Trop J Nat Prod Res, March 2025; 9(3): 1172 - 1178

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Combination of Red Okra and Stevia Ameliorates Oxidative Stress and Inflammation in Secondhand Smoke Rats

Nurina Tyagita[1*], Endang Mahati[2], Azizah H. Safitri[1]

[1]*Biochemistry Department, Faculty of Medicine, Universitas Islam Sultan Agung, Semarang, Central Java, Indonesia*
[2]*Department of Pharmacology and Therapeutics, Faculty of Medicine, Universitas Diponegoro, Semarang, Central Java, Indonesia*

ARTICLE INFO

Article history:
Received 01 February 2025
Revised 10 February 2025
Accepted 21 February 2025
Published online 01 April 2025

Copyright: © 2025 Tyagita *et al*. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

ABSTRACT

Individuals exposed to secondhand smoke (SHS) are at risk of experiencing oxidative stress and inflammation. Red okra and stevia have antioxidant and anti-inflammatory activities. This research aimed to validate the effectiveness of red okra and stevia in improving oxidative stress and inflammation in SHS rats. A posttest-only control group design was used, where 25 Wistar rats were divided into 5 groups including (1) Normal control, (2) SHS group, (3) Red okra group given red okra powder at a dose of 60 mg/200 g BW, (4) Stevia group given stevia powder at a dose of 100 mg/200 g BW, and (5) Kravia group administered with a combination of 60 mg/200 g BW red okra powder and 100 mg/200 g BW stevia powder. All rats, except normal control, were exposed to smoke from 4 cigarettes/day, 5 times/week, for 1 month. Furthermore, oxidative stress parameters measured were glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase, vitamin C, and malondialdehyde (MDA). Inflammation markers were interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), nuclear factor (NF-κβ), NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3), α-klotho, and insulin-like growth factor-1 (IGF-1). Collected data were analyzed using the One-way Analysis of Variance (ANOVA), followed by a post hoc test ($p<0.05$). Results showed that the combination of red okra and stevia significantly enhanced the levels of GPx, SOD, catalase, vitamin C, MDA, IL-6, TNF-α, NF-κβ, NLRP3, α-klotho, and IGF-1 ($p<0.05$) compared to SHS, red okra, and stevia groups. This combination was effective in improving oxidative stress and inflammation in SHS.

Keywords: Secondhand smoke, Red okra, Stevia, Oxidative stress, Inflammation.

Introduction

The number of smokers in Indonesia is found to be increasing persistently. In 2021, the Global Adult Tobacco Survey (GATS) showed that 70.2 million Indonesians (34.5%) used classic cigarette, smokeless products, or heated tobacco. GATS also obtained data on secondhand smoke (SHS) consumers which were significantly more than active smokers/individuals exposed to firsthand smoke (FHS). SHS consists of 20.3 million adults working indoors who are exposed to cigarette smoke (44.8%), 121.6 million exposed to cigarette smoke at home (59.35%), and 56.1 million adults exposed to cigarette smoke while visiting restaurants (74.2%).[1] Free radicals such as reactive oxygen and nitrogen species (ROS and RNS) contained in tobacco smoke can damage macromolecules including lipids, proteins, and nucleic acid, leading to oxidative stress, inflammation, and even cancer.[2] Both FHS and SHS are at risk of experiencing oxidative stress that initiates inflammation through the Toll-like Receptor/TLR signalling pathway and inflammasomes. Inflammation plays a role in cell increasing the production of free radicals.[3]

*Corresponding author. Email: nurinatyagita@unissula.ac.id
Tel: +62-89622188474

Citation: Tyagita N, Mahati E, Safitri AH. Combination of Red Okra and Stevia Ameliorates Oxidative Stress and Inflammation in Secondhand Smoke Rats. Trop J Nat Prod Res. 2025; 9(3): 1172 – 1178 https://doi.org/10.26538/tjnpr/v9i3.37

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Moreover, oxidative stress and insulin-like growth factor/IGF-1 appear to influence each other in inflammatory and degenerative processes of organs.[4] Smoking significantly causes IGF-1 deficiency.[5] α-klotho, an anti-aging protein, is important in oxidative stress because of the ability to induce the expression of antioxidant proteins.[6] The levels of α-klotho are found to be inversely related to smoking.[7] Okra is widely produced in Indonesia with an export volume exceeding the import volume,[8] but not known and used by many of the citizens. Okra from the Malvaceae family has been empirically used for treatment in several parts of the world. This plant is rich in polysaccharides, fiber, and antioxidants, as well as phenolic compounds including quercetin, quercetin 3-O-glucoside (isoquercitrin), rutin, quercetin-3-O-gentibioside, catechin, caffeic acid, and protocatechoic acid.[9] Myricetin and kaempferol found in okra are flavonoids that act as antioxidants to eradicate free radicals and reduce cell oxidative stress.[10] The effect of green okra (*Abelmoschus esculentus* (L.) Moench) on oxidative stress in metabolic disorders has been widely investigated. Green okra infusion water was reported to improve levels of the antioxidant enzyme superoxide dismutase (SOD) in rats injected with streptozotocin.[11] Long-term administration of green okra flavonoids can even reduce oxidative stress and inflammation in Alzheimer's model rats.[12] Meanwhile, anthocyanin provide a red color to red okra (*Abelmoschus esculentus* (L.) Hongjiao) which contains higher antioxidant and quercetin content than green okra.[13] Research on purple/red okra is limited, yet the extract of this plant is found to be better at improving blood glucose levels and malondialdehyde (MDA) oxidants in diabetic rats.[14] Red okra administered to diabetic rats showed improvements in fasting blood sugar levels, insulin, and glucose transporter/GLUT-4,[15] as well as increased antioxidant enzymes SOD and catalase in rats with liver injury. However, the levels of oxidants, MDA, F-isoprostane, and nitric oxide were lower.[13]

