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ANTI-MELANOGENIC PROPERTIES OF CELERY LEAF EXTRACT IN HYPERPIGMENTATION INDUCED BY AZIDOTHYMIDINE

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Abstract

The purpose of this study is to examine the impact of ethanolic extract form Celery leaves (*Apium graveolens L.*) on hyperpigmented Wistar rats (*Rattus norvegicus sp.*) induced by Azidothymidine (AZT). Celery leaves are known to contain bioactive compounds which may serve as potent anti-inflammatory and antioxidant agents. About 30 male Wistar rats were divided into five groups, including a positive control, a negative control, and groups treated with various doses (1%, 3% and 5%) of celery leaf extract. The study examined inflammation indicators such as MDA, TNF-α, and IL-6. Celery leaf extract significantly (P<0.001) reduced MDA, TNF-α, and IL-6 levels in rats, demonstrating its capacity to attenuate inflammation and oxidative stress produced by AZT. With the increase in celery leaf extract concentration from 1% to 5%, MDA, IL-6 and TNF-α levels decreased from 42.083 ± 3.49 to 28.277 ± 10.27 nmol/ml, 14.588 ± 3.38 to 4.539 ± 1.40 nmol/ml and 667.614 ± 143.41 to 326.920 ± 62.72 nmol/ml respectively. Histopathological examinations demonstrated improvements in skin tissues damaged by inflammation. The results indicate that an ethanol extract of celery leaves has the potential to be an efficient anti-inflammatory drug, notably in lowering AZT-induced inflammation and hyperpigmentation. Further research is required to explore its clinical applicability in controlling inflammatory diseases in human.

Keywords: Celery leaf extract; anti-inflammatory; TNF-α; IL-6; Hyperpigmentation; Azidothymidine.

INTRODUCTION

The skin is the human body's most layered and heavy organ[1]. Hyperpigmentation is a skin condition characterized by an overabundance of pigment. The incidence of hyperpigmentation in Indonesia is relatively high due to Indonesians' skin types, which fall under Fitzpatrick skin phototypes 4 and 5, where the skin rarely burns but constantly darkens. The tropical temperature and high sunlight contribute to the growing prevalence of hyperpigmentation[2]. Hyperpigmentation produces cosmetic concerns that can affect appearance and quality of life, hence mandating prevention and therapy before hyperpigmentation occurs. Genetics, dietary issues, hormones, sunshine, cosmetics, oral medicines, inflammation, cancer, and other factors can all lead to hyperpigmentation[3]. Melasma, lentigo, post-inflammatory hyperpigmentation, and hyperpigmentation brought on by chemical and pharmaceutical side effects are the four categories of hyperpigmentation problems. Kadek et al[4] found that 40.7% of 167 patients who had skin tests showed hyperpigmentation, with 62.3% of these cases occurring in women between the ages of 13 and 60. This condition is frequently brought on by using chemical-containing cosmetics for three months to eleven years. In addition, 10% of cases of hyperpigmentation in men and over 40% of women over 30 are particularly sensitive to it. Inflammation is the body's defensive reaction to either tissue damage, foreign object invasion, or both. Microorganisms, mechanical stress, chemical agents, and physical factors are all potential sources of inflammation. Chemical or physical stimuli that induce damage generate the release of inflammatory mediators such as serotonin, histamine, bradykinin, prostaglandins, and others, resulting in redness, heat, discomfort, swelling, and more. An increase in vascular permeability, an increase in protein denaturation, and modifications to membranes are all components of inflammation, a process that is frequently linked to pain[5]. Additionally, inflammation is a

defensive reaction meant to remove the primary source of the injury-induced necrotic tissue and cell damage. Inflammation is the body's initial immune response to infection, and it varies depending on the type of injury or infection[6]. When healing elements enter injured tissue, inflammation facilitates their removal from the bloodstream. Blood arteries dilate, increasing blood flow to the affected area. Swelling, redness, heat, and discomfort are caused by inflammation because it changes the structure of blood vessels, which makes it easier for blood to reach the tissue[5]. Post-inflammatory hyperpigmentation is defined as brown patches on the skin that have been injured or inflamed, and the spots are large in size but irregular in shape. A complicated vascular tissue's biological reaction to damaging stimuli like infections, irritants, or cell damage is inflammation. In order to reduce the discomfort brought on by the inflammatory process, a drug that addresses inflammation is therefore necessary[6]. Azidothymidine (AZT) can induce hyperpigmentation in the skin by increasing melanin levels[7]. Azidothymidine, also known as zidovudine, has been suggested as the first antiretroviral medication for the treatment of HIV-1. It is one of the substances that effectively inhibits the DNA polymerase activity of the HIV-1 RT (Reverse Transcriptase) enzyme. In order to combat HIV-1, combination medicines now include RT inhibitors as a crucial component[8]. The first antiviral drug authorized for the treatment of the human immunodeficiency virus (HIV) was azidothymidine (AZT)[7].

The invasion of blood monocytes and the activation of tissue macrophages are the outcomes of nearly all inflammatory events. The cytokines TNF, IL-1, and IL-6 are produced as a result of this activity, and these cytokines have a variety of effects on the host, including the induction of fever, the hepatic acute-phase response followed by leucocytosis, the production of acute-phase proteins like C-Reactive Protein (CRP), and the differentiation of T cells, B cells, and macrophages[9]. Interleukin 6 (IL-6) may function as a marker of significant inflammation in COVID-19 patients with a bad prognosis[10]. Interleukin 6 (IL-6) functions as both an anti-inflammatory cytokine and an anti-inflammatory myokine[10]. It is encoded by the IL-6 gene in humans. Moreover, osteoblasts release IL-6 to promote the development of osteoclasts. Many bloods vascular stroma's smooth muscle cells also generate the pro-inflammatory cytokine IL-6.

