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Immunomodulatory Potential of Piperine in Rats

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Abstract

Objective: The ultimate goal of this research was to investigate the immunomodulatory potential of piperine, a black pepper alkaloid, on innate and acquired immune responses in Lewis rats.

Materials and Methods: In the first set of experiments, the effects of a one-month oral administration of piperine on the functions of neutrophils and peritoneal macrophages of Lewis rats were investigated. In a separate set of experiments, the effects of piperine gavage on T helper lymphocyte responses *in vivo* and *ex vivo* and their subsets were performed in animals challenged with ovalbumin (OVA).

Results: Oral administration of piperine for one month reduced the adhesion of neutrophils (p=0.04). The levels of nitric oxide (p=0.0001) and oxygen free radicals (p=0.003) were significantly decreased in peritoneal macrophages of rats treated orally with piperine for one month. Peritoneal macrophages obtained from rats treated with piperine at doses of 40 and 80 mg/kg for one month significantly produced lower levels of interleukin (IL)-12 after lipopolysaccharide stimulation (p=0.006). IL-10 level was significantly elevated in lipopolysaccharide-primed macrophages isolated from rats receiving piperine for one month (p=0.03). Piperine significantly reduced the intensity of delayed-type hypersensitivity responses in rats immunized with OVA (p=0.003). *Ex vivo* analysis indicated that oral treatment of piperine increased the expression of GATA3 in OVA-immunized rats (p=0.002). Piperine effectively reduced the expression of T-bet and RORγt mRNA in OVA-immunized rats (p=0.001). Piperine did not alter FOXP3 expression in OVA-immunized rats (p=0.15).

Conclusion: These findings show that piperine is a modulating agent of innate and acquired immune responses.

Keywords: Piperine, neutrophil adhesion, macrophage, Th polarization, immunomodulation

Introduction

The use of effective herbal ingredients in medicine has a long history. Phytochemicals are chemical substances with the biological and medical activity of plant origin (1). These substances mainly have low nutritional value, but they have high clinical potential as a source of medicinal compounds (1,2). Alkaloids are a class of phytochemicals with a relatively high abundance in nature. Biologically, alkaloids are a chemical composition of secondary and heterocyclic metabolites containing a nitrogen atom. Many alkaloids still have medicinal uses due to their therapeutic value (3). Many drugs, such as naloxone, are structural modifications of alkaloid compounds designed by pharmaceutical experts to improve the primary effect of the drug or to attenuate unwanted side effects (3,4).

Black pepper (*Piper nigrum*) is a plant of the *Piperaceae* family, which is known as one of the most common spices around the world. The pungent and distinctive taste of black pepper is mainly due to alkaloid piperine ($C_{17}H_{19}NO_3$) (5-7). Many biological and medical properties, including anti-allergic, anti-microbial, anti-cancer, anti-angiogenic, antioxidant, anti-inflammatory, anti-diabetic, heart protection, anti-obesity, liver protection, anti-aging, anti-epileptic and neuroprotective properties attributed to the piperine alkaloid. Therefore, this alkaloid is a suitable candidate for further studies (5,7,8).

The immune system has an essential role in protecting the body against microbial and parasitic agents and preserving the body's homeostasis. However, improper activation of this system and impaired immune regulation can lead to chronic autoimmune and inflammatory conditions such as

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inflammatory bowel disease and multiple sclerosis (9-11). Current immunosuppressive agents used to treat these diseases include biologics, nonsteroidal anti-inflammatory drugs, corticosteroids, and calcineurin inhibitors. Unfortunately, these agents are all costly and have dangerous side effects such as gastrointestinal damage, lymphoproliferative disorders, bone marrow suppression, hepatotoxicity, renal toxicity, and cardiovascular events (9,10). Therefore, the shortcomings of these current therapies warrant a continued search for more cost-effective and tolerable immunosuppressive and modulating agents. In this regard, phytochemicals may be one of the suitable candidates (10,12).

