RESEARCH ARTICLE

Investigation of Neuroprotective Activity of Extracts of *Asparagus racemosus* Wild. in Acrylamide Induced Neurotoxicity in Adult Zebrafish

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ABSTRACT

The efficacy of an extract from *Asparagus racemosus* seeds in protecting zebrafish neurons from acrylamide-induced neurotoxicity was the focus of this investigation. ACR treatment induced neurotoxicity in zebrafish by reducing glutathione reductase levels by a factor of 3, increasing lipid peroxidation activity by a factor of 3.4, elevating nitrite levels by a factor of 1.7, raising acetylcholinesterase levels by a factor of 3.9, and decreasing total protein levels by a factor of 1.4, when compared to wild-type zebrafish. Vinpocetine demonstrated neuroprotective properties in ACR-induced zebrafish. These results indicate a 2.7-fold increase in glutathione reductase, a 3.4-fold reduction in lipid peroxidation activity, a 1.5-fold decrease in nitrite levels, a 3.2-fold decrease in acetylcholinesterase levels, and a 1.4-fold increase in total protein levels. Zebrafish with inherent genetic traits had similar outcomes. Treatment with solvent seed extracts from *A. racemosus* effectively reduced the damage caused by ACR. Following this intervention, levels of glutathione reductase, lipid peroxides, nitrite, protein, and acetylcholinesterase activity reverted back to levels seen in control group. The ethanolic extract exhibited superior neuroprotective effects compared to the solvent extract in zebrafish treated with ACR. After administering an ethanolic seed extract therapy at a concentration of 440 mg/l, the levels of glutathione reductase rose by a factor of 2.7. Simultaneously, there was a decrease in lipid peroxidation activity by 3.1, nitrite by 1.4, and acetylcholinesterase by 2.7. The protein content of zebrafish exposed to ACR rose by a factor of 1.3. Wild zebrafish had comparable results.

Keywords: Investigation, Neuroprotective Activity, *Asparagus racemosus* Wild., Acrylamide induced, Neurotoxicity, Zebrafish.

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INTRODUCTION

There is a vast and extensive history of using plants as medicinal agents, as all drugs originate from natural sources. The study of plant physiology and the extraction of new therapeutic substances from plants has recently received a lot of attention. Since ancient human civilizations discovered the powerful and adaptable nature of plants as therapeutic assets, their main purpose has been for economic profit.[1,2]

There is a genuine effort to combat drug abuse in developing countries by tapping into the therapeutic properties of plants that native communities have relied on for generations. Moreover, there is a global environmental movement that advocates the notion that herbal medicines have a lower level of danger to human health when compared to modern medications. Furthermore, the growing fascination with plants might be attributed to the fact that a considerable number of potent pharmaceuticals are now derived from plants (Figure 1). Moreover, medicinal tools derived from botanical compounds are very pragmatic. Therefore, some experts believe that the field of plant life may hold the solution to understanding complex human diseases and treating many mysterious illnesses that affect humans.[3,4] Time-honored cultural traditions and rituals have been transmitted from generation to generation. Ancient African medicine, Siddha, Ayurveda, Chinese medicine, and Tibetan medicine all used plant-based drugs extensively. The expectation that the profound botanical knowledge held by our traditional healers would lead to the creation of reliable medicines has not been realized. Hence, labs worldwide are conducting screenings on plants to identify their biological activity, which may possess potential therapeutic benefit. The traditional healer's assertion on the plant's therapeutic capabilities is a vital factor to consider while choosing a plant for this particular study. There are around 370,000 to 500,000 species of higher vegetation on Earth. Hence, the formulation of standards for plant selection in phytotherapeutic research has similar significance to the research process itself. The main factors considered for selection include traditional use, chemical makeup, and targeted assessment of a particular biological function.[5,6]

