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Flying Fish Roe Extract, Serum BDNF, and Recovery After Traumatic Brain Injury in Wistar Rats

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ABSTRACT: Traumatic brain injury (TBI) triggers secondary injury and neurological dysfunction. This exploratory study evaluated Hirundichthys oxycephalus roe extract in relation to serum brain-derived neurotrophic factor (BDNF), neurological recovery, and brain histopathology after experimental TBI. Twelve male Wistar rats underwent modified Marmarou weight-drop injury and were randomized to oral roe extract (200 mg/kg/day) or distilled-water vehicle for 7 days (n = 6/group). Serum BDNF was measured before injury, immediately after injury, and on days 3 and 7. The modified Neurological Severity Score (mNSS) was assessed at baseline, 24 hours, and days 3 and 7; brain histopathology was evaluated on day 7. At day 7, the extract group had lower serum BDNF (255.11 ± 39.95 vs 948.60 ± 14.40 pg/mL; p = 0.002) and mNSS (0.17 ± 0.41 vs 4.00 ± 1.41; p = 0.002) than the vehicle group. Neuronal degeneration, inflammatory-cell infiltration, and gliosis scores were also lower, whereas edema did not differ significantly. In pooled day-7 analyses, serum BDNF correlated positively with mNSS and neuronal degeneration. Roe extract was associated with improved functional and histopathological outcomes accompanied by lower circulating BDNF. These preliminary findings support further investigation, but the small sample and pooled correlations do not establish a mechanism or validate serum BDNF as an independent marker of injury severity.

1. INTRODUCTION

Traumatic brain injury is a major cause of death and disability worldwide, with a substantial and heterogeneous burden across health systems [1]. Its clinical and biological course is shaped not only by the primary mechanical insult but also by secondary processes that include oxidative stress, excitotoxicity, neuroinflammation, blood-brain barrier disruption, and progressive cell death [2,3]. These interdependent mechanisms continue to limit the translation of experimental neuroprotectants into effective therapies [4].

Brain-derived neurotrophic factor is implicated in neuronal survival, plasticity, and recovery after TBI, but its circulating concentration is context dependent and may reflect both adaptive signaling and injury-related release [5]. Serum BDNF should therefore be interpreted alongside functional and structural outcomes rather than as an isolated surrogate of neuroprotection [6]. The modified Marmarou model provides a reproducible experimental platform for examining these linked outcomes after focal brain trauma [7].

Flying fish roe is nutritionally rich in proteins, unsaturated fatty acids, carotenoids, and alpha-tocopherol, constituents with plausible antioxidant and anti-inflammatory relevance [8,9]. Fish roe-derived extracts have also shown antioxidant and anti-

inflammatory bioactivity in preclinical systems [10]. We therefore investigated the effect of H. oxycephalus roe extract on serial serum BDNF, neurological recovery, and brain histopathology in a Wistar rat model of TBI.

2. MATERIALS AND METHODS

2.1 Study Design and Setting

This randomized, parallel-group experimental animal study was conducted from February to August 2026. Animal procedures were undertaken at the Faculty of Medicine, Hasanuddin University; extract preparation, BDNF testing, and histopathological processing were performed in the respective affiliated pharmacology, microbiology, and veterinary pathology laboratories in Makassar, Indonesia.

2.2 Animals, Randomization, and Intervention

Twelve male Wistar rats (Rattus norvegicus), aged 2-3 months and weighing 200-250 g, were obtained from the institutional animal facility and used after veterinary health screening. Animals were eligible if they were healthy, free of visible neurological or behavioral abnormality, and had not undergone any prior experimental procedure; animals with body weight outside the 200-250 g range or signs of illness during acclimatization were excluded. Rats were housed under controlled conditions (temperature 22 ± 2°C, 12-hour light-dark cycle) and acclimatized for 7 days with standard pellet feed and water ad libitum before any procedure. Sample size was based on the resource equation method; for two groups of six animals the error degrees of freedom (E) equaled 10, within the recommended range of 10-20. The twelve animals were allocated 1:1 to the two groups using a computer-generated random number sequence, with allocation concealed from the personnel performing the intervention. All animals received TBI; the intervention group received H. oxycephalus roe extract at 200 mg/kg/day by oral gavage for 7 consecutive days, whereas controls received an equivalent volume of distilled water as vehicle. The study evaluated a single dose without a dose-ranging comparison. The extract was freshly suspended in distilled water immediately before each administration. The first dose was given after the immediate post-injury blood sample had been collected. No animal died or met an exclusion criterion.