Some of the immunomodulatory effects of piperine have been discussed in recent studies (13-15). For example, piperine has been reported to reduce lymphocytic infiltration and inflammation in rats with carrageenan-induced arthritis (13). In the experimental model of asthma in mice, piperine consumption has reduced the amount of interleukin (IL)-4 production (14). Piperine has impaired the migration of dendritic cells and the activation of T lymphocytes by dendritic cells (14). Also, *in vitro* studies have shown that proliferation and cytokine production in human peripheral blood mononuclear cells were inhibited after treatment with piperine (15). Nevertheless, there is no direct evidence regarding the immune system modifying effect following daily piperine intake. In the first series of the experiments in the present study, the effects of oral piperine intake for one month on T helper (Th) lymphocyte responses and their master transcript factors in Lewis rats immunized with ovalbumin (OVA) have been evaluated. OVA, the main egg white protein, is a non-toxic and non-reactive T lymphocyte-dependent antigen (9). OVA is generally used as a model protein to study antigen-specific immune responses in animals (9,11). In addition, the effect of oral uptake of piperine for one month on the activity of peripheral blood neutrophils and peritoneal macrophages was investigated in a separate series of tests.

Materials and Methods

Chemicals

RNX-Plus solution for total RNA isolation was prepared from Synaclone (Tehran, Iran). SYBR Premix Ex Taq II reverse transcription kit was purchased from TAKARA (China). Glucose and cholesterol measurement kits were obtained from Pars Azmon (Iran). Piperine and other reagents were ordered from Sigma-Aldrich Corporation (St. Louis, MO, USA).

Animals

Male Lewis rats (150 to 160 g- 6 weeks) were purchased from the Darou-Paksh company in Iran. The rats were kept at 23°C with a light/dark cycle of 12 light to 12 h dark. Rats had ad libitum access to standard laboratory animal chow and water. All protocols of animal care and experiments were strictly followed by the regulations of our university as well as the provisions of the Declaration of Helsinki on Experiments on Laboratory Animals.

Evaluation of Innate Immunity

In the first set of experiments, male Lewis rats were randomly allocated into the following four groups (n=5): vehicle (olive oil)-treated rats, and piperine-treated mice (20, 40, and 80 mg/kg). Piperine was dissolved in olive oil. The gavage volume was 1 mL. Treatment with piperine was continued orally for 30 consecutive days.

Evaluation of Biochemical and Hematological Parameters

The blood samples were gathered from the retro-orbital plexus of rats into heparinized vials and were monitored for red blood cells (RBC) and white blood cells (WBC) counts. The sera were isolated and used to monitor glucose, cholesterol and total antioxidant capacity (TAC). The RBC and WBC counts were estimated by visual means using the Neubauer counting chamber. Glucose and cholesterol were measured according to the instructions of the commercial kits manufactured by Pars Azmoun (Iran). The method of Benzie and Strain was used to evaluate the TAC of serum samples (16). In brief, the working solution was prepared by mixing buffer acetate with 2,4,6-tri-2-pyridinyl-1,3,5-triazine solution in HCl. Then, FeCl3 was added and mixed. The serum sample (8 µL) and working solution (240 µL) were mixed and incubated for 10 min at room temperature. The optical density of samples was red at 532 nm. TAC was reported as mmol/L.

Neutrophil Adhesion Test

The blood samples were obtained from the retro-orbital plexus of rats into heparinized vials and were evaluated for differential leukocyte count. Then, the samples were cultured with 80 mg nylon fibers/mL for 15 min at 37°C and were analyzed again for differential leukocyte count. This formula was used to calculate the adhesion percentage of neutrophils:

Neutrophil adhesion % = [(Nu-Nt) /Nu]x100

Nu is the neutrophil index of untreated blood samples, and Nt is the neutrophil index of treated blood samples (17).

Macrophage Isolation and Evaluation

Cervical dislocation was used to euthanize the rats after taking blood from the retro-orbital of the animals. Then, 25 ml of ice-cold RPMI-1640 culture medium was inoculated into the peritoneal cavity of the rats. The peritoneal fluid was aspirated after massaging the rats' abdomens.

The isolated fluids were centrifuged at 600 g for 10 minutes at 4°C. The harvested cells were counted with trypan blue dye, and 100 microliters of live cell suspension ($2x10^6$ cells/mL) were placed in 96-well microplates. The microplate was incubated for 40 minutes at 37°C and 5% CO_2 to provide enough time for the adhesion of macrophages to the bottom of the wells. Non-adherent cells, which were mainly lymphocytes, were removed by vigorous washing (18). The viability of macrophages obtained during the study by the trypan blue exclusion method was never below 95%.

