



RESEARCH ARTICLE

Effect of *Moringa oleifera* leaf extract on salivary gland damage in Sjögren's syndrome mice model [version 1; peer review: awaiting peer review]

Agus Joko Susanto ¹, Bambang Purwanto¹, Ambar Mudigdo², Brian Wasita²

¹Department of Internal Medicine, University of Sebelas Maret, Surakarta, Indonesia, 57126, Indonesia

²Department of Anatomical Pathology, University of Sebelas Maret, Surakarta, Indonesia, 57126, Indonesia

V1 First published: 07 Nov 2022, 11:1263
<https://doi.org/10.12688/f1000research.126929.1>

Latest published: 07 Nov 2022, 11:1263
<https://doi.org/10.12688/f1000research.126929.1>

Open Peer Review

Approval Status AWAITING PEER REVIEW

Any reports and responses or comments on the article can be found at the end of the article.

Abstract

Background

Sjögren's syndrome is a chronic autoimmune disease characterized by lymphocytic infiltration and inflammation of the exocrine glands, especially the lacrimal and salivary glands. *Moringa oleifera* (MO) leaves are rich in polyphenols and flavonoids which have antioxidant activity which is also shown when extracted with ethanol. This study aimed to probe the effect of *Moringa oleifera* leaf extract on malondialdehyde (MDA), interleukin-17 (IL-17), matrix metalloproteinase-9 (MMP-9), and caspase-3 levels and salivary gland damage in Sjögren's syndrome mice model.

Methods

Thirty-two samples were divided into four treatment groups: 200 mg/kg BW MO-ethanol leaf extracts with 1.23 mg/kg BW dexamethasone group (T2), 1.23 mg/kg BW dexamethasone alone group (T1), normal control group/C- (without induction of Ro antigen (SSA) and extract of MO-ethanol), and negative group/C+ (with induction of Ro antigen (SSA) on day 42). MDA, IL-17, MMP-9, and caspase-3 levels and salivary gland epithelium damage (histopathological changes) were measured 14 days post-Ro antigen (SSA) induction. The method used to measure MDA level was Thiobarbituric Acid Reactive Substance (TBARS) while IL-7 and MMP-9 were ELISA. Some of the salivary gland was used for histological preparations using the paraffin method without Harris Hematoxylin-Eosin (HE) staining. Then for the examination of caspase-3, we used the standard procedure of immunohistochemically staining. The salivary gland epithelium damage examination used the HE staining of histological preparation.

Results

There were significant differences in MDA, IL-17, MMP-9, and caspase-3 levels in the group given a 200 mg/kg BW dose of MO-ethanol leaf extract compared to the control group. The administration of the

extract also significantly reduced the degree of necrosis of the salivary gland epithelium.

Conclusions

Moringa oleifera leaf extract reduced MDA, IL-17, MMP9, and caspase-3 levels and salivary epithelial damage.

Keywords

Moringa oleifera leaf extract, Sjögren's syndrome, Salivary gland, Apoptosis



This article is included in the **Plant Science** gateway.

Corresponding author: Agus Joko Susanto (agusjoko.susanto4@gmail.com)

Author roles: **Susanto AJ:** Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Investigation, Methodology, Project Administration, Resources, Software, Validation, Writing – Original Draft Preparation, Writing – Review & Editing; **Purwanto B:** Conceptualization, Supervision, Writing – Review & Editing; **Mudigdo A:** Resources, Supervision, Validation, Visualization, Writing – Review & Editing; **Wasita B:** Methodology, Resources, Supervision, Validation, Visualization, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: This work was supported by Sebelas Maret University, Surakarta, Indonesia [grant number: 254/UN27.22/PT.01.03/2022].

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Copyright: © 2022 Susanto AJ *et al.* This is an open access article distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

How to cite this article: Susanto AJ, Purwanto B, Mudigdo A and Wasita B. **Effect of *Moringa oleifera* leaf extract on salivary gland damage in Sjögren's syndrome mice model [version 1; peer review: awaiting peer review]** F1000Research 2022, 11:1263 <https://doi.org/10.12688/f1000research.126929.1>

First published: 07 Nov 2022, 11:1263 <https://doi.org/10.12688/f1000research.126929.1>

Introduction

Sjögren's syndrome (SS) is a systemic, complex, and multifactorial autoimmune disease. The triggering events of autoimmune disease and the pathophysiology of SS are unknown, but it is thought that both genetic and environmental factors play an important role (Igoe and Scofield 2013). The incidence of SS ranges between 0.01% and 0.72% globally (Brito-Zerón *et al.* 2016). Women are more likely to have SS compared with men, with a percentage value of 9:1 and a mean age of 56 years (Stefanski *et al.* 2017). The prevalence of SS is also found to be higher in the elderly population (Haugen *et al.* 2008). SS predominantly appears in women around or after menopause, although it can develop at any age (Brito-Zerón *et al.* 2009; Haugen *et al.* 2008). In Indonesia, there are no epidemiological data related to SS cases; only systemic lupus erythematosus and rheumatoid arthritis (RA), thus SS does not have adequate data.

Oxidative stress occurs in many autoimmune diseases, along with the excess production of reactive oxygen species (ROS) and reactive nitrogen species, which are related to the inflammatory process. The sources of such reactive species include nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) oxidases (NOXs), the mitochondrial electron transport chain, nitric oxide (NO) synthases, and other enzymes (Smallwood *et al.* 2018). Various cluster of differentiation/CD4+ T cell subsets emerge to contribute to primary Sjögren's syndrome (pSS) pathogenesis, including the T helper (Th) 1, follicular T helper, and T helper 17 (Th17) cells. T helper 17 cells play a role in mucosal barrier physiology and pathogen-associated inflammatory responses. T helper 17 cells producing interleukin 17 (IL-17) are also found in salivary gland lesions and are high in peripheral blood (Verstappen *et al.* 2018). Interleukin 17 promotes the production of metalloproteinase matrix 9 (MMP-9) and harms the mice's corneal barrier (Chauhan *et al.* 2009; de Paiva *et al.* 2009). In SS patients there is an increase in MMP-9 gene expression and plasma levels (Hulkkonen *et al.* 2004). MMP-9 is an important inflammatory mediator involved in SS immunopathogenesis (Pflugfelder SC 2014). B lymphocyte cells also play an important role in the pathogenesis of SS by several mechanisms, namely, as cytokine producers, antigen-presenting cells, and autoantibody secretors (Nocturne and Mariette 2018). Autoantibodies that are characteristic of pSS include anti-Ro/Sjögren's syndrome antigen type A (anti-Ro/SSA) antibodies, which can be detected in 70–100% of patients with SS, and anti-La Sjögren syndrome type B antigen (anti-La/SSB), which has detection rates ranging from 40 to 90% (Wenzel *et al.* 2001). Anti-La antibodies are not always positive, however, the combination of the two is more likely to lead to SS than anti-Ro antibodies alone (Scofield *et al.* 2018). Anti-Ro antibodies are a more specific diagnostic marker and are included on Sjögren's syndrome criteria when compared with anti-La (Shiboski *et al.* 2017).