2.3 Extract Preparation and TBI Induction

Hirundichthys oxycephalus roe was obtained from local fishery sources in South Sulawesi, cleaned, and oven-dried at 40-45°C. The dried roe was ground to a coarse powder and macerated repeatedly in 96% ethanol with periodic renewal of the solvent. The combined filtrate was concentrated at 60°C under reduced pressure and then dried until residual solvent was removed, yielding a semi-solid ethanolic extract. From 915 g of cleaned dried roe, 493.1 g of extract was obtained, corresponding to an extraction yield of 53.89%. The extract had a characteristic fishy odor, an oily texture, and an orange color. The roe of this species has previously been characterized as a source of proteins, unsaturated fatty acids, carotenoids, and alpha-tocopherol [8,9]; batch-specific chemical characterization of the administered extract was not performed. Anesthesia was induced with intraperitoneal ketamine (80 mg/kg) and xylazine (10 mg/kg), and adequate depth was confirmed by loss of the pedal withdrawal reflex before surgery. With the head stabilized, a right parietal craniotomy was performed while preserving the dura intact. A single 20-g weight was dropped from a height of 20 cm through a 2-cm-diameter vertical guide tube onto the exposed dura, delivering an impact of approximately 0.04 J, following the modified Marmarou weight-drop method [7]. After injury, animals were allowed to recover on a warming pad, did not receive post-operative analgesia (deliberately withheld to avoid confounding the mNSS, serum BDNF, and histopathological outcomes), and were monitored according to predefined humane endpoints (open skull fracture, severe hemorrhage, severe complications, signs of severe distress, or death within the first 24 hours after the procedure). No animal required early euthanasia.

2.4 Outcome Assessment

At the four predefined time points (before injury [baseline], immediately after injury and before the first intervention, and on days 3 and 7), 0.5 mL of whole blood was collected from the lateral tail vein into plain tubes without anticoagulant and allowed to clot at room temperature. Serum was separated by centrifugation at 3000 rpm for 15 minutes, aliquoted, and stored at -80°C until batched analysis. Serum BDNF was quantified using a commercial rat BDNF sandwich ELISA kit (catalog no. LS-F3963) strictly according to the manufacturer's protocol; all samples were assayed in duplicate; concentrations were interpolated from a standard curve, absorbance was read at 450 nm, and results were expressed in pg/mL. All samples from both groups were analyzed within a single assay run to minimize inter-assay variability. Neurological function was assessed at baseline, 24 hours,

day 3, and day 7 using the modified Neurological Severity Score (mNSS), a composite battery evaluating motor, sensory, beam-balance, and reflex/abnormal-movement components (total range 0-18; higher scores indicate greater deficits). Assessments were performed by a trained examiner who was unaware of group allocation, using standardized scoring criteria defined before the study. On day 7, after the final assessment, animals were deeply anesthetized with an intraperitoneal anesthetic mix and euthanized in accordance with laboratory-animal ethics guidelines; death was confirmed before the brains were harvested. Coronal blocks of the peri-lesional (right parietal) region were fixed in 10% neutral buffered formalin, processed, paraffin-embedded, sectioned at 4-5 µm, and stained with hematoxylin-eosin. A single pathologist, blinded to group allocation, examined three non-overlapping representative peri-lesional fields per animal at 400× magnification and graded neuronal degeneration, edema, inflammatory-cell infiltration, and gliosis on a semiquantitative 0-3 ordinal scale (0 = absent/normal, 1 = mild, 2 = moderate, 3 = severe). The per-animal value for each parameter was taken as the mean of the three fields.

2.5 Statistical Analysis

Continuous data are presented as mean ± standard deviation and ordinal outcomes as mean ± standard deviation with median where appropriate. Distributional normality was assessed with the Shapiro-Wilk test. Because outcomes were ordinal or not normally distributed and the sample was small, nonparametric methods were used throughout. Between-group comparisons used the exact Mann-Whitney U test at pre-injury, immediate post-injury, day 3, and day 7 for serum BDNF, and at baseline, 24 hours, day 3, and day 7 for mNSS. Histopathological scores were compared between groups on day 7. Temporal profiles are described descriptively; within-group inferential results are not reported. Associations among day-7 outcomes were evaluated with the Spearman rank correlation coefficient using all 12 animals pooled across treatment groups. Effect size for the Mann-Whitney comparisons was calculated as $r = Z/\sqrt{N}$ and interpreted as small (0.1), medium (0.3), or large (0.5). A two-sided $p < 0.05$ was considered statistically significant; no formal correction for multiple comparisons was applied. All p values are unadjusted and the findings are exploratory. Analyses were conducted in IBM SPSS Statistics version 31 for Mac.

