Investigating the Anti-Anxiety Properties of *Ammannia baccifera* L in Zebrafish Behavioural Models

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***Abstract*—Neurodegenerative disorders, characterized by the progressive loss of neuronal structure and function, remain a major global health challenge with limited therapeutic options. Ammannia baccifera L., a traditionally used medicinal plant known for its diverse pharmacological properties, has not been extensively evaluated for its potential neurotoxicity. This study aims to assess the neurodegenerative effects of Ammannia baccifera L. using zebrafish (Daniorerio) as a model organism, due to its well-established genetic, anatomical, and behavioural parallels with human neurobiology. Ethanolic extracts of A. baccifera were administered to adult zebrafish at varying concentrations over a defined exposure period. Behavioral assessments including locomotor activity, anxiety-like responses (using the novel tank diving test), were conducted to evaluate neurological function.**

Anxiolytic activity of the ethanolic whole plant extract of Ammania bacciferaL was performed using Novel Tank Diving Test &Locomotory Activity. All the treated groups showed significant level at P<0.05 and P<0.001 compared with control. These results provide important insights into the safety profile of Ammannia baccifera, emphasizing the need for cautious evaluation of its traditional use and guiding further pharmacological research. This research also shows both herbal and drug-based treatment options, acknowledging the limitations and side effects of current medications and the potential of natural products in managing neurological illness symptoms.

***Keywords*— Neurodegeneration, Ammannia baccifera L, Herbal Medicine, Anxiolytic Activity, Zebrafish Model, etc**

I. INTRODUCTION

The plant based substances for medicinal use which is also termed as phytomedicine or herbal medicine. This is an ancient practice that relies on the knowledge of plants for the treatment, prevention and promotion of health. Natural products especially from medicinal plants are importance sources for drug development in the pharmaceutical industry. Although many plants have been and over-used for their medicinal value, there are still many un-screened species which have potential for new discoveries. Research should put more focus on these less common plants especially with ethno pharmacology and ethno botanogy background. [1]

Ancient medical knowledge has and is still being transferred from one generation to the other and so have many great discoveries aided from herbal or natural resources. Natural Products are very important in the process of drug development. Studies indicate that a certain percentage of FDA sanctioned drugs include those which are naturally obtained or chemically synthesized using components obtained from natural sources. Since ancient times, medicinal plants have been used throughout various countries, which grew roots as traditional herbal medicine, the foundation of modern medicine. Plants have also been known to produce lots of complex defensive chemicals which can function as botanical pesticides designed to target and eliminate human pathogens. [2]

For the pharmaceutical sector, plants offer a significant source of pharmacologically active compounds as many are being researched towards the formulation of new products. Having served in the past as traditional medicine for numerous ailments, these natural resources are now used to manufacture a number of pharmaceutical drugs. Natural goods contain bioactive agents that exert the biological activity against the pathogens. The remnants of ancient medical systems give insight which continues to be harnessed for the exploration of plants to be used for preparing the medicines. [3]

The use of herbal medicine is widespread in many of the developing world's because people depend on traditional healers and go to herbal medicinal shops for their health needs. While herbal remedies coexist with modern medicine, they continue to be popular and are indeed more commonly sold than before, even in developed nations. Unlike in other countries,

however, there are varying degrees of regulation concerning herbal drugs in different countries. [4]

Medicinal plants form a unique group whose compounds assist in the development of drugs. They have been helpful in the advancement of culture and also act as sources of food and medicine. There are estimates that many plant species have been used in traditional medicine for a long time. [5]

Neurodegenerative disorders that consist of the gradual death of neurons and underlies the major physiological processes of the body. Alzheimer's disease (AD),[6] Parkinson's disease (PD) [7, 8] and amyotrophic lateral sclerosis (ALS) [9] are examples of these disorders and are becoming more of a problem along with the aging population. The development of new treatments has their own disadvantages, and conventional drugs will never stop having problems regarding side effects and the use of natural products for these illnesses seems promising. [10, 11, 12]

The use of zebrafish (*Danio rerio*) as a research model. Shrimp are small striped fish that are common in aquariums and are now being used more frequently in laboratories. [13] They present several pros including their economical nature since drug testing is costly, but zebrafish are easy to care for and reproduce. [14]Zebrafish possesses a substantial percentage of the Human genome, so their organs and cells are similar to humans and they possess orthologs in a large percentage of genes associated with human diseases. Their embryos are transparent, which makes watching the development easy, and they are considered in-vitro until 5 days after fertilization, which reduces ethical issues. [15]

II. OBJECTIVES

To prepare and characterize the phytochemical constituents of *Ammannia baccifera* L extract using standard analytical methods.

