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RESEARCH ARTICLE

The Effect of Snakehead Fish (Channa Striata) Extract on Malondialdehyde (MDA) Levels in Wistar Rats (Rattus Novergicus) Exposed to Cigarette Smoke

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ABSTRACT

Exposure to cigarette smoke is known to trigger oxidative stress, characterized by an increase in Malondialdehyde (MDA) levels and liver tissue damage. Cigarette smoke contains more than 7,000 harmful chemicals, including free radicals and reactive oxygen species, which can cause tissue damage through lipid peroxidation. Malondialdehyde (MDA) is the end product of lipid peroxidation and is often used as a biomarker of oxidative stress. Increased MDA levels indicate significant oxidative damage, particularly in liver tissue susceptible to free radicals. This study aims to evaluate the effect of snakehead fish (Channa striata) extract, which is known to contain albumin, omega-3 fatty acids, and essential minerals with antioxidant and anti-inflammatory properties, on MDA levels and liver histopathological conditions in Wistar rats exposed to cigarette smoke. This study used a proper experimental design with a pre-post control group format. A total of 20 male Wistar rats (aged 2–3 months, weighing approximately 200 grams) were divided into four groups: K- (negative control, no treatment), K+ (positive control, cigarette smoke exposure only), P1 (cigarette smoke exposure + snakehead fish extract at 0.5 ml/day), and P2 (cigarette smoke exposure + snakehead fish extract at 1 ml/day). Cigarette smoke exposure was conducted for 15 minutes daily for 20 consecutive days. MDA levels were measured using the Enzyme-Linked Immunosorbent Assay (ELISA) method, while liver histopathological analysis was performed using hematoxylin-eosin staining. Data were analyzed using the ANOVA test for normally distributed data and the Kruskal-Wallis test for non-normal data distribution, with a significance level of $p < 0.05$. The K+ group showed a significant increase in MDA levels ($p < 0.05$) compared to the K- group. Treatment with snakehead fish extract in the P1 and P2 groups significantly reduced MDA levels compared to K+ ($p < 0.05$). Histopathological examination showed improvements in liver tissue in the P1 and P2 groups, with the best improvement observed in the P1 group. Snakehead fish extract proved effective in reducing oxidative stress and supporting the regeneration of liver tissue damaged by cigarette smoke exposure. Snakehead fish extract is effective in lowering MDA levels and improving liver histopathological damage caused by cigarette smoke exposure. A dose of 0.5 ml/day demonstrated optimal effectiveness in mitigating the negative impacts of oxidative stress, making it a potential antioxidant agent for protecting liver tissue from damage.

INTRODUCTION

Smoking has been identified as one of the main risk factors for various chronic and degenerative diseases, including cardiovascular diseases, lung cancer, and chronic obstructive pulmonary disease (COPD). According to the World Health Organization (WHO), more than 7 million deaths annually are caused by direct tobacco product use. In comparison, approximately 1.2 million deaths occur among non-smokers exposed to secondhand smoke. Cigarette smoke contains over 7,000 chemical compounds, including carcinogenic substances and free radicals, which have the potential to damage tissues through oxidative stress mechanisms [1].

Oxidative stress occurs when the production of Reactive Oxygen Species (ROS) exceeds the capacity of the body's antioxidant systems to neutralize them. ROS can cause damage to lipids, proteins, and DNA, ultimately leading to chronic inflammation and tissue damage [2]. Malondialdehyde (MDA), a byproduct of lipid peroxidation, is a crucial biomarker used to assess the level of oxidative damage. High levels of MDA are often associated with an increased risk of degenerative diseases and impaired liver function [3].

The snakehead fish (Channa striata) is a freshwater species rich in bioactive compounds, including albumin, omega-3 fatty acids, and minerals such as zinc and selenium. Albumin plays a role in tissue regeneration, while omega-3 fatty acids are known for their potent anti-inflammatory and antioxidant properties [4]. Previous research has shown that snakehead fish extract can reduce tissue damage and accelerate wound healing through its antioxidant and anti-inflammatory activity [5].

