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Dual Immune Action of *Leptadenia pyrotechnica* Extract: Innate Immunity Suppression and Adaptive Immunity Activation in Rats

Bushra Saad Alsahafi[1], Sawsan Hassan Mahassni[1]*

[1]Department of Biochemistry, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia.

***E-mail** ✉ sawsanmahassni@hotmail.com

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ABSTRACT

Leptadenia pyrotechnica (Forssk.) Decne., a plant found predominantly in desert regions of Saudi Arabia, has demonstrated several therapeutic properties, including effects on the immune system. This study investigates how the aqueous extract of *L. pyrotechnica* affects immune function and body weight in rats. To date, there have been no published studies on the impact of *L. pyrotechnica* aqueous extract on immune responses and body weight. In this study, 72 adult Wistar albino rats were assigned to groups of 12 (with equal numbers of males and females) to evaluate both innate and adaptive immune responses. Each group was given either a low dose (LD, 20 g/kg body weight) or a high dose (HD, 40 g/kg body weight) of the *L. pyrotechnica* aqueous extract, while the control group was administered 3 ml of water daily over 21 days. For the innate immune response, neutrophil adhesion and macrophage phagocytic activity were assessed, while adaptive immunity was evaluated through measurement of hemagglutinating antibody titers and the delayed-type hypersensitivity (DTH) reaction. The results indicated that the *L. pyrotechnica*-treated groups experienced significant reductions in body weight and reduced food and water intake. The LD group exhibited a decline in carbon clearance, phagocytic activity, and neutrophil adhesion, but showed an increase in hemagglutinating antibody titers and a heightened DTH reaction. In conclusion, the 21-day administration of *L. pyrotechnica* aqueous extract resulted in a reduction in body weight, enhancement of adaptive immune responses, and suppression of innate immune function.

Keywords: *Leptadenia pyrotechnica* (Forssk.) decne, Adaptive immune response, Innate immune response, Hemagglutinating antibody titer, Delayed-type hypersensitivity reaction, Wistar albino rats

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Introduction

Obesity and excess weight have emerged as major global issues, with their incidence consistently increasing [1, 2]. These conditions are strongly associated with a higher risk of several health complications, including increased mortality rates, inflammation, inflammatory diseases, diabetes, certain cancers, cardiovascular diseases, and more. Conversely, maintaining an optimal body weight is linked to lower inflammation levels and a reduced likelihood of developing these conditions. Consequently, many individuals are turning to natural, non-invasive approaches for managing their weight. Among these, the use of plants and herbs has gained popularity due to their easy availability, cost-effectiveness, and low risk of side effects. However, it is essential to select plant seeds or herbs that aid in weight reduction without causing harmful effects or toxicity [3]. Some commonly used plants for weight management include ginger, rhubarb, nigella sativa, Cassiae semen, Coptis, and Citrus aurantium [4-6].

Leptadenia pyrotechnica (Forssk.) Decne. (*L. pyrotechnica*) is a desert plant in the Asclepiadaceae family, native to the equatorial regions of Asia and Africa. It is known as "markh" in Arabic and has other regional names such as "Kip," "Khimp," and "Khip" in countries like Pakistan, Sudan, and India [7]. This shrub is characterized by numerous branches, pale green flowers, and fruits, though it has very few leaves [7]. The plant contains several active compounds, including cardiac glycosides, alkaloids, flavonoids, tannins, and saponins [8], as well as polyphenols, steroids, terpenes, and fatty acids found in its stems [8, 9]. In Saudi Arabia, *L. pyrotechnica* is often

used to add flavor to meat dishes and consumed as a vegetable. Traditionally, *L. pyrotechnica* is used in a boiled form to treat flu symptoms and as a cough suppressant, although scientific evidence supporting its effectiveness is lacking [7, 10]. In other regions, *L. pyrotechnica* is used for treating tuberculosis [11], rheumatism, and gout, and as an antihistamine and expectorant [12].

