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Synergistic benefits of Nicotine and Thymol in alleviating experimental rheumatoid arthritis

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ABSTRACT

Purpose: Given to the anti-inflammatory effect of Nicotine and Thymol, this study was done to evaluate the effects of co-administration of Nicotine and Thymol on the clinical aspects, and immunity responses in Freund's complete adjuvant (FCA)-induced RA in Wistar rat.

Methods: The study population contained a total of 50 male Wistar rats with a weight range 150 ± 7 g, which RA was induced through FCA at them. These animals were randomly allocated into five groups (n = 10): RA rats treated with PBS (100 mg/kg orally), RA rats treated with Thymol (100 mg/kg orally), RA rats treated with Nicotine (2.5mg/kg-orally), and RA rats treated with combined Nicotine and Thymol (half doses with each one-orally). All treatments were initiated at day seven p.i. when all rats showed a clinical score of ≥1. Clinical symptoms of the disease were recorded every other day until the day 23 p.i.

Results: Obtained data revealed the combination therapy reduced the severity of the disease and improved weight-gaining more profound than each medication alone. Furthermore, combination therapy caused a reduction in some hematological and biochemical RA parameters, such as Rheumatoid factor, C-Reactive Protein, Nitric oxide, Myeloperoxidase, IL-1, and IL-17 more impressive than each treatment alone. Interestingly, the combination therapy with half doses of Nicotine and Thymol did not have any synergistic advantage in anti-proliferation effect, and therefore immunosuppression side effect compared with using each of agents alone.

Conclusion: Collectively, it is possible that combination therapy can be applied as a beneficial strategy to control RA.

1. Introduction

Rheumatoid arthritis (RA) is known as an inflammatory rheumatic disease that leads to chronic synovial inflammation, then destruction in joints [1]. The epidemiologic research showed that the prevalence of rheumatoid arthritis is 0.5%–1.0%. Approximately 75% of patients with RA own autoantibodies such as rheumatoid factor, proposing that RA is an autoimmune disorder [2].In the inflammatory immune process, activation of immune cells is accelerated through the generation of pro-inflammatory cytokines, and mediators in the synovial membrane, which is responsible for more hurt to bone [3,4]. Albeit macrophages set up the inflammation, activation of various subtypes of T-cells own an important role in the progression of the process [5]. As a result, the interaction between macrophages, cytokines, and T-cells owns a substantial action in the pathological imbalance of the immune response in RA [3,4].

Most of the treatments for RA mitigate pain, decrease joint swelling, and damage [6]. These treatments are based on the use of glucocorticoids and non-steroidal anti-inflammatory drugs, which in high dose and long-term using own many side effects on bone, stomach, kidney, and other organs [1,6]. On the other hand, a relatively large proportion of patients have an inadequate response to conventional disease-modifying anti-rheumatic medications [7]. Therefore, RA patients mainly prefer the alternative, and complementary medicines, especially phytomedicines [1,8]. Fortunately, Freund's complete adjuvant (FCA)-induced arthritis is considered as rat model for studying the different aspects of RA [9,10]. Previous studied has revealed FCA-induced RA in rat shares common characteristics with rheumatoid arthritis in human Characterization of a Freund's complete adjuvant-induced model of chronic arthritis in mice [9–11].

Thymol is an illustrious natural monoterpene phenol, and found plentifully in plants belonging to the *Lamiaceae* family (thymus, Monarda genera, Ocimum, and origanum, and many other medicinal herbs [12]. Thymol was considered for its antioxidant, anti-inflammatory, as well as immune-modulatory impacts [13]. Previous documents indicated that Thymol could regress the lipopolysaccharide-

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induced inflammatory response or airway inflammation in an ovalbumin-induced mouse model of asthma [13]. Thymol has been suggested as a safe alternative for treating autoimmune, and inflammatory diseases [12,13]. Nicotine is a famous parasympathomimetic alkaloid, which is typically detected at a high level in tobacco leaves [14,15]. Nicotine also deactivates an inflammatory response, through decreasing of pro-inflammatory cytokines, and cells [15]. Furthermore, Nicotine declines T cell activation, antibody response, and pro-inflammatory cytokine production [16,17]. These anti-inflammatory effects of Nicotine have been confirmed in different studies [14,15,17].

Due to the complex nature of autoimmune diseases, and also the involvement of multiple cells in their pathogenesis, the treatment of these diseases is rarely effective only via one drug [18,19]. One suitable approach to control complicated disorders like RA is to investigate a logical combination of new medications or existing drugs. Accordingly, the recent study was done to evaluate the effects of simultaneous administration of Nicotine and Thymol on clinical presentation, and immune responses in FCA -induced rheumatoid arthritis in Wistar rats.

2. Materials and methods

2.1. Chemicals

Cell culture media and fetal calf serum (FBS) were obtained from GIBCO/Life Technologies Inc. (Gaithersburg, MD, USA). The ELISA (enzyme-linked immunosorbent assay) kits were purchased PeproTech EC, Ltd. (London, UK). Nicotine, Thymol, and other reagents were procured from Sigma-Aldrich Corporation (St. Louis, MO, USA).

2.2. Animals

A total of 50 male Wistar rats (eight weeks old) were obtained from the faculty of veterinary medicine, Urmia University, Iran. These rats weighed 150 ± 7 g. All rats were kept under the condition of temperature (23 °C ± 1) with a 12-hour light/dark cycle and allowed food and water *ad libitum*. All experimental procedure was carried out by the ethical standards of laboratory laws published by the National Institute of Health Guide. This study was conducted under ethical code “IR, UU.AEC.906/AD3” issued by the Ethics Committee for Laboratory Animals of Urmia University, Iran.