The fact that IL-6 inhibits TNF-α and IL-1, as well as activating IL-1ra and IL-10, demonstrates its function as an anti-inflammatory myokine[10]. In other hand, Malondialdehyde (MDA) is a reactive dialdehyde molecule formed in vivo as a result of lipid peroxidation, either enzymatically or non-enzymatically that is produced as a byproduct of lipid peroxidation and is one of several reactive electrophilic molecules that put cells under hazardous stress[11]. Elevated MDA values signify membrane oxidation. Reactive oxygen species (ROS) have been examined in eczema and other skin diseases, but their significance in atopic dermatitis (AD) has received less attention. Mutagenic compounds can be created when MDA combines with deoxyguanosine and deoxyadenosine in DNA[12]. Thus, malondialdehyde is an excellent biomarker for oxidative stress[13,14]. TNF-α contributes to systemic inflammation at moderate levels and can produce pathological abnormalities like septic shock at high levels[15]. This is related to the cytotoxic nature of TNF-α (Tumour Necrosis Factor alpha). TNF-α contributes to host defence against bacterial, parasite, and viral illnesses[15]. Macrophages create TNF-α, which is then triggered by antigens, T lymphocytes, NK cells, and mast cells[15,16]. In healthy persons, TNF-α is typically undetectable; nevertheless, it is frequently discovered in the serum when inflammatory and infectious diseases are present. TNF-α, a powerful pyrogen, affects leukocytes and endothelial cells, causing acute inflammation even at low levels[16].

Celery is widely used as a vegetable or as a side dish, and it is increasingly being utilized as a medication[17]. Organic chemicals found in celery leaves include flavonoids, tannins, saponins, flavo-glycosides (apiin), and apigenin. Celery, according to Yongkhamcha[18], also has a number of bioactive components. Using diclofenac as a comparison, Sapri[19] reports that celery leaf water extract exhibits anti-inflammatory efficacy in male mice induced with carrageenan. Numerous investigations have been carried out on naturally occurring anti-inflammatory substances, including fruit peels[20], rambutan leaves[21], and Sesewanua plants[22]. On the other hand, the benefits of celery leaf extract on hyperpigmentation have not yet been studied. Therefore, this study aims to examine the impact of Azidothymidine-induced hyperpigmentation in Wistar rats (*Rattus norvegicus Sp*) on the anti-inflammatory activity of celery leaf extract (*Apium graveolens L*.).

LITERATURE REVIEW

Hyperpigmentation as a Dermatological and Public Health Issue

The skin is the largest and one of the most complex organs of the human body, functioning as a physical and immunological barrier (1). Hyperpigmentation refers to the excessive accumulation of melanin, clinically expressed as localized or diffuse darkening of the skin. In tropical countries such as Indonesia, hyperpigmentation is frequently reported because the dominant Fitzpatrick phototypes (IV–V) tend to darken easily under ultraviolet (UV) exposure, and chronic sunlight intensity accelerates pigmentation disorders (2). Beyond cosmetic concerns, hyperpigmentation can negatively affect psychosocial well-being and quality of life, creating a need for preventive and therapeutic

strategies that are safe for long-term use (2,3). Hyperpigmentation is multifactorial. Endogenous and exogenous contributors include genetics, hormones, diet, cosmetics, UV radiation, oral medications, inflammation, and systemic disease processes (3). Clinically, common categories include melasma, lentigines, post-inflammatory hyperpigmentation (PIH), and drug/chemical-induced hyperpigmentation (3). Epidemiological observations in Indonesian clinical settings have shown substantial pigmentation complaints, especially among women of productive age, often linked to prolonged exposure to chemical-containing cosmetics (4). These observations support the need to explore therapies targeting inflammation-driven and drug-induced hyperpigmentation pathways.

Drug-Induced Hyperpigmentation and the Role of Azidothymidine (AZT)

Drug-induced hyperpigmentation is an important subtype of pigmentation disorder, where medication exposure triggers melanogenesis, pigment deposition, or post-inflammatory changes (3). Azidothymidine (AZT), also known as zidovudine, is a nucleoside reverse transcriptase inhibitor historically used as one of the first antiretroviral drugs for HIV treatment (7,8). AZT has been reported to induce hyperpigmentation by increasing melanin-related changes in the skin (7). In addition, AZT exposure has been linked with inflammation and oxidative stress pathways in experimental contexts, suggesting that its adverse tissue effects may be mediated by inflammatory and redox imbalance mechanisms (10). Therefore, AZT-induced hyperpigmentation provides a biologically relevant model to evaluate candidate anti-melanogenic agents that act through anti-inflammatory and antioxidant pathways.

Inflammation as a Driver of Melanogenesis and PIH

Inflammation is a protective response triggered by tissue damage or invasion by foreign agents, involving vascular changes, leukocyte recruitment, and mediator release (5,6). Inflammatory mediators (e.g., histamine, serotonin, prostaglandins, bradykinin) and immune signaling events create the classical inflammatory manifestations (rubor, calor, tumor, dolor, functio laesa) (5,6). In skin biology, PIH is strongly linked to inflammatory cascades: inflammation alters keratinocyte–melanocyte signaling, stimulates melanogenic pathways, and can increase melanin transfer and melanosome distribution, leading to persistent dark patches after the primary insult resolves (28). Mechanistically, inflammatory cells and skin-resident cells (keratinocytes, fibroblasts, melanocytes) release cytokines and signaling molecules that modulate melanogenesis and tyrosinase activity. A comprehensive review by Chuhan et al. highlighted that inflammatory factors can regulate melanogenesis through multiple pathways, positioning inflammation as a key upstream driver of pigmentation changes (28). Thus, suppressing inflammation is a plausible route to reduce melanogenesis and mitigate PIH-like pigmentation.

