EAS Journal of Nutrition and Food Sciences
Abbreviated Key Title:EAS J Nutr Food Sci
ISSN: 2663-1873 (Print) & ISSN: 2663-7308 (Online)
Published By East African Scholars Publisher, Kenya
OPEN ACCESS
Volume-5 | Issue-4 | Jul-Aug; 2023 | DOI:10.36349/easjnfs.2023.v05i04.002

Original Research Article

Leukocyte Parameters and Immune Proteins Evolution during Prevention of Immune Insufficiency with an Extract of *Moringa oleifera* in Wistar Rats

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Article History
Received: 06.06.2023
Accepted: 17.07.2023
Published: 30.07.2023

Journal homepage:
https://www.easpublisher.com

Quick Response Code

Abstract: Immunosuppression is a particular pathology reflecting a weakening of immune system and constituting a factor of exposure to other diseases. It is therefore both a disease and a risk factor. The aim of this work was to prevent a possible immune deficiency by using *Moringa oleifera leaf* extract. Forty eigth albino rats including 24 males and 24 females divided into 12 group weighing between 107g and 140g were used. Different doses of the leaves aqueous extract of *Moringa oleifera* respectively 200 mg/kg bw, 400 mg/kg bw, 800 mg/kg bw and 1600 mg/kg bw were compared with controls subjected to distilled water and 50 mg/kg bw of Levamisole for 14 days. At the end of this 14-day treatment, immunosuppression was achieved by intraperitoneal administration of 5 mg/kg bw of dexamethasone in all rats divided into groups for three days twice a day. Then a two-week observation was made to assess changes of the different immune cells after administration of dexamethasone. During the experiment, five samples were taken on day 1, day 14, day 17, day 21 and day 28. These different samples allowed the determination of hematological and immune proteins parameters. The obtained results showed an improvement in the immune status on day 14 with a highly significant increase (p<0,001) in white blood cells, a non-significant increase in neutrophils, lymphocytes, monocytes and immature granulocytes unlike the control subjected to distilled water. The administration of dexamethasone on day 17, total leukocytes in rats of group V and VI decreased in significantly. Contrarily controls and doses of 200 and 400 mg/kg bw. From day 21 to day 28, the levels of the various immune cells increased significantly. In conclusion, 800 mg/kg bw and 1600 mg/kg bw of aqueous extract have a good profile to prevent possible immune deficiency.

Keyword: *Moringa oleifera,* Immunodeficiency, Leukocyte parameters, Immune proteins, Dexamethasone, Levamisole.

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1. INTRODUCTION

Immune system is a system of biological structures and processes that protects against diseases. Thus, it can also be considered as a network of cells, tissues and organs that work in synergy to protect the body against infections (Obi *et al.,* 2018). The immune system comprises two lines of defense namely innate immunity and adaptive immunity. Natural killer (NK) cells, complement systems, macrophages, antigen-presenting cells and neutrophils are part of the innate immune system (Renda *et al.,* 2022). The innate or natural immune system is the first line of defense against infections (Thomas and Audrain 2019). The adaptive immune system is made up of T lymphocytes and B lymphocytes. The adaptive response is initiated by the ingestion of a pathogen by an immature dendritic cell in the infected tissue. The immature dendritic cell plays a sentinel role in the immune system (Thomas and

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Audrain 2019). Currently, all over the world, there is an increase in diseases especially infectious diseases that require effective defense mechanisms of the body to control them through the process of immunomodulation (Nfambi *et al.,* 2015).

The upsurge in immuno-suppression has been a real public health problem worldwide for several decades. This immuno-suppression is caused by several factors, the most important of which are immuno-suppressive drugs, excessive use of drugs such as corticosteroids (Kambou *et al.,* 2015), immuno-suppressive diseases, malnutrition, deficiencies, cancer, particularly metastaticor immune cell infections with viruses such as human immunodeficiency virus (HIV) or human T-lymphotropic virus (Kambou *et al.,* 2015). Also, the immune response decrease at the two extreme ages due to prematurity and decline of body organ functions respectively. Drug treatment of immunity-related disorders requires the use of immuno-modulatory drugs (immunostimulants or immuno-suppressants depending on the nature of the disease). These immuno-modulators have their disadvantages; some are toxic, others are expensive and therefore out of reach of the average citizen. Cheaper and safer alternatives must therefore be found (Peter *et al.,* 2020). Over the past decades, the study of medicinal plants and their traditional use in differents parts of Africa, particularly in Côte d'Ivoire, have aroused growing interest. Today, about 3.3 billion people in emerging countries depend on traditional medicine, which consists mainly of medicinal plants. These plants have numerous pharmacological and nutritional applications that can help prevent life-threatening diseases. Among medicinal plants, *Moringa oleifera* commonly referred to as "Moringa" or drum stick tree in many parts of the world has been considered an alternative remedy (Mehwish *et al.,* 2020). *Moringa oleifera,* also called "miracle tree" or "tree of life" (Fuglie, 2002) is a tree originating from India. Drought-tolerant (Bosch, 2017), *Moringa oleifera* is a plant widely available in tropical and subtropical countries with great economic importance (Foidl *et al.,* 2001). This is a plant with very high nutritional values. *Moringa oleifera* is described as a natural anthelmintic, mildantibiotic, detoxifier, exceptional immune builder. It is used in many countries to treat malnutrition and malaria. According to Dhakad *et al.,* (2019) *Moringa oleifera* is the cheapest and most credible alternative to provide good nutrition, cure and prevent several disorders. However, there is very little information available on the preventive use of *Moringa oleifera* leaves as an immustimulant.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Plant Material

The plant material consisted of the leaves of *Moringa oleifera* which was harvested in the outskirts of the city of Bouaké in the center of Côte d'Ivoire, 342.3 km from the city of Abidjan. This study was carried out in the Physiology, Pharmacology and Pharmacopoeia laboratory of the natural sciences training and research unit.