Roasted green okra ground and served in the form of coffee has an unpleasant taste,[16] but there has been no research on the taste of red okra

© 2025 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

powder. Stevia leaves (*Stevia rebaudiana*) are 100-300 times sweeter than sucrose but contain zero or only a few calories. The sweet taste of stevia leaves originates from the content of stevioside and rebaudioside. Stevia has antioxidant properties, hence the administration improves oxidative status in animals.[17] Mixing stevia leaves with okra powder can provide a sweet taste, improve adherence, and offer antioxidant benefits in counteracting oxidative stress due to cigarette smoke exposure. FHS should quit smoking to prevent oxidative stress and the development of degenerative diseases. On the other hand, SHS forced to inhale cigarette smoke are even at risk of experiencing oxidative stress, inflammation, and degenerative diseases. Consequently, amelioration efforts need to be carried out, such as by administering a combination of red okra powder with stevia leaves. The literature review conducted has not found any other investigations regarding the combination of red okra and stevia leaves in treating oxidative stress and inflammation against SHS. Therefore, this research aims to validate the effectiveness of red okra and stevia combination in ameliorating oxidative stress as well as inflammation in secondhand smoke-exposed rats.

Materials and Methods

Animals

A posttest-only control group design was used on 25 male Wistar rats. This experimental research was conducted in August – December 2024 at 2 places in Indonesia, namely the Biomedical Laboratory, Medical Faculty of Universitas Islam Sultan Agung, Semarang, and the Center of Food and Nutrition Studies, Gadjah Mada University, Yogyakarta. The inclusion criteria used were male white Wistar rats weighing ±200 g and aged ±3 months. The rats were expected not to have any prior treatment and macroanatomical abnormalities.

All rats passed through a 7-day acclimation period to minimize stress and standardize the environment. During this time, the subjects were housed individually in climate-controlled rooms maintained at 18-25°C and 50-70% humidity, with a 12-hour light-dark cycle. Food and water were available ad libitum, while the acclimation period proceeded without incident of animal dropouts or health issues, confirming the suitability of the rats for this study. A total of 25 Wistar rats were randomly divided into 5 groups, each comprising 5 members. These included (1) Normal control, (2) SHS group, exposed to cigarette smoke but not given treatment, (3) Red okra group, exposed to cigarette smoke and given red okra powder at a dose of 60 mg/200 g BW, (4) Stevia group, exposed to cigarette smoke and given stevia powder at a dose of 100 mg/200 g BW, and (5) Kravia group, exposed to cigarette smoke and given a combination of 60 mg/200 g BW red okra powder and 100 mg/200 g BW stevia powder. Each rat was placed in a cage individually during smoke exposure.

Cigarette and Smoking Chamber

The Kretek cigarette used is produced by PT HM. Sampoerna Surabaya, Indonesia. This cigarette comprises a tar content of 39 mg and nicotine of 2.3 mg. Meanwhile, the smoking chambers were constructed from acrylic size 90 x 80 x 40 cm and able to contain 7 individual Wistar rat cages size 25 x 18 x 20 cm. An exhaust fan was placed above the chamber (Figure 1). The burning cigarettes were held and connected to a dynamic suction pump machine by a flexible hose. By creating negative pressure, the pump pulled air through the lit cigarette, generating smoke directed into the acrylic box through the hose.[18] Adjustments to the smoking chamber dimensions may be necessary when the animals used differ significantly in size from Wistar rats.

Figure 1. Smoking chamber with individual cages

Preparation of red okra powder

Mature red okra was collected locally in June 2024 and authenticated by Integrated Biomedical Laboratory Universitas Islam Sultan Agung, Semarang (2FW6+9P3), with specimen number FKSA-PE1-VI24. Red okra was sorted, washed clean, and chopped into smaller sizes. Subsequently, the slices were dried using an oven at a temperature of 40ºC. The dried slices were ground into a fine powder, which was stored in an airtight container to maintain quality, and the dosage of red okra used was 60 mg/200 g rats.[19]

Preparation of stevia leaves powder

Stevia leaves were collected locally in June 2024, and authenticated by Integrated Biomedical Laboratory Universitas Islam Sultan Agung Semarang (2FW6+9P3), with specimen number FKSA-PE2-VI24. Stevia leaves were sorted and washed until clean, then dried using an oven at 40ºC. The dried leaves were ground into a fine powder and stored in an airtight container to maintain quality, and the dosage of stevia leaves used was 100 mg/200 g rats.[20]

Kravia preparation

Red okra powder at a dose of 60 mg/200 g BW/day was mixed with 100 mg/200 g BW/day of stevia leaves powder. Both preparations were dissolved in warm distilled water at a temperature of 50ºC and then administered using an oral tube.

Ethics statement

The research consent was obtained from the Research Bioethics Commission, Faculty of Medicine, Universitas Islam Sultan Agung, Semarang, Central Java, Indonesia (No.300/VIII/2024/Komisi Bioetik).

Experimental design

Adaptation was carried out for 7 days to standardize the lifestyle of rats and prevent stress. During the adaptation period, all treatment groups were provided with standard feed and water ad libitum.

All rats, except normal control, were conditioned to be exposed to smoke from 4 cigarettes/day with a duration of 5 times a week for 1 month. According to the research flow presented in Figure 2, the exposure was conducted during the morning period in a smoking chamber, and treatment was given to Red Okra, Stevia, and Kravia groups in the afternoon.

