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STUDY THE EFFECT OF NANO-PIPERINE IN IMPROVEMENT THE IMMUNITY SYSTEM IN DEXAMETHASONE TREATED MALE RATS

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Abstract

The present study has been designed to investigate wheather Nano-piprine and Vit. C have improving role in immunity system. The study has been conducted on forty-eight mature male Wistar rats (aged 90 days and weighted 150±10 g) have been randomly assigned to 6 equal groups (8 per group) and dosed orally for 30 consecutive day. The first group (C) was given distilled water as a control group. The second group (T1) was given nano-piperine at a dose of 15 mg/kg of B.W. The third group (T2) was given vitamin C at a dose of 25 mg/kg of B.W. The fourth group (T3) was given dexamethasone at a dose of 2mg/kg of B.W. The fifth group(T4) was given both nano-piperine and dexamethasone at the same time. The sixth group (T5) was given both vitamin C and dexamethasone at the same time. After the end of experiment the animals were dissected and blood samples were collected analyzed WBCs and WBCs differentiation. Blood serum samples were separated for assessment of IL-6, IgG, IgA and IgM concentrations. The results illustrated a significant increase ($p \leq 0.05$) in WBCs count in T1 and T2 groups, while decreased in T3 as compared with control group. Also there is increased in WBCs in T4 and T5 groups as compared with T3 group. Also the results revealed there was a significant rise ($p \leq 0.05$) in percentage of lymphocyte and monocyte in T1 and T2 groups while decreased in T3 group as compared with control group. And increased this percentage in T4 and T5 groups as compared with T3 group.

The results indicated there was a significant decrease ($p \leq 0.05$) in percentage of neutrophils in T1,T2 ,T4 and T5,while increased in T3 group.The results registered a significant decrease ($p \leq 0.05$) in concentration of serum IL-6 in T1,T2 and T3 groups as compared with control group, also decrease in T4 and T5 groups as compared with T3 group. Also the results of IgG, IgA and IgM showed a significant decrease ($p \leq 0.05$) in T1,T2,and T3 as compared with control group and appeared a significant decrease in T4 and T5 groups as compared with T3 group.

Key word: Dexamethasone, Immunity, Nano-Piperine, Vit.C.

Introduction

Recently, researchers in medical and biomedicine have focused on the improvement of the immune system (**Whiteside, 2016, Nicholson, 2016, Marshall *et al*., 2018, Cain & Cidlowski,2020; Takeda *et al*., 2020;) .**Based on the complexity of the subject, this study will also improve the immune system through nano-piperine. Immunotherapy can be used to train a patient's immune system to recognize and destroy malignant tumors (**Marshall *et al*., 2018).**These modified immune cells have elicited some remarkable responses in advanced cancer patients.

Adoptive T-cell treatment for cancer employs a transfusion of various mature T-cell subsets to eliminate a tumor and prevent its recurrence (**Yadav *et al.,* 2016)**.Immunotherapy is currently the standard for certain advanced malignancies, such as melanomas and non-small cell lung carcinoma (**Coutinho & Chapman, 2011)**. Recent research indicates that the immune system may also be capable of targeting cerebral lesions outside the blood-brain barrier (**Marshall *et al.,* 2018).** On the other hand, patients with intracranial lesions are typically given corticosteroids prior to initiating immunotherapy to treat cerebral oedema and alleviate the intensity of their symptoms. Additionally, corticosteroids are the first-line treatment for immunotherapy-related side effects such as checkpoint blockade that may arise during or following immunotherapy. While it is well established that corticosteroids have a beneficial effect on adaptive anti-tumor immunity, it is unknown whether their effect on the immune response (**Giles *et al*., 2018).**