2.1.2. Animal Material

Rattus norvegicus rats of the wistar strain weighing between 107 and 140 g, of both sexes, wereu sed for the experiment. These rats were raised in the animal facility of the Physiology, Pharmacology and Pharmacopoeia laboratory of Nangui Abrogoua University.

2.2. Material

2.2.1. Aqueous Extract Preparation

Powdered dried *Moringa oleifera* leaves were used. For the preparation of the aqueous extract, 150 g of *Moringa oleifera* leaf powder were macerate in 500 ml of distilled water for 24 hours on a magnetic stirrer. The maceration obtained was filtered and brought to an oven at 45°C for 72 hours.

2.2.2. Experimental Procedure

The female and male rats were separated and weighed in order to form homogeneous group. Twelve group of four rats including twentyfour females and twentyfour males were made up as follows: group III (200), IV (400), V (800) and VI (1600) made up of eight rats including four males and four females respectively, received 200 mg/kg bw, 400 mg/kg bw, 800 mg/kg bw and 1600 mg/kg bw. Then, another group II (LEV) of rats consisting of eight rats including four males and four females having received 50 mg/kg bw of Levamisole hydrochloride and finally a group I (ED) of eight rats received distilled water. The rats were all fed exclusively with IVOGRAIN brand granules. The adaptation period lasted five days. After these five days, the rats were treated for a period of 14 days with the aqueous extract and the reference molecule. The immuno-suppressive period during which we induced immuno-suppression by administering dexamethasone intraperitoneally at a dose of 5mg/kg of body weight twice a day for three days according to the protocol of Sulaiman *et al.,* (2010). The observation period lasted two weeks.

2.2.3. Samples Collection and Blood Parameters Assays

Five series of samples were taken from the rats fasted the day before during this study. On days 1, 14, 17, 21 and 28. Venous blood samples were taken from the retroorbital sinus of the eye using a sterile Pasteur pipette. According to the method described by Waynforth (1980), each animal was previously anaesthetized under a bell containing cotton soaked in ether for two to three minutes. The animal, removed from this bell, was held in a lateral position with one hand and held by the skin of the neck, between the thumb and the index finger. After disinfecting the eyelid with 70°C alcohol, the end of the pipette was

introduced into the lateral angle of the eye, with a slight rotation untilreaching the venous plexus. Thus, 0.5 to 2 ml of collected blood were immediately collected in tubes containing the anticoagulant ethylene diamine tetra acetic acid (EDTA) for hematological analyzes and in dry tubes for biochemical analyses. The samples were sent to the Pasteur Institute of Cocody (Côte d'Ivoire) for hematological analysis.The samples contained in dry tubes were used to assay the biochemical parameters in the Physiology, Pharmacology and Pharmacopoeia laboratory.

2.2.4. Statistical Analyzes

Statistical analysis of data values was performed with Graph Padprism Version 8.4.3 software (San Diego, California, USA). The results were expressed as the mean followed by the standard error of the mean (M±SEM). The comparison of the means between the control rats and the treated rats was carried out with ANOVA and the likelihood test was carried out with the BONFERRONI test. The various observed proportions of the leukocyte and biochemical parameters were compared by the significance threshold was set at $p < 0.05$.

3. RESULTS

3.1. Homogeneity if Groups

Different groups were made up and harmonized so as to give homogeneous groups. Table I summarizes different groups studied. No significant difference was observed a part from the blood platelets at the start of experiment.

3.2. Effect of Treatment on Blood Parameters before Immunosuppression

The effect of *Moringa oleifera* leaf extract on cellular immunity was evaluated after a 14-day treatment. Values for each group are given as the mean ± standard error of the mean (SEM). The p values are a within-group and group-to- group comparison between periods 1 and 14. Group I received only distilled water, group II received 50 mg/kg bw of Levamisole. Group III, IV, V and VI received 200 mg/kg bw respectively; 400 mg/kg bw; 800 mg/kg bw and 1600 mg/kg bw. Analysis of leukocyte parameters indicates an increase in white blood cells in all group compared to the control who received distilled water. These values initially ranged between 9.29×10^3 cells/µL and 13.4×10^3 cells/µL, settled after treatment in a range of 12.6×10^3 cells/µL to 20.3×10^3 cells/µL. At the end of these treatments, the total white blood cells increased respectively by 3.33; 2.3 and 2.6 compared to those of group I. Polymorpho nuclear neutrophils also experienced a non-significant increase in certain group treated compared to subjects of group I negative control who experienced a non-significant decrease. The results are recorded in Table I.

On the fourteenth day of the study, polymorpho nuclear basophils experienced a decrease in group I ($p<0.001$), group III ($p=0.014$) and group IV ($p<0.001$) unlike group II and group Vin which a non-significant increase was recorded. As for polymorpho nuclear eosinophils, there was a non-significant increase only at the level of group III and group V. With regard to lymphocytes, there is a non-significant increase in all of the group with the exception of group III which experienced a non-significant decrease. On day 14, we recorded an increase in all group and this increase was highly significant in group IV and VI. At the end of the 14-day treatment, in all group, the rate of platelets experienced a non-significant drop, unlike the rate of immature granulocytes, which experienced a highly significant increase in the subjects of group II and III. This increase was observed at the level of groups V and VI.

