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Antioxidant Effects of *Tridax procumbens* L. Ethanol Extract on SOD and MDA Levels in Cigarette Smoke-Exposed Wistar Rats

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ABSTRACT

Cigarette smoke is a source of free radicals that can damage cells and tissues of the respiratory tract. *Tridax procumbens* L. has potential as an antioxidant because it contains flavonoids that play a role in counteracting free radicals. The study aims to determine the potential of *Tridax procumbens* L. as a source of antioxidants against SOD and MDA levels in Wistar rats exposed to cigarette smoke. The study used Wistar rats (25 heads) with 5 treatments consisting of K (aquadest), KN (exposed to cigarette smoke), P1 (exposed to cigarette smoke and given 100 mg/kg BW extract), P2 (exposed to cigarette smoke and given 200 mg/kg BW extract), and P3 (exposed to cigarette smoke and given 300 mg/kg BW extract). Extract administration was carried out on days 1-21 and cigarette smoke exposure was carried out on days 8-21. Treatment is given orally, with the route of administration being through the gastrointestinal tract. Parameters observed consisted of flavonoid content test (by TLC method, total flavonoid content, total phenolic content), body weight, SOD levels (whole blood method), and MDA levels (TBARs method). Data were analyzed by one-way ANOVA test ($P<0.05$) followed by Duncan post hoc test. The results showed that *Tridax procumbens* L. contains flavonoids with an Rf value of 0.23. Body weight showed no significant difference ($P>0.05$), but SOD and MDA levels were different between treatments ($P<0.05$) with the most optimum dose of 300 mg/kg BW. The conclusion shows that a dose of 300 mg/kg BW of ethanol extract of *Tridax procumbens* L. can act as a natural antioxidant from exposure to cigarette smoke in vivo.

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1. Introduction

The number of active smokers in Indonesia continues to increase every year. The Central Bureau of Statistics (BPS) reported that in 2020-2021 there was an increase in the number of smokers from 28.69% to 28.96% (BPS, 2022). This increase needs to be watched out for because cigarette smoke harms health. The World Health Organization (WHO) estimates that 1.2 million passive smokers and 7 million active smokers die (Lucia et al., 2022). The data also shows that around 90% of the human population living in large urban areas breathe air with high toxicant content, such as exposure to cigarette smoke. Cigarette smoke exposure can cause a decrease in healthy air quality (Dylan, 2022).

Cigarette smoke has a variety of gaseous toxic compounds such as carbon monoxide (CO), hydrogen cyanide (HCN), and nitrogen oxides (NO) (Rahma et al., 2019; Mustofa & Fahmi, 2021). Smoking is one of the leading causes of morbidity and mortality in Indonesia, contributing to the increasing prevalence of non-communicable diseases such as heart disease, stroke and cancer. In addition to the health impacts, the economic burden of smoking is significant, including high health care costs and lost productivity due to disability or premature death.

The compound level of toxins in cigarette smoke causes oxidative stress (imbalance between prooxidants and antioxidants) (Hikmah et al., 2021). Oxidative stress due to exposure to cigarette smoke can form lipid radicals that react with oxygen such as peroxyl radicals, peroxides and malondialdehyde (MDA). Lipid peroxidation is a chain oxidation reaction that produces free radicals that initiate further peroxidation and cause the fatty acid chain of the cell membrane to be broken to form MDA (Duwairoh et al., 2018; Septiana & Ardiaria, 2016). MDA compounds can be used as biomarkers of increased lipid peroxides due to free radicals (Theresia et al., 2023). Cigarette smoke can also damage tissues by increasing MDA levels and decreasing superoxide dismutase (SOD) enzyme levels (Adyttia et al., 2014; Setiawan et al., 2016). The SOD enzyme is an endogenous enzyme to reduce free radicals and is the first defense against excessive lipid peroxidation processes in the body (Prayitno et al., 2018; Harun et al., 2017). SOD activity is tightly regulated by cellular redox status and the availability of metal cofactors such as copper and zinc. Suppression of SOD activity leads to accumulation of superoxide radicals, which can cause mitochondrial dysfunction, DNA damage, and ultimately trigger apoptosis or necrosis of cells (Jena et al., 2023).

Therefore, exogenous antioxidants (from outside the body) are needed to help reduce the effects of excessive free radicals (Prayitno et al., 2018). These antioxidants can be found in various compounds in herbal plants. *Tridax procumbens* L. is one of the plants that is easily found in Indonesia and has potential as a source of antioxidants because it contains flavonoids, alkaloids, saponins, tannins, phenolic compounds, and glycosides. Flavonoids are thought to suppress ROS production and enhance the activity of defense enzymes against free radicals to prevent oxidative stress. Flavonoids are a type of exogenous antioxidant that strengthens the body's antioxidant defenses by forming complementary components (Monikasari et al., 2023). The flavonoid content in this plant is 166.7 mg/g (Dattaray, 2022). While *T. procumbens* has been recognized for its antioxidant properties, its effectiveness in models of cigarette smoke-induced oxidative stress has not been extensively studied, making this research one of the first to explore its role in such a context. It is necessary to conduct in vitro and in vivo studies to determine the potential of these plants as sources of antioxidants. This study aims to assess the potential of *T. procumbens* L. as a natural antioxidant in increasing SOD levels and reducing MDA levels in rats exposed to cigarette smoke. An in vivo experimental model was used in which rats were exposed to cigarette smoke for 14 days, followed by assessment of oxidative stress biomarkers (SOD and MDA) in blood serum.

2. Methods

2.1. Research Protocol

This research is a preclinical in vivo experimental study using test animals to determine the effectiveness of *Tridax procumbens* L. on MDA and SOD levels of Wistar rats exposed to cigarette smoke. The research was conducted at the laboratory of Animal Structure and Physiology, Universitas Ahmad Dahlan, Indonesia, by obtaining approval from the UAD Ethics Committee (Approval No. 012307125). *T. procumbens* L. plants were obtained from a community plantation located in Pesantren, Wanayasa, Banjarnegara, Jawa Tengah which was then identified at the Laboratory of Ecology and Systematics, Universitas Ahmad Dahlan (No: 341/Lab.Bio/B/VII/2023) with the scientific name *T. procumbens* L.

