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EXPLORING THE NEUROLOGICAL IMPACT OF AMMANIA BACCIFERA L IN ZEBRAFISH; INSIGHTS INTO NEURODEGENERATION

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ABSTRACT

Introduction: Phytomedicine uses plants for healing and is key to drug development, though many plants are unexplored. Natural products are vital in drug discovery, with many drugs derived from them. Plants produce chemicals that can fight pathogens. Herbal medicine is used globally, but regulations vary. Medicinal plants are important for drugs, food, and medicine. Neurodegenerative diseases like Alzheimer's are increasing, and natural products are a promising treatment avenue. Zebrafish are a useful, cost-effective research model due to their similarity to humans. **Objectives:** This study will analyse the phytochemicals of Ammannia baccifera L. extract, determine its toxic levels in zebrafish, and assess its effects on zebrafish behaviour and neurophysiology, specifically locomotion and anxiety. **Methods:** The study used equipment like a hole board, light/dark tank, and Soxhlet apparatus, and chemicals including dimethyl sulfoxide and scopolamine. Ammania baccifera L. plant material was extracted with 95% ethanol, and adult zebrafish were used as the animal model. The study assessed the extract's acute toxicity in zebrafish and evaluated neurodegenerative effects using tests like the hole board and light/dark tests. Statistical analysis used ANOVA followed by Dunned multiple comparison test. **Results:** The study of oral acute toxicity showed that the ethanolic

extract of Ammannia baccifera L. (EEAB) was toxic to zebrafish at concentrations above 200 mg/L, with complete lethality at 400 and 500 mg/L, while concentrations up to 200 mg/L were non-toxic. In the Hole Board Test, scopolamine increased anxiety, but EEAB treatment, especially at 200 mg/L, reversed this effect, improving exploratory behaviour. The Light and Dark Test indicated that scopolamine induced anxiolytic behaviour, but EEAB had a less pronounced effect, with zebrafish still favoring the dark compartment. **Conclusions:** Neurodegeneration is a complex process leading to neuronal death and brain dysfunction, with diseases like Alzheimer's and Parkinson's becoming increasingly prevalent, necessitating new treatments beyond existing drugs with limitations. This study explored the ethanolic extract of Ammania baccifera L. using behavioural tests and found significant neurodegenerative activity in treated groups, supporting its traditional use, though further research is needed to understand the mechanisms and identify active compounds like alkaloids, flavonoids, and tannins.

1. 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 pharmacologyand ethnobotanogy 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 worlds 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]

Neurodegenerativedisorders 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]

2. 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 and neurophysiological effects of *Ammannia baccifera* L exposure in zebrafish, including changes in locomotion, anxiety-like behaviour.

3. 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, scopolamine, 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]

3.2 Hole board test

Hole board is a method normally used for the screening of prospective anxiolytic drugs. It is based on the hypothesis that the anxiety state of an animal is inversely proportional to its intention to look for baited holes. Initially, the animals are allowed to explore the experimental tank for 15 min. without any external visual cues in the open field and baited holes in the hole board. After habituation for a period of 4 days, training of zebrafish is commenced where only one hole of the whole board is baited. The time required to find the hole with the bait is noted and to avoid fixed directional swimming the fish is released into the tank from various locations. The given dose of Scopolamine in that maximum time consumed to each fish to find the baited hole is 3 min. This experiment tests the spatial cognition of the fish and therefore is an important model for testing of AD novel drug therapies. The parameters observed include the Time spent for zebrafish at centre, Total distance travelled (swam) for zebrafish, Identification of hole and Poke the hole. 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.[18,19]

3.3 Light & Dark Test

This test's primary goal is to gauge anxiety-like behaviours. Drugs like Aluminium Chloride may exacerbate anxiety in neurodegenerative models, as seen by a decrease in light exploration and an increase in dark exploration time.

A tank that is divided: Dark area: Bright area

The Pre-test Acclimatization: Before the test starts, give the zebrafish 3 minutes to get used to the testing tank. This guarantees that the unfamiliar surroundings won't cause them any concern.

Test protocol: Give the zebrafish a predetermined amount of time, usually 3 minutes, to explore the aquarium. The animal is free to roam between the two compartments throughout this period.

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.

Important metrics to track include

1) Time Spent in the Light region: There is an inverse relationship between anxiety levels and the amount of time spent in the light region; a shorter period of time in the light area indicates a higher degree of anxiety.
2) Time Spent in the Dark Area: Since the dark area is a more protected setting, spending more time there indicates greater worry or fear.
3) Number of Transitions between the Light and Dark Areas: Since the zebrafish feels at ease switching between the two zones, more transitions usually signify less anxiety.
4) Latency to Enter the Light region: Anxiety can also be inferred from the zebrafish's latency to enter the lightregion; a longer latency indicates a higher level of anxiety.[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 $p<0.05$.

4. RESULTS AND DISCUSSION

4.1 Oral acute toxicity

The results demonstrate a dose-dependent increase intoxicity 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 thresholddemonstrateacutetoxicity, and therefore, careful dose optimization is crucial for any potential therapeutic applications.

4.2 Hole board test

The Hole Board Test was conducted to evaluate exploratory behaviour and anxiety in the test subjects. The control group (Group Ireceivedpurified water) demonstrated normal exploratory behaviour with a high number of head pokes and low latency to explore. In contrast, the test group (Group IIreceivedscopolamine at a dose of 0.002mg/L) showed a significant reduction in head pokes and a marked increase in latency, indicating heightened anxiety levels. Group III (100mg/L of EEAB)and Group IV(200mg/L of EEAB), which received treatment, displayed a partial or full reversal of these effects. Particularly, Group IV closely resembled the control group, showing increased head pokes and decreased latency. Time spent in the central zone was significantly lower in Group II, while Groups III and IV demonstrated improvement, especially Group IV which spent more time in the centerthan even the control. Similarly, the total distance travelled was lowest in Group II and substantially improved in the treatment groups, suggesting enhanced locomotor and exploratory activity as a result of the treatment.

