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Original Article
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Subcutaneous Injection of Bee Venom in Wistar Rats: effects on blood cells and biochemical parameters

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Objectives: Bee venom (BV) therapy is performed by a bee sting or subcutaneous injection of BV. However, there is not much information on the effect of BV on blood parameters after entering the body. This project aimed to assess the side effects of subcutaneous BV injections in healthy rats by measuring the hematological and biochemical parameters.
Methods: Various amounts of BV, including 100, 200, and 500 (μg/day), were subcutaneously injected into rats for 30 days. The results showed that BV affected the metabolism of the liver, kidney, and glands.
Results: An increase in blood sugar and a decrease in other biochemical parameters, including cholesterol, triglyceride, urea, creatinine AST, ALT, ALP, and phosphorous, were observed. Results also showed increased counts of white blood cells, neutrophils (%), and platelets and decreased levels of red cells, hemoglobin, and hematocrit.
Conclusion: This study demonstrates that BV therapy in medical clinics requires routine care and testing to prevent eventual metabolic and anemia side effects.

Keywords: bee venom, subcutaneous injection, biochemical, hematological

INTRODUCTION

Bee venom (BV) is a toxin from the honey bee, Apis mellifera, and other related species. Venom glands perform biosynthesis and secretion of BV in the abdominal cavity of bees to repel possible enemy attacks [1, 2]. Bee venom contains many enzymes, peptides, nonpeptides, and biogenic amine components [3]. The most common BV peptide components are melittin, apamin, mast cell degranulating (MCD) peptide, secapin, protamine, adolapin, protease inhibitor, tertiapin, and small peptides with less than five amino acids. The most important enzyme components are phospholipase A2, hyaluronidase, acid phosphomonoesterase, lysophospholipase, and α-glucosidase. Also, it includes physiologically active amines (e.g., histamine, dopamine, noradrenaline), amino acids (i.e., aminobutyric acid), carbohydrates, phospholipids, lipids, and volatile compounds [4]. Melittin, which constitutes about 50-60% of BV, acts as a robust anti-inflammatory agent in minimal doses by inducing cortisol production in the body but may cause adverse effects (e.g., inflammation, pain, and itching) in

high doses. It is also a cell lysing peptide, particularly in the red blood cells [1]. Apamin with about 1-3% of BV acts as a mild neurotoxin after entering the body, increasing the production of cortisol in the adrenal gland. Adolapin, comprising 2-5% of the peptides of BV, has cyclooxygenase blocking properties and acts as an anti-inflammatory and analgesic agent [4].

Due to BV's extensive biological properties, bee venom therapy (BVT) has been applied in medicine to treat autoimmune diseases such as multiple sclerosis [4]. Furthermore, its anti-inflammatory and immunosuppressive properties make it special for treating rheumatoid arthritis patients like acupuncture in different clinical trials [5].

BVT is performed by a bee sting or subcutaneous injection of BV. A full venom sac of a bee contains about 0.15 to 0.3 mg BV; however, it is not completely emptied after a sting unless the whole sting apparatus remains on the skin for a time [4]. Nevertheless, in the subcutaneous injection of BV, a determined dose is used as required, with different amounts reported in the literature [4, 6, 7]. Despite the confirmed therapeutic benefits of BV, there have also been reports of unwanted or unexpected side effects, such as systemic and nonspecific reactions or skin problems [8], because it contains various allergenic or immunogenic substances [9].

This study aimed to assess the side effects of subcutaneous BV injection in healthy rats by measuring the hematological and biochemical parameters.

MATERIALS AND METHODS

1. Chemicals and animals

BV was purchased from Asgharpoor Honey and Bee Products Co. (Iran). It was collected from healthy and approved hives from the Apis mellifera strain by electrical stimulation using the protocol suggested by Benton et al. [10]. Sampling tubes containing EDTA (Ethylenediaminetetraacetic acid) anticoagulant (1.5 mg/mL whole blood) were prepared from FL Co. (Italy) for collecting whole blood as ready to use. Biochemistry kits were prepared from Pars Azmoon Co. (Iran).

Thirty-five male Wistar rats weighing ~200 g were used in this study and kept according to the Standard Laboratory Animal Guidelines. The Ethical Committee of Torbat Heydariyeh University of Medical Sciences approved the experimental protocols of (IR.THUMS.REC.1400.003).

