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Effect of Snakehead Fish Extract Supplementation in Protein Status, Oxidativestress Status, Antioxidant Status, and Clinical Outcome of Acute Ischemic Stroke

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Abstract— In acute ischemic stroke, reperfusion paradoxically leads to heightened free radical formation, inducing oxidative stress that exacerbates brain damage. Malondialdehyde (MDA) reflects lipid peroxidation and oxidative stress, while total antioxidant status (TAS) indicates defense against damage. Serum proteins like albumin, transthyretin, retinol binding protein, and transferrin play multifunctional roles in neuroprotection and antioxidation. This study investigates the impact of snakehead fish extract (SFE), rich in proteins, on protein levels, TAS, malondialdehyde, and stroke severity. SFE's potential to modulate these factors offers insights into stroke management. By enhancing antioxidant capacity and deciphering protein influences, this research contributes to strategies countering oxidative damage and advancing acute stroke interventions.

Keywords— acute ischemic stroke, oxidative stress, protein modulation, snakehead fish extract

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I. Introduction

In acute ischemic stroke, particularly during the ischemic phase coinciding with reperfusion or re-oxygenation, an upsurge occurs in the generation of free radicals characterized by high reactivity. These radicals can engage in peroxidation reactions with membrane lipids, proteins, and DNA, rendering the brain susceptible to oxidative stress arising from ischemia and reperfusion. The ischemic phase triggers the production of a substantial quantity of free radicals through various mechanisms. Identifying these mechanisms presents an opportunity for novel therapeutic strategies during the post-ischemic interval. Notably, the primary outcome of the reaction between free radicals and phospholipids is the formation of malondialdehyde (MDA), a biomarker indicative of lipid peroxidation and oxidative stress. Stroke incidents result in a significant elevation in MDA levels. However, when assessing oxidative stress, the measurement of total antioxidant status (TAS) is considered more holistic and representative than that of individual antioxidants. TAS reflects the cumulative protective capacity of existing antioxidants against oxidative damage. In cases of acute stroke triggered by oxidative stress, TAS levels undergo a significant reduction. Consequently, bolstering antioxidant capacity, both individual and overall, subsequent to acute stroke may serve as a protective measure against the detrimental consequences of heightened free radical production during ischemia and reperfusion.

Numerous proteins found in serum exhibit multifunctional attributes, manifesting neuroprotective effects and antioxidant properties within the context of the ischemic cascade. During the onset of the acute phase, alterations in protein levels become evident, encompassing proteins like albumin, prealbumin, transthyretin, retinol binding protein, and transferrin.

Notably rich in protein content, the snakehead fish (Channa striata) serves as a focal point of this study. The objective is to evaluate the impact of snakehead fish extract (SFE) supplementation on protein status, as gauged through assessments of albumin (Alb), transthyretin (TTR), transferrin (TFR), and retinol binding protein (RBP) levels. The study framework encompasses measurements of total antioxidant status (TAS) as an indicator of antioxidant capacity, malondialdehyde (MDA) measurements to gauge oxidative stress, and evaluations of clinical stroke utilizing the National Institutes of Health Stroke Scale (NIHSS) output scores.

II. Literature Study

Several studies have delved into the nutritional aspects of fish extracts and their potential impact on stroke outcomes.

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Apriliani et al. (2016) investigated the influence of gabus fish extract supplementation on serum zinc levels and clinical outcomes in acute ischemic stroke patients[2]. Similarly, Kasim et al. (2017) explored the effects of albumin fish extract supplementation on nutritional status and immunity in stroke patients[14]. Dziedzic et al. (2004) highlighted the predictive value of serum albumin levels for ischemic stroke outcomes[11].