To determine dose-dependent toxicological thresholds of *Ammannia baccifera* L extract in zebrafish to establish safe and neurotoxic ranges.

To evaluate the behavioural anxiolytic effects of *Ammannia baccifera* L exposure in zebrafish, including changes in locomotion, anxiety-like behaviour.

III. METHODOLOGY

Equipment and materials utilized in the research include a whole board arena apparatus, a light and dark tank, a heating mantle, a Soxhlet apparatus, a locomotory tank apparatus, and a novel tank apparatus. Chemicals utilized are dimethyl sulfoxide, ethanol, and aluminium chloride.

The plant material, *Ammania baccifera L.*, was taxonomically identified and verified by a botanist. The plant material was extracted using both Soxhlet apparatus and cold maceration methods with 95% ethanol as solvent. The extracts were concentrated, kept in the dark at low temperature, and percentage yield, colour, and consistency were recorded. The ethanolic whole plant extract was subjected to preliminary phytochemical investigation.

Adult zebrafish were employed as the animal model and were purchased from a certified fish vender. The zebrafish were kept in aerated tanks with controlled temperature (28°C) and photoperiod (12h light: 12h dark), and water quality was ensured. [16]

3.1 Oral Acute Toxicity of *Ammania baccifera* L

Acute toxicity of ethanolic extract of plant samples of *Ammania baccifera* L was tested for acute toxicity in the zebrafish model (*Danio rerio*) as per the OECD guidelines 203. The fish were exposed to the test substance preferably for a period of 96 hours. Mortalities were recorded at 24, 48, and 96 hours, and the concentrations which kill 50% of the fish were recorded.

The fish were inspected after 24, 48, 72, and 96 hours. Concentrations of 100, 200, 300, 400 and 500 mg/L were selected as effective concentrations for performing the main toxicity tests of the plant extracts of different concentrations.

The fish were exposed to the sample based on a static exposure regime. For every experiment, 10 healthy fishes were directly transferred into each prepared concentration. Control groups (10 fishes) were also included for each treatment. The mortalities were recorded at 24, 48, 72, and 96 hours before exposure, and the LD50 values were calculated. Fish were considered dead if there is no visible movement and upon touching of the caudal peduncle produces no reaction. Dead fishes were removed when mortalities are recorded. LD50 was determined based on the concentration of the test substance in water which killed 50% of a test batch of fish within a particular period of exposure was observed. [17]

3.2Novel Tank Diving Test

The zebrafish were divided into four different groups namely Group I(Control), Group-II

(Scopolamine treated), Group III (Test 1) and Group IV (Test 2). Each group consists of 10 adult zebrafish were used.

After given dose of Aluminium chloride to induce and for treatment ethanolic extract *Ammania baccifera* L. of three adult zebrafish from each group were individually placed in three novel tank apparatus (measurements 25cm width × 12 cm length × 20 cm height) filled three quarters full with aquarium treated water. The tank was divided horizontally into two equal sized regions to indicate top and bottom region. The zebrafish swimming behaviour was recorded for 3 minutes using a side-mounted camera. The parameters observed include the number of entries and time spent (s) in the bottom area, number of entries and time spent (s) in the top area, the distance travelled (mm) and maximum speed (mm/s) of the zebrafish. The zebrafish exhibits anxiety by a decrease number of entries in the top region as well as a longer latency in reaching the top region. [18, 19]

3.3 Locomotory Activity:

The zebrafish were divided into four different groups namely Group I(Control), Group-II (Scopolamine treated), Group III (Test 1) and Group IV (Test 2). Each group consists of 10 adult zebrafish were used.

The toxic group received seven days of treatment with 100 mg/L and 200 mg/L of *Ammania Baccifera* L. The fish motions were assessed using a modified version of Xia's technique. We filled a tank (measurements 25cm width × 12 cm length × 20 cm height) with 80% water for our study. The tank was now separated into four sections, each of which was covered with a clear plastic sheet. Fish are now placed in the tank individually, and their motions, including swimming behaviour, distance travelled, and mean speed, are recorded for three minutes. Mortality is also noticed when the fish migrate from one segment to another throughout the 3 min video observation. [19, 20]

3.4 Statistical Analysis

All the values were statistically analysed by one-way analysis of variance [ANOVA] followed by Dunned multiple comparison test. Data from distilled water treated animals were used as the control and data from scopolamine treated animals were used as positive control treated animals. All values are expressed as mean +S.E.M. Results were regarded as significant at $p<0.05$.