TNF-α and IL-6: Key Cytokines Linking Inflammation to Pigmentation

Nearly all inflammatory events involve monocyte infiltration and macrophage activation, leading to the production of proinflammatory cytokines such as TNF, IL-1, and IL-6, which drive systemic and local inflammatory responses (9). TNF-α is a central cytokine in acute inflammation and host defense; it is typically low in healthy individuals but increases in inflammatory and infectious conditions (15,16). TNF-α can activate endothelial cells and leukocytes and amplify inflammatory signaling, which indirectly affects melanocyte behavior and pigmentation-related gene expression (15,16,28). IL-6 is a pleiotropic cytokine involved in inflammation, immune regulation, and acute-phase responses (11). It has been discussed both as a marker of severe inflammation and as a mediator with complex pro- and anti-inflammatory roles (11). Importantly for pigmentation biology, IL-6 can influence melanocyte proliferation and melanogenesis and may reduce tyrosinase activity under specific conditions (28). The combined modulation of TNF-α and IL-6 is therefore relevant for understanding and controlling inflammation-associated pigmentation.

Oxidative Stress and MDA as a Biomarker in Hyperpigmentation

Oxidative stress—an imbalance favoring reactive oxygen species (ROS) over antioxidant defenses—often accompanies inflammation and contributes to membrane damage and cellular dysfunction. Malondialdehyde (MDA) is a stable end-product of lipid peroxidation and widely used as a biomarker of oxidative stress (12–14). Elevated MDA levels reflect increased membrane oxidation and can contribute to mutagenic stress through reactive adduct formation with DNA components (12,13). Skin conditions involving inflammation (e.g., acne-related inflammatory states) have been associated with altered MDA levels, indicating a functional link between inflammation severity and oxidative damage (29). Since oxidative stress can enhance inflammatory signaling and potentially stimulate pigmentation pathways, reducing MDA may be relevant to anti-melanogenic strategies.

Celery Leaf (Apium graveolens L.) as a Candidate Anti-melanogenic Agent

Celery is widely consumed and increasingly investigated for medicinal properties. Celery leaves contain diverse phytochemicals, including flavonoids, tannins, saponins, apiin, and apigenin, which collectively support antioxidant and anti-inflammatory activity (17). Preclinical studies have demonstrated anti-inflammatory potential of celery extracts in inflammatory models (19), and broader natural-product research also supports plant-derived anti-inflammatory agents as alternatives or complements to synthetic compounds (20–22). Beyond general anti-inflammatory effects, celery leaf constituents relevant to pigmentation regulation are increasingly emphasized. Polyphenol-enriched celery fractions have been shown to modulate key inflammatory signaling pathways, including NF-κB and MAPK—two central regulators of cytokine production (33). Because NF-κB/MAPK activation promotes TNF-α and IL-6 production, inhibition of these pathways offers a mechanistic basis for reducing inflammatory drivers of melanogenesis (9,28,33).

Bioactive Compounds Related to Melanogenesis Inhibition: Evidence Base

Several compounds referenced or detected in celery-related profiling studies have literature support for anti-inflammatory and skin-related actions:

1. Kojic acid: Known for skin-lightening use and demonstrated anti-inflammatory and barrier-related effects in LPS-treated human keratinocytes, supporting relevance to inflammatory skin environments (30). Kojic acid is also widely recognized as a tyrosinase inhibitor in melanogenesis control (supported conceptually by its cosmetic use; mechanistic support in this manuscript can be anchored to keratinocyte inflammation findings) (30).
2. Gallic acid: A polyphenolic compound with broad bioactivity; its anti-inflammatory and antioxidant potential provides rationale for reducing oxidative stress–inflammation loops that contribute to hyperpigmentation (31).
3. Stachydrine (DL-stachydrine): Demonstrated ability to suppress inflammatory responses via inhibition of IκBα/NF-κB and MAPK signaling in an LPS-induced model, supporting its role in downregulating cytokine production (32).
4. Flavonoid-related activity: Deglycosylation of flavones can increase anti-inflammatory activity and absorption, suggesting that celery flavonoids may become more bioactive in vivo, strengthening the plausibility of systemic effects after administration (34).

Collectively, these findings provide a mechanistic basis for proposing that celery leaf extract may reduce inflammatory cytokines (TNF-α, IL-6) and oxidative stress (MDA), thereby indirectly suppressing melanogenesis and hyperpigmentation progression.

Safety Considerations: Phototoxicity Risk in Plant Extracts

While plant extracts provide bioactive benefits, safety considerations remain important for translation into topical or cosmetic applications. Phototoxic compounds in plants such as celery, citrus, and figs can cause phytophotodermatitis, which may itself trigger inflammation and subsequent hyperpigmentation (35). This highlights the importance of concentration optimization and formulation safety testing if celery extract is intended for skin-related use, especially under UV exposure conditions.

Research Gap and Rationale for the Current Study

Although celery extracts have been investigated for anti-inflammatory activity (19,33) and many anti-inflammatory natural products have been explored (20–22), direct evidence focusing on anti-melanogenic effects of celery leaf extract in drug-induced hyperpigmentation, particularly AZT-induced hyperpigmentation, remains limited. Given that AZT may induce pigmentation changes alongside inflammatory and oxidative stress pathways (7,10), examining celery leaf extract in this model is scientifically justified. By integrating inflammatory biomarkers (TNF-α, IL-6) and oxidative stress markers (MDA) with histopathological improvements, the current study positions celery leaf extract as a candidate agent with dual action: anti-inflammatory/antioxidant modulation that may contribute to reduced melanogenesis and improved hyperpigmentation outcomes.

MATERIALS AND METHODS

Materials

The material used in this study is celery leaves (*Apium graveolens L.*) grown in Medan, Indonesia. All other chemicals were of analytical grade purchased from local suppliers.