Several investigations have focused on the effects of SFE on antioxidant status and its clinical implications. Kurnianto and Retnaningsih (2015) delved into the impact of SFE on total antioxidant status among acute ischemic stroke patients with a history of hypertension[15]. The work by Apriliani and Pudjonarko (2016) examined the effects of gabus fish extract on serum zinc levels and clinical outcomes in acute ischemic stroke patients[3]. Abidin et al. (2017) investigated the influence of SFE on serum arginine levels and neurological clinical outcomes in acute ischemic stroke patients[1]. Deyanningtyas et al. (2018) explored the effects of Channa striatus extract on serum DHA levels and National Institutes of Health Stroke Scale (NIHSS) scores in acute ischemic stroke patients[9].

Antioxidant and oxidative stress studies have also been conducted. Retnaningsih et al. (2017) explored the correlation between elevated plasma malondialdehyde levels and infarct volume in acute ischemic stroke patients[24]. Churriyyatul Anam et al. (2018) investigated the impact of glutathione supplementation on MMP-9 levels and infarct area in rat models of acute ischemic stroke[6].

Further investigations have examined broader clinical outcomes and correlations. Retnaningsih (2017) studied the association between absolute neutrophil count and mortality in bacterial pneumonia following acute ischemic stroke[24]. The work by Retnaningsih (2014) correlated plasma malondialdehyde level elevation with infarct volume on brain MSCT and Barthel Index among acute ischemic stroke patients[25]. Ummah et al. (2016) analyzed the neutrophil-to-lymphocyte ratio as an outcome indicator in acute ischemic stroke. Rahmawati et al. (2016) investigated the influence of zinc supplementation on clinical outcomes among acute ischemic stroke patients[22]. Aritonang et al. (2018) explored the relationship between serum neuron-specific enolase levels and neurological clinical outcomes in acute ischemic stroke patients[4].

In the realm of traditional extracts, Haruan fish (Channa striatus spp.) gained attention for its neuroregenerative properties (Mohd Shafri et al., 2011; Mohapatra et al., 2017) [18,19]. Studies by Deyanningtyas et al. (2018) and Kurnianto and Retnaningsih (2015) examined the influence of Channa striatus extract on DHA levels, NIHSS scores, and antioxidant status in acute ischemic stroke patients with a history of hypertension[9,16].

Antioxidant profiles have been a focus as well. Cherubini et al. (2000) explored the antioxidant profiles and early outcomes in stroke patients[5]. Hertantows et al. (2014) investigated the effects of snakehead fish extract supplementation on protein status, antioxidant levels, oxidative stress, and stroke outcomes[13].

The potential risks and benefits of consuming fish were evaluated by Du et al. (2012), who assessed nutrient and contaminant levels in various fish species and their implications for human health[10]. Additionally, the multifaceted effects of fish on human health were explored by Li and Hu (2009), encompassing nutritional and sustainability considerations[17].

These studies collectively contribute to a comprehensive understanding of the potential therapeutic roles of fish extracts, particularly snakehead fish extract, in influencing nutritional status, antioxidant profiles, oxidative stress, and clinical outcomes in acute ischemic stroke patients. Further research could provide deeper insights into the underlying mechanisms and therapeutic implications of these findings.

III. Material & Method

This study encompassed a cohort of 61 patients diagnosed with acute ischemic stroke, segregated into two distinct groups: the treatment group and the control group. The treatment group received a daily dosage of 15 g of snakehead fish extract (SFE), while the control group received a placebo. Administration commenced promptly within 72 hours of stroke onset and persisted for one week. It is noteworthy that all participants received standard treatment aligned with the hospital's standard operating procedures (SOP).

The preparation of SFE was executed using a vacuum and temperature control methodology. The variables under scrutiny included albumin, transthyretin, transferrin, and retinol binding protein levels. Additionally, malondialdehyde (MDA) and total antioxidant status (TAS) were measured via the Elisa method. Clinical outcomes were quantified using the National Institutes of Health Stroke Scale (NIHSS) scores.

