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RESEARCH ARTICLE

The Effect of Snakehead Fish Extract (Channa striata) on Superoxide Dismutase (SOD) Levels and Lung Histopathology of Wistar Rats (Rattus norvegicus) Exposed to Cigarette Smoke

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ABSTRACT

Exposure to cigarette smoke triggers oxidative stress, leading to a decrease in Superoxide Dismutase (SOD) levels and lung tissue damage. Snakehead fish extract (Channa striata) contains albumin and essential fatty acids (omega-3) that act as natural antioxidants, anti-inflammatory agents, and tissue-healing agents. This study aims to evaluate the effects of snakehead fish extract on SOD levels and lung histopathology improvement in rats exposed to cigarette smoke. This experimental study employed a Pre-post Control Group Design using 20 Wistar rats (Rattus norvegicus) divided into four groups. Group K- (standard control) received no treatment, K+ was exposed to cigarette smoke, KP1 was exposed to cigarette smoke and administered 0.5 ml of snakehead fish extract, and KP2 was exposed to cigarette smoke and given 1 ml of snakehead fish extract. SOD levels were measured using a spectrophotometric method, and lung histopathology analysis was performed with hematoxylin-eosin staining. Data were analyzed using the Paired Sample T-test and Kruskal-Wallis test with a significance level of $p < 0.05$. The results showed that the K+ group experienced a significant decrease in SOD levels ($p = 0.043$) and more severe lung histopathological damage compared to the negative control group (K-). Treatment with snakehead fish extract at doses of 0.5 ml (KP1) and 1 ml (KP2) significantly increased SOD levels ($p < 0.05$) compared to the K+ group. The 0.5 ml dose (KP1) demonstrated optimal effectiveness in increasing SOD levels (0.3594 ± 0.254) and improving lung histopathology structure (3.6 ± 0.5) damaged by cigarette smoke exposure. Snakehead fish extract effectively increased SOD levels and improved lung histopathology damage induced by cigarette smoke exposure. The 0.5 ml/kg BW dose showed the best effectiveness, balancing antioxidant and anti-inflammatory activities, resembling near-normal conditions. Snakehead fish extract has the potential to be a protective therapy against oxidative stress and lung tissue damage.

INTRODUCTION

Exposure to cigarette smoke has been proven to be a significant source of free radicals that cause oxidative stress in the body. Cigarette smoke contains various toxic compounds, including Reactive

Oxygen Species (ROS) and Reactive Nitrogen Species (RNS), which can damage macromolecules such as lipids, proteins, and DNA.[1]

Free radicals in cigarette smoke can accelerate cellular damage caused by oxidative stress. The target molecules damaged by free radicals include DNA, lipids, and proteins.[2] Harmful chemical components in gaseous and volatile forms in cigarettes cause repeated genetic mutations.[3] Furthermore, the combination of genetic mutations and DNA damage can lead to genetic instability, which contributes to cancer development.[4]

Additionally, exposure to oxidant chemicals in cigarette smoke is associated with a reduction in endogenous antioxidant levels in systemic compartments, further exacerbating oxidative stress during smoking.[5] Oxidative stress induced by cigarette smoke plays a significant role in the pathogenesis of various chronic diseases, such as chronic obstructive pulmonary disease (COPD), lung cancer, and atherosclerosis.[6] Therefore, understanding the chemical composition of cigarettes and their impact on oxidative stress is crucial for the prevention and management of smoking-related diseases.[7]

As a key antioxidant enzyme, SOD (Superoxide Dismutase) catalyzes the conversion of superoxide radicals (O_2^-) into hydrogen peroxide (H_2O_2) and oxygen (O_2), which are more stable and further degraded by catalase or glutathione peroxidase to prevent the formation of highly reactive hydroxyl radicals (OH•).[8] A decrease in SOD activity has been associated with increased oxidative stress, contributing to lung tissue damage due to cigarette smoke exposure.[9] Therefore, enhancing SOD activity through antioxidant supplementation is a potential approach to reduce oxidative stress damage and repair damaged tissues.