© 2025 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

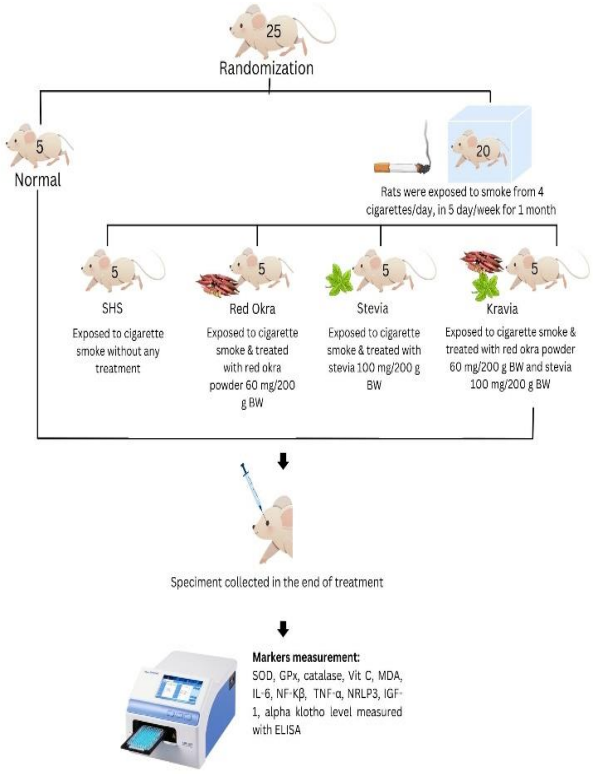

Figure 2. Research flow

Parameters measurement

Blood samples collected from the ophthalmic vein of the rats on the final day of treatment and analyzed by blind experimenters. Oxidative stress markers measured included levels of the antioxidant enzymes glutathione peroxidase-1/GPx, superoxide dismutase/SOD, catalase, plasma vitamin C, and MDA. Meanwhile, inflammatory markers examined were interleukin-6 (IL-6), nuclear factor/NF-kβ, tumor necrosis factor-alpha (TNF-*α*), NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3), which were measured along with levels of the hormone IGF-1 and *α*-klotho. Parameter measurements were performed with the ELISA method using commercially available ELISA kits for each specific biomarker. The procedure for measuring each marker level was conducted based on the instructions of the manufacturer. This included GPx (Cat. No ER0274 FineTest, Wuhan, China), SOD (Cat. No. K335-100 BioVision, Kampenhout, Belgium), Catalase (Cat. No ER0264 FineTest, Wuhan, China), plasma Vitamin C (Cat. No MBS3808794 MyBioSource, Southern California, San Diego (USA)), MDA (Cat. No. ER1878, FineTest, Wuhan, China), IL-6 (Cat. No. ER0042, FineTest, Wuhan, China), TNF-*α* (Cat. No. ER1393, FineTest, Wuhan, China), NF-kβ (Cat. No. ER1186, FineTest, Wuhan, China), NLRP3 (Cat. No. RE3548R, Reed Biotech LTD, Wuhan, China), IGF-1 (Cat No. ER0030, FineTest, Wuhan, China), and *α*-Klotho (Cat No. ER0658, FineTest, Wuhan, China). The results were read at a wavelength of 450 nm using an ELISA reader and presented as GPx (pg/ml), SOD (%), catalase (mIU/ml), MDA (nmol/mL), IL-6 (pg/mL), TNF-*α* (pg/mL), NF-κβ (ng/ml), NLRP3 (ng/L), IGF-1 (pg/mL), and *α*-Klotho (pg/mL).

Statistical analysis

The obtained average levels of GPx, SOD, catalase, vitamin C, MDA, IL-6, NF-kβ, TNF-*α*, NRLP3, IGF-1, and *α*-klotho were inputted and processed using the GraphPad Prism 9 Software, Boston, MA, USA. One-way Analysis of Variance (ANOVA) was conducted and the data were declared significant at $p<0.05$, followed by an LSD post hoc test.

Results and Discussion

Secondhand smokers tend to experience oxidative stress.[21] SHS rats in this research were proven to have the lowest levels of antioxidant enzymes GPx, catalase, SOD, and vitamin C, as well as the highest MDA (Table 1 and Figure 3). Previous case control study found longterm smoking caused an increase in oxidants level, MDA and 8-OHdG, and a decrease in antioxidants enzymes, namely catalase, SOD, and GPx.[22]

Table 1. Average GPx, SOD, catalase, vitamin C, MDA, IL-6, NF-kβ, TNF-α, NRLP3, IGF-1, and α-klotho levels after treatment

Variable	Group					p-value
	Normal	SHS	Red Okra	Stevia	Kravia	
Oxidative stress markers						
SOD (%)	84.64 ± 3.92	24.64 ± 3.69	57.68 ± 3.46	68.12 ± 2.29	74.49 ± 3.49	0.001[a,*]
GPx (pg/ml)	57.6 ± 0.75	24.05 ± 0.96	44.73 ± 0.96	46.85 ± 0.89	52.05 ± 0.84	0.001[a,*]
Catalase (mIU/ml)	12.17 ± 0.13	3.07 ± 0.51	8.53 ± 0.15	9.97 ± 0.37	12.01 ± 0.23	0.001[a,*]
Vitamin C (mg/dl)	1.7 ± 0.08	1.21 ± 0.09	1.37 ± 0.04	1.38 ± 0.08	1.53 ± 0.06	0.001[a,*]
MDA (nmol/ml)	1.5 ± 0.29	11.86 ± 0.27	4.08 ± 0.29	3.07 ± 0.13	2.24 ± 0.23	0.001[a,*]
Inflammation markers						
IL-6 (pg/ml)	42.94 ± 0.44	70.31 ± 2.34	50.71 ± 0.38	47.86 ± 0.55	45.91 ± 0.86	0.001[a,*]
TNF-α (pg/ml)	7.02 ± 0.19	22.36 ± 1.23	9.14 ± 0.2	7.97 ± 0.22	7.04 ± 0.43	0.001[a,*]
NF-κβ (ng/ml)	39.19 ± 1.24	257.2 ± 9.38	54.53 ± 2.56	47.24 ± 2.05	41.69 ± 3.89	0.001[a,*]
NRLP3 (ng/ml)	0.31 ± 0.01	7.79 ± 0.23	2.99 ± 0.08	1.97 ± 0.05	1.08 ± 0.03	0.001[a,*]
IGF-1 (pg/ml)	24.43 ± 0.48	44.39 ± 0.76	40.3 ± 1.56	33.91 ± 1.17	26.44 ± 0.57	0.001[a,*]
α-klotho (pg/ml)	515.0 ± 19.90	175.0 ± 13.18	280.0 ± 20.07	358.3 ± 18.63	435.0 ± 16.03	0.001[a,*]

