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A Pilot Study: Ground and Aqueous Extract of *Leptadenia pyrotechnica* Modulate the Immune System Affecting White Blood Cell Counts and Increasing Red Blood Cell Counts

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Abstract

Leptadenia pyrotechnica (Forssk.) Decne. (LP) is used in local folk medicine for the treatment of different ailments. There are no published studies on the hematological and immune system effects of LP. This study is the first to determine the acute toxicity of LP extract and the effects of ground and aqueous LP extracts on red blood cell (RBC), and total and differential white blood cells counts in rats. Ground LP mixed with the regular diet at 25%, 50% and 75% LP weight/feed weight was administrated to six young adult Wistar albino rats (one female and one male rat per concentration), while control rats were fed the regular feed, daily for one week. Aqueous LP extracts were orally gavaged at 3, 9, 15 and 20 g/kg body weight to 16 rats (two female and two male rat per concentration), while control rats were gavaged with water, daily for two weeks. Blood samples were collected for the determination of blood cells counts and percents. Results were compared with the controls. The extract was safe up to a dose of 40 g/kg body weight. Rats that consumed ground LP had significantly higher neutrophil and monocyte counts and percents, and lower lymphocyte percent and eosinophil counts and percents. Rats that consumed extracts had significantly higher RBC counts and neutrophil counts and percents, and lower lymphocyte percent and eosinophil counts and percents. Therefore, ground and extracts of LP affect blood cells counts and may modulate the immune system, while only the extract increases RBC counts.

Keywords: *Leptadenia pyrotechnica*, Lymphocytes, Neutrophils, Red blood cells, White blood cells

1. Introduction

Leptadenia pyrotechnica (Forssk.) Decne. (LP), a desert plant of the family Asclepiadaceae, is a valuable medicinal plant. It is known as "markh" in Arabic, and it grows in equatorial regions of Asia, Africa and in the sandy plains in the nations of the Western Arabian Gulf (Khasawneh *et al.,* 2015). It is a shrub, with a height of 0.5 to 2.6 meters, that has many green branches but no leaves. The season of flowering and fruiting begins in August and lasts to the end of January (Verma *et al.,* 2014). There are five chief active compounds found in all parts of LP, namely cardiac glycosides (Youssef Moustafa *et al.,* 2009), alkaloids, flavonoids, tannins and saponins (Munazir *et al.,* 2015). The stem contains polyphenolic compounds (Mohammad *et al.,* 2011; Preet and Chand Gupta, 2018), steroids, terpenes, fatty acids (Youssef Moustafa *et al.,* 2007), and other chemical compounds

LP is used locally in Saudi Arabia and other Arab countries to add flavor to some foods and as a vegetable. Its stem has been used in traditional medicine to treat several diseases, including tuberculosis (using the water extract) (Patel *et al.,* 2014); smallpox, psoriasis and diabetes (using the sap of young stems) (Verma *et al.,* 2014); dermatitis, kidney disorders, cough, urinary retention, constipation, abortion and cancer (Bhabootra, 2016). Although there is no scientific evidence, LP may have some effects on the immune system since it is used for the treatment of wounds, (Upadhyay *et al.,* 2007), rheumatism (Bhabootra, 2016), the common cold, eczema and other skin diseases (Katewa and Galav, 2006).

The immune system is one of the most important and complex systems in the body, affecting other systems of the body and general health. The main function of the immune system is to protect the body from pathogens, while the functional failure of the immune system results in many diseases (Rasheed *et al.,* 2016). The immune system is made up of different cells, molecules, and organs. The two main types of immune responses mediate their effects and actions through different molecules and cells. The cells that are important in innate immunity are most of the white blood cells (WBC), such as neutrophils, monocytes, eosinophils and basophils, while in the adaptive immunity the lymphocytes are the major WBC (Hillion *et al.* 2019). Therefore, it is possible to modify the activity of the immune response by affecting the counts of WBC by natural plants, which has received wide attention recently.

Presently, most suppressive immune modulators, such as chemotherapeutic drugs, have cytotoxic effects that limit their repeated use (Kumolosasi *et al.,* 2018). On the other hand, there are many plants and seeds that have been used in folk and traditional medicinal systems worldwide

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for thousands of years for the enhancement of the immune system. Some of these medicinal plants and seeds are black seeds, garden cress seeds, soybean, curcumin and Moringa oleifera (Osman, Shayoub and Babiker, 2012; Ghori *et al.,* 2018). Most medicinal plants that have immunomodulatory activities are considered harmless, thus they can be used as alternative medicines for the treatment of a variety of diseases (Ghori *et al.,* 2018) and for their useful effects (Mahassni and Al-Reemi, 2013; ; Mahassni and Khudauardi, 2017; Mahassni and Bukhari, 2019; ; Mahassni and Munshi, 2019; Mahassni and Nabulsi, 2020).

