UNIVERSA MEDICINA

pISSN: 1907-3062 / eISSN: 2407-2230

Univ Med 2024;43:272-279

DOI: https://doi.org/10.18051/UnivMed.2024.v43.272-279

ORIGINAL ARTICLE

Ethanol extract of *Abrus precatorius* L. leaves diminishes inflammatory responses in nicotine-treated human gingival fibroblasts: an in vitro study

Raden Roro Widorini Kesumaningtias[1], Indra Kusuma[1,2], Yulia Suciati[1,3], and Wening Sari[1,4]

[1]Magister of Biomedical Science Program, School of Postgraduate, Universitas Yarsi, Jakarta, Indonesia
[2]Department of Physiology, School of Medicine, Universitas Yarsi, Jakarta, Indonesia
[3]Department of Biochemistry, School of Medicine, Universitas Yarsi, Jakarta, Indonesia
[4]Department Pharmacology, School of Medicine, Universitas Yarsi, Jakarta, Indonesia

wening.sari@yarsi.ac.id

Date of first submission, November 7, 2023
Date of final revised submission, October 8, 2024
Date of acceptance, October 17, 2024

Cite this article as: Kesumaningtias RRW, Kusuma I, Suciati Y, Sari W. Ethanol extract of *Abrus precatorius* L. leaves diminishes inflammatory responses in nicotine-treated human gingival fibroblasts: an in vitro study. Univ Med 2024;43:272-279

ABSTRACT

BACKGROUND
Nicotine induces oxidative stress in human gingival fibroblasts (HGF) and stimulates the production of cytokines that trigger inflammation. *Abrus precatorius* L. (AP) leaves contain antioxidants with anti-inflammatory properties that can prevent the formation of free radicals and reduce tissue damage due to inflammation. This study aimed to determine the effect of ethanolic extract of AP leaves (EAP) on interleukin (IL-6) levels and cyclooxygenase-2 (COX-2) gene expression in gingival fibroblasts exposed to nicotine.

METHODS
Cells were randomized into six treatment groups and clustered into the non-treatment control group (NTC), solvent control (SC), nicotine control (NC), and groups treated with nicotine and EAP at doses of 9.375 µg/mL, 18.75 µg/mL, and 37.5 µg/mL, respectively, for 24 hours. IL-6 levels were examined using the Elisa method, while COX-2 gene expression was assessed using PCR. Data were analyzed using Oneway ANOVA and the Kruskal Wallis test.

RESULTS
IL-6 levels and COX-2 expression were considerably higher in the nicotine control group. Conversely, the cell groups treated with nicotine and EAP had substantially decreased levels of both inflammatory markers IL-6 and COX-2 ($p=0.029$) across all EAP dose levels compared to the nicotine control group. The highest reduction in response was observed at the dose of 9.375 µg/mL EAP.

CONCLUSION
These results highlight the potential of *Abrus precatorius* L. in relieving nicotine-induced inflammation in smokers. By suppressing the production of inflammatory mediators IL-6 and COX-2 in HGF, EAP presents a promising avenue for further in vitro research.

Keywords : *Abrus precatorius* Linn., gingival fibroblasts, nicotine, IL-6, COX-2

INTRODUCTION

Smoking can cause inflammatory and autoimmune disorders, including genetic and epigenetic changes, increased oxidative stress, and free radical generation. These effects can increase the proliferation of B and T cells, reduce the generation and activity of immuno-suppressive T regulatory (Treg) cells and their autoantibodies, and increase the expression of pro-inflammatory mediators, such as IL-1β, IL-6, IL-8, and TNFs. Smoking has been shown to influence the concentration and activity of specific white blood cells, particularly leukocytes, which are linked to higher levels of the inflammatory markers C-reactive protein (CRP) and IL-6. IL-6 is involved in many biological processes, such as inflammation and immunological regulation, and an imbalance in immune regulation of the IL-6/IL-6 receptor axis can lead to different inflammatory diseases.[1,2]

Smoking also is strongly correlated with oral health, such as periodontal disease, delayed wound healing, and oral cancer. Periodontal disease is characterized by gingival inflammation of the dental tissues.[3] Chemicals in cigarettes, such as nicotine, tar, carbon monoxide, benzene, and hydrogen cyanide, can cause gingival inflammation. Nicotine is a bioactive component with the highest concentration in cigarette tobacco derivatives and is known as an addictive ingredient.[2,4,5]

