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Research Article

ABDALA, a preventive vaccine against SARS-CoV-2, is safe in *Chlorocebus aethiops sabaeus* monkeys

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Abstract

Background: The ABDALA vaccine is a subunit vaccine developed for preventing SARS-CoV-2 infection and the progression of COVID-19 to severe forms, using as an active pharmaceutical ingredient a recombinant version of the SARS-CoV-2 spike protein receptor-binding domain (RBD) expressed in *Pichia pastoris*. The aim of the paper was to describe the safety profile of the ABDALA vaccine in monkeys (Cholorocebus aethiops sabaeus). For such aim, the vaccine was administered to the monkeys intramuscularly six times every 14 days, following the same scheme used in clinical trials done against COVID-19. Animals were allocated to four groups: Placebo, ABDALA Low-dose (50 µg per animal), ABDALA High-dose (100 µg per animal), and Reversion (100 µg per animal) to be submitted to a clinical, hematological and serum biochemical evaluation. Histopathological assessment of all tissues and organs was also conducted.

Results: As the main results, all animals survived and negative effects were not detected during animal clinical evaluations. The body weight and rectal temperature exhibited no variations and hematological and serum biochemical parameters showed no alterations associated with ABDALA administration. Finally, the histopathological study confirmed the proliferation of spleen white pulp due to ABDALA administration.

Conclusions: Therefore, results strongly suggest ABDALA does not cause toxic effects or damage in the organs of *Chlorocebus aethiops sabaeus* monkeys, indicating that it is a promising and safe novel vaccine to prevent SARS-CoV-2 infection in humans and the progression of COVID-19 to severe forms.

Introduction

COVID-19 is the disease caused by the coronavirus SARS-CoV-2. The World Health Organization (WHO) first learned of the existence of this virus on December 31, 2019; when it was informed of a group of cases of "viral pneumonia" declared in Wuhan [1]. This disease has a high transmission capacity and caused more than six million deaths worldwide. At that moment, the availability of a safe and effective vaccine was compulsory to allow the control of the disease.

Most vaccine candidates have focused on inducing antibody responses against the trimeric SARS-CoV-2 spike protein (S), a class I fusion protein that facilitates binding to the

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angiotensin-converting enzyme 2 receptor (ACE2) and triggers virus-cell-membrane fusion. In that sense, a variety of vaccine approaches and formulations are being pursued to target SARS-CoV-2 protein S, including nucleic acid (RNA and DNA) vaccines, human replication-defective adenoviral vaccines, SARS-CoV-2 completely inactivated and recombinant subunit protein vaccines [2.3].

On July 8, 2021, there were 184 COVID-19 vaccine candidates in preclinical development and 105 in human clinical trials [4]. Within them, recombinant subunit protein vaccines were the most common approach (one-third of candidates). Recombinant protein and peptide subunit vaccines use either whole protein or specific regions of the protein containing key B- and T-cell epitopes combined with adjuvants that are taken up by antigen-presenting cells (APC), and processed and presented to B and T cells. They are relatively stable and highly safe vaccines [5,6].

The Center for Genetic Engineering and Biotechnology (CIGB) developed the ABDALA vaccine, a subunit vaccine against SARS-CoV-2 based on a recombinant version of the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein that awakens an immune response and prevents infection and the progression of the disease to severe forms [7].

The ABDALA received Authorization for Emergency Use from the Cuban Regulatory Authority for the prevention of COVID-19 and the progression of the disease to severe forms in July 2021. Regarding this, an ABDALA repeated-dose toxicity study conducted in Non-Human Primates (NHP), as part of the preclinical safety program undertaken, is described in this paper, where two dose levels of the ABDALA vaccine were administered and their effects on behavior, body weight, temperature, hematological, serum biochemical parameters and histopathology of all tissues and organs measured.

Materials and methods

Product under study (ABDALA)

ABDALA is a liquid formulation containing recombinant RBD (50 µg), aluminum hydroxide gel (0.60 mg), disodium hydrogen phosphate (0.50 mg), sodium dihydrogen phosphate dihydrate (0.50 mg), sodium chloride (8.5 mg), tiomersal (0.05 mg) and water for injection, presented in hermetically sealed bulbs [8].

Justification of ABDALA administration route

The intramuscular (IM) route was chosen, because it is the administration route that will be used in human clinical trials. Animals were IM dosed in the deltoid muscle of the arms.

Animals

Twenty adult and healthy NHP (10 males) of *Chlorocebus aethiop sabaeus* species were used. These monkeys were captive-born in Cuba. The average body weight measured at the beginning of the study was 4.99 kg (males) and 3.68 kg (females). The age of monkeys ranged from 3 to 5 years old at the beginning of the study. Hematological and serum biochemical parameters' values were within the physiological range according to species and sex.