Dexamethasone is a class of corticosteroid hormones derived from cholesterol that play a critical role in homeostasis, development, metabolism, and the immune system. When used as the first line of treatment for conditions such as multiple sclerosis and asthma caused by inflammation, synthetic GCs like dexamethasone have powerful immunosuppressant and anti-inflammatory characteristics (**Kim *et al.,* 2020).** Vitamin C has been referred to as L-ascorbic acid since it was discovered as a scurvy treatment. Vitamin C was coined to replace "fat-soluble vitamin A" and "water-soluble vitamin B." The name also encompasses L-dehydroascorbic acid, which the body rapidly converts to L-ascorbic acid (**Ahmed *et al.*, 2020).** Vitamin C is widely popular among the general public due to its antioxidant properties. It does, however, play a far broader role, which will be investigated in this examination. Vitamin C levels in the body are abnormally high compared to other vitamins, indicating this (**Devaki & L R, 2017)**. Vitamin C is present in the human body at approximately 20 mg/kg. Daily human needs are exceedingly difficult to quantify because of the variety of factors that influence them, including their physiological state, stress level, and pre-existing diseases. Recommendations vary greatly around the globe, ranging from 40 to 120 mg/day. This amount of vitamin C has been estimated to be required by people. Piperine's pharmacological activation could only be accomplished with a trace amount of the chemical, which limited its research and utilisation. The amount of piperine incorporated in nanoparticles was calculated (as a percentage of the total weight used in the preparation technique). This gold standard was used to determine the ability of nanoparticles to embed (**Quijia *et al*., 2021).**

Materials and methods

1-Experimental animals

The study has been conducted on adult male rats at the department of physiology, College of Veterinary Medicine, Al-Qadisiya University during the period extended from October, 2021 to June, 2022. Forty-eight mature male Wister rats (aged 90 days and weighted 150±10 g) have been randomly assigned to 6 equal groups (8 per group) and dosed for 30 consecutive day,) and dosed for 30 consecutive day. The first group (C) was given distilled water orally as a control group. The second group (T1) was given nano-piperine at a dose of 15 mg/kg of B.W orally**(Ren *et al.,*2019**). The third group (T2) was given vitamin C at a dose of 25 mg/kg of B.W orally(**Aldahmash and El_Nagar 2014)**.The fourth group (T3) was given dexamethasone at a

dose of 2mg/kg of B.W orally**(Henry *et al*.,1989)**.The fifth group(T4) was given both nano-piperine and dexamethasone at the same time. The sixth group (T5) was given both vitamin C and dexamethasone at the same time**.**

Male rats have been monitored throughout the experimental periods.

After the end of treatments all animals were anaesthesized (by injection of 0.3ml ketamine + 0.1 ml of xylazine/ kg B.W ip),then dissected and blood samples were obtained from abdominal vein in non-heparinized tubes and heparinized tube to analyzed WBCs and WBCs differentiation. Blood serum samples were separated for assessment of IL-6, IgG, IgA and IgM concentrations.

Statistical Analysis:

Results were expressed as mean ± standard error of the mean (SEM). Comparisons were performed using one way analysis of variance (ANOVA). Differences were considered to be significant at the level of P≦0.05. All statistical analysis by (SPSS Institute, Inc., USA) (Joda, 2008).

Results

WBCs and WBCs differentiation

The results illustrated in figure (1) showed a significant difference (p≤0.05) between experimental groups in WBCs count represented by increasing in the WBCs count in T1, T2 groups (7.66±0.018, 6.53±0.100) respectively and decreased in T3 (3.29±0.021) as compared with control group (5.13±0.010). these results also indicated have was increasing in WBCs count in T4 and T5 groups (5.50±0.03, 4.75±0.02) respectively as compared with T3 group, also T4 & T5 recorded insignificant difference between each other.

- **Lymphocytes (%):**

In figure (2) showed there was the highest significant (p≤0.05) percentage of lymphocyte was recorded in T1 male rats (71.68±0.8 % respectively) while T3 group((33.12±0.36 %) male rats recorded the lowest lymphocytes percentage as compared with control group (65.71±0.05) . Moreover there was a significant difference (p≤0.05) represented by increasing in lymphocyte percentage in T4 and T5 groups (63.08±0.38, 64.77±0.065 %) respectively as compared with T3 group. Also there was a significant decrease in lymphocyte percentage in T4 as compared with T5 group.

- **Neutrophils (%):**

The results indicated there was a significant decrease (p≤0.05) in percentage of neutrophils in T1 and T2 (22.2±0.06, 15.54±0.17 %) as compared with control group (26.32±0.02%).on the other hand, T3 group recorded the highest significant(p≤0.05) percentage among the other experimental groups (36.21±0.20 %) Also there was a remarkable low in T4 and T5 groups (24.86±0.28, 16.01±0.21 %) compared with T3 group.