Our previous research revealed that nicotine triggers oxidative stress in human gingival fibroblasts, as indicated by an increased level of malondialdehyde, a lipid peroxidation product, and increased expression of Nrf2, the transcription factor that plays an essential role in overcoming oxidative stress.[6] Free radicals generated by nicotine increase oxidative stress, inflammatory response, and cytokine release in periodontal ligament fibroblasts.[7] The inflammatory response can increase cytokines and inflammatory mediators IL-1β, IL-6, and IL-8, and activate the expression of tumor necrosis factor-alpha (TNF-α) and cyclooxygenase-2 (COX-2) genes, which can induce periodontal tissue damage.[8,9]

Arachidonic acid is released from the cell membrane upon oxidative damage to cells. It is critical to the induction of inflammatory response because certain enzymes, such as cyclooxygenases (COX-1 and COX-2) and lipoxygenase (LPO), convert arachidonic acid to inflammatory mediators, e.g., prostaglandins and leukotrienes, respectively. The inflammatory mediators act as signaling molecules to recruit neutrophils and macrophages to the damaged site. The recruited macrophages release cytokines, which further recruit other neutrophils and macrophages, creating a cyclic process leading to the elevation of various cytokine and chemokine levels.[10]

Smoking habits in most people are difficult to stop even though the negative impacts of smoking are intensively campaigned. Another alternative is to develop natural ingredients that can help reduce the negative impacts of smoking, especially on oral health. *Abrus precatorius* L. (AP), known as *saga* in Indonesia, is a plant widely used traditionally as a medicine in many countries. [11] This plant is often used as a remedy for mouth ulcers. It also reportedly has other therapeutic effects such as anti-fungal, anti-inflammatory, anti-bacterial, anti-tumor, anti-diabetic, and anti-proliferative, including anti-inflammation, and healing of ulcers, wounds, and scratches. [12] Research states that AP leaves contain flavonoids, isoflavanquinones, abruquinone B, phenolic saponins, steroids, and glycosides, which are proven to have potent antioxidant, anti-inflammatory, and hepatoprotective activities. [13]

As previously explained, nicotine can trigger inflammation and oxidative stress, while AP leaves have anti-inflammatory and antioxidant effects. Therefore, assessing whether the AP leaf extract can suppress the inflammatory response in nicotine-treated human gingival fibroblasts is necessary. The present study is expected to support the development of AP as a candidate for anti-inflammatory therapy through its effects on interleukin-6 (IL-6) levels and mRNA expression levels of cyclooxygenase-2 (COX-2) in nicotine-treated gingival fibroblasts.

METHODS

Research design

This was an in vitro study with a post-test-only control group design conducted at the Laboratory of Stem Cell Research Centre and the Laboratory of Molecular Biology Research Centre of YARSI University Jakarta, Indonesia, between June 2022 and January 2023.

Materials

The materials used were Dulbecco's Modified Eagle's medium (DMEM) (Gibco, Invitrogen), fetal bovine serum 10% [FBS] (Gibco, Invitrogen), Antibiotics-Antimycotics 1% (Gibco, Invitrogen), phosphate-buffered saline

[PBS] (Gibco, Invitrogen), RNA Extraction Kit (Zymo Research), SensiFAST SYBR® No-ROX One-Step RT-PCR Kit (Meridian Bioscience), PCR product primers (Genetics Science), IL-6 (Human) ELISA Kit (MyBioSource), nicotine (Merck), dimethyl sulfoxide (DMSO) (SIGMA-Aldrich), ethanol (Merck) as solvent for extract preparation, and dried AP leaves.

Extraction of AP leaves.

AP leaves were obtained from Cibodas Farm - Purwakarta, West Java. Plant determination was carried out by "Herbarium Bogoriense" Center for Biosystematics and Evolution Research, Cibinong - Bogor, West Java, with letter No. B-706/V/DI.05.07/3/2022, identifying the plant as *Abrus precatorius* Linn.

AP leaves in the form of dry simplicia were ground to a coarse powder, after which the leaves were macerated by soaking them in 96% ethanol for 24 hours. The filtered liquid was evaporated in a rotary evaporator at 40 ^{0}C for 24 hours.

Cell culture

Human gingival fibroblasts were obtained from the bio-repository of YARSI University Stem Cells Research Centre. Cells were grown in T-75 cell culture flasks with complete DMEM medium containing 10% FBS and 1% Antibiotics-Antimycotics and put into an incubator at 37°C and 5% CO_2. Confluent cells were harvested and plated on 6- or 24-well plates, as required, and treated.

