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Immunostimulating and Nutritional Effect of a Phytomedicament (lprd33) on Leukocyte Parameters in Sistar Strain Rats

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Abstract **Original Research Article**

The present study was carried out in the context of highlighting the nutritional and immunostimulant effect of a phytomedicine (LPRD33) on leukocyte parameters in Wistar strain rats. 16 rats aged twelve (12) weeks and weighing between 100-150g for males and females were used. These rats after being immunocompromised induced by cyclophosphamide (Endoxan, Bayër, France) were fed with increasing doses of (77, 154 and 308 mg/kg bw) of LPRD33. Effects of the different doses of LPRD33 were then evaluated. No visible signs or mortality were observed in the rats during the 14 days of experimentation based on the observation of clinical signs following the administration of the LPRD33 product. Results, however, showed that administration of LPRD33 at doses of 77, 154 and 308 mg/kg bw caused an increase in the number of disturbed white blood cells, lymphocytes, monocytes and neutrophils, which clearly shows the stimulating properties of LPRD33. Indeed, the 308 mg/kg bw dose of LPRD33 promoted a better rate of blood cell recovery. This study shows that LPRD33 contains bioactive substances (secondary metabolites) that can stimulate immune cells. Therefore, it could be used as a nutrient and immunostimulating agent for strengthening immune capacity and body well-being.

Keywords: LPRD33, Nutritional, traditional medicine,leukocyte parameters, immune system, immunostimulatory activity.

INTRODUCTION

Diet-derived immune system is a remarkably adaptive defense system that protects the body against pathogens as diverse as viruses, bacteria, fungi and parasites. It is composed of a multitude of cells and molecules making up a dynamic network capable of specifically recognizing and eliminating a large number of foreign microorganisms (1Bergereau, 2010). However, it happens that this system is unable to recognize and eliminate pathogens. This inefficiency leads to the weakening of the immune defense, hence the appearance of opportunistic diseases (1Bergereau, 2010). Immunosuppression is caused by several factors, most important of which are certain anticancer treatments, excessive use of medications such as corticosteroids and immunosuppressive diseases (2 Fofana, 2004). Of all these factors, infection caused by the human immunodeficiency virus (HIV) is by far the most worrying. It would be the occurrence of other so-called opportunistic infections that aggravate immunodepression especially in developing countries (3Kouassi, 1990; 4 Gentilini *et al.*, 1995; 5 Kra, 2001). Faced with these numerous complications due to taking these so-called modern drugs, humans have developed more ingenious strategies which consist of using natural products like nutrient to help the immune system. According to the World Health Organization, 80% of populations in developing countries use medicinal plants to combat several diseases and ensure their health care (6WHO, 2002, 7Elkhanne *et al.*, 2017; 8Gisèle, *et al.*, 2018; 9Ezeigwe *et al.*, 2020). Furthermore, studies conducted in several regions of Côte d'Ivoire have reported that more than 90% of the population uses traditional plants for their primary care (10 Manda *et al.*, 2017). Some of these studies have reported that herbal recipes may be responsible for strengthening the immune system (11Hariri *et al.*, 2011). Unfortunately, these recipes are used without scientific evaluation of their therapeutic properties. It is therefore important to scientifically validate traditional recipes in order to contribute better therapeutic management of the health of

populations well←being (12 Koffi, 2003). This is the case of the phytomedicine LPRD33, a product designed in Korhogo and marketed in several cities in Côte d'Ivoire. This traditional plants extract is said to be used in the treatment of various pathologies including strengthening the CD4 count and regenerating the immune system. However, despite the high use of this product, no study on its effectiveness and safety has ever been conducted. It is that context this study proposes to evaluate nutritional and immunostimulant effect of this product in rats made immunocompromised.

MATERIALS AND METHODS

Animal Material

Animal material used consists of rats of the species Rattus norvegicus of Wistar strains aged twelve (12) weeks and weighing between 100-150 g. They were supplied by the breeding farms of the Pasteur Institute of Côte d'Ivoire. Rats were housed in plastic cages with stainless steel covers. They were kept at a temperature of 25°C ± 2 and subjected to an alternation of 12 hours of light and 12 hours of darkness for 7 days. They also had free access to standard IVOGRAIN chow and water. Animal handling procedures were conducted according to good laboratory practices defined by (13 Ognika *et al.*, (2021).