SHS : Secondhand Smoke-exposure group

[a]Analyzed using a One-way ANOVA test
[*]Statistically significant at $p<0.05$

The combination of red okra and stevia/Kravia showed better results than the Red Okra or Stevia itself in antioxidant enzyme SOD, GPx, catalase, vitamin C, and MDA levels, even approaching the SOD, GPx, catalase, and vitamin C levels in normal group (Table 1 and Figure 3).

© 2025 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

The administration of red okra in previous research increased catalase and SOD activities but decreased MDA.[23]

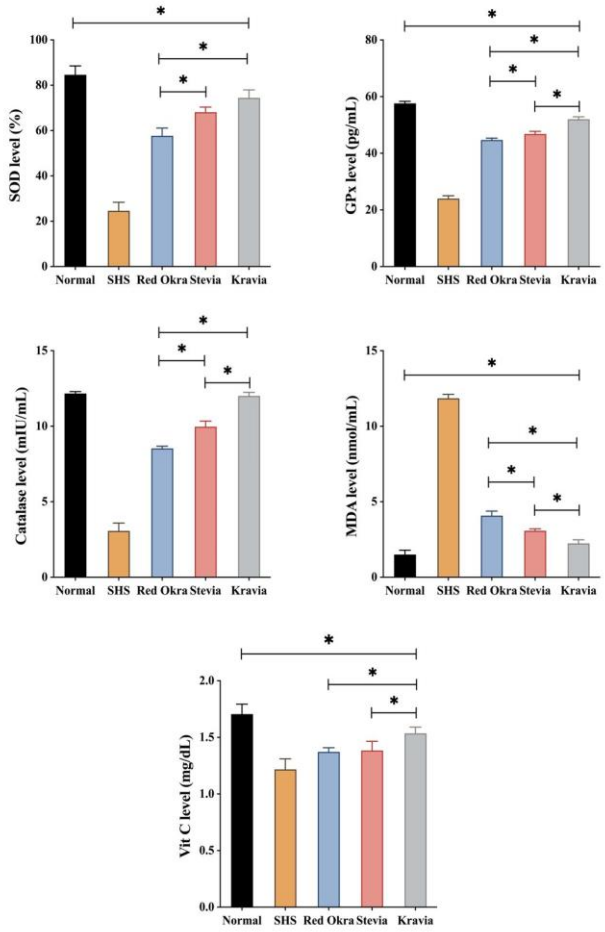

Figure 3. Oxidative stress markers: SOD, GPx, Catalase, Vitamin C, and MDA levels after treatment (*p<0.05)

However, green okra infusion administered to type 2 diabetes mellitus model rats successfully improved SOD.[11] Red okra contains anthocyanins absent in green okra or stevia leaves, which act as reducing agents and donate electrons to free radicals. The darker the color of the food containing anthocyanins correlates to the stronger antioxidant capacity of the material. Red okra also contains carotenoids, which interact with nuclear factor erythroid 2–related factor 2 (NFE2L2/nrf-2), a transcription factor for endogenous antioxidant enzymes including GPx, SOD, and catalase.[24]

Another research reported upregulation of NFE2L2 mRNA expression after administering 200 mg/kg of stevia residue extract, which was found to affect antioxidants through activation of the Akt/Nrf2/HO-1 pathway.[25] Research on cells provided with stevioside, a content in stevia, and exposed to diquat, showed reduced levels of SOD and MDA as well as upregulation of SOD, catalase, and GPx activities compared to those given only diquat.[26] Stevia is also rich in flavonol compounds, including quercetin and kaempferol.[17] The combined effects of flavonols from red okra and stevia are thought to exert a synergistic action, particularly in mitigating oxidative stress in rats exposed to SHS. Secondhand smokes also induce inflammation.[27] SHS group also has the highest level of inflammation markers (IL-6, TNF-α, NF-κβ, and NRLP3) of all (Table 1 and Figure 4). Al-tameemi et al. proved that IL-6 and TNF-α levels in smokers were higher than in non-smokers.[28]

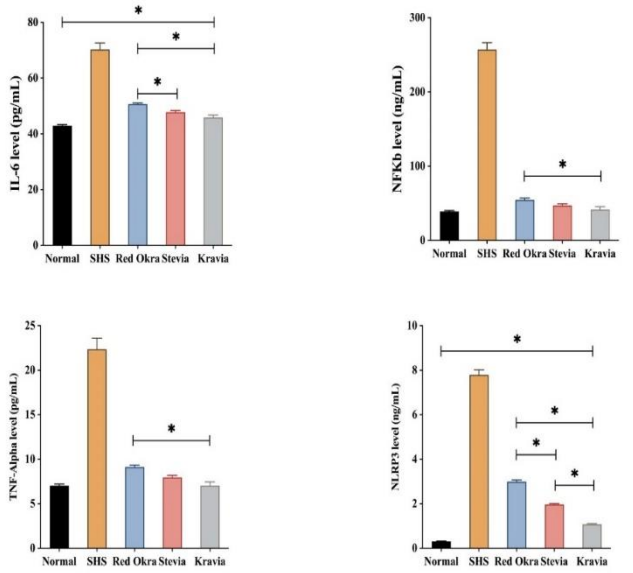

Figure 4. Inflammation markers: IL-6, NF-κβ, TNF-α, and NRLP3 levels after treatment (*p<0.05)

