AUCTORES
Globalize your Research

Clinical Medical Reviews and Reports

Thigita A Pandaleke *

Open Access

Research Article

Orthosiphon Aristatus Alleviates Skin Barrier Through Cytokines Regulation in DNCB- Induced Atopic Dermatitis Balb/C Mice Model

Kusworini Handono [1], Dhelya Widasmara [2], Hani Susianti [1], Thigita A Pandaleke [3*]

[1] Departement of Clinical Pathology, Faculty of Medicine, Brawijaya University, Kota Malang, Jawa Timur 65145, Indonesia.

[2] Department of Dermatology and Venereology, Faculty of Medicine, Brawijaya University, Kota Malang, Jawa Timur 65145, Indonesia.

[3] Doctoral Program of Medical Science, Brawijaya University, Kota Malang, Jawa Timur 65145, Indonesia.

***Corresponding Author:** Thigita A Pandaleke, Departement of Clinical Pathology, Faculty of Medicine, Brawijaya University, Kota Malang, Jawa Timur 65145, Indonesia.

Received Date: January 05, 2023; **Accepted Date:** January 23, 2023; **Published Date:** January 31, 2023

Citation: Kusworini Handono, Dhelya Widasmara, Hani Susianti, Thigita A Pandaleke (2023), Orthosiphon Aristatus Alleviates Skin Barrier Through Cytokines Regulation in DNCB- Induced Atopic Dermatitis Balb/C Mice Model, *Clinical Medical Reviews and Reports*, 5(3); **DOI:10.31579/2690-8794/165**

Abstract

Background: to analysed the effect of Orthosiphon aristatus on repairing skin lesions by regulating cytokines such as IgE, IL4, IL22, PGE2, NO in DNCB-induced Atopic Dermatitis BALB/C Mice Model

Methods: used BALB/C Mice which were sensitized by DNCB for 21 days to developed atopic dermatitis model. Mice were administered oral Orthosiphon aristatus extract once daily (on last 14 days after 7 days of sensitization). The doses given were divided into 6 groups: 17.5 mg/kgbw, 35 mg/kgbw, 70 mg/kgbw, and 140 mg/kgbw. We analysed the levels of cytokines such as IgE, IL4, IL22, PGE2 obtained from the blood. Additionally, we also measured morphological skin lesion severity to confirmed the amelioration effect clinically.

Results: Administration of Orthosiphon aristatus extract reduced the skin lesion severity in all intervention groups. The clinical improvement was supported by decrease of IgE, IL4, IL22, and PGE2 in dose dependent manner.

Conclusion: Orthosiphon aristatus alleviates DNCB-induced Atopic Dermatitis BALB/C Mice Model clinically through downregulating of IgE, IL4, IL22, PGE2, NO levels.

Keywords: orthosiphon aristatus; atopic dermatitis; 2,4-dinitrochlorobenzene; IgE; IL4; IL22; PGE2; NO

Introduction

Atopic Dermatitis (AD) is a chronic skin inflammation, recurrent, characterized by itching, arising in certain predilection sites, and associated with other atopic diseases such as allergic rhinitis and asthma. The clinical manifestations of AD are primarily characterized by pruritus, erythematous lesions in acute and lichenification in chronic state, and dry skin. Itch-scratch cycle in AD also triggers secondary infection [1]. The etiology of atopic dermatitis is not fully understood. It might be multifactorial, such as genetic, destruction of skin barrier, immunological, environment like food and aeroallergen. All of those induce a pathological pathway including inflammation and oxidative stress [2].

Atopic dermatitis begins with the destruction of the skin barrier. Destruction to the skin barrier causes increased production of keratinocyte cytokines such as Thymic Stromal Lymphoprotein (TLSP), IL33, IL1, IL6, IL8, and TNFα. These cytokines are able to stimulate formation of Th2, secrete IL4, IL13, and IL5, and produce IgE by B cells, increasing the expression of endothelial cell adhesion molecules, and eosinophil cell proliferation [3, 4]. Increase of IgE serum levels were found in the majority of AD cases and has a significant relationship with severity of AD [5]. Therefore, IgE is one of the criteria for diagnosing AD [3, 4]. In addition, Th2 and IL22 cells were reported to be involved in the inflammatory mechanism in AD. The cytokine IL22 is reported to cause epidermal hyperplasia and inhibit keratinocyte differentiation and filaggrin formation. Fillaggrin plays a role in the formation of Natural Moisturizing Factor (NMF) which keeps the skin moist. Interestingly, IL22 production is influenced by prostaglandin secretion. Previous studies reported an increase in Prostaglandin (PG) D2 and PGE2 in AD lesions followed by an increase in IL22 production [6, 7]. Robb et al (2018) showed that the DNCB-sensitized DA rats' model had EP4 deficiency

and increased IL22 production by T cells in lymph nodes [8]. PGD2 is major prostanoid produced by activated mast cells. The binding of PGD2 to the CTH2 receptor induces chemotaxis of Th2 cells, eosinophils, and basophils which exacerbates the inflammatory process in AD [6]. In addition to the inflammatory process, oxidative stress also plays a role in the mechanism of AD. ROS and NOS which increase either acutely or chronically cause damage to keratinocytes through the destruction of DNA, enzymes and cell membrane structures, thereby reducing the integrity of the skin barrier. Apart from originating from environmental free radicals, oxidative stress is also generated through inflammatory processes. During the inflammatory process, inflammatory cells such as macrophages secrete pro-inflammatory cytokines and nitric oxide (NO) [9, 10]. Therefore, nitric oxide (NO) is an important parameter of skin damage due to oxidative stress and inflammation in AD patients. Based on these mechanisms it can be concluded that effective therapy in AD is by repairing the skin barrier, inhibiting the inflammatory process, and reducing oxidative stress.