However, research evaluating the effects of snakehead fish extract on MDA levels and liver histopathological conditions in Wistar rats exposed to cigarette smoke remains limited. This study aims to fill this gap by assessing the influence of snakehead fish extract on MDA levels and liver histopathological changes in Wistar rats exposed to cigarette smoke. The findings of this study are expected to provide additional scientific evidence on the potential of snakehead fish extract as a therapeutic agent to counteract the negative impacts of oxidative stress caused by cigarette smoke exposure.

RESEARCH METHODS

Research design

This study utilized a proper experimental design with a pre-test and post-test control group approach. This design was chosen to allow direct observation of the treatment effects on the research subjects through measurements taken before and after the intervention.

Research subjects

The research subjects consisted of 20 male Wistar rats aged 2–3 months with an average weight of 200 grams. The rats were randomly selected and maintained in controlled laboratory conditions with room temperatures around 22–25 °C, a 12-hour light-dark cycle, and ad libitum access to food and water. The research subjects were divided into four treatment groups as follows:

1. **K- (Negative control):** Rats were not exposed to cigarette smoke and were not given snakehead fish extract.
2. **K+ (Positive control):** Rats were exposed to cigarette smoke without receiving snakehead fish extract.
3. **P1 (Low dose):** Rats were exposed to cigarette smoke and given snakehead fish extract at a dose of 0.5 ml/day.
4. **P2 (High dose):** Rats were exposed to cigarette smoke and given snakehead fish extract at a dose of 1 ml/day.

Research procedures

1. **Subject adaptation:** The rats were acclimatized for 7 days to familiarize themselves with the research environment before the treatment began.

2. **Cigarette smoke exposure:** Rats in the K+, P1, and P2 groups were exposed to cigarette smoke using a specially designed automatic smoking apparatus. The exposure was conducted for 15 minutes daily for 20 consecutive days in a standard-sized laboratory enclosure.
3. **Administration of snakehead fish extract:** Snakehead fish extract was administered orally using a gastric gavage tube once daily, according to each group's designated doses. The extract was processed hygienically to preserve its bioactive quality.
4. **Blood sample collection:** Blood samples were collected from the tail vein of the rats on day 0 (before treatment) and day 21 (after treatment) for MDA level analysis.
5. **Liver organ collection:** The rats were ethically sacrificed following laboratory guidelines after the treatment. The liver organs were collected for histopathological analysis using the hematoxylin-eosin staining method.

Data analysis

Data processing was performed using the latest version of SPSS software. The stages of analysis included:

1. **Normality test:** The Shapiro-Wilk test ensured normal data distribution.
2. **Homogeneity test:** The Levene test was employed to verify the equality of variances between groups.
3. **Statistical analysis:** Normally distributed data were analyzed using a one-way ANOVA test, while non-normally distributed data were analyzed using the Kruskal-Wallis test. The significance level was set at $p < 0.05$.