Statistical analysis encompassed paired t-tests, independent sample t-tests, Mann-Whitney U tests, Wilcoxon correlations, Spearman's rho, and a Backward Stepwise (Wald) procedure. These analytical approaches facilitated the comprehensive exploration of the data and elucidation of potential associations and outcomes.

IV. Data Analysis

The presentation of values followed the format of mean ± standard deviation (SD) for data exhibiting a normal distribution, rather than the median. The determination of normality was predicated upon the Shapiro-Wilk test. To assess the statistical significance of inter-group disparities, paired t-tests and independent sample t-tests were employed. Subsequent post hoc analysis involved the utilization of the Mann-Whitney U and Wilcoxon tests. In instances where correlations involving multiple groups were investigated, inferential examinations were conducted through Spearman's rho analysis and the Backward Stepwise (Wald) approach. Statistical analyses were executed utilizing dedicated software, with a confidence threshold of $p < 0.05$ defining significance.

V. Result

The study comprised a selection of 61 participants, drawn from a pool of 199 individuals afflicted with ischemic stroke. Upon randomization, the treatment group consisted of 32 subjects, while the control group comprised 29 subjects. Notably, the treatment group encountered 10 dropouts and 1 fatality, while the control group experienced 6 dropouts and 2 fatalities. Evaluation of subject demographics, including age, level of education, occupational type, and marital status, revealed no statistically significant differences between the two groups. Specifically, the mean age for the treatment group was 58.3 ± 9.39 years, while the control group exhibited a mean age of 56.7 ± 6.166 years (p = 0.513).

Analysis of the results indicated a noteworthy disparity in albumin mean levels between the two groups following treatment (3.87 ± 0.525 vs. 3.35 ± 0.663, p = 0.008). Furthermore, a significant distinction was observed in the changes of albumin mean levels (0.08 ± 0.356 vs. -0.47 ± 0.699, p = 0.002). In the treatment group, albumin levels demonstrated stability or increase, in contrast to the control group where a decline in albumin levels appeared evident.

There was a significant difference in the change of TTR after treatment between treatment group and control group (88.823 ± 87.2595 vs. 2.346 ± 3,7884, p = 0,0001).

Post-treatment analysis unveiled a higher mean transferrin (TFR) level in the treatment group compared to the control group (124.89 vs. 78.18). Remarkably, a substantial and statistically significant elevation was observed within the treatment group (increase by 63.80, p = 0.0001). Moreover, the contrast in alterations of TFR levels following treatment between the treatment and control groups was also deemed statistically significant (63.801 ± 51.36 vs. 3.452 ± 7.12, p = 0.0001).

Contrarily, no notable disparity surfaced in retinol binding protein (RBP) levels between the treatment and control groups. The initial mean malondialdehyde (MDA) level prior to treatment was higher in the treatment group (187.34 vs. 105.75). However, following treatment, the treatment group exhibited a lower MDA level than the control group (76.63 vs. 96.40). Remarkably, a significant distinction was discerned in the alterations of MDA levels post-treatment within the treatment and control groups (110.70 ± 79.35 vs. 9.056 ± 87.25, p = 0.0001).

Prior to treatment, the mean total antioxidant status (TAS) level was higher in the treatment group (1.45 compared to 1.36). Following treatment, the treatment group continued to display a higher TAS level relative to the control group (1.49 compared to 1.35). In terms of serum albumin level (SAT) changes, a significant difference emerged between the treatment group and control group after treatment (0.0339 ± 0.091 vs. 0.0060 ± 0.036, p = 0.075).