Therapeutic modalities currently available are systemic, topical, or phototherapy. The main purposes of the therapy are reduced the severity, prevent infection, and control in long term. Pharmacological therapy includes corticosteroids to treat inflammation, antibiotics for bacterial infections, antihistamines for pruritic symptoms, and calcineurin inhibitors to prevent the spread of eczema and reduce inflammation. Additionally, immunomodulation and phototherapy also been reported to have significant therapeutic effect [11, 12]. Long-term use of systemic corticosteroids in AD patients can cause side effects such as metabolic (hyperglycemia, hypertension, hyperlipidemia), gastrointestinal (gastric ulcers, gastritis, pancreatitis, ulcerative colitis), and Cushing's syndrome. Meanwhile, topical corticosteroids can have side effects on the skin such as skin atrophy, striae, purpura, telangiectasia, hypertrichosis, hypopigmentation, acneiform eruptions, and perioral dermatitis [1]. Antimetabolite groups such as mycophenolate mofetil, azathioprine can cause myelosuppression, especially in elderly users and those with kidney disorders, besides that, antimetabolite groups are also not recommended for use in pregnant women (Category D) [1]. Therapies that are currently being developed such as biologic agents have good effects but are expensive and require multiple injections, making them difficult to give to pediatric patients, and are less curative [13]. Therefore, there is demand of therapeutic modalities with minimal side effects and cost effectiveness, such as the use of bioactive compound from nature like Orthosiphon aristatus. Orthosiphon aristatus has antioxidant and anti-inflammatory activity which is played by phenolics, flavonoids, diterpenes, triterpenes [14, 15]. An in vitro study on murine macrophages conducted by Laavaola et al reported that the chloroform extract from Orthosiphon aristatus leaves, especially the eupatorine and sinestein contents, inhibited iNOS expression and reduced NO and PGE2 production, thereby reducing IL22 levels produced by Th cells [16]. Orthosiphon aristatus ethanol extract and its bioactive compounds (ursolic acid) has the most prominent inhibitory ability by suppressing LPS-induced production of NO and prostaglandin E2 (PGE2) by inhibiting ROS formation while reducing iNOS and COX2 expression in RAW 264.7 cells [17]. Therefore, Orthosiphon aristatus is a potential candidate for effective atopic dermatitis therapy with minimal side effects and cost effectiveness. This study uses Orthosiphon aristatus as an intervention for therapy of DNCB-induced atopic dermatitis BALB/c mice model. This study showed the clinical symptoms of AD pre and post intervention, and analszed serum IgE, NO, IL4, IL22, PGE2, and NO levels as parameters of atopic dermatitis. Additionally, this is the first study that observed the effect of Orthosiphon aristatus in atopic dermatitis.

Methods

Animals and experimental design

This research was conducted at the research and animal handling laboratory, Brawijaya University, Malang. This study used 36 BALB/C mice (aged 6 weeks) with 15–20 grams in weight. This model adopted a previous study (Son et al., 2019) which used 2,4 dinitrochlorobenzene (DNCB) to sensitize and made an atopic dermatitis model. Mice was maintained in a cage with room temperature (22 ± 20C), 40–60% of humidity for acclimatization in a week. They were maintained with food and drink ad libitum with a 12:12 hour dark-light cycle. After a week of acclimatization, their back hair was shaved and started to sensitize the skin with 200 µL of DNCB 1% daily for 7 days. Afterwards, they were sensitized with DNCB 0,5% for 2 weeks (three times a week), while they were given oral Orthosiphon aristatus leaves extracted once daily. These were divided into six groups (@6 mice), such as negative control (healthy mice), positive control (AD mice), P1 (17,5 mg/kgbw), P2 (35 mg/kgbw), P3 (70 mg/kgbw), and P4 (140 mg/kgbw). Finally, at day 22nd, they were sacrificed and their heart blood was drawn to examine atopic dermatitis markers including IgE, NO, IL4, IL22, PGE2, and NO.

HPLC analysis of Orthosiphon aristatus leaves extract

This study used Orthosipon aristatus leaves extract for experimental study. The extract powder mixed with diluted water until the final volume became 1500 uL. Next, put it in a vortex with 2000 rpm for 2 minutes, then spindown at 6000 rpm for 2 minutes. After that, the supernatant was collected using filter syringe 0,22 um. The HPLC analysis was performed using a Thermo Scientific Dionex Ultimate 3000 RSLCnano with micro flow meter, set up with Hypersil GOLD aQ 50 x 1 mm x 1.9 u particle size. Analytical flow rate of the LC procedure was 40 uL/minute. The procedure was running for 30 minutes in a 300 C of column temperature. The HPLC analysis was paired to High Resolution Mass Spectrometry (Full scan 17500 resolutions) and used Compound Discoverer with mzCloud MS/MS Library as data analysis software.

Clinical Symptoms Measurement

Clinical symptoms were observed and scored at pre and post experimental therapy according to the criteria [18], such as erythema/hemorrhage, edema, excoriation/erosion, and scaling/dryness. Each clinical symptom scored from 0 to 3 (none, 0; mild, 1; moderate, 2; and severe, 3), and the maximum score is 12. The higher score indicated the severity.

