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Original Article

Immunological, hematological and biochemical benefits of adjuvant nigella sativa to pharmacotherapy in immune thrombocytopenic purpura patients

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Abstract: Background: Patients having acute and chronic immune thrombocytopenic purpura (ITP) suffer from thrombocytopenia, decreased T regulatory lymphocytes (causing immunological tolerance) and abnormal biochemistry (increased inflammatory mediators, autoimmunity, and oxidative stress). Nigella sativa is a prophetic medicine remedy rich in pharmacological ingredients exerting anti-inflammatory and thrombocytosis effects that may improve the biochemistry of ITP patients. Objective: To evaluate the benefits of nigella sativa to ITP patients. Methods: Ethical committee approval and patients' consents were gained and complied with the declaration of Helsinki. Children's parents or legal guardians provided informed consent. 15 acute ITP patients received steroids or Eltrombopag olamine (revolade) for one month then complete blood count was done in addition to counting CD4 T helper cells and Treg cells using flow cytometry. Then, same patients continued the same treatments plus adjuvant 2 grams/day grinded nigella sativa seeds (taken orally added to foods or drinks) for the next month followed by doing the same blood tests. Another 15 patients having chronic ITP received same treatments and investigations. Results:

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In acute ITP patients on steroids or revolade, platelets count was low (29.26±5.88 × 10^3/cc) by the end of the first month and significantly increased to (275±33 × 10^3/cc) (P<0.001) upon adding adjuvant nigella sativa treatment for one month. Likewise, platelets count was low (28.86±5.88 × 10^3 platelets/cc) in chronic ITP patients by the end of the first month and significantly increased to (267±26 × 10^3 platelets/cc) (P<0.001) upon adding adjuvant nigella sativa treatment for one month. Nigella sativa treatment significantly increased the percentage of CD4 T-helper lymphocytes (P<0.05) and Tregs (P<0.01) in both acute and chronic ITP patients. Conclusion: Adjuvant nigella sativa potentiated pharmacological treatments, exerted potent thrombocytosis effects and increased immunological tolerance in acute and chronic ITP patients possibly due to its anti-inflammatory, immunomodulatory and biochemical effects. Nigella sativa is a recommended adjuvant natural remedy to ITP patients. This study is registered among our hematological studies using nigella sativa in treating hematological diseases in ClinicalTrials.gov. Trial ID: NCT02816957.

Keywords: Immune thrombocytopenic purpura, nigella sativa, CD4(+)CD25(+) Treg cells, CD4 T-helper cells

Introduction

Immune thrombocytopenic purpura (ITP) causes decreased platelets count in both children and adults due to antibody-mediated platelets destruction and decreased platelets formation. ITP is one of the most common bleeding disorders in children, with an incidence of approximately 4-5/100.000/year [1, 2]. Typical clinical features of ITP include purpura, bruises, petechiae, nasal bleeding, oral bleeding, bleeding gums, bloody urine or stool, and vomiting with blood. A head injury that causes bleeding could be fatal. The diagnostic standard for ITP is complete blood count (CBC) to determine the extent of anemia and thrombocytopenia in addition to assaying the proinflammatory cytokines. Severe thrombocytopenia is the characteristic laboratory finding [1, 2].

Biochemical disturbances in Immune Thrombocytopenic Purpura (ITP) center on immune dysregulation, leading to autoantibodies against platelets, altered T-cell responses (like Th1/Th17 dominance and Treg dysfunction), cytokine imbalance (increased TNF-α, IFN-γ, IL-2, IL-17; decreased IL-10, TGF-β), and metabolic shifts in platelets (glycolysis, fatty acid use) affecting their function, alongside impaired platelet production due to autoantibody-mediated megakaryocyte damage [2]. ITP is largely influenced by inflammation as other autoimmune disorders. Immune system imbalances cause platelets self-attack, which creates an inflammatory environment marked by the elevated levels of pro-inflammatory cytokines as IL-1β, IL-18, TNF-α, and IL-21, and associated abnormal immune cell subsets. Interleukin-37 was reported to reduce inflammation and impair the phagocytosis of platelets in ITP [2, 3]. Other causes of ITP may include viral infections (as chicken pox), some medications and vaccines. In ITP, the platelets count is usually less than 100,000 (normal range: 150,000 platelets/cc to 450,000 platelets/cc). Peripheral smear and bone marrow aspiration may be needed to establish the diagnosis. While corticosteroids enhance platelets count in many individuals, a large number of patients still relapse once therapy is stopped [1-4].