Experimental design

The fibroblasts were divided into six groups with the following treatments: i) non-treatment control (NCT) group that received complete DMEM medium only; ii) nicotine control (NC) group, which received DMEM medium and 5 mM nicotine; iii) solvent control group, which received DMEM medium, DMSO 0.01%, and 0.01% ethanol; and iv) 3 treatment groups, which received nicotine 5 mM and ethanolic extract of *Abrus precatorius* L. leaves (EAP) in doses of 9.375 µg/mL (EAP1), 18.75 µg/mL (EAP2), and 37.5 µg/mL (EAP3), respectively.

Enzyme-linked immunosorbent assay (ELISA) for IL-6 levels

Gingival fibroblasts comprising 5 x10^4 cells were grown in 24-well plates until they reached 80% confluence. After receiving treatment with *Abrus precatorius* extract, the human gingival fibroblasts underwent mitosis for 24 hours, were then centrifuged, and the supernatant was taken and treated with IL-6 antibody according to MyBioSource procedures. The result was read with an ELISA reader at a wavelength of 450 nm within 10 minutes after adding the the stop solution to each well.

Quantitative real-time (qRT)-PCR for analysis of COX-2 mRNA expression

Gingival fibroblasts of 3 x10^5 were grown in a 6-well plate until they reached 80% confluence. RNA extraction was performed using the scraper technique, and isolates were extracted using the RNA Extraction Kit (Zymo Research), following the procedures of the RT PCR kit SensiFAST™ SYBR® No-Rox One-Step Kit (Meridian Bioscience). Examination of COX-2 mRNA expression and housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) using primers was conducted as follows:

Gene name	Primer sequences	Length of product
GAPDH	Forward 5’ -CCACCCATGGCAAATTCCATGGCA-3’ Reverse 5’ -TCTAGACGGCAGGTCAGGTCCACC-3’	580 bp
COX-2	Forward 5’ - ATGAGATTGTGGAAAAATTGCT -3’ Reverse 5’ -GATCATCTCTGCCTGAGTATC -3’	310 bp

RT-PCR reaction volume of 20 µl containing 4 µl RNA and 1.6 µl forward and reverse primers 10 µM, and 14.4 µl mixed reaction. One step temperature setting on the ROCHE Light Cycler Series 480 template is reverse transcription at 45°C for 10 minutes, pre-incubation at 95°C for 10 minutes, amplification with 45 cycles (denaturation at 95°C for 10 seconds, annealing at 58°C for 10 seconds, and extension at 72°C for 25 seconds). The melting curve was acquired through the temperatures of 95°C for 5 seconds, 65°C for 1 minute, and 97°C for continuous temperature

from 65°C to 97°C in acquisition mode, respectively and finally a cooling temperature of 40°C for 10 seconds. The confirmation test was done using 2% agarose gel electrophoresis in 0.5% Tris-acetate-EDTA buffer and the result visualized by ethidium bromide staining under UV light (Figure 1).

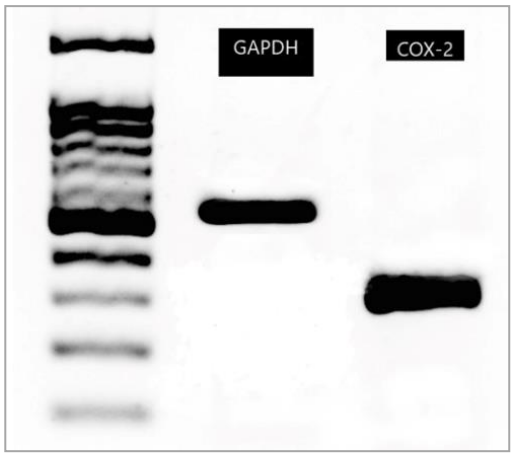

Figure 1. Visualisation of COX-2 and NADPH genes by ethidium bromide staining under UV light in 2% agarose gel electrophoresis and 0.5% Tris-acetate-EDTA buffer

Statistical analysis

The acquired data were initially checked for normality and homogeneity. The one-way ANOVA test was performed if a normal and homogeneous data distribution was obtained, followed by a second test with Least Significant Difference (LSD) to determine differences between treatments. The Kruskal-Wallis nonparametric test was used if the data distribution was not normal and homogenous; if there was a significant difference in the between-group mean, the Mann-Whitney test was applied. The differences were considered significant when $p<0.05$.

Ethical clearance

The research ethics committee of Research Institute Universitas YARSI approved this study under approval No. 182/KEP-UY/BIA/VI/2022.

RESULTS

A standard curve was created as a reference to determine IL-6 levels. Based on the IL-6 standard curve, a linear regression was generated with the formula y= mx + b (y= absorbance (optical density/OD) and x= antigen concentration). The regression analysis showed a linear pattern in the relationship between OD value and antigen concentration. Linear regression equation relationship on the standard curve was y = 0.0021 x + 0.0323, with y as absorbance (OD) and x as concentration (pg/mL) with a high correlation (R2 = 0.9944) (Figure 2).

