



Effects of Ethanol Extract of *Mikania micrantha* Kunth Leaves on the Hematological Profile of Male Rats (*Rattus norvegicus* L.) Exposed to Cigarette Smoke

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Abstract: This study aimed to evaluate the effects of *Mikania micrantha* Kunth leaf extract on erythrocyte count, hemoglobin concentration, hematocrit, leukocyte count, and platelet count in male rats. A completely randomized design (CRD) was employed using 20 male rats randomly allocated into five experimental groups. The normal control group received no treatment for 30 days, whereas the positive control group was exposed to cigarette smoke from three cigarettes per day for 30 days. The treatment groups (P1, P2, and P3) were exposed to the same amount of cigarette smoke for 30 days and were administered *Mikania micrantha* Kunth leaf extract at doses of 100, 200, and 300 mg/kg body weight, respectively, for 23 days. The experimental procedures included phytochemical screening and measurement of hematological parameters, including erythrocyte count, hemoglobin concentration, hematocrit, leukocyte count, and platelet count, using a hematology analyzer. The data were analyzed using one-way analysis of variance (ANOVA), followed by Duncan's multiple range test. The results showed that administration of *Mikania micrantha* Kunth leaf extract significantly affected erythrocyte count, hemoglobin concentration, and hematocrit ($p < 0.001$), as well as leukocyte count ($p < 0.001$) and platelet count ($p = 0.001$). Among the tested doses, 300 mg/kg body weight produced hematological parameter values that most closely approximated those of the normal control group.

Keywords: *Mikania micrantha* Kunth leaves; cigarette smoke; hematological profile

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INTRODUCTION

Blood is a vital component of the body that plays essential roles in oxygen and nutrient transport, maintenance of homeostasis, and immune defense. Cigarette smoke contains thousands of chemical constituents, including numerous toxic, oxidizing, and carcinogenic compounds that may disrupt these physiological functions. Chronic exposure to cigarette smoke can promote oxidative stress, inflammation, and cellular injury, which may subsequently alter hematological parameters, including erythrocyte count, hemoglobin concentration, hematocrit, leukocyte count, and platelet-related indices (Selly et al., 2021). Epidemiological and genetic evidence further indicates that cigarette smoking is associated with increased leukocyte counts and higher hemoglobin and hematocrit values, supporting the use of hematological parameters as sensitive indicators of systemic responses to tobacco exposure (Pedersen et al., 2019; Pedersen et al., 2020). Therefore, evaluation of hematological parameters is relevant for assessing systemic physiological disturbances associated with cigarette smoke exposure (Jiwantoro & Jannah, 2025).

The adverse effects of cigarette smoke are closely linked to oxidative stress, which develops when the production of reactive oxygen species exceeds the capacity of endogenous antioxidant defense systems. This imbalance can damage cellular lipids, proteins, and DNA and contribute to inflammation and tissue dysfunction

(Cipollina et al., 2022). Evidence from smokers also demonstrates increased oxidative stress biomarkers and altered antioxidant defenses, indicating that tobacco exposure can disturb redox homeostasis at both systemic and cellular levels (Khudhur et al., 2025). Cigarette smoke may therefore affect blood cells through a combination of oxidative injury, inflammatory signaling, hypoxia-related responses, and altered cellular turnover. These mechanisms provide a biological rationale for investigating natural products rich in antioxidant compounds as potential agents for attenuating cigarette smoke-induced hematological alterations.

Mikania micrantha Kunth, a member of the family Asteraceae, is a medicinal plant known to contain a wide range of bioactive constituents. Traditionally, it has been used for several health conditions, including gastric disorders, respiratory complaints, jaundice, dysentery, and rheumatism (Chetia et al., 2014; Raka et al., 2019). In addition to its ethnomedicinal uses, *M. micrantha* has been reported to exhibit antioxidant, anti-inflammatory, antimicrobial, antidiabetic, antiproliferative, and other biological activities (Perawati et al., 2019; Andriani et al., 2020). More than 150 phytochemicals, including phenolics, flavonoids, terpenes, terpene lactones, glycosides, and essential oils, have been identified in *M. micrantha*, highlighting its pharmacological potential.