2.3. Induction of RA and clinical evaluations

RA was induced in rats by intradermal injection into the hind paw with 0.1 mL of Complete Freund's adjuvant (CFA), containing 10 mg/ml of killed *Mycobacterium* [20].

The volume of the non-injected hind paw was monitored every other day by an electronic water plethysmograph. The severity of the disease was determined by using the following scoring system: 4 = Complete swelling of the whole leg and inability to bend it; 3 = Swelling of the ankle; 2 = Erythema and swelling of paws; 1 = Erythema of the toe; 0 = Normal paw. All evaluations were made every Monday during the study. Evaluations were done by three independent observers at each examination and the average of the measurements was reported. Therefore, the maximum arthritis score can be 12 (The arthritis index was evaluated only for the non-injected paws). Moreover, the changes in weight of each rat were recorded every other day after immunization.

2.4. Treatment of rats

All therapies were initiated when all rats had developed signs of edema in the non-injected hind paw. Rats were randomly allocated into the 5 equal groups: RA rats treated with PBS, RA rats treated with Thymol (100 mg/kg-orally), RA rats treated with Nicotine (2.5mg/kg-orally), RA rats treated with combined Nicotine and Thymol (half doses with each one-orally) and normal control rats (these rats were given an equal volume of water at the same time). Therapies were continued at day 23 post immunization when the rats were euthanized. These dosages were selected in accordance with the previous studies on some rat inflammatory models [14,21].

2.5. Serum evaluations

Before animal sacrifice, blood was drawn from the abdominal aorta, and centrifuged at 3500 rpm for 15 min at 4 °C. The serum was frozen at −80 °C until analyzed. The levels of TNF-α, IL-1, IL-6, IL-17, and IFN-γ in the serum were measured by ELISA kits for cytokine assays following the manufacturer's instructions (BD, UK).

Moreover, the serum level of nitric oxide (NO) was determined by nitric oxide measuring kit (Sigma, USA). Also, rheumatoid factor (RF) and C-reactive protein (CRP) levels were measured by RF and CRP measuring kits (BD, UK), respectively.

The activity of myeloperoxidase (MPO) enzyme in collected sera was determined by method Pulli et al., [36]. Briefly, 10 µL of sample was added to 80 µL H_2O_2, and 0.75 mM 3,3′,5,5′-Tetramethylbenzidine (TMB) (2.9 mM TMB, 14.5% DMSO, 150 mM phosphate buffer, pH 5.4). After incubation for 5 min at 37 °C, the change in absorbance was measured at 450 nm. The outcomes were presented as U/L over 5 min, whereby one unit of the enzyme was described as the content of MPO degrading 1 nM H_2O_2 per minute at 37 °C.

2.6. Lymphocyte proliferation index

After bleeding from the rats, the spleens were removed under sterile conditions. Each spleen tissue was sliced and crushed in 5 ml of RPMI-1640 medium containing 05% FBS. The spleens were passed through a filter with a swelling of 0.02 ml and the cell suspension was centrifuged at 2000 rpm for 10 min. In order to remove the red blood cells, 5 ml of ACK buffer [containing 8.29 g ammonium chloride, 1 g potassium bicarbonate and 32.2 g EDTA] was added to the cellular deposition. After the cell counting process, the cell suspension was prepared in 1×10^6 cell/ml of each sample. For each sample, three replicates were considered in the presence of 50 µl of phytohemagglutinin (1 mg/ml) and three replicates without phytohemagglutinin. In the three wells, RPMI empty environment was used as blank. After 72 h’ incubation of the samples in the oven with 5% CO2, 25 µl of MTT (5 mg/ml) was added to each well. Following adding the 100 µl of DMSO, the color intensity was determined at 495 nm, and the stimulation index was calculated according to the following equation: stimulation index = OD (with the presence of PHA)-OD (blank)/OD (without the presence of PHA)-OD (blank).

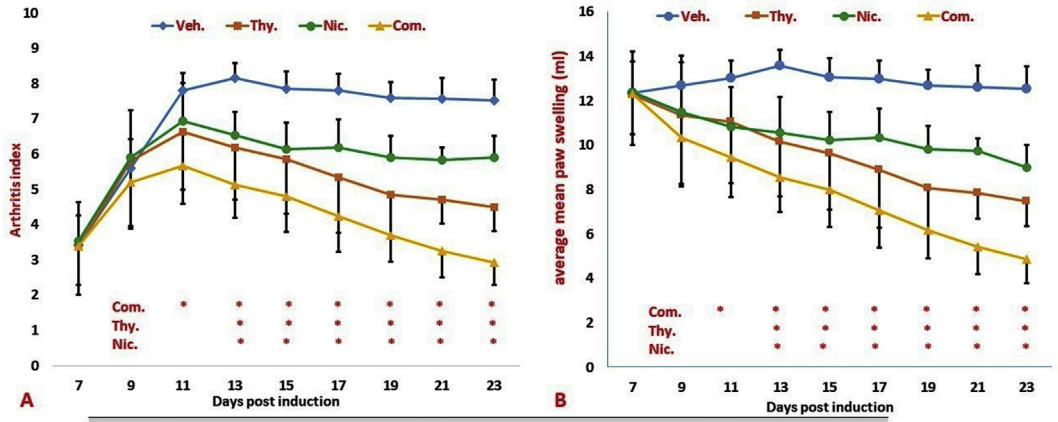
2.7. Statistical analysis

The statistical analysis was performed using the SPSS software program, version 22 (IBM Co., USA). One-way analysis of variance (ANOVA) followed by Tukey's range test for comparison of means of different groups. In the research, the level of statistical significance was set at $p < 0.05$.