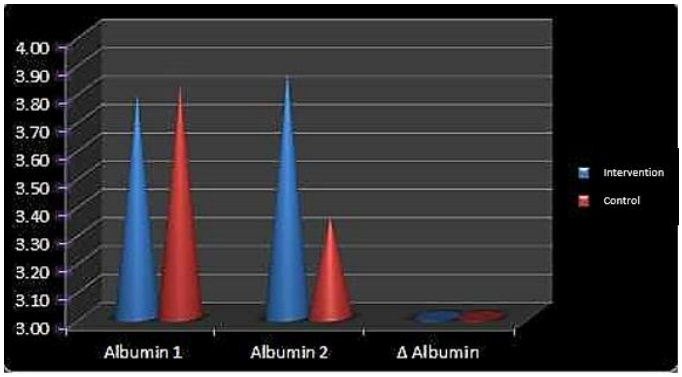
Remarkably, noteworthy correlations emerged between heightened protein status and NIHSS scores, total antioxidant status (TAS), as well as malondialdehyde (MDA) levels, exhibiting statistical significance (P < 0.05, r 0.40-0.59). However, no substantial correlation surfaced between NIHSS scores and either MDA or TAS. Similarly, no significant correlation was discerned between MDA and TAS.

Employing a multivariate logistic regression approach, specifically the Backward Stepwise method (Wald), the study substantiated that certain variables played a pivotal role in impacting post-treatment outcomes, particularly National Institutes of Health Stroke Scale (NIHSS) scores. It was demonstrated that the alteration in transthyretin (TTR) levels significantly influenced the enhancement of outcomes (p = 0.016; OR = 5.200 and 95% CI = 1.367 to 19.774 -OR). This underscores the vital role of TTR in the improvement of post-treatment NIHSS scores.

VI. Discussion

Hypoalbuminemia is a hallmark of malnutrition and systemic inflammatory vascular disease, leading to reduced albumin concentrations due to diminished protein synthesis and an escalated catabolic state. This deficiency often signals heightened morbidity risk. Protein and energy malnutrition (PEM) impairs mechanisms underlying ischemic stroke and hampers recovery progress. PEM transforms the expression of neuron plasticity-associated genes, impacting post-ischemia recovery mechanisms. Amid ischemic stroke, protein synthesis within the neuron's penumbra region is suppressed, and this inhibition correlates with the gradual decline of a dying cell's metabolic energy. The revival of protein synthesis after transient focal ischemia and the endurance of organ tissue rely on efficacy and recirculation mechanisms.

Snakehead fish extracts (SFE) wield a potent effect in elevating albumin levels, evident from test outcomes demonstrating a noteworthy disparity in the mean albumin level changes between the treatment and control groups (p = 0.002).



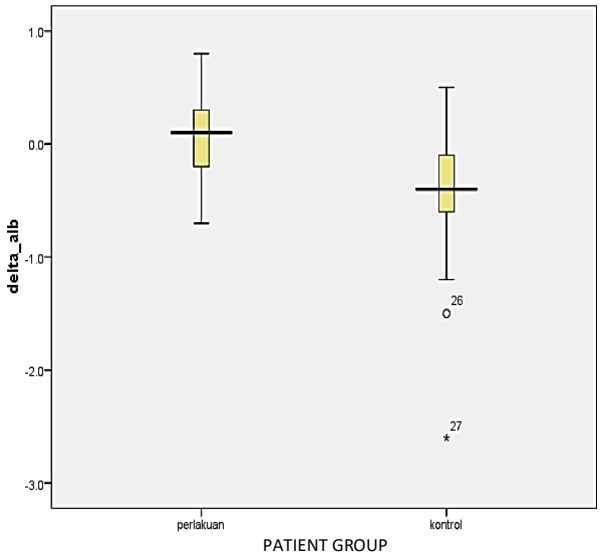
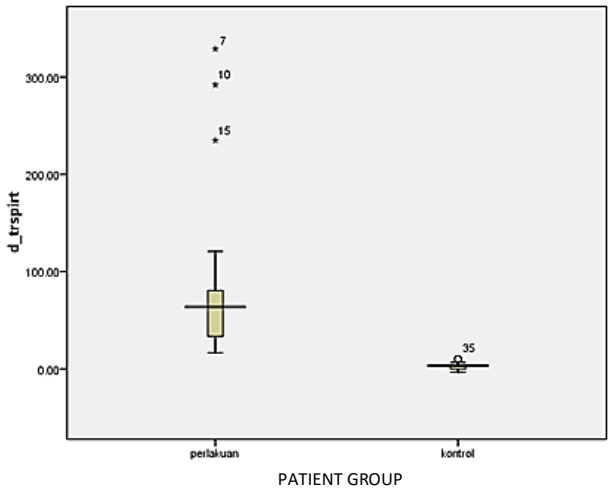