Phytomédicament

Phytomedicine to be tested is obtained from several plants "LPRD 33". This traditional plants extract used in the treatment of various pathologies including the strengthening of the immune system was obtained from a naturopath practicing in the city of Korhogo.

Method of extraction of phytomedicine LPRD33

LPRD33 was supplied as a decoction by a traditional medicine practitioner who produces and markets it. Decoction comes in the form of a brown liquid contained in a 1 liter white plastic bottle. The decoction was filtered using a funnel containing filter paper. Solution obtained was also filtered using a funnel containing cotton. Filtrates obtained were dried in an oven at 44°C for 5 days to obtain dry extracts. The powders obtained were weighed and then allowed the determination of the concentration of the phytomedicine.

Induction of immunosuppression

For this study, induction of immunosuppression was made according to the method of 14Llanos *et al.*, (2011). 16 rats were divided into four (4) groups of four (4) rats, including one (1) control group and three (3) test groups. These 12-week-old rats were previously fasted for 24 hours before the induction of immunosuppression, and kept in an experimental room at 22 ± 2 °C and 70 ± 5% ambient humidity. Distribution of rats was homogeneous according to sex. After fasting, initial sample (day 1) was taken in order to obtain reference values for leukocyte parameters.

Subsequently, four (4) batches received a first dose by intraperitoneal injection at a concentration of 0.3 ml/100g bw of cyclophosphamide on the first day and the fourth day. animals were observed for seven (07) days while waiting for the onset of immunosuppression.

After seventh day of observation, the rats underwent a second blood sample, the samples were then sent to the laboratory for blood analyses. After analysis and comparison of the results with those of the reference values, it turned out that immunosuppression was completely established.

Determination of the different doses of phytomedicine "LPRD33"

Different doses of the treatment were determined using the dosage indicated on the recipe. This dosage from the practitioner is one glass of 120 mL capacity in two doses per day for an adult weighing 70 kg. Thus, to determine the concentration of the phytomedicine, 50 mL were dried in an oven. After drying, 1.134 g of the dry extract was used to determine the different doses of the treatment using the following expression: -Let's determine the daily therapeutic dose.
X: Refers to the daily therapeutic dose for a 70kg man.

50 ml ⟶ 1,134g
240 ml ⟶ X g

X=5.44g of the daily therapeutic dose of a 70 kg man, i.e. 5.44g/70kg of daily consumption.

- Let's define the different doses :

- Dose 1 (Low dose) = 5.44g/70 kg = 77 mg/kg
- Dose 2 (Medium dose) = 77×2 = 154 mg/kg
- Dose 3 (High dose) = 154×2 = 308 mg/kg

Daily therapeutic dose is therefore 77 mg/kg of body weight. For the study, the therapeutic dose of 77 mg/kg was chosen as the low dose.

Feeding rats with the phytomedicine LPRD33

All test groups were fed immediately after the onset of immunosuppression with the phytomedicine LPRD33.

- Lot1 (control batch): immunocompromised rats received only 1 ml of distilled water;
- Lot2 (test batch): immunocompromised rats received by gavage the dose of 77mg/kg bw of LPRD33;
- Lot3 (test batch): immunocompromised rats received by gavage the dose of 154mg/kg bw of LPRD33;
- Lot4 (test batch): immunocompromised rats received by gavage the dose of 308mg/kg bw of LPRD33.

After different doses of the LPRD33 extract administration, the influence of the different doses administered was assessed from the hematological data. Animals were observed in orderrecording of clinical signs during the 14 days of experimentation.

Blood sampling techniques

During the study, the caudal puncture technique was used during the immunosuppression induction tests and at the end of the experiment for the purpose of carrying out hematological tests.

Hematological tests

Quantitative evaluation of the leukocyte lineage, three blood samples were taken on day 0 (D0), day 7 (D7) and day 14 (D14). Blood collected in the EDTA tubes was used to perform the blood count. Thus, white blood cells, blood platelets and hematocrits were quantified using a URIT 3000R hematological machine. but in the context of this, only the leukocyte parameters were taken into account.