The antioxidant potential of *M. micrantha* is particularly relevant to the present study. Phytochemical investigations have identified multiple phenolic constituents in this species with substantial free-radical-scavenging activity in DPPH, ABTS, and FRAP assays (Dong et al., 2017). Several isolated compounds, including caffeic acid, *p*-coumaric acid, hydroquinone, and other phenolic derivatives, have demonstrated antioxidant activities comparable with or greater than those of ascorbic acid in selected *in vitro* assays (Dong et al., 2017). These findings provide stronger species-specific evidence for the antioxidant potential of *M. micrantha* than general assumptions based solely on the presence of flavonoids or phenolic compounds.

Previous studies have shown that antioxidant-rich plant extracts may attenuate hematological and inflammatory disturbances associated with cigarette smoke exposure. For example, Tariq et al. (2025) reported that olive-derived extracts modulated hematological parameters in cigarette smoke-exposed animals. However, evidence from other plant species cannot be directly extrapolated to *M. micrantha*, because phytochemical composition, antioxidant potency, bioavailability, and biological effects may differ among plant extracts. Despite increasing evidence regarding the phytochemical and antioxidant properties of *M. micrantha*, direct experimental studies evaluating its effects on the hematological profile under cigarette smoke exposure remain limited. This represents an important research gap, particularly because hematological alterations provide measurable systemic outcomes of oxidative and inflammatory stress.

Therefore, this study aimed to evaluate the effects of ethanol extract of *Mikania micrantha* Kunth leaves on the hematological profile of male rats (*Rattus norvegicus* L.) exposed to cigarette smoke. It was hypothesized that administration of *M. micrantha* leaf extract would attenuate cigarette smoke-associated alterations in erythrocyte count, hemoglobin concentration, hematocrit, leukocyte count, and platelet count, with greater effects at higher extract doses.

METHOD

Study Site and Period

The study was conducted from August to September 2025. Animal housing and experimental treatments were carried out at the Biology Laboratory, Faculty of Science and Technology, Universitas Islam Negeri Sumatera Utara (UINSU). Plant

identification was performed at Herbarium Medanense, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara (FMIPA-USU). Phytochemical screening was conducted at the Chemistry Laboratory of Universitas Sumatera Utara, whereas flavonoid and antioxidant analyses were performed at the Chemistry Laboratory of the Polytechnic of Chemical Industry Technology. Hematological analyses, including erythrocyte count, hemoglobin concentration, hematocrit, leukocyte count, and platelet count, were conducted at the Medical Hematology Laboratory, Universitas Sumatera Utara Hospital.

Experimental Design

This experimental study used a completely randomized design (CRD). A total of 20 male rats (*Rattus norvegicus* L.) weighing 150–200 g were acclimatized for 7 days before the experiment. The animals were randomly allocated into five groups, with four rats in each group. The experimental groups are presented in Table 1.

Table 1. Experimental groups (KN, KP, P1, P2, and P3)

Code Group	Treatment
KN	Negative control Rats received standard feed and drinking water for 30 days.
KP	Positive control Rats received standard feed and drinking water and were exposed to cigarette smoke for 30 days without administration of <i>Mikania micrantha</i> leaf extract.
P1	Treatment 1 Rats received standard feed and drinking water and were exposed to cigarette smoke for 30 days. <i>Mikania micrantha</i> leaf extract was administered at 100 mg/kg body weight/day from day 8 to day 30.
P2	Treatment 2 Rats received standard feed and drinking water and were exposed to cigarette smoke for 30 days. <i>Mikania micrantha</i> leaf extract was administered at 200 mg/kg body weight/day from day 8 to day 30.
P3	Treatment 3 Rats received standard feed and drinking water and were exposed to cigarette smoke for 30 days. <i>Mikania micrantha</i> leaf extract was administered at 300 mg/kg body weight/day from day 8 to day 30.