Macrophages were incubated for 20 min with 0.1% nitro-blue tetrazolium (NBT) and 100 ng/mL tetradecanoylphorbol acetate (TPA) to evaluate the respiratory burst potential of these cells. To remove the unused NBT dye, the samples were washed twice. The regenerated dye was extracted by dioxin following the respiratory burst of macrophages. The optical intensity was recorded at 520 nm by a microplate reader (18,19).

Macrophages were treated with lipopolysaccharide (LPS) (100 ng/mL) for 6 hours and then cell-free supernatants were analyzed by the Griess method to measure the amount of nitric oxide (18). Briefly, the supernatant was incubated for 10 minutes with Gries's reagent (3% phosphoric acid, 0.1% naphthyl ethylenediamine, and 0.1% sulfanilamide) in the dark at room temperature. The amount of optical density at the wavelength of 540 nm was read by a microplate reader. By drawing the standard curve, the concentration of nitrite was estimated (20). The supernatant obtained from macrophage cells stimulated with LPS was also used to monitor the levels of IL-10 and IL-12 cytokines by the ELISA method.

Evaluation of Acquired Immunity

Immunization with Ovalbumin

In a set of experiments, 25 male Lewis rats were divided into five groups, each consisting of five rats. OVA (at a concentration of 2 mg/mL in PBS) was emulsified in an equal volume of complete Freund's adjuvant to immunize rats. Then, 100 microliters of this emulsion was injected subcutaneously into the shaved back of the rats. Rats were boosted 14 days later with the same concentration of OVA as the initial challenge in incomplete Freund's adjuvant (IFA) (9). The negative control group was injected with normal saline according to the schedule of the other groups and orally received olive oil as a placebo. OVA-immunized rats were orally treated with olive oil as a vehicle or different doses of piperine (20, 40, and 80 mg/kg) from two days before the immunization to one week after the second immunization. Piperine was dissolved in olive oil. The gavage volume was 1 mL.

Splenocyte Isolation and q-PCR Assay

Spleens of rats were removed on the 20th day after immunization under aseptic conditions. The spleens were fragmented and passed across a wire mesh with a diameter of 20 µm. The obtained cell suspension was centrifuged at 2000 rpm for 10 minutes. The mononuclear cells were isolated by Ficoll-Hypaque density gradient. Contamination with RBC was removed using ACK lysis buffer. Cell suspension ($2x10^6$/mL in RPMI-1640 medium with 10% fetal calf serum) was incubated in 96-well plates and pulsed with 100 µg/mL OVA for 72 hours (9). Total RNA was extracted from splenic populations stimulated with OVA or medium using RNX-Plus solution according to the manufacturer's instructions and used to analyze the expression of T-bet, RORγt, Foxp3, and GATA3. The isolated RNA was used to make complementary DNA. The SYBR Green kit was used to administrate polymerase chain reaction amplification in triplicate, according to the manufacturer's instructions. The reference gene in this study was the *HPRT* gene. The sequences of primers for mRNA amplification are shown in Table 1. Final data were reported as relative fold change from non-immunized control group values.

Assessment of Delayed Type Hypersensitivity (DTH) Reaction

The intensity of the delayed-type hypersensitivity reaction was measured according to the conventional method described previously. In short, 48 hours before splenocyte isolation from OVA-immunized rats, 100 µL OVA (1 mg/mL in equal volumes of PBS and IFA) was injected subcutaneously in the left foot pad of each rat. At the same time, the same volume of phosphate-buffered saline was inoculated into the right foot pad of the rats as

Table 1. Primer sequences used for cytokine assay by real-time PCR assays

Gene name	5'-3' Forward primer	5'-3' Reverse primer
GATA3	TTCCTGTGCGAACTGTCAGACCA	CCTTTTTGCACTTTTTCGATTTGCTA
T-bet	GCCAGGGAACCGCTTATATG	GACGATCATCTGGGTCACATTGT
RORγt	AGTGTAATGTGGCCTACTCCT	GCTGCTGTTGCAGTTGTTTCT
Foxp3	GTA CAG CCG GAC ACA CTG C	GCT GAC TTC CAA GTC TCG TGT
GAPDH	ACCACAGTCCATGCCATCAC	TCCACCACCCTGTTGCTGTA

PCR: Polymerase chain reaction

a negative control. The thickness of the footpad before the bleeding time was measured with a digital caliper. The below formula was used to record the intensity of the DTH reaction:

DTH%= [(Thickness of left footpad)_(Thickness of right footpad)/(Thickness of right footpad)] *100 (9).