Enzyme-linked immunosorbent assay (ELISA)

For assessing the atopic dermatitis markers such as IgE, NO, IL4, IL22, and PGE2, the blood was collected from the heart and serum separated by centrifugation. The markers were measured using an Enzyme-Linked Immunosorbent Assay (ELISA) Kit from Elabscience E-EL-M3034 for IgE, E-BC-K035-M for NO, E-EL-0034 for PGE2, E-EL-M2446 for IL22, and BT LAB E0051Mo for IL4. The optical density (OD) (ng/ml) value was calculated in two replicates by the ELISA reader machine with standard curve for quantification formula.

Statistical analysis

The statistical analysis was performed using SPSS v11.5. Data were collected and presented as mean ± SD. One-way ANOVA was used to comparation test and Tukey HSD as a post hoc test, with $P < 0.05$ was considered statistically significant.

Results

Effect of Orthosipon aristatus Leaves Extract on Skin Lesion Severity

Firstly, we examined the effect of Orthosipon aristatus leaves extract on atopic dermatitis symptoms. Sensitization of DNCB on BALB/C mice group showed AD-like signs and symptoms such as erythematous, pruritus, erosion or excoriation, scratching, and dryness of the skin. Meanwhile no alteration was observed for both control groups. The severity of skin lesion scores of intervention groups was significantly reduced after six weeks ($P = 0.000$) (Figure. 1).

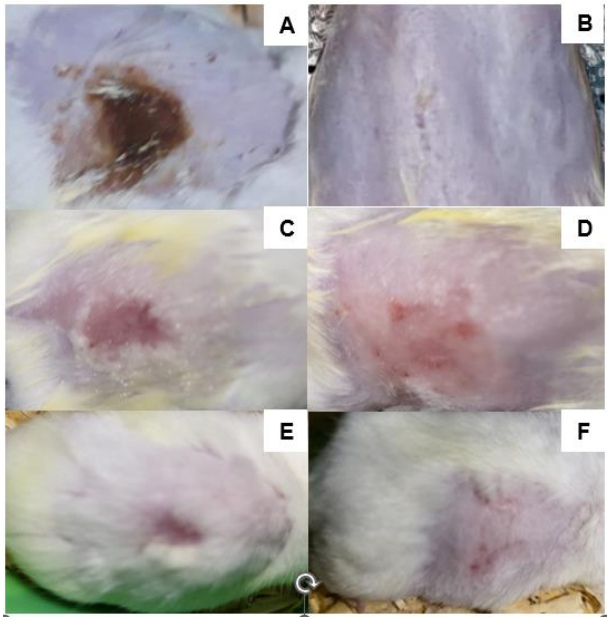

Figure 1: Severity of Skin Lesion Score. **A.** Positive Control Group. **B.** Negative Control Group. **C.** P1 Intervention Group. **D.** P2 Intervention Group. **E.** P3 Intervention Group. **F.** P4 Intervention Group.

Effect of Orthosipon aristatus Leaves Extract on IgE Serum Levels

The mean of IgE levels among six groups showed significant difference (p-value = 0.000<∝) (Fig. 2). The mean IgE level between the positive control group (10286 ± 4018 ng/mL) and the P1 treatment group (7305 ± 2702 ng/mL) showed no significant difference. Therefore, the administration of Orthosipon aristatus leaf extract 175 mg/kgBW can be said not to be enough for reducing the average IgE level in BALB/c mice with AD significantly. However, there was a significant difference in mean IgE levels between the positive control group (10286 ± 4018 ng/mL) and the P2 treatment group (4580 ± 758 ng/mL), P3 (2599 ± 575 ng/mL), and also with P4 (1418 ± 276 ng/mL). This reflected that the administration of Orthosipon aristatus leaf extract at doses of 35 mg/kg, doses of 7 mg/kg, and doses of 140 mg/kg in BALB/c mice with AD was able to reduce the average IgE level. There is no significant difference in the mean IgE level between the P1 (7305 ± 2702) and P2 (4580 ± 758) groups. Even though it was very clear that the mean IgE levels in the P2 group was lower than the IgE levels in the P1 group. It showed a 35 mg/kgBW of Orthosipon aristatus leaf extract given to BALB/c mice with AD was able to reduce the average IgE level faster than a dose of 17,5 mg/kgBW, but both doses have same ability to reduce IgE levels.

The mean IgE level between the positive control group (10286 ± 4018 ng/mL) and the P1 treatment group (7305 ± 2702 ng/mL) showed no significant difference. Therefore, the administration of Orthosipon aristatus leaf extract 175 mg/kgBW was not enough for reducing the average IgE level in BALB/c mice with AD significantly. However, significant difference in mean IgE levels was shown between the positive control group (10286 ± 4018 ng/mL) and P2 treatment group (4580 ± 758 ng/mL), P3 (2599 ± 575 ng/mL), and also with P4 (1418 ± 276 ng/mL). This reflected that the administration of Orthosipon aristatus leaf extract at doses of 35 mg/kg, doses of 7 mg/kg, and doses of 140 mg/kg in BALB/c mice with AD was able to reduce the average IgE level. There is no significant difference in the mean IgE level between the P1 (7305 ± 2702) and P2 (4580 ± 758) groups. Even though it was very clear that the mean IgE levels in the P2 group was lower than the IgE levels in the P1 group. It showed a 35 mg/kgBW of Orthosipon aristatus leaf extract given to BALB/c mice with AD was able to reduce the average IgE level faster than a dose of 17,5 mg/kgBW, but both doses have same ability to reduce IgE levels.