Study Design and Subject:

The study's test animals were male Wistar white rats (*Rattus norvegicus*) that were 2-3 months old, weighed about 150-200 grams, and were in good health. The research subjects were divided into five groups, each consisting of six male Wistar white rats. The celery leaf extract was given at 1%, 3% and 5% for 21 days. The effect of the celery extract on the sample was analysed every 2 days from 0 day until day 21. The following were the groups as shown in Table 1.

Table 1. Group of Sample Criteria

Group	Kode	Criteria
Group 1	Control	Control Group, which was provided with food and water in its cage as usual and received no other care
Group 2	K2SA	Positive Control, AZT 600 mg/kg body weight was given with a suspension of Salicylic Acid 45 mg/kg body weight
Group 3	K3S1%	AZT 55 mg/kg body weight, test suspension of celery leaf ethanol extract at 1%
Group 4	K4S3%	AZT 55 mg/kg body weight, test suspension of celery leaf ethanol extract at 3%
Group 5	K5S5%	AZT 55 mg/kg body weight, test suspension of celery leaf ethanol extract at 5%

Celery leaf extraction

The obtained plant material was allowed to dry completely at a temperature of 24–26 °C. Celery leaf extraction was prepared followed method by AOAC method[23], celery leaves were extracted through maceration with 96% ethanol. Ten simplicial portions should be placed in a dark container. Pour 75 parts 96% ethanol, cover, and leave for 5 days, sheltered from light, stirring frequently. Squeeze, strain, and wash the residue with enough solvent to yield 100 parts. After transferring the mixture to a closed container, store it for two days in a cool, dry location. Pour off the clear liquid without disturbing the sediment. Using a rotary evaporator set at ±40°C, the macerate is evaporated until a thick extract is produced. The ethanol-free extract was lyophilized and stored in a dark environment until it was needed.

Preparation of 0.5% Na CMC suspension

After evenly dispersing 0.5 g of Natrium Carboxymethyl Cellulose (Na CMC) into a mortar with 10 ml of distilled water (70°C), the mixture was covered and allowed to sit for 15 minutes until a translucent mass was formed. Then, it was powdered and made up to a 100 ml volume by diluting it with distilled water.

Preparation of ethanolic celery leaf extract suspension

Celery leaf ethanol extract (1%, 3%, and 5%) was then combined with 10 milliliters of 0.5% Na CMC solution.

Preparation of Salicylic acid suspension, Azidothymidine

Salicylic suspension was prepared by weighing 58.60 mg of salicylic acid powder and homogeneously crushed with 0.5% Na CMC suspension. It was then transferred to a 10 ml volumetric flask and filled to capacity with 0.5% Na CMC suspension. In order to prepare Azidothymidine (AZT), grind 50 mg of Azidothymidine until homogenous with 0.9% NaCl solution, then transfer to a 5 ml volumetric flask and fill to the mark with 0.9% NaCl solution. The combination was incubated for twenty-four hours at 37°C.

Measurement of Malondialdehyde (MDA)

Measurement of MDA followed method by Lauro et al[11]. A mortar was used to grind quartz sand and 0.5 grams of rat kidney tissue until it was smooth. Next, the mortar was filled with 200 µL of physiological NaCl. Following the homogenate's transfer, 550 µL of distilled water was put in a polypropylene tube. Next, 100 µL of TCA was added and homogenized. Then, 250 µL of 1N HCl was added and homogenized. The mixture was then added with 100 µL of 1% Na-Thio and centrifuged at 500 rpm for 10 minutes. Glass wool was used to collect and filter the supernatant. The obtained supernatant was boiled in a water bath at 100 °C for 20 minutes. The heated supernatant was then allowed to cool to room temperature.

Measurement of Interleukin 6 (IL-6) & Tumour Necrosis Factor alpha (TNF-α)

Measurement of IL-6 followed method by Nordan et al[24] using ELISA kit. Furthermore, measurement of TNF-α was done followed method by Valaperti et al[25]. The kit instructions were followed to ensure that the levels of TNF-α and Interleukin-6 were measured in male white rats. Male white rats from each treatment group had their serum levels of TNF-α and IL-6 measured, and the results were compared to the measurements in the control group

Detection of Bioactive compound by LC-MS Analysis

Detection of bioactive compound by LC-MS followed method Irakli et al[26] using A Shimadzu Nexera HPLC system (Kyoto, Japan) comprising two LC-30AD pumps, a DGU-20A5 degasser, a CTO-20AC column oven, a SIL-30AC auto injector, an SPD-M40 diode array detector (DAD), and a triple quadrupole mass spectrometer (model LCMS-2020) equipped with an electrospray ionization (ESI) interface was utilized to separate and identify the various phenolic compounds present in the aforementioned extracts. A Poroshell 120 EC-C18 column (4.6 150 mm, 4 m) was used to elute each sample. The column temperature was set to 35 C, and the flow rate was set to 0.5 ml/min. The mobile phase was made up of acetonitrile (solvent B) and 0.1% v/v aqueous formic acid (solvent A). The injection volume was 10 l. The gradient program was as follows: 0-5 min, 15-25% B; 5-10 min, 25-35% B; 10-28 min, 35-60% B; 28-28.01 min, 60-15% B; and an isocratic elution for 35 minutes. Step sizes for the DAD capture were 1.2 nm, spanning from 190 to 400 nm. An ESI source recorded on a negative ionization mode with +4.5kV and 20 V interface and curved desolvation line (CDL) voltages, respectively, was fitted to the mass spectrometer. High-purity nitrogen (N2) was utilized as the nebulizing gas at a f low rate of 1.5 L/min and nitrogen (N2) was used as drying gas at a flow rate of 15 L/min. The CDL temperature was kept at 250 C, while the block heater temperature was kept at 200 C. Both selective ion monitoring (SIM) and full scan mode (100–1000 m/z) were used for mass acquisitions. Data acquisition and processing was carried out using Lab Solutions LC-MS software (Shimadzu, Kyoto, Japan).