- **Monocytes (%):**

The results of the percentage of monocyte showed there was a significant increase (p≤0.05)in T1 and T2 groups (5.09±0.003, 5.71±0.07) respectively, while decreased in T3 group recorded the lowest monocytes percentage (2.85±0.05%) as compared with control group (4.68±0.007%). Moreover the percentage of monocyte increased in T4 and T5 groups(4.07±0.04, 4.55±0.135)

respectively as compared with T3 group.

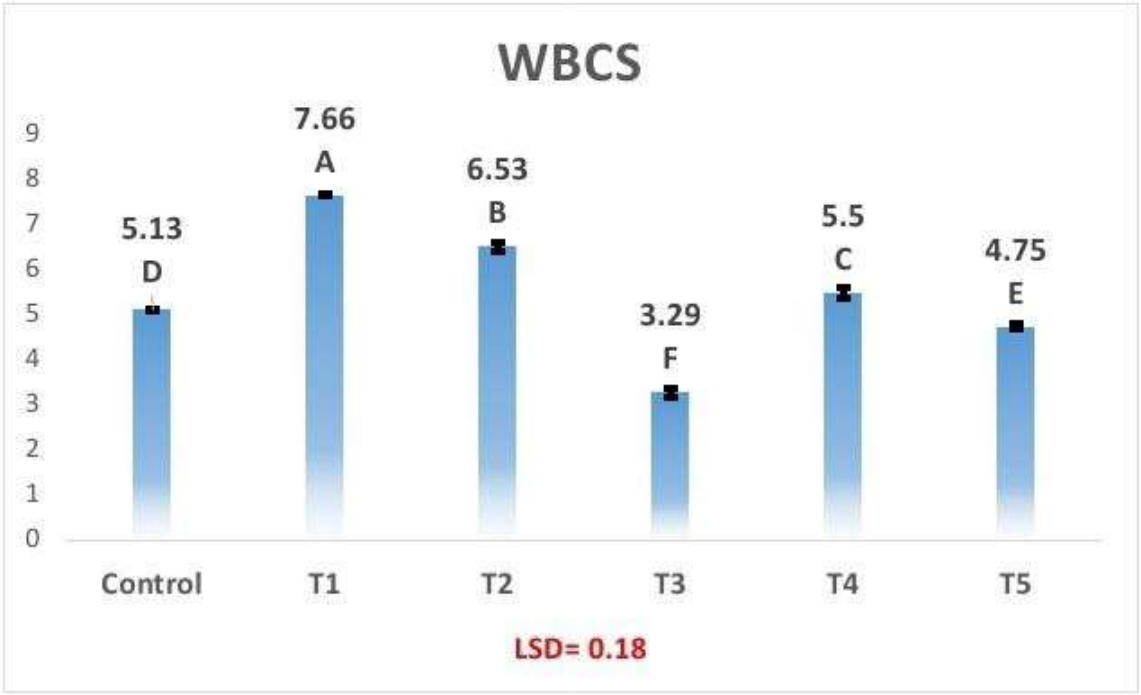

Figure 1: Effect of nano-piprine, dexamethasone and vit. C on WBCs

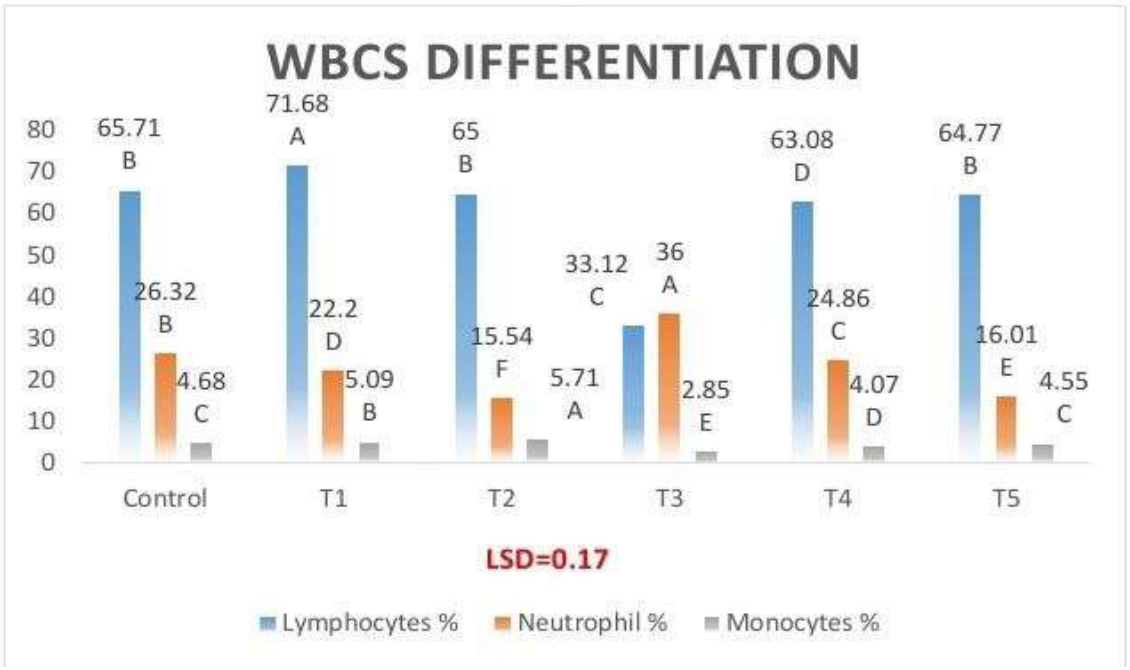

Fig 2: Effect of nano-piprine, dexamethasone and vit. C on WBCs Differentiation

Serum IL-6 concentration

The results illustrated in figure (3) showed a significant difference ($p \leq 0.05$) in serum IL-6 concentration between the all experimental groups. T1, T2 and T3 groups showed significant decrease ($p \leq 0.05$) (2.83±3.13±0. , 3.13±0.08 , 3.81±0.37 %) respectively as compared with control group(8.18±0.11%) and also there was a significant difference ($p \leq 0.05$) represented by decrease in serum IL-6 percentage in T4 and T5 groups(1.75±0.09,1.98±0.23%) respectively as compared with T3 group.(3.81±0.37%).