Monkey handling and husbandry

Monkeys were exposed to 22 - 29 °C throughout the whole study and housed individually in stainless steel cages (90 cm× 60 cm× 60 cm). Under these conditions, animals saw, heard, and smelled other NHPs of the same species. Monkeys were maintained on a 12/12 h light/dark cycle, under an environmental enrichment regime with toys and foraging enrichment for 2 h per day. Monkeys were fed twice daily, with fresh fruits and a commercial diet (granulated formula CMQ 1600 ALYco, certified by the National Center for Laboratory Animal Breeding (CENPALAB), Havana, Cuba; containing 25 % protein, 3.5 % crude fat and 3.8 % crude fiber) at a rate of 150 - 300 g per monkey, according to respective ages and body weights. Water was provided *ad libitum* and monkeys were cared for following the guidelines of the American Association for the Accreditation of Laboratory Animal Care (AAALAC). The study was conducted under approval of the CIGB Animal Care and Use Committee, protocol number: CICUAL/CIGB/21015.

Monkey clinical evaluation

Firstly, monkeys underwent a pre-selection to be included in the study, where a skin test for tuberculin was performed. In addition, monkeys were treated with Ivermectin (200 µg/kg subcutaneously, in the interscapular region of the back). During the study (pre-acceptance process, ABDALA administration period, and reversion time), monkeys underwent daily clinical observations. Additionally, a thorough clinical examination was performed, including examination of the skin, hair, mucous membranes (conjunctiva, nasal, oral, auditory, genital, and rectal), lymphatic, genitourinary, digestive, respiratory, cardiovascular, and nervous systems. The body temperature and weight of monkeys were measured. All specimens were subjected to bacteriological and parasitological studies, taking samples of rectal, vaginal, preputial, auricular, and oral mucosal exudates and fecal samples. Hematological and serum biochemical parameters were also measured. Only clinically healthy monkeys without behavioral alteration (stereotyped movements, aggression, self-harm, apathetic/depressed, over-grooming, drinking urine, eating faeces, etc) were included in the study (inclusion criteria). The body weight was weighted with a Sartorius scale (EB Model, Sartorius, Goettingen, Germany). Temperature was measured with a mercury bulb thermometer. To perform both measurements, monkeys were sedated with an IM injection of ketamine hydrochloride (ketamine - 50, Liorad, 10 mL, 50 mg/mL) into the biceps femoris muscle at a dose of 10 mg/kg body weight. This procedure was performed according to the Program for the Use and Handling of Laboratory Animals for Experimentation and Control of Biotechnological Products, CIGB, Cuba.

Study design

Animals were randomly allocated into four groups: Placebo, Low-dose, High-dose, and Reversion. Placebo and Reversion

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groups consisted of four monkeys each (two males per group) while the Low-dose and High-dose groups consisted of six monkeys each (three males per group) [Table 1]. Monkeys received six inoculations of ABDALA by IM route at a rate of one dose every 14 days during 12 weeks and were immediately euthanized, with the exception of the Reversion group. Animals of this group were euthanized 12 weeks later.

The Low-dose group received 0.5 mL (one bulb), 50 µg of Active Pharmaceutical Ingredient (API) of ABDALA per animal. The High-dose and Reversion groups received 1 mL (two bulbs), 100 µg of API of ABDALA per animal. In the Reversion group, the reversion of possible alterations or lesions detected in animals 12 weeks after the end of the ABDALA inoculations was analyzed. ABDALA doses were selected taking into account requirements established by WHO for vaccines [9], carefully considering the maximum allowable volume according to the species and administration route. The lowest dose level was considered the one established for clinical use: 50 µg. A higher level of 2 x the component of the vaccine candidate was also included, in order to guarantee a wide safety margin.

During the study, food and water avidity were analyzed. Clinical and behavioral measures were also carried out and pathological findings in all organs underwent macro and microscopic observations.

Body weight, body temperature, and different hematological and serum biochemical parameters were carried out at the beginning and at the end of the study.

Blood sample collection from animals

Blood samples were collected prior to the first vaccine administration and at weeks 11 and 22 (Reversion Group) of the study. After an overnight fasting period (14-16 h), monkeys were sedated with an IM injection of ketamine hydrochloride (ketamine-50, Liorad, 10 mL, 50 mg/mL) into the biceps femoris muscle at a dose of 10 mg/kg body weight. Four milliliters of blood were collected from the femoral veins using 21 G × 1/2 gauge needles and 10 mL syringes. Samples were distributed into 1 mL- and 3 mL aliquots, respectively. The 1 mL aliquot was transferred to tubes containing EDTA as an anticoagulant for the hematological parameter determination. On the other hand, 3 mL aliquots were stored in plastic tubes without anticoagulant for the biochemical parameter determination. Aliquots were allowed to clot at room temperature for 30 - 60 min, and sera were separated by centrifugation at 1600 xg for 15 min in a centrifuge 5810 (Eppendorf, Hamburg, Germany). Individual serum samples were stored in polypropylene tubes at -20 °C until the moment of the analysis.