Another research found that chronic SHS exposure increased inflammatory cytokines such as IL-6 and TNF-α in the lungs. SHS is a heterogeneous mixture of mainstream and sidestream tobacco smoke containing a large number of toxic substances. Exposure to cigarette smoke amplifies oxidative stress, which triggers vascular inflammation characterized by increased levels of inflammatory cytokines such as IL-17A, IL-6, IL-1β, and TNF-α.[27] Reactive oxygen species (ROS) produced by cigarette smoke trigger cellular oxidative stress which can damage lipid layers, proteins, and DNA, leading to an inflammatory signalling cascade.[29]

The combination of red okra and stevia tends to show improvements in inflammatory markers better than administering only red okra or stevia. IL-6, NF-κβ, TNF-α and NLRP3 in Kravia group were better than red okra, and stevia groups (Table 1 and Figure 4). Previous research found that ethanol extract of red okra could be given to rats induced by N-methyl- N-nitrosourea (MNU).[30] Flavonoids act as antioxidants and anti-inflammatories, corresponding with a literature review that found a tendency for inflammatory mediators, including C-reactive protein (CRP), IL-1β, IL-6, and TNF-α to decrease in concentration after okra administration.[31] Flavonoids contained in okra are capable of minimizing neuroinflammation, as evidenced by a decrease in levels of NF-κβ, TNF-α, and IL-1β.[12] Myricetin contained in okra,[10] suppresses the key factor in the production of inflammatory mediators.[32] Moreover, red okra has higher quercetin levels compared to green okra.[14] Quercetin inhibits the activity of NLRP3, an inflammasome that activates several inflammatory mediators. The production of inflammatory molecules, cyclooxygenase/COX-2, NF-κβ, activator protein 1 (AP-1), mitogen-activated protein kinase (MAPK), reactive nitric oxide synthase (NOS), and C-reactive protein (CRP) is reduced by quercetin. This compound upregulates Toll-like Receptor/TLR-4, initiating the suppression of inflammatory cytokines such as IL-6, and TNF-α.[33]

Previous research found that stevia administration could reduce IL-1β and TNF-α levels.[34] Stevia leaves contain polyphenol compounds, a by-product of the natural sweetener known as steviol glucoside. Polyphenols from stevia have anti-inflammatory activity, which is not comparably strong as polyphenols from epigallocatechin.[35] These leaves contain more than 30 different steviol glycosides, specifically including stevioside and rebaudioside.[36] Stevia inhibits NF-κβ/NLRP3 activation through AMPK/SIRT1 pathway and modulates JAK2-STAT3 and Nrf2 signalling pathways.[37] Meanwhile, the anti-inflammatory activity of stevioside is obtained from the mechanism of AMPK pathway activation, which inhibits inflammation mediated by

© 2025 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

NF-κβ/IRF5.[38] Stevioside prevents inflammation through the activation of Nrf2 transcription factor response.[39] Rebaudioside A maintains Nrf2 expression and downregulates pro-inflammatory pathway genes NF-κβ, TGF-β1, Smad7, and MMP-13 proteins.[40] Similarly, stevioside can downregulate the secretion and expression of pro-inflammatory genes such as IL-6, IL8, and TNF-α as well as reduce the phosphorylation levels of NF-κβ, an inhibitor of nuclear factor kappa B (Iκβ), and extracellular signal-regulated kinase (ERK1/2) complex.[26] The combination of flavonoids and polyphenols in red okra and stevia enhances the anti-inflammatory activity in SHS.

α-klotho levels in SHS rats were the lowest among all groups (Table 1 and Figure 5).

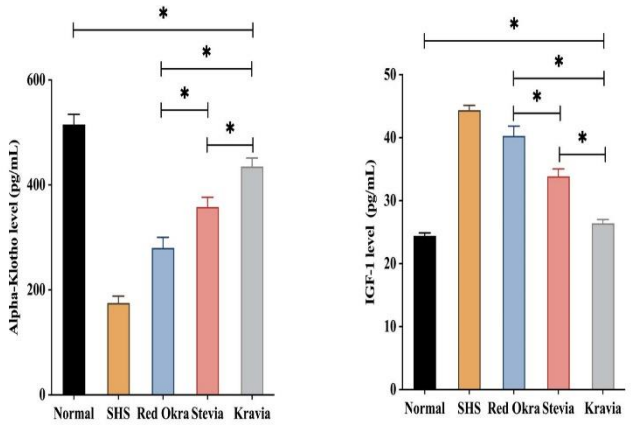

Figure 5. α-klotho and IGF-1 concentration after treatment.

There was an age-related decline in the levels of α-klotho, an anti-aging protein. Cellular senescence encompasses a series of deleterious processes, including chronic inflammation, fibrosis, metabolic dysregulation, DNA damage, mitochondrial dysfunction, protein imbalance, autophagic failure, ROS production, NAD+ depletion, and stem/progenitor cell dysfunction. ROS generated from burned tobacco can trigger the activation of inflammatory genes. The downstream effects of inflammation from the release of cytokines such as TNF, IFN-γ, and IL-6, lead to decreased α-klotho gene expression, further worsened by oxidative stress. The α-klotho levels in this study are consistent with the cross-sectional analysis in the United States population that proves smoking habits play a role in shortening life expectancy. Individuals with a smoking habit are found to have lower α-klotho levels than non-smokers.[7]

Research on α-klotho is still lacking, specifically those related to cigarette use, or natural ingredients containing flavonoids. However, an investigation found that higher dietary oxidative balance score (OBS) and lifestyle enhanced α-klotho levels, showing a significant association between OBS and α-klotho.[41] Kravia group possessed a better level of α-klotho compared to Red Okra, Stevia, and SHS groups (Table 1 and Figure 5). The combination of red okra and stevia leaves contains active substances with high antioxidant activity, including polyphenols and flavonoids, which can scavenge free radicals and reduce oxidative stress in cells.[13] Further research is needed to validate the synergistic effectiveness of the active substances in red okra and stevia combination, such as polyphenols and flavonoids.