Research studies on LP are limited (Alqasoumi *et al.,* 2012; Munazir *et al.,* 2012; Khasawneh *et al.,* 2015), with the alcoholic extract being the form that has been used. In addition, there is only one study (Alsahafi and Mahassni, 2021) on the effects of LP aqueous extracts on the immune system, where it was found to suppress innate immune responses and enhance adaptive immune responses. The specific active components in LP that may be responsible for these effects are probably the alkaloids, which have immunostimulant activity, and the flavonoids, which possess anti-inflammatory and immunomodulatory effects. Other studies have been done on the antioxidant (Alqasoumi *et al.,* 2012) and antibacterial activities (Munazir *et al.,* 2012), and antiatherosclerotic and hypolipidemic effects (Jain *et al.,* 2007) of the alcoholic extracts of LP. Additionally, the LP alcoholic extracts have been shown to have anticancer effects against human breast cancer cells (Khasawneh *et al.,* 2011) and colon cancer cells (Khasawneh *et al.,* 2015).

It is important to ensure the safety and efficacy of medicinal plants before utilizing them for the treatment of diseases and ills. A critical step to ensure the validity of medications is to perform acute toxicity testing in animal models. All toxicity studies on LP have been done on alcoholic extracts only (Watafua and Geidam, 2014; Rasheed *et al.,* 2016). The present study is the first to carry a toxicity study on the LP aqueous extract.

Thus, there are no published research studies using ground or aqueous extracts of LP, none on their effects on the immune system cells, and only one published study on the effects of LP aqueous extracts on the immune responses in rats (Alsahafi and Mahassni, 2021). This study aimed to determine the non-toxic doses of the aqueous LP extract, by using the acute toxicity assay, and the suitability of using ground or water extract of LP in rats. Using the optimal LP form (either ground or water extract), we aimed to investigate the effects of LP on the immune system cell counts and red blood cell (RBC) counts. This may aid in determining the best method for human use for the treatment of different ailments and if, in fact, LP affects the counts of cells of the immune system and red blood cells.

2. Materials and methods

2.1. Collection of LP stems and preparation of the LP aqueous extract

Young LP stems were collected from the Khulais governorate, Makkah, Saudi Arabia during the third week of September 2020. The plants collected was verified by a taxonomist to be *leptadenia pyrotechnica* (Forssk.) Decne. The stems were cleaned thoroughly under running water and finally rinsed with distilled water.

The aqueous extract was prepared by boiling 500 g of young LP stems, that have been cut into small pieces, in 1 L of water for 5 minutes as per the previously described folk medicine method (Patel *et al.,* 2014). The hot water extract was filtered using cotton balls. The yield of the LP extraction was 0.476 ml extract/g LP (47.6%). Subsequently, the LP extract was allowed to air dry for two days resulting in a semisolid precipitate that is green to brown in color. The yield of the precipitate was 0.60 g LP precipitate/ml LP extract (60%). Finally, this precipitate was stored in an airtight bottle at 4ºC for about one week. Fresh batches were prepared weekly.

2.2. Preparation of ground LP diets

Fresh young LP stems were collected and allowed to air dry for one week. The dry LP stems and the pellets of the standard animal diet (Grain Silos and Flour Mills Organization, Jeddah, KSA) were separately ground into powder using a seed grinder. Three different concentrations of ground LP (25%, 50% and 75%) were mixed with the ground animal diet separately. Subsequently, these diets were manually formed into pellets, by the addition of a small amount of water, and they were allowed to air dry in the shade for three days.

2.3. Acute toxicity assay of LP extract

Acute toxicity was determined for the LP aqueous extract according to the Organization for Economic Co-operation and Development (2001) Guidelines for testing of chemicals: acute oral toxicity–acute toxic class method-guideline 423, Paris.

Healthy female adult Wistar albino rats that were used for the assay were allowed to acclimate in the laboratory for one week. After this adaption period, rats were weighed (weights ranged 190-210 g), and they were divided into four groups with three rats for each group. The rats were denied feed for a fasting period of 12 hours. Subsequently, the control group rats were orally administered with a 3 ml of water once. The three remaining groups [toxicity concentrations groups (TC)] were each administered with a single dose of dried LP extract, dissolved in 3 ml of water, at concentrations of 5, 20 and 40 g LP/kg body weight. The rats were monitored daily for 14 days for any signs of toxicity. The body weights for all rats were measured before and after the start of the test. In addition, the feed and water intakes were measured daily for each group.