Sjögren's syndrome primarily affects the salivary and tear glands. In the ACR/EULAR classification criteria for the diagnosis of primary Sjögren's syndrome, the presence of focal lymphocytic sialadenitis in the labial salivary glands and a focal score ≥ 1 foci/4 mm has the highest score, which is 3. The diagnosis of Sjögren's syndrome is established if the total score is ≥ 4 .

Currently, there is no effective drug for the management of the etiology of SS (Shen *et al.* 2019; Vivino *et al.* 2019). Therapeutic approaches are limited to topical and systemic to treat sicca and systemic symptoms, with the aim of improving quality of life (Carsons *et al.* 2017; Shih *et al.* 2017). Although diagnostic criteria and guidelines for the management of SS have been developed, gaps remain with respect to effective specific therapies and their impact on patients (Romão *et al.* 2018). The imbalance of cytokines and their pathological effects is one aspect that can be a target for therapy (Sambataro *et al.* 2017).

Moringa oleifera (MO) is reported to have anti-inflammatory, antimicrobial, antioxidant, anticancer, cardiovascular, hepatoprotective, anti-ulcer, diuretic, antiurolithiasis, and anthelmintic functions (Farooq *et al.* 2012). *M. oleifera* leaf extract, ripe or still soft, exhibits strong antioxidant activity against free radicals, prevents oxidative damage to key biomolecules, and provides significant protection against oxidative damage. Furthermore, various animal safety studies involving *M. oleifera* leaf extracts have shown a high level of safety (Mahmood *et al.* 2010; Stohs and Hartman 2015). The ethyl acetate fraction of *M. oleifera* extract in *in vitro* studies contained high levels of phenols and antioxidant activity. This fraction can inhibit the production of cytokines by macrophages *in vitro* triggered by cigarette smoke extracts, such as tumor necrosis factor (TNF), interleukin-6 (IL-6), and interleukin-8 (IL-8). In addition, it inhibits the expression of *RelA*, which is a gene involved in the NF- κ B (activated B-cell kappa-light-chain-enhancing nuclear factor) p65 inflammatory signaling pathway (Kooltheat *et al.* 2014).

As aforementioned, *M. oleifera* leaves have antioxidant activity, so there is a need for research to assess whether there is any effect of *M. oleifera* (Kelor) leaf extract on salivary gland damage and the levels of IL-17, MMP-9, malondialdehyde (MDA), epithelial necrosis, and caspase-3 levels in primary SS model rats.

Methods

Sample and study design

An experimental laboratory technique with a post-test only control group design was performed. The sampling method used in this study was purposive sampling. Male mice, BALB/c strain, aged 8–10 weeks, body weight (BW) 26–27.5 g, no physical disability, and normal activity were the inclusion criteria. Mice that died during the treatment period was the exclusion criteria. Sample size was determined by Federer's formula $[(n - 1) (t - 1) > 15]$, so the obtained minimum samples number was six mice for each group. Two mice were added to each group to anticipate mice death, so that the final sample size was 32.

Thirty-two samples were divided into four treatment groups: 200 mg/kg BW MO-ethanol leaf extracts with 1.23 mg/kg BW dexamethasone group (T2), 1.23 mg/kg BW dexamethasone alone group (T1), normal control group/C- (without induction of Ro antigen (SSA) and extract of MO-ethanol), and negative group/C+ (with induction of Ro antigen (SSA) on day 42). The treatment in the form of dexamethasone and MO-ethanol leaf extracts given for 14 days. MDA, IL-17, MMP-9, and caspase-3 levels and salivary gland epithelium damage (histopathological changes) were observed 14 days post-Ro antigen (SSA) induction. The method used to measure MDA level was Thiobarbituric Acid Reactive Substance (TBARS) while IL-7 and MMP-9 were ELISA. Some of the salivary gland was used for histological preparations using the paraffin method without Harris hematoxylin–eosin (HE) staining for the examination of caspase-3 with the standard procedure of immunohistochemical staining. The salivary gland epithelium damage examination used the HE staining of histological preparation.

Histopathological preparations were observed and scored according to the following categories:

Score 0: No necrosis

Score 1: Mild necrosis

Score 2: Moderate necrosis

Score 3: Severe necrosis.

Interventions of BALB/c mice, histopathological preparations for epithelial necrosis, and histopathological reading of epithelial necrosis were conducted at the Experimental Animal Care Center (PAU UGM, Yogyakarta), Histology and Cell Biology Laboratory (Faculty of Medicine, UGM, Yogyakarta), and Anatomical Pathology and Histology Laboratory (Faculty of Medicine, UNS Surakarta), respectively. The authors were unaware of the allocation group so that all the mice were handled, monitored and treated in the same way while conducting the experiment.

SS mice model

This mice model was conducted for 42 days. BALB/c mice were immunized with a short peptide of 60-kD Ro antigen (SSA) that triggered an immune response and formed anti-Ro antibodies. There was a decrease in mouse salivary flow and T lymphocyte infiltration (both CD4+ and CD8+ T cells), in immunized mice similar to SS in humans (Scofield *et al.* 2018). BALB/c mice have a similar response to the nucleotide-binding and oligomerization domain/NOD-like receptor gene, *C3H/HeJ*. The *C3H/HeJ* gene has implications for the pathogenesis of SS (Kim *et al.* 2017; Sellers 2017). The average BW of BALB/c mice at 8, 9, and 10 weeks old was 26.2 ± 1.4 , 27.0 ± 1.4 , and 27.4 ± 1.4 g, respectively. Mice were reared on a diet containing 6% fat according to the LabDiet® 5K52 feed formulation.

Mice were kept in four cages made of plastic tubs covered with wire at the top. The conditions during acclimatization and treatment were controlled in a fixed environmental range, namely in a room that had 12 hours of light and 12 hours of darkness with a room temperature ranging from 23–26°C with the aim that the test animals could adapt according to the animal's biological time and the conditions to be occupied during the experiment. Temperature, water supply, the number of mice in the cage, and the change of husks were all done the same for all groups of mice. Adaptation to mice with care in cages with a size of $28 \times 30 \times 12$ cm so that they can move freely and not be stressed.

At the end of the study, euthanasia and removal of salivary glands were performed. We made 3 mm horizontal incision on the skin placed 1 mm below the ear lobe to expose the glands underneath. After identifying the parotid gland, we used the curved forceps to pull the gland out, used a scalpel to separate the gland from the surrounding tissue. The tissue was then put into a container containing 10% neutral buffer formalin. The sample was then made preparations with Harris Hematoxylin Eosin staining.

Moringa oleifera leaf ethanol extract

The leaf extracts were washed with tap water and dried at 24°C for a day and subsequently in an oven for two consecutive days at 45°C. The extracts were then ground using a mechanical blender and stored in a vacuum container. *M. oleifera* leaves were completely crushed using 90% ethanol (ethanol: distilled water, 9:1) and then put into a shaking aspirator bottle for 3 days at 24°C. The residue was filtered using Whatman filter paper No. 1, and the filtrate was condensed using a rotary evaporator at 40°C. The condensed residue softened and became dark green in color, and was then freeze-dried. The freeze-dried extracts were weighed, and stored in closed containers, before being properly labeled and stored at -20°C. Administration of *M. oleifera* leaf extract in ethanol at 200 mg/kg BW for 14 days showed anti-inflammatory activity (Both *et al.* 2017; Karthivashan *et al.* 2016).