2.2. Preparation of *Tridax procumbens* L. Ethanol Extract

T. procumbens L., weighing 10 kg, was dried using an oven at a temperature of 50°C for 2 days. The dried plants were then blended, resulting in 723 g of simplisia. The simplisia was subsequently filtered and macerated using 96% ethanol for 7 days. The maceration results were concentrated using a water bath at 40°C, yielding a semi-solid thick extract weighing 28 g. The extract was then stored in a refrigerator at a temperature of -4°C. A sample of 96% ethanol extract of *T. procumbens* L. plant, weighing 100 mg, was taken and added to 1 ml of ethanol. The mixture was then homogenized using

a vortex and subjected to sonication for 60 minutes. Maceration was carried out for 24 hours, followed by centrifugation to obtain a supernatant. A 20 µl aliquot of the sample was spotted onto a silica gel 60 F_{254} TLC plate. The plate was placed in a chamber saturated with a mobile phase consisting of n-hexane:ethyl acetate:formic acid (6:4:0.1). The plate was eluted to the solvent front and the spots were observed under UV light at wavelengths of 254 nm and 366 nm. Quercetin was used as a comparator in the TLC analysis. The retention factor (Rf) values of the sample and quercetin standard were calculated by dividing the distance traveled by the compound by the distance traveled by the solvent front, allowing comparison of compound mobility and identity.

2.3. Total Flavonoid Content (TFC) by UV-Vis Spectrophotometry

The preparation of the 400 ppm quercetin solution was carried out by weighing 10 mg of quercetin, which was then placed into a 25 mL volumetric flask, dissolved with ethanol, sonicated, and filled with ethanol up to the mark. The 10% (b/v) $AlCl_3$ solution was prepared by weighing 10 g of $AlCl_3 \cdot 6H_2O$, placing it in a 100 mL volumetric flask, dissolving it with 10 mL of aquabidest, sonicated, and then filled with aquabidest to the mark and homogenized. The 1 M sodium acetate solution was prepared by weighing 8.2 g of sodium acetate, placing it in a 100 mL volumetric flask, dissolving it with 10 mL of aquabidest, sonicated, and then filled with aquabidest to the mark and homogenized.

For the determination of operating time, 1250 µL of the 400 ppm quercetin solution was pipetted into a 10 mL volumetric flask to obtain a 50 ppm solution. Ethanol was added to the mark and the solution was homogenized. Then, 0.5 mL of the 50 ppm quercetin solution was transferred into a 5 mL measuring flask, added with 1.5 mL of ethanol, 0.1 mL of 10% $AlCl_3$, and 1 mL of 1 M sodium acetate, followed by aquabidest up to the mark. The solution was measured at a wavelength of 438 nm for 3600 seconds.

For the determination of the maximum wavelength, 1250 µL of the 400 ppm quercetin solution was pipetted into a 10 mL volumetric flask (to obtain a 50 ppm solution), filled with ethanol to the mark, and homogenized. A 0.5 mL aliquot of this solution was placed into a 5 mL measuring flask, added with 1.5 mL of ethanol, 0.1 mL of 10% $AlCl_3$, and 1 mL of 1 M sodium acetate, followed by aquabidest to the mark. The mixture was incubated at room temperature for the determined operating time and its absorption was measured in the 400–600 nm wavelength range.

To prepare the calibration curve, various volumes of the 400 ppm quercetin solution (six variations to yield six different concentrations) were pipetted into separate 10 mL volumetric flasks, filled with ethanol to the mark, and homogenized. Then, 0.5 mL of each 50 ppm quercetin solution was placed into 5 mL flasks, added with 1.5 mL ethanol, 0.1 mL of 10% $AlCl_3$, and 1 mL of 1 M sodium acetate, followed by aquabidest to the mark. These mixtures were incubated at room temperature for the set time and their absorbances were measured at the maximum wavelength.

For the determination of sample content, 100.07 mg of sample was weighed and placed into an Erlenmeyer flask, followed by the addition of 2 mL ethanol. The mixture was vortexed and sonicated for 1 hour. After centrifugation, the filtrate was transferred into a 10 mL volumetric flask. This extraction process was repeated three times, and the combined extract was filled with ethanol to the mark. Then, 0.5 mL of the sample solution was transferred to a 5 mL measuring flask, added with 1.5 mL ethanol, 0.1 mL of 10% $AlCl_3$, and 1 mL of 1 M sodium acetate, followed by aquabidest to the mark. The mixture was incubated at room temperature for the set time and the absorbance was measured at the previously determined maximum wavelength.

2.4. Total Phenolic Content (TPC) by UV-Vis Spectrophotometry

The preparation of the 400 ppm gallic acid master solution was carried out by dissolving 10 mg of gallic acid in 0.5 mL of methanol p.a., which was then placed in a 25 mL volumetric flask, filled with aquabidest to the mark, and homogenized. A 1% NaOH solution was prepared by weighing 1 gram of NaOH, placing it into a 100 mL volumetric flask, adding 80 mL of aquabidest, sonicating until completely dissolved, followed by the addition of aquabidest to the mark, and then homogenized. The 7.5% Folin-Ciocalteu reagent was prepared by pipetting 7.5 mL of Folin-Ciocalteu reagent into a 100 mL volumetric flask, adding aquabidest to the mark, and homogenizing the mixture.

