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Assessment of immunostimulatory activity of *Spirulina platensis* in rabbits (*Oryctolagus cuniculus*) in Côte d'Ivoire

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ABSTRACT

SPIRULINA is the powder of microscopic algae, *Spirulina platensis* (cyanobacteria) produced and consumed for centuries in the world and in Africa for its nutritional and pharmacological properties. Since scientific research has not been conducted on this cyanobacterium produced and consumed in Côte d'Ivoire, we decided to assess its immunostimulatory activity in rabbits (*Oryctolagus cuniculus*). To reach this purpose, SPIRULINA was administered intraperitoneally at doses of 100 mg / kg-bw, 250 mg / kg-bw and 700 mg / kg-bw to experimental groups and normal saline to the control group of rabbits. Haematological analysis, dosage of total serum proteins according to Biuret method and electrophoresis of these proteins by days D_0, D_7, D_{14} and D_{21} showed on the one hand an increase from day D_7 of leukocyte profile (white blood cells, lymphocytes, neutrophils), total proteins levels, alpha 1 globulin, alpha 2 globulin, beta globulin, gamma globulin and on the other hand, a decrease of serum albumin level. These variations of immune cells during experimentation demonstrated the immunostimulatory activity of SPIRULINA at all used doses. However, this activity is dose-dependent and more valuable at dose of 700 mg / kg-bw of SPIRULINA.

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INTRODUCTION

The upsurge of immunosuppression is a real public health issue in the world for several decades. This immunosuppression is caused by several factors and the most important are: immunosuppressive drugs, excessive use of medications such as corticosteroids, immuno-depressing disease, malnutrition, deficiencies, cancer, especially metastatic or infections of immune cells by viruses such as Human Immunodeficiency Virus (HIV) or Human T Lymphotropic Virus [1].

Among these factors, infection with HIV is by far the most worrying as it causes opportunistic infections that worsen immunosuppression especially in developing countries [2-4].

In Côte d'Ivoire, the nutritional status of vulnerable populations is sharply deteriorated during socio-political crisis. The most common form of these nutritional disorders remains chronic malnutrition with prevalence ranging from 30% to 40% in some regions of the country. As for the acute malnutrition, the situation is appalling with a prevalence of 7.1%, with regional disparities and an emergency rate of 10% [5].

UNICEF helps to prevent the worst effects of malnutrition by providing funds to countries. This international organization helps in distribution of essential micronutrients to strengthen the immune system, such as iron and vitamin A during vaccination campaigns or through fortified food [6]. All actions taken seem to be insufficient and people in developing countries continue to be immunocompromised. In this context, some insufficiently valued practices should be considered. Thus, *Spirulina platensis* (Oscillatoriaceae) commonly known as SPIRULINA or blue-green algae from cyanobacteria order is traditionally used for centuries and known for its nutritional and therapeutic properties [7-13].

The presence of gamma-linolenic acid in SPIRULINA is considered as a precursor of chemical mediators of the inflammatory and immune responses [13].

Spirulina platensis rich in lipid compound sulfoquinovosyl diacylglycerol (SQDG) (sulfolipids) demonstrated its ability to inhibit reverse transcriptase of HIV 1 and HIV 2 by *in vitro* experiment while the latter is naturally resistant to this class of molecules [14].

Furthermore, several studies have been conducted on this microalgae to demonstrate its many pharmacological properties [15-18].

To our knowledge, little research has been done on pharmacological effects of *Spirulina platensis* produced and consumed in Côte d'Ivoire [19].

This study aims to assess the change of some blood cells and serum proteins involved in immune function. It seems specifically necessary to conduct investigations in rabbits that consist in:

- Assessing the influence of different doses of SPIRULINA on leukocytes, lymphocytes and neutrophils;
- Determining the effect of different doses of SPIRULINA on the evolution of serum total protein;
- Studying the effect of SPIRULINA different doses on the variation of electrophoretic different fractions of serum total protein patterns;
- Indicating blood parameters which experienced possible changes during experimentation;
- Setting the dose of SPIRULINA which better improved rabbit immune system;
- Pointing out the treatment period, which improves the immune status of rabbits.