Statistical analysis

The normality test used was the Shapiro–Wilk test. Numerical data were subsequently analyzed using ANOVA, then processed using the Tukey HSD (Honest Significant Difference) *post hoc* test for normally distributed and homogeneous data and the Games–Howell *post hoc* test for non-homogeneous data. The Kruskal–Wallis test was conducted on an abnormal distribution and continued with Mann–Whitney *post hoc* test. Independent t-tests were performed to compare the treatment group with other groups. The statistical analysis used was SPSS 22 for windows and a p value < 0.05 was considered statistically significant.

Results

MO-ethanol extract administration on MDA levels in experimental animal model of SS

Differences in MDA levels based on groups can be seen in Table 1. The C(–) control group had the lowest MDA level (1.55±0.2 mg/ml), whereas the C(+) group had the highest MDA level (10±0.5 mg/ml). Based on a one-way ANOVA, there was a significant difference in MDA levels with C(–) as reference (p < 0.05). Levene’s test value was p = 0.03, and the Games–Howell *post hoc* test was conducted (Table 2).

The difference in MDA levels with the T2 group as a reference compared to other groups is shown in Table 3. It exhibits the significant difference in MDA levels of the T2 treatment group post-Ro (SSA) antigen induction when compared to the C(–), C(+), and T1 groups (p < 0.001). This was the most striking difference between the T2 and the C(+) cluster with

Table 1. Differences in MDA levels based on groups.

Groups	MDA levels
C(–)	1.55±0.2
C(+)	10±0.5
T1	5.38±0.43
T2	2.98±0.32
p value	<0.001**

C(–)=Not given Ro (SSA) antigen or MO-ethanol extract.

C(+)=Only given Ro (SSA) antigen.

T1=Dexamethasone dose 1.23 mg/kg BW.

T2=MO-ethanol extract 200 mg/kg BW and dexamethasone 1.23 mg/kg BW.

**=Statistically significant at the 1% level (p < 0.001).

Table 2. Post hoc test for analyzed MDA levels post-Ro (SSA) induction in various groups.

	MDA			
	C(–)	C(+)	T1	T2
p value	Reference category	<0.001**	<0.001**	<0.001**
95% CI	Reference category	–9.05 to –7.87	–4.34 to –3.32	–1.82 to –1.04
Mean difference	Reference category	–8.46	–3.83	–1.43

C(–)=Not given Ro (SSA) antigen or MO-ethanol extract.

C(+)=Only given Ro (SSA) antigen.

T1=Dexamethasone dose 1.23 mg/kg BW.

T2=MO-ethanol extract 200 mg/kg BW and dexamethasone 1.23 mg/kg BW.

**=Statistically significant at the 1% level (p < 0.001).

Table 3. Independent t-test of MDA levels post-induction of Ro (SSA) with the T2 group as reference compared to other groups.

T2 group	MDA level post-Ro (SSA) antigen induction		
	C(-)	C(+)	T1
F value	3.19	3.61	0.46
t value	-10.83	33.64	12.75
p value	<0.001**	<0.001**	<0.001**
95% CI	-1.71 to -1.15	6.58-7.47	1.99-2.80

C(-)=Not given Ro (SSA) antigen or MO-ethanol extract.
 C(+)=Only given Ro (SSA) antigen.
 T1=Dexamethasone dose 1.23 mg/kg BW.
 T2=MO-ethanol extract 200 mg/kg BW and dexamethasone 1.23 mg/kg BW.
 **=Statistically significant at the 1% level (p<0.001).

p<0.001 (95% CI 6.58-7.47). It is clear that the T2 group was more effective in reducing MDA levels post-Ro (SSA) antigen induction than the other groups.

MO-ethanol extract administration on IL-17 levels in experimental animal model of SS

Differences in IL-17 levels based on groups can be seen in Table 4. Based on a one-way ANOVA, there was a significant difference in IL-17 levels with C(-) as reference (p<0.05). Levene’s test value was p=0.92. The Tukey HSD *post hoc* test was subsequently conducted (Table 5).

Table 5 shows that IL-17 levels post-Ro (SSA) antigen induction in the C(+), T1, and T2 groups were significantly different from the C(-) control group with p<0.001. This showed effective treatment in reducing IL-17 levels. Differences in IL-17 levels between the T2 group and the other groups is shown in Table 6. The table demonstrates that there is a significant difference in IL-17 levels of the T2 treatment group post-Ro (SSA) antigen induction when

Table 4. Differences in IL-17 levels based on groups.

Groups	IL-17 levels
C(-)	30.94±2.69
C(+)	82.04±3.52
T1	65.93±3.15
T2	40.95±3.28
p value	<0.001**

C(-)=Not given Ro (SSA) antigen or MO-ethanol extract.
 C(+)=Only given Ro (SSA) antigen.
 T1=Dexamethasone dose 1.23 mg/kg BW.
 T2=MO-ethanol extract 200 mg/kg BW and dexamethasone 1.23 mg/kg BW.
 **=Statistically significant at the 1% level (p<0.001).

Table 5. Post hoc test for analyzed IL-17 levels post-Ro (SSA) induction in various groups.

	IL-17			
	C(-)	C(+)	T1	T2
p value	Reference category	<0.001**	<0.001**	<0.001**
95% CI	Reference category	-5.75 to -5.49	-2.32 to -1.85	1.23 to -0.90
Mean difference	Reference category	-5.62	-2.08	-1.06

C(-)=Not given Ro (SSA) antigen or MO-ethanol extract.
 C(+)=Only given Ro (SSA) antigen.
 T1=Dexamethasone dose 1.23 mg/kg BW.
 T2=MO-ethanol extract 200 mg/kg BW and dexamethasone 1.23 mg/kg BW.
 **=Statistically significant at the 1% level (p<0.001).

Table 6. Independent t-test of IL-17 levels post-induction of Ro (SSA) with the T2 group as reference compared to other groups.

T2 group	IL-17 level post-Ro (SSA) antigen induction		
	C(-)	C(+)	T1
F value	0.52	0.013	0.02
t value	-6.67	24.17	15.54
p value	<0.001**	<0.001**	<0.001**
95% CI	-13.24 to -6.78	37.44-44.74	21.53-28.42

C(-)=Not given Ro (SSA) antigen or MO-ethanol extract.
 C(+)=Only given Ro (SSA) antigen.
 T1=Dexamethasone dose 1.23 mg/kg BW.
 T2=MO-ethanol extract 200 mg/kg BW and dexamethasone 1.23 mg/kg BW.
 **=Statistically significant at the 1% level (p<0.001).

compared with the C(-), C(+), and T1 groups with p<0.001. The most significant difference was between the T2 and C(+) groups with p<0.001 (95% CI 37.44-44.74). It can be concluded that the T2 group was more effective in reducing IL-17 levels post-Ro (SSA) antigen induction than the other groups.