For the determination of operating time, 1250 µL of the 400 ppm gallic acid solution was pipetted into a 10 mL volumetric flask, filled with methanol to the mark, and homogenized. Then, 1 mL of

this solution was taken, mixed with 5 mL of 7.5% Folin-Ciocalteu reagent, shaken, and allowed to stand for 8 minutes. Afterward, 4 mL of 1% NaOH solution was added, and the mixture was homogenized. The absorbance was measured over a time range of 0–90 minutes at a wavelength of 730 nm.

For the determination of the maximum wavelength, 1250 µL of the 400 ppm gallic acid solution was pipetted into a 10 mL volumetric flask, filled with methanol to the mark, and homogenized. Then, 1 mL of the solution was mixed with 5 mL of 7.5% Folin-Ciocalteu reagent, shaken, and allowed to stand for 8 minutes. Into the mixture, 4 mL of 1% NaOH solution was added, and the solution was homogenized, incubated at room temperature for the specified operating time, and the absorbance was measured in the wavelength range of 600–800 nm.

To prepare the calibration curve, several volume variations of the parent gallic acid solution (six different volumes to obtain six different concentrations) were pipetted into 10 mL volumetric flasks, filled with methanol to the mark, and homogenized. From each of these solutions, 1 mL was taken, mixed with 5 mL of 7.5% Folin-Ciocalteu reagent, shaken, and allowed to stand for 8 minutes. Then, 4 mL of 1% NaOH solution was added, and the solution was homogenized, incubated at room temperature for the determined operating time, and the absorbance was measured at the previously obtained maximum wavelength.

For the determination of sample content, 100.15 mg of the sample was weighed, mixed with 2 mL of methanol, vortexed, and sonicated for 15 minutes. The solution was then filtered and centrifuged. The filtrate was transferred into a 10 mL volumetric flask. This extraction procedure was repeated three times, and the combined extracts were filled with methanol to the mark and homogenized. From this solution, 1 mL was taken, mixed with 5 mL of 7.5% Folin-Ciocalteu reagent, shaken, and allowed to stand for 8 minutes. Subsequently, 4 mL of 1% NaOH solution was added, the mixture was homogenized, incubated at room temperature for the appropriate time, and the absorbance was measured at the determined maximum wavelength.

2.5. Animal Treatment and Body Weight Measurement

A total of 25 male Wistar rats (*Rattus norvegicus* Berkenhout, 1769), approximately 2 months old and weighing around 150 g, were used in the study. The rats were acclimatized for 11 days and were intensively maintained in cages measuring 50 cm × 40 cm × 15 cm at a temperature of approximately 26°C. The animals were then divided into five treatment groups, with each group consisting of five rats. The groups included: K (administered aquadest), KN (exposed to cigarette smoke), P1 (exposed to cigarette smoke and administered 100 mg/kg BW of extract), P2 (exposed to cigarette smoke and administered 200 mg/kg BW of extract), and P3 (exposed to cigarette smoke and administered 300 mg/kg BW of extract). The number of replicates for the test animals was determined using the Federer formula.

Cigarette smoke was supplied using a specially designed smoking chamber, which also served as a cage for the experimental animals. The chamber was made from a plastic box measuring 50 cm × 35 cm × 30 cm, with a pipe installed on one side to deliver cigarette smoke. The smoking chamber was equipped with three holes for connection to the smoking pump and twenty holes for air exchange.

The cigarette smoke exposure process began with the introduction of smoke into the chamber using a smoking pump until the chamber was fully filled. The pump was constructed from a modified aquarium air pump. All treatment groups and the negative control group were exposed to cigarette smoke using a total of three cigarettes per group per day for 14 consecutive days. The exposure was carried out every morning at 09.00 WIB. After the introduction of smoke, a 10-minute pause was given to allow the male Wistar rats to inhale the remaining smoke in the chamber before being returned to their respective cages.

The cigarettes used in the exposure were clove cigarettes containing 34–65 mg tar, 1.9–2.6 mg nicotine, and 18–28 mg CO (Setiawan et al., 2022). A total of three cigarettes per group were used for the 14-day exposure period. The ethanol extract of *T. procumbens* L., dissolved in 1 mL of distilled water, was administered orally for 21 days. The body weight of each rat was measured on days 0, 7, 14, and 21 using a digital scale. The average body weight was used to calculate the appropriate extract dosage for each treatment group (Setiawan et al., 2022).

2.6. Blood Sampling

On the 22nd day, blood samples were collected from the rats to obtain serum. The sampling process was initiated by anesthetizing the rats using 10% ether. Once the rats were fully unconscious, blood samples were taken from the orbital sinus using a microhematocrit. The microhematocrit was directed at a 45° angle toward the orbital sinus area and inserted until it penetrated the outer skin. The rats were then tilted, allowing the blood to drip, which was subsequently collected into a collection tube. A total volume of 1.5 mL of blood was obtained from each rat. The blood samples were immediately centrifuged at 3000 rpm for 10 minutes to separate the serum. Following sample collection, rats were humanely euthanized using cervical dislocation, in accordance with institutional ethical guidelines.

2.7. Analysis of SOD Levels

The sample used for the measurement of SOD activity was whole blood. The examination of SOD activity was carried out using the colorimetric method. The procedure for measuring SOD activity can be seen in table 1.

Table 1. Procedure for analyzing SOD activity

	Sample	Blanko 1	Blanko 2	Blanko 3
Whole blood	20 µl	-	20 µl	-
ddH_2O	-	20 µl	-	20 µl
Reagent solution Water Soluble Tetrazolium reagent solution	200 µl	200 µl	200 µl	200 µl
Buffer dilution solution	-	-	20 µl	20 µl
Enzyme reagent solution	20 µl	20 µl	-	-

All solutions were homogenized and incubated at 37◦C for 20 minutes, then absorbance was measured at a wavelength of 450 nm using a microplate reader. The formula for calculating SOD activity (%) (Nufus et al., 2020):

$$\text{SOD Activity (\%)} = \frac{(\text{A blanko 1} - \text{A blanko 3}) - (\text{A sample} - \text{A blanko 2})}{(\text{A blanko 1})} \times 100\% \quad (1)$$

2.8. Analysis of MDA Levels

The sample used for the measurement of MDA levels was blood plasma. Plasma MDA levels were determined using the Thiobarbituric Acid Reactive Substance (TBARs) method. The procedure for measuring MDA activity can be seen in Table 2.