3. RESULTS AND DISCUSSION

3.1 Study Cohort and Serum BDNF

All 12 randomized animals completed follow-up and were analyzed. No statistically significant between-group difference was detected in pre-injury BDNF; baseline mNSS was 0 in both groups. BDNF increased immediately after TBI in both groups; thereafter, concentrations declined toward baseline in extract-treated animals but rose progressively in vehicle-treated animals. Between-group differences were significant from day 3 onward, with large effect sizes (Table 1 and Figure 1).

3.2 Neurological and Histopathological Outcomes

Both groups developed neurological deficits at 24 hours, with no significant between-group difference. By day 7, mNSS was significantly lower in the extract group, and five of six treated animals had returned to a score of 0 (Table 2 and Figure 2). Histopathological scoring also favored the extract group for neuronal degeneration, inflammatory-cell infiltration, and gliosis, whereas edema was not significantly different (Table 3). Representative hematoxylin-eosin micrographs showed relatively preserved tissue architecture in treated animals (Figure 3).

Table 1. Serum BDNF concentrations by study group

Time point	TBI + flying fish roe extract (n = 6)	TBI + vehicle (distilled water; n = 6)	p value	Effect size r
Pre-injury	164.25 ± 26.24	141.26 ± 22.44	0.132	0.46
Immediate post-injury	346.13 ± 38.40	302.03 ± 1.32	0.065	0.55
Day 3	306.07 ± 30.19	473.71 ± 5.47	0.002	0.83
Day 7	255.11 ± 39.95	948.60 ± 14.40	0.002	0.83

Values are mean ± standard deviation, pg/mL. Exact Mann-Whitney test; r=effect size.

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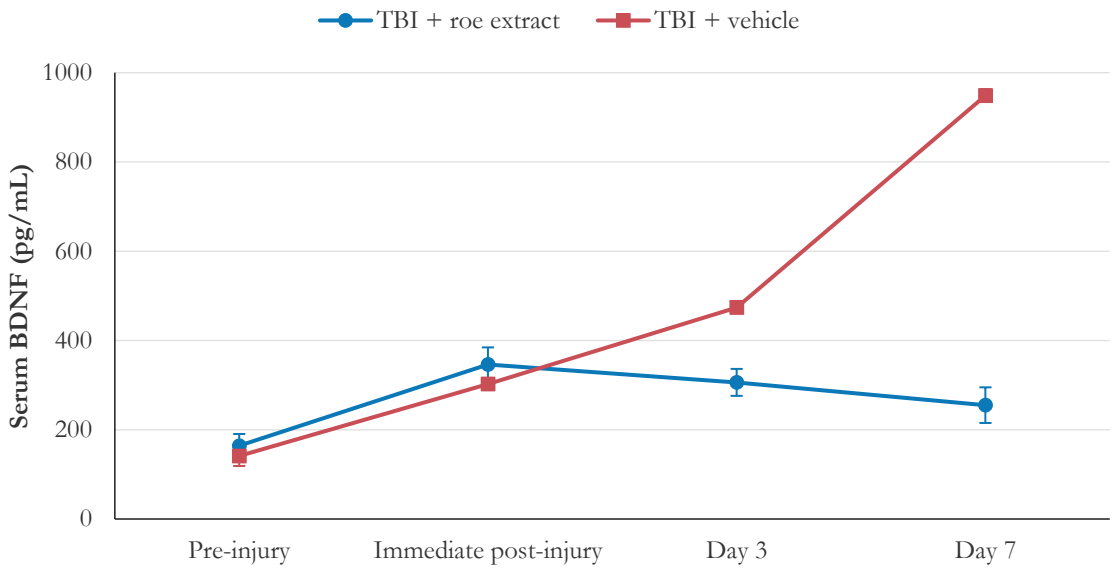

Figure 1. Temporal serum BDNF profile after TBI. Error bars indicate standard deviation.