MO-ethanol extract administration on MMP-9 levels in experimental animal model of SS

Differences in MMP-9 levels based on groups can be seen in Table 7. According to a one-way ANOVA, there was a significant difference in MMP-9 levels with C(-) as reference (p<0.05) and Levene's test value p=0.28, and then the Tukey HSD *post hoc* test was conducted (Table 8). Table 8 shows that MMP-9 levels post-Ro (SSA) antigen induction in the C(+), T1, and T2 groups were significantly different from the C(-) control group with p<0.001. This showed effective treatment in reducing MMP-9 levels. Differences in MMP-9 levels between the T2 group and the other groups are revealed in Table 9.

Table 7. Differences in MMP-9 levels based on groups.

Groups	MMP-9 levels post-Ro (SSA) induction
C(-)	7.68±0.67
C(+)	30.71±1.15
T1	15.71±1.28
T2	11.08±0.84
p value	<0.001**

C(-)=Not given Ro (SSA) antigen or MO-ethanol extract.
 C(+)=Only given Ro (SSA) antigen.
 T1=Dexamethasone dose 1.23 mg/kg BW.
 T2=MO-ethanol extract 200 mg/kg BW and dexamethasone 1.23 mg/kg BW.
 **=Statistically significant at the 1% level (p<0.001).

Table 8. Post hoc test for analyzed MMP-9 levels post-Ro (SSA) induction in various groups.

	MMP-9			
	C(-)	C(+)	T1	T2
p value	Reference category	<0.001**	<0.001**	<0.001**
95% CI	Reference category	-24.40 to -21.64	-9.41 to -6.64	-4.78 to -2.02
Mean difference	Reference category	-23.02	-8.03	-3.4

C(-)=Not given Ro (SSA) antigen or MO-ethanol extract.
 C(+)=Only given Ro (SSA) antigen.
 T1=Dexamethasone dose 1.23 mg/kg BW.
 T2=MO-ethanol extract 200 mg/kg BW and dexamethasone 1.23 mg/kg BW.
 **=Statistically significant at the 1% level (p<0.001).

Table 9. Independent t-test of MMP-9 levels post-induction of Ro (SSA) with the T2 group as reference compared to other groups.

T2 group	MMP-9 level post-Ro (SSA) antigen induction		
	C(+)	C(+)	T1
F value	0.92	0.68	1.21
t value	-8.96	39.04	8.58
p value	<0.001**	<0.001**	<0.001**
95% CI	-4.21-2.59	18.55-20.70	3.47-5.78

C(-)=Not given Ro (SSA) antigen or MO-ethanol extract.
 C(+)=Only given Ro (SSA) antigen.
 T1=Dexamethasone dose 1.23 mg/kg BW.
 T2=MO-ethanol extract 200 mg/kg BW and dexamethasone 1.23 mg/kg BW.
 **=Statistically significant at the 1% level (p<0.001).

Table 9 displays that there are significant differences in MMP-9 levels of the T2 treatment group post-Ro (SSA) antigen induction when compared to the C(-), C(+), and T1 groups with p<0.001. The most significant difference was between the T2 and C(+) groups with p<0.001 (95% CI 18.55-20.70). It can be seen that the T2 group was more effective in reducing MMP-9 levels post-Ro (SSA) antigen induction than the C(-), C(+), and T1 groups.

MO-ethanol extract administration on caspase-3 levels in experimental animal model of SS

Differences in caspase-3 levels based on groups can be seen in Table 10. Based on a one-way ANOVA, there was a significant difference in caspase-3 levels with C(-) as reference (p<0.05) and Levene's test value p=0.01. The Games-Howell *post hoc* test was subsequently conducted (Table 11).

Table 11 shows that caspase-3 levels post-Ro (SSA) antigen induction in the C(+), T1, and T2 groups were significantly different from the C(-) control group with p<0.001. This showed effective treatment in reducing caspase-3 levels. Differences in caspase-3 levels between the T2 group and the other groups is shown in Table 12. Table 12 shows that there

Table 10. Differences in caspase-3 levels based on groups.

Groups	Caspase-3 levels
C(-)	1.77±0.43
C(+)	7.39±0.11
T1	3.85±0.2
T2	2.83±0.14
p value	<0.001**

C(-)=Not given Ro (SSA) antigen or MO-ethanol extract.
 C(+)=Only given Ro (SSA) antigen.
 T1=Dexamethasone dose 1.23 mg/kg BW.
 T2=MO-ethanol extract at a dose of 200 mg/kg BW and dexamethasone at a dose of 1.23 mg/kg BW.
 **=Statistically significant at the 1% level (p<0.001).

Table 11. Post hoc test for analyzed caspase-3 levels post-Ro (SSA) induction in various groups.

	Caspase-3			
	C(-)	C(+)	T1	T2
p value	Reference category	<0.001**	<0.001**	<0.001**
95% CI	Reference category	-5.75 to -5.49	-2.32 to -1.85	1.23 to -0.90
Mean difference	Reference category	-5.62	-2.08	-1.06

C(-)=Not given Ro (SSA) antigen or MO-ethanol extract.
 C(+)=Only given Ro (SSA) antigen.
 T1=Dexamethasone dose 1.23 mg/kg BW.
 T2=MO-ethanol extract 200 mg/kg BW and dexamethasone 1.23 mg/kg BW.
 **=Statistically significant at the 1% level (p<0.001).

Table 12. Independent t-test of caspase-3 levels post-induction of Ro (SSA) with the T2 group as reference compared to other groups.

T2 group	Caspase-3 level post-Ro (SSA) antigen induction		
	C(-)	C(+)	T1
F value	4.52	0.23	1.98
t value	-20.67	72.93	11.74
p value	<0.001**	<0.001**	<0.001**
95% CI	-1.17-9.52	4.43-4.69	0.83-1.21

C(-)=Not given Ro (SSA) antigen or MO-ethanol extract.
 C(+)=Only given Ro (SSA) antigen.
 T1=Dexamethasone dose 1.23 mg/kg BW.
 T2=MO-ethanol extract 200 mg/kg BW and dexamethasone 1.23 mg/kg BW.
 **=Statistically significant at the 1% level (p<0.001).

are significant differences in caspase-3 levels of the T2 treatment group post-Ro (SSA) antigen induction when compared to the C(-), C(+), and T1 groups with p<0.001. The most significant difference was between the T2 and C(+) groups with p<0.001 (95% CI 4.43-4.69). It can be concluded that the T2 group had more effective results in reducing caspase-3 levels post-Ro (SSA) antigen induction than the other groups.

Effect of MO-ethanol extract on salivary gland epithelial necrosis in experimental animal model of SS

A total of 11 mice were induced by Ro (SSA) antigen, and their salivary glands were collected. These samples were prepared with Harris hematoxylin and eosin staining and evaluated using a scoring system, which can be seen in Figure 1. Differences in salivary gland epithelial necrosis based on groups can be seen in Table 13. Table 13 shows that there is a significant difference in salivary gland epithelial necrosis scores between various groups with p<0.001, and then a Mann-Whitney *post hoc* test was conducted (Table 14).

Table 14 shows that dexamethasone administration, with or without MO-ethanol extract, was more effective in preventing damage, upon histopathological examination, to salivary gland epithelial necrosis 14 days post-induction of Ro antigen (SSA). There was also a significant difference after being given MO-ethanol extract at a dose of 200 mg/kg BW in reducing the degree of salivary gland epithelial necrosis.