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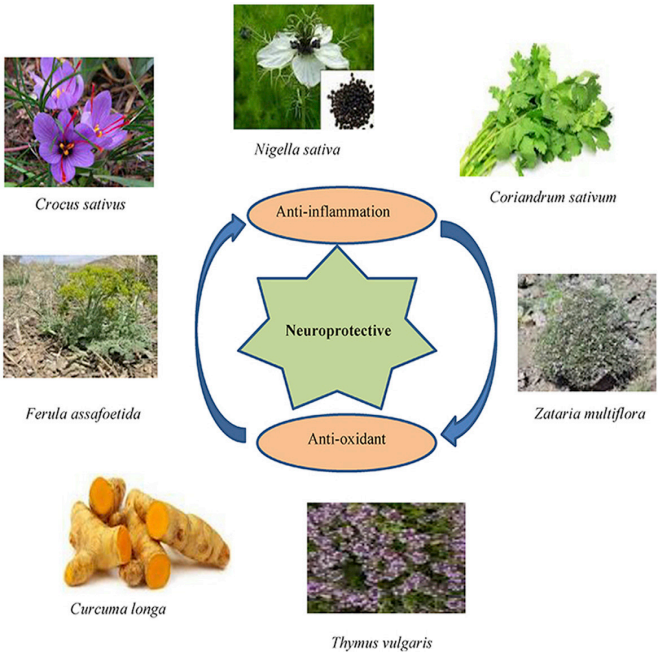

Figure 1: Plants as remedy for neurodegenerative diseases

Figure 2: Plant of *A. racemosus*

MATERIAL AND METHODS

Chemicals

In our experimental procedures, we utilized a range of chemicals and reagents of analytical grade. These included sodium dodecyl sulfate, ethylene diamine tetra acetic acid, nicotinamide adenine dinucleotide phosphate, glutathione disulfide, 5,5'-dithiobis(2-nitrobenzoic Acid), ethanoic acid, hydrogen peroxide, 2-thiobarbituric acid, acetylthiocholine iodide, normal butanol , pyridine, disodium hydrogen phosphate, potassium dihydrogen orthophosphate, vinpocetine, and acrylamide.

Collection and Authentication

Specimens of *A. racemosus* were harvested from botanical gardens. The Director of the A.B.S. Botanical Conservation, Research and Training Center officially recognized and verified the plant *A. racemosus*, which belongs to the Asparagaceae family.[7]

Preparation of Plant Extract

The *A. racemosus* spores were acquired from a medicinal plant garden (Figure 2). An expert botanist confirmed the accuracy of the plant. The seeds of the plant were obtained from a voucher specimen and dried in a different oven at a temperature of 45°C. A mechanical grinder was used to pulverize the dried seeds into a fine powder. A soxhlet device was used to extract 52 grams of seed grain, using ethanol as the solvent and 500 mL of water. Evaporation in a water bath at room pressure concentrated the filtrate.[8]

Animals

Scientific study relies on non-human organisms known as model organisms to better understand and investigate various biological processes. One common tropical fish is the little zebrafish, scientifically known as Daniorerio. This South Asian species is native to the rivers of Nepal, Bhutan, Northern Pakistan, and Northern India. Zebrafish are often used as model vertebrates in scientific research because of their small size, minimal care needs, and transparent embryos. The organism's exceptional regenerative capacity is noteworthy, and researchers have genetically engineered it to produce a wide array of transgenic variations.[9,10] The zebrafish is named because the horizontal blue stripes that extend around its body and tentacles.

Experimental Phase

A solution of freshly manufactured acrylamide exposure (0.75 mM) in distilled water was made. Seven distinct groups of seven fish were formed as

Group (I)

Control group (Fed by water)

Group (II)

ACR (induced by acrylamide)

Standard group (III)

Standard acrylamide (0.75 mM) + Vinpocetine 30 mg/liter test.

Group (IV)

Acrylamide (0.75mM) +Aqueous seed extract of *Asparagus racemosus* 200Mg/ liter

Test group (V)

Acrylamide (0.75 mM) +Aqueous seed extract of *A. racemosus* 440 mg/ liter

Test group (VI)

Acrylamide (0.75 mM) + Ethanolic seed extract of *A. racemosus* 200 mg/liter

Test group (VII)