3. Results

3.1. Clinical evaluations

The inflammatory reactions in the joints environment is an essential clinical symptom in RA and its animal models [22]. Treatment protocols were started at day 7 post-immunization when individual rats showed an arthritis index of ≥1. The peak of paw swelling recorded on day 13 post adjuvant immunization (Fig. 1A and B). Both administrations of Nicotine and Thymol could significantly suppress the arthritis index hind paw swelling of RA rats, compared to vehicle-treated RA rats



C

Group	average arthritis index	average mean paw swelling (ml)	Mean body weight (g)
Veh.	7.36±0.50	12.86±0.25	171.45±7.25
Com.	3.95±0.54*	8.01±0.34*	160.06±5.43*
Thy.	5.25 ±0.61*#	9.39±0.65*#	151.23±6.14*
Nic.	6.37±0.748*#&	10.92±0.47*#&	140.86±5.97*#&

Fig. 1. Evaluation of clinical features in RA rats. RA rats received Nicotine and Thymol alone and in combination as detailed under Materials and Methods. Combined of half doses of Nicotine and Thymol attenuated clinical features of RA vigorous than intervention with either medication alone at optimal doses. A) arthritis index, B) paw swelling arthritis index and C) alteration of average mean paw swelling, average arthritis index and mean body weight. Findings were presented as mean ± S.D. (*p < 0.05 versus PBS-treated RA rats; #p < 0.05 versus combined-treated RA rats; &p < 0.05 versus Thymol-treated RA rats). (Veh., Vehicle-treated RA group; Thy., Thymol-treated RA group; Nic., Nicotine-treated RA group; Com., Combination-treated RA group).

(Fig. 1A and B and C). Albite, the reduction of both indices in the RA group treated with half doses of Nicotine and Thymol in combination was more evident than RA rats treated with each medication at its full doses (Fig. 1 A, B and C). Evaluation of paw swelling data and arthritis index showed that treatment with Nicotine and Thymol in combination led to a significant reduction of paw swelling index from day 11, compared to vehicle-treated RA rats. This beneficial effect was initiated from day 13 p.i. in both Nicotine and Thymol-treated rats (Fig. 1A and B). Also, data analysis showed that Thymol had more profound benefit in the reduction of average mean paw swelling and average arthritis index compared to Nicotine (Fig. 1C).

As shown in Fig. 1C, an increment in body weight was registered after injection of CFA in rats. Our results indicated that all therapeutic groups had improved the weight-gaining during treatment. The highest improvement was observed in the combination therapy and Thymol groups. It can be concluded that treatment with Thymol has more profound benefits in regressing the weight-gaining in RA rats than treatment with Nicotine) Fig. 1C (.

3.2. Changes in biochemical factors

In this research, all treatments significantly decreased the levels of CRP, RF, NO and MPO activity in rats with rheumatoid arthritis. The highest reduction of CRP and MPO was recorded for combination therapy with half doses of Nicotine and thymol compared to therapy with each individual medication at its full doses (Fig. 2 A and B). Based on the observations, the highest reduction in RF and the nitric oxide level was registered for the combination therapy and Thymol groups (Fig. 2C and D). Accordingly, Thymol has been able to decline the content of RF and nitric oxide more than Nicotine (Fig. 2C and D). In summary, the co-administration of Nicotine and Thymol resulted in lower CRP and MPO than individual Nicotine and Thymol treatments, which reflects the synergistic effect of Nicotine and Thymol against CRP and MPO (Fig. 2).

3.3. Effect of treatment on pro-inflammatory cytokine levels

As Fig. 3 shows, all medications led to a significant diminish in the content of these cytokines in the sera samples of rats with RA compared to the vehicle-treated RA rats. Our results revealed the highest decrease in IL-1 and IL-17 level occurred in the group receiving the combination therapy with Nicotine and Thymol at half doses compared to RA rats received monotherapy at full doses. Statistical analysis demonstrated that combination therapy didn't have any synergistic benefit in reducing TNF-α, IL-6 and IFN-γ compared to RA tats treated individually with these medications. Also, Thymol has been able to regress the levels of IL-1β and IL-6 more than Nicotine (Fig. 3). Moreover, there was no significant discernment in IFN-γ and TNF-α levels between RA animals received each of the treatment protocols (Fig. 3).

Collectively, the combination of Nicotine and Thymol resulted in lower IL-1β and IL-17 than individual Nicotine and Thymol treatments, which reflects the synergistic benefits of Nicotine and Thymol against IL-1β and IL-17 (Fig. 3).

3.4. Splenocytes proliferation

All treatment groups significantly decreased lymphocyte proliferation in spleen cells (Fig. 4). As a result, it revealed that combination therapy didn't have any synergistic benefit in anti-proliferative and immunosuppressive effect compared to rats received each agent alone (Fig. 4).