The highest IGF-1 levels were observed in SHS group (Table 1 and Figure 5), but previous research detected lower levels of IGF-1, adiponectin, and leptin in smokers compared to non-smokers.[5] Another research found that carcinogens contained in tobacco caused overexpression of IGF which was important for lung cancer carcinogenesis in the early stages due to being capable of transforming exposed cells. Cigarette smoke activates IGF-1R, leading to an increase in IGF-1 activity due to activation of the biochemical signalling pathway and induction of DNA mutations for IGF-1 bound to the receptor which is crucial in the development of lung cancer. The IGF-1/IGF-1R receptor signalling pathway affects the growth and survival of cancer cells. High IGF-1R expression reflects a poor prognosis and resistance to treatment,[42] as well as initiates metastasis and the development of lung tumor microenvironments.[43] IGF-1R is even considered a potential biomarker in the early prediction of drug response and disease course in non-small cell lung cancer patients.[44] The activation of this receptor promotes cell survival and proliferation while providing a mechanism to inhibit apoptosis.[42]

The IGF-1 level in Kravia group also showed better results compared to Red Okra, Stevia, and SHS groups (Table 1 and Figure 5). The group that received red okra and stevia in a single preparation or combined form experienced suppressed IGF-1 levels than SHS group. Furthermore, flavonoids such as kaempferol, myricetin, and quercetin contained in okra act as adjuvant cancer therapy by modulating apoptosis, cell cycle, DNA repair, and senescence pathways. The results showed that downregulation of anti-apoptotic proteins, including Bcl-2, Mcl-1, and Bcl-xL, along with upregulation of pro-apoptotic proteins such as BAK, BAX, and BID occurred in the administration of flavonoids.[45] Stevioside in stevia potentially induces intrinsic apoptosis in bladder cancer cells excluding normal cells,[46] therefore, it is thought to downregulate IGF-1 which instigates anti-apoptotic cancer development.

The limitations of this study include the lack of histological examination of the lungs, heart, and liver, which are primary organs affected by cigarette smoke exposure. Additionally, the long-term effects of the combined administration of red okra and stevia on oxidative stress as well as inflammation induced by cigarette smoke exposure remain unexplored. Stevia and red okra contain high antioxidants as well as anti-inflammatory properties, but more research should be conducted to determine the optimization of the proper combination dose. There is a need to verify the potential of red okra preparations in combination with stevia as an antidote to oxidative stress, inflammation, and cancer, specifically in SHS. The simple preparation process makes this concoction a potentially valuable resource for individuals continually exposed to cigarette smoke, particularly in promoting health and well-being.

Conclusion

In conclusion, the results showed that the combination of red okra and stevia powder was effective in improving oxidative stress and inflammation caused by secondhand smoke exposure. Further research regarding the interaction of active substances present in the combined two natural ingredients, as well as dose optimization, should be performed to verify the potential as an antidote to oxidative stress and inflammation specifically in passive smokers. The effect of the combined administration of red okra and stevia on the histological changes in organs affected by secondhand smoke exposure would need more investigation. The potential health benefits of combining red okra and stevia for human health, especially in the field of secondhand smoke exposure, is an important area for future investigation.

Conflict of Interest

The authors declare no conflicts of interest.

Author's Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgments

Funding assistance was received from the Indonesian Ministry of Education, Culture, Research, and Technology through a grant with contract number 33/B.1/SA-LPPM/VI/2024 on 13 June 2024.

References

1. GATS. Gats|Global Adult Tobacco Survey Fact Sheet Indonesia 2021 Gats Objectives. Fact Sheet Indonesia. 2021.
2. Caliri AW, Tommasi S, Besaratinia A. Relationships Among