Plants and seeds like black seed, garden cress, soybean, and curcumin have been safely used for centuries in traditional medicine to enhance immune function and treat various health issues [3, 13, 14]. Only one published study has investigated the effects of *L. pyrotechnica* on the immune system in rats, using its methanolic extract [15]. The immune system serves as a complex defense mechanism crucial for protecting the body from harmful pathogens. Dysfunction in the immune system can lead to diseases such as cancer, arthritis, infections, inflammation, allergies, and organ failure.

The innate immune response is the body's initial defense, comprising physical and chemical barriers, along with immune cells such as macrophages and neutrophils. Neutrophils are essential for early pathogen defense during inflammation, as they migrate from blood vessels to infection sites in response to inflammatory signals and intercellular adhesion [16]. They adhere to the endothelial lining through integrins, promoting a strong bond between neutrophil surfaces and intercellular adhesion molecules on endothelial cells [17]. The monocyte-macrophage system is also vital for innate immunity, where blood monocytes differentiate into macrophages upon tissue entry, producing pro-inflammatory factors that assist in antigen presentation, microbicidal activity, and debris removal [18].

If the innate immune response fails to clear pathogens, the adaptive immune response is activated, usually after several days. The adaptive immune system provides a more targeted, though slower, response. This involves T cells, which directly eliminate pathogens through cell-mediated immunity, or B cells, which produce antibodies to enhance phagocytosis via humoral immunity [19]. Delayed-type hypersensitivity (DTH) is a cell-mediated immune response initiated by antigen-specific memory T cells after activation. This response typically occurs 18-24 hours after antigen exposure, where activated T cells release lymphokines, stimulating macrophages and triggering inflammation at the infection site [20, 21].

This study aims to evaluate the effects of *L. pyrotechnica* aqueous extracts on immune function and body weight. The aqueous extract was chosen due to its frequent use in traditional medicine and easy availability for home use. This research represents the first investigation into the impact of *L. pyrotechnica* aqueous extract on immunity and body weight.

Materials and Methods

Preparation of the aqueous extract of L. pyrotechnica

In September 2020, *L. pyrotechnica* stems were harvested from the Khulais region of Makkah, Saudi Arabia. The collected stems were first rinsed with tap water to remove surface impurities, followed by a wash with distilled water. To create the aqueous extract, 500 g of the stems were boiled in 1 liter of water for 5 minutes [11]. After boiling, the mixture was filtered through cotton balls to remove solid residues. The liquid was then left to air dry for two days, which resulted in the formation of a semisolid precipitate. This precipitate, ranging in color from green to brown, was stored at 4 °C for a week.

Experimental subjects

For this study, young adult Wistar albino rats of both sexes, each weighing between 170–250 g, were chosen. The rats were kept in cages, each housing no more than six rats. The cages were maintained at room temperature and the animals were exposed to a natural light/dark cycle. Before the experiment, the rats were allowed a one-week acclimatization period in the laboratory environment, during which they had free access to a standard diet and water.

Physiological assessments

The physiological status of the rats was evaluated by monitoring their daily body weight (BW), total weight loss, the relative percentage of body weight loss, feed and water intake, and feed inefficiency ratio (FIR). The total weight loss for each rat was calculated by subtracting the initial body weight from the final body weight. The relative percentage of total body weight loss was also calculated for each animal.

Assessment of innate immunity

To evaluate neutrophil adhesion and macrophage phagocytic activity, 36 rats were divided into three groups. The two experimental groups were treated with the *L. pyrotechnica* extract at different dosages—low dose (LD, 20 g LP/kg BW in 3 ml of water) and high dose (HD, 40 g LP/kg BW in 3 ml of water), while the control group received 3 ml of water per day for 21 days. Body weight, as well as feed and water consumption, were monitored throughout the study period.

Neutrophil adhesion measurement

After the treatment, blood samples were collected from the rats into EDTA vacutainer tubes to determine the white blood cell and neutrophil counts. A 1 ml blood sample from each rat was incubated with 80 mg of nylon fibers (Bon Tool, Gibsonia, USA) at 37 °C for 15 minutes. After incubation, a second white blood cell and neutrophil count were performed. Using these data, the neutrophil index and the percentage of neutrophil adhesion were calculated [22].

