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# The Anti-Inflammatory Effects of the Nutrition Bio-Shield (NBS) Supplement Intake on Adjuvant-Induced Rheumatoid Arthritis in Rat

## Sıçan Modellerinde Nutrition Bio-Shield (NBS) Takviyesi Alımının Adjuvan Kaynaklı Romatoid Artrit Üzerindeki Anti-Enflamatuvar Etkileri

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### Abstract

**Objective:** The immunological characteristics of rheumatoid arthritis (RA) have led to the use of a wide range of immunosuppressive agents in its treatment procedure; however, the available treatments tend to be merely alleviative in many cases. In this respect, this study attempts to assess the therapeutic potential of the nutrition bio-shield (NBS) supplement, which has inhibitory effects on lymphocytes in the treatment of RA.

**Materials and Methods:** In this study, 25 male Wistar rats were induced by RA using Freud's complete adjuvant. Out of these rates, 15 were orally treated by NBS supplement at relevant concentrations (12.5, 25, and 50 mg/kg) for 30 days. Then, blood samples were obtained from all the cases in order to evaluate the serum levels of rheumatoid factor (RF), erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP).

**Results:** Obtained results indicated that when rats were treated with the supplement, the serum levels of ESR and CRP, i.e. two indicators of the inflammatory responses, significantly declined compared to the control group. Noticeably, it was found that the supplement successfully restored the level of RF to the normal level in all of the treated rats, with the maximal effects observed at 50 mg/kg ($p<0.05$).

**Conclusion:** Altogether, this study proposed that the NBS supplement could restore blood RF to the normal level and eliminate the symptoms of RA in the animal models. As a suggestion, this supplement could be integrated into the treatment protocol of RA; however, further analysis is required to check its therapeutic value.

**Keywords:** Rheumatoid arthritis, nutrition bio-shield supplement, rheumatoid factor, serologic tests, anti-inflammatory agents, micronutrients

### Öz

**Amaç:** Romatoid artritin (RA) immünolojik özellikleri, tedavi prosedüründe çok çeşitli immünosüpresif ajanların kullanılmasına yol açmıştır. Ancak mevcut tedaviler çoğu durumda sadece hafifletici olma eğilimindedir. Bu bağlamda, bu çalışma, RA tedavisinde lenfositler üzerinde inhibitör etkileri olan nutrition bio-shield (NBS) verilmesinin tedavi edici etkisini değerlendirmeyi amaçlamaktadır.

**Gereç ve Yöntem:** Bu çalışmada, 25 erkek Wistar sıçanı, Freund's complete adjuvant kullanılarak deneysel RA oluşturuldu. Bunlardan 15'ine, 30 gün boyunca uygun konsantrasyonlarda (12,5, 25 ve 50 mg/Kg) NBS verildi. Daha sonra tüm olgulardan romatoid faktör (RF), eritrosit sedimentasyon hızı (ESR) ve C-reaktif protein (CRP) serum düzeylerini değerlendirmek için kan örnekleri alındı.

**Bulgular:** Elde edilen sonuçlar, sıçanlar takviye ile tedavi edildiğinde, enflamatuvar yanıtların iki göstergesi olan ESR ve CRP serum seviyelerinin, kontrol grubuna kıyasla önemli ölçüde düştüğünü gösterdi. Dikkat çekici bir şekilde, tedavi edilen tüm sıçanlarda takviyenin, 50 mg/kg'de gözlemlenen maksimum etkilerle, RF seviyesini normal seviyeye başarıyla geri getirdiği görüldü ($p<0.05$).

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**Sonuç:** Bu çalışma, NBS takviyesinin hayvan modellerinde kan RF'yi normal seviyeye getirebileceğini ve RA semptomlarını ortadan kaldırabileceğini göstermiştir. Bir öneri olarak, bu ek RA'nın tedavi protokolüne entegre edilebilir. Bununla birlikte, bu uygulamanın tedavi edici etkisini araştırmak için daha fazla çalışmalara gereksinim vardır.