Preparation of *Mikania micrantha* Leaf Extract

Mikania micrantha Kunth leaves were collected, cleaned, and initially air-dried at room temperature for several days. The leaves were subsequently dried in an oven for 24 h at a temperature not exceeding 50°C (Ujjwal Kanti Dev & Department, 2015). The dried leaves were then ground into a fine powder.

Maceration was performed using 70 g of powdered *M. micrantha* leaves and 8.4 L of 70% ethanol at a plant material-to-solvent ratio of 1:120. This ratio corresponded to 70 g of powdered material in 8,400 mL of 70% ethanol. After maceration, the mixture was filtered using filter paper to separate the filtrate from the plant residue. The filtrate was collected in a beaker and concentrated using a rotary evaporator. The resulting extract was subsequently diluted with 0.5% carboxymethyl cellulose (CMC) according to the required treatment doses.

The extract was subjected to phytochemical screening for flavonoids, saponins, tannins, alkaloids, and triterpenoids. Flavonoids were tested using 5% FeCl₃, Mg + HCl, or H₂SO₄ reagents. Positive reactions were indicated by the formation of a black colloid with FeCl₃, a pink, orange, or yellow solution with Mg + HCl, or a yellow-orange solution with H₂SO₄.

For the saponin test, the sample was immersed in methanol (CH₃OH) for approximately 24 h in a sealed container and then filtered. Distilled water was added to the filtrate, and the mixture was shaken. The formation of stable foam was considered a positive indication of saponins. For tannin detection, the sample was

immersed in methanol for approximately 24 h, filtered, and tested with 5% FeCl_3 . The formation of a black colloid indicated the presence of tannins.

For alkaloid screening, the sample was immersed in ethanol ($\text{C}_2\text{H}_5\text{OH}$) for approximately 24 h, filtered, and subsequently tested using Mayer, Dragendorff, or Bouchardat reagents. A yellowish-white precipitate with Mayer reagent, a brick-red precipitate with Dragendorff reagent, or a brown precipitate with Bouchardat reagent was considered a positive reaction. For triterpenoid screening, the sample was immersed in chloroform (CHCl_3) for approximately 24 h, filtered, and tested using Salkowski or Liebermann–Burchard reagents. A brick-red solution with Salkowski reagent or the formation of a red-violet ring with Liebermann–Burchard reagent indicated the presence of triterpenoids. Antioxidant activity was subsequently evaluated using the DPPH assay by measuring the percentage inhibition at different extract concentrations.

Preparation and Treatment of Experimental Animals

A total of 20 male Wistar rats (*Rattus norvegicus* L.), aged 2–3 months and weighing 150–200 g, were used in this study. Before the animals were introduced, the cages were cleaned, lined with an adequate amount of rice husk bedding, and equipped with food and drinking-water containers. The cages were maintained in a well-lit, well-ventilated area away from excessive noise.

The rats were acclimatized for 1 week to minimize stress associated with the new environment and to reduce the risk of metabolic disturbances. During the acclimatization period, the animals had free access to food. Only healthy rats, characterized by normal body weight and the absence of hair loss, were included in the experiment. After acclimatization, the animals were divided into five groups of four rats each: one positive control group, one negative control group, and three treatment groups.

Cigarette Smoke Exposure

Cigarette smoke exposure was used as a source of free radicals in this study. Kretek cigarettes (brand X), containing 2.3 mg nicotine per cigarette, were used at a dose of three cigarettes per day. Each exposure lasted 15 min and was conducted at 10:00 a.m. Western Indonesian Time (WIB) for each cage over a period of 30 days (Dewanto et al., 2017).

Rats assigned to cigarette smoke exposure were placed in a modified exposure chamber measuring 50 × 40 × 20 cm, with an internal volume of approximately 40 L and a chimney installed at the top. Each chamber housed four rats according to the experimental grouping. Cigarette smoke was delivered using a water pump. Two tubes were installed at the top of the chamber: one served as the smoke inlet, whereas the other held the cigarette and was adjusted to match the cigarette diameter. The chamber was covered during exposure, while a small opening was maintained to allow air circulation.

