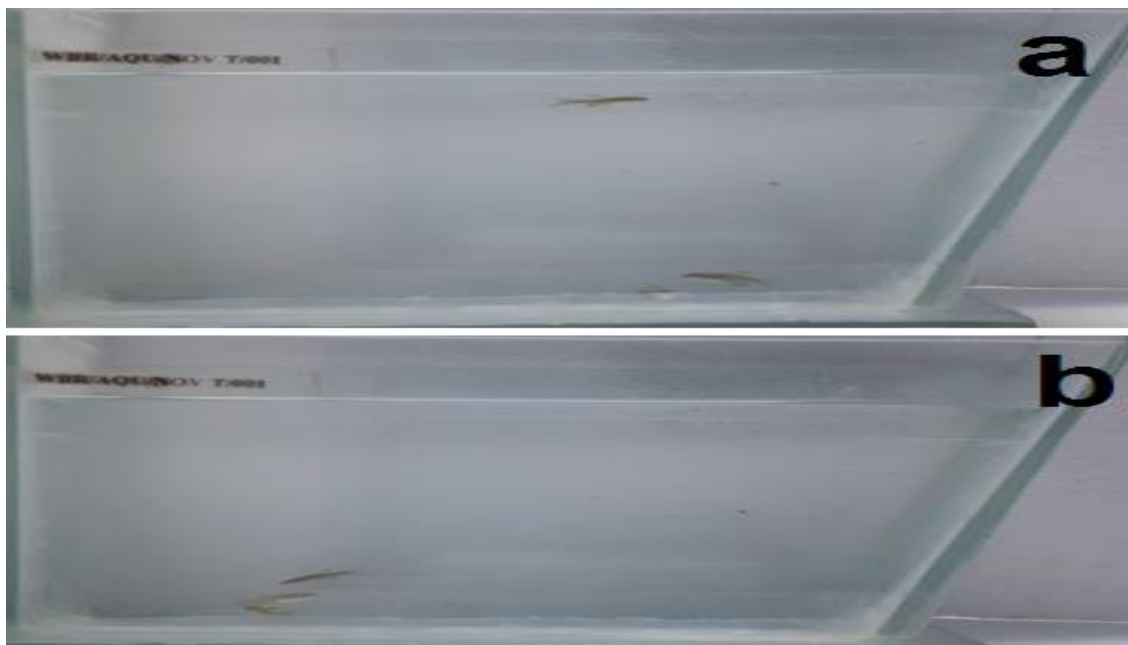


Fig 1.8. Untreated depressed group; Untreated depressed group.



Discussion

Acute toxicity tests on fish organs such as the brain, gills, liver, and kidney found no signs of harm. In the tank, zebra fish are known for their fast movements and shoaling. They tried to move around in the tank and were quite adventurous. The extract's antidepressant properties were investigated in zebra fish by observing their shoaling behaviour. Overall, the extract did not appear to have considerable anti-anxiety or antidepressant effect, but it did boost the activity of the conventional medicine.

Summary & Conclusion

Anxiety is an unpleasant condition of tension, discomfort, or uneasiness, as well as a dread that frequently has no explanation. The most common mental illnesses are anxiety disorders. The current study uses zebra fish models to investigate the anti-anxiety and antidepressant properties of the herb *Abelmoschus pugnens*. Anxiety and depression are two disorders that have shown to be difficult to treat with medications due to adverse effects and the potential for addiction. This suggests that more study is needed in this area to isolate and define an anti-anxiety and antidepressant medication derived from this plant.

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