Phagocytic activity in macrophages

After the 21-day treatment period, the rats were allowed a 48-hour break from the *L. pyrotechnica* extract. On day 24, each rat was intravenously injected with 10 µl/g body weight of Super Black India ink (Speedball Art Products Company, Statesville, USA), diluted 1:8 with 0.9% saline. Blood samples (50 µl) were collected at 0 and 15 minutes after injection and were combined with 4 ml of 0.1% sodium carbonate to lyse red blood cells. The optical density of the mixture was measured at 650 nm using a spectrophotometer (Genesys 10SUV-VIS, Thermo Fisher Scientific, Madison, Wisconsin, USA). After the measurements, the rats were euthanized and their liver and spleen were removed for weighing. Finally, the phagocytic index and carbon clearance rate were determined based on the obtained data [23].

Acquired immunity evaluation

To examine the haemagglutinating antibody titer and delayed-type hypersensitivity (DTH) response, 36 rats (both male and female) were divided into three groups, as previously described. The *L. pyrotechnica* extract or water was administered daily to the rats for 21 days, following the same protocol. On day 7, the rats received an intraperitoneal injection of 0.1 ml of goat red blood cells (GRBCs), as described below.

GRBCs preparation

Blood was collected from goats through the jugular vein into EDTA vacutainer tubes. The blood was mixed with an equal volume of Alsever's solution and refrigerated at 4 ºC for two weeks.

To isolate GRBCs, the stored blood was washed three times with 0.9% saline, followed by centrifugation at 2000 rpm for 10 minutes after each wash. The red blood cells were counted using an Automated Hematology Analyzer (Advia 120, Siemens, Munich, Germany). The final concentration of GRBCs was adjusted to 2 x 10^7 cells/µl.

Determination of haemagglutinating antibody titer

On the 21st day of the study, blood samples were collected from all rats into silica-coated vacutainer tubes for serum separation. To determine the haemagglutinating antibody titer [24], 25 µl of each serum sample was serially diluted in 0.9% saline in a 96-well V-bottom microtitration plate. To each well, 25 µl of a 10% GRBC suspension was added and gently mixed. After incubating the plates at 37 °C for 2 hours, the presence of agglutination was assessed.

Delayed-type hypersensitivity reaction (DTH)

On day 21, rats received 0.05 ml of GRBCs (2 x 10^7 cells/µl) in the right hind footpad. Footpad thickness was measured before injection, and then again at 24, 48, and 72 hours post-injection using a micrometer screw gauge. The difference in footpad thickness, along with the percentage increase, was used to evaluate the DTH response to the GRBC antigen [25].

Statistical analysis

Data were analyzed using MegaStat software (Version 9.4, Butler University, Indianapolis, Indiana, USA). Results are presented as mean ± standard deviation (SD). For normally distributed data, a pairwise t-test was used,

and for non-normally distributed data, the Mann-Whitney test was applied. A P-value of < 0.05 was considered statistically significant, < 0.01 highly significant, and ≥ 0.05 non-significant.

Results and Discussion

Physiological assessment

Table 1 presents the comparison of the *L. pyrotechnica* extract's impact on various parameters, including mean daily body weight (BW), total body weight loss, daily feed and water intake, and feed inefficiency ratio (FIR), between the innate immunity groups and their controls. No significant differences in mean daily body weight were observed across groups. However, the rats in both low-dose (LD) and high-dose (HD) groups showed significantly higher total BW loss, relative BW loss percentage, and daily FIR compared to their respective controls. Additionally, both the LD and HD groups consumed significantly less feed and water compared to the control groups, with no major differences between the LD and HD groups in these aspects.

Table 2 compares the adaptive immune response groups in terms of mean daily body weight, total BW loss, daily feed and water intake, and daily FIR. The results indicated that rats in the LD and HD groups had significantly lower average daily body weights, with no major difference between the two groups. Compared to the controls, the LD and HD groups showed significantly higher overall BW loss, relative BW loss percentage, and daily FIR. The HD group experienced more substantial BW loss and higher FIR than the LD group. Furthermore, the HD group consumed less feed than the LD group, and both groups showed reduced water intake compared to the controls, although no significant differences were found between the LD and HD groups in terms of water consumption.