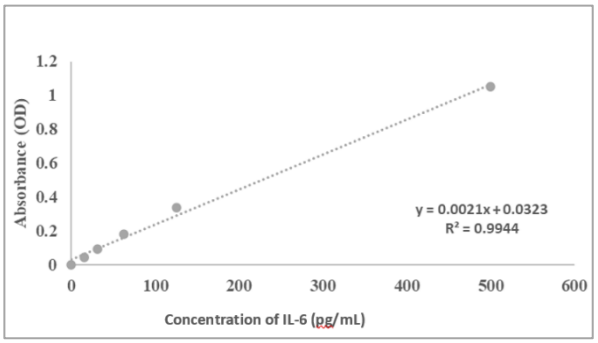

Figure 2. Standard curve of IL-6 concentration (pg/mL) in ELISA; OD: optical density

The results showed that IL-6 levels were lower in the cell groups that received nicotine and EAP at all dose levels compared to the nicotine control group. The difference was significant in cells that received extract doses of 9.375 µg/mL and 37.5 µg/mL (p=0.029) (Figure 3).

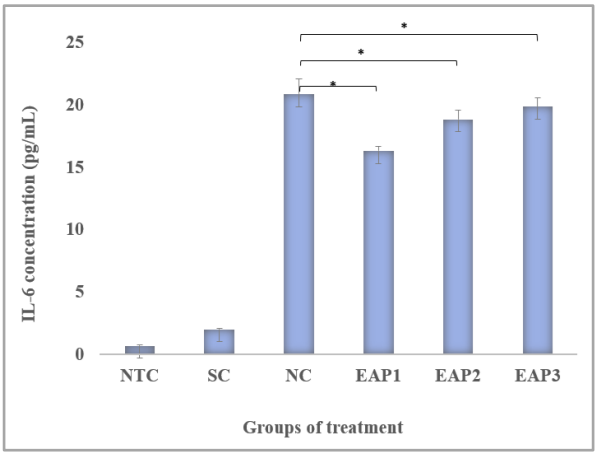

Figure 3. The concentration of IL-6 (pg/mL) on HGF after 24 hours of nicotine and EAP exposure. Cells seeded in 24-well culture plates at a density of 5 x10^4 cells/well. Data was presented as mean ± SD and analyzed by the Kruskal-Wallis test with the Mann Whitney post-analysis, * indicates $p<0.05$. NTC: non-treatment control; CS: solvent control; NC: nicotine control; EAP1: nicotine and EAP 9.375 µg/mL; EAP2: nicotine and EAP 18.75 µg/mL; EAP3: nicotine and EAP 37.5 µg/mL

The same pattern was shown when COX-2 mRNA expression was examined (Figure 4). The group that received nicotine and EAP at doses of 9.375 µg/mL, 18.75 µg/mL, and 37.5 µg/mL expressed significantly lower COX-2 mRNA than the nicotine control group (p=0.029) (Figure 4).

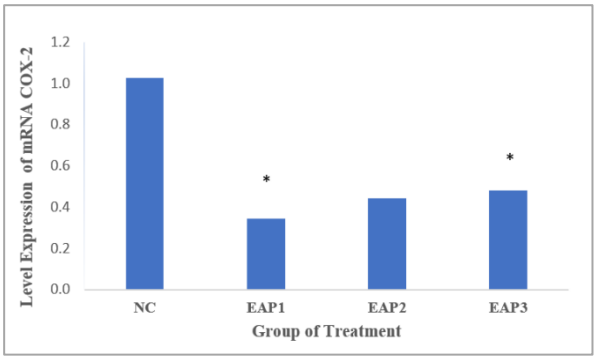

Figure 4. The expression of COX-2 on HGF after 24 hours of nicotine and EAP exposure. Cells seeded in 6-well culture plates at a density of 3 x10^5 cells/well. Data was presented as mean ± SD and analyzed by One way of ANOVA with LSD post-analysis, *indicates $p < 0.05$. NTC: non-treatment control; CS: solvent control; NC: nicotine control; EAP1: nicotine and EAP 9.375 µg/mL; EAP2: nicotine and EAP 18.75 µg/mL; EAP3: nicotine and EAP 37.5 µg/mL