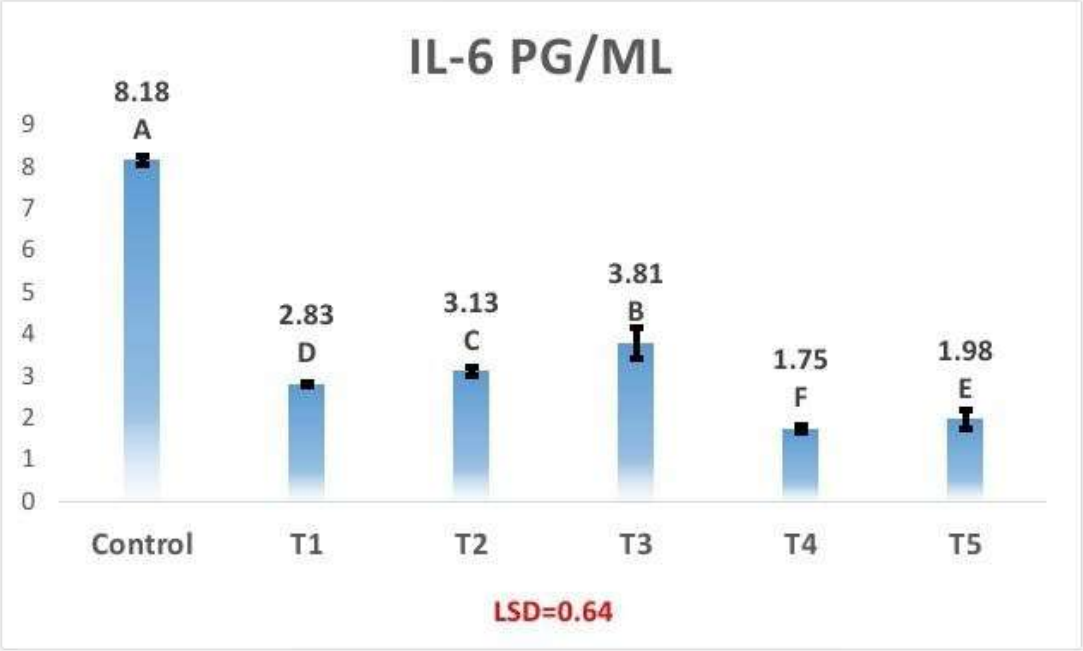

Figure 3: Effect of nano-piprine, dexamethasone and vit. C on IL-6

Immunoglobulins concentration

The results illustrated in figure (4) showed a significant difference ($p \leq 0.05$) between experimental groups in IgG represented by decreasing in the IgG in T1, T2 and T3 groups (42.57±0.22, 48.67±0.23, 37.81±0.42) respectively as compared with control group (53.97±0.27). these results also indicated there was decreased in IgG in T4 and T5 groups (36±0.42, 32.13±0.17) as compared with T3 group and with another groups.

The results illustrated in figure (4) showed a significant difference ($p \leq 0.05$) between experimental groups in IgA represented by decreasing in the IgA in T1, T2 and T3 groups (7.20±0.10, 9.99±0.11, 8.22±0.07) respectively as compared with control group (12.37±0.01). these results also indicated have was insignificant difference in IgA of T4 and T5 groups (8±0.01, 8.33±0.17) as compared with T3 group .

The results illustrated in figure (4) showed a significant difference ($p \leq 0.05$) between experimental groups in IgM represented by decreasing in the IgM in T1, T2 and T3 groups (8.81±0.08, 11.49±0.10, 10.37±0.12) respectively as compared with control group (14.89±0.03). on the other hand these results also indicated have was decreased in IgM in T4 and T5 groups (8.14±0.01,

8.97±0.06) as compared with T3 group and with another groups.

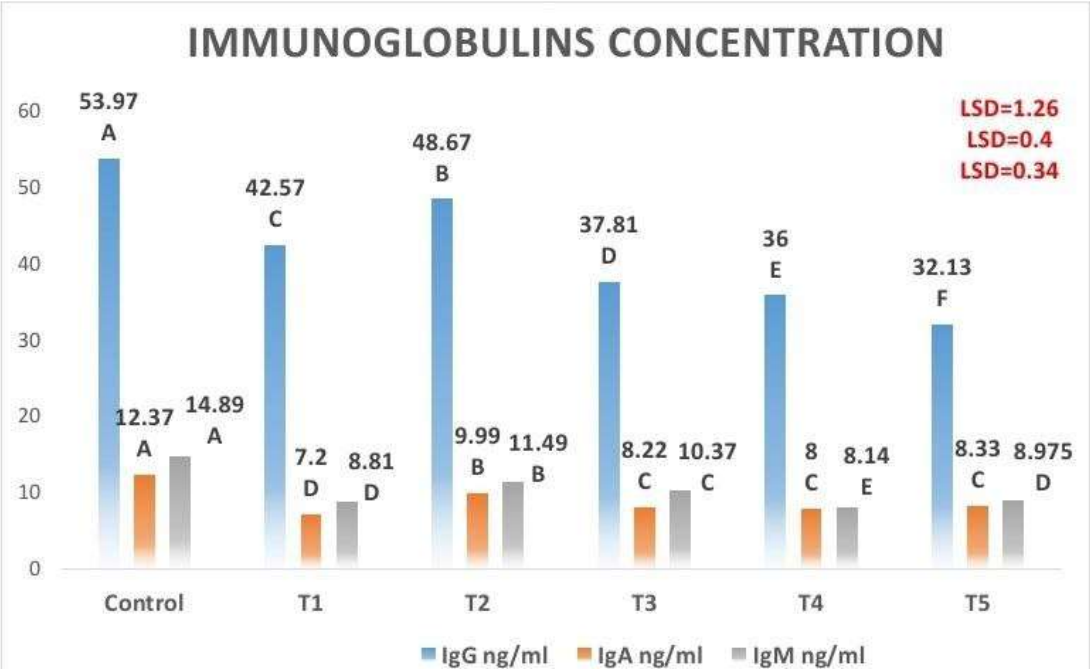

Fig 4: Effect of nano-piprine, dexamethasone and vit. C on Immunoglobulins concentration