MATERIALS AND METHODS

Plant material

The plant material was made up of dry powder of *Spirulina platensis* provided by SAP (Société Agro-Piscicole) of Lamé in the Department of Adzopé (Côte d'Ivoire) where the production unit is settled.

In fact, SPIRULINA is produced in ponds covered with greenhouses. The harvest consists of filtering a part of the culture on a linen cloth (mesh diameter of 40 µm) after opening the valves connecting the ponds to the laboratory. The biomass obtained is drained, dewatered, pressed, weighed, extruded and then dried in a dehumidifier at 45°C. Once dried, SPIRULINA is ground using a grinder to obtain a powder. This powder is also used for manufacturing tablets and capsules.

Animal material and products administration

Young-adult rabbits (aged from 2 to 3 months) of both sexes, belonging to *Oryctolagus cuniculus* species [20] from leporidae family [21], with a mean weight of 1.65 ± 0.14 kg, were fed with pellets provided by Ivograin® with free access to tap water.

Animal did not receive any medication and were acclimatized for a week in the Faculty of Pharmaceutical and Biological Sciences pet room of Felix Houphouet Boigny University (Côte d'Ivoire).

For our study, four groups of rabbits were used, each consisting of three animals: a control group and three groups treated with different doses of *Spirulina platensis* powder, prepared with normal saline (0.9% NaCl) as solvent.

After acclimatization, the administration of products was performed by intraperitoneal route for three consecutive days using a 5 cc syringe and the injected volume was 1 ml [22].

The control group received normal saline while the other three groups received SPIRULINA at doses of 100 mg / kg-bw, 250 mg / kg-bw and 700 mg / kg-bw.

Experimental procedures and protocols used in this study were approved by the ethical committee of University Félix Houphouët Boigny. These guidelines were in accordance with the internationally accepted principles for laboratory use and care.

Blood Sampling and determination of haematological and biochemical parameters

Blood samples were performed each morning on fasting rabbits with 5 cc syringes at the marginal ear vein [23] in tubes containing anticoagulant (EDTA) on days D_0, D_7, D_{14} and D_{21} for haematological analysis and in dry tubes for electrophoresis and the determination of total serum proteins. Samples for electrophoresis and quantification of the total serum proteins were centrifuged at a speed of 3000 revolutions per minute for 5 minutes and the sera obtained were aliquoted to be stored at – 20°C in the freezer.

Haematological analyses were achieved using an automatic brand analyzer (Sysmex KX21-N) at the immuno-haematology laboratory of Cocody University Hospital. The numbers of white blood cells, lymphocytes and neutrophils for all groups of rabbits were considered.

The total protein determination was performed according to Biuret method [24] by an automatic biochemical analyzer (Lyasis); protein electrophoresis was performed on a band of cellulose acetate at central laboratory of Treichville University Hospital. This technique enabled the determination of albumin, alpha 1 globulin, alpha 2 globulin, beta globulin and gamma globulin.

Statistical analyses

Statistical analyses of data were performed using the software Graph Pad Prism 5.01 (San Diego California, USA).

The results were expressed as means ± standard deviation. Changes of parameters subjected to our study were observed by performing comparison tests of means by an analysis of variance (ANOVA ONE WAY), using a post hoc Tukey test. A probability level $P < 0.05$ was chosen for the significance of all analyses.

RESULTS

Variation of blood cells

Total white blood cells

The mean values of white blood cells during treatments are shown in Table 1. No significant difference ($P > 0.05$) between values of white blood cells in all groups of rabbits was observed by day D_0 (before administration of products). A very highly significant ($P < 0.0001$) increase of white blood cells number in rabbits of groups 3 and 4 respectively treated with SPIRULINA doses of 250 mg / kg bw and 700 mg / kg-bw was recorded by day D_7. Concerning group 2, treated with the dose of 100 mg / kg-bw, the increase of white blood cell number was not statistically significant ($P > 0.05$).