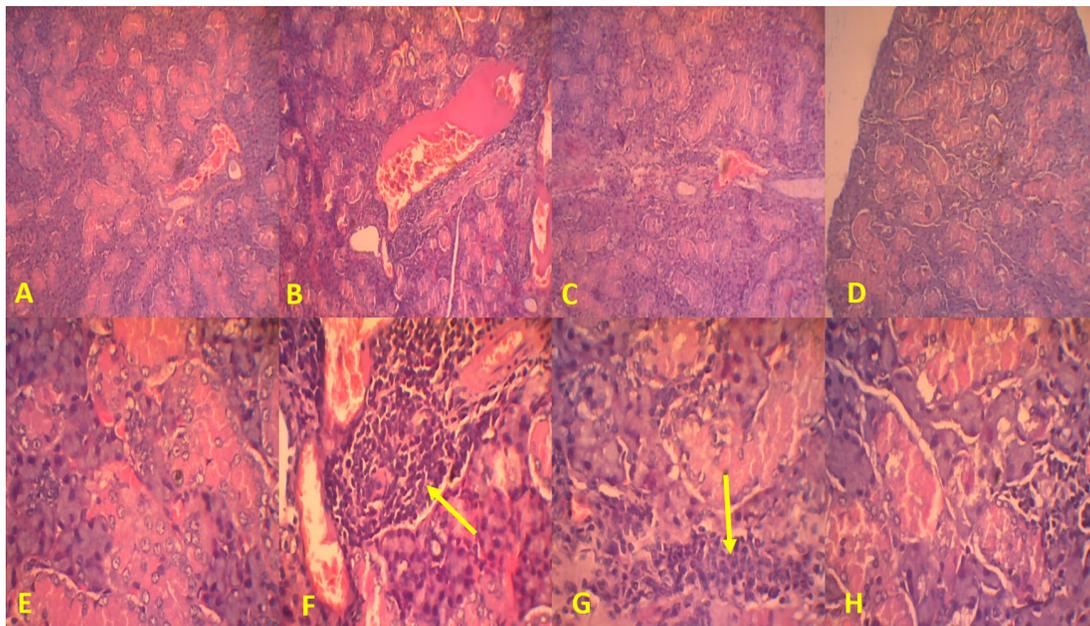


Figure 1. Hematoxylin-eosin staining of the submandibular gland showing a score of 0, 3, 2, and 1 in the normal group (A and E), negative control group (B and F), T1 group (C and G), and T2 group (D and H), respectively. (A, B, C, and D) Magnification at 100x and (E, F, G, and H) 400x. Arrows indicate lymphocytic infiltration.

Table 13. Differences in salivary gland epithelial necrosis based on groups.

Groups	Score of salivary gland epithelial necrosis, n (%)			
	0	1	2	3
C(-)	8 (100)	0 (0)	0 (0)	0 (0)
C(+)	0 (0)	0 (0)	5 (62.5)	3 (37.5)
T1	0 (0)	0 (0)	5 (62.5)	3 (37.5)
T2	0 (0)	4 (50)	4 (50)	0 (0)
p value	<0.001 **			

C(-)=Not given Ro (SSA) antigen or MO-ethanol extract.

C(+)=Only given Ro (SSA) antigen.

T1=Dexamethasone dose 1.23 mg/kg BW.

T2=MO-ethanol extract 200 mg/kg BW and dexamethasone 1.23 mg/kg BW.

**=Statistically significant at the 1% level (p<0.001).

Table 14. Post hoc test for analyzed score of salivary gland epithelial necrosis post-Ro (SSA) induction in various groups.

Groups	Score of salivary gland epithelial necrosis post-Ro (SSA) antigen induction p value		
	T1	T2	C(+)
T1			
T2	0.01*		
C(+)	1.00	0.01*	
C(-)	<0.001**	<0.001**	<0.001**

C(-)=Not given Ro (SSA) antigen or MO-ethanol extract.

C(+)=Only given Ro (SSA) antigen.

T1=Dexamethasone dose 1.23 mg/kg BW.

T2=MO-ethanol extract 200 mg/kg BW and dexamethasone 1.23 mg/kg BW.

*=Statistically significant at the 5% level (p<0.05).

**=Statistically significant at the 1% level (p<0.001).

Discussion

The pathogenesis of SS involves autoantigen presentation, B and T cells, and autoantibody-mediated mechanisms of tissue injury (anti-SSA/Ro and anti-SSA/La antibodies). This mechanism causes cell fragmentation. TNF- α triggers endothelial cell adhesion molecules such as E-selectin that in turn trigger polymorphonuclear (PMN) cell arrest, which also involves IL-17 (microcirculation) and initiation of inflammatory responses (MMP-9 secretion, lysozyme, and caspase-3 pathways). PMN cells, especially neutrophils, trigger the activation of MMP-9 that degrades collagen leading to basement membrane damage. Neutrophils also secrete lysozyme enzymes that can affect mitochondrial oxygenation and the GMP cycle, triggering epithelial necrosis. The inflammatory process makes cells swell, so that the cell membrane ruptures, which leads to epithelial necrosis. Caspase-3 activation, which acts as an executor, promotes DNA fragmentation and ends with cell death through apoptosis. Basement membrane damage, epithelial necrosis, and epithelial cell apoptosis result in destruction to the salivary and lacrimal glands. Macrophages and lymphocytes are the main source of IL-6, especially in the inflammatory process. Together with IL-1 and TNF- α , IL-6 is able to activate T cells, induce an acute inflammatory response, and increase C-reactive protein (CRP) synthesis by hepatocytes. CRP is one of the inflammatory mediators that lowers nitric oxide synthase levels in the endothelium, which encompass endothelial dysfunction. NF κ B has activity in initiating the inflammatory process and increasing proinflammatory cytokines (TNF- α), adhesion molecules, and NADPH transcriptions. Angiotensin II increases ROS production through NOX stimulation via a type 1 receptor (AT1R/Angiotensin II type 1 receptor). Angiotensin II and TNF- α have activity to stimulate NF κ B activation in ROS-dependent pathways, which can further increase the production of cytokines and other proinflammatory chemokines. The formation of more ROS than antioxidants causes oxidative stress. MDA is used as a reference for the emergence of indicators of oxidative stress (Both *et al.* 2017; Brito-Zerón *et al.* 2009; Nakamura *et al.* 2018; Pflugfelder SC 2014; Verstappen *et al.* 2018).

M. oleifera leaves exhibit antioxidant activity due to their high polyphenol content. They exhibit strong antioxidant activity against free radicals, thus prevent oxidative damage to major biomolecules and provide protection against oxidative damage. *M. oleifera* leaf extract is a prospective indicator of oxidative stress according to decreasing serum MDA levels. This would prevent the apoptotic process that is characterized by a decrease in caspase-3 levels in serum,

and epithelial necrosis followed by a decrease in serum MMP-9 and IL-17 levels so that lacrimal and salivary gland damage can be minimized (Charoensin 2014; Verma *et al.* 2009).