Table I: Uniform distribution of groups and administration effect of plant extract on initial leukocyte and thrombocytes parameters

Parameters	Périods	Group I ED	Group II LEV	Group III (200)	Group IV (400)	Group V (800)	Group VI (1600)	P values
White bloodcells (10^3/µL)	J1	10,70±0,92	12,00±0,77	11,20±0,73	9,29±0,75	13,40±0,72	11,60±0,79	P=0,052
	J14	13,70±1,05	16,50±0,23	12,60±0,32	19,30±0,31	20,30±0,38	19,40±0,62	P=0,006
	P values	P=0,099	P< 0,001	P=0,112	P< 0,001	P=0,004	P< 0,001	
Neutrophils (10^3/µL)	J1	2,00±0,58	2,76±0,01	2,40±0,29	3,00±0,41	2,98±0,25	2,67±0,37	P=0,285
	J14	1,26±0,72	3,68±0,58	2,91±0,74	4,38±0,69	4,13±0,26	3,58±0,45	P=0,151
	P values	P=0,467	P=0,187	P=0,558	P=0,163	P=0,032	P=0,191	
Basophils (10^3/µL)	J1	0,15±0,00	0,03±0,01	0,07±0,01	0,09±0,05	0,03±0,01	0,05±0,02	P=0,023
	J14	0,03±0,01	0,04±0,01	0,02±0,01	0,04±0,01	0,05±0,01	0,05±0,01	P=0,890
	P values	P<0,001	P=0,454	P=0,014	P<0,001	P=0,101	P< 0,999	
Eosinophils (10^3/µL)	J1	0,71±0,01	0,64±0,01	0,13±0,02	0,35±0,11	0,25±0,08	0,32±0,11	P=0,001
	J14	0,25±0,11	0,34±0,08	0,21±0,01	0,17±0,07	0,50±0,14	0,18±0,09	P=0,171
	P values	P=0,013	P=0,022	P=0,018	P=0,219	P=0,199	P=0,375	
Lymphocytes (10^3/µL)	J1	10,30±0,68	7,80±0,06	12,60±0,83	5,68±0,04	9,86±0,58	7,77±0,63	P=0,001
	J14	10,60±1,30	11,50±1,67	8,48±1,32	14,10±0,21	14,8±1,02	14,8±0,54	P=0,426
	P values	P=0,848	P=0,091	P=0,057	P< 0,001	P=0,014	P=0,001	
Monocytes (10^3/µL)	J1	0,83±0,01	0,80±0,05	0,25±0,03	0,16±0,04	0,24±0,02	0,10±0,01	P=0,001
	J14	1,59±0,53	0,86±0,26	1,00±0,04	5,63±0,12	0,81±0,14	0,83±0,01	P=0,001
	P values	P=0,223	P=0,832	P< 0,001	P< 0,001	P=0,015	P< 0,001	

Parameters	Périods	Group I ED	Group II LEV	Group III (200)	Group IV (400)	Group V (800)	Group VI (1600)	P values
Immature Granulocytes (10^3/µL)	J1	0,02±0,01	0,05±0,01	0,00±0,00	0,09±0,01	0,01±0,01	0,01±0,01	P=0,001
	J14	0,01±0,01	0,08±0,07	16,8±0,36	0,08±0,04	0,05±0,02	0,03±0,01	P=0,001
	P values	P=0,349	P< 0,001	P< 0,001	P=0,842	P=0,143	P=0,250	
Thrombocytes (10^3/µL)	J1	868±27,23	923±63,6	808±28,8	553±38,66	773±86,5	823±35,74	P=0,002
	J14	774±34,40	850±77,10	706±45,20	716±46,60	725±65,40	728±30,90	P=0,424
	P values	P=0,099	P=0,506	P=0,130	P=0,055	P=0,681	P=0,115	

3.3. Evolution of Leukocyte and Platelet Parameters after Immunosuppression

The administration of dexamethasone induced a reduction in white blood cells in all group but this reduction was significant in group I, II, III and IV having received respectively distilled water, Levamisole, 200mg/kg bw and 400 mg/kg bw of the aqueous extract. No significant difference was observed for group V and VI. The level of circulating neutrophils increased in most group with the exception of group IV which recorded a very significant decrease (p = 0.004). The number of polymorpho nuclear basophils decreased in all group subjected to different concentrations of the extract tested and this reduction was highly significant (p<0.001) in group IV. As for the control group, we noted an increase and this increase was highly significant at the level of the negative control group. At the same time, we recorded a drop in the rate of polymorpho nucleare osinophils. This decrease was very significant at the level of group III. The administration of dexamethasone induced a decrease in all groups studied in the concentrations of total lymphocytes and monocytes on day 17. As for immature granulocytes, a decrease was observed in the group that received no treatment as well as group III, IV and V. However, an increase was noted in the group that received Levamisole and in group VI that received the dose of 1600 mg/kg bw. Regarding blood platelets, a decrease was observed on day 17 in group I, II and V, unlike group III, IV and VI which experienced an increase.