Test Principles

Cell counting principle of the URIT-3000 is based on impedance variation. Thus, when a quantity of 10 µL of whole blood is aspirated, the cells pass through a calibrated orifice. Direct current field is applied on either side of this orifice. Since the cell does not have the same conductivity as the medium surrounding it, its passage through the orifice causes a modification of the current established between the two electrodes. This current difference is recorded, which allows them to be counted each time the cell passes. Moreover, since the current difference is proportional to the volume of the particle, the latter is measured at the same time as the count. The volume is therefore a criterion for differentiating blood cell populations since platelets, red blood cells and leukocytes have distinct volumes most of the time.

In the white blood cell measurement chamber, the sample remains for 10 seconds and then the size and number of leukocytes are determined by impedancemetry.In the red blood cell measuring chamber, the size and number of erythrocytes and thrombocytes are also determined by the same method. From the measured values, the other parameters are calculated in the microprocessor of the machine. During the incubation time, erythrocytes are dissolved under the influence of lysis and hemoglobin is released and then transformed into methemoglobin. A part of the sample from this chamber is introduced into the hemoglobin flow cuvette. Hemoglobin concentration is measured spectrophotometrically (15 Kraus, 1980).

Statistical treatment

Statistical analysis of the values and graphical representation of the data were performed using Graph PadPrism 8 software (San Diego, California, USA). The statistical difference between the results was performed using analysis of variances (ANOVA), followed by the Tukey-Kramer multiple comparison test, with a significance threshold of $p < 0.05$ for the expression of the results. All values are presented as mean ± SEM (Standard Error of the Mean).

RESULTS AND DISCUSSION

Results

Induction of immunosuppression

Immunosuppression induction in rats the results of are reported in (Table I). These results indicate a significant decrease after induction of immunosuppression in rats.

In batch 1, the significant decrease in leukocytes observed on day 0 is 6.57 ± 0.81 103/mm3 against 16.05 ± 2.71 103/mm3 on day 7, lymphocytes is 4.30 ± 0.78, against 14.05 ± 2.46% on day 7, monocytes is 0.77 ± 0.12, against 1.18 ± 0.28% on day 7, and the number of neutrophils is 0.37 ± 0.06%, against 0.58 ± 0.31% on day 7.

In batch 2, the significant decrease in leukocytes observed on day 0 is 7.63 ± 1.01 103/mm3, compared to 15.43 ± 1.76 on day 7, lymphocytes is 5.57 ± 0.58%, compared to 10.15 ± 1.03% on day 7, monocytes is 0.63 ± 0.09%, compared to 3.1 ± 0.12% on day 7, and the number of neutrophils is 1.1 ± 0.20%, compared to 2.68 ± 0.33% on day 7.

In batch 3, the significant decrease in leukocytes observed on day 0 is 5.18 ± 0.79 103/mm3, compared to 13.25 ± 2.58 103/mm3 on day 7, lymphocytes is 3.58 ± 0.42%, compared to 9.83 ± 0.73% on day 7, monocytes is 0.58±0.22%, compared to 2.28±0.55% on day 7, and the number of neutrophils is 0.95 ± 0.22%, compared to 2.15 ± 0.81% on day 7.

In batch 4, the significant decrease in leukocytes observed on day 0 is 3.6 ± 0.27, compared to 14.35 ± 1.46 on day 7, lymphocytes is 2.68 ± 0.93%, compared to 10.63 ± 1.65% on day 7, monocytes is 0.48 ± 0.12%, compared to 2.05 ± 0.54 on day 7, and the number of neutrophils is 1.26 ± 0.19, compared to 1.68 ± 0.66% on day 7.

Table I: Evolution of leukocyte parameters after cyclophosphamide induction in rats

Batchs	Paramètres leucocytaires	0 (day)	7(day)
batch 1	Leucocytes (103/mm3)	16,05±2,71	6,57±0,81$^{\#\#}$
	Lymphocytes (%)	14,05±2,46	4,30±0,78$^{\#\#}$
	Monocytes (%)	1,18±0,28	0,77±0,12$^{\#\#}$
	Neutrophiles (%)	0,58±0,31	0,37±0,06
batch 2	Leucocytes (103/mm3)	15,43±1,76	7,63±1,01$^{\neq}$
	Lymphocytes (%)	10,15±1,03	5,57±0,58
	Monocytes (%)	3,1±0,12	0,63±0,09$^{\#\#}$