Table 2. Modified Neurological Severity Score by study group

Time point	TBI + flying fish roe extract (n = 6)	TBI + vehicle (distilled water; n = 6)	p value	Effect size r
Baseline	0.00 ± 0.00 [0]	0.00 ± 0.00 [0]	1.000	0.00
24 hours	7.67 ± 1.63 [8]	6.33 ± 1.63 [6]	0.180	0.41
Day 3	3.33 ± 1.86 [3]	4.17 ± 1.17 [4]	0.394	0.28
Day 7	0.17 ± 0.41 [0]	4.00 ± 1.41 [4]	0.002	0.86

Values are mean ± standard deviation [median]. Exact Mann-Whitney test.

Table 3. Day-7 histopathological scores

Parameter	TBI + flying fish roe extract (n = 6)	TBI + vehicle (distilled water; n = 6)	p value	Effect size r
Neuronal degeneration	1.50 ± 0.55 [1.5]	3.00 ± 0.00 [3]	0.002	0.90
Edema	1.50 ± 0.55 [1.5]	2.00 ± 0.63 [2]	0.240	0.40
Inflammatory-cell infiltration	1.33 ± 0.52 [1]	2.33 ± 0.52 [2]	0.026	0.71
Gliosis	1.17 ± 0.41 [1]	2.00 ± 0.00 [2]	0.015	0.81

Values are mean ± standard deviation [median]. Scores range from 0 to 3. Exact Mann-Whitney test.

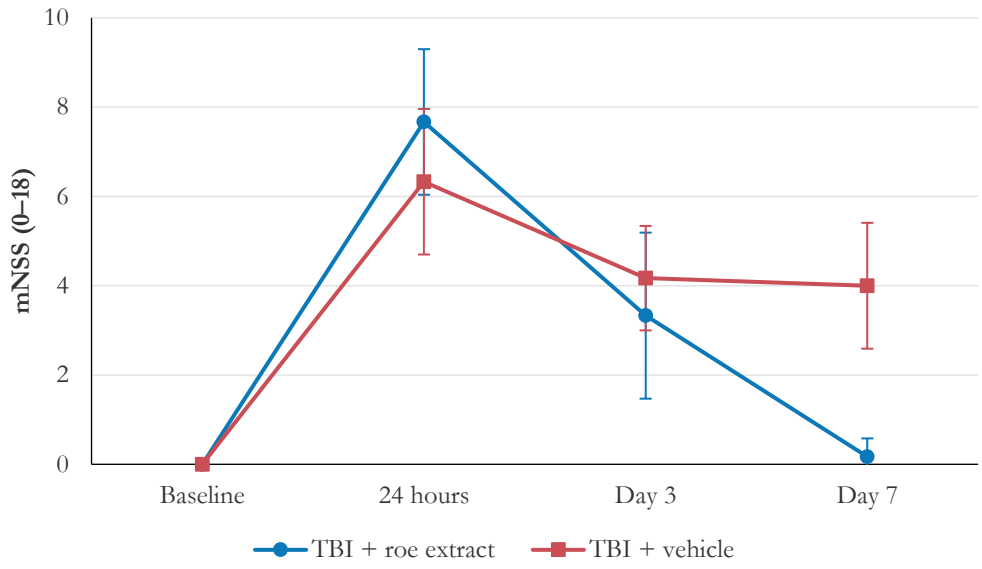

Figure 2. Temporal mNSS profile after TBI. Lower scores indicate better neurological function; error bars indicate standard deviation.

3.3 Correlation Analysis

At day 7, serum BDNF correlated positively with mNSS and neuronal degeneration. mNSS also correlated strongly with gliosis and neuronal degeneration, supporting agreement between functional and structural outcomes (Table 4).

Table 4. Spearman correlations among day-7 outcomes

Variable 1	Variable 2	rho	p value
Serum BDNF	mNSS	0.828	<0.001
Serum BDNF	Neuronal degeneration	0.785	0.002
Serum BDNF	Gliosis	0.661	0.019
Serum BDNF	Inflammatory-cell infiltration	0.618	0.032
mNSS	Gliosis	0.890	<0.001
mNSS	Neuronal degeneration	0.816	0.001

All correlations used n=12. Positive coefficients indicate that higher serum BDNF or worse neurological deficit co-occurred with higher tissue-injury scores.

3.4 Discussion

The present study demonstrates that *H. oxycephalus* roe extract was associated with a more favorable recovery profile after experimental TBI. The improvement in mNSS together with lower scores for neuronal degeneration, inflammatory-cell infiltration, and gliosis indicates that the observed effect was not limited to one outcome domain. Instead, functional recovery was accompanied by preservation of tissue architecture, supporting a biologically coherent neuroprotective response. Although no statistically significant between-group differences were detected in pre-injury BDNF or 24-hour mNSS, nonsignificance in this small sample does not establish equivalence in initial injury severity.