Antiplatelet autoantibodies are present in ITP as a result of loss of tolerance. Tregs, or regulatory T cells, are crucial for maintaining the peripheral tolerance since they are positive for both the clusters of differentiation (CD4+ & CD25+). Peripheral Tregs levels decrease in ITP patients. Functional defects in Tregs may contribute to the breakdown of self-tolerance in patients with chronic ITP [4]. Decreased numbers and functions of Treg cells might cause immune regulation dysfunction in ITP [5]. Peripheral self-tolerance is induced and maintained in part by the immunoregulatory CD4(+) CD25(+) T cells via stopping potentially autoreactive T-cells. Patients with ITP who were in the severe phase and those who tested positive for anti-glycoprotein IIb-IIIa antibody had considerably less CD4(+)CD25(+) T cells [6].

Current treatments directed to ITP are diverse (**Table 1**) aiming to avoid bleeding, induce remission, and improve health-related quality of life. Corticosteroids are currently the usual first-line therapy [1-7]. Eltrombopag is an agonist that binds to the thrombopoietin receptors causing a lower incidence of bleeding and protecting adults and children from severe ITP [1-7]. Rituximab is a monoclonal anti-CD20 antibody that causes peripheral B-cell depletion and acts as an anti-neoplastic therapy for lymphoma. Rituximab caused platelets counts to be more than 50 × 10^9/l in up to 50-60% of ITP patients, with a projected five-year sustained response in 25-30% [1-7]. Usually, the

Table 1. Current pharmacological treatments lines for ITP patients [1-7]

Early treatments	1. Steroids 2. Intravenous immunoglobulins
Second line treatments	1. Monoclonal antibodies: Rituximab 2. Thrombopoietin Receptor Agonists: eltrombopag, avatrombopag, romiplostim 3. Chemotherapy: • Azathioprine • Cyclophosphamide • Cyclosporine A • Vinca alkaloids 4. Surgery: splenectomy
Treatment of non-responsive cases	1. Surgery: splenectomy 2. Monoclonal antibodies: Alemtuzumab 3. Chemotherapy 4. Hematopoietic stem cells transplantation. 5. Sirolimus: is a mammalian target of rapamycin (mTOR) inhibitor [1-7]

prognosis of ITP treatment is good apart from few rare unresponsive and resistant cases.

Nigella sativa (black seeds) is a natural medicinal plant and is a recommended remedy in prophetic medicine. Many published studies recommended nigella sativa as an adjuvant anti-oxidant and anti-inflammatory agent [8-12]. Nigella sativa oil significantly increased platelets counts both in normal rats [8] and Trypanosoma-infected rats [9]. This encouraged us to investigate benefits of nigella sativa to ITP patients.

Nigella sativa extracts remain available over-the-counter as a dietary supplement in multiple forms including seeds (grinded to powder as done in this study), liquids, powders and capsules purported to be helpful for digestive health and to boost energy. It can be used in different ways: adding the grinded seeds to foods or natural honey with chewing, administering nigella sativa oil orally or by inhalation as we previously reported when treating COVID-19 patients [10]. Nigella sativa can be obtained from pharmacies, herbal shops, and groceries. No report is there on the effects of adding nigella sativa to ITP drugs as anti-CD20 monoclonal antibodies, mTOR inhibitors, or eltrombopag but nigella sativa intake is associated with increased platelets count possibly due to its antioxidant and anti-inflammatory merits. To the best of the authors' knowledge, there is no reported studies on using nigella sativa in treating ITP. Nigella sativa was reported to alleviate quinine-induced thrombocytopenia (associated with decreased levels of antioxidants and trace metals). Nigella sativa aqueous seed extract for 12 days resulted in an increase in platelets count (1.59-fold) and serum levels of antioxidants as catalase, ascorbic acid, and bilirubin. Moreover, nigella sativa pre-treatment was useful in increasing the levels of micronutrients; iron, nickel and cobalt when compared to quinine-induced effects [11]. In another study, administration of nigella sativa seeds resulted in a significant increase in the phagocytic and intracellular killing activities of polymorphonuclear leukocytes of patients. It increased the production of interferon, protected normal cells from the effects of harmful invaders and helped destroying tumor cells. Nigella sativa potentiated the functions of the immune system [12].

In this study, we investigated the effects of a short-term treatment using nigella sativa as an adjuvant to current ITP therapies on ITP-induced thrombocytopenia and decreased Tregs.

Patients and methods

The study objectives

The aim of this study was to evaluate the possible platelet-protective effects and immunological benefits of nigella sativa in treating children having ITP.