**Anahtar Kelimeler:** Romatoid artrit, besin desteği, romatoid faktör, serolojik testler, anti-enflamatuvar ajanlar, mikrobesinler

## Introduction

In rheumatoid arthritis (RA), the autoimmune response against articular tissues is the first mechanism proposed for the pathogenesis of the disease (1). It has been suggested that genetic and environmental factors could develop the disease (2,3). Environmental factors including infections, diet, and lifestyle may activate the genetic drivers of RA to stimulate the innate and adaptive arms of the immune system in order to produce inflammatory mediators namely tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), and IL-6 (4). It has been declared that while environmental factors could propel innate immune cells to the articular space, genetic abnormalities affect T-lymphocytes repertoire selection and antigen presentation, which could shift the balance between the osteoblasts and osteoclasts, leading to bone erosion and joint deformity (5). These two important symptoms influence a patient's quality of life (5). The results of previous study revealed that the prevalence of depressive symptoms and anxiety resulting from physical disability was high in RA patients, which could, in turn, influence their social lives (6). Although numerous decades have passed since the first description of RA and a progress has been made in the understanding of the disease pathogenesis, the diagnosis of this complication and the treatment strategies for the patients have not run into any changes.

Nutrition bio-shield (NBS) supplement is a herbal dietary supplement derived from wheat grains (NBS Organic Company, Turkey). It has been stated that the wheat germ contains considerable amounts of tocopherol, policosanol, phytosterol, riboflavin, thiamin, and niacin (7). Furthermore, in another study, it was found that the NBS supplement was able to stimulate the immune system upon changing the balance of neutrophils to lymphocytes (8). Since 2010, the European League Against Rheumatism and the American College of Rheumatology have elected rheumatoid factor (RF), erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) for serological diagnosis of RA (9). By making some modifications to the treatment, the treatment of this diseases begins shortly after its detection; to this end, immunosuppressive agents like non-steroidal anti-inflammatory drugs and glucocorticoids are used (10). Recently, TNF blockers have found their way into the treatment protocol of RA, as either a single agent or in combination with immunosuppressive drugs. Despite their impressive success, several side effects, like the high risk of infections, have restricted the clinical usage of such agents in RA patients (11). IL-6 inhibitors, rituximab, and abatacept (T cell inhibitor) are other therapeutic agents that have been under evaluation in pre-clinical studies (12). In addition to these synthetic drugs, herbal and natural medicines also consist a fertile ground for treating RA (13,14). Since many of these therapies have proven efficient in ameliorating the disease symptoms without serious toxicity, much attention has been drawn to this field so far. The current study attempts to assess the NBS supplement's therapeutic potential in the case of RA-induced rats.

## Materials and Methods

### Animal and Ethical Statements

Twenty-five male Wistar rats with the estimated weights of 200-250 g were purchased from Institute of Medicinal Plants, ACECR, Karaj, Iran. They were kept in special cages at 22-25°C and provided with food and water in 12 hours of light and darkness. They were transferred to the laboratory one week before the experiment to adapt them to the new conditions. The protocol was investigated and confirmed by the Institutional Review Board of Islamic Azad University of Mashhad (IR.IAU.MSHD.REC.1398.233).

### Induction of Rheumatoid Arthritis (RA) in Rats

To induce the RA model, rats received xylazine ketamine for anesthesia and then, 0.2 cc Freud's complete adjuvant (FCA) was injected into their knees (15). It should be noted that one group of animals consisting of 5 rats was only injected with 10 mg of normal saline and it remained as the control group. RA-induced rats were then divided into four groups (each having five rats) for further analysis.

### Treatment of RA-Induced Rats with the NBS Supplement

In order to measure the therapeutic value of the supplement, three groups of RA-induced rats received oral treatment containing different NBS supplement concentrations (12.5, 25, and 50 mg/kg) in the form of gavage for 30 days after induction of RA. The ingredients of the NBS supplement are shown in Table 1. After 30 days, xylazine ketamine was used to anesthetize the rats and 3-5 mL of blood was sampled from their hearts to assess the

serum levels of ESR using the Westergren method (16), CRP, and RF (each evaluated three times to ensure the results are exact). Of note, one of the rat groups did not receive any supplement concentrations and was considered as the negative control.