Tabel 2. Procedure for analyzing MDA activity

	Sample	Standart	Blangko
H_3PO_4	750 µl	750 µl	750 µl
TBA	250 µl	250 µl	250 µl
Sample	50 µl	-	-
Standart	-	50 µl	-
Aquadest	-	-	50 µl
Aquadest	450 µl	450 µl	450 µl

All the above solutions were mixed and put into a water bath with a temperature of 100 ° C for 60 minutes. After the sample, standard, and blank came out of the water bath they put into an ice bath. Next, Sep-Pak C18 Column was prepared and 5 ml methanol was inserted and then discarded. Aquades 5 ml is inserted and then discarded, 5 ml sample is inserted and then discarded. Aquadest 4 ml was inserted and then discarded. Methanol 4 ml is inserted and then accommodated. Then read with a spectrophotometer with a wavelength of 532 nm (Nufus et al., 2020).

2.9. Data Analysis

All data parameters were analyzed using one way ANOVA ($P < 0.05$) and followed by a post hoc test using Duncan's test ($P < 0.05$) to determine significant differences between treatments.

3. Results and Discussion

3.1. Flavonoid Test of *Tridax procumbens* L. Ethanol 96% Extract

The results showed that *T. procumbens* L. [italic] extract contained flavonoid compounds. Table 3 shows the results of qualitative testing using TLC, as well as quantitative flavonoid and phenolic content determined by TFC and TPC.

Table 3. Results of flavonoid content analysis of 96% ethanol by thin layer chromatography (TLC), total flavonoid content (TFC), and total phenolic content (TPC) methods.

Sample	*Tridax procumbens* L. extract.
Test parameters	Flavonoid Kualitatif
Results	+
Methods	TLC
RF Flavonoid	0.23; 0.34; 0.59; and 0.87
Test parameters	Total Flavonoid Content (TFC)
Methods	UV-Vis Spectrophotometry
Results	67.036 ± 0.248 ppm
Test parameters	Total Phenolic Content (TPC)
Methods	UV-Vis Spectrophotometry
Results	134.695 ± 0.903 ppm

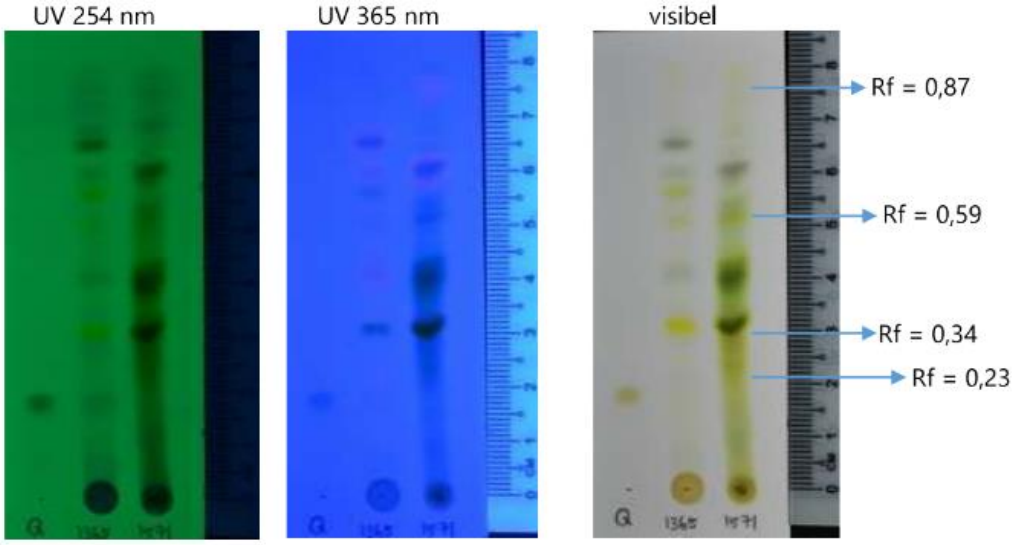

Figure 1. Thin-layer chromatography (TLC) profile of 96% ethanol extract of *Tridax procumbens* L. observed under UV 254 nm, UV 365 nm, and visible light. Lane Q represents the quercetin standard; lane 1571 represents the ethanol extract of T. procumbens L. The extract produced four visible spots with Rf values of 0.23, 0.34, 0.59, and 0.87. The spot with Rf = 0.23 corresponds closely to that of quercetin, indicating the presence of flavonoids in the extract.

Qualitative testing of 96% ethanol extract of *T. procumbens* L. was carried out using thin layer chromatography (TLC) method, eluted using mobile phase n-hexane: ethyl acetate: formic acid (6:4:0.1). Then the spots were observed at UV254 nm and UV366 nm. The TLC analysis used quercetin as standard. The results of the Rf value will be compared with the Rf value of the standard quercetin levels can be seen in figure 1. The 96% ethanol extract of *T. procumbens* L. multistage maceration has 4 spots, one of which has an Rf value of 0.23 which is relatively the same as the Rf value of quercetin standard so that the 96% ethanol extract of *T. procumbens* L. also contains flavonoids. Quantitative testing of 96% ethanol extract of *T. procumbens* L. carried out with TFC obtained results of 67.036 ± 0.248 ppm while TPC obtained results of 134.695 ± 0.903 ppm.