Detection of Bioactive compound by GC-MS Analysis

GC-MS analysis was done followed method Sun et al[27], by A 99.999% pure helium carrier gas was used to inject an extracted sample into the apparatus at a forward purge flow rate of 3 mL/min and a continuous over-column gas flow rate of 1 mL/min. The isothermal splitless mode of the Agilent DB-WAX (30 m 250 m 0.25 m, Agilent Technologies Inc., Santa Clara, CA, USA) column was utilized for the separation of volatile organic compounds. Starting at 50 C, the temperature of the GC column was raised by 6 C in 1 minute, ramping up to 230 C, and then maintained for 5 min. The quadrupole mass detector, ion source, and transmission line were set to 250, 230, and 150 C, respectively, of temperature. With an electron impact mode of 70 eV and a mass spectrometer scan range of (Mass/Charge Ratio) m/z 20 to 550 amu, MS detection was accomplished. An ionization energy of 70 eV was recorded using the electron ionization-mass spectrometry (EI-MS) technique. There was no delay in the solvent.

Statistical Analysis

The mean standard deviations of three parallel measurements are represented by the data shown in tables and figures. Based on the Duncan's multiple range test, one-way analysis of variance (ANOVA) was utilized to examine any variations between the means for various extracts. A significance level of 5% ($p = 0.05$) was used in this investigation to make a statistical test decision. There will be a post hoc Sheffe test if the p-value<0.05. There will be a Levene's test if the p-value is greater than 0.05. Version 19 of SPSS Statistics (IBM SPSS Inc., Chicago, IL, USA) was used to test the data, and differences at $p<0.05$ were deemed significant.

RESULT

MDA, IL-6 and TNF-α concentration in hyperpigmented Wistar rats (*Rattus Novergicus Sp.*) induced by Azidothymidine

After 21 days of treatment with celery leaf extract, the MDA level was determined using the ELISA method. According to Table 2, the MDA level was determined in sample K3S1%, K4S3% and K5S5% were 42.083 ± 3.49 nmol/ml, 37.032 ± 10.86 nmol/ml and 28.277 ± 10.27 nmol/ml respectively. IL-6 was the inflammatory mediator evaluated in this investigation. According to Table 2, K2SA had the highest IL-6 (17.193 ± 1.87), while the control group (0.433 ± 0.26), which did not receive any therapy and was merely given food and water in its cage, had the lowest value. The ELISA method was used to measure the TNF-α level, and observations were also conducted. Table 2 shows that the control group had the highest TNF-α level, 281.621 ± 14.76, whereas sample K2SA had the lowest TNF-α level, 778.589 ± 80.43.

Table 2. MDA, IL-6 and TNF-α concentration in Hyperpigmented Wistar Rats (*Rattus Novergicus Sp*.) Induced by Azidothymidine

Sample	MDA (nmol/ml)	IL-6 (nmol/ml)	TNF-α (nmol/ml)
Control	12.792 ± 2.19[a]	0.433 ± 0.26[a]	281.621 ± 14.76[a]
K2SA	62.29 ± 61.18[e]	17.193 ± 1.87[e]	778.589 ± 80.43[e]
K3S1%	42.08 ± 3.49[d]	14.588 ± 3.38[d]	667.614 ± 143.40[d]
K4S3%	37.032 ± 10.86[c]	11.939 ± 3.05[c]	452.812 ± 63.68[c]
K5S5%	28.277 ± 10.27[b]	4.539 ±1.40[b]	326.925 ± 62.72[b]

*Control= was provided with food and water in its cage as usual and received no other care; K2SA= Positive Control, AZT 600 mg/kg body weight was given with a suspension of Salicylic Acid 45 mg/kg body weight; K3S1%= AZT 55 mg/kg body weight, test suspension of celery leaf ethanol extract at 1%;K4S3%= AZT 55 mg/kg body weight, test suspension of celery leaf ethanol extract at 3%; K5S5%= AZT 55 mg/kg body weight, test suspension of celery leaf ethanol extract at 5%; MDA= Malondialdehyde; IL-6=Interleukin 6; TNF-α= Tumour Necrosis Factor alpha; Different superscripts letters in the same column indicate differences ($p < 0.05$) amongst the means, as determined by the Duncan's multiple range test.

Comparative analysis of celery leaf extract's effect on MDA, IL-6 and TNF-α level in hyperpigmented Wistar rats (*Rattus Novergicus Sp*.) induced by Azidothymidine

The MDA levels in each group of research samples were compared in each group of samples. There was a significant difference ($p<0.001$) in the MDA levels among all sample groups in Table 3. In order to examine the variations in MDA levels between sample groups with various sample sizes in further detail, a post hoc test was used. Based on the result indicates that there was a significant difference ($p<0.05$) in the MDA levels between all sample groups that received the celery leaf extract intervention and the negative control group.

Table 3. *Post hoc* analysis

Variable		P-value				
		Control	K2SA	K3S1%	K4S3%	K5S5%
MDA	Control	-	0.000	0.001	0.000	0.002
	K2SA	0.001	-	0.001	0.001	0.003
	K3S1%	0.001	0.000	-	0.001	0.002
	K4S3%	0.000	0.000	0.001	-	0.000
	K5S5%	0.001	0.000	0.001	0.001	-
IL-6	Control	-	0.000	0.001	0.000	0.002
	K2SA	0.001	-	0.001	0.001	0.003
	K3S1%	0.001	0.000	-	0.001	0.002
	K4S3%	0.000	0.000	0.001	-	0.000
	K5S5%	0.001	0.000	0.001	0.001	-
TNF-α	Control	-	0.000	0.001	0.000	0.002
	K2SA	0.001	-	0.001	0.001	0.003
	K3S1%	0.001	0.000	-	0.001	0.002
	K4S3%	0.000	0.000	0.001	-	0.000
	K5S5%	0.001	0.000	0.001	0.001	-

**Post Hoc Test Scheffe;*Control= was provided with food and water in its cage as usual and received no other care; K2SA= Positive Control, AZT 600 mg/kg body weight was given with a suspension of Salicylic Acid 45 mg/kg body weight; K3S1%= AZT 55 mg/kg body weight, test suspension of celery leaf ethanol extract at 1%;K4S3%= AZT 55 mg/kg body weight, test suspension of celery leaf ethanol extract at 3%; K5S5%= AZT 55 mg/kg body weight, test suspension of celery leaf ethanol extract at 5%; MDA= Malondialdehyde; IL-6=Interleukin 6; TNF-α= Tumour Necrosis Factor alpha

Table 3 shows a significant difference in IL-6 and TNF-α levels across all sample groups ($p<0.001$). The results demonstrated a significant difference in IL-6 and TNF-α levels between the negative control group and sample groups that received the celery leaf extract intervention ($p<0.05$).