Table II: Changes of immune cells after immunosuppression

Parameters	Périods	Group I ED	Group II LEV	Group III (200)	Group IV (400)	Group V (800)	Group VI (1600)	P values
White bloodcells (10^3/µL)	J14	13,70±1,05	16,50±0,23	12,60±0,32	19,30±0,31	20,30±0,38	19,40±0,62	P=0,006
	J17	3,95±0,46	12,00±0,01	10,40±0,01	9,12±0,40	18,30±4,07	17,20±2,11	P=0,001
	P values	P=0,001	P< 0,001	P=0,002	P< 0,001	P=0,656	P=0,002	
Neutrophils (10^3/µL)	J14	1,26±0,72	3,68±0,58	2,91±0,74	4,38±0,69	4,13±0,26	3,58±0,45	P=0,151
	J17	2,08±0,83	3,76±3,60	6,29±3,08	0,16±0,05	15,00±3,47	15,50±1,66	P=0,001
	P values	P=0,496	P=0,984	P=0,346	P=0,004	P=0,035	P=0,002	
Basophils (10^3/µL)	J14	0,03±0,01	0,04±0,01	0,02±0,01	0,04±0,01	0,05±0,01	0,05±0,01	P=0,890
	J17	0,10±0,01	0,10±0,01	0,01±0,01	0,005±0,01	0,02±0,01	0,01±0,01	P=0,380
	P values	P< 0,001	P=0,013	P=0,349	P=0,015	P=0,025	P=0,099	
Eosinophils (10^3/µL)	J14	0,25±0,11	0,34±0,08	0,21±0,01	0,17±0,07	0,50±0,14	0,18±0,09	P=0,171
	J17	0,01±0,00	0,005±0,01	0,06±0,02	0,00±0,00	0,11±0,04	0,01±0,01	P=0,007
	P values	P=0,088	P=0,015	P=0,003	P=0,059	P=0,059	P=0,124	
Lymphocytes (10^3/µL)	J14	10,60±1,30	11,50±1,67	8,48±1,32	14,10±0,21	14,80±1,02	14,8±0,54	P=0,426
	J17	1,38±0,34	1,15±0,21	3,05±0,62	3,33±0,57	2,70±0,65	1,44±0,58	P=0,026
	P values	P=0,002	P=0,004	P=0,020	P< 0,001	P< 0,001	P=0,001	
Monocytes (10^3/µL)	J14	1,59±0,53	0,86±0,26	1,00±0,04	5,63±0,12	0,81±0,14	0,83±0,01	P=0,001
	J17	0,48±0,31	0,28±0,19	0,98±0,25	0,57±0,18	0,47±0,14	0,23±0,08	P=0,202
	P values	P=0,144	P=0,143	P=0,940	P< 0,001	P=0,162	P=0,002	
Immature Granulocytes (10^3/µL)	J14	0,01±0,01	0,08±0,07	16,80±0,36	0,08±0,04	0,05±0,02	0,03±0,01	P=0,001
	J17	0,34±0,35	0,12±0,13	0,11±0,09	0,00±0,00	0,01±0,01	0,64±0,49	P=0,491
	P values	P=0,754	P=0,795	P< 0,001	P=0,157	P=0,225	P=0,285	
Thrombocytes (10^3/µL)	J14	774±34,40	850±77,10	706±45,20	716±46,60	725±65,40	728±30,90	P=0,424
	J17	539±66,50	363±44,50	939±77,60	850±84,00	585±85,60	785±93,10	P=0,002
	P values	P=0,035	P=0,005	P=0,060	P=0,235	P=0,264	P=0,592	

3.4. Evolution of Differents Immune Cells during Eventual Reversibility

3.4.1. Evolution of White Blood Cells

Figure 1.A shows the effect of dexamethasone on total leukocyte count after 14 days treatment. This rate was between 3.95×10^3 cells/µL and 18.30×10^3 cells/µL on day 17. After administration of dexamethasone, a decrease in the level of leukocytesis observed in group I having received only distilled water; group III, V and VI having received respectively 200 mg/kg, 800 mg/kg and 1600 mg/kg of the aqueous extract of *Moringa oleifera* leaves on day 21. On the other hand, an increase in the level of total leukocytes was observed at the level of group II and IV subjected to Levamisole and at a dose of 400 mg/kg bw respectively. Means varied to be on day 21 between

3.85×10^3 cells/µL and 16.50×10^3 cells/µL. On day 28, a gradual decrease is observed in all group and this progressive decrease was very significant in the rats of group VI. Day 28 means decreased to range from 2.13×10^3cells/µL to 13.50×10^3cells/µL.

3.4.2. Evolution of neutrophils.

The variations of the neutrophil averages are given by figure 1.B. The figure tells us that on day 17 the averages were between $0.16.10^3$cells/µL and $15.50.10^3$cells/µL. after a 14-day treatment with the aqueous extract of *Moringa oleifera*, dexamethasone induced a progressive decrease in neutrophil counts in group III and VI from day 21 to day 28. In this same period, an increase in this parameter was observed in group I, II and IV. This increase was highly significant ($p<0.001$) in group IV having received 400 mg/kg bw of the aqueous extract of *Moringa oleifera*. For group I, II and IV, a decrease was also observed on day 28.

3.4.3. Evolution of Eosinophils

Figure 1.C shows the evolution of the polymorpho nuclear eosinophil count from day 17 to day 21 and from day 28 after administration of dexamethasone in all the rats of the different group. With the exception of group III, a gradual increase in the level of polymorpho nuclear eosinophils is observed and this increase was highly significant ($p<0.001$) in the group subjected to Levamisole and very significant in the rats of group III and V having received the aqueous extract of Moringa leaves at a dose of 200 mg/kg bw and 800 mg/kg bw.

3.4.4. Evolution of Basophils

Figure 1.D shows change in the level of polymorpho nuclear basophils after induction of immunosuppression on day 17 in all the experimental group. On days 21 and 28, an increase in this level is observed in group II, III and IV compared to group I. This increase was highly significant ($p<0.001$) in the rats of group II and III and not significant at the level of group IV.