© 2025 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

Smoking, Oxidative Stress, Inflammation, Macromolecular Damage, and Cancer. Mutat Res. 2021: 1;787:108365.
3. Ramos-González EJ, Bitzer-Quintero OK, Ortiz G, Hernández-Cruz JJ, Ramírez-Jirano LJ. Relationship Between Inflammation and Oxidative Stress and its Effect on Multiple Sclerosis. Neurologia (Engl Ed). 2024; 39(3):292–301.
4. Herrera ML, Champarini LG, Oliveros AL, Bellini MJ, Hereñú CB. Potentialities of IGF-1 for Regulating Oxidative Stress in Neuroinflammation and Neurodegeneration: Theoretical Review. Explor Neuroprot Ther. 2024; 4:442–458.
5. Erlandsson MC, Medina RD, Silfverswärd ST, Bokarewa MI. Smoking Functions as a Negative Regulator of IGF1 and Impairs Adipokine Network in Patients with Rheumatoid Arthritis. Mediators Inflamm. 2016;(3082820). https://onlinelibrary.wiley.com/doi/10.1155/2016/3082820
6. Donate-Correa J, Martín-Carro B, Cannata-Andía JB, Mora-Fernández C, Navarro-González JF. Klotho, Oxidative Stress, and Mitochondrial Damage in Kidney Disease. Antioxidants (Basel). 2023; 12(2):239.
7. Du R, Tang X, Jiang M, Qian S, Yang L, Tong X, Huang W. Association between cigarette smoking and serum alpha klotho levels among US adults over 40-years-old: a cross-sectional study. Sci Rep. 2023; 9:13(1)19519.
8. Tridge. Fresh Okra. Overview of Fresh Okra Market in Indonesia. 2025.
9. Nikpayam O, Safaei E, Bahreini N, Saghafi-Asl M. The Effects of Okra (*Abelmoschus esculentus* L.) Products on Glycemic Control and Lipid Profile: A Comprehensive Systematic Review. J Funct Foods. 2021; 87:104795.
10. Prabhune A, Sharma M, Ojha B, Prabhune CA. *Abelmoschus esculentus* (Okra) Potential Natural Compound for Prevention and Management of Diabetes and Diabetic Induced Hyperglycemia: Review. Int J Herb Med. 2017; 5(52):65–68.
11. Tyagita N, Utami KP, Zulkarnain FH, Rossandini SM, Pertiwi NP, Rifki MA, Safitri HA. Okra Infusion Water Improving Stress Oxidative and Inflammatory Markers on Hyperglycemic Rats. Bangladesh J Med Sci. 2019; 18(4):748–752.
12. Yan T, Liu B, Wang N, Liao Z, Wu B, He B, Jia Y. The flavonoids of Okra Insulates Against Oxidative Stress, Neuroinflammation and Restores BDNF Levels in Aβ1-42 Induced Mouse Model of Alzheimer's Disease. Exp Gerontol. 2021; 147:111263.
13. Wahyuningsih SPA, Mwendolwa AA, Winarni D, Anggreini RW, Mamuaya BKK. Protective Effect of Red Okra (*Abelmoschus esculentus* (L.) Moench) Pods against Sodium Nitrite-Induced Liver Injury in Mice. Vet Med Int. 2021; 2021:6647800.
14. Damayanthi E, Rimbawan R, Handharyani E. Potential of okra (*Abelmoschus esculentus* L.) Extract to Reduce Blood Glucose and Malondialdehyde (MDA) Liver in Streptozotocin-Induced Diabetic Rats. J Gizi dan Pangan. 2018; 13(1):47–54.
15. Tyagita N, Mahati E, Safitri AH. Superiority of Purple Okra (*Abelmoschus esculentus*) to Green Okra in Insulin Resistance and Pancreatic β Cell Improvement in Diabetic Rats. Folia Med (Plovdiv). 2021; 63(1):51–58.
16. Akinoso R, Adegoroye EO, Sanusi MS. Effects of Roasting on Physicochemical Properties and Fatty Acids Composition of Okra Seed Oil. Meas Food. 2023; 9:100076.
17. Papaefthimiou M, Kontou PI, Bagos PG, Braliou GG. Antioxidant Activity of Leaf Extracts from *Stevia rebaudiana* Bertoni Exerts Attenuating Effect on Diseased Experimental Rats: A Systematic Review and Meta-Analysis. Nutrients. 2023; 15:15.
18. Kozma R de las H, Alves EM, Barbosa-de-Oliveira VA, Lopes FDTQ dos S, Guardia RC, Buzo HV, de Faria CA, Yamashita C, Junior MC, Frei F, Ribeiro-Paes MJdO, Ribeiro-Paes JT. A new experimental model of cigarette smoke-induced emphysema in Wistar rats. J Bras Pneumol. 2014; 40(1):46–54.
19. Abbas AY, Muhammad I, Abdulrahman MB, Bilbis LS. Antioxidant effect of ex-maradi okra fruit variety (*Abelmuscus esculentus*) on Alloxan-Induced Diabetic Rats. Trop J Nat Prod Res. 2020; 4(3):105–12.
20. Abo Elnaga NIE, Massoud MI, Yousef MI, Mohamed HHA. Effect of Stevia Sweetener Consumption as Non-Caloric Sweetening on Body Weight Gain and Biochemical's Parameters in Overweight Female Rats. Annals Agric Sci. 2016; 61(1):155–163.
21. Kuang H, Feng J, Li Z, Tan J, Zhu W, Lin S, Pang Q, Ye Y, Fan R. Volatile Organic Compounds from Second-Hand Smoke May Increase Susceptibility of Children Through Oxidative Stress Damage. Environ Res. 2022; 207(112227).
22. Joshi B, Singh S, Sharma P, Mohapatra T, Kumar P. Effect of Cigarette Smoking on Selected Antioxidant Enzymes and Oxidative Stress Biomarkers. J Clin Diagn Res. 2020; 14(10):19–23.
23. Wahyuningsih SPA, Savira NII, Anggraini DW, Winarni D, Suhargo L, Kusuma BWA, Nindyasari F, Setianingsih N, Mwendolwa AA. Antioxidant and Nephroprotective Effects of Okra Pods Extract (*Abelmoschus esculentus* L.) against Lead Acetate-Induced Toxicity in Mice. Scientifica (Cairo). 2020;2020.
24. Speer H, D'Cunha NM, Alexopoulos NI, McKune AJ, Naumovski N. Anthocyanins and Human Health—A Focus on Oxidative Stress, Inflammation and Disease. Antioxidants. 2020; 9(5):1–13.
25. Zhao L, Yang H, Xu M, Wang X, Wang C, Lian Y, Mehmood A, Dai H. Stevia Residue Extract Ameliorates Oxidative Stress in d-Galactose-Induced Aging Mice via Akt/Nrf2/HO-1 Pathway. J Funct Foods. 2019; 52:587-595
26. Xu Q, Liu M, Chao X, Zhang C, Yang H, Chen J, Zhou B. Stevioside Improves Antioxidant Capacity and Intestinal Barrier Function while Attenuating Inflammation and Apoptosis by Regulating the NF- κ B / MAPK Pathways in Diquat-Induced Oxidative Stress of IPEC-J2 Cells. Antioxidants (Basel). 2023; 12(5):1070.
27. Bhat TA, Kalathil SG, Bogner PN, Miller A, Lehmann P V, Thatcher TH, Phipps RP, Sime PJ, Thanavala Y. Secondhand Smoke Induces Inflammation and Impairs Immunity to Respiratory Infections. J Immunol. 2018; 200(8):2927–2940.
28. Al-tameemi SA, Hameed NJ, Gomes KB, Abid HA. Cigarette Smoking Increases Plasma Levels of IL-6 and TNF-α. Baghdad J Biochem Appl Biol Sci. 2022;3(1).
29. Addissouky TA, El Sayed IET, Ali MMA, Wang Y, El Baz A, Elarabany N, Khalil AA. Oxidative Stress and Inflammation: elucidating Mechanisms of Smoking-Attributable Pathology for Therapeutic Targeting. Bull Natl Res Cent. 2024; 48.
30. Pramudya M, Dewi FRP, Wong RW, Anggraini DW, Winarni D, Wahyuningsih SPA. Anti-cancer Activity of an Ethanolic Extract of Red Okra Pods (*Abelmoschus esculentus* L. Moench) in Rats Induced by N-methyl-N-nitrosourea. Vet World. 2022; 15(5):1177–1184.
31. Mahdavi AM, Javadivala Z, Ahmadian E. Effects of Okra (*Abelmoschus esculentus* L) on Inflammatory Mediators: A Systematic Review of Preclinical Studies. Food Funct. 2022;21(13):3159–69.
32. Imran M, Saeed F, Hussain G, Imran A, Mehmood Z, Gondal TA, El-Ghorab A, Ahmad I, Pezzani R, Arshad MU, Bacha U, Shariarti MA, Rauf A, Muhammad N, Shah ZA, Zengin G, Islam S. Myricetin: A Comprehensive Review on its Biological Potentials. Food Sci Nutr. 2021; 9(10):5854–5868.
33. Aghababaei F, Hadidi M. Recent Advances in Potential Health Benefits of Quercetin. Pharmaceuticals. 2023; 16(7):1020.
34. Cebeci E, Katirci E, Karhan M, Korgun ET. The Immunomodulator Effect of *Stevia Rebaudiana* Bertoni Mediated by TNF-α And IL-1β in Peripheral Blood in Diabeticrats. Food Sci Nutr. 2024; 12:7581–90.
35. Myint KZ, Zhou Z, Shi Q, Chen J, Dong X, Xia Y. Stevia