Most natural antioxidant sources come from plants that contain phenolic compounds in all parts of the plant body, both roots, flowers, and leaves. Phenolic compounds have a structure that easily donates hydrogen or electrons to acceptors, such as reactive oxygen species or peroxyl groups from fats, so that they can reduce the activity of oxygen and peroxyl radicals. Phenolic or polyphenolic

compounds can be flavonoids that have the ability to convert or reduce free radicals and also as anti-free radicals. Flavonoids also have an important role in human life, including as antioxidants, antimutagenic, antineoplastic, and vasodilator activities (Chopipah et al., 2021).

When flavonoid compounds react with free radicals, they form new radicals that are stabilized by the resonance effect of aromatic nuclei (anti-radical scavenging). The effectiveness of the compound in capturing free radicals is increased by the presence of an ortho-dihydroxy system on the B ring, double bonds at C2 and C3 that are conjugated with carbonyl groups on the C ring and hydroxy groups at C3 and C5 on the ring. Flavonoids are divided into several groups, namely chalcones, flavones, flavonols, and isoflavones based on the carbon atoms in ring C that bind ring B as well as the degree of unsaturation and oxidation of ring C (Arnanda & Nurwada, 2019). Quercetin is a flavonoid compound that is found in many plants. Besides having very strong antioxidant activity, quercetin also has other biological activities such as antiviral, antibacterial, anti-inflammatory, and anticancer (Widyasari et al., 2019).

The activity of flavonoids as antioxidants is based on their ability to directly seek out and collect reactive oxygen species and then chelate free radicals by directly donating hydrogen atoms or by single electron transfer. Another mechanism of action of flavonoids as exogenous antioxidants is through the chelation of transition metal elements because flavonoids have chelating properties, which are activated to bind metal ions in the human body to prevent them from being accessible for oxidation, such as quercetin compounds used for chelation of metal ions namely Fe2+ and Cu2+ which play an important role in free radical formation (Liu & Guo, 2015). Flavonoids can also act as inhibitors of enzymes useful for the formation of free radical compounds such as xanthine oxidase, lipoxygenase, protein kinase C, and cyclooxygenase. Induction of endogenous antioxidant enzymes is another mechanism of action of flavonoids (Arnanda & Nurwada, 2019).

3.2. Body Weight

Observation of body weight aims to determine changes in body weight in rats affected by the administration of 96% ethanol extract of *T. procumbens* L. and exposure to cigarette smoke. In addition, body weight weighing is also useful for monitoring the health of experimental animals. Table 4 shows the body weight of Wistar rats that received 96% ethanol extract of *T. procumbens* L. and were exposed to cigarette smoke from day 0 to 21.

Table 4. Body weight of Wistar rats that received 96% ethanol extract of *T. procumbens* L. and were exposed to cigarette smoke.

Variable	Treatment	Day			
		0	7	14	21
Body weight (in g)	K	151.67 ± 20.00[a]	158.67 ± 22.14[a]	161.67 ± 20.23[a]	187.33 ± 21.36[a]
	KN	158.00 ± 31.74[a]	147.33 ± 14.43[a]	151.33 ± 16.28[a]	172.00 ± 19.97[a]
	P1	152.00 ± 12.16[a]	160.33 ± 9.60[a]	169.00 ± 11.35[a]	182.66 ± 4.16[a]
	P2	152.00 ± 10.58[a]	157.67 ± 12.05[a]	164.66 ± 10.78[a]	175.00 ± 9.64[a]
	P3	153.67 ± 12.66[a]	165.67 ± 5.50[a]	188.00 ± 17.34[a]	182.00 ± 5.19[a]

Notes : a superscript letters on the same line indicate there is no significant difference between treatments (P > 0.05). K (distilled water), KN (cigarettes), P1 (100 mg/KgBB dose of extract and cigarettes), P2 (200 mg/KgBB dose of extract and cigarettes), and P3 (300 mg/KgBB dose of extract and cigarettes).

Body weight weighing was carried out to see changes in body weight in experimental animals caused by the administration of 96% ethanol extract of *T. procumbens* L. and exposure to cigarette smoke. Based on the test results for 21 days of administration of 96% ethanol extract of *T. procumbens* L. and exposure to cigarette smoke, the body weight of rats had no significant difference (P <0.05) between treatment groups. Chemical compounds that are harmful to the body such as CO, tar, nicotine, cyanide, ammonia, and dozens of other toxic compounds contained in cigarettes. Nicotine in cigarette smoke can reduce appetite and reduce body weight (Setiawan et al., 2022). However, this shows that 96% ethanol extract of *T. procumbens* L. does not inhibit weight loss due to exposure to cigarette smoke. The body weight of Wistar rats showed a gradual increase in body weight (Setiawan et al., 2022).

3.3. Superoxide Dismutase (SOD) Levels

The results showed that mathematically, the dose that could increase SOD levels the highest was a dose of 300 mg/KgBB. Figure 2 shows the diagram of SOD levels of Wistar rats after getting 96% ethanol extract of *T. procumbens* L. plants.

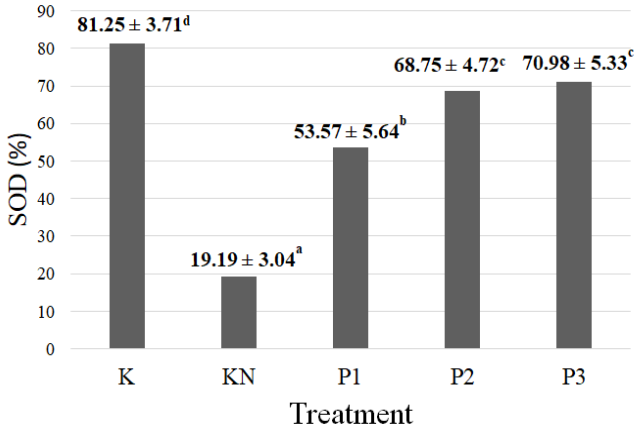

Figure 2. Superoxide dismutase (SOD) activity (%) in Wistar rats following 21-day administration of 96% ethanol extract of *T. procumbens* L. and exposure to cigarette smoke. K: control (distilled water), KN: negative control (cigarette smoke), P1: 100 mg/kg BW extract + cigarette smoke, P2: 200 mg/kg BW extract + cigarette smoke, P3: 300 mg/kg BW extract + cigarette smoke. Bars represent mean ± SD (n = 5). Superscript letters (a–d) indicate significant differences between groups based on one-way ANOVA followed by Duncan's post hoc test ($P < 0.05$).