Statistical Evaluation Methods

After ensuring the normal distribution of all data by the Kolmogorov-Smirnov assay, the data were analyzed using a one-way analysis of variance (ANOVA) and Tukey's post hoc test. A p-value of 5% or less was considered statistically significant. In the figures, the results were represented as mean ± standard deviation.

Results

Oral Intake of Piperine for One Month Increased the Total Antioxidant Capacity of Serum in Rats

Based on the statistical analysis, receiving oral piperine for one-month dose did not affect the number of RBCs, WBCs, and serum glucose and cholesterol in rats (p=0.11, Table 2). However, serum TAC levels were dose-dependently increased in rats receiving piperine compared to the those of sera isolated from the control group (p=0.01, Table 2).

The Oral Intake of Piperine for One Month Reduced the Inflammatory Reactions of The Innate Immune System

Oral administration of piperine for one-month dose-dependently reduced the adhesion of neutrophils compared to the adhesion potential of neutrophils obtained from untreated rats (p=0.04, Figure 1). On average, after oral administration of piperine, the percentage of adhesion of neutrophils obtained from untreated rats was 1.4, 1.64, and 1.95 times higher than rats receiving piperine in doses of 20, 40, and 80 mg/kg, respectively (Figure 1).

The production of nitric oxide and oxygen free radicals in the population of macrophages isolated from the peritoneum of rats that were orally treated with piperine for one month showed a significant decrease in a dose-dependent manner compared to the macrophages obtained from control rats (Figure 2 A and B). The analysis of the findings showed a statistically significant difference in the measurement of nitric oxide production (p=0.0001) and the ability to produce oxygen free radicals (p=0.003) in the isolated macrophages that were challenged with LPS and TPA, respectively, among different groups (Figure 2 A and B).

Peritoneal macrophages obtained from rats that treated with 40 and 80 mg/kg piperine for one month significantly produced lower levels of IL-12 after stimulation with LPS compared to macrophages obtained from control rats (p=0.006, Figure 2 C). The level of IL-12 production between macrophages obtained from rats that were treated with 20 mg/kg of piperine for one month and rats in the control group did not show a significant difference after stimulation with LPS (p=0.07, Figure 2 C). In contrast, IL-10 secretion was significantly increased in LPS-primed macrophages gathered from rats receiving piperine for one month compared to macrophages isolated from untreated rats LPS (p=0.03, Figure 2 D). Intergroup comparison revealed no significant statistical difference between groups that received different doses of piperine (p=0.08, Figure 2 D).

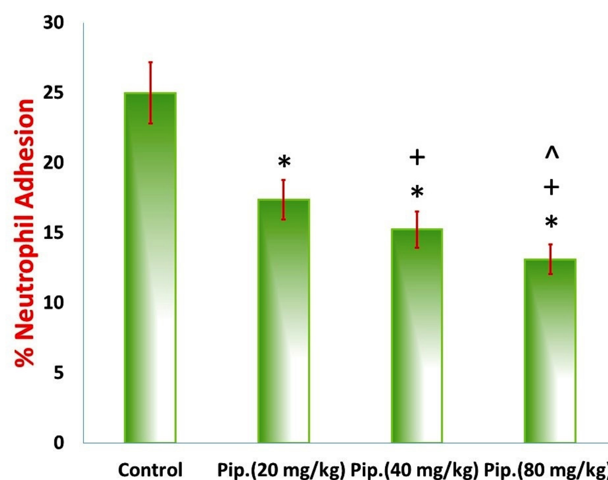

Figure 1. Evaluation of adhesion potential of neutrophils. Peripheral blood neutrophils of rats were isolated one month after daily gavages with piperine, and the adhesion potential of neutrophils was checked according to the protocol mentioned in the methods section. The results were presented as mean ± standard deviation (*: p<0.05 versus control rats, +: p<0.05 versus rats orally received 20 mg/kg piperine (pip.), ^: p<0.05 versus rats orally received 40 mg/kg piperine).