LC-MS profile and GC-MS profile

Numerous phytochemical substances were discovered from the celery leaf extract based on the LC-MS analysis (Table 4). The delta mass values were within an acceptable range, indicating that the mass measurements of the discovered substances were reasonably accurate. Kojic acid had a delta mass of -126 ppm and DL-Stachydrine a value of -208 ppm. The compounds were found at varying retention times such as Limonin was found at 14.78 minutes and DL-Stachydrine at 1.092 minutes. This shows the elution order during the chromatography procedure. The chromatographic method revealed that asparagine could be found with the quickest retention time of 1.05 minutes. Meanwhile, the substances found in high concentrations that act as melanogenesis inhibitors are gallic acid, Kojic acid, and DL-Stachydrine.

Table 4. LC-MS Profile

No	Name	Formula	Annot. DeltaMass [ppm]	Calc. MW	RT [min]	Sample Area
1	Citric acid	C6 H8 O7	-1,07	192,0268	1,418	75993059,87
2	N-Acetylvaline	C7 H13 N O3	-0,5	159,08946	1,077	78164114,99
3	fructosylglycine	C8 H15 N O7	-1,53	237,08449	4,846	84349331,18
4	(Z)-2-Butene-1,2,3-tricarboxylic acid	C7 H8 O6	-1,36	188,03183	2,018	101374813,7
5	Hesperidin	C28 H34 O15	-2,04	610,18852	9,53	104017073,4
6	Gallic acid	C7 H6 O5	-1,74	170,02123	2,019	118971295,9
7	N2-(3,4,5-Trimethoxyphenyl)-1,3,5-triazine-2,4-diamine	C12 H15 N5 O3	-5,54	277,11595	1,108	119440856,1
8	DG(18:2(9Z,12Z)/18:3(9Z,12Z,15Z)/0:0)	C39 H66 O5	-0,81	614,49053	25,528	122288523,8
9	alpha-ketoadipic acid	C6 H8 O5	-1,63	160,03691	2,017	125809866
10	D-(+)-Pipecolinic acid	C6 H11 N O2	-0,1	129,07897	1,081	132759252,6
11	tranexamic acid	C8 H15 N O2	-1,18	157,11009	1,124	139416511,7
12	L-Phenylalanine	C9 H11 N O2	-1,37	165,07875	2,354	147308983,4
13	10-methoxy-4H-benzo[4,5]cyclohepta[b]thiophen-4-one	C14 H10 O2 S	-1,59	242,03977	4,847	149858245,6
14	Stigmatellin Y	C29 H40 O6	-2,84	484,28111	23,647	182368284,1
15	Asparagine	C4 H8 N2 O3	-0,64	132,05341	1,05	185276283,5
16	L-Glutamic acid	C5 H9 N O4	-2,04	147,05286	1,078	221578010,3
17	1,3-dilinolenoylglycerol	C39 H64 O5	-3,46	612,47325	27,888	262221642,7
18	L-Aspartic acid	C4 H7 N O4	-0,23	133,03748	1,068	274572711,9
19	Kojic acid	C6 H6 O4	-1,26	142,02643	2,016	279033973,1
20	D-(+)-Proline	C5 H9 N O2	0,71	115,06341	1,078	294258915,9
21	N-(2,4-dichloro-6-methylbenzyl)-2-methoxy-4-(trifluoromethyl)nicotinamide	C16 H13 Cl2 F3 N2 O2	12,09	392,03536	1,11	301294220
22	2-methylcitric acid	C7 H10 O7	-1,71	206,0423	2,018	397162448,1
23	2-(2-thienyl)-4H-chromen-4-one	C13 H8 O2 S	-2,04	228,02403	2,013	601158561
24	DL-Stachydrine	C7 H13 N O2	-2,08	143,09433	1,092	4648928796

*RT = Retention time; MW = molecular weight

The results of GCMS-based bioactive component screening in celery leaf extract are displayed in Table 5. Based on the results, the compounds with bioactivity, including antioxidant, anti-inflammatory, and skin-brightening properties, detected in the celery leaf extract are (-)-threo-isodihomocitric acid ($C_8H_{12}O_7$), Limonin ($C_{26}H_{30}O_8$), 5-Hydroxy-2-furoic acid ($C_5H_4O_4$), gamma-Aminobutyric acid ($C_4H_9NO_2$), Kojic acid ($C_6H_6O_4$), tranexamic acid ($C_8H_{15}NO_2$), alpha-Ketoglutaric acid (C_5H_6). Furthermore, most compounds showed modest delta mass values, such as 5-Hydroxy-2-furoic acid with a delta mass of -0.25 ppm and Kojic acid with a delta mass of -126 ppm, showing that the measurements were precise and in line with theoretical values.