Acrylamide (0.75 mM) + Ethanolic seed extract of *A. racemosus* 440 mg/liter

Experimental Protocol

Seven fish were carefully chosen from main tank and placed separately in seven separate 5-liter tanks, each labeled with

one of the seven group names indicated earlier. The fish in the control group tank were fed on a daily basis, following the same feeding regimen as the main tank. Six distinct 1-liter tanks were selected and designated as ACR exposure tanks. A solution of 0.7 mM concentration of ACR was prepared by dissolving it in 1 liter of water. The solution was then stored in the 6 tanks. The fish from the 5-liter tanks of the other 6 groups, except the control group, were moved into their corresponding ACR exposure tanks. Individuals were subjected to ACR for a duration of 30 minutes. Subsequently, fish were relocated back to their individual 5-liter aquariums, where they received daily feeding. The same exposure method was conducted for a continuous duration of three days. Subsequently, in order to treat fish with ACR-induced symptoms, two extracts of AR and a conventional medication were used. Five separate 1-liter tanks were allocated and labeled based on the treatment groups. The therapy medications were dissolved in 1 liter of water at appropriate quantities and stored in the 5 tanks. The fish that were stimulated were moved from the 5-liter tanks of the remaining 5 treatment groups (except the control group and ACR group) to their corresponding 1-liter treatment tanks. They had a 60-minute exposure to therapeutic medications. Subsequently, the fish were relocated back to their individual 5-liter aquariums, where they received daily feeding. A consistent treatment protocol was implemented for three consecutive days.[11-13]

Neurobehavioral Assays

Although not as advanced as those seen in rat models, the range and intricacy of paradigms developed to evaluate adult zebrafish behavior now include tasks that involve both cognitive and sensorimotor abilities. There are zebrafish-specific protocols that have been developed by either developing new methodologies or adapting old ones that were initially designed for rats to evaluate zebrafish behavior. Regularly, rudimentary swimming and capture/consumption experiments may provide valuable insights. Advanced behavioral assessments have been used to measure less significant changes in behavior. These tasks include the assessment of many behaviors such as aggression, anxiety, addiction, learning and memory, movement, social responses, anti-predatory behavior, and mate selection. These activities have been used in studies pertaining to drug abuse, drug development, and exposure to toxins. However, behavioral testing often focuses on measuring endpoints during the embryonic or larval stages. The predominant method for evaluating the influence of developmental exposure on embryos or larvae is to quantify their activity during alternate photoperiods, such as their swimming distance or velocity. During the light cycle, the typical zebrafish will exhibit more distance and speed in swimming compared to the dark cycle. Below are concise explanations and clarifications of some widely used cognitive and sensorimotor tests conducted on adult zebrafish. The following are encompassed: social responses, anti-predatory behavior, startle response/habituation learning, and spatial discrimination learning.[14-16]

Light/dark preference test

It was performed in order to evaluate behavior of scototaxis. All five groups were surveyed, and pertinent factors from the foundational research were considered. A partition partitions it in half, ensuring that each half contains an equal number of black-and-white vertical components (Figure 3).

Numerical values seven and fourteen. Filtered water was used to fill the testing apparatus to 80% of its capacity. Following that, each zebrafish was transferred to the light compartment for a period of acclimatization, which lasted for one to two minutes, with sliding door remaining closed. A period of five minutes was devoted to observing the zebrafish's movements subsequent to the raising of the sliding door.

Novel tank diving test

The primary approach to thoroughly evaluating the site preference tendencies of zebrafish is through the utilization of the new tank diving test (NTDT). This examination method has been formally approved for gauging the locomotor activity and anxiety-related behaviors of zebrafish. The testing apparatus comprises a transparent trapezoidal aquarium with a volume of 1.5 liters. The exact measurements of the tank-15.2 cm in height, 7.1 cm in width, 27.9 cm in height at the top, and 22.5 cm in height at the bottom-are set against a white backdrop. Notably, a demarcating line along the tank's outside walls divides it into two virtual horizontal pieces, top and bottom, that are equally sized. The aquarium is situated on a stable and even surface. Evaluation of zebrafish behavior during the NTDT involves tracking number of transitions, duration (measured in seconds), and latency toward upper zone (also in seconds) (Figure 4). Behavioral analysis is facilitated through utilization of automated video monitoring hardware and software (ANY-Maze, Stoelting Co. USA, Version 6.1).[17,18]