4. Discussion

Complementary and alternative medicines for the treatment of rheumatoid arthritis (RA), is gaining more attraction due to its lower side effects, and its potential to cure RA [11,23,24]. Of note, the principal aim of combination therapy is that the combination leads to a significant reduction in the clinical features better than treatment with either medication alone. The results of the current study revealed that the combined Nicotine and Thymol at half doses caused a significant reduction in the severity of the established RA more profound than full doses of either medication alone. Also, it is essential that each pharmacologic substance used for combination therapy shows a tolerable safety margin, and doesn't promote additional toxicities when prescribed in combination. In the current survey, no obvious adverse event

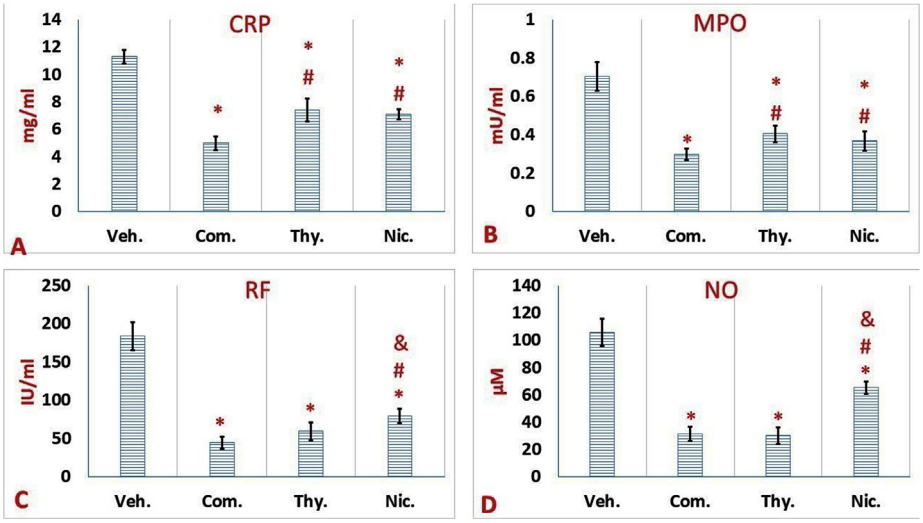


Fig. 2. Biochemical changes in the sera of RA rats. The results indicated that combination therapy with half doses of Nicotine and Thymol could synergistically reduce the levels of CRP (A) and myeloperoxidase activity (B) more profound than full doses of each medication alone. Moreover, the highest reduction in RF (C) and nitric oxide (D) levels were recorded for the combination therapy and Thymol treated groups. Data were reported as mean ± S. D. (*p < 0.05 versus PBS-treated RA rats; #p < 0.05 versus combined-treated RA rats; &p < 0.05 versus Thymol-treated RA rats). (Veh., Vehicle-treated RA group; Thy., Thymol-treated RA group; Nic., Nicotine-treated RA group; Com., Combination-treated RA group).

was noticed in experimental groups. In this regard, the co-administration of Thymol and Nicotine improved weight-gaining in animals, which suggests no toxic effects of the combination of the two compounds. More importantly, the combination therapy with Nicotine and Thymol did not show any synergistic or additive superiority in anti-proliferation effect, and therefore immunosuppression effect compared to using each of medications alone. Furthermore, combination therapy caused a reduction in some hematological and biochemical RA parameters, such as CRP, MPO, IL-1, and IL-17 more impressive than each treatment alone.

Of note, cigarettes boost autoimmune risk like RA, but not through nicotine. It has been discovered that cholinergic anti-inflammatory pathway via α7 nicotinic acetylcholine receptor activation plays an essential, and interesting role to control the chronic inflammation like RA or multiple sclerosis [14,25]. Similar to our results, the beneficial effects of nicotine in ameliorating RA have been documented in some previous studies [26,27]. Nevertheless, there is no/or limited information about the role of Thymol on RA. Our results demonstrated for the first time that therapeutic treatment with Thymol could reduce the signs of the animal model of RA.

Based on our data, Nicotine, Thymol and their combination insignificantly cause a decrease in the levels of pro-inflammatory and inflammatory cytokines. Our results also showed that combination therapy with Thymol and Nicotine reversed established RA more than each medication alone, at least partly by suppressing pro-inflammatory

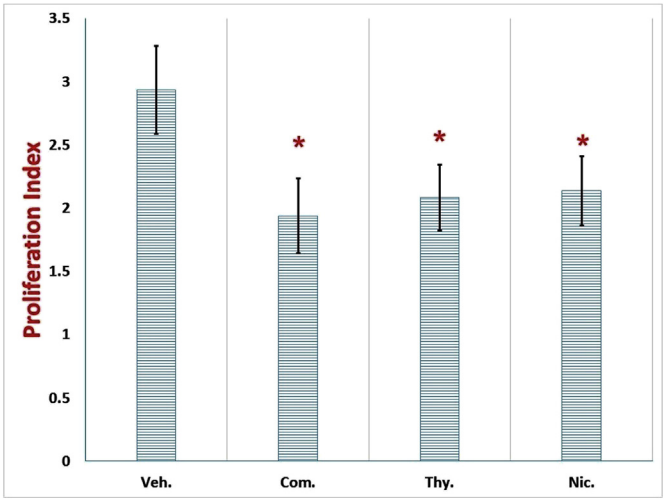


Fig. 4. Effect of medications on splenocyte proliferation index. Proliferation index of splenocytes was checked by MTT assay as presented under Materials and Methods. Combination therapy didn't show any synergy in anti-proliferative and immunosuppressive effect. Results were shown as mean ± S.D. (*p < 0.05 versus PBS-treated RA rats). (Veh., Vehicle-treated RA group; Thy., Thymol-treated RA group; Nic., Nicotine-treated RA group; Com., Combination-treated RA group).