By day D_{14}, a highly significant ($P < 0.001$) increase of white blood cells number of group 2 and a very highly significant ($P < 0.0001$) increase of groups 3 and 4 was observed.

By day D_{21}, an increase of white blood cells number was obtained in all treated groups of rabbits with different doses of SPIRULINA, which differences with reference to day D_0 were statistically significant ($P < 0.05$), highly significant ($P < 0.001$) and very highly significant ($P < 0.0001$) for the groups 3, 2 and 4 respectively.

As for the control group (lot1) treated with normal saline, no statistically significant difference ($P > 0.05$) of white blood cells number were observed throughout our experimentation.

Total lymphocytes

The mean values of lymphocytes during treatments are shown in Table 2.

No statistically significant difference ($P > 0.05$) between the values of lymphocytes in all groups of rabbits was noted by day D_0 (before the administration of products).

Normal saline, doses of 100 mg / kg-bw and 250 mg / kg-bw of SPIRULINA did not induce any statistically significant differences ($P > 0.05$) in the number of lymphocytes of groups 1, 2 and 3 respectively during treatments. On the contrary, the dose of 700 mg / kg-bw caused a significant increase ($P < 0.05$) of lymphocytes number for group 4 by days D_{14} and D_{21}.

Changes of serum proteins

Total protein

The mean values of total serum proteins during treatments are summarized in Table 4.

No statistically significant difference ($P > 0.05$) between the values of total serum proteins in all groups of rabbits was observed by day D_0 (before the administration of products).

Total protein in the control group (group 1) and in group 2, treated with 100 mg / kg-bw of SPIRULINA experienced no statistically significant change during treatments.

By day D_7, total protein levels in groups treated by SPIRULINA doses of 250 mg / kg-bw and 700 mg / kg-bw resulted in significant increases ($P > 0.05$ and $P < 0.0001$, respectively) then ($P < 0.0001$ and $P < 0.001$, respectively) on day D_{14}.

By day D_{21}, increases of serum total protein are very highly significant ($P < 0.0001$) in treated groups with doses of 250 mg / kg-bw and 700 mg / kg-bw of SPIRULINA.

Albumin

The mean values of serum albumin during treatment are presented in Table 5.

No statistically significant difference ($P > 0.05$) between the values of serum albumin in all rabbits groups was obtained by day D_0 (before the administration products).

No statistically significant difference ($P > 0.05$) of serum albumin rate for group 2 treated with the dose of 100 mg / kg-bw of SPIRULINA was recorded during the experimentation.

By day D_7, any SPIRULINA dose induced statistically significant difference ($P > 0.05$) of serum albumin rates in all groups of rabbits.

By day D_{14}, only the SPIRULINA dose of 700 mg / kg-bw brought about a highly significant ($P < 0.001$) decrease of serum albumin.

Until the day D_{21}, decreases in serum albumin rates were significant ($P < 0.0001$, $P < 0.05$) in SPIRULINA treated groups with 700 mg / kg-bw and 250 mg / kg-bw, respectively.

Alpha 1 globulins

The mean values of alpha 1 globulins during treatments are shown in Table 6.

Apart from the SPIRULINA dose of 700 mg / kg-bw which induced a significant increase ($P < 0.05$) of alpha 1 globulin rate, other doses did not result in significant changes of the serum protein fraction during treatments.

Alpha 2 globulins

The mean values of alpha 2 globulins during treatments are shown in Table 7.

By days D_7 and D_{14} only the dose of 700 mg / kg-bw of SPIRULINA induced a significant increase ($P < 0.05$) of alpha 2 globulins rate in group 4.