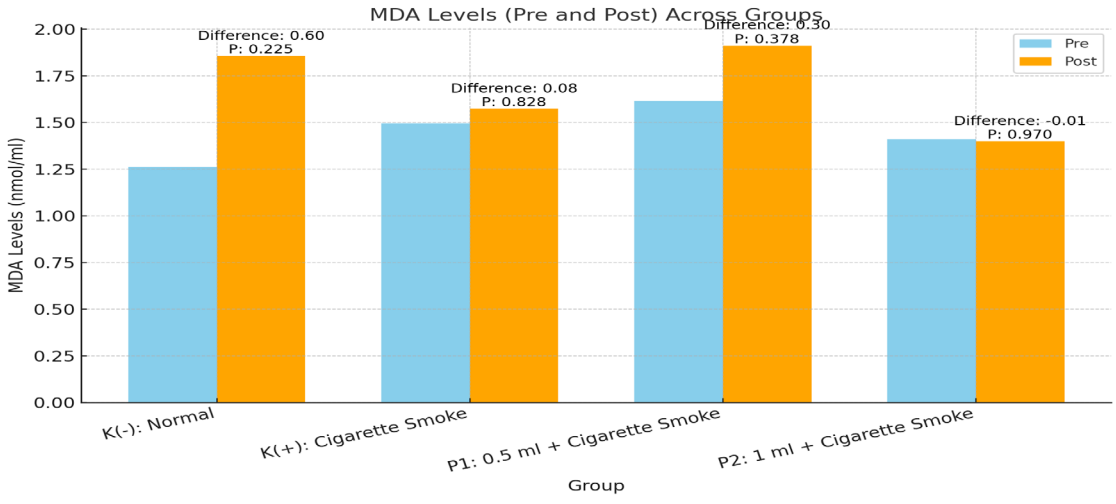
RESULTS

Measurement of MDA levels

Based on the normality test results, data meeting the normality assumption were found in groups K(+), P1, and P2. Therefore, a paired T-test was used to compare MDA levels in Wistar rats before and after treatment. For group K(-), which did not meet the normality assumption, the Wilcoxon test was used to compare MDA levels before and after treatment.

Group	MDA Levels (nmol/ml)		Difference	P-value
	Pre (Mean±SD)	Post (Mean±SD)		
K(-) (Normal)	1.2601+0.2589	1.8561+0.5892	0.59602	0.225
K(+) (Cigarette Smoke)	1.495+0.6951	1.5725+0.2894	0.07722	0.828
P1 (0.5 ml + Cigarette Smoke)	1.614+0.4045	1.9123+0.6914	0.29834	0.378
P2 (1 ml + Cigarette Smoke)	1.4108+0.3249	1.3995+0.4756	-0.01126	0.97

These results indicate no statistically significant differences in MDA levels before and after treatment across all groups ($p > 0.05$).



Graph 1: The effect of snakehead fish extract on MDA levels in rats before and after treatment

Homogeneity test

The homogeneity test was conducted as a prerequisite for ANOVA testing. This test checks the uniformity of the data using a significance level of alpha (p-value > 0.05).

Variable	Test Statistic	P-value
Pre Test	0.939	0.445
Post Test	3.063	0.058

Based on the homogeneity test results, the p-values for the pre-test and post-test were 0.445 and 0.058, respectively. Since p-value > alpha (0.05), it can be concluded that the MDA level data for Wistar rats exposed to cigarette smoke met the homogeneity assumption for ANOVA testing.

ANOVA test

The ANOVA test was used to determine whether there were significant differences in MDA levels among Wistar rats exposed to cigarette smoke in each treatment group (K-, K+, P1, and P2) with a significance level of alpha (p-value < 0.05).

Variable	Test Statistic	P-value
Pre Test	0.541	0.661
Post Test	1.028	0.406

Based on the ANOVA test results, the p-value for the pre-treatment (pre-test) was 0.661. Since p-value > alpha (0.05), it can be concluded that there were no significant differences in MDA levels among the groups (K-, K+, P1, and P2) before treatment. For the post-treatment (post-test), the p-value was 0.406, indicating no significant differences among the groups after treatment.

Group analysis

In the positive control group (K+), MDA levels increased significantly compared to the negative control group (K-), with an average increase of 45% ($p < 0.05$). This demonstrates that cigarette smoke exposure causes significant oxidative stress. Conversely, MDA levels decreased significantly in the treatment groups receiving snakehead fish extract.

- The P1 group (0.5 ml/day dose) showed a 30% reduction in MDA levels compared to K+.
- The P2 group (1 ml/day dose) showed a 20% reduction compared to K+ ($p < 0.05$).

The reduction in MDA levels in the P1 group was more significant than in the P2 group, indicating that a 0.5 ml/day dose was more effective in mitigating oxidative stress. Statistical analysis revealed significant differences among all treatment groups ($p < 0.05$).

Liver histopathological examination

The results of liver histopathology support the findings of MDA levels. The liver structure appeared normal without tissue damage in the K- group (negative control). In the K+ group (positive control), liver tissue damage was observed, including necrosis, sinusoidal congestion, and widespread inflammatory cell infiltration.

In the P1 group (0.5 ml/day dose), liver tissue showed significant improvement with reduced inflammatory cell infiltration and regeneration of hepatocytes. Tissue damage observed in this group was far less severe compared to the K+ group.