In four treatment groups, P1 (7305 ± 2702 ng/mL), (P2 (4580 ± 758 ng/mL), P3 (2599 ± 575 ng/mL), and P4 (1418 ± 276 ng/mL) showed no significant difference. Decrease of IgE level was along with an increase in the dose of Orthosipon aristatus leaf extract given to BALB/c mice with AD.

Furthermore, it can be concluded that BALB/c mice with AD show high levels of IgE. In addition, administration of Orthosipon aristatus leaf extract was shown to reduce the average IgE level in BALB/c AD mice, and the significant doses started with 35 mg/kg.

Effect of Orthosipon aristatus Leaves Extract on IL4 Serum Levels

The mean of IL4 levels among six groups showed that there was a significant difference (Fig. 3). This is indicated by the p-value = 0.000<∝. The mean IL4 levels between the positive control group (780 ± 94 ng/L) and the P1 treatment group (598 ± 61 ng/L) showed a significant difference. This means that the administration of Orthosipon aristatus leaf extract 175 mg/kgBW can be said to be able to reduce the average IL4 level in BALB/c mice with AD. Likewise, a significant difference was shown in the mean IL4 levels between the positive control group (780 ± 94 ng/L) and the treatment groups P2 (490 ± 32 ng/L), P3 (322 ± 31 ng/L), and P4 (222 ± 56ng/L). The mean value of IL4 levels in the P2, P3, and P4 treatment groups was lower than the mean IL4 levels in the positive control group. This means that the administration of Orthosipon aristatus leaf extract at a dose of 35 mg/kg, at a dose of 70 mg/kg, and at a dose of 140 mg/kg, can be said to be able to reduce the average IL4 level in BALB/c mice with AD. This also means that the average IL4 level will decrease as the dose of Orthosipon aristatus leaf extract is given to BALB/c mice with AD. However, no significant difference in the mean IL4 levels between the P3 (322 ± 31 ng/L) and P4 groups (222 ± 56 ng/L). Even though, it was obvious that the mean IL4 level in the P4 group was lower than the IL4 level in the P3 group. This means that a dose of 140 mg/kgBW of Orthosipon aristatus leaf extract given to BALB/c mice with AD was able to reduce the average IL4 level faster than a dose of 70 mg/kgBW, but both doses had the same ability.

It can be concluded that BALB/c mice with AD show high levels of IL4. In addition, administration of Orthosipon aristatus leaf extract at a dose of 17,5 mg/kg, at a dose of 35 mg/kg, at a dose of 70 mg/kg, and at a dose of 140 mg/kg, was shown to be able to reduce the average IL4 level in AD model BALB/c mice, and the effective dose starting from a dose of 17,5 mg/kgBB.

Effect of Orthosipon aristatus Leaves Extract on IL22 Serum Levels

The mean of IL22 levels among six groups showed significant difference (Figure. 4).

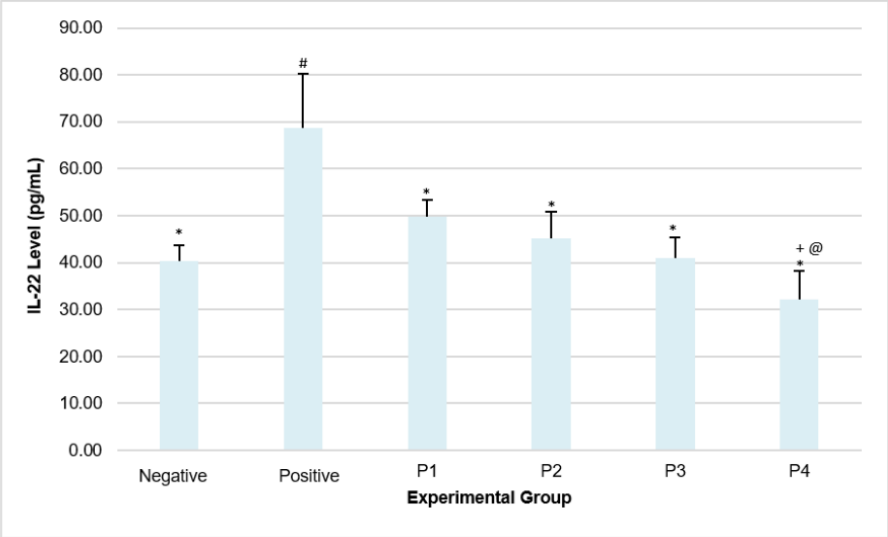

Figure 4: Measurements of IL22 Serum Levels. Data are expressed as mean ± SD. *P<0.05 compared with Positive, #P<0.005 compared with Negative, +P<0.005 compared with P1, @P<0.005 compared with P2

This is indicated by the p-value = 0.000<∝. Significant difference was shown in the mean IL22 levels compared with positive control and P1 groups. This means that the administration of 17,5 mg/kgBW Orthosipon aristatus has been able to reduce IL22 level in BALB/c mice with DA. Levels of IL22 between the positive control group (68.72 ± 11.48 pg/mL) and the treatment group P2 (45.18 ± 5.63 pg/mL), P3 (41.05 ± 4.28 pg/mL), and P4 (32.13 ± 6.08 pg/mL) were also shown a significant difference. Along with increasing doses, the mean value of IL22 levels showed a decreasing trend compared to the positive control group. However, significant difference in the mean IL22 level between the P1 treatment group (49.87 ± 3.57 pg/mL) and the P2 treatment group (45.18 ± 5.63 pg/mL) and P3 (41.05 ± 4.28 pg/mL), but it was significantly different with P4 (32.13 ± 6.08 pg/mL). Meanwhile, no significant difference between the P3 and P4 groups, even though the IL22 level in P4 was lower than in P3. Therefore, it can be concluded that the administration of Orthosipon aristatus leaf extract was able to reduce IL22 levels in DNCB-induced BALB/c AD mice with a starting dose of 175 mg/kg, and the effect is increased with administration 140 mg/kg of doses.