### Statistical Analysis

The serological tests were performed in triplicate and the outcome was yielded in the form of the mean ± standard deviation. In addition, the Kolmogorov-Smirnov test was adopted to confirm the normality of data distribution. The One-Way ANOVA test via IBM-SPSS software was also employed to gauge the data significance with a favorable probability level of $p<0.05$. The Tukey Post-hoc test was used to perform Post-hoc analysis with One-Way ANOVA.

## Results

To evaluate whether the application of the NBS-supplement treatment to RA-induced rats could ameliorate the inflammatory responses, we first evolved an RA model in rats by injecting FCA into their knees. Analysis of their blood samples revealed that the number of inflammatory parameters such as ESR, CRP, and RF increased in the RA-induced rats in comparison to the control group (FCA-untreated rats) (Table 2). Then, RA-induced rats were treated by the NBS supplement (12.5, 25, and 50 mg/kg) for a month. Our results demonstrated that the NBS supplement could not only robustly diminish the levels of ESR and CRP, but also significantly reduce the levels of RF in the treated rats (Table 2). Although the ESR and CRP levels of rats in group 1 (treated with 12.5 mg/kg of the NBS) reduced in comparison with the negative control group, they remained higher than normal (Figure 1). Maximum effect was observed in the group treated by the 50 mg/kg NBS supplement.

The conducted ANOVA test results point to a statistically significant difference among ESR, CRP, and RF levels of groups [(ESR: $F(4.20)=88.92$, p-value=0.00), (CRP: $F(4.20)=121.88$, p-value=0.00), (RF: $F(4.20)=147.71$, p-value=0.000]. ESR, CRP, and RF of RA-induced mice were statistically significantly lower after treatment by any dosage of the NBS supplement compared to the negative control (untreated) group.

Mean ESR in group 3 was statistically considerably lower than that in groups 1 and 2 ($p<0.001$ and $p=0.002$, respectively). However, no statistically significant difference was found between group 1 (12.5 mg/kg of the NBS) and group 2 (25 mg/kg of the NBS) ($p=0.172$). The mean CRP in groups 2 and 3 was statistically and significantly lower than that in group 1 ($p=0.001$ and $p=0.003$, respectively). No statistically significant difference was found between group 2 (25 mg/kg of the NBS) and group 3 (50 mg/kg of the NBS) ($p=0.997$). Finally, RF level was statistically significantly lower in group 3 than that in groups 1 and 2 ($p<0.001$ and $p=0.001$, respectively). However, no noticeable difference between groups 1 and 2 was observed ($p=0.279$). All results of the ANOVA and the Tukey Post-hoc tests are shown in Table 3.

## Discussion

Being among the devastating chronic inflammatory diseases, RA represents one of the oldest autoimmune diseases described. The data of a cohort study done in the USA illustrated that from every five patients

**Table 1.** Ingredients of the nutrition bio-shield dietary supplement.[1]

| Composition | Amount | Minerals | Amount | Vitamins | Amount |
|---|---|---|---|---|---|
| Moisture % | 8.4 | Total phosphorus % | 0.44 | B1 (mg/g) | 0.66 |
| Total ash % | 1.8 | Potassium % | 2.31 | B2 (mg/g) | 0.28 |
| Fiber % | 11.26 | Sulfur % | 0.28 | B3 (mg/g) | 2.7 |
| Digestible nutrients % | 61.9 | Magnesium % | 0.32 | B5 (mg/g) | 0.89 |
| Carbohydrate g/100g | 42.53 | Calcium % | 1.67 | B6 (mg/g) | 0.89 |
| Gross energy kcal/kg | 4300 | Boron % | 0.62 | C (mg/g) | 52.40 |
| Ether extract % | 7.2 | Iron (mg/kg) | 241 | A (IU) | 530.0 |
| Crude protein % | 20.6 | Manganese (mg/kg) | 49.80 | D (IU) | 483.0 |
| Sugar % | 3.7 | Zinc (mg/kg) | 26.9 | E (mg) | 0.97 |
| Cellulose % | 6.0 | Copper (mg/kg) | 13.6 | K (µg) | 63.60 |
| Omega-3 fatty acids (mg/g) | 48.42 | | | | |
| Omega-6 fatty acids (mg/g) | 60.62 | | | | |
| Omega-9 fatty acids (mg/g) | 22.16 | | | | |