2.4. Pilot study design and collection of blood samples

Twenty-eight healthy Wistar albino rats (with an equal number of female and male rats), at weights of about 170-250 g, were used for this study. Rats were obtained from the in-house animal facility of the King Abdulaziz University, Jeddah, Saudi Arabia. Rats were given free access to water and the regular laboratory animal feed (Grain Silos and Flour Mills Organization, Jeddah, Saudi Arabia), and they were maintained at room temperature and exposed to the natural light-dark cycle. After an adaption period of one week, eight rats were equally divided into four groups (LPG 1, LPG 2, LPG 3, and control), with one female and one male rat per group. The groups were administered ground LP mixed with the regular rat diet at three different concentrations (25%,

50%, 75% ground LP), while the control group was given the regular diet without any LP. Fresh food was given daily for an experimental period of one week.

For the LP aqueous extract groups (LPE 1, LPE 2, LPE 3, LPE 4, and control), 20 rats were equally divided into the five groups, with two females and two males per group. Each group of rats was gavaged with one of four different concentrations of the extract (3, 9, 15, and 20 g/kg, respectively), while the control rats were gavaged with 3 ml of water, daily for 2 weeks. On the 7th and 14th days, whole blood samples were withdrawn from the rats, under anesthesia with diethyl ether, from the retro orbital plexus using heparinized capillary tubes. Blood samples were collected in ethylenediamine tetraacetic acid vacutainer test tubes for the determination of the total and differential WBC and RBC counts.

2.5. Complete and differential blood counts

Total and differential WBC and RBC counts were done manually at King Fahd Center for Medical Research, Jeddah, Saudi Arabia. RBC and WBC were counted by using a hemocytometer under 40x magnification. Blood samples were diluted (1:20) with Turk's solution for the WBC counts and Hayem's solution (1:200) for the RBC counts. The differential WBC counts were counted under 100x magnification by using frosted slides and Rapi-diff II stain (Atom Scientific, Cheshire, Hyde, United States of America).

2.6. Statistical analysis

The MegaStat (Version 9.4, Butler University, Indianapolis, Indiana, United States of America) statistical program was used for the analysis of the data. The data were expressed as mean ± standard deviation (SD). The pairwise *t*-test was used for the significance testing between groups for all of the parameters. The statistical difference was considered significant for $P < 0.05$, highly significant for $P < 0.01$ and non- significant for $P \geq 0.05$.

3. Results

3.1. Acute toxicity assay of LP extract

There was no evidence of toxicity for the extract doses used, and thus the extract is considered safe up to a dose of 40 g/kg body weight. Body weight, water and feed intakes for the groups are show in Table 1. Compared to the control group, there were no significant differences in the mean final and initial body weights, and water and feed intakes for the acute toxicity groups. Additionally, there were no significant differences between the mean final and initial body weights for all groups.

Table 1: Initial and final body weights, water and feed intakes, and toxicity signs for all experimental groups.

Variables	Groups	n	Mean ± SD	*P*-value [a]	*P*-value [b]
Initial body weight (g)	Control	3	200.01 ± 12.58		0.069 (NS)
	TC 1	3	201.00 ± 11.94	0.846 (NS)	0.836 (NS)
	TC 2	3	204.45 ± 15.01	0.700 (NS)	0.880 (NS)
	TC 3	3	212.33 ± 7.64	0.265 (NS)	0.188 (NS)
Final body weight (g)	Control	3	204.00 ± 7.28		
	TC 1	3	203.21 ± 11.39	0.831 (NS)	
	TC 2	3	205.00 ± 15.01	0.887 (NS)	
	TC 3	3	213.53 ± 8.74	0.351 (NS)	
Water intake (ml)	Control	3	81.68 ± 8.88		
	TC 1	3	83.00 ± 6.43	0.544 (NS)	
	TC 2	3	81.65 ± 8.12	0.759 (NS)	
	TC 3	3	80.00 ± 6.43	0.647 (NS)	
Feed intake (g)	Control	3	62.48 ± 1.24		
	TC 1	3	61.35 ± 2.56	0.399 (NS)	
	TC 2	3	62.12 ± 1.33	0.247 (NS)	
	TC 3	3	60.92 ± 3.41	0.247 (NS)	

Pairwise *t*-test was used for the significance testing; a: between the groups and the control; b: between final and initial body weights for each group

TC: Toxicity concentration group; NS: Non-significant; SD: Standard deviation

3.2. Pilot study

3.2.1. Complete and differential blood counts for the ground LP groups

Table 2 shows the mean total WBC and RBC counts for the ground LP groups. It is apparent that there were no significant differences in the mean counts of WBC and RBC for each group compared with the respective control.