Table 2. Evaluation of biochemical and hematological parameters

Factor / Group	RBC ($\times 10^2$ µL)	WBC ($\times 10^3$ µL)	Glucose (mg/dL)	Cholesterol (mg/100 mg/dL)	TAC (mmol/L)
Control	5.1 ± 1.5	4.9 ± 0.5	95.2 ± 5.4	92.3 ± 7.0	845.7 ± 99.4
Pip. (10 mg/kg)	5.4 ± 0.6	5.2 ± 0.4	97.2 ± 3.8	92.3 ± 4.7	970.4 ± 134.6^{*+}
Pip. (20 mg/kg)	5.6 ± 0.6	5.4 ± 0.7	96.8 ± 4.6	92.0 ± 5.6	1125.0 ± 141.7$^{*+\wedge}$
Pip. (40 mg/kg)	5.4 ± 0.7	5.1 ± 0.7	96.5 ± 4.1	93.2 ± 7.2	1365.0 ± 139.6$^{*+\wedge\$}$

The results were presented as mean ± standard deviation (*: p<0.05 versus Control rats, $^+$: p<0.05 versus rats orally received 20 mg/kg piperine (pip.), $^\wedge$: p<0.05 versus rats orally received 40 mg/kg piperine)

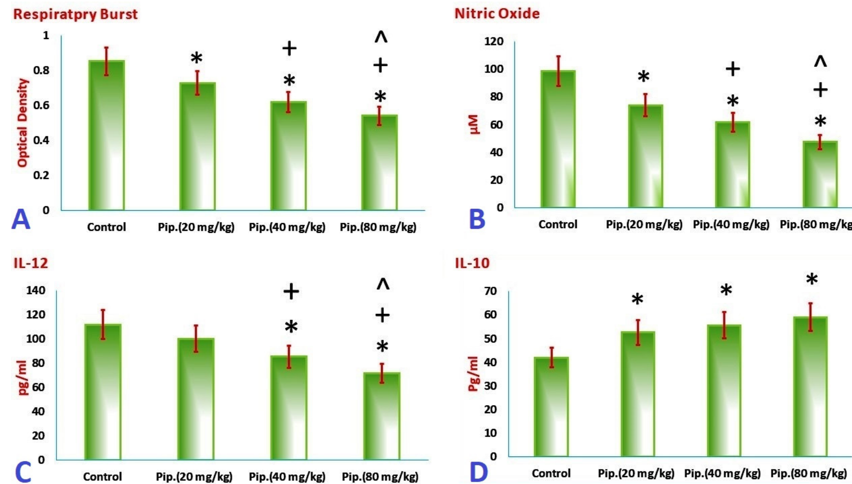

Figure 2. Evaluation of inflammatory functions of peritoneal macrophages. One month after daily piperine gavages, the peritoneal macrophages of the rats were isolated to analyze the respiratory burst (A), and production of nitric oxide production (B), interleukin (IL)-12 (C), and IL-10 (D). The results were presented as mean ± standard deviation (*: p<0.05 versus control rats, +: p<0.05 versus rats orally received 20 mg/kg piperine (pip.), ^: p<0.05 versus rats orally received 40 mg/kg piperine).

Oral Administration of Piperine for One Month Was Effective in Modulating Adaptive Immune Responses Following OVA Challenge

When examining the severity of the DTH reaction, the mean value for rats challenged with OVA alone was taken as 100%. According to research findings, oral intake of piperine significantly reduced the intensity of DTH responses in rats immunized with OVA compared to DTH responses in unimmunized rats (p=0.003, Figure 3). The analysis of the findings indicated that the mean intensively of DTH reaction in OVA-immunized rats was 1.88, 2.08, and 2.37 times higher than those receiving piperine at doses of 20, 40, and 80, respectively (Figure 3).