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diagnosed with RA, one would lose the ability to work in five years following the diagnosis, suggesting that apart from economic costs, RA could have a heavy toll on patients' life quality (7). The results of the molecular and immunological investigations pointed to the role of both self-reactive B and T lymphocytes in producing a complex of cytokines, chemokines, and especially anti-bodies (Ab) that accumulate in the joints, thus harming the normal function and structure of articular tissues (17). It is obvious that T lymphocytes, produce some inflammatory cytokines such as IL-17 which could attract other inflammatory cells and mediators into the joints, thus orchestrating more inflammatory responses which, in turn, leads to the activation of osteoclasts (18).

Given the immunologic characteristics of RA, thus far, numerous immune-based therapeutic options have been recommended for treating the patients; however, none of them ensures complete recovery (19). Due to the success of many natural and herbal products in providing medicine

**Table 2.** The alteration in serological factors in healthy and rheumatoid arthritis-induced rats that responded to the supplementation.

| **Control group (healthy rats)** | | |
|---|---|---|
| **Serological factors** | **Statistical results** | |
| | **Mean ± standard error** | **Mean difference compared to reference range[f] ± standard error** |
| ESR[a] (mm/hr) | 6.8 ± 3.5 | 15.2 ± 3.5 |
| CRP[b] (mg/L) | 1.7 ± 0.4 | 4.3 ± 0.4 |
| RF[c] (U/mL) | 3.9 ± 2.0 | 16.1 ± 2.0 |
| **Negative control (RA[d]-induced rats without any treatment)** | | |
| **Serological factors** | **Statistical results** | |
| | **Mean ± standard error** | **Mean difference compared to reference range[f] ± standard error** |
| ESR (mm/hr) | 28.8 ± 1.7 | -6.8 ± 1.7 |
| CRP (mg/L) | 8.1 ± 0.3 | -2.1 ± 0.3 |
| RF (U/mL) | 25.1 ± 1.7 | -5.1 ± 1.7 |
| **Group 1 (RA-induced rats received 12.5 mg/kg of the NBS[e] supplement)** | | |
| **Serological factors** | **Statistical results** | |
| | **Mean ± standard error** | **Mean difference compared to reference range[f] ± standard error** |
| ESR (mm/hr) | 23.3 ± 1.6 | -1.3 ± 1.6 |
| CRP (mg/L) | 7.0 ± 0.7 | -1.0 ± 0.7 |
| RF (U/mL) | 18.6 ± 1.5 | 1.4 ± 1.5 |
| **Group 2 (RA-induced rats received 25 mg/kg of the NBS supplement)** | | |
| **Serological factors** | **Statistical results** | |
| | **Mean ± standard error** | **Mean difference compared to reference range[f] ± standard error** |
| ESR (mm/hr) | 20.3 ± 0.3 | 1.7 ± 0.3 |
| CRP (mg/L) | 5.5 ± 0.4 | 0.5 ± 0.4 |
| RF (U/mL) | 16.7 ± 0.4 | 4.3 ± 0.4 |
| **Group 3 (RA-induced rats received 50 mg/kg of the NBS supplement)** | | |
| **Serological factors** | **Statistical results** | |
| | **Mean ± standard error** | **Mean difference compared to reference range[f] ± standard error** |
| ESR (mm/hr) | 14.6 ± 1.2 | 7.4 ± 1.2 |
| CRP (mg/L) | 5.4 ± 0.2 | 0.6 ± 0.2 |
| RF (U/mL) | 12.1 ± 0.9 | 7.9 ± 0.9 |

[a]: Erythrocyte sedimentation rate
[b]: C-reactive protein
[c]: Rheumatoid factor
[d]: Rheumatoid arthritis
[e]: Nutrition bio-shield
[f]: Reference range for ESR, CRP and RF is ≤22, ≤6 and ≤20, respectively, ESR: Erythrocyte sedimentation rate, CRP: C-reactive protein, RF: Rheumatoid factor

for a broad spectrum of diseases, especially those with inflammatory basics, these compounds have received significant attention for RA treatment (20). Chinese herbs seem to be successful in inhibiting the development of RA by halting the generation of some inflammatory cytokines, namely TNF-α, IL-1, IL-17, and matrix metalloproteinases-3 (21,22). Red ginseng is another herbal compound that has, reportedly, improved the RA symptoms in mice by hampering the STAT3 signaling axis in T helper 17 lymphocytes (23). Based on the promising results of the herbal medicines, the objective of the current research was to assess the therapeutic value of the NBS supplement in ameliorating the RA condition in Wistar rats.