The results showed that *T. procumbens* L. plant extract has phytochemical compounds that can increase blood SOD levels in rats exposed to cigarette smoke. Phytochemical compound tests show that *T. procumbens* L. plants, especially the stems and leaves, contain many flavonoid compounds that act as antioxidants. According to Dewi & Ulfah (2021), SOD levels in the blood play an important role as a defense against free radicals. Based on the results of testing SOD levels, it is known that the levels of *T. procumbens* L. extract at the three doses used can increase blood SOD levels. Mathematically, the dose that can increase SOD levels is the highest dose of 300 mg/KgBB. SOD levels in the control showed high results because they were in normal conditions. While the negative control SOD levels in the blood were very low without being given *T. procumbens* L. The data of the test results of SOD levels can be seen in figure 2.

Superoxide dismutase (SOD) is an enzymatic antioxidant that protects tissues from free radical-induced oxidative damage. SOD catalyzes the reduction reaction of superoxide anion radical (O_{2*}) to hydrogen peroxide (H_2O_2) and oxygen (O_2). The SOD enzyme is an endogenous defense to protect any free radicals that enter the body. When the number of free radicals entering the body exceeds the maximum amount of SOD enzyme work, the use of more SOD enzymes so that the work of the SOD enzyme is disrupted, and causes a decrease in SOD levels in the body (Nufus et al., 2020).

3.4. Malondialdehyde (MDA) Levels

The results showed that mathematically, the dose that can reduce MDA levels is a dose of 300 mg/KgBB. Figure 3 shows the diagram of MDA levels of Wistar rats after getting 96% ethanol extract of *T. procumbens* L. plants.

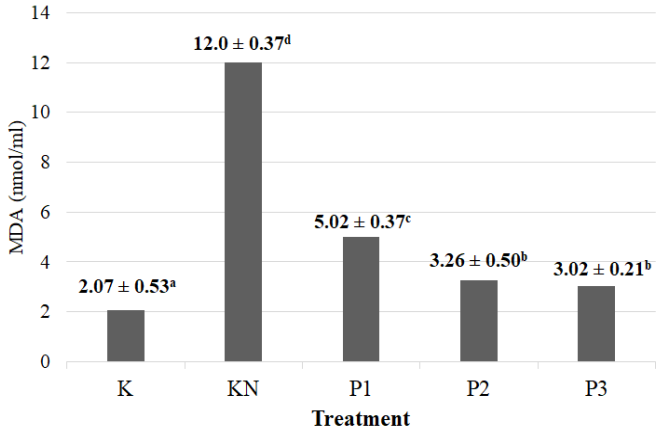

Figure 3. Malondialdehyde (MDA) levels (nmol/mL) in Wistar rats following administration of 96% ethanol extract of *Tridax procumbens* L. and exposure to cigarette smoke. K: control (distilled water), KN: negative control (cigarette smoke only), P1: 100 mg/kg BW extract + cigarette smoke, P2: 200 mg/kg BW extract + cigarette smoke, P3: 300 mg/kg BW extract + cigarette smoke. Data are expressed as mean ± SD (n = 5). Superscript letters (a–d) indicate significant differences between groups according to one-way ANOVA followed by Duncan's post hoc test ($P < 0.05$).

The results showed that *T. procumbens* L. plant extract has phytochemical compounds that can reduce blood MDA levels in rats exposed to cigarette smoke. Phytochemical compound tests show that *T. procumbens* L. plants, especially the stems and leaves, contain many flavonoid compounds that act as antioxidants. According to Dewi & Ulfah (2021), MDA levels show the levels of free radicals in the body. Based on the results of testing MDA levels, it is known that *T. procumbens* L. extract at the three doses used can reduce MDA levels in the blood and the dose that can significantly reduce MDA levels is a dose of 300 mg/KgBB. MDA levels in the control showed low results because they were in normal conditions. While in the negative control, MDA levels in the blood were very high without the administration of *T. procumbens* L. The data on the results of testing MDA levels can be seen in figure 3.

Under normal circumstances, free radicals in the body are formed slowly and slowly. When free radicals enter the body beyond the working capacity of endogenous defenses, the body will experience oxidative stress. This is characterized by an excessive increase in lipid peroxidation. The result of lipid peroxidation is MDA, so when there is an increase in lipid peroxidation, it can cause an increase in MDA levels in the body (Zaenal et al., 2022, Triandhini et al., 2013). MDA in the body is formed as a result of oxidative stress conditions, which is an imbalance between the formation of reactive oxygen species (ROS) and the presence of antioxidants, where free radicals are higher than antioxidants. Excess hydroxyl radicals and peroxynitrite can attack lipoprotein cell membranes and form lipid peroxides and produce MDA (Kusuma, 2015).

Nicotine contained in cigarettes is a secondary metabolic product included in the class of true alkaloids derived from the synthesis of nicotinic acid. If nicotine consumption is carried out continuously it will cause addiction. Nicotine enters the body through the respiratory tract, digestive tract, and skin. If someone smokes, nicotine enters the body through the respiratory tract to get into the blood, nicotine enters through the pulmonary circulation and then will be carried to the brain. In the brain there are nicotine receptors called Nicotinic Cholinergic Receptors (nicotinic acetylcholine receptors or nAChRs). Nicotine binding on the surface between 2 subunits on this receptor will open a pathway that allows sodium or calcium ions to enter, the entry of these 2 ions will activate the calcium channel voltage which makes more calcium intake. The effect caused by the entry of calcium ions is the release of neurotransmitters, and dopamine is one of the neurotransmitters released. Before dopamine is released, nicotine first activates glutamine, which functions as a neurotransmitter that works in helping the release of dopamine and the release of y-aminobutyric acid (GABA) which can inhibit the active dopamine (Bachman et al., 2024).