These findings are in line with the current understanding that secondary brain injury is driven by interacting mechanisms rather than by a single pathway. Oxidative stress, mitochondrial dysfunction, excitotoxicity, inflammatory activation, blood-brain barrier disruption, and progressive neuronal death can collectively extend tissue damage after the primary insult [2,3]. Therefore,

interventions with antioxidant and anti-inflammatory properties may be particularly relevant because they can influence several components of the secondary-injury cascade simultaneously. The improved functional and histopathological profile in the extract group is also consistent with Chen et al. (2023) [11], who reported that activation of neuroprotective signaling after TBI promoted neuroregeneration and improved functional outcomes in mice.

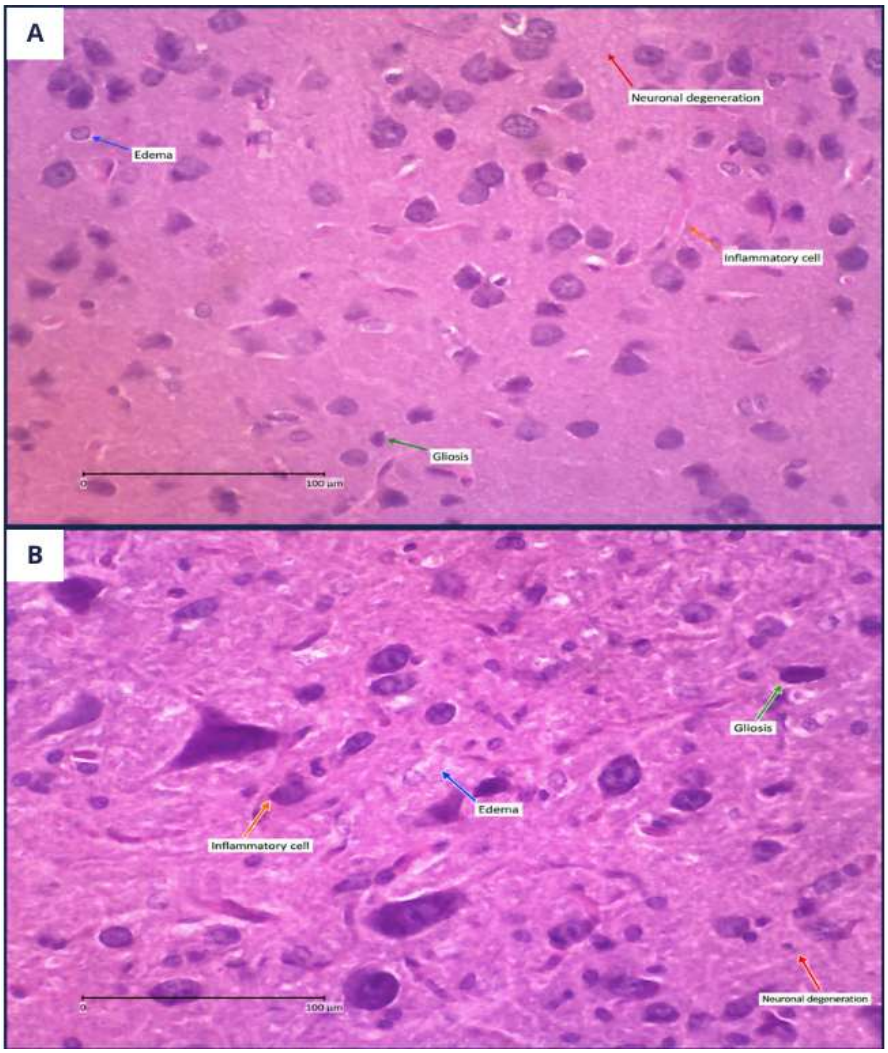

Figure 3. Representative photomicrographs of peri-lesional brain tissue on day 7 after traumatic brain injury. (A) The flying fish roe extract group shows relatively preserved tissue architecture with less prominent neuronal degeneration, inflammatory-cell infiltration, and gliosis. (B) The vehicle-control group shows more extensive tissue injury, including neuronal degeneration, inflammatory-cell infiltration, edema, and gliosis. Sections were stained with hematoxylin-eosin and examined at 400× magnification. Red arrows indicate neuronal degeneration, blue arrows indicate edema, orange arrows indicate inflammatory cells, and green arrows indicate gliosis. Scale bar = 100 µm.