2. Preparation of BV solutions

A stock BV solution was prepared by desolvation of BV powder in normal saline at a concentration of 5,000 μg/mL and diluted to 2,500 and 500 μg/mL.

3. Experimental design

In addition to the untreated blank group, rats were divided into 4 groups and treated daily for 30 days. In placebo control, 0.2 mL of normal saline was injected subcutaneously between two shoulders, and in three other groups, prepared BV solutions were injected (i.e., 100, 500, and 1,000 μg/day).

4. Blood sampling

After 30 days, at least 4 mL of whole blood was taken directly from the heart after deep anesthesia; 1.5 mL was collected into the EDTA anti-coagulant tubes for hematological parameters, and 2.5 mL into free anti-coagulant tubes for biochemical parameters. The serums were separated after coagulation by centrifugation at 3,000 rpm, room temperature, 10 min.

5. Measurement of hematological and biochemical parameters

Biochemical parameters of serum, including blood glucose (Glu), cholesterol (Ch), triglyceride (Tg), urea (Ur), creatinine (Cr), AST (aspartate aminotransferase or SGOT, serum glutamic-oxaloacetic transaminase), ALT (alanine aminotransferase or SGPT, serum glutamic pyruvic transaminase), ALP (alkaline phosphatase), calcium (Ca), and phosphorus (Ph) were measured using an auto-analyzer (BT 1500, Biotechnica Co., Italy).

Whole blood was evaluated for hematological parameters (complete blood count [CBC]) by an automated cell counter (XP-300, Sysmex Co., Japan). CBC included the number of white blood cells (WBCs), percentage of neutrophils (%Neu), red blood cells (RBCs), and their indexes, including mean cell volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and red cell distribution width (RDW). Hemoglobin (Hb), hematocrit (Hct), and platelet counts (Plts) were also obtained.