Our results showed that when rats were treated with the NBS supplement for 30 days, the serum levels of ESR and CRP, two indicators of the inflammatory responses, significantly dropped compared to the control group. In agreement with our finding, it was also reported that herbal supplements (such as hydrogenated curcuminoid) could restore the serum levels of both ESR and CRP in RA patients (24). However, regarding the reference ranges of ESR and CRP, no significant differences in ESR and CRP levels were observed in group 1, in which the rats were given 12.5 mg/kg of the NBS supplement. Although this dosage of the NBS was not entirely effective in reducing the ESR and CRP levels to normality, other dosages could make the best of lowering it to normal with a significant mean difference (Table 2).

Since both of ESR and CRP are not specific only to RA and their levels could be altered due to other mediating factors (25), we also investigated the effects of the NBS supplement on the serum RF level, which is one of the best indicators of RA in the patients. Noticeably, we found that the supplement not only successfully diminished the level of RF, but also restored it to the normal level in all treated rats (concentration).

Bayat et al. (26) reported that the herbal dietary supplement had suppressive effects on the number of

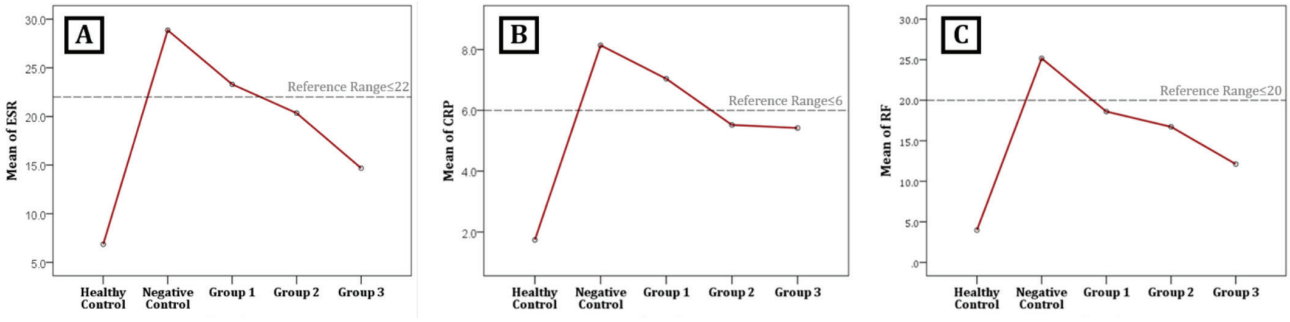


**Figure 1.** Comparison between mean of ESR (part A), CRP (part B) and RF (part C) levels amongst five different rat groups. Healthy control: Not rheumatoid arthritis (RA)-induced rats; negative control: RA-induced rats without any treatment; group 1: RA-induced rats received 12.5 mg/kg of the nutrition bio-shield (NBS) supplement; group 2: RA-induced rats received 25 mg/kg of the NBS supplement, group 3: RA-induced rats received 50 mg/kg of the NBS supplement. The dashed line shows the reference range threshold of each parameter.