Discussion

Due to vitamin C activity in the differentiation and proliferation of B lymphocytes and its antioxidant function, vitamin C supplementation in diets may influence antibody-mediated immune responses. Several studies have revealed that vitamin C enhances immunological responses mediated by antibodies (**Dudhatra *et al*., 2012)**. Showing that Dexamethasone can promote the accumulation and degranulation of leukocytes at the site of inflammation **(Merkel *et al*., 2017)**. The rise in cell count may have been caused by the migration of eosinophils, basophils, monocytes, and lymphocytes from circulation to lymphoid tissues or a combination of both. In male rats treated with Dexamethasone, the percentages of total leukocytes, lymphocytes, monocytes, and eosinophils were dramatically decreased, whilst the percentages of neutrophils were significantly raised. This result was consistent with those of **Soto-Piña *et al*. (2019)**, which demonstrated that glucocorticoids decreased T-lymphocyte numbers and activity, and those of **Gan *et al*. (2019)**, which demonstrated that fluorescent is othiocyanate-labelled lymphocytes were redistributed. Rats treated with Dexamethasone exhibited peripheral blood neutrophilia and lymphopenia, eosinopenia, monocytopenia, and basopenia. These consequences may have been caused by oxidative damage to biological structures. Pro-oxidant conditions predominate, either because of an increase in free radical production due to excessive oxidative stress or a poor scavenging capacity in the body, which may result in a decrease in lymphocytes due to the immunosuppressive effect of glucocorticoids. This imbalance has been connected to the pathogenesis of various diseases caused by toxins **(Chen *et al*., 2021).** Nano-piperine proteins have

been demonstrated to boost IL-6 production by lymphocytes in cultures with or without allogenic cells, which may explain the extract's ability to reduce Dexamethasone's negative effects **(Nounou *et al*., 2010).** Dexamethasone's inhibitory effect on neutrophil function, particularly in those that have undergone priming of activation via phagocytosis or nitric oxide release, or stress that alters neutrophil function, may account for the observed decrease in phagocytic activity of cells in rats treated with the steroid in the current study. Male rats treated with nano-piperine exhibited increased phagocytic activity because nano-piperine stimulates immune cells and boosts the immunological potential's activity (**Janakiraman *et al*., 2018**). It has been demonstrated that vitamin C has anti-inflammatory properties. Vitamin C supplementation has been demonstrated to reduce oxidative stress-induced inflammation by decreasing the generation of inflammatory cytokines. Vitamin C supplementation dramatically decreased IL-6 expression levels in spleens subjected to dexamethasone-induced oxidative stress **(Salihu *et al*., 2022).** Changes in Cellular and Antibody-Mediated Responses resulted in a study by **Alli *et al*., (2014**); dietary vitamin C supplementation was found to have immunomodulatory effects on the immune response of rats. According to the findings of this study, vitamin C supplementation significantly increased IL-6 production in splenocytes, which stimulated T cell growth and differentiation. An in vitro study that explored the effect of vitamin C treatment on heterophil function against Staphylococcus microorganisms also yielded intriguing results. Treatment with vitamin C increased the bactericidal activity of heterophils, indicating the possibility of preventing staphylococcal infections via vitamin C supplementation (**Mrityunjaya *et al*., 2020)**. serum IgG levels were considerably higher in control groups and vitamin C groups. According to research, systematic sensitization with allergen followed by repeated airway exposure leads to the highest levels of IgG and eosinophil infiltration. Bronchial asthma and elevated IgG levels are connected. Antigen-specific T helper cells in the lung are hypothesised to become activated, secrete IL-6, and contribute to the development of this disease **(Safaeian & Zabolian, 2014).** Rats in T4 groups and T5 groups had considerably lower serum IgG concentrations than rats in nano-piperine groups and dexamethasone groups. A study examining the relationship between dietary vitamin C intake, serum IgG concentrations, and atopy discovered that higher vitamin C intake was related to higher serum IgG concentrations than vitamin E and a reduced frequency of allergen sensitisation. These findings may explain the observed protective effect of vitamin E in the diet against asthma development **(Merkel *et al*., 2017).** Vitamin C administration inhibited the immunological response by raising interferon- and lowering IL-6 in cultured primary splenocytes, as demonstrated by prior studies on piglets who were not supplemented with vitamin C and exhibited an increase in serum IgG. These results support the use of megadose vitamin C to treat soy allergy **(Brusini *et al*., 2020).**

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