Table 1: Experimental Design of the safety study of the ABDALA vaccine in Non-Human Primates.

Group	Treatment	Number of animals by gender	Doses of ABDALA Antigen quantity (µg)
I	Placebo	2 males 2 females	-
II	ABDALA Low dose	3 males 3 females	50
III	ABDALA High dose	3 males 3 females	100
IV	ABDALA / Reversion	2 males 2 females	100

Hematological and serum biochemical parameter study

The hematological parameter study included the analysis of the total leukocyte count (WBC) and differential leukocyte count (measured as a percentage of white blood cells) including lymphocytes (LYMPHO %), neutrophils (NEUTRO %), monocytes (MONO %), eosinophils (EO %) and basophils (BASO). Total erythrocyte count (RBC), hemoglobin concentration (HGB), hematocrit percentage (HCT), mean corpuscular volume (MCV), mean hemoglobin concentration (MCH), mean corpuscular hemoglobin concentration (MCHC) and total platelet count (PLT) were also analyzed (Table 2). Blood samples were evaluated on a Nihon Kohden hematology analyzer (Celltac model MEK6450J; Nishiochiai, Shinjuku-ku, Japan). A differential leukocyte count (measured as a percentage of white blood cells) including LYMPHO %, NEUTRAL %, MONO %, EO %, and BASO % was performed by staining peripheral blood slides with Giemsa reagent, and cells were counted using an optical microscope equipped with an immersion lens (VistaVision, MO 000004, Zeiss, Germany).

Serum biochemical parameters were analyzed on a Cobas Integra 400 PLUS automated analyzer (Roche Diagnostic Systems) including evaluation of alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyltranspeptidase (GGT), alkaline phosphatase (ALP) creatinine (CREA), uric acid (UA), urea (UR), total protein (TP), albumin (ALB), A/G ratio, glucose (GLU), triglycerides (TG), cholesterol (CHOL), direct bilirubin (BIL-D), total bilirubin (BIL-T), calcium (CA), phosphorus (PHOS), cholinesterase (CHE) and pancreatic amylase (P-AMY) (Table 3).

Electrocardiography

Electrocardiograms (ECG) (limb leads I, II, and III, and augmented leads aVR, aVL, and aVF) were recorded in all animals during the pre-treatment period, after the first and last treatment, and at the end of the recovery period, always 10 - 15 min after inoculation. The procedure was performed using a diagnostic electrocardiograph (Cardisuny D300, Fukuda M-E. Tokyo, Japan). The tracings were assessed for gross changes, indicative of cardiac electrical dysfunction. The potential presence of abnormalities involving heart rate, sinus, and atrioventricular rhythm or conductivity was determined.

Ophthalmoscopy examinations

Funduscopic (indirect ophthalmoscopy) and biomicroscopic (slit lamp) examinations were performed for all animals during the pre-treatment period and at the end of treatment. The biomicroscopic study of the eyes of each animal was observed using a slit lamp (SL-130; Carl Zeiss Meditec AG, Germany).

Euthanasia

Monkeys were euthanized by intravenous administration of 200 mg/kg sodium thiopental (Sodium Thiopental-500,

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Table 2: Hematological and Serun biochemical parameters analyzed in the study.

Parameters	Abbreviation	Unit
White Blood Cells	WBC	10^3/µL
Red Blood Cells	RBC	10^6/µL
Hemoglobin	HGB	g/dL
Hematocrit	HCT	%
Mean Corpuscular Volume	MCV	fL
Mean Corpuscular Hemoglobin	MCH	Pg
Mean Corpuscular Hemoglobin Concentration	MCHC	g/dL
Platelet Count	PLT	10^3/µL
Neutrophils percentage	NEUTRO%	%
Lymphocytes percentage	LYMPHO%	%
Monocytes percentage	MONO%	%
Eosinophils Percentage	EO%	%
Basophils Percentage	BASO%	%
A / G Index	A/G	
Alanine aminotransferase	ALT	UI/L
Aspartate aminotransferase	AST	UI/L
Glutamyltranspeptidase	GGT	UI/L
Alkaline phosphatase	ALP	UI/L
Creatinine	CREA	mg/dL
Total Protein	TP	g/dL
Albumin	ALB	g/dL
Glucose	GLU	mmol/L
Cholesterol	CHOL	mg/dL
Total Bilirubin	TB	mmol/L
Direct Bilirubin	BIL-D	mmol/L
Pancreatic amylase	AMY-P	U/L
Calcium	CA	mg/dL
Cholinesterase	CHE	U/L
Phosphorus	PHOS	mg/dL
Triglycerides	TRIG	mg/dL
Urea	UR	mg/dL
Uric Acid	UA	mg/dL

Table 3: Mean of the body weight measured in animals of both sexes by experimental groups.