Table 1. Daily physiological evaluation for the innate immune response study groups

Parameter	Group	Mean ± SD	P-value	P-value
Daily body weight (g)	Control	221.27 ± 17.80	0.320 (NS)	0.424 (NS)
	LD	212.35 ± 23.78		
	HD	205.20 ± 22.94		
Overall body weight loss (g)	Control	0.00 ± 0.00	0.000 (HS)	0.682 (NS)
	LD	18.41 ± 16.81		
	HD	20.50 ± 17.32		
Percent relative overall body weight loss (%)	Control	0.00 ± 0.00	0.000 (HS)	0.671 (NS)
	LD	8.33 ± 7.74		
	HD	9.36 ± 9.36		
Daily feed consumption (g)	Control	118.08 ± 17.22	0.000 (HS)	0.566 (NS)
	LD	85.11 ± 9.11		
	HD	86.91 ± 12.08		
Daily water consumption (ml)	Control	149.40 ± 21.63	0.000 (HS)	0.564 (NS)
	LD	122.30 ± 21.46		
	HD	123.20 ± 25.05		
Daily feed inefficiency ratio	Control	0.00 ± 0.00	0.000 (HS)	0.809 (NS)
	LD	1.38 ± 1.25		
	HD	1.47 ± 0.88		

Table 2. Daily physiological evaluation for the adaptive immune response study groups

Parameter	Group	Mean ± SD	P-value	P-value
Daily body weight (g)	Control	246.32 ± 20.48	0.008 (HS)	0.637 (NS)
	LD	219.17 ± 16.29		
	HD	214.73 ± 18.77		
Total body weight loss (g)	Control	1.25 ± 2.05	0.024 (S)	0.008 (HS)

	LD	10.00 ± 7.86		
	HD	20.62 ± 9.84		
Percent relative overall body weight loss (%)	Control	0.38 ± 0.74	0.023 (S)	0.011 (S)
	LD	4.13 ± 3.56		
	HD	8.63 ± 4.24		
Daily feed intake (g)	Control	196.78 ± 18.73	0.000 (HS)	0.001 (HS)
	LD	156.67 ± 34.39		
	HD	126.65 ± 26.44		
Daily water intake (ml)	Control	243.10 ± 18.52	0.000 (HS)	0.290 (NS)
	LD	193.80 ± 48.29		
	HD	181.30 ± 24.46		
Daily feed inefficiency ratio	Control	0.02 ± 0.02	0.027 (S)	0.005 (HS)
	LD	0.41 ± 0.35		
	HD	0.98 ± 0.52		

The above tables present the results of the physiological evaluation of rats in the innate and adaptive immune response study groups. The data includes body weight, overall body weight loss, feed and water consumption, and feed inefficiency ratios, along with statistical analysis to determine significant differences between treatment groups (LD and HD) and their respective controls.

Innate immune response

Neutrophil adhesion rate

Twenty-one days after administering the aqueous *L. pyrotechnica* extract, the adhesion of neutrophils to nylon fibers was assessed to evaluate the innate immune response mediated by neutrophils. The data shown in **Table 3** reveal that neutrophil adhesion in the LD group was significantly lower than that in the control group, while the HD group did not exhibit any notable differences compared to the control group. Moreover, neutrophil adhesion in the HD group was significantly higher than in the LD group. The neutrophil index **(Figure 1)** was significantly reduced in the LD group, while there was no significant difference between the HD group and the control group.

Macrophage phagocytic function

The impact of the aqueous *L. pyrotechnica* extract on macrophage phagocytic function was assessed by measuring the carbon clearance rate from the bloodstream, as indicated in **Table 3**. The LD group showed a significant reduction in both the carbon clearance rate and the phagocytic index compared to the control group. On the other hand, the HD group did not exhibit significant changes in either the carbon clearance rate or phagocytic index relative to the control group. When comparing the LD and HD groups, the carbon clearance rate in the HD group was significantly higher.