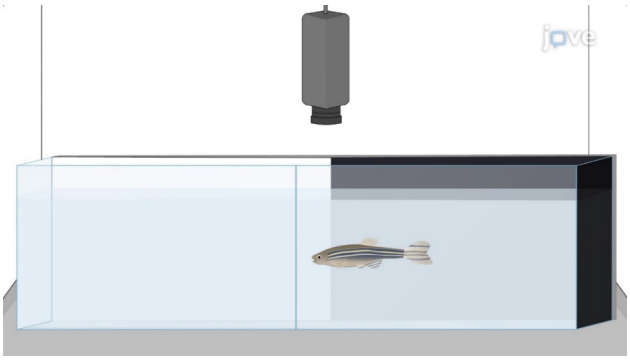

Figure 3: Light/dark preference test (LDPT)

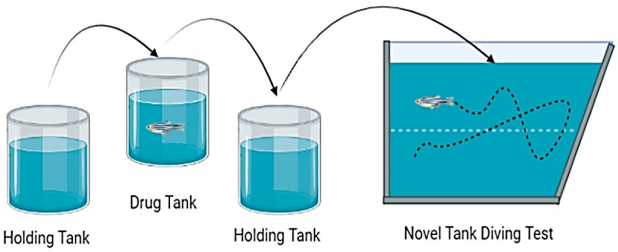

Figure 4: Novel tank diving test

T-maze test

The T-maze device, consisting of a transparent T-shaped acrylic box with a center piece of 10 by 10 cm and three arms measuring 20 cm each, was used to assess cognitive abilities related to learning and memory (Figure 5). During the exploratory test, both treated and untreated animals were placed in the bottom arm of the T-maze for two days without any previous information about the circumstances before the training phase. For five days, the zebrafish were trained for the experiment by eating food in the blue region for fifteen minutes every day. Each zebrafish was placed in the lower leg and watched for six minutes as it ate in the blue zone as part of the experiment.[19]

RESULT AND DISCUSSION

Neurobehavioral Assay

Light/dark preference test

Zebrafish naturally exhibit a preference for darkness, as scototaxis evaluation is used to measure their aversion to darkness and desire for light, which serves as a marker for stress. Graphs and typical traces of LDPT are included in the given data, which shows how the naïve and control groups' behaviors changed due to exposure to overcrowding (OC) stress and waterborne B[a]P. Animals exposed to both overcrowding stress and waterborne B[a]P exposure exhibited a higher frequency of transitions to light compared to animals that were naïve or served as controls ($F4,35 = 26.70$, $p < 0.05$). Latency to enter light zone reduced progressively with repeated treatments ($F4,35 = 23.01$, $p < 0.05$), and amount of time spent in light zone considerably increased. This suggests a departure from the natural predilection for dark areas in both the naïve and control groups ($F4,35 = 116.6$, $p < 0.05$). While the stress caused by OC resulted in a little divergence associated to acquaintance to B[a]P alone, when both were present, it caused significant changes in behavior across many test measures (Figure 6).

Novel tank diving test

The sample graphs and graphical NTDT tracks showed that different groups behaved differently according to the parameters. The administration of B[a]P decreased the time it took for OC stress to reach the top zone ($F4,35 = 83.25$, $p < 0.05$) and substantially enhanced the frequency of transitions and the amount of time expended in top zone compared to the naïve and control group ($F4,35 = 44.30$, $p < 0.05$; $F4,35 = 74.00$, $p < 0.05$). The aforementioned behavioral finding highlights the significant impact of OC stress in causing impaired movement in zebrafish when exposed to B[a]P (Figure 7).