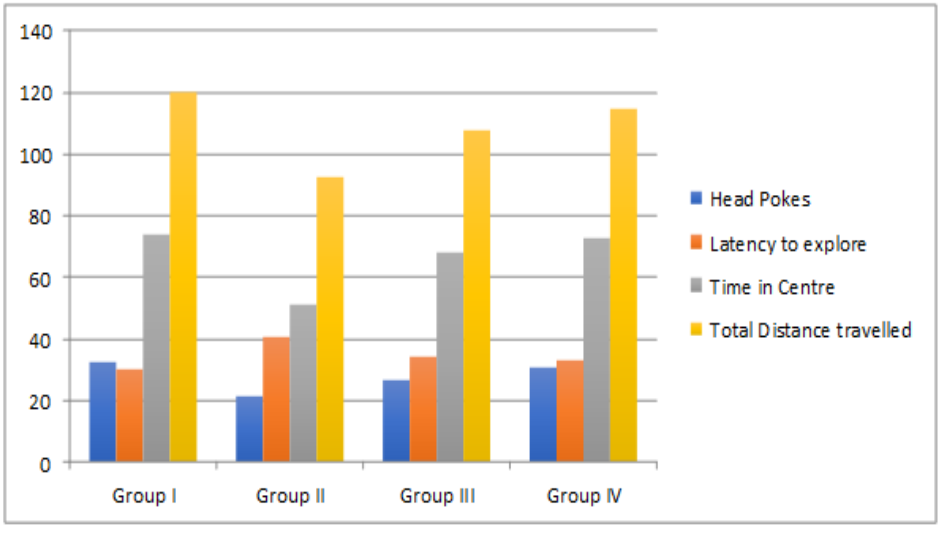

Fig. No. 1: Histogram for hole board test.

4.3 Light and Dark test

This test is a standard model for assessing anxiety-related behaviour, where a preference for the dark compartment indicates higher anxiety. Thecontrol group (Group Ireceived purified water) spent more time in the dark compartment and less in the light, as expected. Group II (Group IIreceived scopolamine at a dose of 0.002mg/L) exhibited a significant shift, spending more time in the light compartment, which may indicate anxiolytic behaviour. However, this shiftwas not as pronounced in GroupIII (100mg/L of EEAB) and GroupIV(200mg/L of EEAB), which spent slightly more time in the light than control but still favoredthe dark. These results suggest that while the test drug induced some level of anxiolysis, its effect was more pronounced at standard treatment doses and less so with experimental treatments.

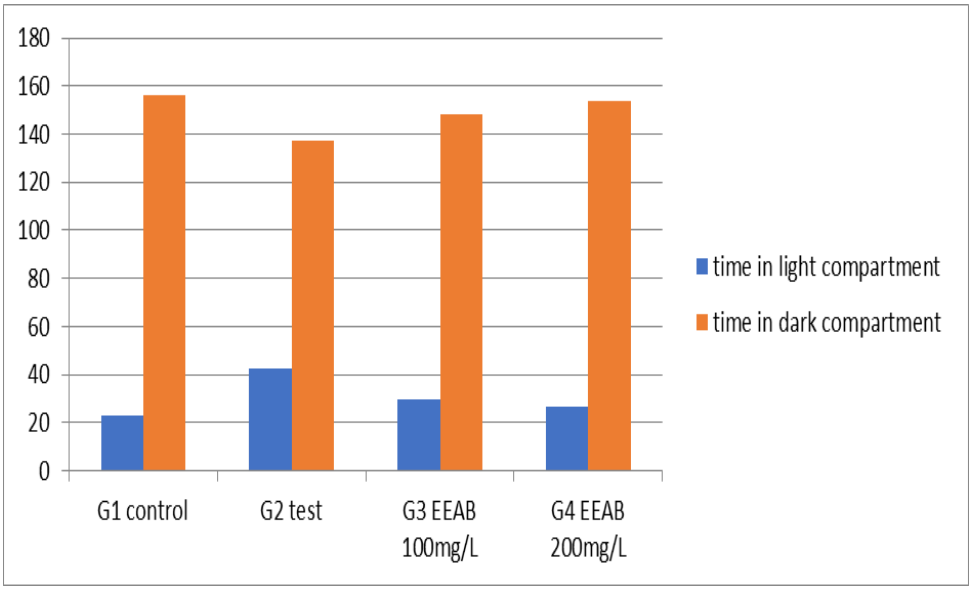

Fig. No. 2: Histogram for Light & Dark test.

5. CONCLUSION

Neurodegeneration is a complex process causing neuronal death and resulting in damage and dysfunction in the brain and spinal cord. It involves oxidative stress, axonal transport deficits, protein aggregation, calcium deregulation, mitochondrial dysfunction, abnormal neuron-glial interactions, neuroinflammation, DNA damage, and aberrant RNA processing. Neurodegenerative diseases, such as Alzheimer's (AD), Parkinson's (PD), Huntington's (HD), and amyotrophic lateral sclerosis (ALS), are characterized by misfolded protein accumulation and neurodegeneration in specific neurons.

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 of the ethanolic extract of *Ammania baccifera* L. using methods like the Hole board and Light & Dark tests. 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.

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