By day D_{14}, the doses of 100 mg / kg-bw and 250 mg / kg-bw of SPIRULINA did not create significant changes ($P > 0.05$) of alpha 2 globulins rate in groups 2 and 3 respectively.

By day D_{21}, alpha 2 globulins rate in groups 3 and 4 respectively treated by doses of 250 mg / kg-bw and 700 mg / kg-bw of SPIRULINA increased very significantly ($P < 0.001$) and very highly ($P < 0.0001$), respectively.

As for the control group (group 1), treated with normal saline, no significant difference ($P > 0.05$) of alpha 2 globulins rate was observed during the experimentation.

Beta globulins

The mean values of beta globulins during treatments are reported in Table 8.

By day D_7, no significant difference ($P > 0.05$) of beta globulins rates for groups submitted to all doses of SPIRULINA was recorded.

By day D_{14}, SPIRULINA doses of 250 mg / kg-bw and 700 mg / kg-bw provoked a very significant increase ($P < 0.001$) of beta globulin rates for groups 3 and 4.

By day D_{21}, doses of 250 mg / kg-bw and 700 mg / kg-bw of SPIRULINA induced a significant ($P < 0.05$) and a very highly significant ($P < 0.0001$) increases of beta globulins rates for groups 3 and 4, respectively.

However, beta globulins rates experienced no significant differences ($P < 0.05$) in the control group (group 1) and in group 2 treated with normal saline and 100 mg / kg-bw of SPIRULINA, respectively.

Gamma globulins

The mean values of gamma globulins during treatments are shown in Table 9.

No significant change of gamma globulins rates of groups submitted to all doses of SPIRULINA was observed by day D_7.

By day D_{14}, SPIRULINA doses of 250 mg / kg-bw and 700 mg / kg-bw induced a highly significant ($P < 0.001$) of gamma globulins rates for groups 3 and 4.

By day D_{21}, SPIRULINA doses of 250 mg / kg-bw and 700 mg / kg-bw produced significant increases ($P < 0.05$, $P < 0.0001$) of gamma globulins rates for groups 3 and 4, respectively.

However, rates of these immunoglobulins experienced no significant change ($P > 0.05$) in the control group (group 1) and in group 2 treated with normal saline and 100 mg / kg-bw of SPIRULINA respectively.

DISCUSSION

The objective of this study is to evaluate the immunostimulatory activity of *Spirulina platensis* in rabbits.

The evaluation of *in vivo* immunostimulant activity enables us to confirm the effectiveness of our product by the study of changes in immune parameters during the experimentation.

In this study, we took into account variation of blood immune cells (white blood cells, polymorphonuclear neutrophils and lymphocytes) and serum immune parameters (total protein, albumin, alpha 1 globulins, alpha 2 globulins, beta globulins and gamma globulins).

Before administration of products in all groups of rabbits, the mean number of white blood cells, lymphocytes and neutrophils, despite being heterogeneous, are not significantly different within each parameter on the one hand and do not differ significantly from the values found by other authors on the other hand [25-30]. These values show that in a homogeneous population of rabbits, the number of white blood cells is not a fixed value but varies within a certain range compatible with the normal life of rabbits [25].

From the day D_7, our results indicated significant increases in immune haematological parameters (white blood cells, lymphocytes) in all the treated rabbits groups with different doses of SPIRULINA. For doses of 250 mg / kg-bw and especially that of 700 mg / kg-bw, those increases were greater by days D_7 and D_{14} from which they decreased gradually.

As for neutrophils, the increases were more valuable by day D_7 for doses of 250 mg / kg-bw and 700 mg / kg-bw and then gradually decreased.

According to these results, we can say in agreement with other authors [31-33] that SPIRULINA stimulates the immune system with the front line proliferation of white blood cells. This immunostimulation is dose-dependent. It activates macrophages and NK cells. It induces the production of antibodies, and also activates T and B cells [34]. It has immune modulating effects by increasing the production of cytokines (interleukin-4 (IL-4), interferon-γ (IFN-γ), interleukin-2 (IL-2) and natural killer (NK) [15].