Figure 1. Snake Head Fish Extract (SFE) Supplementation in early ischemic stroke improved protein status Albumin

While albumin remained stable or increased in the treatment group, it decreased in the control group before and after treatment. Based on these findings, it can be deduced that SFE administration can effectively sustain stable albumin levels or prevent their decline in stroke patients. The considerable increase in albumin levels among stroke patients in this study is attributed to SFE's remarkably high albumin content (30.2%).

Albumin and transferrin, as hepatic transport proteins, play crucial roles. While transferrin's half-life is shorter than albumin's, both serve as indicators of nutritional status. Changes in albumin levels can be anticipated earlier by monitoring transferrin fluctuations. In this study, the administration of **SFE led to increased transferrin levels**, suggesting an enhancement in retinol transport during ischemic stroke.



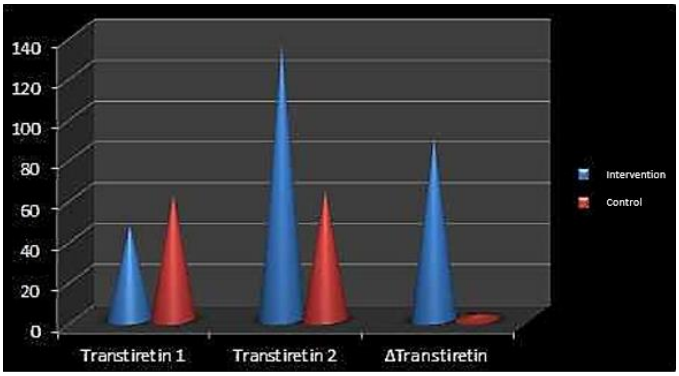


Figure 2. Snake Head Fish Extract (SFE) Supplementation in early ischemic stroke improved protein status Transferin

Furthermore, this study demonstrated that the supplementation of **SFE significantly lowered MDA levels** compared to the control group. MDA serves as a marker of lipid oxidative damage, with the aldehyde component implicated in a majority of pathophysiological processes associated with oxidative stress in cells and tissues. It also represents the end product of lipid peroxidation. During the propagation phase of lipid peroxidation, SFE acts as a scavenger of peroxyl and alkoxyl radicals, thereby safeguarding lipids. Beyond lipid peroxidation stages, the study **establishes SFE's ability to significantly increase TAS levels** ($P < 0.05$). Plasma TAS comprises various antioxidant defenses, including enzymatic and non-enzymatic systems. Enzymatic antioxidants, characterized by low molecular weight proteins, mitigate oxidative damage through catalytic reactions that detoxify free radicals. The mechanism behind the TAS elevation could stem from SFE's direct antioxidative activity (scavenging and chelation), its modulatory impact on endogenous antioxidants, or a combination of both factors.

Test results reveal a notable decrease in NIHSS scores, particularly pronounced in the treatment group compared to the control group, signifying a significant difference in the reduction of NIHSS scores between the two groups. These findings underscore the **administration of SFE's capacity to substantially enhance patients' neurology within 7 days of stroke onset**. Notably, in this study, **NIHSS scores after 1 week averaged a decrease to 5.6.**

Furthermore, the study identified **significant correlations between lowered NIHSS scores and elevated protein status** (albumin, transthyretin, and transferrin levels, except for retinol binding protein). The NIHSS total score holds potential as a tool for determining the necessary hospital stay duration for acute stroke patients to receive appropriate treatment. For each incremental point in the total NIHSS score, the length of hospital stay is projected to increase by approximately 1 day.