Ethical statement

The present study was performed after gaining an ethical committee approval of Tanta Faculty of medicine (approval code # 31485/04/17) that complied with the declaration of Helsinki. A parent or legal guardian of the children provided informed consent. International ethical standards and applicable local regulatory gui-

delines were followed without causing any physical, psychological, social, legal, economic, or any other anticipated risks to study's participants. The study kept participants' privacy. The study's investigators were responsible for keeping the data confidentiality. Each subject got a unique identifier code. This study is registered among our hematological studies using nigella sativa in treating hematological diseases in ClinicalTrials.gov. Trial ID: NCT02816957.

Patients grouping

This prospective study included 30 children with ITP attending the hematology unit of the pediatric department at Tanta University Hospital (Gharbeya governorate, Egypt) during the study period. 30 children proved clinically to have ITP where the diagnosis was confirmed by relevant blood tests. The children were allocated into 2 groups: (acute ITP vs chronic ITP), each included 15 children. The basis for grouping is the duration of ITP either acute or chronic. In both groups, lack of response to steroids or revolade for 30 days was the indication to add adjuvant nigella sativa for the next 30 days (from day 31 to day 60).

Group I: 15 children with acute ITP (who failed to respond to steroids or revolade for 30 days) were given grinded nigella sativa seeds (taken orally added to foods or drinks) at a dose of 2 grams/day for one month (7 of them were taking Eltrombopag olamine (Revolade) and another 8 were taking steroids).

Group II: 15 children with chronic ITP (who failed to respond to steroids or revolade for 30 days) were given grinded nigella sativa at a dose of 2 grams/day taken orally added to foods or drinks) for one month (11 of them were taking Revolade and the other 4 children were receiving steroids).

Inclusion criteria

1. ITP children more than 3 years (children and young adults). 2. ITP children with acute or chronic ITP. 3. ITP children willing to participate in the study.

Exclusion criteria

Patients with secondary thrombocytopenia or ITP children who were not willing to participate in the study.

Procedures

All patients were subjected to clinical assessment and laboratory investigations: (1) Complete blood count (CBC): Thrombocytopenia or decreased platelets count is defined as platelets count less than 100,000/μL. (2) CD4, CD25, and Regulatory T cells (Tregs) counts were assessed (using flow cytometry) before and one month after adjuvant nigella sativa therapy.

Flow cytometry

Beckman Coulter automated cell counter (CA, USA) was used to count complete blood picture components (RBCs, WBCs and platelets) in addition to differential leucocytic count. Whole blood was subjected to flow cytometry to assess the proportions of lymphocyte subsets as was previously reported [13]. For immunostaining, phycoerythrin (PE)- and fluorescein isothiocyanate (FITC)-conjugated mouse monoclonal antibodies against CD4 and CD25 were purchased (BD Biosciences PharMingen, San Diego, CA, USA). Isotype-matched mouse antibodies were used as control antibodies. Briefly, whole blood (150 μl) was stained with labeled antibodies at 4°C for 30 minutes in the dark. After immunostaining, the red blood cells were lysed using a TQ Prep system (Beckman Coulter, Miami, FL) and expression of CD4 and CD25 on the gated lymphocytes was analyzed using WinMDI software (EPICS XL; Beckman Coulter Inc.). Soluble CD25 concentrations in plasma were determined using a specific enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's guidelines (R & D Systems, Minneapolis, MN). All samples and standards were assayed in triplicates. Isolation of human peripheral T-Lymphocyte cells was done for in vitro analysis. The percentage of CD4 T helper lymphocytes and (CD4+)(CD25+)(Treg cells) were determined.

Statistical analysis

Data collection was done and was followed by data analysis using SPSS software version 22 and presented as (Mean ± standard error of mean). Microsoft excel was used to draw the figures as bar charts. Paired samples t test was used to compare the results between the experimental groups. Significant statistical indi-

Table 2. Demographic data of the studied patients

	Acute ITP	Chronic ITP	*p* value	Statistical significance
Age				
Mean ± SD	7.9±2.54	6.77±2.77	0.174	Not significant
Range	3:12	3:13		
Sex				
Male	7 (46.67%)	8 (53.33%)	>0.9999	Not significant
Female	8 (53.33%)	7 (46.67%)		
Anthropometric measurements				
Weight (kg)	23.80±7.5	23.21±7.55	0.812	Not significant
Height (cm)	125.40±16.81	121.80±16.81	0.562	Not significant
BMI (kg/m^2)	14.91±1.82	15.35±2.08	0.535	Not significant

Fisher's exact test for the association between gender and ITP status (acute or chronic) =1 (>0.05) i.e., non-significant.