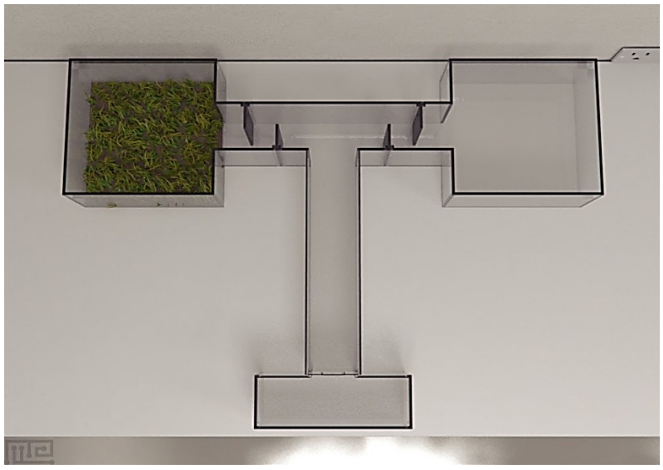

Figure 5: T-maze apparatus

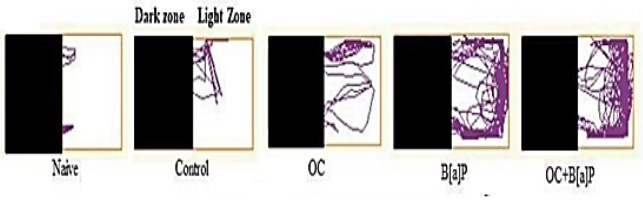

Figure 6: LDPT

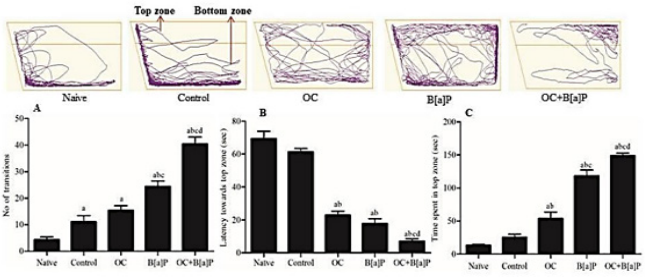

Figure 7: NTDT

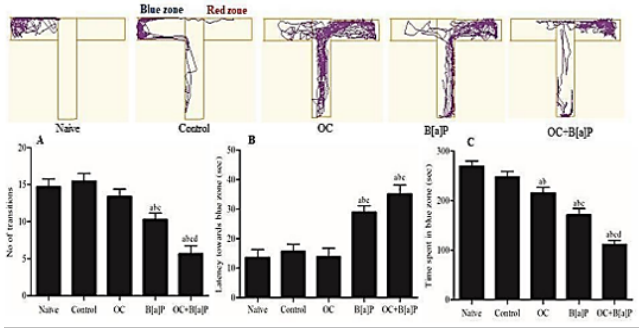

Figure 8: T-maze test

T-maze test

Subsequent to food bait training, learning and memory tests were conducted. In contrast to the treatment groups, the control and naïve zebrafish groups swiftly reached the blue zone, showing that their memory and recognition abilities were strong ($F4,98 = 11.54$, $p < 0.05$). The amount of entries and time spent in the blue zone were both drastically reduced when animals were subjected to both B[a]P and OC stress, indicating that animals exposed to B[a]P showed some memory loss compared to animals treated with both substances. A significance level of $p < 0.05$ was used in the statistical analysis, which produced an F-value of 4.145 = 14.96 (Figure 8).

Biochemical Estimations

Estimation of glutathione reductase activity

Hydroethanolic extracts of *A. racemosus* at a concentration of 100 mg/kg significantly restored the activity ($p < 0.001$). The formulation of the extracts also showed a substantial level of significance ($p < 0.001$) when compared to the 6-OH group (Table 1).

Glutathione peroxidase

In comparison to the control group, the 6-OH group exhibited a significantly lower concentration of glutathione peroxidase in the striatum ($p < 0.001$). The hydroethanolic extracts of *A. racemosus* at 100 mg/kg significantly and dose-dependently boosted the decrease in glutathione peroxidase production ($p < 0.001$). In comparison to the 6-OH group, the formulation of extracts likewise showed a highly significant level of significance ($p < 0.001$) (Table 2).

Thiobarbituric acid reactive substance

The variation in TBARS levels among the treatment groups is displayed in Table. In the 6-OH group, TBARS content in the substantianigra was noticeably higher than in the control group ($p < 0.001$). The hydroethanolic extracts of *A. racemosus* at a dosage of 200 mg/kg significantly and dose-dependently decreased the increases in TBARS production ($p < 0.001$). In comparison to the 6-OH group, the formulation of both extracts showed a highly significant level of significance ($p < 0.001$) (Table 3).