Indeed, the presence of macromolecules with high molecular weight [35] contained in SPIRULINA especially proteins, polysaccharides and glycoproteins [36-41] stimulates immunity by inducing a significant rise of the rate of immune cells. The penetration of an antigen in the body causes a first response evidenced by the increase of mono-macrophage cells number in order to capture and eliminate the antigen [42]. The more the size and the number of antigens are important, the more they induce significant immunity.

The increase of lymphocyte number indicated by our results is due to the presence of the antigen which stimulated the transformation of B cells into plasma cells, antibody producing cells [42].

Before administering the products to all groups of rabbits, our results indicated mean rates of total protein, albumin, alpha 1 globulins, alpha 2 globulins, beta globulins and gamma globulins in accordance with those of Ouedraogo [29] and Fofana [22], but different from those of Abba *et al*.[43] which showed rates of serum total protein ranging from 39.83 ± 2.50 g / l to 41.77 ± 5.45 g / l and rates of gammaglobulins ranging from 1.70 ± 0.70 g / l to 2.22 ± 0.60 g / l.

Overall, during the experimentation, we observed a significant improvement of neutrophils, lymphocytes, total protein, alpha 1 globulins, alpha 2 globulins, beta globulins and gamma globulins profiles and a regression of albumin rate for all treated groups with different doses of SPIRULINA. Acquired immunity from day D_7 continued until day D_{21}, however, the best immune status extended until D_{14}.

The immune system was better enhanced with SPIRULINA dose of 700 mg / kg-bw which indicated better improvement of immune cells during treatments.

Changes of these serum immune parameters are dose-dependent and confirm the immunostimulant activity [23] of SPIRULINA in rabbits.

Indeed, an immunogenic molecule also causes an increase of serum total protein rate with its various fractions, except for the rate of albumin that decreases [22, 23, 29].

Antibody production by plasma cells from B cells accounted for the increase rate of total globulins. These observations can be explained on the one hand by the fact that albumin serves as a transport protein for many molecules including gamma globulins, which increase entails inevitably the decrease of albumin rate by deflection of amino acids to immunoglobulins synthesis [44,45] and on the other hand, the increase of gamma globulins rate, integral part of total globulins, causes an increase of the latter.

Our investigations concerning the immunostimulatory activity of *Spirulina platensis* corroborate the studies of Haney *et al.* [4-6] and Yapi *et al*. [47] which indicated that this

cyanobacterium has an immunostimulant effect respectively in tilapia (*Oreochromis niloticus*) and an effect on total protein concentration increase in burn patients.

Furthermore, the immunostimulatory effect of SPIRULINA is due to its composition in gamma-linolenic acid, vitamins and various minerals, phycocyanin [13] and sulfolipids [14].

Our research findings could explain the empirical use of SPIRULINA by humans.

CONCLUSION

Further to this study which aims to assess the immunostimulatory activity of *Spirulina platensis* in rabbit, we can say that this microalgae made and used in Côte d'Ivoire has actually immunostimulatory effects. These properties of this cyanobacteria were demonstrated during our experimentations by the stimulation of haematological immune cells and serum parameters.

These immunostimulatory effects of SPIRULINA would be beneficial to patients infected with HIV who know immunosuppression and nutritional imbalance.

In order to refine our research, further clinical studies on people living with HIV will be conducted and the mitogenic activity of immune cells will be assessed *in vitro*. It would also be necessary to attempt to potentiate the immunostimulatory activity of SPIRULINA by conducting chromatographic fractionation and by investigating on the physiological mechanism of immunostimulatory activity of SPIRULINA.

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COMPETING INTERESTS

The authors declare no conflict of interest.