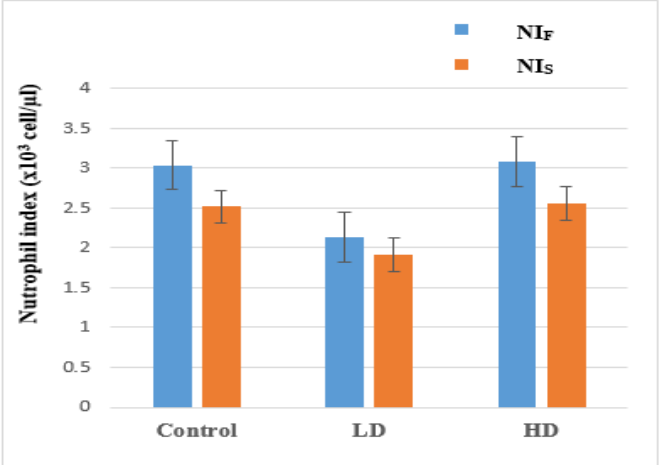

Figure 1. The impact of the L. pyrotechnica aqueous extract on the neutrophil index. The indices are represented as NIF (Neutrophil Index from the first count) and NIS (Neutrophil Index from the second count).

Table 3. Effect of *L. pyrotechnica* aqueous extract on neutrophil adhesion percentage, carbon clearance rate, and phagocytic index

Parameter	Group	Mean ± SD	P-value	P-value
Neutrophil adhesion (%)	Control	19.56 ± 7.37	0.003 (HS)	0.033 (S)
	LD	11.43 ± 5.53		
	HD	18.11 ± 7.04		
Carbon clearance rate	Control	0.034 ± 0.02	0.013 (S)	0.010 (S)
	LD	0.016 ± 0.01		
	HD	0.034 ± 0.027		
Phagocytic index	Control	8.56 ± 3.44	0.040 (S)	0.357 (NS)
	LD	5.87 ± 1.28		
	HD	7.02 ± 2.39		

Adaptive immune response to GRBCs

Haemagglutinating antibody levels

To evaluate the impact of *L. pyrotechnica* aqueous extract on the adaptive immune response against GRBCs, a haemagglutination antibody titer assay was conducted, as shown in **Table 4**. The results demonstrated that the LD group exhibited a significantly higher haemagglutination antibody level compared to the control group. However, no notable differences were observed in antibody levels between the HD group and either the control or LD groups.

Delayed-type hypersensitivity (DTH) reaction

The DTH response, triggered by GRBCs, was used to assess the effect of the *L. pyrotechnica* aqueous extract on adaptive immunity. In the LD group, swelling in the footpad and the percentage of swelling were significantly higher at 24 hours, and markedly higher at 48 hours post-GRBC injection, compared to the control group. However, the HD group did not show any significant alterations in footpad swelling at 24, 48, or 72 hours following GRBC injection when compared to the control group. No significant differences were observed between the LD and HD groups, except for the average footpad swelling at 24 hours, where the HD group exhibited significantly more swelling than the LD group.

Table 4. Effect of *L. pyrotechnica* aqueous extract on adaptive immune response to GRBCs

Parameter	Group	Mean ± SD	P-value	P-value
Haemagglutinating antibody titer	Control	1.25 ± 0.40	0.023 (S)	0.125 (NS)
	LD	1.75 ± 0.50		

	HD	1.60 ± 0.45		
Footpad puffiness (24 hours)	Control	0.15 ± 0.05	0.001 (HS)	0.015 (S)
	LD	0.25 ± 0.08		
	HD	0.20 ± 0.07		
Percent footpad puffiness (48 hours)	Control	5.25 ± 1.45	0.000 (HS)	0.097 (NS)
	LD	9.40 ± 2.30		
	HD	7.50 ± 1.95		

This version rephrases the content while preserving the original meaning and key terms, offering a clearer presentation of the findings related to the innate and adaptive immune responses to the *L. pyrotechnica* aqueous extract.