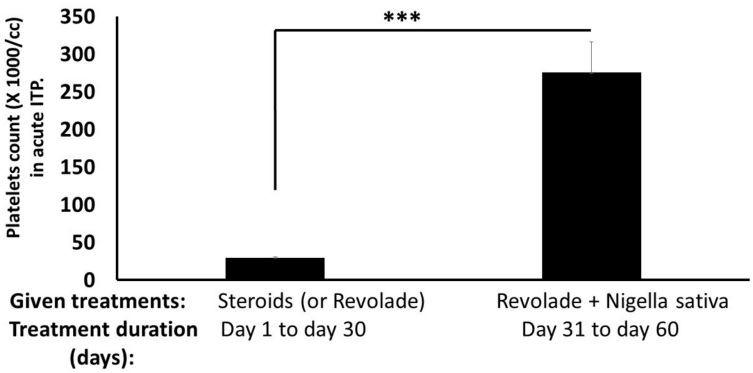

Figure 1. Adjuvant nigella sativa treatment significantly (P<0.001) and maximally increased platelets count in acute ITP patients. Platelets count increased 9 times after adding nigella sativa to regular treatments. *** means P<0.001.

cators were used e.g., * indicated P<0.05, ** indicated P<0.01 and *** indicated P<0.001 to compare the study results before and after adding adjuvant nigella sativa treatment. Fisher's exact test was used to determine if there's a significant association between gender and status of ITP (acute or chronic) in this small sample study size using SPSS for analyzing data in 2 × 2 contingency tables. *p* value was considered significant at <0.05 to suggest that a real relationship exists, while a non-significant *p* value means insufficient evidence for a link.

Results

Study participants have similar demographic data

The investigated participants (in acute and chronic ITP) were almost of the same age group with no significant differences (P=0.174). Fisher's exact test for the association between gender and ITP status (acute or chronic) = 1 (P>0.05) (**Table 2**). Moreover, no significant differences occurred as regard weight (P=0.812), height (P=0.562) or BMI (P=0.535) (**Table 2**).

Effects of nigella sativa treatment on acute ITP patients

In acute ITP patients, nigella sativa treatment (for one month) exerted a significant thrombocytosis effect (P<0.001) via significantly increasing the number of platelets count from 29.2±5.88 to 275.33±182.5 platelets/μl (P< 0.001) (**Figure 1**). Nigella sativa treatment significantly increased the percentage of (CD4+) (CD25+) (Treg cells) (P<0.001) (**Figure 2**) and the percentage of CD4 T helper lymphocytes (P<0.05) (**Figure 3**). In acute ITP children receiving the current treatments (steroids or revolade) added to nigella sativa, platelet counts increased significantly and subsequently clinical purpura dramatically improved.

Effects of nigella sativa treatment on chronic ITP patients

The one-month nigella sativa intake as an adjuvant treatment had a substantial thrombocytosis impact on chronic ITP patients by significantly raising the platelets count from 28.8712.73 to 267.2786.1 platelets/μl (P< 0.001) (**Figure 4**). The percentage increase of (CD4+)(CD25+) lymphocytes (Treg cells) was significant after taking nigella sativa (P<0.01)

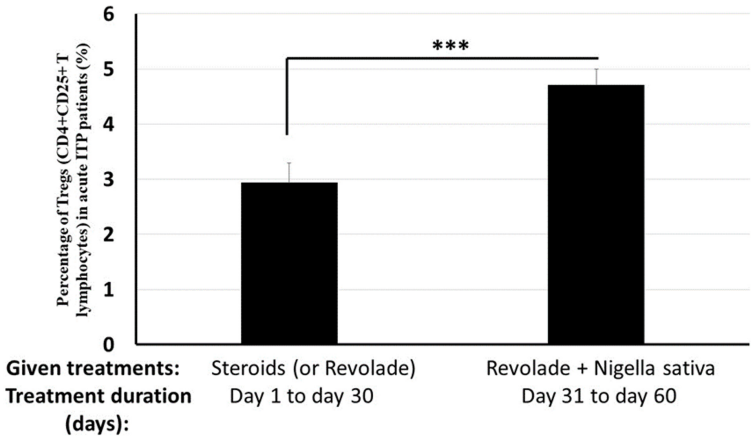

Figure 2. Adjuvant nigella sativa treatment significantly (P<0.001) increased the percentage of Tregs (CD4+CD25+ T lymphocytes) in acute ITP. This (in addition to the anti-inflammatory benefits of nigella sativa) may help restoring immunological tolerance and eliminating the causes of platelets destruction. *** means P<0.001.