Results in Table 3 show the effects of ground LP on the differential counts for the groups compared with the control. The percent and absolute count of neutrophils was significantly higher for the LPG 3 group compared with the respective control. Lymphocytes percent for the LPG 3 group was significantly lower compared with the control. The percent and absolute count of monocytes were highly significantly higher for the LPG 3 group compared with the respective control. Eosinophils percent and absolute counts for all the experimental groups were highly significantly lower compared with the respective control. All other group comparisons were not significantly different compared to the respective controls.

Table 2: Mean total WBC and RBC counts for the ground LP groups.

Parameter	Group	n	Mean ± SD	*P*-value
WBC	Control	2	10.22 ± 1.09	
($\times 10^3$ cell/µl)	LPG 1	2	11.75 ± 3.04	0.696 (NS)
	LPG 2	2	12.07 ± 0.74	0.637 (NS)
	LPG 3	2	11.22 ± 6.47	0.796 (NS)
RBC	Control	2	9.61 ± 1.86	
($\times 10^6$ cell/µl)	LPG 1	2	0.69 ± 8.40	0.570 (NS)
	LPG 2	2	9.76 ± 0.31	0.651 (NS)
	LPG 3	2	9.50 ± 1.41	0.796 (NS)

Pairwise *t*-test was used for the significance testing between the groups and the control

WBC: White blood cells; RBC: Red blood cells; LPG: Ground LP group; NS: Non-significant; SD: Standard deviation

Table 3: Differential mean WBC counts and percents for the ground LP groups.

Parameter	Group	n	Mean ± SD	*P*-value
Neutrophils	Control	2	2.27 ± 0.20	
($\times 10^3$ cell/µl)	LPG 1	2	2.16 ± 0.50	0.905 (NS)
	LPG 2	2	2.55 ± 0.06	0.771 (NS)
	LPG 3	2	4.34 ± 2.60	0.049 (S)
Neutrophils	Control	2	23.50 ± 4.95	
(%)	LPG 1	2	24.50 ± 0.71	0.846 (NS)
	LPG 2	2	31.00 ± 2.83	0.195 (NS)
	LPG 3	2	38.50 ± 7.78	0.036 (S)
Lymphocytes	Control	2	6.92 ± 1.17	
($\times 10^3$ cell/µl)	LPG 1	2	6.25 ± 1.09	0.455 (NS)
	LPG 2	2	5.47 ± 0.46	0.120 (NS)
	LPG 3	2	5.66 ± 1.78	0.171 (NS)
Lymphocytes	Control	2	70.00 ± 5.77	
(%)	LPG 1	2	71.50 ± 2.89	0.729 (NS)
	LPG 2	2	66.00 ± 2.31	0.363 (NS)
	LPG 3	2	56.50 ± 9.81	0.007 (HS)
Monocytes	Control	2	0.24 ± 0.03	
($\times 10^3$ cell/µl)	LPG 1	2	0.33 ± 0.21	0.315 (NS)
	LPG 2	2	0.20 ± 0.03	0.649 (NS)
	LPG 3	2	0.52 ± 0.09	0.006 (HS)
Monocytes	Control	2	2.50 ± 0.58	
(%)	LPG 1	2	3.05 ± 1.73	0.294 (NS)
	LPG 2	2	2.50 ± 0.58	0.294 (NS)
	LPG 3	2	5.50 ± 1.73	0.006 (HS)
Eosinophils	Control	2	0.33 ± 0.13	
($\times 10^3$ cell/µl)	LPG 1	2	0.05 ± 0.05	0.000 (HS)
	LPG 2	2	0.04 ± 0.04	0.000 (HS)
	LPG 3	2	0.09 ± 0.07	0.002 (HS)
Eosinophils	Control	2	3.50 ± 1.73	
(%)	LPG 1	2	0.50 ± 0.58	0.001 (HS)
	LPG 2	2	0.50 ± 0.58	0.001 (HS)
	LPG 3	2	0.80 ± 0.50	0.002 (HS)
Basophils	Control	2	0.00 ± 0.00	
($\times 10^3$ cell/µl)	LPG 1	2	0.00 ± 0.00	1.000 (NS)
	LPG 2	2	0.00 ± 0.00	1.000 (NS)
	LPG 3	2	0.00 ± 0.00	1.000 (NS)
Basophils	Control	2	0.00 ± 0.00	
(%)	LPG 1	2	0.00 ± 0.00	1.000 (NS)
	LPG 2	2	0.00 ± 0.00	1.000 (NS)
	LPG 3	2	0.00 ± 0.00	1.000 (NS)