		I / Placebo	II/ Low Dose	III/ High Dose	IV/ Reversion
		Mean	Mean	Mean	Mean
Males	Day 0	4.11a	4.91a	5.62a	4.06a
	Day 28	4.67a	4.60a	5.71a	3.98a
	Day 56	-	-	-	4.09a
Females	Day 0	3.07a	3.41a	3.64a	2.90a
	Day 28	3.14a	3.35a	3.47a	2.76a
	Day 56	-	-	-	2.97a

AICA, 500 mg) after sedation with ketamine hydrochloride (ketamine-50, Liorad, 10 mL, 50 mg/mL) following recommendations of the Program for the Use and Handling of Laboratory Animals for Experimentation and Control of Biotechnological Products, CIGB, Cuba.

Macroscopic and histopathological evaluation

Macroscopic observation of all organs was performed during necropsy. For histopathological evaluation, samples were taken from the adrenal glands, thymus, lungs (with bronchi and bronchioles), heart, liver, spleen, kidneys, testes, prostate, brain, pituitary gland, uterus (cervix and oviducts), ovaries, pancreas, thyroid and parathyroid gland, lymph nodes, mammary glands, salivary glands, skeletal muscle, lymph nodes, bone marrow, trachea, aorta, esophagus, stomach, small and large intestine, ureters, urinary bladder, epididymis, seminal vesicle, vagina, peripheral nerves, eyes and optic nerve, spinal cord, larynx, tongue and site of application (skin and nasopharyngeal mucosa samples). The total and relative weight of all organs was determined [10]. Samples were placed in 4 % neutral formalin and processed following the kerosene embedding method [10]. They were stained with Eosin-Hematoxylin and observed under a Carl Zeiss simple microscope at 40 X and 100 X magnification [12]. Photomicrographs were taken with a Canon Power Shot digital camera (Canon, Japan). All the animals in each experimental group were included in the morphometric study of the spleen. Photographs of the organ were digitized with a digital camera (Nikon) at an average distance of 15 cm. A digital analysis of the 100 x image of the histological smears was performed, using the morphometric parameters that quantify the size and shape of the germinal centers (where diameter length, and perimeter were taken into account). Image software version 1.36b [14] (Wayne Rasband, National Institutes of Health, USA), available at http://rsbweb.nih.gov/ij/index.html, was used.

A morphometric study of spleens was performed in two animals of Placebo, High-dose, and Reversion groups. The area and perimeter of the periarteriolar zone of the lymphoid follicles of 3 fields (40X magnification) of the organ were determined in these animals, using the ImageJ® 1.43u program [14]. The administration site microscopic analysis and the irritability evaluation were performed according to the ISO / WD 10993-10 standard [15].

Additionally, the microscopic analysis of the liver, kidney, lung, and cerebral cortex of the animals of the different experimental groups, was carried out by means of an electronic microscope. The samples were viewed under the MIRA3-TESCAN Scanning Electron Microscope (Kohoutovice, Czech Republic), using a transmission detector with 3 nm resolution (according to the manufacturer). Each sample was fixed in 3.2 % glutaraldehyde for 1 hour at 4 °C and post-fixed at 1 % in osmium tetroxide for 1 hour at 4 °C. Subsequently, it was washed with 0.1 M PBS at pH 7.2 and dehydrated in increasing concentrations of ethanol (30 %, 50 %, 70 % and 100 %), for 10 minutes each time, at 4 °C. Embedding was performed and ultrathin sections were made with an RMC ultramicrotome, 40-50 nm thick, placed on 400 -hole nickel grids. After making the ultrathin sections and placing them on the grids, they were contrasted with saturated uranyl acetate and lead citrate. A total of 10 photomicrographs per sample were analyzed at different magnifications.

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Statistical analysis

The descriptive statistics were firstly calculated for all studied parameters (means or medians, standard deviation, and ranges according to age and sex by experimental group) and the statistical analysis of the measurements obtained from each animal throughout the study was performed using the SPSS Statistics software, version 26 for Windows (2020). The Shapiro-Wilk test was used to verify the assumption of normality of variables to be included in the analysis, and the Levene test was applied to verify homoscedasticity (variance homogeneity) among experimental samples. For the analysis of the differences among groups, the one-way analysis of variance test (simple ANOVA) was used when data met the aforementioned assumptions or the Kruskal-Wallis for independent samples when data, even after transformations did not satisfy normality and homoscedasticity assumptions. The Student's t-test for dependent samples was used to compare measurements collected by the group at the beginning and end of the study when data followed a Gaussian distribution. When data did not follow this distribution, the Wilcoxon test was used. The Mann-Whitney U test for independent samples was used to determine the significance of the difference in the presentation of the most prominent microscopic lesions. The significance level (a) used in all analyses was 0.05 %.