DISCUSSION

Cigarettes include a variety of substances that can stimulate the generation of free radicals and create oxidative stress, which is damaging to the body. Active and passive smokers have significantly decreased total antioxidant capacity and higher total oxidant levels than non-smokers, indicating that smoking lowers endogenous antioxidant defenses and increases oxidative stress.[14] Smokers have higher levels of the inflammatory mediator IL-6 and lower levels of the endogenous antioxidant superoxide dismutase (SOD) compared to non-smokers.[15] It was also discovered that the expression of IL-6 and COX-2 was ten times higher in smokers than in non-smokers.[16]

Nicotine, one of the active chemicals in cigarettes, has been demonstrated to raise ROS levels in the cytosol, causing oxidative stress in numerous cell line cultures.[17] A study revealed that nicotine influences the development of periodontitis via inflammation and osteoclastogenesis. Nicotine administration in experimental animals enhances the expression of TNF-α and COX-2, which can cause periodontal inflammation and alveolar bone loss. Nicotine exposure in cocultures of periodontal ligament cells and CD4+ T cells significantly reduces cell viability and increases apoptosis, which is accompanied by increased expression of the matrix metalloproteinases MMP1 and MMP3, as well as the cytokines IL-1β, IL-6, IL-17, IL-21, and chemokine CXCL12.[18]

Cyclooxygenase-2 (COX-2) is an inducible enzyme in immune cells synthesizing pro-inflammatory prostaglandins (particularly PGE2) from arachidonic acid. Nicotine stimulates cancer cell proliferation, whereas cigarette smoke inhibits gingival fibroblasts' normal tissue healing function. [19] Oxidative stress in mouse lungs led to elevated STAT1α protein levels, iNOS and COX-2 gene expression, and lung tissue oxidative stress. Similarly, STAT1 has been demonstrated to generate pro-inflammatory cytokines and chemokines that promote cancer via the iNOS and COX-2 genes. Furthermore, STAT1 promotes skin tumors by upregulating iNOS and COX-2 genes.[20] Nicotine has been shown to enhance gastric cancer cell proliferation by upregulating COX-2. Nicotine stimulates protein kinase C (PKC) expression via β-adrenergic receptors (β-AR), leading to ERK1/2 phosphorylation and COX-2 expression. This leads to increased prostaglandin and VEGF production, which regulates neovascularization for nutrient supply and promotes tumor cell proliferation. COX-2 also suppresses the P53-P21/P27 signaling pathway and instead raises the expression of cyclin D1/cyclin-dependent kinase 4/6 (CDK4/6), therefore facilitating the cell cycle from G1 to S phase and promoting the proliferation of gastric cancer cells.[21]

Our study investigated the effects of EAP on IL-6 and COX-2 expression in nicotine-exposed gingival fibroblasts. The results revealed increased IL-6 levels and COX-2 mRNA expression in the nicotine control group. Studies indicate that nicotine causes gingival keratinocytes and human gingival fibroblasts to produce cytokines such as IL-1β, IL-6, and IL-8, which cause inflammation and tissue damage. [9,22] Nicotine administration has been shown in vivo and in vitro to boost COX-2 expression, resulting in increased release of pro-inflammatory prostaglandin E2 and thromboxane A2, which can promote tumor growth and angiogenesis.[18]

AP plants comprise up to 54 different types of flavonoid chemicals, which are classified as flavones, flavonols, flavanones, chalcones, isoflavones, anthocyanidins, isoflavones, and isoflavanquinones.[23] The leaves contain flavonoids such as pinitol, triterpene glycosides, glycyrrhizin, and alkaloids such as hepaphotine, precatorin, abrin, and choline. Triterpene glycosides include abrusosides A, B, and C and tree glycosides derived from aglycones of the abrutigenin and cycloartane types. Other active chemicals identified in AP leaves include

abruslactone A, abrusgenic acid triterpenes, methyl abrusgenate liquiritigenin-7-diglycoside, liquiritigenin-7-monoglycoside, toxifolin-3-glucoside, and vitexin flavonoid. Glycyrrhizin gives the leaves a sweet taste, and its concentration can reach 10%.[24]

The compounds contained in AP are widely known to have antioxidant effects. The study of Vijayan et al. revealed that the methanol extract of AP leaves has significant antioxidant potential by inhibiting the free radicals 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS), as well as the antioxidant enzyme activity of catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD).[25] Another study discovered that the ethanol extract of AP dramatically boosted the overall antioxidant capacity of plasma, liver, and kidney while causing less histological tissue injury in STZ/nicotinamide-induced diabetic rats compared to the control group.[26] The macerating of AP leaves in coconut oil at a concentration of 500 µg/mL effectively neutralized ABTS radicals, with a 78.13% inhibition rate. GC-MS analysis detected n-hexadecanoic acid, octadecane, and tetracosane in the macerated AP. Molecular docking reveals that these compounds have a high affinity for TNF-α, indicating their potential as anti-inflammatory agents.[25]