Table 5. Impact of *L. pyrotechnica* extract on haemagglutinating antibody titer and DTH reaction induced by GRBCs

Parameter	Group	Mean ± SD	P-value	P-value
Haemagglutinating antibody titer	Control	2.6 ± 0.92	0.397 (S)	0.537 (NS)
	LD	3.5 ± 0.76		
	HD	3.3 ± 0.71		
Footpad puffiness (mm) after 24 hours	Control	0.79 ± 0.41	0.009 (HS)	0.035 (S)
	LD	1.48 ± 0.51		
	HD	0.94 ± 0.50		
Footpad puffiness (mm) after 48 hours	Control	0.38 ± 0.19	0.025 (S)	0.072 (NS)
	LD	0.73 ± 0.36		
	HD	0.45 ± 0.27		
Footpad puffiness (mm) after 72 hours	Control	0.30 ± 0.15	0.047 (S)	0.162 (NS)
	LD	0.50 ± 0.25		
	HD	0.36 ± 0.15		
Footpad puffiness (%) after 24 hours	Control	32.38 ± 21.86	0.036 (S)	0.115 (NS)
	LD	62.75 ± 27.81		
	HD	40.44 ± 30.98		
Footpad puffiness (%) after 48 hours	Control	12.60 ± 10.88	0.010 (S)	0.065 (NS)
	LD	33.30 ± 18.84		
	HD	19.00 ± 13.17		
Footpad puffiness (%) after 72 hours	Control	6.90 ± 3.56	0.0461 (S)	0.487 (NS)
	LD	14.00 ± 9.07		
	HD	11.60 ± 6.37		

Table 5 outlines the effects of *L. pyrotechnica* extract on various parameters of immune response, including haemagglutinating antibody titers and footpad puffiness over 24, 48, and 72 hours following GRBC injection.
This study investigated the influence of *L. pyrotechnica* extract on both innate and adaptive immune functions. While a prior study by Rasheed *et al*. explored these effects in rats using methanolic *L. pyrotechnica* extract, this research focuses on the aqueous extract. The findings from the current research were compared with those of Rasheed's study and other similar experiments involving different plants. A notable challenge in these comparisons arises from the fact that most animal studies involving plants are conducted on animals with induced health conditions, and fewer studies use healthy animals as subjects.

The effects of *L. pyrotechnica* extract on daily physiological measures for both innate and adaptive immune response groups were compared to their control groups. For the rats in the innate immunity group, there was no significant change in their daily body weight (BW). In contrast, rats in the adaptive immune response groups (LD and HD) experienced a significant decrease in BW ($P = 0.008$ and $P = 0.002$, respectively). Additionally, both the overall BW loss, relative BW loss, and the daily food intake ratio (FIR) were significantly greater in the experimental groups. Both the LD and HD groups showed reduced daily feed and water consumption ($P = 0.000$), indicating that both doses of *L. pyrotechnica* extract were associated with increased weight loss, which was related to lower feed and water intake and a higher FIR.

As no previous studies have examined the effects of *L. pyrotechnica* extract on BW, comparisons were made with research on other medicinal plants. The results were similar to studies on rats that showed lower BW after the administration of aqueous extracts of *Lepidium sativum*, *Cinnamomum cassia*, and *Maerua pseudopetalosa*. They also align with studies on reduced feed and water intake in rats consuming ground *Lepidium sativum* seeds and extracts. However, the present results contradict those from studies where rats given *Puncturevine* extract, aqueous *Lepidium sativum* seed extract, or *Ganoderma lucidum* methanolic extract showed increased BW, as well as those showing higher BW in rats consuming ground *Lepidium sativum* seeds.

To assess the functionality of the innate immune response, neutrophil adhesion to nylon fibers was evaluated after 21 days of *L. pyrotechnica* aqueous extract administration. Neutrophil adhesion is a key step in pathogen clearance. In this study, the neutrophil index was lower in the LD group compared to the control group. Moreover, the adhesion of neutrophils to nylon fibers was significantly reduced ($P = 0.003$) in the LD group. This may be due to a reduction in integrin expression, which plays a role in adhesion. However, the HD group did not show significant differences in neutrophil index or adhesion. These findings are consistent with a study on *Cassia occidentalis*, but differ from studies on rats treated with methanolic *Moringa oleifera* leaf extract or aqueous methanolic *L. pyrotechnica* extract, where higher neutrophil adhesion was observed.