Effect of Orthosipon aristatus Leaves Extract on PGE2 Serum Levels

The results of this study showed significant difference of PGE2 levels in positive control group (8468 ± 1757 pg/mL) and the P1 treatment group (6457 ± 1051 pg/mL). The mean value of PGE2 levels in the P1 was lower than the mean PGE2 levels in the positive control group. This means that the administration of Orthosipon aristatus leaf extract 175 mg/kgBW has been able to reduce the average PGE2 level in BALB/c mice with AD. The same was shown in the P2 treatment group (3202 ± 509ad pg/mL), P3 (1686 ± 242ad pg/mL), and P4 (1510 ± 246d pg/mL). The trend of decreasing PGE2 levels continued with increasing doses (Figure. 5).

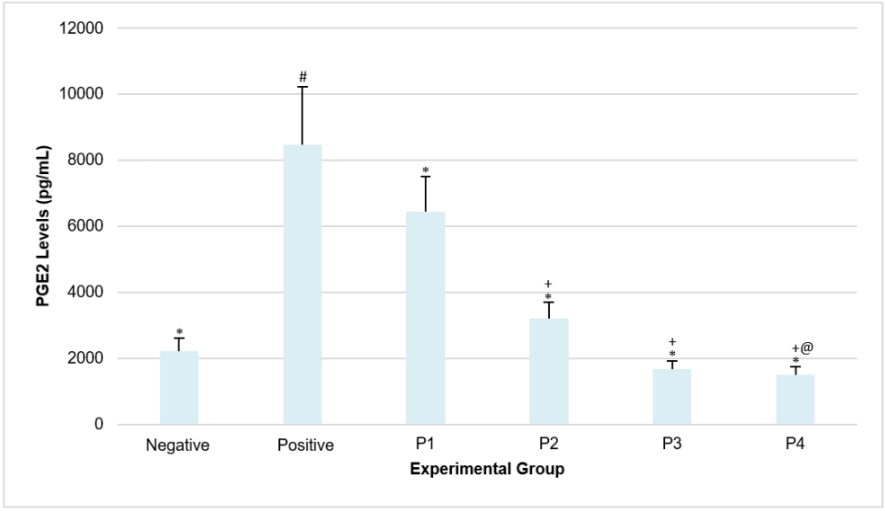

Figure 5: Measurements of PGE2 Serum Levels. Data are expressed as mean ± SD. *P<0.05 compared with Positive, #P<0.005 compared with Negative, +P<0.005 compared with P1, @P<0.005 compared with P2

PGE2 level in P1 treatment group (6457 ± 1051 pg/mL) compared with P2 treatment group (3202 ± 509 pg/mL), P3 (1686 ± 242ad pg/mL), and P4 (1510 ± 246d pg/ mL) was significant difference. However, no significant difference in P2 treatment group (3202 ± 509ad pg/mL) and the P3 group

(1686 ± 242ad pg/mL) and also with P4 (1510 ± 246d pg/mL). It was shown also in P3 treatment groups (1686 ± 242ad pg/mL) and P4 (1510 ± 246d pg/mL). In other words, doses of 35 mg/kg, doses of 70 mg/kg, and doses of 140 mg/kg were considered to have the same ability to reduce the average PGE2 level in BALB/c AD mice.

Effect of Orthosipon aristatus Leaves Extract on NO Serum Levels

NO levels between the positive control group (1.718 ± 0.356 uM) and the P1 treatment group (1.186 ± 0.308 uM) showed a significant difference. Positive control group (1.718 ± 0.356 uM) compared with the other treatment groups, P2 (0.931 ± 0.263 uM), P3 (0.423 ± 0.088a uM), and P4 (0.260 ± 0.046 uM) was shown significant difference (Figure. 6). 005 compared with P1, @P<0.005 compared with P2

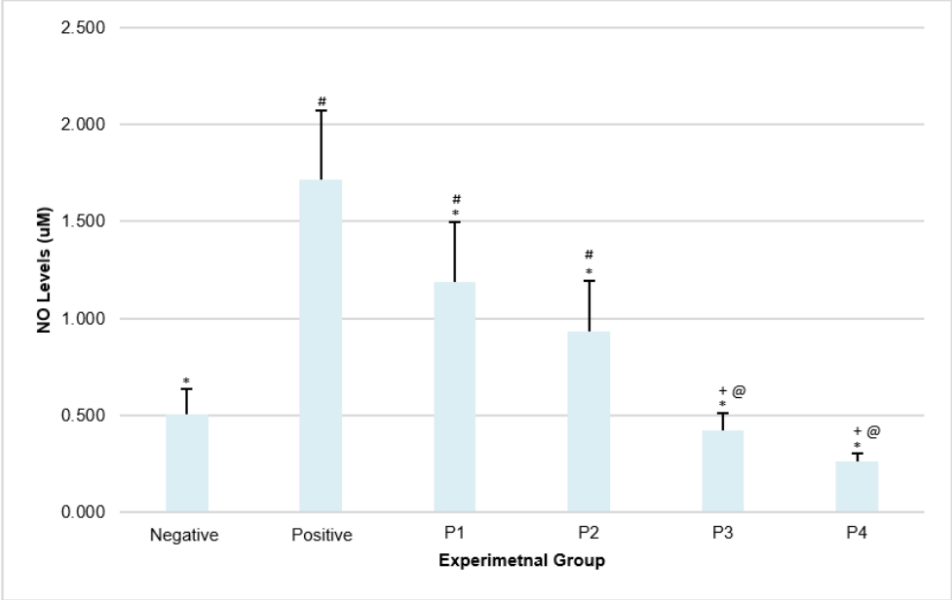

Figure 6: Measurements of NO Serum Levels. Data are expressed as mean ± SD. *P<0.05 compared with Positive, #P<0.005 compared with Negative, +P<0.005 compared with P1, @P<0.005 compared with P2