3.4.5. Change in Total Lymphocyte

Figure 1.E shows the evolution of the total lymphocyte count following the induction of immunosuppression in all group. On day 17, the total lymphocyte count in all group varied from 1.15×10^3cells/µL to 3.33×10^3cells/µL. On day 21, this rate was around $1.96.10^3$cells/µL to 6.70×10^3cells/µL. This rate rose on day 28 to 1.61×10^3cells/µL and 10.50×10^3cells/µL. The level of total lymphocytes increased significantly ($p<0.001$) at the level of group IV, V and V compared to the control group.

3.4.6. Evolution of Monocyte

Figure 1.F shows the variation in the level of monocytes after the administration of dexamethasone on the 17[th]day. In all the group treated with the different doses of the aqueous extract of the leaves of *Moringa oleifera*, an increase in the level of monocytes is observed compared to the controls. This rate was between 0.28×10^3cells/µL and 0.98×10^3cells/µL on the 17[th]day, the rate varied to reach levels of 0.19×10^3cells/µL to 1.73×10^3cells/µL on the 28th day.

3.4.7. Evolution of Immature Granulocytes

The presence of immature granulocytes or their granulocyte precursors reflects a state of myelemia. The presence or absence of immature granulocytes is a biomarker that indicates the reaction of the bone marrow to infection, sepsis, inflammation, stress, leukemia the response to stimuli. Immature granulocytes therefore means all the cells of the marrow composed of metamyelocytes, myelocytes and promyelocytes. During our experiment, a part from group II and group III which recorded a significant increase ($P<0.001$), no significant variation was observed on day 14 compared to the control group I ED. Induction of immunosuppression induced a decrease in immature granulocytes to the level of group III (200), group IV and group V (800) compared to control group I ED. This decrease was highly significant ($P < 0.001$) at the group III (200). During the observation phase, the rate of immature granulocytes experienced a gradual decrease to the level of group II LEV and group VI (1600) on day 28.

3.4.8. Evolution of Blood Platelets

Figure 1.H shows us the evolution of trombocytes level between day 17 and day 28 after administration of dexamethasone. We can also see a non-significant decrease in group I and III, on the other hand in group II and V, a significant increase was observed during this period. At the level group IV and VI there was a decrease on day 21 then this rate increased on day 28.

3.4.9. Evolution of Inflammatory Proteins

Tables III et IV show the course of inflammatory proteins in all test subjects. The plasma protein (α1-globulin, α2-globulin and β-globulin) allowed us to explore the inflammatory condition of subjects in different group. On day 14 there was a decrease in the rate of α1-globulin of all the treated group compared to the control which increased in a non- significant way (P=0,228) with exception of group V which have known a non-significant increase (P= 0,437). As for α2- globulin, on the 14th day there was an increase in group II LEV, IV (400), V(800) and VI (1600) compared to control group I ED. This increase has been highly significant ($P<0.001$) at group VI (1600). For β-globulin, we recorded an increase in all the treated abd control group. This increase was highly significant ($P<0.001$) in group I ED and V (800). On the 17th day of test, administration of dexamethasone caused an increase non-significant α1-globulin in LEV group II, III(200), IV(400) and VI(1600) compared to the control group I ED which have known non-significant decrease (P= 0,348). On the 28th day of

observation, this rate increased in group V(800) and VI(1600) in the same way as group I ED. Unlike in the group IV(400) there was a gradual decrease on the 28th day. As far as concerned α2-globulin level, we can see an increase in group IV(400) and V(800) compared to the control I ED group, on the other hand we noted a decrease at the group level II LEV, III (200) and VI(1600) which decreased significantly(P<0.001). The rate of β-globulin was increased in all group treated compared to the group I control ED on day 17.

However, this rate gradually during the observation at the level of group II LEV, IV(400), V(800) and VI(1600) unlike the group I ED on 28th day.