Results

Animal clinical and behavioral analysis

No monkey died during the trial and no clinical or behavioral changes were detected in the studied monkeys. During the study, all animals gained weight with modest growth, regardless of the treatment group to which they belonged and their sex (Image 1, Table 1). No affectations were detected throughout the trial in the avidity for water and food and behavioral variables. The rectal temperature of all monkeys was within the physiological range for the species (38.0 - 39.5 °C) [16]. The study of the administration site showed the absence of adverse effects and temperature variations in the inoculation area.

Ophthalmological evaluation

No ocular changes were associated with the IM injection of ABDALA for 12 weeks at doses up to 100 µg by animal.

Electrocardiography

All animals showed sinus rhythm and therefore, the succession of the QRS complexes (ECG rhythm) was regular. The heart rates calculated were similar across all experimental groups according to one-way ANOVA ($p > 0.05$). The morphology of the P, QRS, and T waves showed common patterns or wave morphology. Negative T waves, the pattern of this species *Cholorocebus aethiops sabaeus*, were manifested equally in all experimental groups regardless of treatment (Image 2-6).

Hematological and serum biochemical parameters

The result of the hematological and serum biochemical parameters performed by sex was as follows. After six

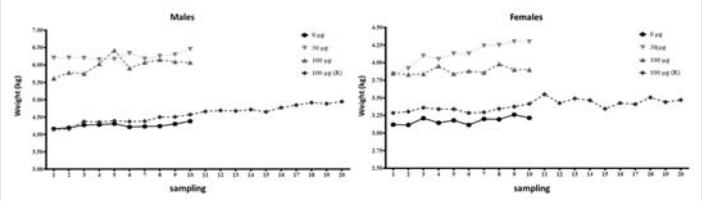

Image 1: Medians of the body weight by group and sex (kg). No significant differences were detected between the groups (Kruskal-Wallis test, $p < 0.05$).

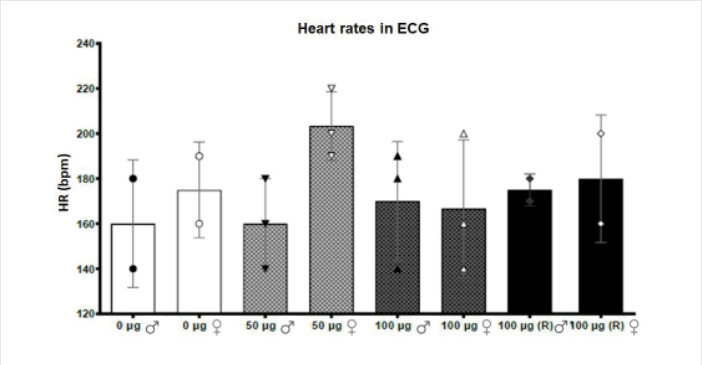

Image 2: Frequency values in electrocardiogram by groups and sex, subjected to different dose levels. The ordinate values represent the means. No significant differences were found between the groups. One-way ANOVA ($p > 0.05$).

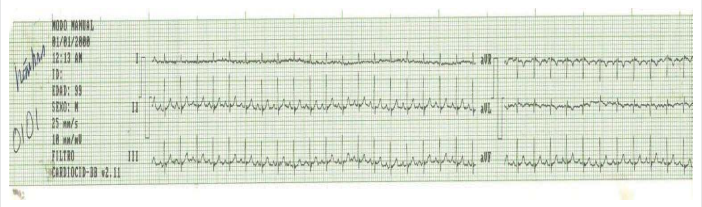

Image 3: Electrocardiographic recording of an animal of group I.

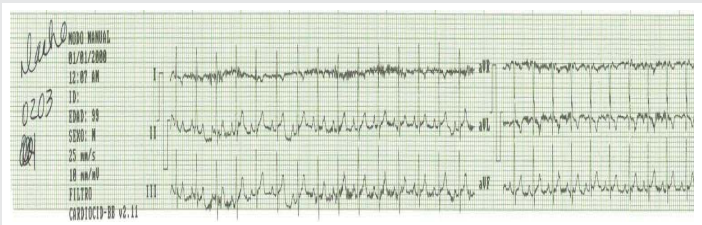

Image 4: Electrocardiographic recording of an animal of group II.

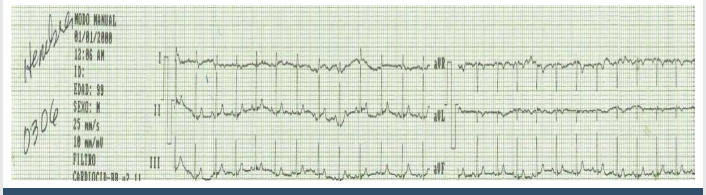

Image 5: Electrocardiographic recording of an animal of group III.