Batchs	Paramètres leucocytaires	0 (day)	7(day)
	Neutrophiles (%)	2,68±0,33	1,1±0,20
Batch 3	Leucocytes (103/mm3)	13,25±2,58	5,18±0,79$^{\neq}$
	Lymphocytes (%)	9,83±0,73	3,58±0,42$^{\neq}$
	Monocytes (%)	2,28±0,55	0,58±0,22$^{\neq}$
	Neutrophiles (%)	2,15±0,81	0,95±0,22$^{\neq}$
Batch 4	Leucocytes (103/mm3)	14,35±1,46	3,6±0,27$^{\neq}$
	Lymphocytes (%)	10,63±1,65	2,68±0,93$^{\neq}$
	Monocytes (%)	2,05±0,54	0,48±0,12$^{\neq}$
	Neutrophiles (%)	1,68±0,66	1,26±0,19

D0: Before immunosuppression; D7: After immunosuppression; ≠: Significant decrease (p<0.05), ≠≠: Highly significant decrease (p<0.01), ≠≠≠: Very highly significant decrease (p<0.001).

Effect of LPRD33 aqueous extract on leukocytes

Results in Table IV indicate the variation in leukocyte count during the experiment.

On day 7, that is, after induction of immunosuppression, a significant decrease (p<0.05) in the leukocyte rate was observed in rats from batches 1 to 4 with a reduction rate of batch 1 (-59.07%), batch 2 (-50.55%), batch 3 (-60.91%) and batch 4 (-74.91%).

At the end of treatment with LPRD33 (day 21), a highly significant increase (p<0.01) in the leukocyte count was observed in rats of batch 2 receiving the dose of 77 mg/kg bw. This variation was +58.19%, compared to +29.38% for the control.

Table 3: Evolution of body mass of rats during treatments

Poids (g)	0(day)	7(day)	21(day)
Batch 1 (témoin) Variation	86,25±21,08	73±21,07 **-15,36%^{a}**	75,75±21,43 **+3,78%^{a}**
Batch 2 (77 mg/kg p.c) Variation	85±16,10	73±15,47 **-14.12%^{a}**	94±14,75 **+28,77%^{a}**
Batch 3 (154 mg/kg p.c) Variation	81,25±11,01	69,75±10,40 **-14,15%^{a}**	99±12,73 **+41.94%^{a}**
Batch 4 (308 mg/kg p.c) Variation	**77**±0,63	65,25±10,50 **-15,26%^{a}**	99±8,35 **+51,72%^{a}**

D0: Initial weight; D7: After immunosuppression; D14: 1st week of treatment; D21: 2nd week of treatment; a: Rate of variation compared to day D14, *: Significant increase (p<0.05), **: Highly significant increase (p<0.01), ***: Very highly significant increase (p<0.001

Table 4: Effect of LPRD33 on leukocyte count

Leucocytes	0(day)	7(day)	21(day)
Batch 1(Témoin) **Variation**	16,05±2,71	6,57±0,81 **-59,07%$^{a\neq}$**	8,5±0,70 **+29,38%^{a}**
Batch 2 (77 mg/kg p.c) **Variation**	15,43±1,76	7,63±1,1 **-50,55%$^{a\neq}$**	12,07±2,08 **+58,19%^{a**}**
Batch 3 (154 mg/kg p.c) **Variation**	13,25±2,58	5,18±0,79 **-60,91%$^{a\neq}$**	11,43±0,81 **+120.66%^{a***}**
Batch 4 (308 mg/kg p.c) **Variation**	14,35±1,46	3,6±0,27 **-74,91%$^{a\neq}$**	9,83±1,46 **+173,06%^{a***}**

D0: Before immunosuppression; D7: After immunosuppression; D21: After treatment; a: Rate of change compared to day D14, *: Significant increase (p<0.05), **: Highly significant increase (p<0.01), ***: Very highly significant increase (p<0.001).

III-1-4-2-Effect of aqueous extract of LPRD33 on lymphocytes

Results shown in Table V indicate the changes in lymphocyte counts after induction of immunosuppression and after LPRD33 treatment.

On day 7, a highly significant decrease (p<0.01) in the lymphocyte count was observed in batch 1 with a reduction rate of -69.40%.

Similarly, a significant decrease (P<0.05) in the lymphocyte count was observed in batch 3 and 4 with a reduction rate of -63.58% and -74.79% respectively.