A study reported that the ethyl acetate extract of AP seed suppressed expression of COX-2, iNOS, NF-κB, IL-1β, and TNF-α proteins and reduced gastric ulcer score significantly in hydrochloric acid/ethanol (HCl/EtOH)-induced ulcers in rats.[26] Another study discovered that an ethanol extract of AP stem bark possesses nephroprotective properties against gentamicin-induced kidney injury. Histopathological examination of the kidneys in the extract-treated rats revealed significantly fewer regions of inflammation, fatty degeneration, blood vessel congestion, tubular necrosis, and glomerular atrophy than in the control group. It was also explained that the rats who received the extract had considerably lower kidney damage markers, oxidative stress, and inflammatory responses than the control group.[27]

Glycyrrhizin, one of AP's active chemicals, interacts in silico with the Tyr385 and Tyr348 residues of the COX-2 protein, forming a complex structure with a binding energy of -7.7 kcal/mol. The affinity and effectiveness of the protein and ligand receptor docking increase as the binding energy decreases. The presence of chemical interactions with amino acids in the active pockets, as well as low binding energy values, show that glycyrrhizin has high affinity and steric compatibility with the COX-2 protein, implying that glycyrrhizin is a promising inhibitor of the COX-2 protein.[28] In a mouse model of colorectal cancer caused by azoxymethane (AOM)/dextran sodium sulfate (DSS), the research discovered that COX-2, IL-6, and TNF-α proteins were upregulated. However, the expression of the three proteins was much reduced in cancer cells treated with glycyrrhizin compared to the control group.[29]

Vitexin, another active component in AP, is recognized to have anti-inflammatory properties by modulating COX-1, COX-2, and PGE2. In macrophages, vitexin and isovitexin reduced COX-1 and COX-2 mRNA expression. In in-silico research, it was revealed that vitexin had the highest fitness score against COX-2 and COX-1 enzymes, as well as tactical hydrogen bonding against both. Vitexin demonstrated moderate inhibition more towards COX-2 than COX-1 enzyme, and the flavonoids interacted with hydrogen bonding more at the cyclooxygenase catalytic sites, just like celecoxib, the recognized COX-2 inhibitor.[30]

In our study, nicotine and EAP treatment significantly decreased IL-6 levels and COX-2 mRNA expression in gingival fibroblasts at concentrations of 9.375 µg/mL, 18.75 µg/mL, and 37.5 µg/mL, compared to the nicotine control group, suggesting that EAP can diminish the impact of nicotine on the production of the inflammatory mediators IL-6 and COX-2. These findings are also pertinent to investigations on active agents in AP that can inhibit the activation of inflammatory mediators.

The lowest dose of EAP, 9.375 µg/mL, resulted in the most significant reduction in both parameters (IL-6 level and COX-2 mRNA expression). This effect is assumed to be linked to EAP's biphasic response to both inflammatory parameters. The biphasic reaction, also known as hormesis, is characterized by a stimulatory response at low dosages and an inhibitory response at high concentrations. Exposure to a chemical in low quantities can stimulate the organism's physiological regulatory function by inducing homeostasis reconstruction. However, at high doses, the chemical affects homeostasis and the organism's compensatory mechanisms, potentially causing harm.[31]

Considering that IL-6 has a hormesis effect, determining the appropriate dose of EAP requires

further research. Low IL-6 secretion is associated with anti-inflammatory, antioxidant, and proliferative effects, whereas high systemic IL-6 levels can induce pro-inflammatory, pro-oxidant, and pro-fibrotic responses.[32]

The present study revealed that EAP can reduce IL-6 levels and COX-2 mRNA expression in nicotine-induced gingival fibroblast cells, suggesting that it perchance expands as an alternative supplement to prevent or minimize the adverse effects of nicotine on smokers' oral health. The limitation of this study is that it was conducted in vitro, which limits the analysis of the two inflammatory markers, IL-6 and COX-2. Further research is needed to elaborate on the effects of EAP on the activation of NF-κβ and IL-10 by the inflammatory response and the dose range that can effectively reduce IL-6 and COX-2 expression.

CONCLUSION

This study found that exposure of HGF to nicotine increased IL-6 levels and COX-2 expression. The administration of EAP at three dose levels considerably lowered both inflammatory markers. The most effective dose of EAP was 9.375 µg/mL. This research will aid in the creation of supplements that can reduce the detrimental effects of nicotine on smokers' periodontal tissue. Further research is needed to determine the effect of EAP on the activation of NF-κβ and IL-10 during the inflammatory response, as well as the dose range that effectively inhibits IL-6 and COX-2 production.