Administration of *Mikania micrantha* Leaf Extract

Mikania micrantha Kunth leaf extract was administered at three doses: 100, 200, and 300 mg/kg body weight. The dose administered to each animal was adjusted according to body weight, which ranged from 180 to 200 g. A 0.5% CMC suspension was prepared by dissolving 0.5 g of CMC powder in 100 mL of distilled water and heating the mixture on a hot plate until a homogeneous suspension was obtained. The extract was then suspended in 0.5% CMC before administration.

The extract was administered orally by gavage at 4:00 p.m. WIB from day 8 to day 30 of the experimental period. The amount administered was calculated according

to the average body weight of each rat to ensure accurate dosing. For example, for a rat weighing 200 g and receiving an extract dose of 100 mg/kg body weight, the required daily dose was calculated as follows:

$$\text{Extract dose} = \frac{200 \times 100}{1000} = 20 \text{ mg/rat}$$

For four rats over 5 days:

$$20 \text{ mg} \times 4 \text{ rats} \times 5 \text{ days} = 400 \text{ mg} = 0.4 \text{ g}$$

The required volume of 0.5% CMC was calculated as:

$$2 \text{ mL} \times 4 \text{ rats} \times 5 \text{ days} = 40 \text{ mL}$$

Thus, for the 100 mg/kg body weight dose, 0.4 g of *M. micrantha* ethanol extract was mixed with 40 mL of 0.5% CMC. A rat weighing 200 g received 2 mL of the extract suspension orally each afternoon for 23 days. The original protocol states that administration began after ethylene glycol treatment because the maximum fluid volume that could be administered was 1% of body weight.

Blood Collection and Hematological Analysis

Blood samples were collected after anesthesia and before animal termination. Approximately 2 mL of blood was obtained from the orbital sinus and transferred into EDTA-containing tubes. The samples were subsequently transported to the Hematology Laboratory of Universitas Sumatera Utara Hospital for determination of erythrocyte count, hemoglobin concentration, hematocrit, leukocyte count, and platelet count.

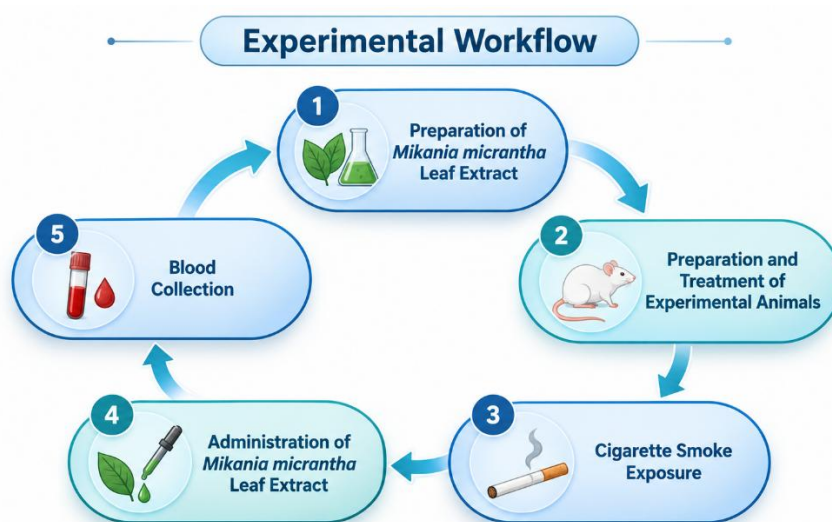


Figure 1. Blood collection from experimental rats

RESULTS AND DISCUSSION

Effects of *Mikania micrantha* Ethanol Extract on Erythrocyte Count, Hemoglobin Concentration, and Hematocrit in Rats Exposed to Cigarette Smoke

The effects of *Mikania micrantha* ethanol extract on erythrocyte count, hemoglobin concentration, and hematocrit are presented in Table 2.