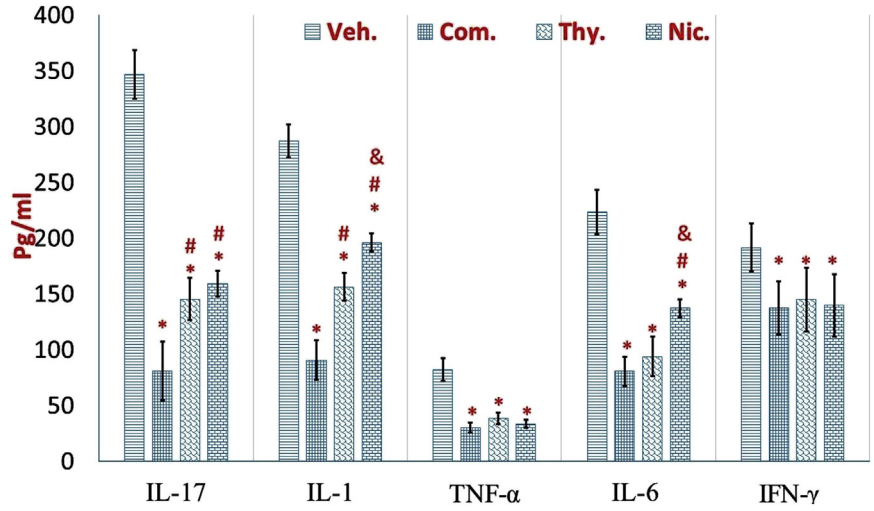


Fig. 3. Effect of treatment regimens on the production of inflammatory cytokines in the splenocyte population. The most profound decrease in IL-1 and IL-17 level was recorded in the group receiving the combination therapy. Albite, combination therapy didn't have any synergy in reducing TNF-α, IL-6 and IFN-γ compared to RA tats treated individually. Furthermore, Thymol has been able to decrease the levels of IL-1β and IL-6 more than Nicotine. The values were presented as mean ± S.D. (*p < 0.05 versus PBS-treated RA rats; #p < 0.05 versus combined-treated RA rats; &p < 0.05 versus Thymol-treated RA rats). (Veh., Vehicle-treated RA group; Thy., Thymol-treated RA group; Nic., Nicotine-treated RA group; Com., Combination-treated RA group).

cytokines, including IL-17, and IL-1 more than monotherapy.IL-17, as a key pro-inflammatory cytokine, plays an important role in the inflammation of RA. Since there is a relationship between the level of IL-17, and RA activity, its level can be determined as a degree of disease [6,28]. IFN-γ significantly expressed in inflammatory synovial membrane in RA patients, and is involved in increasing other inflammatory mediators such as IL-6, and TNF-α [29,30]. In close agreement with our findings, Yu [31] proved that Nicotine decreases the levels of IL-17, and IFN-γ. Similarly, it was registered a reduction in the levels of IL-17, and IFN-γ due to Thymol administration [17]. It has been well registered that there is a significant relationship between the level of Th17 cytokine, as an upstream cytokine over IFN-γ, and the severity of joint inflammation in patients with RA [32]. Collectively, it seems that IL-17 cells are more pathogenic than IFN-γ. In this regard, many common drugs, and even new medications, such as Infliximab (anti-TNF-α antibody), are not well suited to changing the level of IL-17 in patients with rheumatoid arthritis [33]; therefore, reduction in the level of IL-17 in the combination therapy group has a potential benefit. Furthermore, overproduction of TNF-α increases the expression of IL-1, and IL-6 cytokines, stimulate collagenase, generate matrix-degrading enzymes and facilitate the progress in arthritic erosions [34,35]. It has been ruled out that thymol significantly reduces the expression of genes responsible for pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6 [36,37]. In the current survey, all treatment protocol caused a significant decrease in the levels of TNF-α, IL-1β, and IL-6 in the sera samples of rats with RA compared to the vehicle-treated RA rats. Albite, the combination therapy caused the highest decrease in the level of IL-1β compared to other monotherapy. The IL-1 inflammatory cytokine is considered as a key intermediary in RA, as it increases the generation of matrix metalloproteinase in the synovial membrane [38], as well as the destruction of bone [39]. Also, IL-1 reduces the regeneration process via suppressing matrix synthesis. IL-1 also induces several inflammatory genes at both systemic and local levels. Therefore, the decline of the level of IL-1 cytokine owns an essential role in reducing inflammation and clinical symptoms [38].

The capability of both Nicotine and Thymol to decline lymphocyte proliferation may limit the number of potentially pathologic T cells. According to these results, Kalra et al. found that Nicotine in the laboratory could reduce the proliferation of lymphocytes in human [40]. Meeran et al. also revealed that Thymol reduces the proliferation of lymphocytes in a dose-dependent manner [41]. Notably, our study indicated that the combination therapy with Nicotine and Thymol did not have any synergistic advantage in anti-proliferation effect and therefore immunosuppression side effect compared with using each of agents alone.

It is clear that the upregulated level of MPO activity is one of the foremost diagnostic tools of oxidative and inflammatory stress biomarkers in autoimmune diseases like RA [42]. NO-dependent nitrative tissue injury has been shown in several rheumatic diseases, such as RA [43].Nicotine has also been reported to inhibit the activation of neutrophils, and infiltrate into the region of inflammation, resulting in the lower activity of the MPO enzyme in an animal model of colitis [44]. A former documents showed that nicotine markedly could inhibit LPS-induced mRNA expression of inducible nitric oxide synthase in rat intestinal mesothelial cells [45]. Thymol was also recognized that declines activity of MPO enzyme and nitric oxide in acetic acid-induced colitis [12,46]. Our results showed that combination therapy with a half dose of Nicotine and Thymol could reduce the blood levels of MPO more pronounced than each medication at full doses. Furthermore, Thymol and combination therapy could regress the level of NO more impressive that treatment with nicotine. However, the level of NO did not show any significant differences between Thymol and combination therapy groups.