Snakehead fish extract (Channa striata) has been recognized as a natural antioxidant agent rich in albumin, essential amino acids, omega-3 fatty acids, and selenium, which play a crucial role in accelerating tissue regeneration and enhancing SOD activity.[10,11] Albumin is a plasma protein that maintains osmotic pressure, accelerates protein synthesis and stimulates fibroblast proliferation to repair damaged tissues.[12] The omega-3 and omega-6 fatty acids in snakehead fish also have anti-inflammatory effects, helping to reduce inflammation and accelerate wound healing through collagen synthesis and cell regeneration stimulation.[13] Additionally, zinc (Zn) and selenium (Se) in snakehead fish support the activity of antioxidant enzymes such as SOD and glutathione peroxidase, which protect cells from free radicals.[14]

Previous research has shown that oral administration of snakehead fish extract can increase Superoxide Dismutase (SOD) levels and reduce malondialdehyde (MDA) levels, which are markers of oxidative damage, as well as improve the histopathological structure of lung tissues damaged by cigarette smoke exposure.[11] With its bioactive components that support antioxidant activity and tissue regeneration, snakehead fish extract emerges as a potential natural therapy to protect lung tissues from damage caused by free radicals induced by cigarette smoke exposure.

This study aims to evaluate the effects of snakehead fish extract on Superoxide Dismutase (SOD) levels and lung histopathology in Wistar rats (Rattus norvegicus) exposed to cigarette smoke. The findings are expected to provide scientific evidence supporting using snakehead fish extract as a therapeutic agent to counteract oxidative stress and repair lung tissue damage.

Snakehead fish extract (Channa striata) contains bioactive compounds such as albumin, essential amino acids, and omega-3 fatty acids, which are natural antioxidants. These compounds can repair tissue damage and enhance antioxidant activity. However, the optimal dosage for protecting lung tissues from cigarette smoke exposure has not been fully explored.

This study aims to analyze the effects of snakehead fish extract in improving lung tissue damage and increasing SOD levels in rats exposed to cigarette smoke.

RESEARCH METHODS

Research Design:

This experimental study uses a Pre-post-test control group design with a completely randomized design (CRD).

Research Subjects:

Before treatment, the animals were placed in prepared cages and fed generally for one week as an adaptation period. The test animals were randomly divided into four groups, five per group. The groups were assigned as follows:

a K- (Normal Control): Provided with standard feed and water daily without any exposure to cigarette smoke.

b K+ (Cigarette Smoke Exposure): Provided with standard feed and water daily and exposed to cigarette smoke for 14 days.

c KP1 (Minimal Dose + Cigarette Smoke): Exposed to cigarette smoke and administered 0.5 ml/gBW/day of snakehead fish extract orally.

d KP2 (Maximum Dose + Cigarette Smoke): Exposed to cigarette smoke and administered 1 ml/gBW/day of snakehead fish extract orally.

Measurement of SOD Levels:

The data obtained were analyzed for homogeneity and distribution. SOD levels were measured using a spectrophotometric method with a SOD ELISA kit, both before and after treatment. The examination was conducted at the HUMRC Laboratory, Hasanuddin University Hospital.

Histopathological Examination:

Lung tissue samples were collected, fixed in formalin, and analyzed using hematoxylin-eosin staining to observe the extent of tissue damage. The analysis was performed at the Anatomical Pathology Laboratory, Hasanuddin University Veterinary Hospital.

Materials:

The materials used for the preparation and testing of the antioxidant effects of snakehead fish extract (Channa striata) on experimental rats include:

Snakehead fish extract (Channa striata) was obtained from Onoiwa MX, a herbal medicine PT Natura Nuswantara Nirmala produced. The main ingredient in Onoiwa is snakehead fish extract (Channa striata), which is packaged in sachets as a liquid supplement with doses of 0.5 ml/gBW/day and 1 ml/gBW/day.

The SOD ELISA kit, used for antioxidant measurement, was procured from the HUMRC Laboratory, Hasanuddin University Hospital, Makassar.