Pairwise *t*-test was used for the significance testing between the groups and the control

LPG: Ground LP group; S: Significant; HS: Highly significant; NS: Non-significant; SD: Standard deviation

3.2.2. Complete and differential blood counts for the LP extract groups

The mean total WBC and RBC counts for the LP groups after 7 and 14 days compared with the respective controls are shown in Table 4. After 7 days, no significant differences were found between the mean WBC counts for all experimental groups and the control. On the other hand, the mean RBC counts were highly significantly higher for the LPE 4 group compared with the control. For the mean counts after 14 days, the WBC counts showed no significant differences between the groups and the control. However, the mean RBC counts were significantly higher for the LPE 2 and LPE 3 groups and highly significantly higher for the LPE 4 group compared to the control.

Table 5 shows the mean differential counts for the LP extract groups after 7 and 14 days, compared with the control. It was found that after 7 days only the mean neutrophil and eosinophil counts and percents showed significant differences compared to the respective controls. The absolute mean counts for neutrophils, compared to the control, for the LPE 3 and LPE 4 groups were significantly higher. On the other hand, the mean percent of neutrophils was significantly higher for the LPE 4 group compared with the control. Mean eosinophil counts were significantly lower for the LPE 1, LPE 2 and LPE 3 groups compared with the control. The mean percent count for eosinophils was significantly lower for the LPE1 and LPE 2 groups compared to the control. All other group comparisons were not significantly different compared to the respective controls.

Results for effect of LP extract on differential counts for the groups after 14 day (Table 5) were fewer than after 7 days. The only significant differences were found for mean neutrophil counts and percents, and mean lymphocyte percents and only for the LPE 4 group compared to the control. The mean absolute count and percent of neutrophils for the LPE 4 group were highly significantly higher compared to the respective controls. The mean lymphocyte percent for the LPE 4 group was highly significantly lower compared to that of the control.

Table 4: Mean total WBC and RBC counts for the LP extract groups after 7 and 14 days.

			7th day		14th day	
Parameter	Group	n	Mean ± SD	*P*-value	Mean ± SD	*P*-value
WBC	Control	4	13.44 ± 1.07		15.85 ± 1.32	
($\times 10^3$ cell/µl)	LPE 1	4	15.48 ± 9.37	0.282 (NS)	16.26 ± 3.47	0.488 (NS)
	LPE 2	4	14.88 ± 3.89	0.442 (NS)	15.96 ± 3.35	0.575 (NS)
	LPE 3	4	10.15 ± 0.72	0.092 (NS)	15.10 ± 4.61	0.817 (NS)
	LPE 4	4	10.45 ± 2.96	0.123 (NS)	14.25 ± 3.65	0.595 (NS)
RBC	Control	4	8.06 ± 0.952		8.05 ± 1.04	
($\times 10^6$ cell/µl)	LPE 1	4	7.91 ± 0.593	0.892 (NS)	10.50 ± 1.39	0.050 (NS)
	LPE 2	4	9.80 ± 2.458	0.119 (NS)	11.11 ± 1.56	0.023 (S)
	LPE 3	4	9.36 ± 1.859	0.235 (NS)	10.95 ± 1.36	0.017 (S)
	LPE 4	4	11.03 ± 0.552	0.012 (HS)	11.88 ± 2.43	0.004 (HS)

Pairwise *t*-test was used for the significance testing between the groups and the control

WBC: White blood cells; RBC: Red blood cells; LPE: LP aqueous extract group; S: Significant; HS: Highly significant; NS: Non-significant; SD: Standard deviation

Table 5: Differential mean WBC counts for the LP extract groups after 7 and 14 days.