Conflict of Interest

The Authors declare no conflict of interest.

Acknowledgement

The authors would like to thank the Stem Cells Research Centre Universitas YARSI for providing the human gingival fibroblasts.

Funding

We express our gratitude to the YARSI Foundation for funding this research through an internal grant with contract number 099/INT/UM/WR II/UY/V/2022.

Author Contributions

WK and IK conducted the experiment, WS and YS designed the experiment and analyzed the data. All authors contributed equally to the writing of the manuscript. All authors have read and approved the final manuscript.

Data Availability Statement

The data used to support the findings of this study is available from the corresponding author upon request.

Declaration of Use of AI in Scientific Writing

Nothing to declare.

REFERENCES

1. Elisia I, Lam V, Cho B, et al. The effect of smoking on chronic inflammation, immune function and blood cell composition. Sci Rep 2020;10:19480. doi: 10.1038/s41598-020-76556-7
2. Wang H, Chen H, Fu Y, et al. Effects of smoking on inflammatory-related cytokine levels in human serum. Molecules 2022;27:3715. doi: 10.3390/molecules27123715.
3. Holliday RS, Campbell J, Preshaw PM. Effect of nicotine on human gingival, periodontal ligament and oral epithelial cells: a systematic review of the literature. J Dent 2019;86:81-8. doi: 10.1016/j.jdent.2019.05.030..
4. Javed F, Kellesarian S V., Sundar IK, Romanos GE, Rahman I. Recent updates on electronic cigarette aerosol and inhaled nicotine effects on periodontal and pulmonary tissues. Oral Dis 2017;23:1052-57. doi: 10.1111/odi.12652.
5. Soleimani F, Dobaradaran S, De-la-Torre GE, Schmidt TC, Saeedi R. Content of toxic components of cigarette, cigarette smoke vs cigarette butts: a comprehensive systematic review. Sci Total Environ 2022;813:152667. doi: 10.1016/j.scitotenv.2021.152667.
6. Azmi S, Hadi RS, Kusuma I, Suciati Y, Sari W. Nicotine reduces cell viability and induces oxidative stress in human gingival fibroblasts. Univ Med 2024;43:20–30. DOI: https://doi.org/10.18051/UnivMed.2024.v43.20-30.
7. Nguyen TT, Huynh NNC, Seubbuk S, Nilmoje T, Wanasuntronwong A, Surarit R. Oxidative stress induced by Porphyromonas gingivalis lysate and nicotine in human periodontal ligament fibroblasts. Odontology 2019;107:133-41. doi: 10.1007/s10266-018-0374-1.
8. Chang CH, Han ML, Teng NC, et al. Cigarette smoking aggravates the activity of periodontal disease by disrupting redox homeostasis- an observational study. Sci Rep 2018;8:11055. doi: 10.1038/s41598-018-29163-6.
9. Bozkurt SB, Nielsen FH, Hakki SS. Boric acid reverses nicotine-induced cytokine expressions of human gingival fibroblasts. Biol Trace Elem Res 2023;201:1174-80. doi: 10.1007/s12011-022-03243-1.
10. Caliri AW, Tommasi S, Besaratinia A. Relationships among smoking, oxidative stress,