Conversely, **no significant correlations emerged between the decline in NIHSS scores and MDA levels or total antioxidant status**. This absence of correlation corresponds to the lack of significant associations between the decrease in

MDA levels and total antioxidant status, indicating intricate interactions beyond the scope of these parameters.

VII. Further Work

While this study provides valuable insights into the potential benefits of snakehead fish extract (SFE) in mitigating the impact of acute ischemic stroke, there remain avenues for further exploration to enhance our understanding and clinical applications.

Firstly, investigating the mechanisms underlying the observed increase in albumin and transferrin levels following SFE administration could offer deeper insights into the nutritional and neuroprotective effects of SFE. Exploring the specific pathways and interactions involved in retinol transport enhancement would be particularly informative.

Additionally, delving into the broader effects of SFE on oxidative stress and antioxidant defenses warrants attention. Further studies could elucidate the precise antioxidative mechanisms of SFE, including its potential modulation of endogenous antioxidant systems, which could contribute to its observed effects on MDA levels and total antioxidant status.

To establish a more comprehensive understanding of SFE's impact, longitudinal studies with larger cohorts could provide insights into the long-term effects of SFE supplementation on neurology, protein status, and stroke outcomes. This could shed light on the sustainability and lasting benefits of SFE-based interventions.

Furthermore, exploring potential interactions between SFE and conventional stroke treatments could pave the way for integrated therapeutic approaches that optimize patient outcomes. Finally, elucidating the underlying biological pathways through which SFE exerts its effects would be instrumental in developing targeted interventions and enhancing the clinical management of acute ischemic stroke.

VIII. Conclusion & Suggestion

A. Conclusion

The supplementation of Snakehead Fish Extract (SFE) to patients during the early acute phase of ischemic stroke, spanning up to day 7, yielded notable findings:

1. A substantial increase in protein status was observed, as indicated by the significant elevation of albumin, transthyretin, and transferrin levels, although retinol binding protein levels remained unaffected.
2. A significant reduction in oxidative stress status, quantified by malondialdehyde (MDA) levels, was observed.
3. A noteworthy rise in total antioxidant status (TAS) levels was detected.
4. The outcome of acute ischemic stroke, as assessed by National Institutes of Health Stroke Scale (NIHSS) scores, exhibited a significant improvement.

Analysis of the correlation test outcomes yielded the following insights:

1. A significant correlation emerged between the reduction in National Institutes of Health Stroke Scale (NIHSS) scores and the augmentation of protein status, encompassing albumin, transthyretin, and transferrin levels, with the exception of retinol binding protein levels.
2. A noteworthy correlation was identified between the decline in NIHSS scores and the reduction in malondialdehyde (MDA) levels, as well as the increase in total antioxidant status (TAS).
3. However, no significant correlation was observed between the reduction in malondialdehyde levels and the increase in total antioxidant status.

B. Suggestion

The implications drawn from this study are outlined as follows:

1. Medical practitioners and healthcare professionals can consider utilizing Snakehead Fish Extract (SFE) as a potential neuroprotective therapy for the treatment of acute ischemic stroke.
2. For the benefit of academic researchers and scholars, the study recommends:
    a. Exploring further research avenues to investigate the impact of inflammation, immunology, and apoptosis in the context of acute ischemic stroke.
    b. Conducting similar investigations involving other study variables, spanning from the first day to the seventh day or beyond, to attain a more comprehensive understanding of the dynamic changes associated with acute ischemic stroke.
    c. Undertaking additional research endeavors focused on assessing other variables that contribute to the clinical outcomes of acute ischemic stroke, particularly those connected with the immune system, inflammation, and apoptosis pathways.
3. For the general public, it's worth noting that Snakehead Fish Extract (SFE) could serve as a potential supplementary option to facilitate the recovery process for individuals affected by ischemic stroke.

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