Test Animals:

The test animals used were male Wistar rats (Rattus norvegicus), aged 8–10 weeks, weighing 150–200 grams, with 20 rats obtained from the Veterinary Hospital Laboratory, Hasanuddin University, Makassar.

Statistical Analysis:

The Superoxide Dismutase (SOD) levels were analyzed using the Paired Sample T-Test and Kruskal-Wallis test with a significance level of $p < 0.05$. Meanwhile, the lung histopathology data were analyzed semi-quantitatively using a scoring method, followed by statistical analysis with the

Kruskal-Wallis test to determine the significance of differences between the control and treatment groups.

RESULTS

Effects of Snakehead Fish Extract on SOD Levels in Rats Before and After Treatment

Based on the analysis results in Table 1, the mean SOD levels before treatment (Pre) indicate no significant differences among the groups K-, K+, KP1, and KP2. However, after treatment (Post), there were substantial differences in the average SOD levels among the K+, KP1, and KP2 groups compared to the K- group.

Table 1. Differences in Mean SOD Levels in Rats Before and After Treatment

Group	SOD Level (ng/dL) (Mean ± SD)	
	Pre	Post
K- (normal)	1.2546 ± 0.1396	1.3304 ± 0.1213[a]
K+ (Cigarette Smoke Exposure)	1.3188 ± 0.6608	1.0170 ± 0.1888[b]
KP1(0.5 ml Extract + Smoke)	1.2126 ± 0.2482	1.5720 ± 0.2196[b]
KP2 (1 ml Extract + Smoke)	1.0179 ± 0.3121	1.4481 ± 0.1402[b]

Notes: There are significant differences between groups with different superscripts (a, b) in the same column (variable) ($p < 0.05$).

Table 2. Effects of Snakehead Fish Extract on SOD Levels in Rats Before and After Treatment

Group	SOD Level (ng/dL) (Mean ± SD)		p-Value
	Pre	Post	
K- (normal)	1.2546 ± 0.1396	1.3304 ± 0.1213	0.519
K+ (Cigarette Smoke Exposure)	1.3188 ± 0.6608	1.0170 ± 0.1088	0.043
KP1(0.5 ml Extract + Smoke)	1.2126 ± 0.2482	1.5720 ± 0.2196	0.034
KP2 (1 ml Extract + Smoke)	1.0179 ± 0.1121	1.4481 ± 0.1402	0.048

Notes: A paired Sample T-Test was used. Significant differences are indicated by $p < 0.05$.

Interpretation:

The K+ group (exposed to cigarette smoke without treatment) showed a significant decrease in SOD levels ($p = 0.043$), confirming the impact of oxidative stress induced by cigarette smoke exposure.

The KP1 (0.5 ml extract) and KP2 (1 ml extract) groups showed significant increases in SOD levels ($p = 0.034$ and $p = 0.048$, respectively) compared to the K+ group, demonstrating the antioxidant effects of snakehead fish extract.

The KP1 group exhibited the highest increase in SOD levels, indicating the optimal effectiveness of the 0.5 ml dose in enhancing antioxidant activity and potentially balancing oxidative stress and inflammation.

Based on the analysis results in Table 4, there was a significant decrease in Superoxide Dismutase (SOD) levels in the K+ group compared to the standard control group (K-). The reduction in SOD levels in the K+ group (-0.3017 ± 0.245) reflects the impact of oxidative stress caused by exposure to cigarette smoke, which contains free radicals and toxic compounds. In contrast, the groups treated with snakehead fish extract (KP1 and KP2) showed a significant increase in SOD levels ($p < 0.05$) compared to the K+ group.