CRP, an acute phase reactant, reflects different pathological processes driven by the underlining chronic inflammation [47]. It has been documented that the injection of CFA in rat model leads to a significant elevation in the level of rheumatoid factor (RF) [10].Both factors are important in terms of tracking RA [48], so reducing their level is a good predictor. In the recent study, the highest reduction in RF was recorded for the combination therapy and Thymol. Furthermore, the synergistic effect of Nicotine and Thymol was observed against CRP. In agreement with our results, it has been showed that thymol [36], and Nicotine [49]reduces the level of inflammatory biomarkers such as CRP.

5. Conclusion

Our investigation demonstrated for the first time that therapeutic treatment with Thymol could reduce the signs of the animal model of RA. Moreover, combination therapy with a half dose of Nicotine and Thymol led to an improvement in clinical and laboratory indices of RA more profound than treatment with each medication alone. Therefore, co-administration of these substances can be considered as a potential approach to control RA. However, further exploration is required to identify the exact mechanism of action and to identify the synergistic role of Nicotine and Thymol components.

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Declaration of competing interest

The authors declare that they have no conflicts of interest.

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References

[1] T. Pan, T. Cheng, Y. Jia, P. Li, F. Li, Anti-rheumatoid arthritis effects of traditional Chinese herb couple in adjuvant-induced arthritis in rats, J. Ethnopharmacol. 205 (2017) 1–7, https://doi.org/10.1016/J.JEP.2017.04.020.
[2] Y. Okada, S. Eyre, A. Suzuki, Y. Kochi, K. Yamamoto, Genetics of rheumatoid arthritis: 2018 status, Ann. Rheum. Dis. 78 (2019), https://doi.org/10.1136/annrheumdis-2018-213678 446 LP-453.
[3] G.R. Burmester, E. Feist, T. Dörner, Emerging cell and cytokine targets in rheumatoid arthritis, Nat. Rev. Rheumatol. 10 (2014) 77–88, https://doi.org/10.1038/nrrheum.2013.168.
[4] D.E. Furst, P. Emery, Rheumatoid arthritis pathophysiology: update on emerging cytokine and cytokine-associated cell targets, Rheumatology 53 (2014) 1560–1569, https://doi.org/10.1093/rheumatology/ket414.
[5] S.P. Vyas, A.K. Hansda, R. Goswami, Rheumatoid arthritis: “melting pot” of T helper subsets, Int. Rev. Immunol. 0 (2019) 1–20, https://doi.org/10.1080/08830185.2019.1621865.
[6] I.B. McInnes, G. Schett, Pathogenetic insights from the treatment of rheumatoid arthritis, The Lancet 389 (2017) 2328–2337, https://doi.org/10.1016/S0140-6736(17)31472-1.
[7] D. Patel, A. Shelbaya, R. Cheung, J. Aggarwal, S.H. Park, J. Coindreau, Cost-effectiveness of early treatment with originator biologics or their biosimilars after methotrexate failure in patients with established rheumatoid arthritis, Adv. Ther. 36 (2019) 2086–2095, https://doi.org/10.1007/s12325-019-00986-7.
[8] J.K. Rao, K. Mihaliak, K. Kroenke, J. Bradley, W.M. Tierney, M. Weinberger, Use of complementary therapies for arthritis among patients of rheumatologists, Ann. Intern. Med. 131 (1999) 409–416, https://doi.org/10.7326/0003-4819-131-6-199909210-00003.
[9] N.L. Chillingworth, L.F. Donaldson, Characterisation of a Freund's complete adjuvant-induced model of chronic arthritis in mice, J. Neurosci. Methods 128 (2003) 45–52, https://doi.org/10.1016/S0165-0270(03)00147-X.
[10] O.A.M.A. El-Gaphar, A.M. Abo-Youssef, A.A. Abo-Saif, Effect of losartan in complete freund's adjuvant –induced arthritis in rats, Iran. J. Pharm. Res. (IJPR) 17 (2018) 1420–1430.
[11] G.V. Bihani, S.R. Rojatkar, S.L. Bodhankar, Anti-arthritic activity of methanol extract of Cyathocline purpurea (whole plant) in Freund's complete adjuvant-induced arthritis in rats, Biomed. Aging Pathol. 4 (2014) 197–206, https://doi.org/10.1016/J.BIOMAG.2014.04.007.
[12] P. Tahmasebi, S. Meysam, A. Froushani, N.A. Ahangaran, Thymol Has Beneficial Effects on the Experimental Model of Ulcerative Colitis, (2019), pp. 1–13.
[13] M.F. Nagoor Meeran, H. Javed, H. Al Taee, S. Azimullah, S.K. Ojha,