Parameter	Group	7th day			14th day	
		n	Mean ± SD	*P*-value	Mean ± SD	*P*-value
Neutrophils	Control	4	1.48 ± 0.38		1.42 ± 0.27	
(×10³ cell/µl)	LPE 1	4	1.55 ± 0.12	0.798 (NS)	1.29 ± 0.56	0.798 (NS)
	LPE 2	4	1.82 ± 0.45	0.158 (NS)	1.62 ± 0.17	0.407 (NS)
	LPE 3	4	2.57 ± 0.40	0.000 (HS)	2.06 ± 1.15	0.143 (NS)
	LPE 4	4	2.91 ± 0.08	0.000 (HS)	2.47 ± 0.70	0.021 (HS)
Neutrophils (%)	Control	4	19.70 ± 3.86		22.33 ± 2.31	
	LPE 1	4	24.00 ± 2.16	0.313 (NS)	25.50 ± 3.70	0.282 (NS)
	LPE 2	4	24.80 ± 6.95	0.238 (NS)	26.50 ± 4.04	0.163 (NS)
	LPE 3	4	26.00 ± 5.85	0.076 (NS)	27.00 ± 4.00	0.145 (NS)
	LPE 4	4	30.80 ± 9.88	0.016 (S)	34.33 ± 3.86	0.001 (HS)
Lymphocytes (×10³ cell/µl)	Control	4	5.40 ± 0.26		4.55 ± 0.26	
	LPE 1	4	4.84 ± 0.83	0.611 (NS)	4.60 ± 1.28	0.957 (NS)
	LPE 2	4	5.05 ± 1.48	0.754 (NS)	4.13 ± 1.30	0.661 (NS)
	LPE 3	4	6.95 ± 0.68	0.169 (NS)	4.85 ± 1.44	0.773 (NS)
	LPE 4	4	6.98 ± 2.85	0.162 (NS)	4.29 ± 0.99	0.781 (NS)
Lymphocytes (%)	Control	4	72.66 ± 2.06		72.00 ± 2.16	
	LPE 1	4	72.66 ± 0.96	1.000 (NS)	71.80 ± 5.65	0.936 (NS)
	LPE 2	4	71.25 ± 5.56	0.755 (NS)	68.50 ± 5.62	0.272 (NS)
	LPE 3	4	70.25 ± 6.13	0.604 (NS)	66.77 ± 3.69	0.108 (NS)
	LPE 4	4	64.70 ± 12.23	0.111 (NS)	59.80 ± 4.11	0.001 (HS)
Monocytes (×10³ cell/µl)	Control	4	0.44 ± 0.18		0.29 ± 0.14	
	LPE 1	4	0.20 ± 0.12	0.053 (NS)	0.26 ± 0.06	0.821 (NS)
	LPE 2	4	0.27 ± 0.06	0.150 (NS)	0.27 ± 0.15	0.840 (NS)
	LPE 3	4	0.30 ± 0.22	0.246 (NS)	0.40 ± 0.12	0.186 (NS)
	LPE4	4	0.34 ± 0.14	0.386 (NS)	0.35 ± 0.13	0.363 (NS)
Monocytes (%)	Control	4	6.00 ± 2.45		4.50 ± 1.73	
	LPE 1	4	3.00 ± 1.63	0.063 (NS)	4.50 ± 1.91	0.847 (NS)
	LPE 2	4	3.00 ± 0.00	0.063 (NS)	4.25 ± 2.63	0.847 (NS)
	LPE 3	4	3.00 ± 2.16	0.063 (NS)	5.00 ± 1.43	0.565 (NS)
	LPE 4	4	3.50 ± 3.00	0.115 (NS)	5.00 ± 1.43	0.565 (NS)
Eosinophils (×10³ cell/µl)	Control	4	0.10 ± 0.04		0.03 ± 0.02	
	LPE 1	4	0.02 ± 0.03	0.022 (S)	0.02 ± 0.04	0.873 (NS)
	LPE 2	4	0.02 ± 0.03	0.012 (S)	0.01 ± 0.03	0.657 (NS)
	LPE 3	4	0.03 ± 0.04	0.030 (S)	0.02 ± 0.03	0.812 (NS)
	LPE 4	4	0.05 ± 0.04	0.090 (NS)	0.03 ± 0.03	0.949 (NS)
Eosinophils (%)	Control	4	1.66 ± 0.58		0.66 ± 0.58	
	LPE 1	4	0.33 ± 0.58	0.040 (S)	0.50 ± 1.00	0.832 (NS)
	LPE 2	4	0.33 ± 0.50	0.022 (S)	0.50 ± 1.00	1.000 (NS)
	LPE 3	4	0.66 ± 1.15	0.108 (NS)	0.66 ± 1.15	0.832 (NS)
	LPE 4	4	0.66 ± 0.85	0.108 (NS)	1.00 ± 1.15	0.497 (NS)
Basophils (×10³ cell/µl)	Control	4	0.03 ± 0.06		0.03 ± 0.06	
	LPE 1	4	0.00 ± 0.00	0.134 (NS)	0.00 ± 0.00	0.269 (NS)
	LPE 2	4	0.00 ± 0.00	0.134 (NS)	0.05 ± 1.00	0.293 (NS)
	LPE 3	4	0.00 ± 0.00	0.134 (NS)	0.04 ± 0.90	1.000 (NS)
	LPE 4	4	0.00 ± 0.00	0.134 (NS)	0.00 ± 0.00	1.000 (NS)
Basophils (%)	Control	4	0.30 ± 0.50		0.30 ± 0.50	
	LPE 1	4	0.00 ± 0.00	0.134 (NS)	0.30 ± 0.50	1.000 (NS)
	LPE 2	4	0.00 ± 0.00	0.134 (NS)	0.30 ± 0.50	1.000 (NS)
	LPE 3	4	0.00 ± 0.00	0.134 (NS)	0.00 ± 0.00	0.375 (NS)
	LPE 4	4	0.00 ± 0.00	0.134 (NS)	0.00 ± 0.00	0.375 (NS)