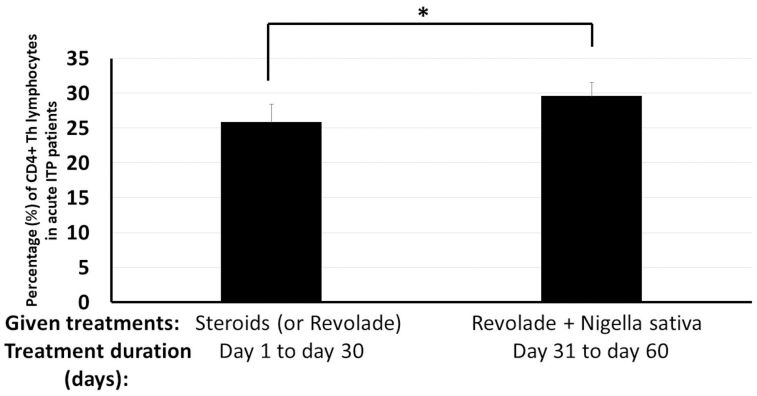

Figure 3. Adjuvant nigella sativa treatment significantly (P<0.05) increased the percentage of T helper lymphocytes in acute ITP patients. * means P<0.05.

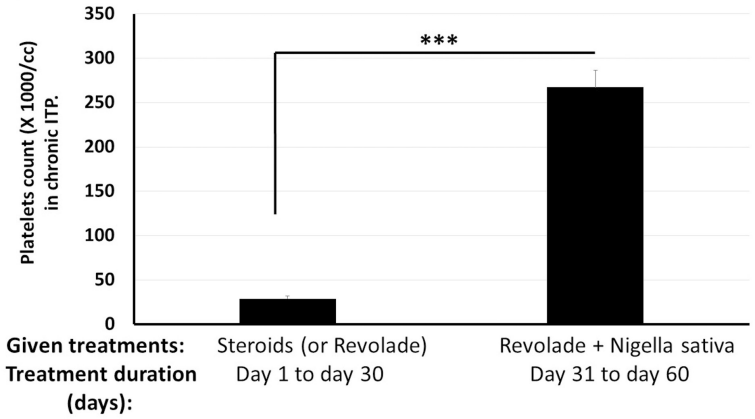

Figure 4. Adjuvant nigella sativa treatment significantly (P<0.001) and maximally increased platelets count in chronic ITP patients. Platelets count increased 9 times after adding nigella sativa to regular treatments. *** means P<0.001.

as an adjuvant treatment (**Figure 5**) and so was the significant increase in CD4 T-helper lymphocytes counts (P< 0.05) (**Figure 6**). In chronic ITP children receiving the current treatments (steroids or revolade) added to nigella sativa, platelet counts increased significantly and subsequently clinical purpura dramatically improved.

Effects of short-term nigella sativa treatment on other parameters of complete blood count

In acute ITP patients, short-term adjuvant nigella sativa treatment (for one month) did not significantly affect hemoglobin percentage, red blood cells count, white blood cells count, hematocrit values, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration or red cell distribution width. Short-term nigella sativa treatment (for one month) in acute ITP patients slightly increased lymphocytes count but that was not significant (P>0.05) (**Table 3**). In chronic ITP patients, short-term adjuvant nigella sativa treatment (for one month) produced similar effects to the same CBC parameters (**Table 4**). Short-term nigella sativa treatment (for one month) in chronic ITP patients slightly increased lymphocytes count but that was not significant (P>0.05) (**Table 4**).

Discussion

Newly diagnosed ITP refers to the hematological and clinical status from the onset of diagnosis to 3 months afterward. Persistent ITP arises if symptoms persist 3 to 12 months after the initial diagnosis. Chr-

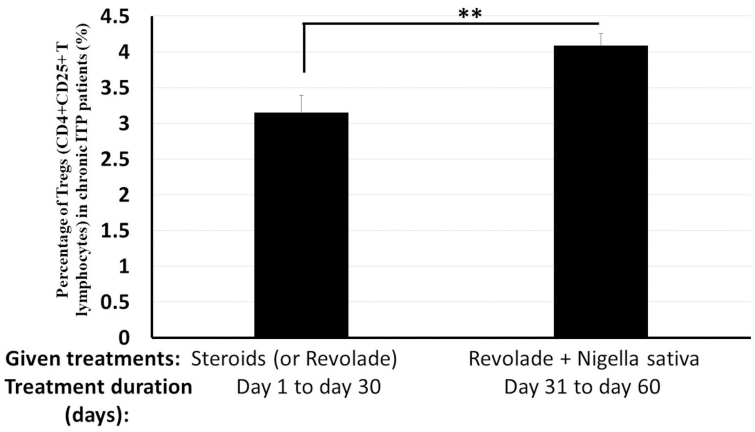

Figure 5. Adjuvant nigella sativa treatment significantly (P<0.01) increased the percentage of Tregs (CD4+CD25+ T lymphocytes) in chronic ITP. This (in addition to the anti-inflammatory benefits of nigella sativa) may help restoring immunological tolerance and eliminating the causes of platelets destruction. ** means P<0.01.