Pharmacological properties and molecular mechanisms of thymol: prospects for its therapeutic potential and pharmaceutical development, Front. Pharmacol. 8 (2017) 1–34, https://doi.org/10.3389/fphar.2017.00380.
[14] S. Khezri, S.M. Abtahi Froushani, M. Shahmoradi, Nicotine augments the beneficial effects of mesenchymal stem cell-based therapy in rat model of multiple sclerosis, Immunol. Investig. 47 (2018) 113–124, https://doi.org/10.1080/08820139.2017.1391841.
[15] S. Pourtayeb, S.M. Abtahi Froushani, Nicotine can modulate the effects of the mesenchymal stem cells on neutrophils, Adv. Med. Sci. 62 (2017) 165–170, https://doi.org/10.1016/J.ADVMS.2016.07.006.
[16] Y.M. Geng, S.M. Savage, L.J. Johnson, J. Seagrave, M.L. Sopori, Effects of nicotine on the immune response. I. Chronic exposure to nicotine impairs antigen receptor-mediated signal transduction in lymphocytes, Toxicol. Appl. Pharmacol. 135 (1995) 268–278, https://doi.org/10.1006/TAAP.1995.1233.
[17] S. Wu, H. Luo, X. Xiao, H. Zhang, T. Li, X. Zuo, Attenuation of collagen induced arthritis via suppression on Th17 response by activating cholinergic anti-inflammatory pathway with nicotine, Eur. J. Pharmacol. 735 (2014) 97–104, https://doi.org/10.1016/J.EJPHAR.2014.04.019.
[18] L. Klareskog, D. van der Heijde, J.P. de Jager, A. Gough, J. Kalden, M. Malaise, E.M. Mola, K. Pavelka, J. Sany, L. Settas, J. Wajdula, R. Pedersen, S. Fatenejad, M. Sanda, Therapeutic effect of the combination of etanercept and methotrexate compared with each treatment alone in patients with rheumatoid arthritis: double-blind randomised controlled trial, The Lancet 363 (2004) 675–681, https://doi.org/10.1016/S0140-6736(04)15640-7.
[19] W. Dai, C. Qi, S. Wang, Synergistic effect of glucosamine and vitamin E against experimental rheumatoid arthritis in neonatal rats, Biomed. Pharmacother. 105 (2018) 835–840, https://doi.org/10.1016/J.BIOPHA.2018.05.136.
[20] C.M. Pearson, Development of arthritis, periarthritis and periostitis in rats given adjuvants, PSEBM (Proc. Soc. Exp. Biol. Med.) 91 (1956) 95–101, https://doi.org/10.3181/00379727-91-22179.
[21] A.R.S. Ribeiro, P.B.F. Diniz, M.S. Pinheiro, R.L.C. Albuquerque-Júnior, S.M. Thomazzi, Gastroprotective effects of thymol on acute and chronic ulcers in rats: the role of prostaglandins, ATP-sensitive K+ channels, and gastric mucus secretion, Chem. Biol. Interact. 244 (2016) 121–128, https://doi.org/10.1016/J.CBI.2015.12.004.
[22] M. Jahan Tigh, S. Abtahi Froshani, N. Afzal Ahangaran, Effect of mesenchymal stem cells treated with 17β-estradiol on the pattern of intrinsic immunity responses in collagen-induced rheumatoid arthritis in wistar rats, Armaghane Danesh 23 (2018) 42–56 http://armaghanj.yums.ac.ir/article-1-1926-en.html , Accessed date: 11 October 2019accessed.
[23] G.R. Burmester, J.E. Pope, Novel treatment strategies in rheumatoid arthritis, The Lancet 389 (2017) 2338–2348, https://doi.org/10.1016/S0140-6736(17)31491-5.
[24] E.H. Choy, A.F. Kavanaugh, S.A. Jones, The problem of choice: current biologic agents and future prospects in RA, Nat. Rev. Rheumatol. 9 (2013) 154–163, https://doi.org/10.1038/nrrheum.2013.8.
[25] C. Ren, Y.L. Tong, J.C. Li, Z.Q. Lu, Y.M. Yao, The protective effect of alpha 7 nicotinic acetylcholine receptor activation on critical illness and its mechanism, Int. J. Biol. Sci. 13 (2017) 46–56, https://doi.org/10.7150/ijbs.16404.
[26] J. Grygielska, F. Raciborski, A. Kłak, J. Owoc, The impact of nutrition and generally available products such as nicotine and alcohol on rheumatoid arthritis – review of the literature, Reumatologia 56 (2018) 121–127, https://doi.org/10.5114/reum.2018.75524.
[27] J.P. Gomes, A. Watad, Y. Shoenfeld, Nicotine and autoimmunity: the lotus' flower in tobacco, Pharmacol. Res. 128 (2018) 101–109, https://doi.org/10.1016/J.PHRS.2017.10.005.
[28] A.N. Burska, A. Thu, R. Parmar, I. Bzoma, B. Samans, E. Raschke, S. Olek, P.G. Conaghan, P. Emery, F. Ponchel, Quantifying circulating Th17 cells by qPCR: potential as diagnostic biomarker for rheumatoid arthritis, Rheumatology (2019) 1–10, https://doi.org/10.1093/rheumatology/kez162.
[29] R. Ulmansky, K. Turjeman, M. Baru, G. Katzavian, M. Harel, A. Sigal, Y. Naparstek, Glucocorticoids in nano-liposomes administered intravenously and subcutaneously to adjuvant arthritis rats are superior to the free drugs in suppressing arthritis and inflammatory cytokines, J. Control. Release 160 (2012) 299–305, https://doi.org/10.1016/J.JCONREL.2011.12.024.
[30] Y. Nozaki, T. Yamagata, M. Sugiyama, S. Ikoma, K. Kinoshita, M. Funauchi, Anti-inflammatory effect of all-trans-retinoic acid in inflammatory arthritis, Clin. Immunol. 119 (2006) 272–279, https://doi.org/10.1016/J.CLIM.2005.11.012.