IV. RESULTS AND DISCUSSION

4.1 Oral Acute Toxicity

The results demonstrate a dose-dependent increase in toxicity of EEAB on zebrafish. Concentrations up to 200 mg/L showed no signs of toxicity, as all fish survived up to 96 hours. However, concentrations from 300 mg/L and above induced increasing mortality, with complete lethality observed at 400 and 500 mg/L by 96 hours. This indicates that the LC_{50} (lethal concentration for 50% mortality) likely lies between 100 mg/L and 200 mg/L under the tested conditions.

The Ethanolic Extract of *Ammannia baccifera* L concentration of 100mg/L & 200mg/L is non-toxic to zebrafish at concentrations up to200 mg/L. However, concentrations above this threshold demonstrate acute toxicity, and therefore, careful dose optimization is crucial for any potential therapeutic applications.

4.2 Novel Tank Diving Test

This test was used to assess anxiety in a novel environment by monitoring movement between the top and bottom of the tank. Group II(Group II received $AlCl_3$ at a dose of 0.2mg/L) exhibited increased latency to reach the top, spent significantly less time at the top, and had fewer entries, all of which are indicative of anxiety-like behaviour. In contrast, Groups III (100mg/L of EEAB) and IV (200mg/L of EEAB) showed improvements across all parameters. Group IV (200mg/L of EEAB) in particular had the highest time spent in the top and reduced bottom-dwelling time, resembling the behaviour of the control group(Group I received purified water). These results further support the anxiolytic potential of the treatment, especially at higher doses.

4.3 Locomotary Test

The locomotory activity of the subjects was assessed based on distance moved, velocity, and predominant vertical position. The control group (Group I received purified water) showed high overall activity and preferred mid-water regions. Group II (Group II received $AlCl_3$ at a dose of 0.2mg/L) exhibited a marked decline in total distance moved and average velocity, as well as a tendency to remain near the bottom or edges, which are typical signs of reduced motor activity and increased anxiety. Treatment with the experimental agent [Groups III (100mg/L of EEAB) and Group IV (200mg/L of EEAB)] led to a significant

improvement in both distance travelled and average speed. Both groups also explored all levels of the environment, indicating a normalization of behaviour and reduced anxiety.

V. CONCLUSION

Anxiolytic activity of the ethanolic whole plant extract of *Ammania baccifera* L was performed using Novel Tank Diving Test & Locomotory Activity. All the treated groups showed significant level at *P=<0.05 and **=P<0.001 compared with control.

The rising prevalence of these diseases has increased the urgency to find effective treatments. While drug-based options exist, their negative effects and inability to halt disease progression highlight the need for alternative treatments. Herbal compounds like curcumin and aloe-Vera show promise due to their antioxidative qualities, but their low absorption rates limit their medicinal use. Further research is needed to validate the efficacy of these natural compounds.

This study investigated the anxiolytic potential of theethanolic extract of *Ammania baccifera* L. using methods like the Novel Tank Diving test and Locomotar test. The treated groups showed significant results compared to the control. The findings support the traditional use of *Ammania baccifera L.*, but further studies are required to understand the precise mechanisms and identify the active compounds responsible for the observed pharmacological activity. The ethanolic extract of *Ammania baccifera* L. demonstrates significant neurodegenerative activity, potentially due to the presence of alkaloids, flavonoids, and tannins.