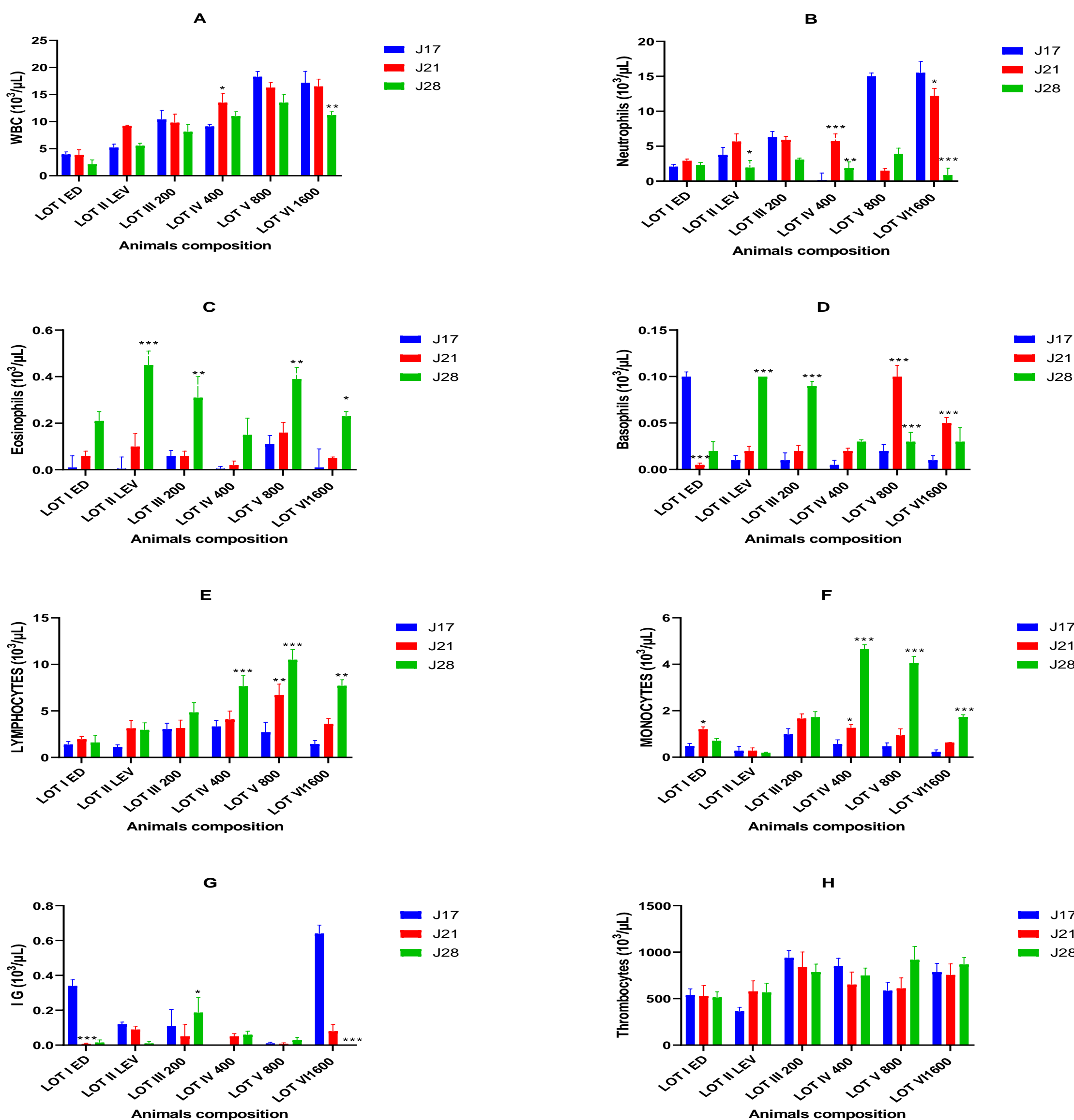

Figure I: White blood cell parameters and thrombocytes evolution during reversibility

Group I ED: group I distilled water. Group II LEV: group II Lévamisole. Group III 200: group III 200 mg/kg pc. Group IV 400: group IV 400 mg/kg pc. Group V 800: group 800 mg/kg pc. Group VI 1600: group VI 1600 mg/kg pc. PNN: neutrophils. PNB: basophils.PNE: éosinophils. G. I.: immatures granulocyts

3.4.10. Evolution of Immunity Proteins

Tables V and VI show the variation in the level of gammaglobulins during the test. The level of gammaglobulins indicates a state of humoral immunity of the rats of the different group studied. The results of our experiment indicate a highly significant increase (P<0.001) in the level of gammaglobulins in the subjects of group I, III and IV and a significant increase (P<0.05) in the subjects of group VI on day 14. On the other hand, this increase was not significant at the level of group II and V compared to day 1 in the same way as group I (ED) control. On day 17 after administration of dexamethasone, we recorded a decrease in this parameter in group I subjected to distilled water, unlike the different treated group where there was an increase in the level of gammaglobulins, which shows the

preventive power *Moringa oleifera* leaf extract. During the observation phase, the serum concentration of γ-globulins decreased from the 17th to the 21st day in all the treated group compared to the control group I ED, then this level increased in a non-significant way on the 28th day of experimentation.

Table III: Uniform distribution of immune proteins and inflammatory parameters

Parameters	Périods	Group I ED	GroupII LEV	Group III (200)	Group IV (400)	Group V (800)	Group VI (1600)	P values
α1-globulin (g/L)	J1	8,60±0,05	8,90±0,05	11,60±0,25	11,30±0,05	10,20±0,03	10,40±0,40	P<0,001
	J14	12,65±2,85	8,60±0,80	10,40±0,10	10,50±1,30	11,45±1,45	8,60±0,90	P=0,394
	P values	P=0,228	P=0,727	P=0,011	P=0,572	P=0,437	P=0,142	
α2-globulin (g/L)	J1	7,80±0,05	8,10±0,06	7,80±0,05	6,00±0,57	8,63±0,05	5,55±1,55	P=0,040
	J14	6,45±0,15	10,25±1,05	6,90±4,00	8,15±3,25	10,12±1,85	23,00±1,20	P=0,003
	P values	P=0,001	P=0,110	P=0,833	P=0,550	P=0,466	P<0.001	
β-globulin (g/L)	J1	10,50±0,05	11,90±0,05	10,50±0,05	11,00±0,57	10,16±0,04	8,15±0,05	P<0,001
	J14	18,30±0,20	13,45±2,15	15,05±1,65	15,45±3,35	17,54±0,51	8,80±0,30	P=0,030
	P values	P<0,001	P=0,416	P=0,051	P=0,261	P<0,001	P=0,099	
γ-globulin (g/L)	J1	0,50±0,06	0,50±0,05	0,001±0,001	0,10±0,01	0,51±0,01	0,55±0,45	P=0,209
	J14	7,80±0,80	4,70±1,60	4,50±0,001	4,95±0,05	3,98±1,30	2,60±0,40	P=0,036
	P values	P<0,001	P=0,059	P<0,001	P<0,001	P=0,056	P=0,027	