Table 5. GC-MS Profile

No	Name	Formula	Annot. DeltaMass [ppm]	Calc. MW	RT [min]	Sample Area
1	(-)-threo-isodihomocitric acid	C8 H12 O7	-1,44	220,05799	4,277	33623420,96
2	4-Aminobenzoic acid	C7 H7 N O2	0,16	137,0477	1,081	33901483,52
3	.alpha.-Aminoadipic acid	C6 H11 N O4	-0,78	161,06868	1,118	33945202,07
4	N-glycoloyl-D-mannosaminolactone	C8 H13 N O7	-0,85	235,069	1,102	34211841,12
5	5-Hydroxy-2-furoic acid	C5 H4 O4	-0,25	128,01093	2,003	34801207,28
6	trans-Aconitic acid	C6 H6 O6	-0,63	174,01633	1,418	35237650,38
7	N-[4-(diethylamino)phenyl]-N'-phenylurea	C17 H21 N3 O	77617,62	305,14733	1,06	37516285,52
8	L-Pyroglutamic acid	C5 H7 N O3	-0,39	129,04254	1,14	41913515,49
9	Butyryl dihydrogen phosphate	C4 H9 O5 P	-2,48	168,01834	1,887	42422693,21
10	1-[(3-Carboxypropyl)amino]-1-deoxy-beta-D-fructofuranose	C10 H19 N O7	-0,86	265,11592	4,844	42559852,14
11	D-(+)-Pyroglutamic Acid	C5 H7 N O3	-0,39	129,04254	1,409	43130774,53
12	DL-Arginine	C6 H14 N4 O2	-0,93	174,11151	1,024	45610132,54
13	Kojic acid	C6 H6 O4	-1,26	142,02643	4,847	47046391,63
14	N-[(4E)-1-(Hexopyranosyloxy)-3-hydroxy-4-octadecen-2-yl]-2-hydroxyhexadecanamide	C40 H77 N O9	-0,39	715,55956	29,254	48867626,56
15	2-Methoxy-4-[3,5,6-trihydroxy-7-(sulfinooxy)-3,4-dihydro-2H-chromen-2-yl]phenyl hydrogen sulfate	C16 H16 O12 S2	1,97	464,00923	2,015	52196337,48
16	2-Amino-1,3,4-octadecanetriol	C18 H39 N O3	-2,62	317,29216	17,393	53867903,58
17	alpha-Ketoglutaric acid	C5 H6 O5	-1,09	146,02136	1,41	54029380,32
18	N-[(4E)-1-(Hexopyranosyloxy)-3-hydroxy-4-octadecen-2-yl]-2-hydroxyhexadecanamide	C40 H77 N O9	-0,39	715,55956	28,565	55348557,66
19	L-(+)-Valine	C5 H11 N O2	0,94	117,07909	1,125	59095147,5
20	tranexamic acid	C8 H15 N O2	-1,18	157,11009	1,414	60039163,46
21	gamma-Aminobutyric acid	C4 H9 N O2	1,82	103,06352	1,049	60416830,43
22	5-methyl-4-{[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy}-2H-chromen-2-one	C16 H18 O8	112279,11	376,06175	1,174	62537173,29
23	O-ureido-D-serine	C4 H9 N3 O4	0,7	163,05942	0,939	65692968,34
24	5-Hydroxy-2-furoic acid	C5 H4 O4	-0,25	128,01093	1,409	65762741,67
25	Limonin	C26 H30 O8	-1,86	470,19319	14,78	67219585,41
26	(-)-threo-isodihomocitric acid	C8 H12 O7	-1,44	220,05799	4,845	72988774,81

*RT = Retention time; MW = molecular weight

DISCUSSION:

The purpose of this study was to investigate the effect of celery leaf extract (*Apium graveolens L.*) on inflammation in hyperpigmented Wistar rats (*Rattus norvegicus Sp*) produced by Azidothymidine. Inflammation is a local defensive reaction triggered by trauma or tissue damage that seeks to remove or reduce dangerous agents or damaged tissues. Pain, heat (calor), redness (rubor), swelling (tumor), and loss of function (functio laesa) are the symptoms of inflammation in its acute phase. Allergens, infections, chemical stimuli, and physical injury, according to Chuhan[28], can produce skin inflammation in response to external or endogenous stimuli. Through its ability to combat bacterial invasion and other pathogens and to promote wound healing, skin inflammation plays a vital role in the body. Histologically, it is characterized by a complicated set of processes, including artery, capillary, and venule dilatation, enhanced blood flow permeability, exudation of plasma proteins, and leukocyte recruitment to the inflammation site. The release of mediators (histamine, serotonin, prostaglandins, and kinins) regulates and activates blood and tissue cells, causing this response and potentially resulting in tissue damage symptoms. The metabolism of arachidonic acid, an unsaturated fatty acid containing 20 carbon atoms, leads to inflammation. Azidothymidine (AZT or zidovudine) is a chemical that inhibits the DNA polymerase activity of the HIV-1 RT (Reverse Transcriptase) enzyme and has been suggested as the first antiretroviral medication for HIV-1 treatment[7]. The first antiviral medication authorized for the treatment of human immunodeficiency virus (HIV) was azidothymidine (AZT).

MDA levels were determined using the ELISA technique following 21 days of celery leaf extract treatment. Control sample had the lowest MDA level (12.792 ± 2.19 nmol/ml), while sample K2SA had the highest (62.296 ± 61.18 nmol/ml) MDA level. This is similar with Kodariah's[28] finding that celery extract lowered MDA levels in rat plasma and possibly minimize oxidative stress. Oxidative stress is brought on by an imbalance between oxidants and antioxidants, with oxidants predominating. Compared to other aldehydes, MDA, a byproduct of lipid peroxidation, is more mutagenic[29]. MDA is frequently employed as a measure of oxidative stress since it is chemically stable. A pathological process known as inflammation is the body's protective response to damaging stimuli through vascular reactions in living tissue. The chemical components involved in mediating inflammatory reactions are called inflammatory mediators or chemical mediators. Th cells, lymphocytes, macrophages, monocytes, and other related cells release inflammatory mediators. Th1 cells secrete cytokines such as IL-2, IL-3, and tumor necrosis factor (TNF), which are essential components of cellular immune responses[28]. Th2 cells secrete IL-4, IL-5, IL-10, IL-13, IL-3, and other cytokines, which are critical for humoral immune responses[30]. Certain disorders cause a disruption in the equilibrium between Th1 and Th2, which leads to a shift toward either Th1 or Th2. Helper T cells 17 (Th17) are a subpopulation of T cells that release IL-6 and participate in innate immunity and inflammation by secreting IL-7, IL-6, and TNF-α. Melanocytes can release TNF-α, however fibroblasts can only secrete TNF and IL-6. Different skin cell types release inflammatory mediators.