Examination of NO levels also showed dose-dependent activity, with an increase in the dose of the extract followed by a decrease in NO levels. NO levels in P1 treatment group (1.186 ± 0.308 uM) compared with P2 treatment group (490 ± 32 uM), P3 (322 ± 31 uM), and also with P4 (222 ± 56 uM), showed a significant difference. Besides that, no significant difference in the mean NO levels between the P3 (0.423 ± 0.088 uM) and P4 (0.260 ± 0.046 uM) groups. In other words, a dose of 70 mg/kgBW and a dose of 140 mg/kgBW are considered to have the same ability to reduce the average NO level in BALB/c AD mice.

Therefore, it can be concluded that the administration of Orthosipon aristatus leaf extract is able to reduce serum NO levels even at a dose of 17,5 mg/kg and the effect will be stronger as the dose is increased.

Discussion

Atopic disease like atopic dermatitis is a chronic inflammatory skin disease associated with other atopic diseases such as allergic rhinitis and asthma [19–22]. It is a genetic predisposition disease and common in pediatric patients. was frequently associated with food allergy at least in 40% of children [23]. The clinical manifestations of AD are primarily characterized by pruritus, erythematous lesions in acute and lichenification in chronic state, also dry skin [1]. Previous study showed that AD patient had sleep disturbance and depression, and also affect the quality of life of patients [18]. This study confirmed that skin lesion severity in positive control was worse than negative control significantly. These symptoms include erythema, hemorrhage, edema, excoriation/erosion, and scaling/dryness. Interestingly, these symptoms were alleviated with administration of Orthosiphon aristatus leaves extract. This study showed that skin lesion was healed after extract administration in dose dependent manner (Fig. 1). Additionally, this study analyzed serum markers to confirmed that beneficial effect of Orthosiphon aristate give impact in molecular level. Therefore, we measured serum levels of IgE, IL22, IL4, PGE2, and NO. These molecules had known play roles in atopic dermatitis pathophysiology.

The etiology of atopic dermatitis is not fully understood. It might be affected by multifactorial, such as genetic, destruction of skin barrier, immunological, environment like food and aeroallergen [2]. All of these risks cause skin barrier destruction through inflammation reaction and oxidative stress. These reactions increase production of keratinocyte cytokines such as TLSP, IL33, IL1, IL6, IL8, and TNFα, which are able to stimulate the formation of Th2, secretes IL4, IL13, and IL5. Additionally, Th2 stimulates B cells to secrete IgE antibodies, which increase expression of endothelial cell adhesion molecules, and eosinophil cell proliferation [3, 4]. On the other hand, inflammation involves hypersensitivity reactions associated with allergens. Furthermore, production of IgE and histamine are closely associated with this mechanism and become one of the diagnostic criteria of AD [3, 4, 21, 22]. In addition, IL22 plays a role in the inflammation reaction of AD. This cytokine causes epidermal hyperplasia and inhibits keratinocyte differentiation and filaggrin formation which disturbs skin moisturizing [6]. Interestingly, IL22 production is influenced by prostaglandin secretion. Previous studies reported an increase in Prostaglandin (PG) D2 and PGE2 in AD lesions followed by an increase in IL22 production [6, 7]. A study showed that the DNCB-sensitized AD rats' model had EP4 deficiency and increased IL22 production by T cells in lymph nodes. PGD2 is the main proteinoid produced by activated mast cells. Its binding to the CTH2 receptor also induces chemotaxis of several immune cells which exacerbates AD inflammation process [6]. This study showed increase of IgE, IL4, IL22, PGE2, and NO levels in BALB/C mice after DNCB sensitizing-induced atopic dermatitis.

Currently, steroids, antihistamines, and immunosuppressives are commonly used to treat AD, but side effects often occur [24]. Recently, complementary and supportive therapy like herbal medicine, have been reported more frequently and globally. Although evidences are limited, some herbal

medicines administered topically and orally effective for treating AD [25]. Orthosiphon aristatus leaves extract is one of important herbal medicines. Previous studies reported that more than 20 phenolic compounds were detected from Orthosiphon aristatus leaves, and the compounds that have pharmacological properties include caffeic acid, rosmarinic acid, sinensetin, eupatorium, and polymethoxylated flavones [14, 26]. Beneficial effects of phenolic compounds in Orthosiphon aristatus includes antioxidant [27], antibacterial [28], antifungal [29], antidiabetic [30], antiinflammatory [17], antimutagenic [31], and antiarthritic [32].