Immunization of Lewis rats with OVA caused a significant increase in the expression of transcription factors T-bet, RORγt, GATA3, and FOXP3 in lymphocytes obtained from the spleen of rats after *ex vivo* stimulation with OVA compared to lymphocytes obtained from non-immunized rats (p<0.05, Figure 4 A-C). After one month of piperine treatment in OVA-immunized rats, T-bet mRNA expression of splenocytes decreased in a dose-dependent manner compared to T-bet mRNA expression of lymphocytes obtained from untreated rats (p=0.02, Figure 4 A). The mean expression level of the T-bet mRNA in rats immunized with OVA was 1.5, 1.78, and 2.13 times higher than those receiving piperine at doses of 20, 40, and 80, respectively (Figure 4 A).

Ex vivo analysis indicated that treatment with piperine increased the expression of GATA3 in rats challenged with OVA compared to untreated immunized rats (p=0.002, Figure 4 A). There was no significant difference between the expression level of the GATA3 in the doses of 40 and 80 mg/kg of piperine (p=0.06, Figure 4 B). The mean

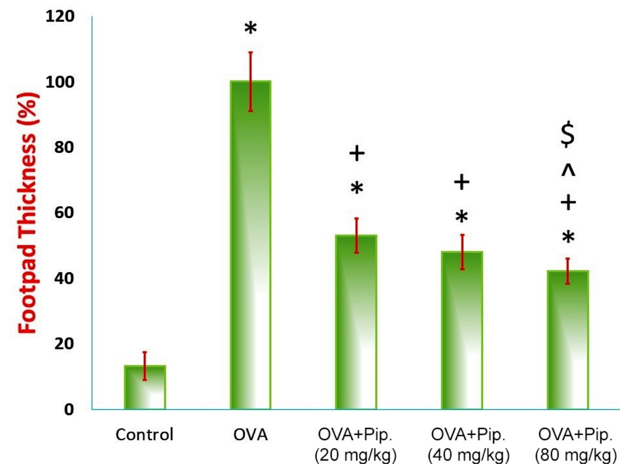

Figure 3. Effects of piperine on DTH response. On the 28th day after immunization, rats received a subcutaneous injection of 50 µL OVA (1 mg/mL in equal volumes of PBS and IFA) into the left hind paw. The same volume of PBS was injected into the right foot pad of the rats as a negative control. The intensity of the DTH reaction was evaluated by a digital caliper after 48 hours. The findings were expressed as mean ± standard deviation (*: p<0.05 versus control rats, +: p<0.05 versus OVA-immunized rats, ^: p<0.05 versus OVA-immunized rats orally received 20 mg/kg piperine (pip.), $: p<0.05 versus OVA-immunized rats orally received 40 mg/kg piperine).
OVA: Ovalbumin, DTH: Delayed type hypersensitivity, IFA: Incomplete Freund's adjuvant

expression level of GATA3 mRNA in rats immunized with OVA and receiving oral piperine at doses of 20, 40, and 80 was 1.25, 1.47, and 1.58 times higher than that of rats challenged with OVA alone (Figure 4 B).

Treatment with piperine effectively reduced the expression of RORγt mRNA in rats challenged with OVA compared to untreated immunized rats (p=0.001, Figure 4 C). The mean expression level of the RORγt mRNA in rats immunized with OVA was 2.07, 2.33, and 3.12 times higher