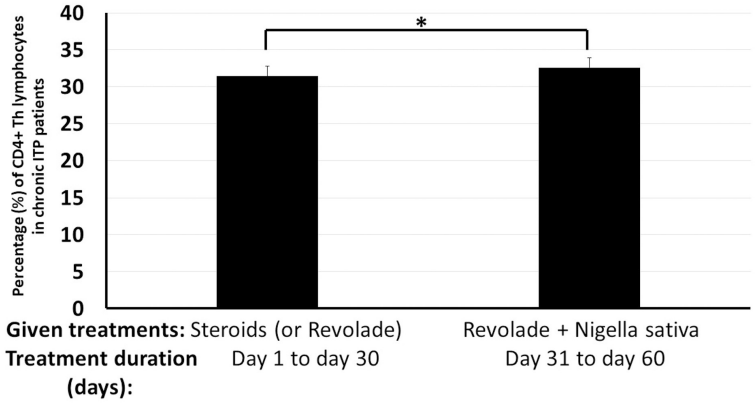

Figure 6. Adjuvant nigella sativa treatment significantly (P<0.05) increased the percentage of T helper lymphocytes in chronic ITP patients. * means P<0.05.

onic ITP indicates continuous symptoms lasting more than 12 months from the initial diagnosis until improvement or resolving the condition. Refractory ITP includes cases that do not improve with splenectomy [1-7, 14-17]. Our data revealed a non-significant association between gender and ITP status (acute or chronic) (**Table 2**).

Immune complex-mediated inflammation may play a role in platelets destruction in both acute and chronic ITP patients and this may justify using steroids to treat their thrombocytopenia. Approximately two-thirds of children with ITP experience a preceding febrile illness. Formation of autoantibodies by epitope spreading, binding of immune complexes, production of platelets expressing viral antigens, and production of antiviral antibodies cross-reactive with platelet antigens have all been linked to ITP [15]. There is evidence of an imbalance between the pro-inflammatory cytokines (interleukin (IL)-6, tumor necrosis factor (TNF)-a, interferon (IFN)-gamma) and the anti-inflammatory cytokines. Treatment with dexamethasone can rebalance the Th1/Th2 axis by increasing the levels of the anti-inflammatory cytokines such as IL-4, IL-10, and TGF-β [16]. Another report supported the role of inflammation in platelets destruction where serum uric acid levels (a mediator of inflammation via enhanced production of inflammatory cytokines) significantly increased in ITP patients and correlated negatively with platelet counts. In ITP patients, high serum uric acid and CRP levels significantly correlated with low platelets count and increased CRP levels. Such inflammation may mediate the low platelet count, and could play a pathophysiological role in the development of ITP [17]. They may explain the therapeutic benefits of nigella sativa in treating ITP owing to its rich content of dozens of natural antioxidant, anti-inflammatory, immunomodulatory and tissue protective ingredients as thymoquinone, carvacrol, thymol, nigellone, limonene, α-pinene, β-pinene and others [10, 18-22]. **Table 1** lists the current pharmacological treatments lines for ITP patients (**Table 1**). In our recently published review article, many prophetic medicine remedies including nigella sativa exerted potent hematological benefits when treating different hematological diseases [23]. In this study, the demographic data showed no significant differences in the mean age between acute ITP group and chronic ITP group or sex where the distribution of males was comparable to females in both the acute ITP and chronic ITP groups (**Table 2**). Our study reported that

Table 3. Complete blood count analysis for acute ITP before and after treatment with nigella sativa