This study is the first study that confirmed beneficial effects of administration Orthosiphon aristatus in atopic dermatitis. This study showed that administration of the Orthosiphon aristatus leaves extract reduced IgE, IL4, IL22, PGE2, NO serum levels. It was parallel with improvement of the skin lesions. It reflected that this extract improved the inflammation and oxidative stress that induced AD. Anti-inflammatory effects of Orthisiphon aristatus extract have been found in previous studies. A study used a 200µg extract and showed the inhibitory effect of TPA (tetradecanoylphorbol)-induced inflammation in mice [33]. A series of experiments showed that Orthisiphon aristatus extract reduced NO production in LPS activated macrophage-like J774.1 cells. Lyckander and Malterud (1992) showed the effect of ethyl acetate from extract and 8 lipophilic flavonoids isolated from Orthosiphon aristatus (leaves) on the arachidonic acid oxidation catalyzed by 15-lipoxygenase [34]. The results showed inhibition effect on 15-lipoxygenase [IC50 value amounting to 0.018% (w/v)] in dose-dependent inhibition manner compared to quercetin (positive control). Additionally, Orthosiphon aristatus showed antioxidant activity based on β-carotene coupled and autooxidised linoleic acid mechanism and were comparable to quercetin and butylated hydroxyanisole [35]. Another study also marked inhibition mechanism of NO, PGE2, and ROS production, including iNOS and COX-2 gene expression in LPS-stimulated RAW 264.7 cells [17]. Even though, further research is still needed to assess the beneficial activity of this extract to control atopic dermatitis. Histological studies may be proposed to assess the degree of improvement at the microscopic structural level. Related to the role of Orthosiphon aristatus extract as atopic dermatitis therapy, both as supporting/adjuvant therapy, still needs further discussion.

Conclusion

We firstly reported the therapeutic effects of Orthosiphon aristatus leaf effect on atopic dermatitis by DNCB-induced AD-like lesion mouse models. Administration of Orthosiphon aristatus extract improved the progression of AD-like lesions severity. Additionally, it was demonstrated that the anti-atopic effects were exerted through down-regulating the serum level of cytokines such as IL4, IL22, IgE, PGE2, and NO. These effects suggested that Orthosiphon aristatus could be a valuable herbal therapy for atopic dermatitis.

Abbreviations

AD- atopic dermatitis

DNCB- 2,4 Dinitrochlorobenzene

IgE- immunoglobulin E

IL- Interleuikin

Inos- Inducible nitric oxide synthase

LPS- Lipopolysaccharides

NO- nitric oxide

NOS- nitrogen oxygen species

PGE- prostaglandin E

ROS- reactive oxygen species

Th- T-helper

TNF- tumor necrosis factor

TPA- tetradecanoylphorbol

TSLP- Thymic Stromal Lymphoprotein

Declarations

Availability of data and materials

The datasets generated during and/or analysed during the current study are available in Figshare: Dataset of Orthosiphon aristatus effect on DNCB-induced Atopic Dermatitis Mice Model. https://doi.org/10.6084/m9.figshare.22776173.

Acknowledgements

Not applicable

Ethics approval and consent to participate

All procedures performed in this experimental study involving animals were approved by the ethical committee of Faculty of Medicine, Brawijaya University with registration number 102-KEP-UB-2021

Consent for publication

Not applicable.

Competing interests

The authors have no confict of interest to declare.

References

1. Kang S, editor. (2019). Fitzpatrick's dermatology. Ninth edition. New York: *McGraw-Hill Education.*
2. Eaton A, Melonson-Wliliams A, Ebele Seals S. Atopic Dermatitis in the Pediatric Population: Pathogenesis, Treatment, and Quality of Life Issues. U S Pharmacist 37:31–4.
3. David Boothe W, Tarbox JA, Tarbox MB. (2017). Atopic Dermatitis: Pathophysiology. *Adv Exp Med Biol.*1027:21–37.
4. Boguniewicz M, Financier L, Guttman-Yassky E, Ong PY, Silverberg J. et al. (2018). Atopic dermatitis yardstick: Practical recommendations for an evolving therapeutic landscape. *Ann Allergy Asthma Immunol.* 120:10–22e2.
5. Vaneckova J, Bukač J. (2016). The severity of atopic dermatitis and the relation to the level of total IgE, onset of atopic dermatitis and family history about atopy. *Food and Agricultural Immunology.* 27:734–741.
6. Yanes DA, Mosser-Goldfarb JL. (2018).Emerging therapies for atopic dermatitis: The prostaglandin/leukotriene pathway. *J Am Acad Dermatol.*78:71–75.
7. Tsuge K, Inazumi T, Shimamoto A, Sugimoto Y. (2019). Molecular mechanisms underlying prostaglandin E2-exacerbated inflammation and immune diseases. *Int Immunol.*31:597–606.
8. Robb CT, McSorley HJ, Lee J, Aoki T, Yu C, et al. (2018). Prostaglandin E2 stimulates adaptive IL-22 production and promotes allergic contact dermatitis. *J Allergy Clin Immunol.*141:152–162.
9. Orita K, Hiramoto K, Kobayashi H, Ishii M, et al. (2011). Inoue M. Inducible nitric oxide synthase (iNOS) and α-melanocyte-stimulating hormones of iNOS origin play important roles in the allergic reactions of atopic dermatitis in mice: Allergic reactions of atopic dermatitis in mice. *Exp Dermatol.*20:911–914.
10. Lee HN, Shin SA, Choo GS, Kim HJ, Park YS, et al. (2017). Anti–inflammatory effect of quercetin and galangin in LPS–