Reduced glutathione (GSH)

In comparison to the control group, the 6-OH dopamine group had a significantly lower GSH content in the substantianigra ($p < 0.001$). The 200 mg hydroethanolic extracts of *A. racemosus* treatment groups recovered the reduction in GSH content more dramatically and dose dependently ($p < 0.001$) than the 6-Oh dopamine treated group. When comparing the 6-OH group to the group using the extract formulation, a highly significant difference was noted ($p < 0.001$) (Table 4).

Superoxide dismutase

In the 6-OH dopamine group, the striatal superoxide dismutase (SOD) activity was noticeably lower than in the control group ($p < 0.001$). Groups treated with 100 mg/kg of hydroethanolic *A. racemosus* extracts had their reduced SOD activity recovered in a dose-dependent manner. The formulation of extracts showed a highly significant level of association ($p < 0.001$) (Table 5).

Catalase

In comparison to the control group, the 6-OH group exhibited a considerably lower concentration of catalase in the striatum ($p < 0.001$). The activity was markedly revived ($p < 0.001$) in hydroethanolic extracts of *A. racemosus* at 100 and 200 mg/kg. In comparison to the 6-OH group, the formulation of extracts likewise showed a highly significant level of significance ($p < 0.001$) (Table 6).

Hematological Studies

The experimental period for the hydroethanolic extracts of *A. racemosus* showed that hematological parameters, including total white blood cell count, hemoglobin, hematocrit, platelet count, differential leukocyte count, and red blood cell count,

Table 1: Results of glutathione reducates activity

S. No.	*Groups*	*Glutathione reducates activity*
1		Mean ± SD
2	I	27.73 ± 1.1992
3	II	5.2184 ± 0.648a***
4	III	9.7272 ± 0.9072b*
5	IV	12.2544 ± 0.9504b***
6	V	15.3084 ± 1.2172b***

Table 2: Results of glutathione peroxidase studies

S. No.	*Groups*	*Glutathione peroxidase activity*
1		Mean ± SD
2	I	11.89 ± 0.9516
3	II	1.7928 ± 0.3564a***
4	III	4.6844 ± 0.594a*
5	IV	6.8384 ± 0.6588a***
6	V	8.2816 ± 0.9504a***

Table 3: Results of thiobarbituric acid reactive substance studies

S. No.	*Groups*	*Thiobarbituric acid reactive substance*
1		Mean ± SD
2	I	6.48 ± 1.1772
3	II	34.02 ± 1.512a***
4	III	24.494 ± 1.35a***
5	IV	18.728 ± 1.134a***
6	V	14.04 ± 0.9594a***

Table 4: Results of reduced glutathione (GSH) studies

S. No.	*Groups*	*Reduced glutathione (GSH) activity*
1		Mean ± SD
2	I	24.30 ± 1.5444
3	II	5.94 ± 0.8208a***
4	III	15.12 ± 1.5984b**
5	IV	22.42 ± 1.8144b***
6	V	25.93 ± 1.7624b***

Table 5: Results of superoxide dismutase studies

S. No.	*Groups*	*Superoxide dismutase activity*
1		Mean ± SD
2	I	7.47 ± 1.0872
3	II	1.4172 ± 0.2592a***
4	III	4.3248 ± 0.5508b*
5	IV	5.4304 ± 0.7904b***
6	V	5.67 ± 0.5832b***

Table 6: Results of catalase activity studies

S. No.	*Groups*	*Catalase activity*
1		Mean ± SD
2	I	8.55 ± 1.0584
3	II	1.5808 ± 0.432a***
4	III	3.52 ± 1.2096b*
5	IV	4.86 ± 0.3456b**
6	V	5.7624 ± 0.4536b***