*ESR: Erythrocyte sedimentation rate, CRP: C-reactive protein, RF: Rheumatoid factor*

**Table 3.** Multiple comparisons of ESR, CRP and RF difference in three groups of RA-induced rats that received different dosages of nutrition bio-shield (NBS) supplement using the Tukey Post-hoc test. P-value ≤0.05 is considered as statistically significant.

| | Mean difference of ESR[a] (mm/hr ± standard error), p-value | | | Mean difference of CRP[b] (mg/L ± standard error), p-value | | | Mean difference of RF[c] (IU/mL ± standard error), p-value | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Group 1[d]** | **Group 2[e]** | **Group 3[f]** | **Group 1[d]** | **Group 2[e]** | **Group 3[f]** | **Group 1[d]** | **Group 2[e]** | **Group 3[f]** |
| Negative control[g] | 5.5 ± 1.2 p=0.002 | 8.5 ± 1.2 p<0.001 | 14.1 ± 1.2 p<0.001 | 1.1 ± 0.3 p=0.015 | 2.6 ± 0.3 p<0.001 | 2.7 ± 0.3 p<0.001 | 6.5 ± 0.9 p=0.000 | 8.4 ± 0.9 p<0.001 | 13.0 ± 0.9 p=0.000 |
| Group 1[d] | - | 2.9 ± 1.2 p=0.172 | 8.6 ± 1.2 p<0.001 | - | 1.5 ± 0.3 p=0.001 | 1.6 ± 0.3 p<0.001 | - | 1.8 ± 0.9 p=0.279 | 6.4 ± 0.9 p<0.001 |
| Group 2[e] | -2.9 ± 1.2 p=0.172 | - | 5.66 ± 1.2 p=0.002 | -1.5 ± 0.3 p=0.001 | - | 0.1 ± 0.3 p=0.997 | -1.8 ± 0.9 p=0.279 | - | 4.6 ±0.9 p=0.001 |
| Group 3[f] | -8.6 ± 1.2 p<0.001 | -5.66 ± 1.2 p=0.002 | - | -1.6 ± 0.3 p<0.001 | -0.1 ± 0.3 p=0.997 | - | -6.4 ± 0.9 p<0.001 | -4.6 ± 0.9 p=0.001 | - |

[a]: Erythrocyte sedimentation rate
[b]: C-reactive protein
[c]: Rheumatoid factor
[d]: RA-induced rats received 12.5 mg/kg of the NBS supplement
[e]: RA-induced rats received 25 mg/kg of the NBS supplement
[f]: RA-induced rats received 50 mg/kg of the NBS supplement
[g]: RA-induced rats without any treatment, ESR: Erythrocyte sedimentation rate, CRP: C-reactive protein, RF: Rheumatoid factor, RA: Rheumatoid arthritis

lymphocytes in Balb/C mice. Their results suggested that upon ten days of treating the mice with the NBS supplement, the number of peripheral lymphocytes diminished significantly in mice, while there was an increase in the number of neutrophils (26). Given this and based on the fundamental roles of lymphocytes in the pathogenesis of RA, it is reasonable to assume that perhaps the favorable therapeutic impacts of the supplement on RA-induced rats result from its reductive effects on the number of lymphocytes. Furthermore, the therapeutic impacts of NBS supplement on the fatty liver disease were observed in the form of reducing the rats' inflammatory responses, shedding more light on the anti-inflammatory effects of the supplement (27).

## Conclusion

In light of obtained findings and based on their interpretation, this study proposed NBS supplement as an herbal dietary supplement that could restore the blood RF of the studied rats to a normal level at any applied dosages. In addition, the supplement had significant restoring effect on the ESR and CRP levels at higher concentrations, especially at 50 mg/kg. To reduce both inflammatory markers and RF, the administration of 50 mg/kg of the NBS supplement managed to produce better anti-inflammatory outcome, meaning that it could be integrated into the treatment protocol of RA. Yet, further analysis is required to investigate its therapeutic potential. For future research, it is suggested that more clinical and histopathological data before and after the intervention of the NBS may open up a new frontier to ensure a better understanding of its therapeutic effects on the RA disease.

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### Ethics

**Ethics Committee Approval:** The protocol was investigated and confirmed by the Institutional Review Board of Islamic Azad University of Mashhad (IR.IAU.MSHD.REC.1398.233).

**Informed Consent:** Not necessary.

**Peer-review:** Externally and internally peer-reviewed.

### Authorship Contributions

Supervision: M.M., Y.E., Fundings: A.K., Concept: M.M., Design: M.M., Data Collection or Processing: Y.S., Analysis or Interpretation: Y.S., M.M., Literature Search: Y.S., M.M., Writing: Y.S., M.M.

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