On the other hand, a non-significant decrease (P>0.05) in the lymphocyte rate was observed in batch 2 with a reduction rate of -45.12%.

After LPRD33 treatment, on 21 day, a very highly significant increase ($p<0.001$) in the variation of the lymphocyte rate was observed in all rats of the different batches of the experiment compared to the controls.

These variations at the level of lot 2 were +93.36%, against +55.81% for the control, of lot 3 were +145.51%, against +55.81% for the control and of lot 4 were +166.04%, against +55.81% for the control.

III-1-4-3-Effect of aqueous extract of LPRD33 on monocytes

Results shown in Table VI indicate a change in the lymphocyte rate of the groups of rats treated during the experiment.

After induction of immunosuppression in rats, a very highly significant decrease ($p<0.001$) in the variation of monocyte levels was observed in batches 1 and 2 compared to controls. This variation is -34.75% and -79.68% respectively for batches 1 and 2.

Furthermore, a significant decrease ($p<0.05$) was noted in batches 3 and 4, the reduction rate of which was -74.56% and -76.59% respectively for batches 3 and 4.

The effect of LPRD33 on monocyte levels 21 days revealed a very highly significant increase ($p<0.001$) in the variation of monocyte levels in all rats from different batches of the experiment compared to controls.

These variations at the level of lot 2 amount to +79.37%, against +27.27% for the control, of lot 3 amount to +94.83%, against +27.27% for the control and of lot 4 amount to +114.58%, against +27.27% for the control.

Table 5: Effect of LPRD33 on lymphocytes

Lymphocytes	0(day)	7(day)	J21(day)
Batch 1 (Témoin) **Variation**	14,05±2,46	4,30±0,78 **-69,40%$^{a\#\neq}$**	6,7±0,37 **+55,81%^{a}**
Batch 2 (77 mg/kg p.c) **Variation**	10,15±1,03	5,57±0,58 **-45,12%^{a}**	10,77±0,35 **+93,36%^{a***}**
Batch 3 (154 mg/kg p.c) **Variation**	9,83±0,73	3,58±0,42 **-63,58%$^{a\neq}$**	8,8±0,34 **+145,81%^{a***}**
Batch 4 (308 mg/kg p.c) **Variation**	10,63±1,65	2,68±0,93 **-74,79%$^{a\neq}$**	7,13±1,30 **+166,04%^{a***}**

J0 : Avant l'immunodépression ; J7 : Après l'immunodépression ; J21 : Après traitement ; a : Taux de variation par rapport au jour J14, * : Augmentation significative ($p<0,05$), ** : Augmentation hautement significative ($p<0,01$)**, *** :** Augmentation très hautement significative ($p<0,001$).

Table 6: Effect of LPRD33 on monocytes

Monocytes	0(day)	7(day)	21(day)
Batch 1 (Témoin) **Variation**	1,18±0,28	0,77±0,12 **-34,75%$^{a\#\#\#}$**	0,98±0,13 **+27,27%^{a}**
Batch 2 (77 mg/kg p.c) **Variation**	3,10±0,12	0,63±0,09 **-79,68%$^{a\#\#\#}$**	1,13±0,10 **+79,37%^{a***}**
Batch 3 (154mg/kg p.c) **Variation**	2,28±0,55	0,58±0,22 **-74,56%$^{a\neq}$**	1,13±0,16 **+94,83%^{a***}**
Batch 4 (308 mg/kg p.c) **Variation**	2,05±0,54	0,48±0,12 **-76,59%$^{a\neq}$**	1,03±0,19 **+114,58%^{a***}**

D0: Before immunosuppression; D7: After immunosuppression; D21: After treatment; a: Rate of change compared to day D14, *: Significant increase ($p<0.05$), **: Highly significant increase ($p<0.01$), ***: Very highly significant increase ($p<0.001$).

III-1-4-4-Effect of aqueous extract of LPRD33 on neutrophils

Table VII shows results of the variation of neutrophil counts during the experiment. Results of immunosuppression on 7 days, indicate a significant decrease ($p < 0.05$) in the variation of the neutrophil rate of batch 3, with a reduction rate of -55.81%. On the other hand, a decrease without significant variation ($P > 0.05$) of the neutrophil rate at the level of batches 1, 2 and 4 was noted. These variations were -36.31%, -58.96%, and -61.31%, respectively for batches 1, 2 and 4.