Table IV: Variation of inflammatory and immunity proteins from D14 to D17

Parameters	Périods	Group I ED	GroupII LEV	Group III (200)	Group IV (400)	Group V (800)	Group VI (1600)	P values
.α1-globuline (g/L)	J14	12,65±2,85	8,60±0,80	10,40±0,10	10,50±1,30	11,45±1,45	8,60±0,90	P=0,394
	J17	9,25±1,45	9,60±0,60	12,30±1,00	17,75±8,75	10,41±2,19	10,40±1,80	P=0,640
	P values	P=0,348	P=0,374	P=0,132	P=0,458	P=0,712	P=0,422	
.α2-globuline (g/L)	J14	6,45±0,15	10,25±1,05	6,90±4,00	8,15±3,25	10,12±1,85	23,00±1,20	P=0,003
	J17	5,95±1,45	5,50±1,40	6,55±1,15	15,95±10,55	19,57±2,55	7,10±1,40	P=0,195
	P values	P=0,749	P=0,053	P=0,937	P=0,519	P=0,040	P<0,001	
.β-globuline (g/L)	J14	18,30±0,20	13,45±2,15	15,05±1,65	15,45±3,35	17,54±0,51	8,80±0,30	P=0,030
	J17	12,00±5,00	20,70±6,00	17,20±3,10	22,35±4,15	24,27±3,54	20,70±7,70	P=0,623
	P values	P=0,276	P=0,319	P=0,573	P=0,265	P=0,133	P=0,197	
γ-globuline (g/L)	J14	7,80±0,80	4,70±1,60	4,50±0,001	4,95±0,05	3,98±1,30	2,60±0,40	P=0,036
	J17	5,05±0,85	11,40±2,20	10,70±0,40	18,80±7,70	18,95±2,30	6,15±0,65	P=0,053
	P values	P=0,078	P=0,069	P<0,001	P=0,146	P=0,005	P=0,010	

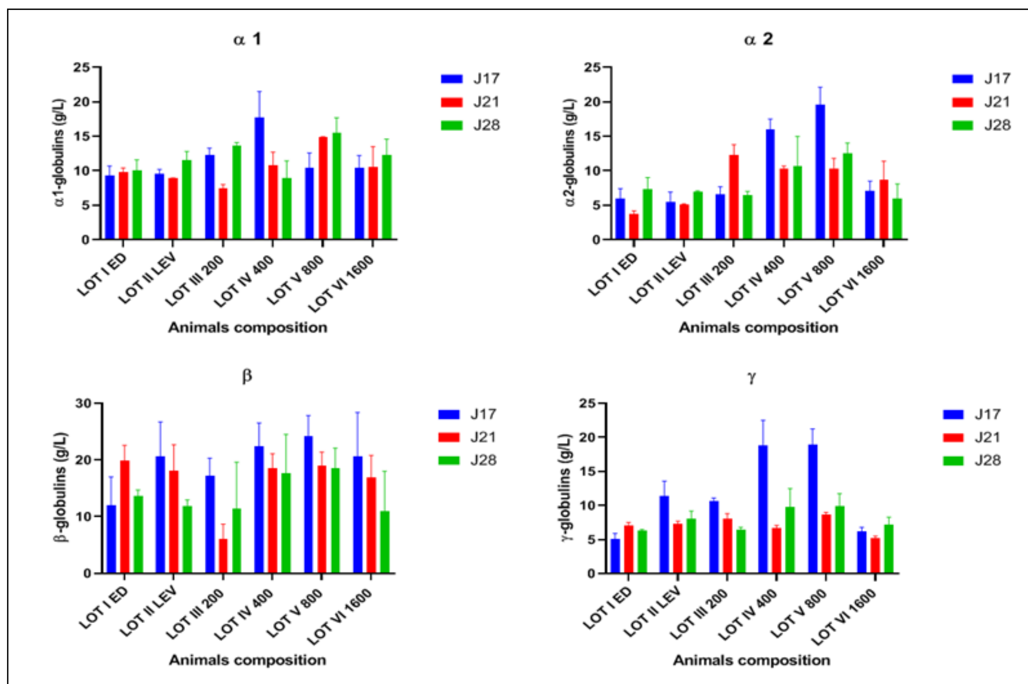

Figure II: Inflammatory and immune parameters evolution during reversibility

Group I ED: group I distilled water. Group II LEV: group II Lévamisole. Group III 200: group III 200 mg/kg pc. Group IV 400: group IV 400 mg/kg pc. Group V 800: group 800 mg/kg pc. Group VI 1600: group VI 1600 mg/kg pc.