Table 1: Evolution of white blood cells number (x10^3 / mm^3) during treatments

Groups (n=3)	Treatments	Days			
		D_0	D_7	D_{14}	D_{21}
Group 1	Normal saline	6.56 ± 0.73^a	7.23 ± 0.68^b	7.86 ± 0.70^b	6.53 ± 0.41^b
Group 2	D100	8.66 ± 1.19^a	11.23 ± 0.80^a	13.16 ± 1.69^a	12.83 ± 0.40^a
Group 3	D250	5.60 ± 1.37^a	11.83 ± 0.47^a	13.10 ± 0.56^a	10.60 ± 0.34^c
Group 4	D700	7.76 ± 0.98^a	13.66 ± 0.76^c	15.36 ±0.81^a	13.73 ± 1.10^a

Means followed by the same letters in the same column are not statistically different at the level of 5%.

Table 2: Evolution of lymphocytes number (x10^3/mm^3) during treatments

Groups (n=3)	Treatments	Days			
		D_0	D_7	D_{14}	D_{21}
Group 1	Normal saline	4.56 ± 1.26^a	5.40 ± 1.21^a	5.16 ± 1.19^a	4.10 ± 0.96^a
Group 2	D100	3.83 ± 1.56^a	5.73 ± 0.64^a	5.23 ± 0.45^a	5.13 ± 0.32^a
Group 3	D250	5.90 ± 1.97^a	7.50 ± 1.32^a	8.56 ± 2.04^b	8.43 ± 1.70^b
Group 4	D700	3.80 ± 0.20^a	8.16 ± 1.60^a	8.46 ± 1.87^b	9.10 ± 0.98^b

Means followed by the same letters in the same column are not statistically different at the level of 5%.

Table 3: Evolution of neutrophils number (x10^3/mm^3) during treatments

Groups (n=3)	Treatments	Days			
		D_0	D_7	D_{14}	D_{21}
Group 1	Normal saline	2.43 ± 0.40^a	2.76 ± 0.25^b	2.53 ± 0.25^b	2.60 ± 0.17^a
Group 2	D100	4.13 ± 0.70^a	8.80 ± 1.51^a	7.50 ± 1.32^{ab}	6.33 ± 0.92^{ab}
Group 3	D250	3.23 ± 0.63^a	7.47 ± 0.64^a	5.40 ± 0.79^a	4.26 ± 0.90^{bc}
Group 4	D700	5.60 ± 2.26^a	10.56 ± 1.72^a	8.23 ± 1.53^a	7.13 ± 0.85^c

Means followed by the same letters in the same column are not statistically different at the level of 5%.

Table 4: Change of serum total protein rate (g / l) during treatments

Group (n=3)	Treatments	Days			
		D_0	D_7	D_{14}	D_{21}
Group 1	Normal saline	64.33 ± 7.63^{a}	65.00 ± 6.55^{a}	58.00 ± 3.46^{c}	54.33 ± 2.08^{c}
Group 2	D100	63.66 ± 4.93^{a}	68.33 ± 4.93^{a}	67.66 ± 2.08^{a}	67.66 ± 3.05^{a}
Group 3	D250	55.66 ± 1.15^{a}	67.66 ± 3.78^{a}	71.00 ± 1.73^{ab}	72.00 ± 1.00^{ab}
Group 4	D700	54.00 ± 1.00^{a}	70.33 ± 1.52^{a}	74.66 ± 0.57^{b}	73.33 ± 1.52^{b}

Means followed by the same letters in the same column are not statistically different at the level of 5%.

Table 5: Change of serum albumin rate (g / l) during treatments

Groups (n=3)	Treatments	Days			
		D_0	D_7	D_{14}	D_{21}
Group 1	Normal saline	32.30 ± 5.30^{a}	29.83 ± 4.92^{a}	30.93 ± 4.87^{a}	29.50 ± 5.46^{a}
Group 2	D100	32.03 ± 3.62^{a}	31.46 ± 1.62^{a}	31.90 ± 3.72^{a}	25.83 ± 3.16^{a}
Group 3	D250	35.43 ± 0.51^{a}	26.93 ± 0.41^{a}	25.60 ± 0.26^{a}	24.36 ± 1.35^{a}
Group 4	D700	37.63 ± 0.90^{a}	28.83 ± 2.45^{a}	25.53 ± 1.49^{a}	21.86 ± 0.21^{a}

Means followed by the same letters in the same column are not statistically different at the level of 5%.