Table 7: Hematological parameters in zebrafish model

Hematological parameter	*Vehical control*	*AR (200 mg dose/kg)*
	Male	Female
Total RBC count ($\times10^6$/mm^3)	9.80 ± 1.57	8.77 ± 1.79
White Blood Cell Count ($\times10^3$/mm^3)	14.78 ± 2.12	10.33 ± 1.56
Hemoglobin (Hb) (g/dl)	17.09 ± 2.09	14.92 ± 1.36
Hematocrit (%)	45.97 ± 1.46	48.78 ± 1.61
Platelets ($\times10^3$/mm^3)	705.97 ± 13.32	1038.63 ± 17.96
Neutrophils (%)	19.22 ± 2.19	22.63 ± 3.35
Lymphocytes (%)	89.16 ± 3.61	83.86 ± 2.64
Eosinophil (%)	2.57 ± 0.51	1.97 ± 0.81
Monocyte (%)	3.35 ± 0.10	0.00 ± 0.00
Basophil (%)	0.00 ± 0.00	1.09 ± 0.00
Creatinine (mg/dl)	0.69 ± 0.00	0.66 ± 0.043
Urea (mg/dl)	16.39 ± 1.56	19.57 ± 2.16
Triglycerides (mg/dl)	58.53 ± 9.74	53.29 ± 6.78
Total cholesterol (mg/dl)	63.06 ± 4.26	56.42 ± 4.05
Total amount of protein (gm/100 mL)	4.24 ± 0.11	5.31 ± 1.02
Albumin protein (gm/100mL)	3.62 ± 0.20	4.01 ± 0.10
AST (IU/L)	129.13 ± 30.94	121.52 ± 25.79
ALT (IU/L)	68.59 ± 5.86	78.42 ± 4.32
ALP (IU/L)	105.6 ± 15.46	112.66 ± 16.52
Total amount of bilirubin (mg/100 mL)	0.34 ± 0.08	0.31 ± 0.12

stayed within the physiological range in both the control and treated groups (Table 7).

CONCLUSION

Study results have confirmed previous assertions on the medicinal qualities of the *A. racemosus* plant, especially its neuroprotective capabilities. The phytochemical study detected flavonoids, phenolics, and tannins in the seed extract of *A. racemosus*. These components are recognized for their notable antioxidant properties. *A. racemosus* extracts may reduce oxidative stress in the zebrafish brain, as shown by the enhanced activity of antioxidant enzymes. The administration of ACR treatment led to the following neurotoxic effects in fish samples when compared to wild-type zebrafish: a threefold increase in lipid peroxidation activity, a threefold decrease in glutathione reductase, a 1.5 times increase in nitrite levels, a threefold increase in acetylcholinesterase, and a 1.5 times decrease in total protein.

Results from various assays showed that zebrafish treated with vinpocetine as a standard medication exhibited significant neuroprotective effects. These effects included a 2.7-fold increase in glutathione reductase, a 3.4-fold increase in lipid peroxidation activity, a 1.5-fold increase in nitrite levels, a 3.2-fold increase in acetylcholinesterase, and a 1.4-fold increase in total protein. The results were in line with what was observed in zebrafish in the wild. We used several solvent seed extracts of *A. racemosus* to reverse the inhibition of glutathione reductase, lipid peroxides, nitrite, protein, and acetylcholinesterase activities caused by ACR. The results were determined to be congruent with those of the control group. The zebrafish that were exposed to ACR showed a far more pronounced response to the ethanolic extract compared to the extract dissolved in the alternate solvent. The findings suggest that zebrafish exposed to ACR showed a 1.3-fold rise in overall protein content after treatment with 440 mg/l of ethanolic seed extract.

In contrast, the levels of the enzymes glutathione reductase, lipid peroxidation activity, nitrite concentration, and acetylcholinesterase reduced by a factor of 2.7, 3.1, and 1.4, respectively. The findings were similar to those seen in wild zebrafish. Jangir *et al*. (2018) reported the presence of brain histological alterations, including inflammation and neuronal degeneration, in the rat model treated with ACR. Similar histological alterations were seen in ACR-exposed zebrafish brain regions in the present study. Pyramidal and glial cell degeneration was one of these alterations. Extracts of *A. racemosus* seeds, whether in an aqueous or ethanolic form, mitigated the decline in neuronal cell density. Results from this study suggest that *A. racemosus* seed extracts, both ethanolic and aqueous, may mitigate ACR-induced oxidative stress and thereby have neuroprotective properties. Prior research on the plant's antioxidant and free radical scavenging properties provide credence to this claim. The results show that *A. racemosus* may be good for you and suggest that it could be used to make new dementia treatments.

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