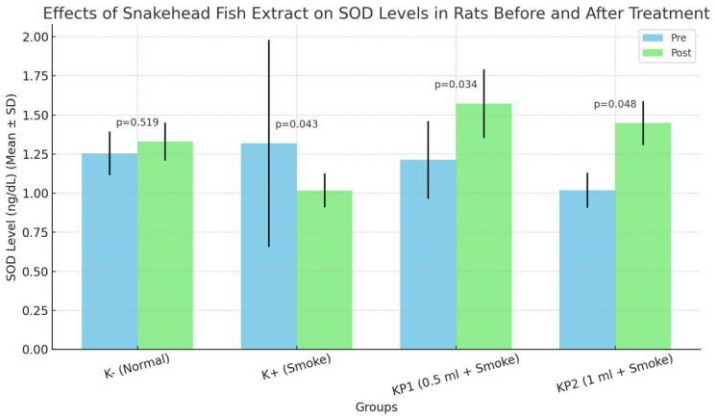

Figure 1. Effects of Snakehead Fish Extract on SOD Levels in Rats Before and After Treatment

The analysis results presented in Table 2 and Figure 1 indicate that snakehead fish extract significantly increased SOD levels in rats, with p-values < 0.05 in the treatment groups (KP1 and KP2). This demonstrates the potential of snakehead fish extract as an agent capable of enhancing the body's response to oxidative stress and improving antioxidant activity.

On the other hand, the positive control group (K+) showed a significant decrease in SOD levels, suggesting that oxidative stress conditions can reduce SOD activity in the absence of supportive interventions. The KP1 group, which received a 0.5 ml dose, exhibited an increase in SOD levels (0.3594 ± 0.254), while the KP2 group, which received a 1 ml dose, showed a more significant increase (0.4302 ± 0.503).

The difference in effectiveness between the doses indicates that, although the 1 ml dose (KP2) resulted in a more significant increase in SOD levels, the 0.5 ml dose (KP1) was considered more stable, with lower variability (lower standard deviation).

Effects of Snakehead Fish Extract on Lung Histopathology in Rats

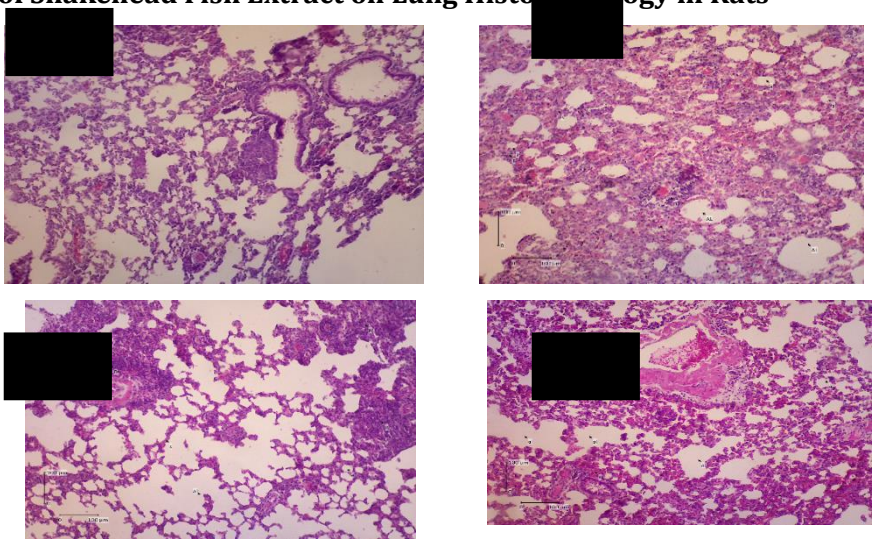

Figure 2. Lung Histopathology Structure in Rats (100x magnification, Hematoxylin-Eosin Staining): K- (Normal Control): No extract and no cigarette smoke exposure, K+ (Cigarette Smoke Exposure): Exposed to cigarette smoke without extract, KP1 (0.5 ml extract + cigarette smoke): Exposed to cigarette smoke and treated with 0.5 ml/gBW/day snakehead fish extract, KP2 (1 ml extract + cigarette smoke): Exposed to cigarette smoke and treated with 1 ml/gBW/day snakehead fish extract.

Histopathological Analysis Results:

Based on Figure 2, the negative control group (K-) exhibited typical alveolar lung structures with no visible damage or presence of alveolar macrophages. In contrast, the positive control group (K+) displayed severe lung tissue damage, including Alveolar macrophage infiltration covering the alveoli, Inflammatory cells characterized by the presence of macrophages and neutrophils, Fibrosis, and alveolar septum destruction leading to atelectasis. In the KP1 group (0.5 ml extract), lung damage appeared less severe, with reduced macrophage infiltration and less inflammation, suggesting a protective effect of the extract. The KP2 group (1 ml extract) also showed improvements, but some inflammatory cells, fibrosis, and alveolar septum destruction were still observed, albeit less severe than the K+ group.