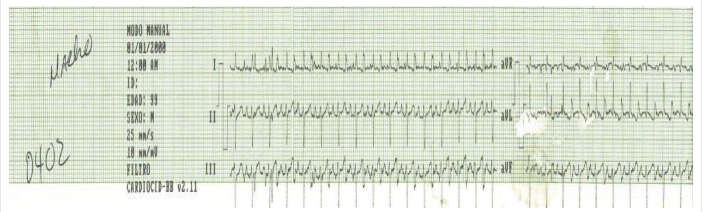

Image 6: Electrocardiographic recording of an animal of group IV.

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immunizations with the Abdala vaccine, only one animal (male, Reversion group) showed HGB and RBC values below the expected physiological ranges. For all animals, of both sexes, the rest of the hematological parameters (WBC, LYMPHO %, NEUTRO %, MONO %, EO %, BASO %, MCV, MCH, MCHC, and PLT) were within the expected physiological range for the species (Tables 4,5). Values of the hematological and serum biochemical parameters obtained for each sex at weeks 0 and 12 were compared among groups in each sampling and in each group in the different samplings (Tables 4,5). In both genders, no statistically significant differences ($p > 0.05$) were confirmed when comparing, using the Kruskal-Wallis test, the initial and final values of the hematological parameters among the different experimental groups. The application of the Wilcoxon Test for related samples did not detect significant differences ($p < 0.05$) between the initial and final values of these parameters in any of the groups (Tables 6,7 for males and females, respectively). Regarding the serum biochemical parameters, also in both genders, no statistically significant differences ($p > 0.05$) were confirmed when comparing, using the Kruskal-Wallis test, the initial and final values among the different experimental groups. The results of the Wilcoxon Test for related samples also showed no significant differences ($p > 0.05$) between the initial and final values of these parameters in each of the groups (Tables 8,9 for males and females, respectively).

Macroscopic and microscopic analysis

The analysis of the absolute weight of the organs showed significant statistical differences in the liver of males between the Placebo and High-Dose groups ($p = 0.0489$), which disappeared when analyzing the relative weight of the organ, therefore it lacks biological significance for the study. In the case of the absolute weights in the female animals, highly significant differences were determined between the lungs of the Placebo group and the group treated with High Dose ($p = 0.0135$), which were maintained in the analysis of the relative weight (Image 7).

Also, significant differences ($p = 0.0028$) were detected in the relative weight of the male spleen, being higher in the High Dose group of ABDALA compared to the Low Dose group. The kidney's relative weight of the Placebo females was significantly larger with respect to the Low Dose group females ($p = 0.0219$). These differences may be associated with the presence of congestion present in an animal from the Placebo group (Image 8).

The most relevant alterations observed during the macroscopic analysis were detected in the administration site and in the thymus. In the subcutaneous cellular tissue of the skin corresponding to the administration site, a slight hemorrhage was observed in one animal of the Placebo group and in two animals of the High-dose Group. Also, in all the treated animals, a focal inflammatory reaction with the prevalence of mononuclear cells was observed, including macrophages, lymphocytes, and plasma cells. In animals treated with the vaccine, the inflammatory reaction was more severe, causing discrete focal atrophy of muscle fibers (Image 9).

In the case of thymus, the atrophy of this organ was detected in seven animals: one from the Placebo group (25 %), and three animals from the Low-dose group and High-dose group respectively (33.3 %).

Hyperplasia and hypertrophy of the white pulp were also observed in the spleen of five animals treated with the High-dose of ABDALA (50 %) (Image 10). These morphological changes were characterized by an increase in follicular lymphoid cells (hyperplasia and hypertrophy of the mantle) and hypertrophy of the germinative centers.

Finally, according to the study conducted using the electronic microscope, all samples obtained from the liver, kidney, lung, and cerebral cortex, showed a normal structure. No evidence of damage or pathological changes was apparent; with Ultrastructure within normal limits (Images 11-14).

Discussion

Currently, WHO has approved the emergency use of 11 vaccines against COVID-19. Two of those vaccines are based on protein subunits, similar to the ABDALA vaccine [17]. ABDALA has an administration schedule of three doses separated by 14 days (28 days for the complete scheme) and has demonstrated its immunogenicity in several animal species [18], as well as its effectiveness in humans [19,20]. Safety is the main advantage of subunit vaccination. Recombinant subunit protein vaccines use specific fragments of a disease-causing agent instead of a whole pathogen to stimulate the immune system. This is an extremely safe method of immunization and can be used for virtually anyone who needs to be vaccinated, regardless of health status [21].