This study showed that administration of 200 mg/kg BW of MO-ethanol leaf extract in SS model mice could significantly reduce MDA levels compared to the negative control group. This is in accordance with Nadimin's study (2016), which aimed to determine the effect of *M. oleifera* extract on MDA levels during pregnancy in Makassar, Indonesia. This study was divided into two denominations: the intervention and the control. It was found that MDA levels in the control group were greater than those in the intervention cluster ($p=0.033$) (Nadimin 2016). The powder form of MO leaves could also reduce MDA levels in pregnant women, with $p=0.028$ (Misrawati and Marliah 2018). Several other studies have also shown that administration of MO-ethanol extract can reduce MDA levels in various diseases (Albrahim and Binobead 2018; Almufazar 2018; Wulandari *et al.* 2017).

This study showed that administration of MO-ethanol leaf extract significantly reduced IL-17 levels. The effect of MO-ethanol leaf extract, which can reduce IL-17 levels, was also useful in cases of inflammation due to ultraviolet-B/UV-B exposure (El Shanawany *et al.* 2019). The study by Ma *et al.* (2018) on psoriasis-induced mice showed a decrease in IL-17 levels ($p<0.05$) when given MO seed extract. A previous study showed that *M. oleifera* significantly reduced serum levels of IgG/Immunoglobulin G, IL-2, and IL-17 in sheep coinfecting with *Fasciola gigantica* and *Clostridium novyi* (Engsuwan *et al.* 2021). Several studies addressing other cytokines including TNF- α , IL-6, IL-8, IL-1 β , IL-10, NO, and PGE2 (prostaglandin E2) have been conducted. Koolheat *et al.* (2014) found that MO can abolish the production of monocyte-derived macrophage factors, such as TNF- α , IL-6, and IL-8 (Xiao *et al.* 2020). Wardhani (2020) found that *M. oleifera* has a hepatoprotective effect by inhibiting TNF- α , IL-1 β , IL-6, and IL-10. *M. oleifera* also inhibits fatty liver disease by inhibiting lipogenesis via the NF- κ B pathway as characterized by decreased LDL-R/low-density lipoprotein receptor, SRB1c, DGAT2/diacylglycerol o-acyltransferase 2, and PPAR γ /peroxisome proliferator activator γ and increased insulin sensitivity (Wardhani 2020).

Xie *et al.* (2021) evaluated the inhibitory effect induced by the alkaloids contained in *M. oleifera* on the proliferative and migratory phases in *in vivo* or *in vitro* methods on human prostate cell cancer (PC3). This study shows that *M. oleifera* will inhibit proliferation and induce cell apoptosis which causes cell cycle arrest. Furthermore, *M. oleifera* suppresses the migration of prostate cancer cells and inhibits the expression of MMP-9 (Xie *et al.* 2020). The study of Xie *et al.* (2020) also showed that western blotting results of *Moringa oleifera* alkaloids extract treatment at 200 g/ml inhibited the expression of MMP-2 ($p<0.05$) and MMP-9 cell migration-associated proteins compared to the control. Both studies were in accordance with the recent study, especially regarding the MO-ethanol leaf extract effect that can reduce MMP-9 levels compared to the control group.

Several studies, which analyzed the anti-inflammatory effect of MO leaf extract on caspase-3 levels, have been conducted. The study of Mousa *et al.* (2019), which aimed to determine the anti-inflammatory effect of MO leaves on thioacetamide intoxicated rats, showed that MO-ethanol leaf extract is able to downregulate caspase-3. The study by Bahr and Farouk (2016), which aimed to determine the hepatoprotective effect of MO leaf extract on experimental animals combined with lornoxicam, obtained significant results in reducing caspase-3 levels ($p<0.05$). Rijal *et al.* (2016) observed changes in caspase-3 expression (apoptosis) in PCG (primary congenital glaucoma) trabecular cell cultures treated with *Moringa oleifera* leaf extract. This study showed a significant change in caspase-3 expression (apoptosis) after administration of methanol extract of *Moringa oleifera* leaves at doses of 20, 30, and 40 g/ml in primary congenital glaucoma trabecular meshwork cell cultures (Wulandari *et al.* 2019). In accordance with these studies, this research showed that MO-ethanol leaf extract could significantly reduce caspase-3 levels.

In this study, it was shown that administration of MO-ethanol leaf extract significantly reduced salivary gland epithelial necrosis scores in the treatment group compared to the control group. It occurred due to the anti-inflammatory mechanism of MO-ethanol. The study conducted by Fatmawati *et al.* (2019), which observed histopathological features of the pancreas in diabetic rats induced by streptozotocin, showed changes in Langerhans insula repair compared to the hyperglycemic control group and also restored weight loss to normal. The study conducted by Kamaliani *et al.* (2018) found that the administration of *M. oleifera* ethanol extract (200 mg/kg BW) in diabetic Wistar rat kidneys causes fatty degeneration compared to the control group. They explained that a dose of 200 mg/kg BW was an optimal dose without causing necrosis. Ijioma *et al.* (2018) also investigated several doses of MO-ethanol leaf extract (200, 400, and 800 mg/kg BW) in the stomach of aspirin-induced rats, which showed epithelial surface protection, characterized by more mucus granules and better results than those of Cimetidine in which patches of intact superficial cells were observed.

This study had several strengths. Firstly, the study proved that MDA, IL-17, MMP-9, and caspase-3 levels and salivary gland epithelium decreased significantly when given MO-ethanol extract compared to those given only Ro (SSA) antigen and dexamethasone. MO-ethanol extract could be a complementary therapy. Secondly, the study was expected to form

the basis for further research. Thirdly, the research will hopefully become a protocol in human clinical trials and could inspire future researchers to conduct research using human samples.

However, the study also had several limitations. Firstly, many other dependent variables such as markers of salivary gland damage (IFN- γ /interferon gamma, IL-6, BAFF/B-cell-activating factor, TGF- β /Transforming Growth Factor- β , LAMP3/Lysosome-associated membrane glycoprotein 3) were not observed. Secondly, the study did not observe any variation in dose of MO-ethanol leaf extract. Further research can be conducted with MO-ethanol leaf extract dose variations to determine minimum and maximum doses that can be given as supportive therapy in SS. Finally, the study method used IHC (immunohistochemistry) and ELISA (enzyme-linked immunosorbent assay) techniques; other techniques such as immunofluorescence could be performed in further research.

Conclusion

Administration of 200 mg/kg BW MO-ethanol extract 14 days post-induction by Ro (SSA) antigen significantly reduced MDA, IL-17, MMP-9, and caspase-3 levels and salivary epithelium damage (histopathological changes). MO-ethanol at a dose of 200 mg/kg BW can inhibit the apoptosis process of SS.

Ethical approval

The experiments were approved by the ethical guidelines by Dr. Moewardi Hospital Ethical Committee (approved number: 178/II/HREC/2022).

Author contributions

Conceptualization, Agus Susanto; Data curation, Agus Susanto, Ambar Mudigdo and Brian Wasita; Formal analysis, Agus Susanto; Funding acquisition, Agus Susanto; Investigation, Agus Susanto and Brian Wasita; Methodology, Agus Susanto; Project administration, Agus Susanto and Brian Wasita; Resources, Agus Susanto and Brian Wasita; Software, Agus Susanto; Supervision, Bambang Purwanto and Ambar Mudigdo; Validation, Ambar Mudigdo and Brian Wasita; Visualization, Brian Wasita; Writing – original draft, Agus Susanto; Writing – review & editing, Agus Susanto and Bambang Purwanto.