4. DISCUSSION

4.1. Evolution of leukocyte and Platelet Parameters

The aim of this work was to evaluate the preventive immunostimulant potential of the aqueous extract of *Moringa oleifera* leaves in rats. This evaluation was carried out on cellular and humoral immunity in rats. The values of the leukocyte and platelet parameters show that the homogeneous population of rats usedis in good health, which is recommended in order to better appreciate the possible modifications during the experiments (Andreu, 2005). The results obtained indicate that the total aqueous extract significantly increases the level of total leukocytes, neutrophils, monocytes, total lymphocytes and immature granulocytes at the doses evaluated compared to the control. Our results are similar to those of Nfambi *et al.,* (2014) and Gupta *et al.,* (2010). These increases are believed to be due to protein amino acids, micronutrients such as iron, zinc, copper, calcium, manganese, magnesium, potassium, sodium, and B vitamins (thiamin, riboflavin, and niacin), E(α-tocopherol) and nicotinamide contained in the total aqueous extract of *Moringa oleifera* and highlighted by several authors (Fahey *et al.,* 2005; Aslam *et al.,* 2005; Anwar *et al.,* 2007). These elements, in particular vitamins B12, B6, C and E, folicacid and riboflavin, are essential for DNA synthesis (Nfambi *et al.,* 2014). Indeed, the iron, zinc, potassium and vitamins B and E contained in the leaves of *Moringa oleifera* are essential for the growth, differentiation and proliferation of immune system cells (Maggini *et al.,* 2007). Copper is involved in many physiological functions. Copper is also necessary for bone growth and strength as well as for the proper functioning of our immune functions (Underwood and Suttle, 1999). Also zinc is an essential trace element for cell growth and differentiation. It plays a major role in strengthening the immune system. The administration of Dexamethasone on day 17 leads to a highly significant decrease in white blood cells, lymphocytes, monocytes, polymorpho nuclear cells and also a decrease in the proportion of hematopoietic stem cells in control rats. Further more, the decrease in these parameters in the treated rats is not significant. This decrease is due to Dexamethasone. This is because Dexamethasone is a glucocorticoid that has many potentially serious adverse effects when used in high doses or over the long term. These results are similar to previous studies where the administration of Dexamethasone after 8 hours significantly reduced the number of white blood cells, lymphocytes, monocytes, platelets, neutrophils, eosinophils and basophils inrats (Yasuhiko *et al.,* 2010). Dexamethasone also depletes peripheral blood lymphocytes and affects immune responses (Wayne *et al.,* 2019). The slight decrease in leukocyte parameters observed in rats treated with the total aqueous extract of *Moringa oleifera* leaves at doses of 800 mg/kg bw and 1600 mg/kg bw, would be due to the preventive capacity of the plant thanks to the substances contained in different parts of the plant. A similar observation has been reported with the effect of the ethanolic extract of *Moringa oleifera* leaves in normal and immuno compromised mice. Pre-treatment with *Moringa oleifera* extract inhibited the bone marrow suppressing effect of cyclophosphamide on phagocyticactivity in mice (Gupta *et al.,* 2010). Another study also demonstrated that *Moringa oleifera* can attenuatemyelo suppression and leukopenia induced by any immunosuppressant in rats (Siddhuraju and Becker 2003). One week after the induction of immunosuppression, we recorded a significant change in white blood cells ($p<0.05$) and a slight increase in all leukocyte parameters of the treated rats compared to those on day 17. Indeed, the results of this study reveal that the use of the aqueous extract of *Moringa oleifera* allows the restoration of all the white blood cells in the treated subjects. This would mean that the aqueous extract of *Moringa oleifera* leaves would strengthen immunity and activate natural resistance mechanisms due to its protein, zinc and selenium content (Nikiéma *et al.,* 2009). It can thus serve as a fortifier and stimulant of the immune system against viral attacks, particularly in subjects living with HIV/AIDS (Shindano *et al.,* 2009; Tété-Bénissan *et al.,* 2012). Fromday 21 to day 28 in the rats of group VI (1600), we recorded a decrease.

4.2. Evolution of the Parameters of the Inflammatory State and Immunity

During inflammatory processes, the levels of α-globulin and β-globulin increase. The increase in the level of γ- globulin in the rats on the 14th day of treatment was less significant compared to the control. This increase could arguably suggest a gradual rise in antibodies as a function of age. These results are in agreement with those of Goullet and Kaufmann (1964) who carried out a quantitative study of the immune response according to age in rats of 3 months, 11 months and 22 months. These authors established that the concentration of antibodies was scalable according to their age. After induction of immunosuppression on the 17th day, all the grouptreatedexperienced an increase in the level of γ- globulin unlike the control, which makes it possible to establish that the aqueous extract inhibited the effect of dexamethasone. This inhibition capacity would be due to the presence of antioxidant chemical compounds. These results agree with those of Gupta *et al.,* (2010) who administered orally at doses of 125, 250 and 500 mg/kg per day for 15 days. The results demonstrated that the extractreduced cyclophosphamide-induced immunosuppression by stimulating both cellular and humoral immunity. What comforts us on the 28th day by

an increase in all the treated subjects unlike the control. Conclusion At the end of the experiment, all the parameters determined made it possible to evaluate the effect of the total aqueous extract of *Moringa oleifera* leaves on cellular and humoral immunity in rats. This study highlighted the preventive immunostimulant properties of *Moringa oleifera* leaves at doses of 800 mg/kg body weight and 1600 mg/kg body weight on haematological parameters. At the end of this work we can encourage the use of *Moringa oleifera* leaves in all people subject to an immune deficiency, in particular the elderly, people with HIV, kidney failure, people with cancer, etc. however it would be very important to determine the preventive mechanism of *Moringa oleifera* leaf extract for wider use.

CONCLUSION

At the end of the experiment, all the parameters determined made it possible to evaluate the effect of the total aqueous extract of *Moringa oleifera*l eaves on cellular and humoral immunity in rats. This study highlighted the preventive immunostimulant properties of *Moringa oleifera* leaves at doses of 800 mg/kg body weight and 1600 mg/kg body weight on haematological parameters. At the end of this work we can encourage the use of *Moringa oleifera* leaves in all people subject to an immune deficiency, in particular the elderly, people with HIV, kidney failure, people with cancer, etc. however it would be very important to determine the preventive mechanism of *Moringa oleifera* leaf extract for wider use.

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Cite This Article: Aboubacar Coulibaly, François Gnaté Monteomo, Désirée Oulai Tagninon, Mathieu Nahounou Bleyere (2023). Leukocyte Parameters and Immune Proteins Evolution during Prevention of Immune Insufficiency with an Extract of *Moringa oleifera* in Wistar Rats. *EAS J Nutr Food Sci, 5*(4), 92-101.