Pairwise *t*-test was used for the significance testing between the groups and the control

LPE: LP aqueous extract group; S: Significant; HS: Highly significant; NS: Non-significant; SD: Standard deviation

4. Discussion

This research study is the first to determine the acute toxicity, RBC counts, and total and differential WBC counts in rats given ground or aqueous extract of LP. The ground and aqueous extract of LP were used since they are the most commonly used forms of LP in humans in folk and traditional medicinal systems, although there is no scientific evidence of their beneficial effects. In addition, they are the most easily accessible forms for self-healing and treatment at home. Additionally, the stem was the only part of the plant used for the study since only the stem is used in folk medicine and, more importantly, the stem contains more active ingredients than other parts of the plant. The acute toxicity assay and the pilot study were carried to determine whether the ground or aqueous extract of LP is more effective at inducing changes in the counts of the immune system cells in rats and to determine the appropriate concentrations to use.

There are no previous scientific studies on the effects of ground or aqueous extracts of LP on the counts of cells involved in the immune system in humans or laboratory animals. On the other hand, only one previous study used the aqueous extract of LP for immune response parameters, although the complete and differential blood counts were not determined (Alsahafi and Mahassni, 2021). In addition, there were only two studies (Watafua and Geidam, 2014; Rasheed *et al.,* 2016) that determined the acute toxicity of the alcoholic extracts of LP, while none used aqueous extracts. Therefore, we were unable to compare any of the current findings with those of other previous researchers.

In the acute toxicity study, it is recommended by the Organization for Economic Co-operation and Development Guidelines (2002) to test any substance in laboratory animals at concentrations of 0.0005 g/kg body weight and up to 2 g/kg body weight. In some circumstances, it is allowed to use concentrations of 2-5 g/kg body weight. Therefore, in this study the toxicity of the LP extract was tested starting at 5 g/kg body weight, which is the recommended upper limit for the doses, and up to 40 g/kg body weight, with no detectable toxicity. This was expected since LP is commonly eaten by humans in large amounts with no adverse reactions. In addition, the rat dose, based on the recommended dose used for humans in folk medicine (Patel *et al.,* 2014), is 1,86 g/kg. Thus, it was decided to use a little more than double this dose (3 g/kg body weight) for the lowest dose administered to the rats and to use 20 g/kg body weight as the highest dose, which is 11 times the rat dose.

In the acute toxicity assay, the mean initial and final body weights, and mean water and feed consumptions were not significantly different between all groups. Furthermore, there were no significant differences between the final and initial body weights for each group. Additionally, no signs of toxicity were observed in rats for all used extract concentrations. The signs of toxicity according to the Organization for Economic Co-operation and Development Guidelines (2002) that are commonly observed might be increased sleep, coma, salivation, mortality, convulsions, diarrhea, tremors, lacrimation, writhing reflex, and any changes in motor activity and patterns of behavior. These results are in agreement with the previous acute toxicity study in mice (Rasheed *et al.*, 2016) using different doses of the methanol LP extract, which found that doses up to 10 g LP/kg body weight were safe. A study (Watafua and Geidam, 2014) that is in disagreement with the present results showed slight toxic effects of the ethanolic LP extract in rats at 50, 100 and 150 mg/kg body weight.

For the pilot study, it was planned that both the ground and aqueous LP extract would be administered for two weeks, but it was observed within the first few days of the experiment that the rats that were given the ground LP were not consuming the expected amount of feed, and they were scattering it in the cage. In addition, their body weights were decreasing daily. As for the rats that were orally gavaged with the LP extract, they were consuming their regular diet normally and their weights were not decreasing. Therefore, it was decided not to continue the ground LP groups for the second week.

For the ground LP groups, the total mean WBC and RBC counts were not significantly different ($P \geq 0.05$) between the groups after one week of feeding. The mean neutrophil and monocyte counts and percents for the rats that were fed the highest percent of LP were significantly higher ($P < 0.05$ and $P < 0.01$) compared to the respective mean counts and percents for the control. The mean lymphocyte percent for the highest ground LP group, and the mean eosinophil counts and percents for all LP groups were significantly lower ($P < 0.01$) compared to the respective mean controls counts and percents.