Table 6: Change of alpha 1 globulins rate (g / l) during treatments

Groups (n=3)	Treatments	Days			
		D_0	D_7	D_{14}	D_{21}
Group 1	Normal saline	0.83 ± 0.30^{a}	0.8 ± 0.87^{a}	0.73 ± 0.58^{b}	0.63 ± 0.15^{b}
Group 2	D100	0.76 ± 0.55^{a}	1.06 ± 0.20^{a}	1.13 ± 0.37^{ab}	1.20 ± 0.43^{c}
Group 3	D250	1.03 ± 0.40^{a}	1.9 ± 0.10^{a}	2.06 ± 0.25^{a}	2.06 ± 0.15^{a}
Group 4	D700	1.00 ± 0.43^{a}	1.53 ± 0.66^{a}	1.8 ± 0.10^{a}	2.20 ± 0.10^{a}

Means followed by the same letters in the same column are not statistically different at the level of 5%.

Table 7: Change of alpha 2 globulins rate (g / l) during treatments

Groups (n=3)	Treatments	Days			
		D_0	D_7	D_{14}	D_{21}
Group 1	Normal saline	5.16 ± 0.57^a	6.06 ± 1.00^a	4.20 ± 1.85^a	4.86 ± 1.53^b
Group 2	D100	4.86 ± 1.85^a	6.50 ± 2.00^a	7.90 ± 0.79^a	8.20 ± 0.75^a
Group 3	D250	5.36 ± 0.55^a	8.26 ± 1.36^a	8.40 ± 0.81^a	10.36 ± 0.11^a
Group 4	D700	4.06 ± 0.55^a	7.80 ± 0.72^a	8.46 ± 0.15^a	11.40 ± 0.60^a

Means followed by the same letters in the same column are not statistically different at the level of 5%.

Table 8: Change of beta globulins rate (g / l) during treatments

Groups (n=3)	Treatments	Days			
		D_0	D_7	D_{14}	D_{21}
Group 1	Normal saline	8.40 ± 1.80^a	8.73 ± 0.49^a	7.80 ± 1.96^b	7.00 ± 0.10^c
Group 2	D100	6.63 ± 0.70^a	8.50 ± 1.35^a	9.03 ± 0.15^a	9.63 ± 1.05^a
Group 3	D250	6.03 ± 0.70^a	10.73 ± 2.68^a	11.73 ± 1.82^a	11.33 ± 1.26^{ab}
Group 4	D700	6.06 ± 0.32^a	9.13 ± 1.05^a	11.86 ± 3.58^a	13.03 ± 0.50^b

Means followed by the same letters in the same column are not statistically different at the level of 5%

Table 9: Change of gamma globulins level (g / l) during treatments

Groups (n=3)	Treatments	Days			
		D_0	D_7	D_{14}	D_{21}
Group 1	Normal saline	14.20 ± 0.78^a	15.13 ± 1.30^a	14.06 ± 0.87^a	12.53 ± 0.45^c
Group 2	D100	14.00 ± 1.11^a	20.26 ± 4.00^a	17.56 ± 3.13^{ab}	18.77 ± 2.51^a
Group 3	D250	14.03 ± 0.40^a	20.33 ± 1.40^a	17.63 ± 2.25^{ab}	22.43 ± 0.63^{ab}
Group 4	D700	12.30 ± 1.11^a	19.73 ± 3.52^a	20.00 ± 1.80^b	24.07 ± 0.40^b

Means followed by the same letters in the same column are not statistically different at the level of 5%.

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