The analysis of lung histopathology data was conducted semi-quantitatively using a scoring method, which was then statistically analyzed to distinguish between the control and treatment groups.

Table 2. Histopathology Score Differences Between Groups

Variable	Group	Mean ± SD	p-Value
Lungs	K- (normal)	3.8 ± 0.4	0.001
	K+ (Cigarette Smoke Exposure)	1.0 ± 0.0*	
	KP1 (0.5 ml extract + smoke)	3.6 ± 0.5*	
	KP2 (1 ml extract + smoke)	2.6 ± 0.5*	

Notes: The Kruskal-Wallis Test was used.

(*) Significant difference compared to positive control (K+) ($p < 0.05$).

Interpretation:

According to Table 2, all groups showed significant differences in histopathology scores compared to the negative control group (K-).

The K+ group showed the most severe damage, confirming the detrimental effects of cigarette smoke on lung tissue.

The KP1 group (0.5 ml extract) exhibited better lung recovery than the KP2 group (1 ml extract), which still showed mild inflammation and fibrosis, although less severe than in the K+ group.

These findings suggest that 0.5 ml of snakehead fish extract provides optimal protection by balancing antioxidant and anti-inflammatory effects, reducing oxidative stress, and promoting lung tissue repair more effectively.

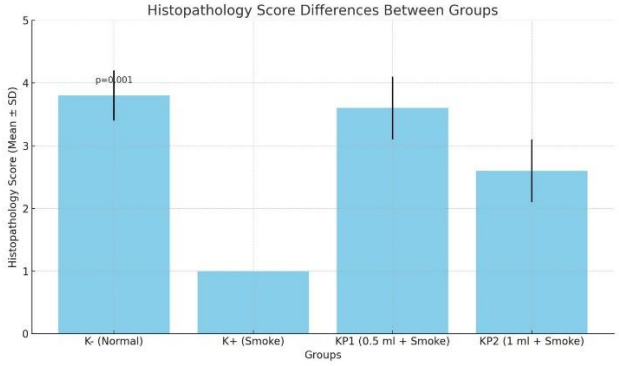

Figure 3. Differences in Lung Histopathology Scores Between Rat Groups

Histopathological analysis of the lungs presented in Table 2, Figures 2, and 3 revealed severe damage in the K+ group, which was significantly improved in the treatment groups that received snakehead

fish extract. The 0.5 ml dose (KP1) demonstrated histopathology scores closer to normal (3.6 ± 0.5) compared to the 1 ml dose (KP2) (2.6 ± 0.5), suggesting that the lower dose provided optimal protection. This effect is likely associated with a balanced anti-inflammatory and antioxidant response at the lower dose, while the higher dose may have triggered secondary inflammation.

DISCUSSION

The results showed that the K+ group, which was exposed to cigarette smoke, experienced a significant decrease in Superoxide Dismutase (SOD) levels compared to the standard control group (K-). This decline is consistent with previous studies by Asri and Rahmawati (2020), which reported that cigarette smoke exposure increases the production of free radicals, such as reactive oxygen species (ROS), leading to oxidative stress and reducing SOD activity, a key antioxidant enzyme.

An increase in SOD levels in the treatment groups KP1 (0.5 ml) and KP2 (1 ml) after the administration of snakehead fish extract confirms the potent antioxidant effects of the extract. Research by Setiawan and Nurdiana (2019) supports these findings, indicating that snakehead fish extract, rich in albumin, omega-3 fatty acids, and essential amino acids, enhances antioxidant enzyme activity by stimulating protein synthesis and promoting cell regeneration damaged by oxidative stress.