As for the LP extract groups after 7 days, the mean total WBC counts were not significantly different ($P \geq 0.05$) between the groups, while the mean RBC counts were significantly higher ($P < 0.01$) for the LPE 4 group compared to the respective control. As for the differential blood counts, the mean neutrophil counts for the LPE 3 and 4 groups ($P < 0.01$) and mean neutrophil percents for the LPE 4 group ($P < 0.05$) were all significantly higher than the respective mean counts and percents for the respective controls. The mean eosinophil counts for the LPE 1, 2 and 3 groups ($P < 0.05$) and mean percents for the LPE 1 and 2 groups ($P < 0.05$) were significantly lower than the respective mean counts and percents for the respective controls.

As for the LP extract groups after 14 days, the mean total WBC counts were not significantly different ($P \geq 0.05$) between the groups, while the mean RBC counts for LPE 2 ($P < 0.05$), LPE 3 ($P < 0.05$) and LPE 4 groups ($P < 0.01$) were significantly higher compared to the means for the respective controls. For the LPE 4 group, the mean neutrophil count and percent ($P < 0.01$) were significantly higher and the mean lymphocyte percent ($P < 0.01$) was significantly lower compared to the respective mean counts and percents for the respective controls.

Therefore, it is apparent that most significant differences were found for the highest concentration of the ground and extract groups. In addition, there were some differences in the types of cells effected by ground LP and its extracts. When comparing the significant changes for the ground and extracts of LP, mean neutrophil and eosinophil counts and percents, and mean lymphocyte percents all showed the same significant differences. On the other hand, mean monocyte counts and percents behaved differently in the ground and extracts groups. In addition, the effects of the extracts were different after one

and two weeks. Thus, the duration of LP consumption is important since different periods lead to different effects.

The high mean neutrophil and monocyte counts for both the ground LP and its extract, compared to the controls, may be due to specific components in the two forms that may have enhancing effects on the immune system. Thus, LP might stimulate the bone marrow to generate and release more neutrophils and monocytes, specifically, into the blood (Ofem, Ani and Eno, 2012). On the other hand, LP may have inhibitory effects on the bone marrow, thus leading to the lower mean lymphocyte and eosinophil percents and counts.

On the other hand, compared to the control, the mean WBC counts were not significantly different at the end of the experimental periods for both ground and extracts of LP, although some of the individual white blood cells were significantly higher while others were significantly lower. Thus, the non-significance found for the WBC counts may be due to the canceling effects of higher and lower counts and percents of the different types of WBC.

The LP extracts may have some effects on the kidney, which might lead to the increased production of erythropoietin leading to increased production of RBC, as found in the current study. In addition, the tannins and flavonoids in PL are known to protect RBC from oxidative damage (Jorum *et al.,* 2016), thereby possibly leading to higher RBC counts. Therefore, as expected and as found in the current findings, the higher the dose of LP extract the higher the mean count of RBC.

Studies on other medicinal plants show similar results to the results of the current study. Ofem *et al.* (2012) used the aqueous extract of *Ocimum gratissimum* in rats for 28 days and found higher RBC and neutrophil counts and lower lymphocyte counts, in agreement with the current findings. A study (Jorum *et al.,* 2016) using varying doses of the methanolic extracts of *Carissa edulis* leaf administered to rats, for different time periods up to 21 days, found significant increases in the monocyte and neutrophil cell counts, in agreement with our findings. On the other hand, in contradiction to our findings of lower counts, they found (Jorum *et al.,* 2016) increased counts for lymphocytes and eosinophils. Finally, they also found increased WBC and basophil cell counts, while in the present findings there were no significant differences. The previous research study of Antai *et al*. (2009) on the ethanolic root extract of *Gonglonema latifolium*, given to rats for 14 days, resulted in a significant increase in the counts of monocytes in agreement with the current study. On the other hand, they found a significant increase in the WBC counts contradicting the no significant change in the present study. Finally, in contradiction to our findings, they found increased eosinophil counts and no significant differences for RBC counts, while we found lower eosinophil counts and higher RBC counts.

5. Conclusions

In conclusion, LP seems to be completely safe for consumption since high doses of the LP extract did not cause any toxic effects in rats. It is apparent that both the ground and extracts of LP modulate both the innate and acquired immune systems, while only the extract may be beneficial for increasing RBC counts. Furthermore, the extract led to different effects for the different durations of consumption. Thus, it may be assumed, pending further studies, that the same effects may be found in humans. It is recommended that further studies be done in rats using higher concentrations of the aqueous extract and investigating other parameters and effects on the major organs of the body.

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Conflicts of interest/Competing interests

Both authors have no conflicts of interest/competing interests.

Availability of data and material

Data is available upon request.

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