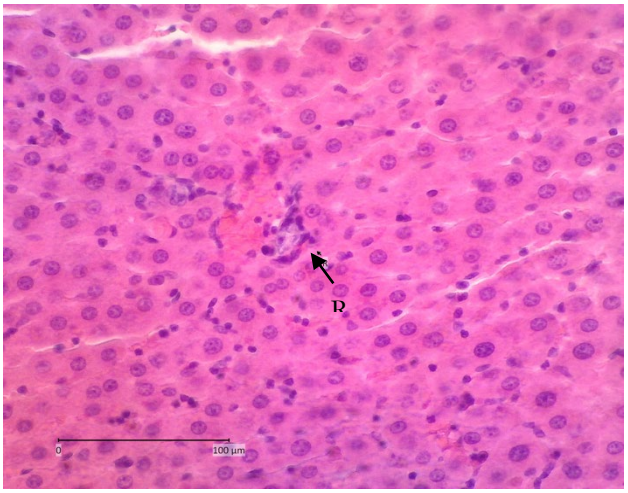
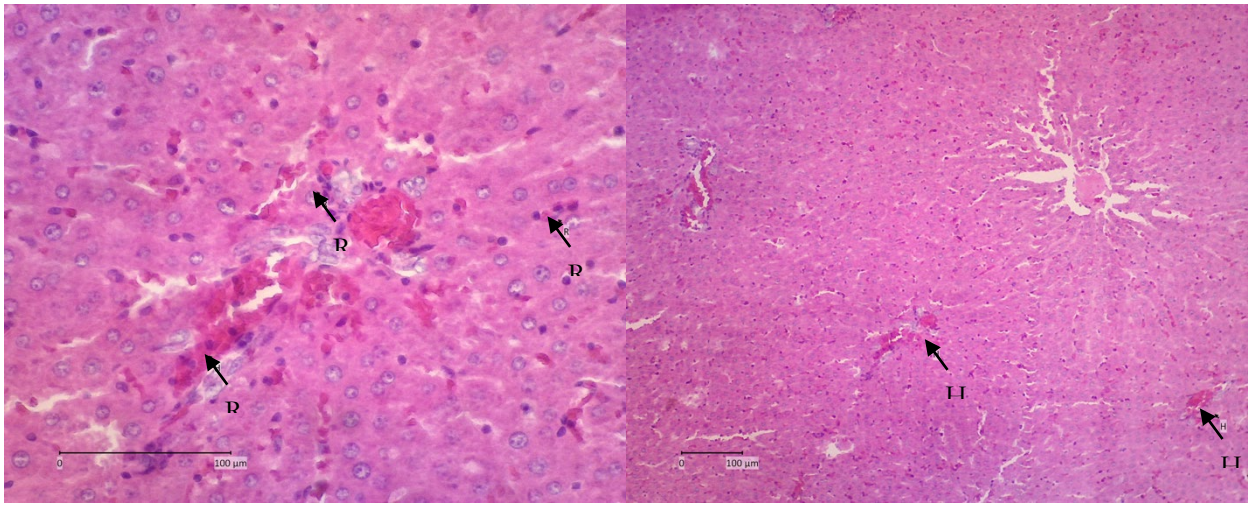


Figure 1: Liver Histopathological Structure in Rats from the P1 Group (0.5 ml + Cigarette Smoke) with 400x Magnification using Hematoxylin-Eosin (HE) Staining