Effect of LPRD33 on the neutrophil count after analysis on 21 day showed a very highly significant increase ($p<0.001$) in the variation of the neutrophil count in all rats of the different batches of the experiment compared to the controls. These variations at the level of batch 2 amount to +66.36%, against +29.38% for the control, of batch 3 amount to +76.84%, against +29.38% for the control and of batch 4 amount to +93.85%, against +29.38% for the control.

Table 7: Effect of LPRD33 on neutrophils

Neutrophile	0(day)	7(day)	21(day)
Batch 1 (Témoin) **Variation**	0,58±0,31	0,37±0,06 **-36,21%^{a}**	0,53±0,12 **+43,24%^{a}**
Batch 2 (77 mg/kg p.c) **Variation**	2,68±0,33	1,1±0,20 **-58,96%^{a}**	1,83±0,43 **+66,36%^{a***}**
Batch 3 (154 mg/kg p.c) **Variation**	2,15±0,81	0,95±0,22 **-55,81%$^{a\neq}$**	1,68±0,43 **+76,84%^{a***}**
Batch 4 (308 mg/kg p.c) **Variation**	1,68±0,66	0,65±0,17 **-61,31%^{a}**	1,26±0,19 **+93,85%^{a***}**

D0: Before immunosuppression; D7: After immunosuppression; D21: After treatment; a: Rate of change compared to day D14, *: Significant increase ($p<0.05$), **: Highly significant increase ($p<0.01$), ***: Very highly significant increase ($p<0.001$).

DISCUSSION

Within the framework of promoting the well-being of the population through food using plants and phytomedicines, a pharmacological study was conducted on "LPRD33″. This improved traditional practisis used in the treatment of various diseases, more specifically for strengthening the immune system. Its effect on blood cells involved in the body's defense was the subject of this study. This study was conducted in two phases, namely, induction of immunodepression and evaluation of the effect of LPRD33 in immunodepressed rats. For the induction of immunosuppression in the present, cyclophosphamide was used which caused a very significant decrease in all leukocyte parameters in rats. This suggests that cyclophosphamide administered to rats caused immunosuppression, thus weakening the body's immunity and well¬bieng. According to 16 Moignet *et al.,* (2012), their decrease is an indicator of the failure of the immune system. These results are in agreement with those of Gbogbo *et al.,* (2022) who confirmed the immunosuppressive effect of this product during a study of the evaluation of the in vivo pathogenicity of Candida species isolated from palm wine and sorghum beer in a murine model in the wistar rat. Regarding the study of the effects of LPRD33, according to (13) Guideline 423 for the testing of chemicals, the lethal dose 50 (LD50) is greater than 5000 mg/kg bw. However, present study shows, doses of LPRD33 used are all less than 5000 mg/kg bw and are calculated according to the dosage proposed by the traditional medicine practitioner (two (02) tea glasses for a 70 kg adult). In view of this guideline, LPRD33 would be non-toxic. According to the results of the leukocyte formula, a significant increase from the 7th to the 21st day of experimentation (D7 to D21) was observed in rats treated in general. For the leukocyte rate, a highly significant increase at the dose of 77 mg/kg bw, and very highly significant at doses of 154 to 308 mg/kg bw was observed. At the level of lymphocytes, monocytes and neutrophils, a very highly significant increase at doses of 77, 154, and 308 mg/kg bw was also noted compared to controls. According to some authors, this high leukocyte rate indicates that the body is fighting against inflammation or a malignant infection (17Bajai *et al.,* (2005); 18 Mouhamed and Azab, (2014). Furthermore, the increase in their rate would be due to the stimulation of the immune system by LPRD33 which would promote the production of immune cells (19 Fahim *et al.,* 2012; 11 Hariri *et al.,* 2011). This suggests that LPRD33 contained substances capable of stimulating the production of white blood cells. These results are consistent with those of 20Diaby *et al.,* (2016) who showed that cadmium sulfate caused an increase in the level of white blood cells in rats. This increase was also revealed by 21Aichour *et al.,* (2017), these authors showed that the immunomodulatory activity of the methanolic extract of leaves and fruits of Capparis spinosa would have a mitigating effect on the bone marrow suppression induced by cyclophosphamide. Which translates into an increase in total white blood cells. Which translates into an increase in total white blood cells. This reveals the possible effect of LPRD33 to restore and strengthen the depleted immune system due to the injection of cyclophosphamide which is observation corroborates that of (22 Shah *et al.,* 2008). Regarding the number of lymphocytes, a significant modification was observed compared to the control. These results are similar to those of 7 Elkhanne *et al.,* (2017) with dietary supplement of zingiber officinalis rhizomes in mice. They corroborate those of 23 Salem. (2005) who showed that certain plants (nigella) have immunostimulatory properties of lymphocytes. This immunostimulation according to 24 Messaouden *et al.,* (2011) would be due to flavonoids which would lead to an increase in interleukins, which is why they are called lymphocyte stimulating factors.