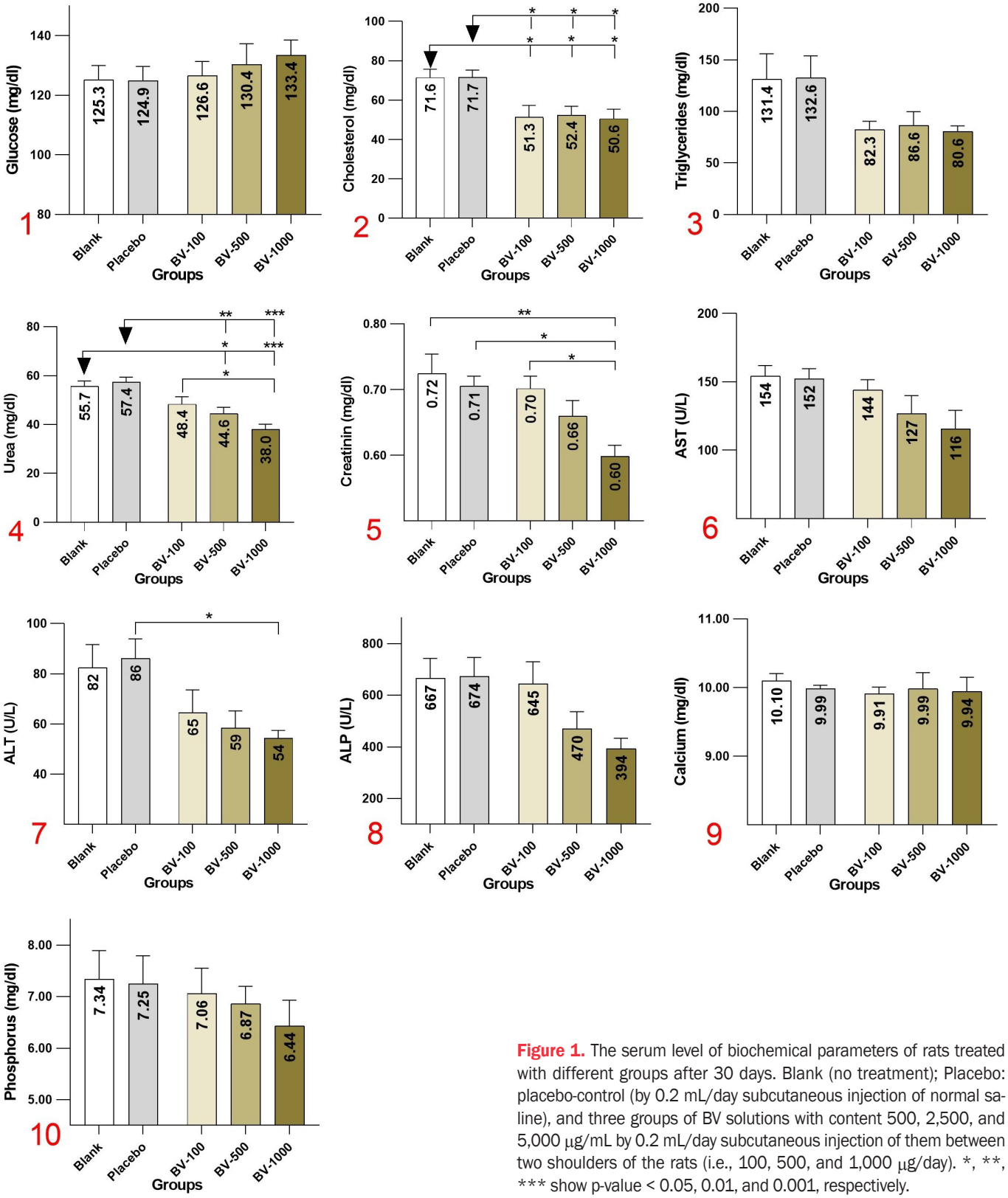

Figure 1. The serum level of biochemical parameters of rats treated with different groups after 30 days. Blank (no treatment); Placebo: placebo-control (by 0.2 mL/day subcutaneous injection of normal saline), and three groups of BV solutions with content 500, 2,500, and 5,000 μg/mL by 0.2 mL/day subcutaneous injection of them between two shoulders of the rats (i.e., 100, 500, and 1,000 μg/day). *, **, *** show p-value < 0.05, 0.01, and 0.001, respectively.

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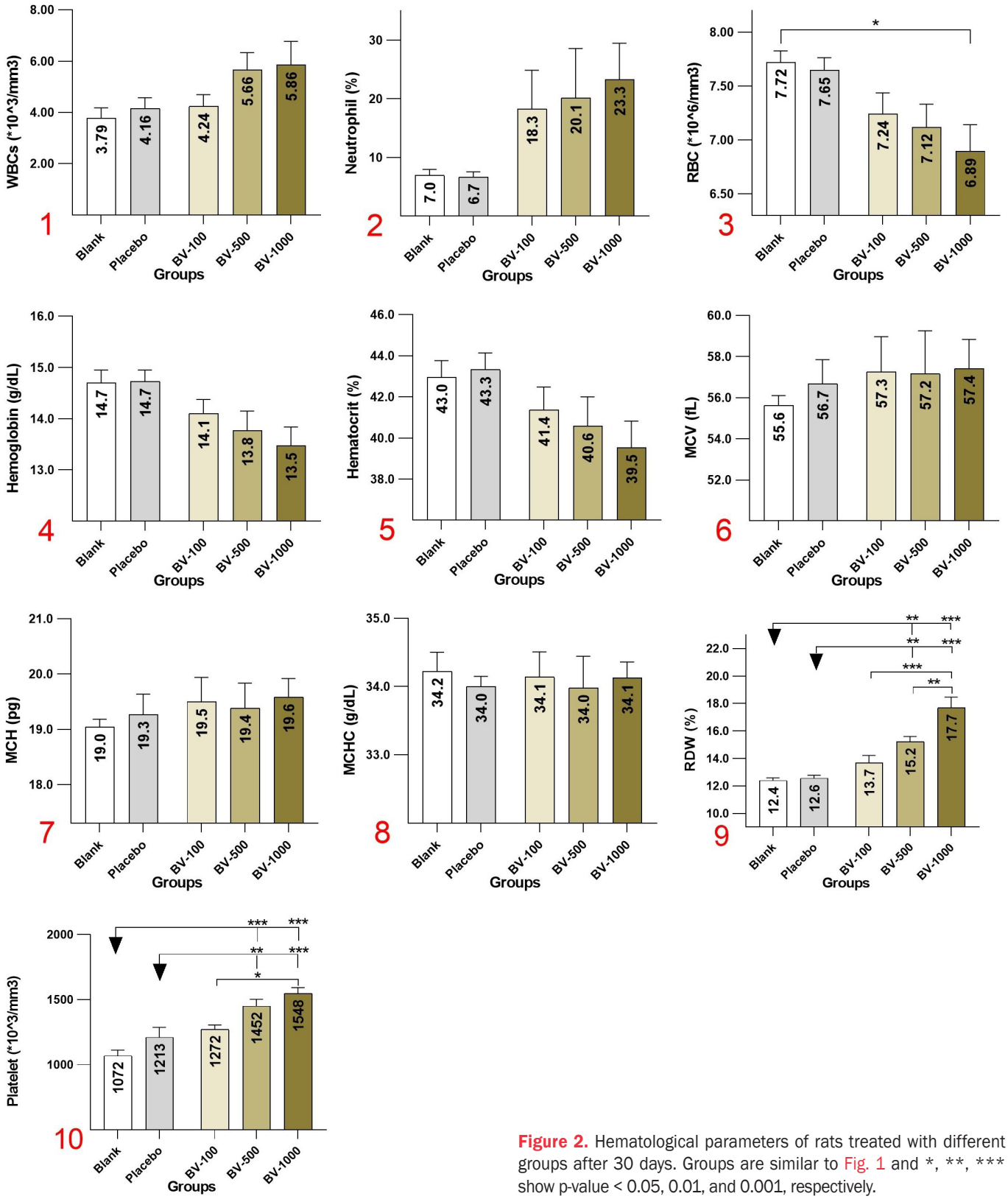