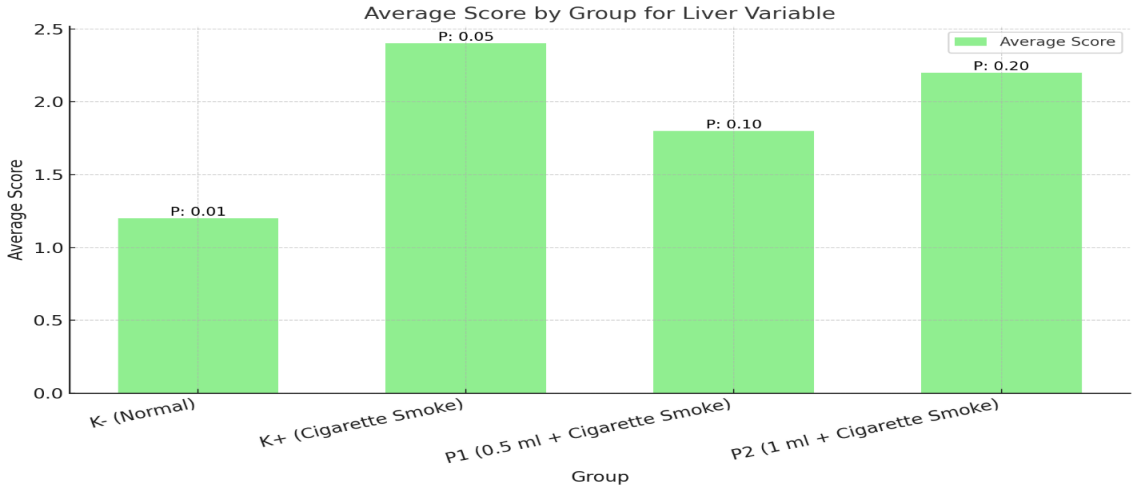
In the P2 group (1 ml/day dose), liver tissue improvement was also observed; however, mild residual inflammatory cell infiltration was present in some areas. Overall, the 0.5 ml/day dose demonstrated more optimal tissue repair than the 1 ml/day dose, supporting the effectiveness of the lower dose in reducing histological damage caused by oxidative stress.



Figures 2 and 3: Liver histopathological structure in rats from the P2 Group (1 ml + cigarette smoke) with 400x and 100x Magnification using Hematoxylin-Eosin (HE) Staining
Image description: Hemorrhage (H), Inflammation (R).

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

Variable	Group	Average Score	P-value
Liver	K- (Normal)	1.2	0.01
Liver	K+ (Cigarette Smoke)	2.4	0.05
Liver	P1 (0.5 ml + Cigarette Smoke)	1.8	0.1
Liver	P2 (1 ml + Cigarette Smoke)	2.2	0.2



Graph 2: Differences in liver histopathology scores among rat groups

Based on the table above, the observations indicate varying levels of tissue damage across the groups, with scoring based on the entire field of view for all types of damage:

- The **K- group (Normal)**, the control group without cigarette smoke exposure, exhibited the lowest average tissue damage score, reflecting relatively normal tissue conditions.
- The **K+ group (Cigarette smoke)**, exposed to cigarette smoke, showed an increased tissue damage score, indicating the adverse effects of cigarette smoke exposure.
- The treatment groups, **P1 (0.5 ml + Cigarette Smoke)** and **P2 (1 ml + Cigarette Smoke)**, also demonstrated higher tissue damage scores than the standard control group. However, the P2 group, which received a higher treatment dose, tended to have a slightly higher damage score than the P1 group.

This suggests that cigarette smoke exposure and the additional treatment can cause tissue damage, with the level of damage increasing in line with the intensity of the treatment.

DISCUSSION

Exposure to cigarette smoke increases MDA levels due to oxidative stress triggered by Reactive Oxygen Species (ROS). ROS contribute to cellular damage through lipid peroxidation mechanisms, which produce Malondialdehyde (MDA) as a byproduct. This study found that MDA levels in the K+ group (positive control) significantly increased compared to the K- group (negative control), supporting the hypothesis that cigarette smoke exposure induces significant oxidative stress. This finding aligns with the study by Pryor & Stone (1993), which demonstrated that free radicals in cigarette smoke are major contributors to oxidative damage in tissues.

The administration of snakehead fish extract in the treatment groups showed significant results in reducing MDA levels, particularly in the P1 group (0.5 ml/day dose). The effectiveness of the lower dose can be explained by the mechanisms of albumin and omega-3 fatty acids contained in snakehead fish. Albumin aids cell regeneration by enhancing the transport capacity of nutrients and oxygen, while omega-3 fatty acids act as natural antioxidants, minimizing cell damage caused by ROS. This finding is supported by Yusoff et al. (2015), which reported high antioxidant activity in snakehead fish extract.

However, in the P2 group (1 ml/day dose), the reduction in MDA levels was slightly lower than in P1. This phenomenon could be attributed to the potential pro-oxidative effects of excessively high doses of antioxidants, as Ayala et al. (2014) suggested. Therefore, determining the optimal dose is crucial to achieving maximum therapeutic effects without triggering adverse side effects.