Data availability statement

Underlying data

Data repository name: [Raw Data Agus Joko Susanto.xlsx]. <https://10.6084/m9.figshare.21388788.org/> (Nadimin 2016).

The project contains the following underlying data:

- [Raw data Agus Joko Susanto] (raw data).

Reporting guidelines

Data repository name: ARRIVE checklist for '[ARRIVE Guidelines-Author Checklist -.pdf]'. <https://10.6084/m9.figshare.21388866.org/> (Albrahim and Binobead 2018).

Data are available under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/) (CC-BY 4.0).

Acknowledgements

The authors thank Fatna Andika Wati and Dyah Rohmania Agustiana for their indispensable assistance in manuscript preparation.

References

Albrahim T, Binobead MA: **Roles of Moringa oleifera Leaf Extract in Improving the Impact of High Dietary Intake of Monosodium Glutamate-Induced Liver Toxicity, Oxidative Stress, Genotoxicity, DNA Damage, and PCNA Alterations in Male Rats.** *Oxidative Med. Cell. Longev.* 2018; **2018**: 1–11.
[PubMed Abstract](#) | [Publisher Full Text](#)

Almuzafar HM: **Effect of Moringa oleifera leaves extract on the oxidative stress and gastric mucosal ulcer induced by indomethacin in rats.** *Afr. J. Biotechnol.* 2018; **17**(3): 51–56.

Bahr HI, Farouk SM: **Comparative Ameliorative Effect of Basil Oil and Moringa oleifera on Lornoxicam- Mediated Histological and Biochemical Alterations in Albino Rat Liver.** *J. Veterinar. Sci. Technol.* 2016; **7**(2): 1–11.

Both T, Dalm VA, van Hagen PM, *et al.*: **Reviewing primary Sjögren's syndrome: beyond the dryness - From pathophysiology to diagnosis and treatment.** *Int. J. Med. Sci.* 2017; **14**(3): 191–200.
[PubMed Abstract](#) | [Publisher Full Text](#)