Table 2. Erythrocyte count, hemoglobin concentration, and hematocrit in experimental groups

Group	Erythrocyte count ($10^6/\mu\text{L}$), mean $\pm$ SD	Hemoglobin (g/dL), mean $\pm$ SD	Hematocrit (%), mean $\pm$ SD
K-	6.98 $\pm$ 0.65 ^a	13.52 $\pm$ 0.62 ^a	39.97 $\pm$ 0.57 ^a
K+	9.24 $\pm$ 0.45 ^c	16.30 $\pm$ 0.82 ^c	46.05 $\pm$ 0.64 ^d
P1	8.77 $\pm$ 0.56 ^c	16.00 $\pm$ 0.42 ^c	44.23 $\pm$ 1.63 ^c
P2	7.86 $\pm$ 0.47 ^b	14.82 $\pm$ 0.57 ^b	41.93 $\pm$ 0.77 ^b
P3	7.08 $\pm$ 0.31 ^a	13.60 $\pm$ 0.52 ^a	39.99 $\pm$ 0.77 ^a
<i>p</i> -value	<0.001	<0.001	<0.001

Before one-way ANOVA was performed, the data were tested for normality using the Shapiro–Wilk test and for homogeneity of variance using Levene’s test. Erythrocyte count, hemoglobin concentration, and hematocrit were normally distributed ($p > 0.05$), and variances were homogeneous across groups ($p > 0.05$). Therefore, the assumptions for parametric analysis were satisfied, and the data were analyzed using one-way ANOVA. Significant differences among the experimental groups were observed for erythrocyte count, hemoglobin concentration, and hematocrit ($p < 0.001$).

The positive control group showed higher erythrocyte, hemoglobin, and hematocrit values than the negative control group. These values also exceeded the reference values stated in this study, namely $6\text{--}7 \times 10^6/\mu\text{L}$ for erythrocytes, 13 g/dL for hemoglobin, and 40% for hematocrit. This finding is consistent with Tariq et al. (2025), who reported increased erythrocyte counts, hemoglobin concentrations, and hematocrit following cigarette smoke exposure. Such increases may reflect a physiological adaptive response to tissue hypoxia through stimulation of erythropoiesis.

The elevation in erythrocyte count, hemoglobin concentration, and hematocrit may be associated with carbon monoxide (CO) in cigarette smoke. CO has an affinity for hemoglobin approximately 200–250 times greater than that of oxygen, resulting in the formation of carboxyhemoglobin (HbCO). This interaction reduces the oxygen-carrying capacity of hemoglobin and can induce tissue hypoxia (Maharani et al., 2021). Cigarette smoke also contains free radicals, including hydroxyl radicals ($\cdot\text{OH}$), which contribute to the formation of reactive oxygen species (ROS) that can damage erythrocyte membranes and cellular lipids (Selly et al., 2021). ROS can initiate lipid peroxidation, disrupt erythrocyte membrane integrity, generate toxic aldehyde products, and ultimately promote hemolysis (Yunita, 2021). Extensive erythrocyte damage may further aggravate hypoxia and stimulate compensatory erythrocyte production to maintain adequate oxygen delivery (Maharani et al., 2021).

In response to hypoxia, the kidneys increase erythropoietin secretion, which stimulates bone marrow erythropoiesis and helps restore oxygen-carrying capacity (Wulandari et al., 2024). Increased erythropoiesis also compensates for damaged erythrocytes. However, excessive erythrocyte production may result in values above the physiological range. An increase in red blood cell mass associated with hypoxic conditions may lead to secondary polycythemia, characterized clinically by elevated hemoglobin and hematocrit levels (Cahyanur & Rinaldi, 2019). Cigarette smoke exposure is also associated with several respiratory disorders, including chronic obstructive pulmonary disease (COPD), asthma, lung cancer, respiratory infections, pneumonia, and tuberculosis (Adeloye et al., 2022).