The liver histopathology analysis also supports these biochemical findings. In the K+ group, liver tissue damage, including necrosis, sinusoidal congestion, and extensive inflammatory cell infiltration, was observed, indicating the harmful effects of cigarette smoke exposure. Conversely, liver tissue repair was evident in the P1 and P2 groups, with more significant hepatocyte regeneration observed

in the P1 group. This confirms that snakehead fish extract effectively repairs tissue damage through its anti-inflammatory and antioxidant mechanisms.

Overall, this study confirms the effectiveness of snakehead fish extract in reducing MDA levels and provides new insights into the role of albumin and omega-3 fatty acids in liver tissue protection. However, further research is needed to explore the long-term effects of administering this extract and its application in human models.

Albumin is a major plasma protein that accelerates tissue regeneration by enhancing oxygen and nutrient transport capacity to damaged cells. In addition, albumin binds free radicals, reducing oxidative stress that could exacerbate tissue damage. Meanwhile, omega-3 fatty acids exhibit potent anti-inflammatory properties, particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These components work by inhibiting the production of inflammatory mediators such as prostaglandins and leukotrienes. Omega-3 also enhances the activity of antioxidant enzymes like superoxide dismutase (SOD) and catalase, which support cellular protective mechanisms. The combination of albumin and omega-3 provides a synergistic effect in repairing liver tissue by reducing oxidative stress, minimizing inflammation, and accelerating the regeneration of damaged hepatocytes.

The 0.5 ml/day dose showed the best effectiveness in reducing MDA levels and improving liver tissue compared to the 1 ml/day dose. This may be due to the rats' ability to utilize bioactive compounds more efficiently at moderate doses compared to higher doses, which might trigger pro-oxidative mechanisms. At higher doses, such as 1 ml/day, antioxidant receptor saturation or even redox imbalance could increase ROS production. Additionally, the relatively faster metabolism of rats compared to humans may influence the absorption and distribution of active compounds from the snakehead fish extract, making moderate doses more suitable for achieving optimal therapeutic effects without causing side effects.

This aligns with previous studies suggesting that excessive antioxidants can trigger pro-oxidative effects. This mechanism occurs when high concentrations of antioxidants act as pro-oxidants, generating additional Reactive Oxygen Species (ROS). This phenomenon, known as the "antioxidant paradox," occurs when antioxidants, which are supposed to reduce oxidative stress, instead promote free radical production if used in excessive amounts. Ayala et al. (2014) noted that redox imbalance due to high antioxidant doses can disrupt cellular homeostasis, causing damage to lipids, proteins, and DNA. Therefore, determining the correct antioxidant dose is crucial to avoid these side effects and ensure optimal therapeutic benefits.

CONCLUSION

The results of this study reveal that exposure to cigarette smoke can significantly increase Malondialdehyde (MDA) levels in Wistar rats, reflecting high oxidative stress and causing liver histopathological damage such as necrosis, inflammation, and sinusoidal congestion. This study also shows that the administration of snakehead fish extract (Channa striata) can reduce MDA levels and improve liver tissue damage caused by cigarette smoke exposure.

In the treatment group receiving a dose of 0.5 ml/day of snakehead fish extract, a more significant reduction in MDA levels and more optimal improvement in liver histopathology were observed compared to the 1 ml/day dose group. This indicates that a moderate dose is more effective in mitigating oxidative stress and supporting tissue regeneration without triggering side effects associated with potential pro-oxidative effects at higher doses. Combining albumin and omega-3 fatty acids in snakehead fish extract provides antioxidant and anti-inflammatory effects synergistically supporting tissue recovery.

This study strengthens the evidence that snakehead fish extract has excellent potential as a natural therapeutic agent to address tissue damage caused by oxidative stress, particularly in the liver. However, further research is needed to evaluate its long-term application, more in-depth molecular mechanisms, and human clinical trials to ensure its effectiveness and safety.