VI. APPENDIX

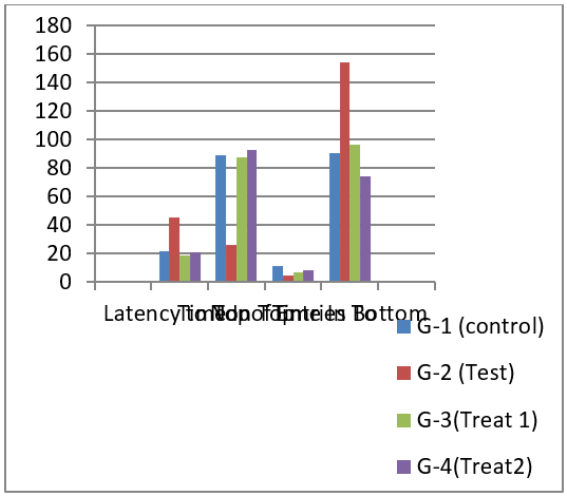


Figure No.1: Histogram for Novel Tank Diving Test

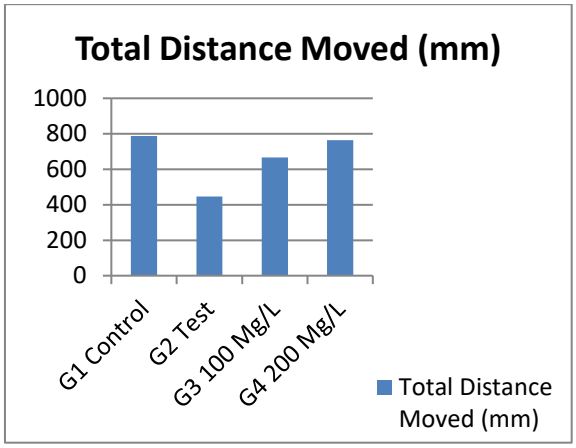


Figure No. 3: Histogram of Locomotory Test Model (Total Distance Moved)

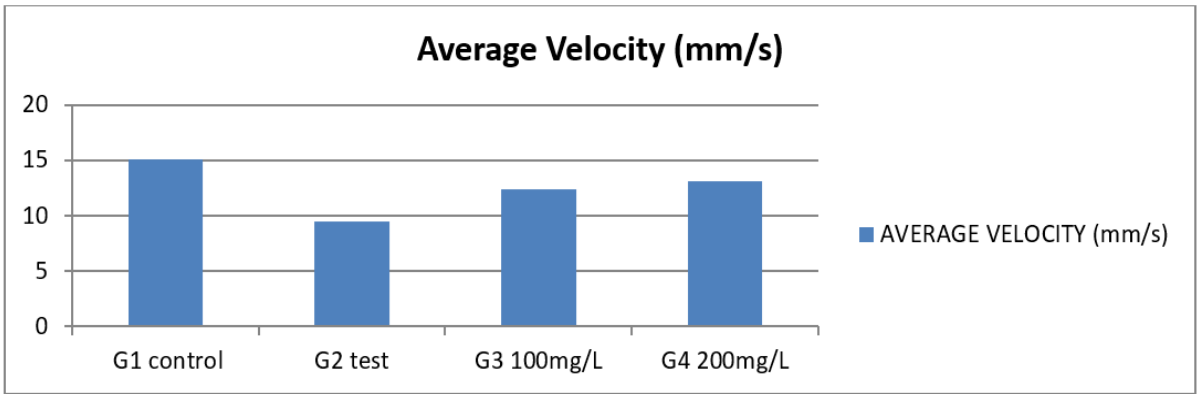


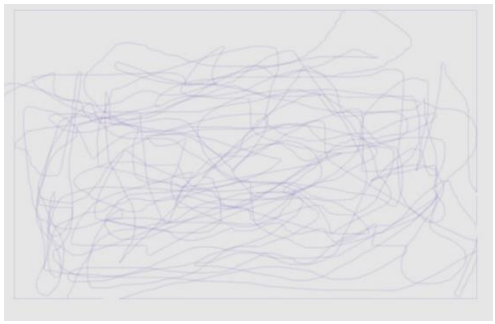
Figure No. 4: Histogram of Locomotory Test Model (Average Velocity)

(A) Control Group

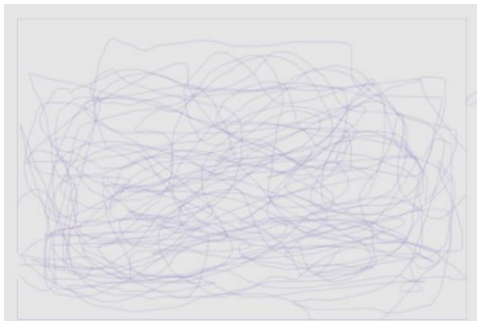
(B) Control Group

(C) Anxiety induced groups

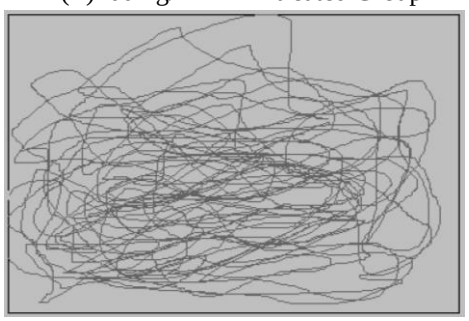
(D) Anxiety induced group



(E)100mg/L EEAB treated Group



(F)100mg/L EEAB treated Group



(G)200mg/L EEAB treated Group

(H)200mg/L EEAB treated Group

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