Post hoc comparisons showed that the erythrocyte count in the negative control group (6.98 ± 0.65) differed significantly from P1 (8.77 ± 0.56) and P2 (7.86 ± 0.47),

but not from P3 (7.08 ± 0.31). A similar pattern was observed for hemoglobin, with the negative control group (13.52 ± 0.62) differing significantly from P1 (16.00 ± 0.42) and P2 (14.82 ± 0.57), but not from P3 (13.60 ± 0.52). Likewise, hematocrit in the negative control group (39.97 ± 0.57) differed significantly from P1 (44.23 ± 1.63) and P2 (41.93 ± 0.77), but not from P3 (39.99 ± 0.77). These findings indicate that the 300 mg/kg body weight dose produced erythrocyte, hemoglobin, and hematocrit values that most closely approached those of the negative control group.

The observed effect may be related to secondary metabolites in *M. micrantha*, particularly flavonoids and polyphenols, which possess antioxidant activity. Flavonoids and polyphenols can donate hydrogen atoms or electrons to neutralize free radicals, thereby limiting lipid peroxidation and protecting erythrocyte membranes and hemoglobin from oxidative damage. Antioxidant mechanisms may also involve regulation of the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway, which contributes to the expression of endogenous antioxidant enzymes, including superoxide dismutase (SOD) (Layal, 2016). The antioxidant activity of *M. micrantha* leaf extract was supported by a DPPH IC_{50} value of $76.80 \mu\text{g/mL}$, indicating substantial free-radical-scavenging activity. Through these antioxidant effects, the extract may help preserve erythrocyte membrane integrity and maintain a balance between erythrocyte production and destruction, thereby contributing to the normalization of erythrocyte count, hemoglobin concentration, and hematocrit (Setiawan, 2024).

Effects of *Mikania micrantha* Ethanol Extract on Leukocyte Count in Rats Exposed to Cigarette Smoke

The effects of *M. micrantha* ethanol extract on leukocyte count are presented in Table 3.

Table 3. Leukocyte counts in experimental groups

Group	Leukocyte Count, mean $\pm$ SD	p-value
K-	12.13 ± 0.53^a	<0.001
K+	16.48 ± 0.59^d	
P1	14.99 ± 0.52^c	
P2	13.83 ± 0.76^b	
P3	12.54 ± 0.39^a	

One-way ANOVA showed significant differences in leukocyte counts among the experimental groups ($p < 0.001$). Duncan's multiple range test at the 5% significance level showed that the negative control group (12.13 ± 0.53) differed significantly from the positive control group (16.48 ± 0.59). The increase in leukocyte count following cigarette smoke exposure may be related to nicotine-induced catecholamine release and inflammatory responses caused by irritation of the respiratory tract. Inflammation of the bronchial airways may also promote an increase in circulating inflammatory markers (Miri-Moghaddam et al., 2014).

Nicotine-containing cigarette smoke can stimulate inflammatory signaling pathways, including those associated with granulocyte colony-stimulating factor (G-CSF) (Elisia et al., 2020). Colony-stimulating factors contribute to the regulation and production of circulating immune cells by the bone marrow (Miri-Moghaddam et al., 2014). Data presented in Appendix 6 showed that absolute lymphocyte counts in the cigarette smoke control group were higher than those of neutrophils, monocytes, eosinophils, and basophils, indicating that lymphocytes were the predominant

leukocyte population. This pattern suggests that cigarette smoke exposure may affect not only the total leukocyte count but also the composition of circulating immune cells.

Chronic irritation of the respiratory tract by toxic constituents of cigarette smoke can stimulate the release of pro-inflammatory cytokines, including TNF- α , IL-1, IL-6, IL-8, and granulocyte-macrophage colony-stimulating factor (GM-CSF). These mediators promote activation and mobilization of immune cells from the bone marrow into the circulation (Andreoli et al., 2015). Although the early inflammatory response primarily involves innate immune cells such as neutrophils and monocytes, persistent exposure may contribute to a transition toward chronic inflammation and increased involvement of adaptive immune responses.