The 0.5 ml dose (KP1) demonstrated a more optimal increase in SOD levels compared to the 1 ml dose (KP2). This result is in line with the findings of Yuliana and Pratiwi (2020), which stated that excessive doses of antioxidants could potentially trigger secondary pro-oxidative effects, making antioxidant protection less effective. Therefore, the 0.5 ml dose may provide an ideal balance between antioxidant and anti-inflammatory effects, contributing to a more efficient increase in SOD levels.

Histopathological analysis showed that the K+ group experienced significant lung tissue damage, including alveolar septal thickening, inflammatory cell infiltration, and edema, consistent with the findings of Wahyuni and Kusuma (2021). Their study reported that oxidative stress caused by cigarette smoke damages cell membranes and connective tissues in the lungs through chronic inflammation.

The treatment groups KP1 (0.5 ml) and KP2 (1 ml) demonstrated significant improvements in lung tissue structure after administering snakehead fish extract. These findings align with the research of Kurniawati and Lestari (2022), which showed that snakehead fish extract accelerates tissue regeneration through its albumin content, promoting fibroblast proliferation and collagen synthesis, essential for wound healing and tissue repair.

The 0.5 ml dose (KP1) demonstrated more optimal histopathological improvement (3.6 ± 0.5) compared to the 1 ml dose (KP2) (2.6 ± 0.5). This result supports the study by Yuliana and Pratiwi (2020), which indicated that lower doses of snakehead fish extract provided better anti-inflammatory effects. In contrast, higher doses might trigger secondary inflammation, slowing tissue regeneration. Additionally, research by Setiawan and Nurdiana (2019) highlighted that high protein extracts may overstimulate cellular metabolism, causing secondary oxidative stress, which can impair tissue healing.

The effectiveness of snakehead fish extract in improving SOD levels and lung histopathology can be attributed to its bioactive compounds, including albumin, essential amino acids, and omega-3 fatty acids. These compounds support tissue regeneration and enhance the endogenous antioxidant system. This study supports previous findings by Asri and Rahmawati (2020) and Setiawan and Nurdiana (2019), demonstrating that snakehead fish extract reduces oxidative stress and repairs damaged tissues by enhancing antioxidant activity and cell regeneration.

The differences in effectiveness between the 0.5 ml (KP1) and 1 ml (KP2) doses suggest that the optimal dose of snakehead fish extract is 0.5 ml. This dose provides better protection by balancing antioxidant and anti-inflammatory effects without triggering excessive inflammatory responses, which may inhibit tissue regeneration.

CONCLUSION

This study concludes that cigarette smoke exposure significantly reduces Superoxide Dismutase (SOD) levels, indicating high oxidative stress in tissues. Administration of snakehead fish extract at doses of 0.5 ml and 1 ml effectively increased SOD levels after treatment. However, the 0.5 ml dose (KP1) proved to be more optimal in enhancing SOD activity, providing adequate protection against oxidative stress and repairing lung tissue.

Additionally, cigarette smoke exposure caused significant lung tissue damage, as evidenced by low histopathology scores in the K+ group. Treatment with snakehead fish extract significantly improved lung tissue structure, especially at the 0.5 ml dose (KP1), which showed the most optimal effectiveness, with results approaching normal conditions (K-). The 1 ml dose (KP2) also promoted improvement, although slightly less effective than the 0.5 ml dose (KP1).

These findings suggest that the 0.5 ml dose provides the best balance between antioxidant and anti-inflammatory effects, supporting the regeneration of lung tissue damaged by cigarette smoke exposure.

Thus, snakehead fish extract has the potential to be a natural antioxidant agent to protect tissues from free radical damage and oxidative stress induced by cigarette smoke exposure. The recommended optimal dose based on this study is 0.5 ml, which provides maximum protection without causing excessive inflammatory responses. This study also serves as a foundation for developing snakehead fish extract as a therapeutic agent to combat oxidative stress and lung tissue damage.

AUTHORS' CONTRIBUTIONS

ND and AS were involved in the conception and planning of the research, ND and MHC performed the data acquisition/collection, and ND and AS calculated the experimental data. They performed the analysis, ND drafted the manuscript and designed the figures, and MHC aided in interpreting the results. All authors took part in a critical revision of the manuscript.

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