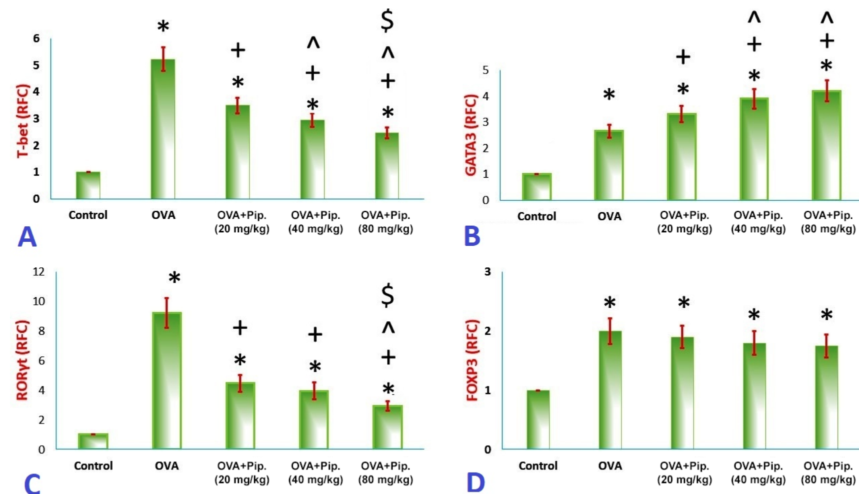

Figure 4. Assessment of *ex vivo* Th polarization changes after treatments. Total mRNA was extracted from spleen cells stimulated with OVA or medium and analyzed for the expression of T-bet (A), GATA3 (B), RORγt (C), and Foxp3 (D). The data were reported as mean ± standard deviation [*: p<0.05 versus control rats, +: p<0.05 versus OVA-immunized rats, ^: p<0.05 versus OVA-immunized rats orally received 20 mg/kg piperine (Pip.), $: p<0.05 versus OVA-immunized rats orally received 40 mg/kg piperine].
OVA: Ovalbumin

than in rats receiving piperine at doses of 20, 40, and 80, respectively (Figure 4 C). The reduction of this factor did not show a statistically significant difference among the groups receiving piperine in doses of 20 and 40 mg/kg (p=0.07, Figure 4 C).

Oral piperine treatment for one month did not alter FOXP3 expression in OVA-immunized rats (p=0.15, Figure 4 D).

Discussion

Neutrophils are considered the frontline soldiers of the innate immune system. Based on the current knowledge, these cells can regulate many processes such as acute damage and repair, development and direction of adaptive immune response, cancer, autoimmunity, and chronic inflammatory processes (21). According to our findings, oral administration of piperine for one month reduced the adhesion potential of neutrophils compared to the adhesion potential of neutrophils obtained from untreated rats. In line with the results of our research, it has been reported that piperine inhibits the adhesion of neutrophils to endothelial cells by suppressing the Nuclear factor kappa-light-chain-enhancer of activated B-cells signaling pathway and activating IkB kinase in endothelial cells (22). At present, scientific literature lacks comprehensive information regarding the impact of piperine on the expression of adhesion molecules and chemotactic receptors in neutrophils. Therefore, further research is warranted to delve deeper into this aspect.

In many immunopathological conditions, abnormal/inappropriate responses of helper T lymphocytes (Th) and innate immune cells, like the macrophages, have been identified as the main culprits (20). Macrophages are the focus of many immune system responses (9). Macrophages are not the only simple "servants" of T or B-cells in acquired immunity. Macrophages begin and conduct immune responses from simple multicellular animals to humans (11). The well-known "double-edged sword" property of the immune system can be ascribed in large part to the unique ability of macrophages to be polarized toward either reparative/anti-inflammatory (M2) or killer/inflammatory (M1) type responses (11,18,23). In a healthy state, alternative type (M2) macrophages preserve homeostasis by assisting to repair and replace lost cells. In the presence of pathogens or altered cells, these cells can transform into classical-type macrophages (M1). In immunopathological conditions, excessive formation of M2 macrophages leads to chronic infections, fibrosis, allergies, and tumors (11,18). M1 macrophages mainly play an important role in autoimmunity, atherosclerosis and other chronic inflammatory conditions (18,23). Recently, piperine has been shown to reduce the expression of intercellular adhesion molecule-1 in macrophages (24). Piperine inhibited the expression of inflammatory mediators like tumor necrosis factor-α, inducible NO synthase, and LPS-induced COX-2 in RAW 264.7 macrophage cells (25,26). Also, piperine suppresses pyroptosis and release of IL-1β in LPS-primed bone marrow-derived macrophages (27). Based on our findings, the production of oxygen free radicals and nitric oxide, as well as the production power of IL-12 in the population of macrophages obtained from the peritoneum of mice that were orally treated with piperine for one month, were significantly reduced, compared to the macrophages isolated from control rats. Instead, piperine treatment increased the potential of macrophages in IL-10

production. M1 macrophages often secrete IL-12, and M2 macrophages secrete IL-10 (11). Overall, these findings indicate the ability of treatment with oral piperine to polarize macrophages towards the M2 anti-inflammatory phenotype.