The biological plausibility of the observed effect may be related to the nutritional composition of flying fish roe. Previous studies have shown that flying fish roe contains proteins, fatty acids, carotenoids, and alpha-tocopherol [8,9]. These compounds may contribute to the regulation of oxidative and inflammatory responses after neural injury. Fish roe-derived extracts have demonstrated antioxidant and anti-inflammatory activity in experimental systems, supporting their potential as marine-derived bioactive products [10]. In particular, fatty-acid components may help maintain neuronal membrane integrity and modulate inflammatory signaling, whereas carotenoids and tocopherol may contribute to protection against oxidative damage.

The lower inflammatory-cell infiltration and gliosis in the extract group are particularly relevant because neuroinflammation and reactive glial responses are central features of secondary injury. Although inflammatory activation initially contributes to

debris clearance and tissue repair, excessive or prolonged activation may sustain neuronal injury and impair recovery. The histopathological pattern observed in this study suggests that the extract may have reduced the intensity of this persistent injury response. This interpretation is compatible with evidence that DHA-related mechanisms can support antioxidant defenses and influence neuroinflammatory signaling in the brain [12,13]. The accompanying reduction in neuronal degeneration is compatible with neuroprotection, but oxidative-stress and inflammatory signaling mechanisms were not directly assessed in this study.

The BDNF findings require interpretation within the broader context of injury severity and recovery. BDNF is widely recognized as an important mediator of neuronal survival, synaptic plasticity, and repair after brain injury [5]. However, circulating BDNF does not exclusively represent regenerative activity because it may also arise from platelets and other peripheral sources and can increase in response to tissue stress or injury burden [6]. In the present study, persistent serum BDNF elevation was associated with worse neurological and histopathological outcomes. This pattern suggests that prolonged elevation during the acute-to-subacute phase may represent an unresolved injury-associated response rather than successful neuroregeneration.

The positive correlations were calculated across both treatment groups and may partly reflect between-group separation. They do not establish an independent relationship between serum BDNF and injury severity, nor show that higher BDNF is inherently harmful. In contrast, the decline toward baseline observed in the extract group occurred alongside improved functional and structural outcomes, without establishing the mechanism underlying this association. Therefore, BDNF should not be interpreted as a stand-alone marker of recovery but should be assessed together with neurological function and tissue-level findings. Overall, the present findings identify *H. oxycephalus* roe extract as a promising marine-derived candidate for further investigation as a multi-target neuroprotective intervention after TBI.

3.5 Limitations

This exploratory study included six male animals per group, a single extract dose, a 7-day follow-up, and no uninjured sham group. The reported sample-size rationale did not include an outcome-specific power calculation. Multiple unadjusted comparisons increase the risk of false-positive findings. Day-7 correlations pooled both groups and may reflect treatment-group separation rather than an independent association with injury severity. Brain BDNF expression, TrkB activation, and biochemical measures of oxidative stress or inflammatory signaling were not reported; mechanistic interpretations therefore remain hypotheses. Batch-specific chemical characterization of the extract and the precise euthanasia method were incompletely reported, limiting reproducibility. Larger, independently replicated studies with standardized extract characterization, fuller procedural reporting, and longer follow-up are needed.

4. CONCLUSION

In this exploratory Wistar rat study, H. oxycephalus roe extract was associated with lower day-7 mNSS and histopathological scores for neuronal degeneration, inflammatory-cell infiltration, and gliosis, accompanied by lower serum BDNF. The findings support further mechanistic and dose-ranging studies, but do not establish clinical efficacy or validate serum BDNF as an independent marker of injury severity.

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AUTHOR CONTRIBUTIONS

MAP: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Visualization, Writing - original draft. AAI: Conceptualization, Methodology, Supervision, Writing - review and editing. RAN: Methodology, Validation, Supervision, Writing - review and editing. MIK: Methodology, Investigation, Validation, Writing - review and editing. NM: Validation, Supervision, Writing - review and editing. W: Validation, Supervision, Writing - review and editing. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare no competing interests.

FUNDING

This research received no external funding.

ETHICS STATEMENT

The protocol was approved by the Health Research Ethics Committee, Faculty of Medicine, Hasanuddin University (No. 964/UN4.6.4.5.31/PP36/2026). Procedures followed the 3Rs and ARRIVE reporting principles [14].

DATA AVAILABILITY STATEMENT

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

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