Nicotine can increase dopamine levels in the brain. If cigarette consumption decreases, there is automatically a decrease in nicotine levels in the body, so the smoker will experience anxiety to be able to consume cigarettes continuously (Triandhini et al., 2013). In addition, nicotine that enters the

body will be metabolized in the liver. Metabolism of iminium can occur through the electron transport pathway with a redox cycle that will produce a radical. Cationic metabolism arises from several pathways, including oxidation of nicotine itself and protonation of myosmine derived from nornicotine through nicotine demethylation. This metabolism requires hydrolysis of nicotine imotine into ketone amines with open chains that will undergo nitrosation to form a toxic nitrosamine. Furthermore, nitrosamine functions as a DNA alkylator and causes the activation of various oxidative damage and radical pathways. Free radicals are involved in cell signaling involving redox processes in the categories of ion transport, neuromodulation, and transcription. When the amount of free radicals is high, toxicity will occur in the body. This toxicity can cause oxidative damage, lipid peroxidation, and DNA formation (Fuadi et al., 2020).

4. Conclusion

The study concluded that the 96% ethanol extract of *T. procumbens* L. significantly increased superoxide dismutase (SOD) levels and reduced malondialdehyde (MDA) levels in Wistar rats exposed to cigarette smoke. This effect was observed at a dose of 300 mg/kgBB, indicating the potential of *T. procumbens* L. as an antioxidant agent in combating oxidative stress caused by cigarette smoke exposure. These findings suggest that the plant extract may have beneficial properties in reducing oxidative damage, which could be explored further for potential therapeutic applications. However, further studies are needed to confirm these effects in different models, including long-term exposure settings and mechanistic analyses of the active compounds involved. Future investigations may also assess the extract's safety profile, bioavailability, and efficacy in human-relevant models to support its potential as a therapeutic antioxidant.

REFERENCES

Adyttia, A., Untari, E. & Wahdaningsih, S. (2014). Efek Ekstrak Etanol Daun *Premna cordifolia* terhadap Malondialdehida Tikus yang Dipapar Asap Rokok. *Pharmaceutical sciences and research*, 1(2):105-115. https://doi.org/10.7454/psr.v1i2.3302.

Arnanda, Q.P. & Nuwarda, R.F. (2019). Review Article, Penggunaan Radiofarmaka Teknesium-99M dari Senyawa Glutation dan Senyawa Flavonoid sebagai Deteksi Dini Radikal Bebas Pemicu Kanker. *Farmaka*, 17(2):236-243.

Bachman, J.L., Kitcher, S.R., Vattino, L.G., Beaulac, H.J., Chaves, M.G., Rivera, I.H., Katz, E., Wedemeyer, C. & Weisz, C.J.C. (2024). GABAergic synapses between auditory efferent neurons and type II spiral ganglion afferent neurons in the mouse cochlea. *bioRxiv*. https://doi.org/10.1101/2024.03.28.587185.

Badan Pusat Statistika. (2022). *Data Persentase Merokok*. URL: https://www.bps.go.id/indicator/30/1435/1/persentase-merokok-pada-penduduk-umur-15-tahun-menurut-provinsi.html. Diakses tanggal 7 Oktober 2023.

Chopipah, S., Solihat, S.S. & Nuraeni, E. (2021). Review: Aktivitas Antioksidan Senyawa Flavonoid pada Daun Benalu, Ktuk, Johar, dan Kajajahi. *Journal of Biological Science*, 1(2):19-26.

Dattaray, D. (2022). Traditional Uses and Pharmacology of Plant Tridax procumbens. *Systematic Review Pharmacy*, 13(7):476-482.

Dewi, M. & Ulfah, M. (2021). Efek Kefir terhadap Kadar Superoksidase Dismutase, Malondialdehyde pada Mencit Balb/C Diinduksi Streptococcus Agalactiae. *Journal of Issues in Midwifery*, 5(3):101-110. https://doi.org/10.21776/ub.JOIM.2021.005.03.1.

Duwairoh, A., Wirjatmadi, B. & Adriani, M. (2018). Pengaruh Ekstrak Bawang Putih Tunggal dalam Penurunan Kadar Malondialdehid (MDA) akibat Pemaparan Rokok Elektronik. *Jurnal Ilmiah Kedokteran Wijaya Kusuma*, 7(2):149-157. https://doi.org/10.30742/jikw.v7i2.436.

Dylan, E. & Mustaram, A. (2022). Rumah Pohon Tambora: Perbaikan Kualitas Udara melalui Filtrasi Polusi Udara Perkotaan di Kawasan Tambora. *Jurnal Sains, Teknologi, Urban, Perancangan, Arsitektur*, 4 (2):1-15. https://doi.org/10.24912/stupa.v4i2.22208.

Fuadi, A., Tangka, J., Martino, Y.A. & Purnomo, Y. (2020). Potensi Ekstrak Etanol Daun Gedi Merah (*Abelmoschus manihot* (L.) Medik) terhadap Kadar Superoxide Dismutase dan Malondialdehyde Jaringan Ginjal Tikus Model Diabetes Melitus. *Jurnal Bio Komplementer*, 7(2):1-8.

Harun1, I., Susanto, H. & Rosidi, A. (2015). Pemberian Tempe Menurunkan Kadar Malondialdehyde (MDA) dan Meningkatkan Aktivitas Enzim Superoxide Dismutase (SOD) pada Tikus dengan Aktivitas Fisik Tinggi. *Jurnal Gizi Pangan*, 12 (3):211-216. https://doi.org/10.25182/jgp.2017.12.3.211-216.