Figure 2. Hematological parameters of rats treated with different groups after 30 days. Groups are similar to Fig. 1 and *, **, *** show p-value < 0.05, 0.01, and 0.001, respectively.

6. Statistical analysis

One-way analysis of variance and Tukey comparison was performed to assess the statistical significance of differences among groups. Results with a p-value < 0.05 were considered statistically significant. Statistical analysis was performed using the SigmaPlot 12.0.lnk software.

RESULTS

Biochemical parameters are shown in Fig. 1. Means ± SE for serum levels of treated groups were increased for blood Glu and decreased for Chol, Tg, Ur, Cr, AST, ALT, ALP, and Ph, dose-dependently. However, significant differences between treated and blank/placebo groups were observed only in Chol, Ur, Cr, and ALT.

Fig. 2 shows the means ± SE for hematological parameters of treated rats using daily subcutaneous BV injection with different doses. WBCs, %Neu, RDW, MCV, and Plts count were increased, and RBC count, Hb, and Hct levels decreased dose-dependently. Besides, indexes of RBCs (MCH and MCHC) were unchanged. Nevertheless, significant differences were observed in RBC count, RDW, and Plts counts between treated and blank/placebo groups.

DISCUSSION

Previous studies have investigated the effect of BV on blood parameters with limited doses in various animal models [11, 12]. According to guidelines on long-term toxicity investigation of herbal medicines and based on the expected period of repeated administration in clinical use (parenteral route of BV), it should be a minimum of 4 weeks followed in rodents with groups receiving at least three different doses. One of six items of observations and examinations which should be performed on the following items are both hematological and biochemical parameters before the start of drug administration and autopsy for studies of less than one month [13]. Accordingly, the current study was designed and carried out.

Many investigations have reported on the mechanism of BV action; however, details after entering the body are uncertain [11]. As BV is multi-component, each component has a minimum of one activity that can synergize with other BV components in the body. For example, cytolytic peptides increase penetration of BV into the deeper layers of skin after biting; these actions can differ depending on their amount [14]. For example, melittin shows an anti-inflammatory effect in minimal doses; however, it possesses an inflammatory effect in higher doses [15].

Furthermore, BV can affect the organs, such as the islets of Langerhans in the pancreas [16], adrenal glands, and as a consequence, their hormones [17]. In the current study, blood Glu levels increased dose-dependently, although reports show that BV reduces Glu in diabetic rats by increasing insulin secretion [18]. Insulin and glucagons are essential hormones in blood sugar regulation [19]. However, also other hormones, such as adrenaline, affect blood Glu. Pain due to injection of BV can induce acute stress, adjusted by the sympathetic adrenergic axis where catecholamines mobilize energy stores, including carbohydrates, as the main parameter of the endocrine stress response [20]. Thus, blood Glu increased, and Chol and Tg blood levels were decreased. Also, the lipoprotein lipase enzyme was activated by insulin and hydrolysis Tg [21].

Literature reports indicate kidney injury, liver damage, and hepatotoxicity by BV therapy [22, 23]. Measuring Ur and Cr blood levels is the simplest way to monitor kidney function; Ur and Cr are regular metabolic waste products. Also, blood level Ur is related to the amount of protein in the diet and liver function, and blood level Cr reflects skeletal muscle mass [24, 25].