inflammation, macromolecular damage, and cancer. Mutat Res Rev Mutat Res 2021;787: 108365. doi: 10.1016/j.mrrev.2021.108365.
11. Perkasa AY. Utilization of the saga plant *Abrus precatorius* L. in Indonesian folk medicine. MAU J Agr Nat 2024:4:1-8. https://doi.org/10.59359/maujan.1311263
12. Kaula BC, Mishra R, Geeta, Kumar S, Mohanty A. Phytoconstituents and ethnopharmacological activities of *Abrus precatorius* L. (Fabaceae): a review. Vegetos 2022;35:869–79. Doi: 10.1007/s42535-022-00397-0.
13. Okoro EE, Maharjan R, Jabeen A, et al. Isoflavanquinones from *Abrus precatorius* roots with their antiproliferative and anti-inflammatory effects. Phytochemistry 2021;187:112743. doi: 10.1016/j.phytochem.2021.112743.
14. Ahmadkhaniha R, Yousefian F, Rastkari N. Impact of smoking on oxidant/antioxidant status and oxidative stress index levels in serum of the university students. J Environ Health Sci Eng 2021;19:1043-6. doi: 10.1007/s40201-021-00669-y.
15. Kumboyono K, Chomsy IN, Hakim AK, et al. Detection of vascular inflammation and oxidative stress by cotinine in smokers: measured through interleukin-6 and superoxide dismutase. Int J Gen Med 2022;15:7319-28. doi: 10.2147/IJGM.S367125.
16. Shanker YR, Surya K, Mani TP, Pathak AK, Ali MA. Transcript levels of COX-2, TNF-α, IL-6 and IL-10 in chronic obstructive pulmonary disease: an association with smoking and severity. Res J Biotech 2022;17:90-7. DOI: 10.25303/1710rjbt90097.
17. Malińska D, Więckowski MR, Michalska B, et al. Mitochondria as a possible target for nicotine action. J Bioenerg Biomembr 2019;51:259-76. doi: 10.1007/s10863-019-09800-z.
18. Zhang W, Lin H, Zou M, et al. Nicotine in inflammatory diseases: anti-inflammatory and pro-inflammatory effects. Front Immunol 2022;13:826889. doi: 10.3389/fimmu.2022.826889.
19. Hashemi Goradel N, Najafi M, Salehi E, Farhood B, Mortezaee K. Cyclooxygenase-2 in cancer: a review. J Cell Physiol 2019;234:5683-99. doi: 10.1002/jcp.27411.
20. Mahmoud AA, Osman Abdel-Aziz H, Elbadr M, Elbadre H. Effect of nicotine on STAT1 pathway and oxidative stress in rat lungs. Rep Biochem Mol Biol 2021;10:429-36. doi: 10.52547/rbmb.10.3.429.
21. Xu Y, Wang J, He Z, et al. A review on the effect of COX-2-mediated mechanisms on development and progression of gastric cancer induced by nicotine. Biochem Pharmacol 2024;220:115980. doi: 10.1016/j.bcp.2023.115980.
22. Ye D, Rahman I. Emerging oral nicotine products and periodontal diseases. Int J Dent 2023;2023:9437475. doi: 10.1155/2023/9437475..
23. Qian H, Wang L, Li Y, et al. The traditional uses, phytochemistry and pharmacology of *Abrus precatorius* L.: a comprehensive review. J Ethnopharmacol 2022;296:115463. doi: 10.1016/j.jep.2022.115463.
24. Aswin RK, Tridiganita IS, Arif NMA, Gavrila AP, Dina DA, Gabrielle AVP. *Abrus precatorius*: a comprehensive insight into the phytochemical, pharmacological, therapeutic activities and safety. J Drug Deliv Ther 2022 ;12:151–7. DOI: https://doi.org/10.22270/jddt.v12i1.5173.
25. Vijayan S, Margesan T. Arthritis alleviation: unveiling the potential in *Abrus precatorius* macerated oil. Future Sci OA 2024;10:FSO981. doi: 10.2144/fsoa-2023-0248.
26. Omoboyowa DA, Omomule OM, Balogun TA, Saibu OA, Metibemu DS. Protective potential of ethylacetate extract of *Abrus precatorius* (Linn) seeds against HCl/EtOH-induced gastric ulcer via pro-inflammatory regulation: In vivo and in silico study. Phytomedicine Plus 2021;1:100145. DOI: 10.1016/j.phyplu.2021.100145.
27. Falayi OO, Oyagbemi AA, Omobowale TO, et al. Nephroprotective properties of the methanol stem extract of *Abrus precatorius* on gentamicin-induced renal damage in rats. J Complement Integr Med 2018;16: /j/jcim.2019.16.issue-3/jcim-2017-0176/jcim-2017-0176.xml. doi: 10.1515/jcim-2017-0176.
28. Saini M, Malik JK. In-silico validation of glycyrrhizin against proinflammatory mediator COX-2: anti-proliferative potential. South Asian Res J Pharm Sci 2023;5:206–12. DOI: 10.36346/sarjps.2023.v05i05.004.
29. Wang G, Hiramoto K, Ma N, et al. Glycyrrhizin attenuates carcinogenesis by inhibiting the inflammatory response in a murine model of colorectal cancer. Int J Mol Sci 2021;22:1–16. doi: 10.3390/ijms22052609.
30. Abdulai IL, Kwofie SK, Gbewonyo WS, Boison D, Puplampu JB, Adinortey MB. Multitargeted effects of vitexin and isovitexin on diabetes mellitus and its complications. Sci World J 2021;2021:6641128. doi: 10.1155/2021/6641128.
31. Wan Y, Liu J, Mai Y, et al. Current advances and future trends of hormesis in disease. NPJ Aging 2024;10:26. doi: 10.1038/s41514-024-00155-3.
32. Forcina L, Franceschi C, Musarò A. The hormetic and hermetic role of IL-6. Ageing Res Rev 2022;80:101697. doi: 10.1016/j.arr.2022.101697.