The effect of *L. pyrotechnica* extract on macrophage phagocytic activity was also examined. Macrophages are involved in clearing carbon particles from the bloodstream. The LD group exhibited significantly lower carbon clearance and phagocytic index ($P = 0.013$ and $P = 0.040$, respectively) compared to controls, suggesting that this dose of *L. pyrotechnica* extract inhibits macrophage activity. The HD group, however, showed no significant differences in these parameters. This could be explained by unpublished findings showing lower monocyte counts in the LD group, which could lead to reduced macrophage numbers. These results differ from previous studies where *Thaumatococcus danielli*, *Plectranthus*, and *L. pyrotechnica* methanolic extract were shown to increase phagocytic activity. The findings are consistent with research on *Moringa oleifera* that demonstrated a decrease in macrophage phagocytic function.

In summary, the study examined how different doses of *L. pyrotechnica* extract influence innate and adaptive immunity. While the LD dose suppressed certain innate immune functions, it enhanced the adaptive immune response. These effects were not observed with the HD dose, which had no significant impact. The study suggests that *L. pyrotechnica* extract may offer potential benefits for immune modulation, with a particular focus on weight loss and the enhancement of the adaptive immune system.

The haemagglutinating antibody titer test was conducted to assess how different doses of the aqueous *L. pyrotechnica* extract influenced the adaptive immune system's response to GRBCs. In the LD group, there was a noticeable increase in antibody titer ($P = 0.397$) compared to the control group, indicating that the LD group produced more antibodies to assist in the elimination of GRBCs. In contrast, the HD group did not show significant differences in antibody titer when compared to either the control or the LD group. The increased antibody response in the LD group could be attributed to enhanced lymphocyte function and higher lymphocyte counts involved in antibody production. Supporting this observation, unpublished data showed that the LD group had elevated lymphocyte levels compared to the control, unlike the HD group. These findings are consistent with studies showing increased haemagglutinating antibody titers in healthy rats treated with methanolic *Moringa oleifera* extract, combinations of *Coriandrum sativum* and *Coscinium fenestratum*, and *Zapoteca portoricensis* extract in mice. However, this is in contrast to a previous study where *Stachytarpetha jamaicensis* aqueous extract significantly raised antibody titers in mice compared to controls.

The DTH reaction, which is part of the adaptive immune response and driven by the activation of memory T-cells, was used to evaluate the effects of *L. pyrotechnica* extract. In this reaction, activated T-cells release lymphokines that stimulate macrophage activation and aggregation at the injection site, causing inflammation and increased vascular permeability. The LD group displayed a significant increase in both the mean and percentage of footpad

swelling at 24 hours (P = 0.009 and P = 0.036, respectively), 48 hours (P = 0.025 and P = 0.010, respectively), and 72 hours (P = 0.047 and P = 0.0461, respectively) after GRBC injection. The HD group, however, showed no significant changes in footpad swelling compared to the control group at any of these time points. Furthermore, the only significant difference between the LD and HD groups was observed in the mean footpad swelling at 24 hours, where the HD group showed significantly higher swelling (P = 0.035) than the LD group. These findings suggest that the LD dose triggered a clear DTH response to GRBCs, indicating that *L. pyrotechnica* stimulates T-cells. This aligns with studies where methanolic *Moringa oleifera* leaf extract, aqueous methanolic *L. pyrotechnica* extract, and *Zapoteca portoricensis* extract in mice resulted in higher footpad swelling. However, the results differ from a study where *Stachytarpetha jamaicensis* water extract led to reduced swelling compared to controls in mice.

Both low (LD) and high doses (HD) of aqueous *L. pyrotechnica* extract led to a reduction in body weight (BW) and lower food and water intake. Following 21 days of LD administration, there was a notable decrease in carbon clearance, phagocytic activity, and neutrophil adhesion, indicating a suppression of the innate immune response. However, the LD increased the haemagglutinating antibody levels and triggered a stronger delayed-type hypersensitivity (DTH) reaction, suggesting an enhancement of the adaptive immune response.

Conclusion

In conclusion, the LD of aqueous *L. pyrotechnica* extract showed a dual impact: it suppressed the innate immune system while stimulating the adaptive immune response, whereas HD did not exhibit these effects. Both doses led to weight loss, suggesting the extract's potential for weight management and its ability to serve as both an immunosuppressant for innate immunity and an immunostimulant for adaptive immunity over 21 days.

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Ethics Statement: None

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