stimulated RAW264.7 macrophages and DNCB–induced atopic dermatitis animal models. *Int J Mol Med* [Internet].
11. Mayba JN, Gooderham MJ. (2017). Review of Atopic Dermatitis and Topical Therapies. *J Cutan Med Surg.*21:227–236.
12. Kusari A, Han AM, Schairer D, Eichenfield LF. (2019). Atopic Dermatitis. *Dermatol Clin.* 37:11–20.
13. Cline A, Bartos GJ, Strowd LC, Feldman SR. (2019). Biologic Treatment Options for Pediatric Psoriasis and Atopic Dermatitis. *Children.*6:103.
14. Hossain MA, Mizanur Rahman SM. (2015). Isolation and characterisation of flavonoids from the leaves of medicinal plant Orthosiphon stamineus. *Arab J Chem.*8:218–221.
15. Adnyana IK, Setiawan F, Insanu M. (2015). From Ethnopharmacology to Clinical Study of Orthosiphon Stamineus Benth. *Int J Pharm Pharm Sci* 5:66–73.
16. Eräsalo H, Hämäläinen M, Leppänen T, Mäki-Opas I, et al. (2018). Natural Stilbenoids Have Anti-Inflammatory Properties in Vivo and Down-Regulate the Production of Inflammatory Mediators NO, IL6, and MCP1 Possibly in a PI3K/Akt-Dependent Manner. *J Nat Prod*. 81:1131–1142.
17. Hsu CL, Hong BH, Yu YS, Yen GC. (2010). Antioxidant and Anti-Inflammatory Effects of Orthosiphon aristatus and Its Bioactive Compounds. *J Agric Food Chem*. 58:2150–2156.
18. Koszorú K, Borza J, Gulácsi L, Sárdy M. (2019). Quality of life in patients with atopic dermatitis. *Cutis.* 104:174–177.
19. Custovic A, Sonntag HJ, Buchan IE, Belgrave D, Simpson A, (2015). Prosperi MCF. Evolution pathways of IgE responses to grass and mite allergens throughout childhood. *J Allergy Clin Immunol.*136:1645–1652e8.
20. Weidinger S, Novak N. (2016). Atopic dermatitis. *The Lancet.*387:1109–1122.
21. Furue M, Chiba T, Tsuji G, Ulzii D, Kido-Nakahara M, et al. (2017). Atopic dermatitis: immune deviation, barrier dysfunction, IgE autoreactivity and new therapies. *Allergology Int.*66:398–403.
22. Kim J, Kim BE, Leung DYM. (2019). Pathophysiology of atopic dermatitis: Clinical implications. *allergy asthma proc.* 40:84–92.
23. Bergmann MM, Caubet JC, Boguniewicz M, Eigenmann PA. (2013). Evaluation of Food Allergy in Patients with Atopic Dermatitis. J Allergy Clin Immunology: *Pract.* 1:22–28.
24. Megna M, Napolitano M, Patruno C, Villani A, Balato A, et al. (2017). Systemic Treatment of Adult Atopic Dermatitis: A Review. *Dermatol Ther (Heidelb).*7:1–23.
25. Kwon CY, Lee B, Kim S, Lee J, Park M, Kim N. (2020). Effectiveness and Safety of Herbal Medicine for Atopic Dermatitis: An Overview of Systematic Reviews. *Evidence-Based Complement Altern Med.*1–15.
26. Ameer OZ, Salman IM, Asmawi MZ, Ibraheem ZO, Yam MF. (2012). Orthosiphon stamineus: Traditional Uses, Phytochemistry, Pharmacology, and Toxicology. *J Med Food.*15:678–690.
27. Yam MF, Basir R, Asmawi MohdZ, Ismail Z. (2007). Antioxidant and Hepatoprotective Effects of Orthosiphon stamineus Benth.: Standardized Extract. *Am J Chin Med.*35:115–126.
28. Ho CH, Noryati I, Sulaiman SF, Rosma A. (2010). In vitro antibacterial and antioxidant activities of Orthosiphon stamineus Benth. extracts against food-borne bacteria. *Food Chem.* 122:1168–1172.
29. Hossain MA, Ismail Z, Rahman A, Kang SC. (2008). Chemical composition and anti-fungal properties of the essential oils and crude extracts of Orthosiphon stamineus Benth. *Ind Crops Prod.*27:328–334.
30. Lokman EF, Saparuddin F, Muhammad H, Omar MH, Zulkapli A. (2022). Orthosiphon stamineus as a potential antidiabetic drug in maternal hyperglycemia in streptozotocin-induced diabetic rats. *Integr Med Res.*8:173–179.
31. Al-Dulaimi DW, Shah Abdul Majid A, Baharetha M, Ahamed H, Faisal MBK, (2022). Al Zarzour SF. Anticlastogenic, antimutagenic, and cytoprotective properties of Orthosiphon stamineus ethanolic leaves extract. *Drug Chem Toxicol.* 45:641–650.
32. Chung YS, Choo BKM, Ahmed PK, Othman I, Shaikh MohdF. (2020). A Systematic Review of the Protective Actions of Cat's Whiskers (Misai Kucing) on the Central Nervous System. *Front Pharmacol*.11:692.
33. Masuda T, Masuda K, Shiragami S, Jitoe A, Nakatani N. (1992). Orthosiphol A and B, novel diterpenoid inhibitors of TPA (12-O-tetradecanoylphorbol-13-acetate)-induced inflammation, from Orthosiphon stamineus. *Tetrahedron.*48:6787–6792.
34. Lyckander IM, Malterud KE. (1992). Lipophilic flavonoids from Orthosiphon spicatus as inhibitors of 15-lipoxygenase. *Swed Pharm Press Stockholm* 4:159–166.
35. Molina MF, Sanchez-Reus I, Iglesias I, Benedi J. Quercetin, (2003). a Flavonoid Antioxidant, Prevents and Protects against Ethanol-Induced Oxidative Stress in Mouse Liver. *Biol Pharm Bull.* 26:1398–1402.

DOI: 10.31579/2690-8794/165