Although most of the vaccines against COVID-19 that received approval for emergency use were safe in clinical trials, the adverse reactions reported were numerous, including fever, headache, fatigue, injection site pain, and nausea [22,23]. Even, as the vaccination campaigns progressed, some other complications occurred in some subjects [24,25]. The possible complications induced by COVID-19 vaccines mainly include the following categories: (1) coagulation dysfunction, such as thrombocytopenia [26,27], (2) heart diseases, such as myocarditis [28,29], (3) immune diseases, such as allergic reactions [30], autoimmune hepatitis [31], and autoimmune thyroid diseases [32], (4) nervous system diseases, such as facial paralysis [33,34] and functional neurological disorders [35], (5) lymphatic system diseases [36] and (6) other diseases, such as Rowell's syndrome [37], macular rash [38], and chilblain-like lesions [39]. Although the incidence of these complications is low, the possible appearance of these elements during Nonclinical safety studies must be studied.

The Nonclinical evaluation of the immunogenicity and safety of vaccines in NHP models provides several advantages for clinical translation. They are non-consanguineous, have greater similarity to humans than rodents in innate immune responses and B- and T-cell repertoires, and allow for the use of clinically relevant vaccine doses [40,41]. Multiple NHP species have been explored to this end, including *Macaca*

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Table 4: Mean and standard deviation of hematological parameters measured in males.