Parameters	Acute ITP		*p* value	Statistical significance
	Pre-Treatment	Post-Treatment		
WBC 10^9/L	8.4±6.33	8.97±6.05	0.3941	Not significant
Lymphocytes 10^9/L	3.78±1.05	4.17±3.36	0.4178	Not significant
Monocytes 10^9/L	0.43±0.21	0.62±0.39	0.145	Not significant
Granulocytes 10^9/L	4.19±1.93	4.18±3.46	0.3948	Not significant
Lymphocytes %	45.36±9.9	46.39±13.1	0.6602	Not significant
Monocytes %	5.42±2.26	6.86±1.87	0.1636	Not significant
Granulocytes %	48.09±10.34	46.75±13.98	0.7045	Not significant
Red Blood Cells million/mm	4.4±0.51	4.3±0.67	0.7513	Not significant
Hemoglobin g/L	10.83±1.34	10.43±1.33	0.329	Not significant
Hematocrit L/L	32.49±3.51	33.24±4.09	0.4926	Not significant
MCV fL	73.85±5.31	76.75±7.49	0.4062	Not significant
MCH (pg)	24.5±2.5	24.23±2	0.8461	Not significant
MCHC (g/L)	31.6±1.25	32.83±1.24	0.0051	Not significant
RDW %	14.25±1.42	13.6±2.76	0.8621	Not significant
PLT platelets (×10^3)/μL	29.2±5.88	275.33±182.5	<0.0001	Sig. (***)
PCT (ng/mL)	0.23±0.1	0.25±0.18	0.5053	Not significant
MPV (fl)	10.9±1.91	10.75±1.53	0.943	Not significant
RDW %	11.52±3.43	14.4±3.65	0.0625	Not significant

*** means P<0.001.

Table 4. Complete blood count analysis for chronic ITP patients before and after treatment with nigella sativa

Parameters	Chronic ITP		*p* value	Statistical significance
	Pre-Treatment	Post-Treatment		
WBC 10^9/L	8.13±2.57	7.91±23.9	0.8943	Not significant
Lymphocytes 10^9/L	2.98±0.77	3.37±0.89	0.9918	Not significant
Monocytes 10^9/L	0.44±0.15	0.49±0.16	0.415	Not significant
Granulocytes 10^9/L	4.4±1.11	4.13±1.76	0.6747	Not significant
Lymphocytes %	38.1±6.88	42.17±15.5	0.9755	Not significant
Monocytes %	5.6±1.37	6.13±2.1	0.0626	Not significant
Granulocytes %	56.84±6.79	51.68±11.48	0.3614	Not significant
Red Blood Cells million/mm	4.85±0.37	4.7±0.38	0.2452	Not significant
Hemoglobin g/L	11.97±0.83	11.65±0.67	0.2123	Not significant
Hematocrit L/L	36.58±2.87	36.51±2.46	0.4923	Not significant
MCV fL	75.56±3.8	77.9±3.91	0.2208	Not significant
MCH pg	24.91±1.32	24.85±1.6	0.7053	Not significant
MCHC g/L	33.11±0.84	32±1.14	0.0123	Not significant
RDW %	13.53±1.18	13.63±0.91	0.6897	Not significant
PLT platelets (×10^3)/μL	28.87±12.73	267.27±86.1	<0.001	Sig. (***)
PCT ng/mL	0.2±0.1	0.2±0.13	0.7357	Not significant
MPV fl	11.19±4.26	9.64±1.28	0.4411	Not significant
PDW %	8.58±3.74	10.8±2.35	0.0393	Not significant

*** means p<0.001.

nigella sativa had a significant thrombocytosis effect via increasing the platelets count in both acute ITP patients (**Figure 1**) and chronic ITP patients (**Figure 4**).

Nigella sativa is a recommended prophetic medicine remedy for treating so many disease conditions. Immunological indicators of nigella sativa's immunomodulatory benefits include increasing the leucocytes count, CD4 count, CD8 count and interferon-gamma [24]. Nigella sativa-induced immunomodulatory effects on T-cells in children with ITP are quite promising. A specialized subgroup of T cells called regulatory T cells (Tregs) function to inhibit the immunological responses, preserve homeostasis and self-tolerance. Tregs have been demonstrated to have the ability to suppress T cells growth and cytokines production, and they are essential for preventing autoimmunity. Treg cells come in a variety of subgroups with various functions. Flow cytometry may typically be used to identify Tregs. The FoxP3 protein, which is located intracellularly, is the most unique marker for these cells. In a typical clinical setting, certain surface markers, such as CD25high (high molecular density) and CD127low (low molecular density), may work as surrogate markers to identify Tregs. Autoimmune diseases may emerge when Treg cells frequency or function are dysregulated. Therapeutic T-cell receptor modulation is thought to be promising in preventing graft rejection [25]. Our study reported that both acute and chronic ITP patients have leukocytosis which usually occurs post-viral infection (**Tables 3** and **4**). Our study reported also that nigella sativa causes a mild lymphocytosis effect in both acute ITP patients (**Table 3**) and chronic ITP patients (**Table 4**). Our data also revealed that in acute ITP patients, adjuvant nigella sativa caused a significant lymphocytosis that occurred in Tregs (**Figure 2**) and T helper cells (**Figure 3**). Likewise, adjuvant nigella sativa treatment in chronic ITP patients exerted a similar lymphocytosis effect in increasing the Tregs (**Figure 5**) and T helper cells (**Figure 6**). This enhances immunological tolerance (to stop platelets destruction) and augments cell-mediated immunity.