In view of these observations LPRD33 could be qualified as a nutritional factor stimulating lymphocyte activity and could contain flavonoids. On the other hand, our results are in contrast to those of 25 Bidie *et al.,* (2016) and 10 Manda *et al.,* (2017) who worked respectively on the total aqueous extract of chrysophyllum perpulchrum and on the Nature remedy, the administration of which in wistar rats did not result in any significant modification of the above-mentioned parameters. This difference in results between several authors can be explained by the difference in components contained in each extract used, the extraction procedure and by the variation depending on the doses studied and the species used. Regarding the monocyte rate, after

treatment, a significant increase was revealed compared to controls. Unlike the study conducted by 26 Oubibete and Slimani. (2017) who showed that the aqueous extract of Artemisia herba-alba would lead to a significant decrease in the monocyte rates of treated patients compared to control subjects. However, the study conducted by 27 Guergour (2018) on female mice treated with a dose of total alkaloids from peganum harmala grains, showed a significant increase in monocytes. Results are in line with that of 27 Guergour (2018). In view of these results, LPRD33 could have alkaloid molecules that would be at the origin of the restoration of monocytes. As for the neutrophil rate, the observation of the results after 14 days of treatment with LPRD33 reveals a significant increase in the neutrophil rate unlike the controls. Our results are consistent with those of 28 Chabbi and Hadjadj, (2014) with the recipe (magic fattening), indicating a significant increase in the number of neutrophils in treated mice compared to controls. This increase can be explained by the fact that glucocorticoids inhibit the ability of neutrophils to adhere to endothelial cells following a reduction in the expression of L-selectin on neutrophils and P-selectin on endothelial cells (29 Krou *et al.*, 2011; 30Guilpain and Jeune, 2012). In the present study, it is important to note that treatment with different doses of LPRD33 to immunocompromised rats for 14 days revealed a significant turnover of leukocyte parameters. This may be explained by the fact that LPRD33 played its role as a stimulator of the immune system (31 Coffi *et al.*, 2014, 22 Shah *et al.*, 2008). Its richness in secondary metabolites strengthens the immune system by the overproduction of hematopoiesis regulatory elements such as CSF (Colonystimulating factor), EPO (Erythropoietin), TPO (Thrombopoietin) by macrophages and bone marrow stromal cells, thus providing a favorable local environment for hematopoiesis (32 Chang-Gue *et al.*, 2003; 33 Udut *et al.*, 2005; 34 Rong *et al.*, 2009). The progressive recovery of immunocompromised rats upon treatment with LPRD33 could be due to the effect of LPRD33, which would guarantee general immunity and well-being of the organism.

CONCLUSION

Present work aimed to evaluate the pharmacological effect of LPRD33 in rats, a product used to strengthen the immune system by monitoring the evolution of hematological parameters. From these investigations, it emerges that the effect of different doses of this extract in immunocompromised rats revealed that the extract contains bioactive substances ; flavonoids and alkaloids, capable of restoring and stimulating immune cells in particular leukocytes, lymphocytes, monocytes and neutrophils. Therefore, it could be used as an immunostimulant agent for strengthening the organism. The dose of 308 mg/kg showed the most beneficial effect, stimulating the overproduction of lymphocytes, leukocytes, monocytes and neutrophils, cells playing a very important role in the stimulation of immune cells. Our results are encouraging, as our doses are lower but more effective compared to studies already done. Nevertheless, in-depth and complementary studies will be necessary to prove the immunostimulant activity of this extract and their total immunity.

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