A delicate balance between T-cells with separate inflammatory and tolerogenic roles is vital to mount a surveilling immune response versus pathogens without compromising immune tolerance to self-antigens (20). $CD4^+$ Th lymphocytes play a central role in regulating immune responses and creating the type of response. Helper T lymphocytes are polarized depending on their microenvironment and develop specific functional patterns. The most famous polarization patterns of T lymphocytes are Th1 [signature cytokines including interferon (IFN)-γ and IL-2], Th2 (signature cytokines including IL-4 and IL-5), and Th17 (signature cytokines including IL-17A and IL-22) and T regulatory lymphocytes (Treg) (signature cytokines including transforming growth factor-β and IL-10) (28). Polarization of Th1 lymphocytes occurs through the expression of the T-bet transcription factor. Th1 responses support the organism animals against most pathogens and are liable for perpetuating auto-inflammatory and autoimmune responses. Polarization of Th2 lymphocytes occurs through the expression of transcription factor GATA3. These lymphocytes are the opposite of Th1 lymphocytes and can protect the organism against versus worm parasites and facilitate resolve cell-mediated inflammation (29). Th17 and Treg lymphocyte responses are mutually regulated. The polarization towards Th17 lymphocytes protects the organism against extracellular bacteria and fungi (20,30). In certain immunopathological conditions, Th17 responses are often elevated, and the immune responses shifts toward an inflammatory phenotype and damages normal cells and tissues (30). Anti-inflammatory mediators expressed by Treg lymphocytes prevent the development of an excessive immune response. RORγt is a master regulator of Th17 cells, and FOXP3 is a master regulator of Treg lymphocytes (30). There is not enough evidence about the effect of piperine on T lymphocytes. Piperine has only been reported to inhibit the proliferation of polyclonal and antigen-specific T lymphocytes without affecting their viability (29). Piperine treatment also regressed the production of IFN-γ, IL-2, and IL-17A, and the generation of cytotoxic effector lymphocytes (31). Here, we showed that the mRNA expression of transcription factors Th1 and Th17 was decreased after one-month oral treatment with piperine in OVA-immunized rats. Meanwhile, the OVA-specific immune responses in rats immunized with albumin continued to deviate towards Th2. However, piperine had no effect on the expression of the master transcript factors of Treg lymphocytes.

Immunity mediated by T lymphocytes plays an essential role in the fight against intracellular microbes (29). However, unbridled responses of cellular immunity play a role in the development and spread of immunopathological conditions (23). DTH is one of the patterns of stereotypic T cell-mediated immune response (19,23). M1 macrophages and Th1/Th17 lymphocytes cooperate to induce this response (19). The findings obtained in this research indicated the reduction of DTH reaction. These findings were in agreement with the findings related to the progress of the anti-inflammatory macrophages and the increase in the polarization of T-lymphocyte responses towards Th2 compared to Th1 and Th7 lymphocytes following the oral treatment of rats with piperine.

Another important finding was that the oral administration of piperine for one month did not cause significant changes in the hematological factors of the number of RBC and WBC and the biochemical levels of glucose and cholesterol. Of course, the TAC of serum increased in rats that received piperine, which may strengthen the ability of these rats to deal with stress caused by inflammation in immunopathological conditions. The antioxidant effects of piperine have been reported before (5,7,8).

Conclusion

As conclusion, one month of oral administration of piperine inhibited the inflammatory function of macrophages and neutrophils in Lewis rats. Also, treatment with piperine caused the deviation of the master transcription factors of T lymphocytes after specific stimulation with antigen from T-bet and RORγt toward GATA3. These findings suggest that piperine is a modulating agent of immune responses. Overall, piperine may be helpful in the control of some immunopathological conditions. Nevertheless, these results are only from a preliminary survey, and it is required to conduct more extensive investigation in different immunopathological conditions.

Ethics

Ethics Committee Approval: It was approved Urmia University and was carried out according -to standard animal experimentation protocol of the Veterinary Ethics Committee of Urmia University (approval number: IR-UU-AEC-3/5, date: 15.12.2023).

Informed Consent: Not necessary.

Authorship Contributions

Surgical and Medical Practices: A.G., Concept: A.G., S.M.A.F., A.T., Design: S.M.A.F., Data Collection or Processing: A.G., Analysis or Interpretation: A.G., A.T., Literature Search: A.G., S.M.A.F., A.T., Writing: E.C., S.M.A.F.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declare that they have no relevant financial.

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