[31] H. Yu, Y.H. Yang, R. Rajaiah, K.D. Moudgil, Nicotine-induced differential modulation of autoimmune arthritis in the lewis rat involves changes in interleukin-17 and anti-cyclic citrullinated peptide antibodies, Arthritis Rheum. 63 (2011) 981–991, https://doi.org/10.1002/art.30219.
[32] S. Alzabin, Effector T cells in rheumatoid arthritis: lessons from animal models, FEBS (Fed. Eur. Biochem. Soc.) Lett. 585 (2011) 3649–3659, https://doi.org/10.1016/J.FEBSLET.2011.04.034.
[33] F. Miyoshi, K. Sato, T. Mimura, Changes in the pattern of cytokine production from peripheral blood mononuclear cells in patients with rheumatoid arthritis treated with infliximab and their relation to plasma arginase activity, Int. J. Rheum. Dis. 21 (2018) 1907–1914, https://doi.org/10.1111/1756-185X.12864.
[34] J.L. Pablos Álvarez, Interleukin 6 in the physiopathology of rheumatoid arthritis, Reumatol. Clínica 5 (2009) 34–39, https://doi.org/10.1016/S2173-5743(09)70086-3 English Edition.
[35] K. Syed Zameer Ahmed, S.S.Z. Ahmed, A. Thangakumar, R. Krishnaveni, Therapeutic effect of Parmotrema tinctorum against complete Freund's adjuvant-induced arthritis in rats and identification of novel Isophthalic ester derivative, Biomed. Pharmacother. 112 (2019), https://doi.org/10.1016/J.BIOPHA.2019.108646 108646.
[36] N. Gholijani, Z. Amirghofran, Effects of thymol and carvacrol on T-helper cell subset cytokines and their main transcription factors in ovalbumin-immunized mice, J. Immunotoxicol. 13 (2016) 729–737, https://doi.org/10.3109/1547691X.2016.1173134.
[37] G. Li-Sha, Z. Jing-Lin, C. Guang-Yi, L. Li, Z. De-Pu, L. Yue-Chun, Dose-dependent protective effect of nicotine in a murine model of viral myocarditis induced by coxsackievirus B3, Sci. Rep. 5 (2015) 1–10, https://doi.org/10.1038/srep15895.
[38] H.E. Barksby, S.R. Lea, P.M. Preshaw, J.J. Taylor, The expanding family of interleukin-1 cytokines and their role in destructive inflammatory disorders, Clin. Exp. Immunol. 149 (2007) 217–225, https://doi.org/10.1111/j.1365-2249.2007.03441.x.
[39] K.-J. Gou, R. Zeng, X.-D. Ren, Q.-L. Dou, Q.-B. Yang, Y. Dong, Y. Qu, Anti-rheumatoid arthritis effects in adjuvant-induced arthritis in rats and molecular docking studies of Polygonum orientale L. extracts, Immunol. Lett. 201 (2018) 59–69, https://doi.org/10.1016/J.IMLET.2018.11.009.
[40] R. Kalra, S.P. Singh, J.C. Pena-Philippides, R.J. Langley, S. Razani-Boroujerdi, M.L. Sopori, Immunosuppressive and anti-inflammatory effects of nicotine administered by patch in an animal model, Clin. Diagn. Lab. Immunol. 11 (2004) 563–568, https://doi.org/10.1128/CDLI.11.3.563-568.2004.
[41] A. Khan, M. Alsahli, A. Rahmani, Myeloperoxidase as an active disease biomarker: recent biochemical and pathological perspectives, Med. Sci. 6 (2018) 33, https://doi.org/10.3390/medsci6020033.
[42] G. Nagy, A. Koncz, T. Telarico, D. Fernandez, B. Érsek, E. Buzás, A. Perl, Central role of nitric oxide in the pathogenesis of rheumatoid arthritis and sysemic lupus erythematosus, Arthritis Res. Ther. (2010) 12, https://doi.org/10.1186/ar3045.
[43] J.K.S. Ko, C.H. Cho, The diverse actions of nicotine and different extracted fractions from tobacco smoke against hapten-induced colitis in rats, Toxicol. Sci. 87 (2005) 285–295, https://doi.org/10.1093/toxsci/kfi238.
[44] S.D. AlSharari, H.I. Akbarali, R.A. Abdullah, O. Shahab, W. Auttachoat, G.A. Ferreira, K.L. White, A.H. Lichtman, G.A. Cabral, M.I. Damaj, Novel insights on the effect of nicotine in a murine colitis model, J Pharmacol Exp Ther. 344 (2013) 207–217, https://doi.org/10.1124/jpet.112.198796.
[45] M. Chamanara, A. Abdollahi, S.M. Rezayat, M. Ghazi-Khansari, A. Dehpour, E. Nassireslami, A. Rashidian, Thymol reduces acetic acid-induced inflammatory response through inhibition of NF-kB signaling pathway in rat colon tissue, Inflammopharmacology (2019), https://doi.org/10.1007/s10787-019-00583-8.
[46] A.C. Bay-Jensen, A. Platt, M.A. Jenkins, M.E. Weinblatt, I. Byrjalsen, K. Musa, M.C. Genovese, M.A. Karsdal, Tissue metabolite of type I collagen, C1M, and CRP predicts structural progression of rheumatoid arthritis, BMC Rheumatol. 3 (2019) 1–10, https://doi.org/10.1186/s41927-019-0052-0.
[47] M. Benito, Review, Acta Scientific Medical Sciences Rheumatoid Arthritis: An Outlook Of The Main Inflammatory Cells And Mediators Involved, And Treatments To Target Inflammation 2 (2018), pp. 66–70 https://www.actascientific.com/ASMS/pdf/ASMS-02-0150.pdf.
[48] M. Slevin, R.S. Iemma, Y. Zeinolabediny, D. Liu, G.R. Ferris, V. Caprio, N. Phillips, M. Di Napoli, B. Guo, X. Zeng, R. AlBaradie, N.K. Binsaleh, G. McDowell, W.H. Fang, Acetylcholine inhibits monomeric C-reactive protein induced inflammation, endothelial cell adhesion, and platelet aggregation; a potential therapeutic? Front. Immunol. 9 (2018) 2124, https://doi.org/10.3389/fimmu.2018.02124.