I. INTRODUCTION

1.1 Background

In 2018, the Indonesian Ministry of Health reported that Indonesia ranked third globally for the highest number of smokers after China and India. Smoking is closely linked to serious diseases such as coronary artery disease (CAD), chronic obstructive pulmonary disease (COPD), cancers in various organ systems, and reproductive health decline (Alexandrov *et al*., 2016; Kovac *et al*., 2015). Smoking-related diseases occur due to chemical compounds in cigarette smoke. Thus, smoking negatively affects not only active smokers but also those exposed to secondhand smoke. Clinical and epidemiological studies have shown that both active and passive smokers are at risk of lung diseases, cardiovascular diseases, and other chronic conditions (Ueno *et al*., 2015; Alberg *et al*., 2013).

Cigarettes contain harmful free radical components that can damage nucleic acids, lipids, and proteins in human cells. This damage disrupts normal cell function, triggers genetic mutations, and causes various diseases (Caliri *et al*., 2021). Prolonged exposure to cigarette smoke increases oxidative stress, disrupting the balance between Reactive Oxygen Species (ROS) production and the body's ability to neutralize them. This oxidative stress not only causes direct cell damage but also triggers inflammation, worsens cellular conditions, and creates a continuous cycle of damage (Alexandrov *et al*., 2016; Alberg *et al*., 2013).

Free radical exposure from cigarette smoke induces physiological changes in the body. Hematological and biochemical profiles can serve as specific indicators to detect these changes. Blood components play vital roles in the body, such as oxygen and nutrient transport, immune protection (antibodies), and metabolic waste removal. Therefore, changes in blood values can indicate body homeostasis and clinical conditions (Danesh *et al*., 2022).

In addition to blood profiles, malondialdehyde (MDA), a compound produced from the peroxidation of unsaturated fatty acids can serve as a biomarker to measure oxidative stress in biological samples (e.g., blood, urine). After production, MDA is metabolized by enzymes and can covalently interact with proteins and nucleic acids. These interactions generate MDA epitopes linked to the activation of pro-inflammatory genes and downstream inflammatory signals, including protein kinase-C, p38-MAPK, ERK1/2, and NF-kB (Cordiano *et al*., 2023).

Previous studies have examined the efficacy of plant extracts as antioxidants and anti-inflammatory agents against cigarette smoke-induced damage (Savira *et al*., 2023; Saputra *et al.*, 2021; Damayanti *et al*., 2020). However, few studies have explored animal-derived compounds as therapeutic candidates. Bioactive components from animals such as fatty acids, proteins, and peptides show promising potential as effective antioxidants and anti-inflammatory agents, offering new opportunities for natural therapeutic approaches (Alves *et al*., 2013).

The Black Soldier Fly (*Hermetia illucens*), commonly referred to as BSF is an insect currently under investigation for its bioactive compounds. Sujai (2023) reported

that BSF extract exhibits stronger antioxidant activity than α-tocopherol. BSF extract inhibits MDA formation and reduces oxidative damage by directly reacting with free radicals before they interact with lipids, thereby breaking the lipid peroxidation chain. It may also act indirectly by suppressing pro-inflammatory cytokines that generate free radicals or by enhancing intracellular antioxidant enzyme activity (Da Pozzo *et al*., 2018).

Budikania *et al*., (2021) characterized BSF pupae phytochemicals and found strong antioxidant activity in n-hexane, ethyl acetate, and methanol extracts. Similar tests using methanol, water, and acetone solvents revealed potent antioxidant activity, with compounds such as flavonoids, indoles, and terpenoids (Gunawan, 2023). Rahayu *et al*., (2024) reported that 100% methanol BSF extract inhibited protein denaturation five times more effectively than 75% or 50% concentrations. Current BSF research primarily focuses on solvent-based extraction (Sujai, 2023; Budikania *et al*., 2021), while oil extraction via pressing remains underexplored a gap this study addresses.

BSF oil is a novel research subject. Afriani *et al*., (2023) identified lauric acid as a major component via Gas Chromatography-Mass Spectrometry (GC-MS). Anuar *et al*., (2023) demonstrated lauric acid's strong antioxidant and antidiabetic properties, including ameliorating male reproductive complications caused by hyperglycemia. Other fatty acids in BSF oil (e.g., linoleic, palmitic, stearic, myristic, oleic acids) show superior antioxidant activity compared to rice bran, olive, and krill oils (Phongpradist *et al*., 2023). BSF oil exhibited the highest DPPH radical scavenging activity (96.61% inhibition) (Phongpradist *et al*., 2023). Afriani *et al*., (2023) also identified 14 fatty acids

in BSF oil with potential anti-inflammatory, antinociceptive, antimicrobial, antiviral, and immunomodulatory properties.

Current research on BSF oil's antioxidant and anti-inflammatory potential is limited, despite its promising bioactive profile. In-depth studies on in vivo efficacy, toxicity, and therapeutic applications particularly for oxidative stress and chronic inflammation (e.g., cigarette smoke exposure) are scarce. This gap presents an opportunity to strengthen the scientific foundation for BSF's medical benefits and clinical applications. This study will analyze BSF oil's bioactive compounds using Gas Chromatography Flame Ionization Detector (GC-FID), which offers higher sensitivity and linearity than GC-MS (Aparicio *et al*., 2018). As natural and sustainable solutions gain prominence, BSF research could redefine approaches to inflammation related diseases.

1.2 Research Question

1. What fatty acids are present in BSF larval oil extracted using the solventless pressing method?
2. What is the effect of BSF larval oil on the blood profile of mice exposed to cigarette smoke?
3. What is the effect of BSF larval oil on MDA levels in the lungs of mice exposed to cigarette smoke?
4. What is the effect of BSF larval oil on the lung histopathology of mice exposed to cigarette smoke?

5. What are the effects of BSF larval oil on body weight gain, and on food and water intake, in mice exposed to cigarette smoke?

1.3 Research Objectives

1. To identify the fatty acids contained in BSF larval oil isolated using a solventless pressing method.
2. To analyze the effect of BSF larval oil on blood profiles of mice exposed to cigarette smoke.
3. To analyze the effect of BSF larval oil on lung MDA levels in mice due to cigarette smoke exposure.
4. To examine the impact of BSF larval oil on lung histopathology and lung organ weight index in mice exposed to cigarette smoke.
5. To assess the influence of BSF larval oil on body weight, food intake, and water consumption in mice.

1.4 Research Benefits

This study is expected to contribute to scientific knowledge on the effects of BSF oil as an antioxidant and anti-inflammatory agent against cigarette smoke exposure in mice.