The lymphocytosis effects of nigella sativa are in quite agreements with our previous reports where nigella sativa caused lymphocytosis effects in COVID-19 patients and patients having other viral diseases [10, 18-22]. It also agreed with our previous report where nigella sativa increased the lymphocytes count in thalassemia children [24]. The lymphocytosis effects of nigella sativa agreed also with the lymphocytosis effects exerted by Al-hijamah, both of which are prophetic medicine remedies (medical knowledge gained from teachings of the Prophet Muhammad Peace Be Upon Him) [26, 27]. Our data also agreed with Feyzi et al. who showed that the immunological benefits of nigella sativa on T lymphocytes take place via activating T lymphocytes and exerting immunomodulatory effects [28]. Our data also agreed with He and Xu who reported potent immunomodulatory effects of nigella sativa on T lymphocytes in chronic diseases where nigella sativa improved asthmatic patients via activating T lymphocytes [29]. Data of this study agreed with and can be understood in light of our previous reports where prophetic medicine remedies (as nigella sativa and Al-hijamah) exert tissue-protective effects via enhancing the antioxidants reserve and ameliorating the tissue-damaging oxidants and inflammation [26, 27, 30-33]. The study is limited by the small sample's numbers and the short duration of using adjuvant nigella sativa (one month).

The authors' point of view strongly recommends nigella sativa intake for treating acute and chronic ITP. Both acute ITP and chronic ITP children receiving the current treatments (steroids or revolade) added to nigella sativa exhibited significantly increased platelet counts with subsequent dramatic improvement in clinical purpura. Our article data is quite novel and there are no previous studies that treated either acute or chronic ITP with adjuvant nigella sativa. So, there are no similar studies in this topic for comparison. In our study, patients with ITP (both acute and chronic) do benefit from a significant thrombocytosis response upon treatment with nigella sativa. Additionally, nigella sativa-induced lymphocytosis effects on (CD4+)(CD25+)(Treg cells) and CD4 T helper lymphocytes will benefit those patients in restoring autotolerance that cures ITP. As a result, nigella sativa is promising in treating ITP by raising the platelets counts, altering T cells, and reestablishing the immunological tolerance. The resultant laboratory and clinical improvements based on adjuvant nigella sativa is expected to be due to the improvements in abnormal serum biochemistry in ITP patients including autoimmunity, and inflammatory mediators (**Table 5**), This agrees with many previous reports in treating other disease condi-

Table 5. Biochemical disturbances in ITP that may improve using adjuvant nigella sativa

Disturbed biochemical parameters in ITP patients
1. Autoantibodies against platelets
2. Inflammatory cytokine imbalance (increased TNF-α, IFN-γ, IL-2, IL-17)
3. Decreased (IL-10, TGF-β)
4. Metabolic shifts in platelets (glycolysis, fatty acid use) affecting their function, alongside impaired platelet production

tions using different natural prophetic medicine antioxidants [31, 34-43]. To avoid their influence on the experimental results, the study recruited the same patients who received Revolade or steroid treatment for a similar period of time with adjuvant nigella sativa. Usually patients who did not respond to Revolade or steroid treatment will be non-responsive on prolonging the treatment duration with the same drugs. Hence, the improvement is largely attributed to adjuvant nigella sativa. To aid in reaching more detailed conclusions on the impact of nigella on the clinical course of ITP, additional research with larger sample sizes and longer durations of intervention are highly recommended. To verify our findings, future multi-center collaboration and the inclusion of kids with secondary thrombocytopenia are necessary.

Conclusion

Adjuvant nigella sativa treatment exerts a potent thrombocytosis effect (a significant increase in platelets count) in children with ITP (both acute and chronic). Nigella sativa also exerted lymphocytosis effects on CD4 T helper lymphocytes and (CD4+)(CD25+)(Treg cells). Hence, nigella sativa is promising in ITP management via increasing platelets count and modulation of T cells and restoring the immunological tolerance. Further studies with bigger sample sizes and longer duration of intervention are quite necessary to help drawing more in-depth conclusions regarding the effects of nigella on the clinical course of ITP. Future multi-center cooperation is required to reproduce our results.

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Disclosure of conflict of interest

None.

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