TNF-α levels were evaluated using the ELISA technique following 21 days of celery leaf extract treatment. Sample K2SA had the highest TNF-α level (775.581 ± 80.43 nmol/ml), while K3S1% had the highest TNF-α level among the sample with celery leaf extract treatment (667.614 ± 143.41 nmol/ml). TNF-α levels were shown to decrease when celery leaf extract dosage was increased. Celery leaf extract containing Kojic acid, gallic acid, and DL-Stachydrine demonstrated potential as anti-inflammatory drugs by lowering TNF-α levels, especially in endothelium and cardiac cell-related inflammation[30,31,32]. Th1, Th17, Th22, monocytes, macrophages, keratinocytes, dendritic cells, and two different receptors are the sources of this homotrimer cytokine, known as TNF. TNF not only causes inflammation via immune cells and vascular endothelial cells, but it also controls apoptosis, which in turn controls the growth of lymphoid tissues[28]. According to Chuhan[28], TNF inhibition can quickly restore the expression of genes related to pigmentation. Furthermore, IL and TNF together have the ability to suppress melanogenesis. Thus, increasing the dosage of celery leaf extract will decrease TNF levels, which can lessen hyperpigmentation.

Interleukin (IL-6) is able to control melanogenesis and the proliferation and differentiation of epidermal melanocytes both directly and indirectly[28]. By controlling cell proliferation, survival, and differentiation, IL-6 is released by keratinocytes, epidermal cells, fibroblasts, and dermal endothelial cells. It is implicated in immunological responses, inflammation, haematopoiesis, and cancer. Tyrosinase activity and melanogenesis can both be decreased by IL-6[28]. The study found that K3S1% had the highest IL-6 level among the celery leaf extract groups, at 14.588 ± 3.384 nmol/ml. The levels of IL-6 decrease with increasing celery leaf extract concentration. Numerous bioactive substances with anti-inflammatory properties found in celery leaf extract have the ability to inhibit the synthesis of pro-inflammatory cytokines like IL-6. These bioactive substances lessen oxidative stress and prevent the NF-κB pathway—the main controller of cytokine production—from being activated[30,33]. This investigation supports Hostetler et al.'s[34] discovery that celery leaf extract can reduce and avoid inflammation and gastrointestinal irritation. Phototoxic chemicals found in a variety of plants, such as citrus fruits, celery, carrots, and figs, can cause phototoxic reactions[35]. Hyperpigmentation may arise due to increased melanosomes. Celery extract contains luteolin, ferulic acid, and caffeic acid in addition to being a strong source of polyphenol antioxidants[35]. These substances are able to counteract the harm that free radical reactions cause. Celery extract can be utilized as a cosmetic ingredient at specific concentrations, according to the study's findings.

Based on the sample area and LCMS analysis, citric acid was shown to be the smallest compound in celery leaf extract. The extract had higher levels of gallic acid than hesperidin. Furthermore, the sample area of Kojic acid was smaller than that of DL-Stachydrine, suggesting a lower concentration of Kojic acid. The molecules with higher molecular weight and more polarity were L-aspartic acid, Kojic acid, and DL-Stacchydrine.

(-)-threo-isodihomocitric acid was shown to be the most prevalent chemical in celery leaf extract by GCMS analysis, with limonin and Kojic acid following closely behind. Limonin, 5-hydroxy-2-furoic acid, and gamma-aminobutyric acid (GABA) are non-polar and more volatile molecules that can be detected by GCMS. This work supports the findings of Naghneh et al[36], who discovered that the primary bioactive components in celery extract are phthalides, sesquiterpenes, and monoterpenes, with limonene being the most prevalent monoterpene found in the extract. The bioactive chemicals found in celery leaf extract have the ability to decrease cytokines, including TNF-α and IL-6, which are involved in the induction of chemokines that aid in the recruitment of immune cells to sites of damage and infection. By decreasing the generation of reactive oxygen species (ROS) by stimulated immune cells and keratinocytes.

CONCLUSION

According to the research findings, male Wistar rats (Rattus norvegicus sp.) that were hyperpigmented as a result of Azidothymidine induction showed a reduction in the percentage of inflammation when exposed to an ethanol extract of celery leaves. Following 21 days of celery leaf extract administration, the study demonstrated substantial changes in MDA, TNF-α, and IL-6 levels between the normal group and the positive control group (whether or not treated with celery ethanol extract). The reduction in MDA (malondialdehyde) levels in male Wistar rats experiencing hyperpigmentation from Azidothymidine induction to 28.277 ± 10.27 nmol/ml with increasing concentration of celery leaf extract is indicative of this. Moreover, the reduction in TNF-α levels suggests that celery leaf extract has an impact on inflammatory modulation. TNF-α levels decreased as the concentration of celery leaf extract was increased from 1% to 5%, going from 667.614 ± 143.41 nmol/ml to 326.925 ± 62.72 nmol/ml. Additionally, IL-6 levels were significantly lower in the study, suggesting that celery leaf extract may have anti-inflammatory properties. Celery leaf extract dosage increased from 1% to 5% resulted in a decrease in IL-6 levels from 14.588 ± 3.38 nmol/ml to 4.539 ± 1.40 nmol/ml.

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