- Brito-Zerón P, Baldini C, Bootsma H, et al.: **Sjögren syndrome**. *Nat. Rev. Dis. Primers*. 2016; **2**(16047).
[Publisher Full Text](#)
- Brito-Zerón P, Soria N, Muñoz S, et al.: **Prevalence and clinical relevance of autoimmune neutropenia in patients with primary Sjögren's syndrome**. *Semin. Arthritis Rheum*. 2009; **38**(5): 389–395.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Carsons SE, Vivino FB, Parke A, et al.: **Treatment Guidelines for Rheumatologic Manifestations of Sjögren's Syndrome: Use of Biologic Agents, Management of Fatigue, and Inflammatory Musculoskeletal Pain**. *Arthritis Care Res. (Hoboken)*. 2017; **69**(4): 517–527.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Charoensin S: **Antioxidant and anticancer activities of Moringa oleifera leaves**. *J. Med. Plant Res*. 2014; **8**(7): 318–325.
- Chauhan SK, El Annan J, Ecoiffier T, et al.: **Autoimmunity in dry eye is due to resistance of Th17 to Treg suppression**. *J. Immunol*. 2009; **182**(3): 1247–1252.
[PubMed Abstract](#) | [Publisher Full Text](#)
- De Paiva CS, Chotikavanich S, Pangelinan SB, et al.: **IL-17 disrupts corneal barrier following desiccating stress**. *Mucosal Immunol*. 2009; **2**(3): 243–253.
[PubMed Abstract](#) | [Publisher Full Text](#)
- El Shanawany EE, Fouad EA, Keshta HG, et al.: **Immunomodulatory effects of Moringa oleifera leaves aqueous extract in sheep naturally co-infected with Fasciola gigantica and Clostridium novyi**. *J. Parasit. Dis*. 2019; **43**(4): 583–591.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Engsuwan J, Waranuch N, Limpeanchob N, et al.: **Anti-inflammatory effect of Moringa oleifera Lam. leaf extract on UVB-irradiated human keratinocytes**. *Songklanakarinn. J. Sci. Technol*. 2021; **43**(3): 774–780.
- Farooq F, Rai M, Tiwari A, et al.: **Medicinal properties of Moringa oleifera: An overview of promising healer**. *J. Med. Plant Res*. 2012; **6**(27): 4368–4374.
- Fatmawati A, Bachri MS, Nurani LH: **Combination Effects of Moringa oleifera Leaf Ethanol Extract and Andrographis paniculata Herb on Blood Glucose Levels and Pancreas Histopathology of Diabetic Rats Induced by Streptozotocin**. *Trad. Med*. 2019; **24**(2): 85–90.
[Publisher Full Text](#)
- Haugen AJ, Peen E, Hultén B, et al.: **Estimation of the prevalence of primary Sjögren's syndrome in two age-different community-based populations using two sets of classification criteria: the Hordaland Health Study**. *Scand. J. Rheumatol*. 2008; **37**(1): 30–34.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Hulkkonen J, Pertovaara M, Anttonen J, et al.: **Matrix metalloproteinase 9 (MMP-9) gene polymorphism and MMP-9 plasma levels in primary Sjogren's syndrome**. *Rheumatology*. 2004; **43**(12): 1476–1479.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Igoe A, Scofield RH: **Autoimmunity and infection in Sjögren's syndrome**. *Curr. Opin. Rheumatol*. 2013; **25**(4): 480–487.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Ijioma SN, Nwaogazi EN, Nwankwo AA, et al.: **Histological exhibition of the gastroprotective effect of Moringa oleifera leaf extract**. *Comp. Clin. Pathol*. 2018; **27**(2): 327–332.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Kamaliani BR, Setiasih NLE, Winaya IBO: **Gambaran Histopatologi Ginjal Tikus Wistar Diabetes Melitus Eksperimental yang Diberikan Ekstrak Etanol Daun Kelor**. *Buletin Veteriner Udayana*. 2018; **11**(1): 71–77.
- Karthivashan G, Kura AU, Arulselvan P, et al.: **The modulatory effect of Moringa oleifera leaf extract on endogenous antioxidant systems and inflammatory markers in an acetaminophen-induced nephrotoxic mice model**. *PeerJ*. 2016; **4**: e2127–e2118.
[Publisher Full Text](#)
- Kim SK, Choe JY, Lee GH: **Enhanced expression of NLRP3 inflammasome-related inflammation in peripheral blood mononuclear cells in Sjögren's syndrome**. *Clin. Chim. Acta*. 2017; **474**: 147–154.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Kooltheat N, Sranujit RP, Chumark P, et al.: **An ethyl acetate fraction of Moringa oleifera Lam. Inhibits human macrophage cytokine production induced by cigarette smoke**. *Nutrients*. 2014; **6**(2): 697–710.
[Publisher Full Text](#)
- Mahmood KT, Mugal T, Haq IU: **Moringa oleifera: a natural gift-A review**. *Int. J. Pharm. Sci. Res*. 2010; **2**(11): 775–781.
- Misrawati, Marliah: **Pengaruh Pemberian Tepung Daun Kelor Pada Ibu Hamil Terhadap Kadar Malondialdehid (MDA)**. *Jurnal Ilmiah Kesehatan Sandi Husada*. 2018; **10**(1): 48–54.
[Publisher Full Text](#)
- Mousa AA, El-Gansh H, Eldaim M, et al.: **Protective effect of Moringa oleifera leaves ethanolic extract against thioacetamide-induced hepatotoxicity in rats via modulation of cellular antioxidant, apoptotic and inflammatory markers**. *Environ. Sci. Pollut. Res. Int*. 2019; **26**(31): 32488–32504.
[Publisher Full Text](#)
- Nadimin: **The Influence Provision of Moringa Leaf Exctracy (Moringa Oleifera) against the Level of Mda (Malondialdehyde) in Pregnant Women**. *IJSBAR*. 2016; **27**(3): 48–56.
- Nakamura H, Horai Y, Shimizu T, et al.: **Modulation of Apoptosis by Cytotoxic Mediators and Cell-Survival Molecules in Sjögren's Syndrome**. *Int. J. Mol. Sci*. 2018; **19**(8): 1–19.
- Nocturne G, Mariette X: **B cells in the pathogenesis of primary Sjögren syndrome**. *Nat. Rev. Rheumatol*. 2018; **14**(3): 133–145.
[Publisher Full Text](#)
- Pflugfelder SC: **What causes dryness in Sjögren's syndrome patients and how can it be targeted?**. *Expert. Rev. Clin. Immunol*. 2014; **10**(4): 425–427.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Romão VC, Talarico R, Scirè CA, et al.: **Sjögren's syndrome: state of the art on clinical practice guidelines**. *RMD Open*. 2018; **4**: 1–8.
[Publisher Full Text](#)
- Sambataro D, Sambataro G, Dal Bosco Y, et al.: **Present and future of biologic drugs in primary Sjögren's syndrome**. *Expert. Opin. Biol. Ther*. 2017; **17**(1): 63–75.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Scofield RH, Fayyaz A, Kurien BT, et al.: **Prognostic value of Sjögren's syndrome autoantibodies**. *J Lab Precis Med*. 2018; **3**: 1–11.
[Publisher Full Text](#)
- Sellers RS: **Translating Mouse Models**. *Toxicol. Pathol*. 2017; **45**(1): 134–145.
[Publisher Full Text](#)
- Shen L, He J, Kramer JM, et al.: **Sjögren's Syndrome: Animal Models, Etiology, Pathogenesis, Clinical Subtypes, and Diagnosis**. *J. Immunol. Res*. 2019; **2019**: 1–3.
[Publisher Full Text](#)
- Shiboski CH, Shiboski SC, Seror R, et al.: **International Sjögren's Syndrome Criteria Working Group. 2016 American College of Rheumatology/European League Against Rheumatism Classification Criteria for Primary Sjögren's Syndrome: A Consensus and Data-Driven Methodology Involving Three International Patient Cohorts**. *Arthritis Rheumatol*. 2017; **69**(1): 35–45.
[Publisher Full Text](#)
- Shih KC, Lun CN, Jhanji V, et al.: **Systematic review of randomized controlled trials in the treatment of dry eye disease in Sjogren syndrome**. *J. Inflamm. (Lond)*. 2017; **14**(26): 1–11.
[Publisher Full Text](#)
- Smallwood MJ, Nissim A, Knight AR, et al.: **Oxidative stress in autoimmune rheumatic diseases**. *Free Radic. Biol. Med*. 2018; **125**: 3–14.
[Publisher Full Text](#)
- Sreelatha S, Padma PR: **Antioxidant activity and total phenolic content of Moringa oleifera leaves in two stages of maturity**. *Plant Foods Hum. Nutr*. 2009; **64**(4): 303–311.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Stefanski AL, Tomiak C, Pleyer U, et al.: **The Diagnosis and Treatment of Sjögren's Syndrome**. *Dtsch. Arztebl. Int*. 2017; **114**(20): 354–361.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Stohs SJ, Hartman MJ: **Review of the Safety and Efficacy of Moringa oleifera**. *Phytother. Res*. 2015; **29**(6): 796–804.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Verma AR, Vijayakumar M, Mathela CS, et al.: **In vitro and in vivo antioxidant properties of different fractions of Moringa oleifera leaves**. *Food Chem. Toxicol*. 2009; **47**(9): 2196–2201.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Verstappen GM, Corneth O, Bootsma H, et al.: **Th17 cells in primary Sjögren's syndrome: Pathogenicity and plasticity**. *J. Autoimmun*. 2018; **87**: 16–25.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Vivino FB, Bunya VY, Massaro-Giordano G, et al.: **Sjogren's syndrome: An update on disease pathogenesis, clinical manifestations and treatment**. *Clin. Immunol*. 2019; **203**: 81–121.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Wardhani TM: **Pemanfaatan Tanaman Kelor (Moringa oleifera, Lam.) sebagai Sumber Terapi Preventif dan Kuratif pada Pasien Perlemakan Hati dengan Sindrom Metabolik**. *SCRIPTA SCORE Scientific Medical Journal*. 2020; **1**(2): 1–11.
- Wenzel J, Gerdson R, Uerlich M, et al.: **Antibodies targeting extractable nuclear antigens: historical development and current knowledge**. *Br. J. Dermatol*. 2001; **145**(6): 859–867.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Wulandari LP, Santoso B, Purwanto B: **Kadar Malondialdehid tikus model Sindroma Ovarium Polikistik dengan daun kelor (Moringa oleifera)**. *Jurnal Biosains Pascasarjana*. 2017; **19**: 224–236.
[Publisher Full Text](#)

Wulandari LR, Umiati S, Sujuti H: **Protective effect of methanol extract of Kelor (*Moringa oleifera*) leaves on Glutathione Peroxidase (GPx) levels in trabecular meshwork cell culture of primary congenital glaucoma patients.** *Eurasia J. Biosci.* 2019; **13**: 839–844.

Xiao X, Wang J, Meng C, *et al.*: ***Moringa oleifera* Lam and its Therapeutic Effects in Immune Disorders.** *Front. Pharmacol.* 2020; **11**: 1–9.
[Publisher Full Text](#)

Xie J, Luo F, Shi CY, *et al.*: ***Moringa oleifera* Alkaloids Inhibited PC3 Cells Growth and Migration Through the COX-2 Mediated Wnt/ β -Catenin Signaling Pathway.** *Front. Pharmacol.* 2020; **11**: 1–12.
[Publisher Full Text](#)

Xie J, Peng L, Yang M, *et al.*: **Alkaloid Extract of *Moringa oleifera* Lam. Exerts Antitumor Activity in Human Non-Small-Cell Lung Cancer via Modulation of the JAK2/STAT3 Signaling Pathway.** *Evid. Based Complement. Alternat. Med.* 2021; **2021**: 1–12.

The benefits of publishing with F1000Research:

- Your article is published within days, with no editorial bias
- You can publish traditional articles, null/negative results, case reports, data notes and more
- The peer review process is transparent and collaborative
- Your article is indexed in PubMed after passing peer review
- Dedicated customer support at every stage

For pre-submission enquiries, contact research@f1000.com

F1000Research