Under chronic inflammatory conditions, lymphocytes, particularly T and B cells, contribute to sustained immune responses to repeated antigenic stimulation. Cigarette smoke contains numerous irritating and antigenic particles that can persistently stimulate pulmonary tissue and promote lymphocyte recruitment and activation. Repeated stimulation may increase lymphocyte proliferation, differentiation, and the frequency of activated and memory-cell subsets. Piaggese et al. (2021) reported increased frequencies of several T-cell subsets, including activated CD8⁺ T cells and memory cells, among active smokers, suggesting a shift toward chronic inflammation and adaptive immune activation. Oxidative stress caused by cigarette smoke may further enhance inflammation through activation of signaling pathways such as MAPK and NF- κ B, thereby increasing the production of pro-inflammatory mediators and sustaining immune-cell recruitment (Andreoli et al., 2015). Liang et al. (2021) similarly reported that cigarette smoke exposure was associated with increased systemic inflammatory cells, including lymphocytes and monocytes.

The predominance of lymphocytes relative to other leukocyte populations may therefore reflect persistent antigen exposure, progression from acute to chronic inflammation, and sustained activation of adaptive immunity. Under these conditions, T and B lymphocytes may be continuously produced, activated, and maintained in the circulation, whereas neutrophil and monocyte responses may be more transient. Increased lymphocyte activity has also been implicated in the pathogenesis of COPD and lung cancer (Andreoli et al., 2015).

The positive control group showed an increased leukocyte count (16.48 ± 0.59) compared with the negative control group. Cigarette smoke contains numerous harmful chemicals capable of generating free radicals (Selly et al., 2021). These radicals can damage cellular membranes in the respiratory tract and vasculature, particularly through lipid peroxidation (Maharani et al., 2021). Such cellular injury can initiate inflammatory responses in the respiratory tract and promote the production and release of leukocytes from the bone marrow into the circulation (Miri-Moghaddam et al., 2014). These findings are consistent with Shakeela Begum et al. (2017), who reported an increase in leukocyte count from 8.25 ± 0.85 to 13.7 ± 2.02 following cigarette smoke exposure.

Post hoc analysis showed that the leukocyte count in the negative control group (12.13 ± 0.53) differed significantly from P1 (14.99 ± 0.52) and P2 (13.83 ± 0.76), whereas no significant difference was observed between the negative control and P3 (12.54 ± 0.39). Thus, the 100 and 200 mg/kg body weight doses did not restore leukocyte counts to values comparable with the negative control, whereas the 300 mg/kg body weight dose produced values that most closely approached the normal-control level.

This effect may be related to antioxidant metabolites, particularly flavonoids. Flavonoids can directly neutralize free radicals by donating hydrogen atoms and can

reduce ROS formation by chelating metal ions such as iron, which participate in radical-generating reactions. Through these antioxidant mechanisms, flavonoids may reduce oxidative stress and limit cellular damage caused by cigarette smoke exposure. At the systemic level, these effects may contribute to stabilization of leukocyte counts (Rithio Chandraca Islamy, 2020). The antioxidant potential of *M. micrantha* extract was further supported by its IC₅₀ value of 76.80 µg/mL (Appendix 3). Reducing oxidative stress may help maintain more physiological leukopoiesis and support leukocyte function and survival (Borghi et al., 2023). Similar findings were reported by Shakeela Begum et al. (2017), who showed that polyphenol-rich green tea (*Camellia sinensis*) reduced cigarette smoke-associated leukocytosis.

Effects of *Mikania micrantha* Ethanol Extract on Platelet Count in Rats Exposed to Cigarette Smoke

The effects of *M. micrantha* ethanol extract on platelet count are presented in Table 4.

Table 4. Platelet counts in experimental groups

Group	Platelet count (mm ³), mean ± SD	p-value
K-	1211.50 ± 326.54 ^d	0.001
K+	674.25 ± 81.32 ^a	
P1	738.00 ± 28.85 ^{ab}	
P2	952.25 ± 59.58 ^{bc}	
P3	1139.00 ± 124.55 ^{cd}	

One-way ANOVA demonstrated significant differences in platelet counts among the experimental groups ($p = 0.001$). Duncan's post hoc test showed that the negative control group (1211.50 ± 326.54) differed significantly from the positive control group (674.25 ± 81.32), indicating a substantial reduction in circulating platelet numbers following cigarette smoke exposure. This reduction may be associated with cigarette smoke-induced oxidative stress and subsequent damage to platelet membranes.