© 2025 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

Polyphenols, Their Antimicrobial and Anti-Inflammatory Properties, and Inhibitory Effect on Digestive Enzymes. Molecules. 2023; 28(22):7572.
36. Peteliuk V, Rybchuk L, Bayliak M, Storey KB, Lushchak O. Natural sweetener stevia rebaudiana: Functionalities, Health Benefits and Potential Risks. Excli J. 2021; 20:1412–1430.
37. Mehmood A, Althobaiti F, Zhao L, Usman M, Chen X, Alharthi F, Soliman MM, Shah AA, Murtaza MA, Nadeem M, Ranjha MMAN, Wang C. Anti-inflammatory Potential of Stevia Residue Extract Against Uric Acid-Associated Renal Injury in Mice. J Food Biochem. 2022; 46(10):e14286
38. Wei F, Zhu H, Li N, Yu C, Song Z, Wang S, Sun Y, Zheng L, Wang G, Huang Y, Bao Y, Sun L. Stevioside Activates AMPK to Suppress Inflammation in Macrophages and Protects Mice from LPS-Induced Lethal Shock. Molecules. 2021;26(4):858.
39. Casas-Grajales S, Ramos-Tovar E, Chávez-Estrada E, Alvarez-Suarez D, Hernández-Aquino E, Reyes-Gordillo K, Cerda-Gracia-Rojas CM, Camacho J, Tsutsumi V, Lakshman MR, Muriel P. Antioxidant and Immunomodulatory activity Induced by Stevioside in Liver Damage: *In vivo, in vitro* and *In silico* Assays. Life Sci. 2019; 224:187–196.
40. Casas-Grajales S, Reyes-Gordillo K, Cerda-García-Rojas CM, Tsutsumi V, Lakshman MR, Muriel P. Rebaudioside A Administration Prevents Experimental Liver Fibrosis: An *In Vivo* And *In Vitro* Study of the Mechanisms of Action Involved. J Appl Toxicol. 2019; 39(8):1118–31.
41. Wang K, Jiang Z, Zhou Q, Tang H. The associations Between Oxidative Balance Score and Serum Klotho Level in the U.S. Population Aged 40–79 Years. Sci Rep. 2024; 14(1):1–12.
42. Shahid A, Santos SG, Lin C, Huang Y. Role of Insulin-like Growth Factor-1 Receptor in Tobacco Smoking-Associated Lung Cancer Development. Biomedicines. 2024; 12(3):563.
43. Alfaro-Arnedo E, López IP, Piñeiro-Hermida S, Canalejo M, Gotera C, Sola JJ, Roncero A, Peces-Barba G, Ruiz-Marinez C, Pichel JG. IGF1R Acts as a Cancer-Promoting Factor in the Tumor Microenvironment Facilitating Lung Metastasis Implantation and Progression. Oncogene. 2022; 41(28):3625–3639.
44. Sgrò P, Ceci R, Lista M, Patrizio F, Sabatini S, Felici F, Sacheti M, Bazzuchi I, Duranti G, Di Luigi L. Quercetin Modulates IGF-I and IGF-II Levels After Eccentric Exercise-Induced Muscle-Damage: A Placebo-Controlled Study. Front Endocrinol. 2021; 12:745959.
45. Ponte LGS, Pavan ICB, Mancini MCS, Silva LGS da, Morelli APMBS, Brandemarte M, Bezerra RMN, Simabuco FM. The Hallmarks of Flavonoids in Cancer. Molecules. 2021; 26(7):2029.
46. Chen C, Na X, Wang L, Yu R. High-throughput screening identifies stevioside as a potent agent to induce apoptosis in bladder cancer cells. Biochem Pharmacol. 2022; 203:115166.

© 2025 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License