Hikmah, M., Nurdiana & Sari, N. (2021). Pengaruh Infuse Water Lemon (*Citrus limon*) terhadap Kadar Malondialdehyde (MDA) Plasenta pada Tikus (*Rattus novergicus*) Bunting yang Dipapar Uap Rokok Elektrik. *Journal of Issues in Midwifery*, 5 (1): 1-9. https://doi.org/10.21776/ub.JOIM.2021.005.01.1.

Jena, A.B., Samal. R.R., Bhol, N.K. & Duttaroy, A.K. (2023). Cellular Red-Ox system in health and disease: The latest update. *Biomedicine & Pharmacotherapy*, 162. https://doi.org/10.1016/j.biopha.2023.114606.

Kusuma, A.S. W. (2015). The Effect of Ethanol Extract of Soursop Leaves (*Annona muricata* L.) to Decreased Levels of Malondialdehyde. *Jurnal Majority*, 4(3):14-18.

Liu, Y. Z. & Guo, M. Q., (2015). Studies on Transition Metal-Quercetin Complexes Using Electrospray Ionization Tandem Mass Spectrometry. *Molecules*, 20:8583-8594.

Lucia, S., Yetiani, N., Suwarni, L., Rusmitasari, H., Maretalinia, M. & Suyitno, S. (2021). The Determinants of Adolescent Smokers in Indonesia. *International Journal of Public Health Science*, 11(3):808-814. https://doi.org/10.11591/ijphs.v11i3.21510.

Monikasari, Widyastiti, N., Mahati, E., Syauqy, A. & Al-Baarri, A. (2023). Pengaruh Pemberian Ekstrak Bekatul Beras Hitam (*Oryza sativa* L. Indica) terhadap Kadar MDA, SOD dan Trigliserida pada Tikus Diabetes Mellitus Tipe 2. *Aceh Nutrition Journal*, 8(1):129-138. https://doi.org/10.30867/action.v8i1.731.

Mustofa, S. & Fahmi, Z. (2021). Efek Protektif Kardiovaskular Ekstrak Rhizophora Apiculata Berbagai Pelarut pada Tikus yang Dipaparkan Asap Rokok. *Jurnal Unila*, 5(1):7-15.

Nufus, I., Lisdiana, Marianti, A. & Peniati, E. (2020). Pengaruh Nikotin dalam Rokok Elektrik Terhadap Kadar MDA dan SOD pada Darah Tikus. *Journal Life Science*, 9(2):161-170.

Prayitno, S., Kusnadi, J. & Murtini, E. (2018). Pengaruh Ekstrak Etanol 90% Daun Sirih Merah terhadap Kadar Malondialdehid (MDA) dan Superoksida Dismutase (SOD) Mencit Tikus yang Dipapar Asap Rokok. *Journal Of Chemistry*, 6(1):1-9. https://doi.org/10.18860/al.v6i1.5018.

Rahma, F., Ardiaria, M., & Panunggal, B. (2019). Pengaruh Pemberian Ubi Jalar Ungu (*Ipomoea Batatas* L. Poir) terhadap Kadar Leukosit Total Tikus Wistar Jantan (*Rattus Norvegicus*) yang Dipapar Asap Rokok. *Journal Of Nutrition College*, 8(2):65-72. https://doi.org/10.14710/jnc.v8i2.23815.

Septiana, W., & Ardiaria, M. (2016). Efek Pemberian Seduhan Kulit Buah Naga Merah (*Hylocereus Polyrhizus*) terhadap Kadar Malondialdehyde (MDA) Tikus Sprague Dawley Dislipidemia. *Jurnal Of Nutrion College*, 5(4):344-352.

Setiawan, D., Tjahyono, K. & Afifah, D. (2016). Pemberian Kecambah Kacang Kedelai terhadap Kadar Malondialdehid (MDA) dan Superoxide Dismutase (SOD) tikus Sprague Dawley Hiperkolesterolemia. *Jurnal Gizi Klinik Indonesia*, 13(1):20-26. https://doi.org/10.22146/ijcn.22815.

Setiawan, H., Hikmawati, A., Wulandari, S.W., Malinda, E.V. & Putra, I.L.I. (2022). Effect of *Calina papaya* Leaves Extract on Respiratory Tract in Cigarette Smoke Exposed Rats. *Jurnal Biodjati*, 7(2):189-198. https://doi.org/10.15575/biodjati.v7i2.19068.

Theresia, C., Simorangkir, S. & Nababan, K. (2023). Pengaruh Pemberian VCO (*Virgin Coconut Oil*) Terhadap Kadar MDA (Malondialdehid) Tikus Putih Jantan Galur Wistar yang Terpapar Asap Rokok. *Jurnal Majalah Kedokteran Andalas*, 46(1): 1-9. https://doi.org/10.56260/sciena.v1i2.33.

Triandhini, R. R., Mangimbulude, J. C. & Karwur, F. F. (2013). Merokok dan Oksidasi DNA. *Sains Medika*, 5(2):120-127.

Widyasari, E.M., Sriyani, M.E., Daruwati, I., Halimah, I. & Nuraeni, W. (2019). Karakteristik Fisiko-Kimia Senyawa Bertanda 99M Tc-Kuersetin. *Jurnal Sains dan Teknologi Nuklir Indonesia*. 20(1):9-18. http://dx.doi.org/10.17146/jstni.2019.1.1.4108.

Zaenal, N., Ariani, N. & Zahiroh, I. (2022). Effect of Soursop Fruit Juice (*Annona Muricata* Linn) on Malondialdehyde (MDA) Levels of Placenta of White Rat Strain Wistar (*Rattus novergicus*) Pregnant with Exposure to Cigarette Smoke. *Jurnal Multidisiplin Madani*, 2(7):2999-3006. https://doi.org/10.55927/mudima.v2i7.607.