Prolonged cigarette smoke exposure may adversely affect bone marrow function, particularly under conditions of high exposure, thereby reducing platelet production. In addition, oxidative stress and endothelial dysfunction induced by chronic cigarette smoke exposure may accelerate platelet activation and consumption, resulting in lower circulating platelet counts (Khudhur et al., 2025).

Nicotine may also enhance platelet activation and aggregation and promote intravascular thrombus formation. Excessive platelet aggregation can exacerbate endothelial dysfunction and inflammation, thereby increasing platelet consumption during coagulation (Fauziansyah et al., 2023). Consequently, even when thrombopoiesis continues, platelet consumption may exceed platelet production, resulting in a reduction in circulating platelet numbers (Wu et al., 2025). Consistent with this mechanism, the positive control group showed a mean platelet count of 674.25 ± 81.32, which was lower than the reference range cited in this study (1,000–1,500 × 10³/mm³).

Post hoc analysis showed that platelet count in the negative control group (1211.50 ± 326.54) differed significantly from P1 (738.00 ± 28.85) and P2 (952.25 ± 59.58), whereas P3 (1139.00 ± 124.55) did not differ significantly from the negative control. Platelet counts increased progressively with increasing extract dose, from 738.00 ± 28.85 at 100 mg/kg body weight to 952.25 ± 59.58 at 200 mg/kg body weight

and 1139.00 ± 124.55 at 300 mg/kg body weight. Therefore, among the tested doses, 300 mg/kg body weight produced the platelet response most closely resembling the physiological condition observed in the negative control group.

The increase in platelet count following *M. micrantha* treatment may be associated with the antioxidant activity of the extract, which had an IC_{50} value of 76.80 $\mu\text{g/mL}$ (Appendix 3). By reducing oxidative stress, antioxidant compounds may help preserve the integrity of megakaryocyte and platelet membranes and support normal thrombopoiesis (Sumantri et al., 2020). *M. micrantha* contains secondary metabolites, including flavonoids and phenolic compounds, that possess free-radical-scavenging activity (Dong et al., 2017). Phenolic compounds can donate hydrogen atoms to free radicals, generating more stable phenoxyl radicals, whereas flavonoids can suppress singlet oxygen and other reactive oxygen species, thereby reducing oxidative damage to platelets (Syukriah et al., 2022).

Hasibuan & Ifandi (2023) further suggested that flavonoids may contribute to wound healing through effects on platelet activation and hemostatic processes. Supporting evidence from Tariq et al. (2025) also indicates that flavonoid-containing olive extracts may influence thrombopoietic responses under cigarette smoke exposure. Taken together, these findings suggest that the flavonoid and phenolic constituents of *M. micrantha* may contribute to the recovery of platelet counts by attenuating oxidative damage and supporting platelet production and survival.

CONCLUSION

Ethanol extract of *Mikania micrantha* Kunth leaves significantly modulated hematological parameters in male rats (*Rattus norvegicus* L.) exposed to cigarette smoke. Cigarette smoke exposure increased erythrocyte count, hemoglobin concentration, hematocrit, and leukocyte count while reducing platelet count. Administration of the extract produced a dose-dependent tendency toward the values observed in the normal control group. Among the tested doses, 300 mg/kg body weight produced erythrocyte, hemoglobin, hematocrit, leukocyte, and platelet values that were closest to those of the normal control group and were not significantly different from the normal control for these parameters. These findings indicate that *M. micrantha* leaf extract has potential to attenuate cigarette smoke-associated hematological alterations, with 300 mg/kg body weight showing the most favorable response among the doses evaluated.

RECOMMENDATION

Further studies are recommended to investigate the underlying mechanisms by which *Mikania micrantha* leaf extract affects hematological parameters under cigarette smoke exposure. Future research should also consider additional biomarkers of oxidative stress and inflammation, broader dose ranges, and larger sample sizes to strengthen the interpretation of the observed effects. Potential limitations and experimental factors that may influence the results should also be carefully evaluated in subsequent studies.

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