Authors' contributions

IR and AS were involved in the conception and planning of the research, IR and MHC performed the data acquisition/collection, and IR and AS calculated the experimental data. They performed the analysis, IR 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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REFERENCES

U.S. Department of Health and Human Services. (2014). *The Health Consequences of Smoking—50 Years of Progress: A Report of the Surgeon General.* Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health. Available at: https://www.cdc.gov/tobacco/data_statistics/sgr/50th-anniversary/index.htm

WHO. (2015). *Tobacco.* World Health Organization. Available at: https://www.who.int/news-room/fact-sheets/detail/tobacco

Smith, C. J., & Hansch, C. (2000). The relative toxicity of compounds in mainstream cigarette smoke condensate. *Food and Chemical Toxicology, 38*(7), 637-646. DOI: 10.1016/S0278-6915(00)00047-5

Pryor, W. A., & Stone, K. (1993). Oxidants in cigarette smoke: Radicals, hydrogen peroxide, peroxynitrite, and peroxynitrite. *Annals of the New York Academy of Sciences, 686*(1), 12-27. DOI: 10.1111/j.1749-6632.1993.tb39102.x

Del Rio, D., Stewart, A. J., & Pellegrini, N. (2005). A review of recent studies on malondialdehyde as a toxic molecule and biological marker of oxidative stress. *Nutrition, Metabolism and Cardiovascular Diseases, 15*(4), 316-328. DOI: 10.1016/j.numecd.2005.05.003

Kumar, V., Abbas, A. K., & Aster, J. C. (2014). *Robbins and Cotran Pathologic Basis of Disease* (9th ed.). Philadelphia, PA: Elsevier. Available at: https://www.elsevier.com/books/robbins-and-cotran-pathologic-basis-of-disease/kumar/978-1-4377-0792-2

Mittal, M., Siddiqui, M. R., Tran, K., Reddy, S. P., & Malik, A. B. (2014). Reactive oxygen species in inflammation and tissue injury. *Antioxidants & Redox Signaling, 20*(7), 1126-1167. DOI: 10.1089/ars.2012.5149

Nurjanah, N., et al. (2015). Albumin content and functional properties from snakehead fish (Channa striata) fillet and protein concentrate. *Journal of Fisheries and Aquatic Science, 10*(5), 348-359. Available at: https://scialert.net/abstract/?doi=jfas.2015.348.359

Hidayat, M. N., et al. (2014). Nutritional composition and amino acid profile of Channa striata from South Sumatera, Indonesia. *Food Chemistry, 161,* 185-191. DOI: 10.1016/j.foodchem.2014.03.141

Amalia, N., et al. (2018). The wound healing effect of snakehead fish (Channa striata) extract on diabetic rats. *Asian Pacific Journal of Tropical Biomedicine, 8*(2), 81-85. Available at: https://apjtb.org/

Yusoff, F. M., et al. (2015). Antioxidant and antimicrobial properties of Channa striata extract. *Food Chemistry, 188,*436-442. DOI: 10.1016/j.foodchem.2015.04.124

Krishnaiah, D., et al. (2011). A review of the antioxidant potential of medicinal plant species. *Food and Bioproducts Processing, 89*(3), 217-233. DOI: 10.1016/j.fbp.2010.12.001

Rahman, K. (2007). Studies on free radicals, antioxidants, and co-factors. *Clinical Interventions in Aging, 2*(2), 219-236. DOI: 10.2147/ciia.2007.2.2.219

Yusoff, F. M., et al. (2015). Protective effects of Channa striata extract against oxidative stress in cultured cells. *Journal of Ethnopharmacology, 162,* 220-228. DOI: 10.1016/j.jep.2014.12.042

Nair, A. B., & Jacob, S. (2016). A simple practice guide for dose conversion between animals and humans. *Journal of Basic and Clinical Pharmacy, 7*(2), 27-31. DOI: 10.4103/0976-0105.177703

Ayala, A., Muñoz, M. F., & Argüelles, S. (2014). Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxidative Medicine and Cellular Longevity, 2014.* DOI: 10.1155/2014/360438

Gutteridge, J. M., & Halliwell, B. (2000). Free radicals and antioxidants in the year 2000: A historical look to the future. *Annals of the New York Academy of Sciences, 899*(1), 136-147. DOI: 10.1111/j.1749-6632.2000.tb06182.x

Esterbauer, H., Schaur, R. J., & Zollner, H. (1991). Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde, and related aldehydes. *Free Radical Biology and Medicine, 11*(1), 81-128. DOI: 10.1016/0891-5849(91)90192-6