Moreover, aspartate and alanine aminotransferases (AST and ALT) catalyze the repartition of nitrogen between amino acids and corresponding oxoacids, participating in protein metabolism and gluconeogenesis. Therefore, AST and ALT are commonly used for liver disease diagnosis [26]. ALP enzyme is a hydrolase concentrated in the liver, bile duct, and kidney, and liver disorders are almost associated with an ALP increase [27]. Therefore, the presence of renal and liver diseases can increase these levels. However, our results showed a decrease in Ur, Cr, AST, ALT, and ALP. It seemed that subcutaneous injection of BV up to 1,000 μg/day after 30 days could not interfere with kidney and liver function.

Furthermore, regulated Ca and Ph blood levels depend primarily on three hormones: parathyroid, calcitonin, and 1,25-dihydroxy vitamin D [28]. Ca and Ph homeostasis is controlled by absorption in the gut, glomerular filtration, renal tubular reabsorption, and bone formation and resorption [29]. Some studies have reported the reducing effect of BV on Ca and Ph serum levels after entry into the bloodstream [30] or the effect of melittin on decreasing Ca levels of equine skeletal muscle [31]. However, our results showed that BV only decreased Ph serum

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level dose-dependently, without affecting Ca level.

WBCs are part of the immune system that protects the body against alien agents, and neutrophils are one type. Total WBC count provides essential information on health status [32]. Studies show that immunosuppressive and anti-inflammatory properties of BV reduced WBC count and %Neu via the effects on pro-inflammatory cytokines [33]. The effect of BV on the WBC count has been investigated in animal models of formaldehyde-induced arthritis [12], complete Freund's adjuvant-induced arthritis [34], and type II collagen-induced arthritis [35]. These studies state that WBC count and %Neu was reduced by BV treatment after inducing inflammation in models, while our result showed an increase of WBC count and %Neu in healthy rats dose-dependently.

RBCs are flexible biconcave cells containing Hb, and anemia is the deficiency of Hb that can be assessed by measuring RBCs, Hb, HCT, and RBC indices [36]. Indices of RBCs help elucidate the etiology of anemia. MCV defines the size of RBCs, MCH quantifies the amount of Hb per RBC, MCHC indicates the amount of Hb per unit volume, and RDW can be quantified distribution width of RBCs and is known as variation in the size or anisocytosis [37]. de Jonge et al. [38] showed that hemolysis caused a decrease in RBC count and Hct level; however, a high degree of hemolysis led to decreased RBC count, Hct and MCV, and an increase in RDW, MCH, and MCHC. The lytic effect of melittin of BV on RBCs has also been proved [1]. In the current study, RBC count, Hb concentration, and Hct percentage were decreased with increasing concentrations of BV injection, while MCV and RDW were increased. However, MCH and MCHC remained normal. Thus, our study shows that BV can lead to hemolytic anemia without significant change in MCH and MCHC with minor incremental changes in MCV.

The Plts are anuclear fragments in the blood, which act in the coagulation process. Hemolytic diseases, such as hemolytic uremic syndrome and sickle cell anemia, are often associated with hypercoagulability and increased baseline Plts; thus, Plts directly activated by free Hb in circulation blood [38]. Results showed the Plts count increased with increasing concentrations of injected BV.

CONCLUSION

This study demonstrated that subcutaneous BV injection in rats for 30 days affected the liver, kidney, and glands. The outputs observed were an increase/decrease in biochemical parameters, including blood sugar, Chol, Tg, Ur, Cr, AST, ALT, ALP, and Ph. Also, BV injection affected the hematological parameters, including WBCs, %Neu, RBCs, Hb, Hct, MCV, RDW, and Plts. Among these metabolic effects, decreases of Ur, Cr, and Chol and their hemolytic impact on red blood cells are critical. Therefore, using BV to treat (autoimmune) diseases in medical clinics requires periodic and regular care and testing to prevent eventual metabolic and anemia side effects.

AUTHORS' CONTRIBUTIONS

YY and MA conceived and designed the experiments and wrote the manuscript. SMH and MJMP performed the experiments. MRHN analyzed the data. SMH and MA helped perform the analysis with constructive discussions. MO revised the manuscript and participated in the interpretation of the results. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declared no potential conflicts of interest concerning this article's research, authorship, or publication.

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DATA AVAILABILITY

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials. Raw data are available from the corresponding author upon reasonable request.

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