	Physiological range		Placebo	Low-Dose	High-Dose	Reversion	Reversion (after reversion time; 12 weeks)
			Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
WBC	4.5 – 14.3	Day 0	8.9 ± 2.4	6.3 ± 3	8.9 ± 2.4	5.5 ± 1.5	-
		ASAA	6.6 ± 4.7	6.3 ± 3	5.8 ± 1.2	6 ± 1.5	4.2 ± 0.8
LYMPHO %	58 – 78	Day 0	69 ± 3	69 ± 4	69 ± 3	69 ± 5	-
		ASAA	71 ± 3	69 ± 4	69 ± 3	71 ± 3	59 ± 30
MONO %	0 – 3	Day 0	2 ± 0	1 ± 1	2 ± 0	2 ± 1	-
		ASAA	1 ± 0	1 ± 1	1 ± 0	1 ± 1	2 ± 0
NEUTRO %	21 – 40	Day 0	29 ± 3	30 ± 4	29 ± 3	30 ± 4	-
		ASAA	28 ± 2	30 ± 4	30 ± 3	29 ± 4	40 ± 30
EO %	0 – 1	Day 0	0 ± 0	0 ± 1	0 ± 0	0 ± 0	-
		ASAA	1 ± 1	0 ± 1	0 ± 1	1 ± 1	0 ± 0
BASO %	0 – 0	Day 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	-
		ASAA	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
RBC	6.8 – 9.6	Day 0	8.60 ± 0.61	6.93 ± 0.5a	8.60 ± 0.61	8.20 ± 0.80	-
		ASAA	6.15 ± 0.2	6.93 ± 0.5	6.82 ± 0.7	6.87± 1.3	5.47 ± 0.33
HGB	11.4 – 15.6	Day 0	14.5 ± 0.4	14.7 ± 1.8a	14.5 ± 0.4	13.1 ± 0.9	-
		ASAA	12.3 ± 0	14.7 ± 1.8	13.8 ± 0.6	13 ± 2.1	11 ± 0.1
HCT	34.8 – 47.8	Day 0	44.3 ± 1.2	49.2 ± 5.5a	44.33 ± 1.2	40 ± 2.8	-
		ASAA	41.8 ± 0.1	49.2 ± 5.5	46.7 ± 2.9	46 ± 7.7	38.1 ± 0.1
MCV	63 – 69	Day 0	66 ± 2.2	71 ± 3*a	66 ± 2.2	66 ± 0	-
		ASAA	68 ± 1	71 ± 3	69 ± 3	67 ± 1	70 ± 4
MCH	14.9 – 18.1	Day 0	16.67 ± 1.1	21.2 ± 1*a	16.67 ± 1.1	15.95 ± 0.5	-
		ASAA	20 ± 0.4	21.2 ± 1	20.3 ± 1.1	19 ± 0.6	20.2 ± 1.6
MCHC	23.4 – 26.4	Day 0	25.23 ± 0.9	29.9 ± 0.6*a	25.23 ± 0.9	24.25 ± 0.6	-
		ASAA	29.4 ± 0	29.9 ± 0.6	29.5 ± 0.5	28.3 ± 0.3	28.9 ± 0.6
PLT	66 – 569	Day 0	372 ± 114	280 ± 62a	372 ± 114	249 ± 41	-
		ASAA	311 ± 93	280 ± 62	326 ± 21	288 ± 27	332 ± 11
A/G	1.64 – 4.13	Day 0	2.7 ± 0.3	2.9 ± 0.8	2.4 ± 0.4	3.4 ± 0.4	-
		ASAA	3 ± 2.1	3.4 ± 1	2.7 ± 0.1	4 ± 0	4 ± 0.2
ALT	0 - 96	Day 0	152 ± 1	82 ± 24	99 ± 55	65 ± 3	-
		ASAA	39 ± 18	53 ± 15	19 ± 1	32 ± 7	17.05 ± 5.44
ALB	43.4 – 58.1	Day 0	50 ± 5	55 ± 5	49 ± 1	52 ± 4	-
		ASAA	40 ± 6	48 ± 3	45 ± 3	50 ± 0	56 ± 1
ALP	0 - 796	Day 0	307 ± 365	251 ± 260	95 ± 29	340 ± 192	-
		ASAA	492 ± 548	274 ± 346	80 ± 30	516 ± 312	440 ± 363
AST	12 - 151	Day 0	159 ± 6	88 ± 45	102 ± 50	62 ± 7	-
		ASAA	120 ± 56	78 ± 29	88 ± 11	53 ± 13	49.8 ± 4.50
BIL-D	0 – 0.92	Day 0	0 ± 0	0 ± 0	0.6 ± 0.7	0.4 ± 0.5	-
		ASAA	0.2 ± 0.3	0.6 ± 0.2	0.5 ± 0.3	0.1 ± 0.1	0.20 ± 0
BIL-T	1.99 – 2.91	Day 0	0 ± 0	0.4 ± 0.5	1.3 ± 1.1	0.3 ± 0.1	-
		ASAA	1.6 ± 1	2 ± 1.1	1.3 0.6±	1.2 ± 1	2.7 ± 1
CA	1.8 - 10	Day 0	2.38 ± 0.29	2.45 ± 0.19	2.13 ± 0.26	2.33 ± 0.13	-
		ASAA	2.05± 0.13	2.20 ± 0.23	2.06 ± 0.10	2.24 ± 0.09	2.29 ± 0.04
CHOL	1.31 – 3.69	Day 0	2.88 ± 1.26	2.37 ± 0.26	3.08 ± 0.62	2.24 ± 0.21	-
		ASAA	2.86 ± 0.72	2.25 ± 0.30	2.71 ± 0.51	2.49 ± 0.23	2.29 ± 0.12
CREA	31 - 72	Day 0	44 ± 11	66 ± 18	58 ± 9	53 ± 22	-
		ASAA	47.5 ± 21.9	65 ± 15.7	50.3 ± 7.8	43.5 ± 13.4	54.6 ± 17.7
GGT	53 - 174	Day 0	137 ± 27	107 ± 49	116 ± 43	98 ± 13	-
		ASAA	128 ± 39	121 ± 52	118 ± 46	92 ± 23	67 ± 45
GLU	3.54 – 6.84	Day 0	4.79 ± 0.33	4.98 ± 1.07	4.35 ± 0.56	4.69 ± 1.46	-
		ASAA	3.76 ± 0.32	6.40 ± 1.51	4.92 ± 0.61	5.28 ±0.37	4.40 ± 1.17
PHOS	1.25 – 2.41	Day 0	1.57 ± 0.74	1.71 ± 0.12	1.80 ± 0.29	1.84 ± 0.06	-
		ASAA	2.02 ± 0.55	1.69 ± 0.13	1.52 ± 0.54	1.92 ± 0.15	2.07 ± 0.46
TP	58.1 – 81.9	Day 0	68.8 ± 4.5	74.9 ± 9.5	70 ± 2.1	67.3 ± 2.4	-
		ASAA	57.3 ± 3.7	62.7 ± 7.6	61.6 ± 3.9	62.5 ± 0.2	70.2 ± 2.6
TRIG	0.24 – 0.76	Day 0	0.55 ± 0.049	0.65 ± 0.135	0.61 ± 0.101	0.43 ± 0	-
		ASAA	0.40 ± 0.02	0.46 ± 0.06	0.52 ± 0.06	0.49 ± 0.15	0.37 ± 0.01
UA	0 - 19	Day 0	1 ± 1	0 ± 0	9 ± 9	13 ± 13	-
		ASAA	1 ± 1	4 ± 6	1 ± 1	0 ± 0	31 ± 6
UR	4.9 – 10.3	Day 0	6.6 ± 1.9	7.9 ± 2.5	4.7 ± 0.4	8.1 ± 3.1	-
		ASAA	6.1 ± 0.3	8 ± 2.9	7.5 ± 1.4	8.4 ± 1.5	8.4 ± 1.8

Legend: ASAA: After Six ABDALA Administrations.

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Table 5: Mean and standard deviation of hematological parameters measured in females.

	Physiological range		Placebo	Low-Dose	High-Dose	Reversion	Reversion (after reversion time; 12 weeks)
			Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
WBC	5.7 – 16.5	Day 0	10.3 ± 5.1	8.9 ± 2.3	9.7 ± 4.5	12.4 ± 0.8	-
		ASAA	4.3 ± 0.9	6.5 ± 1	8 ± 0.8	6.2 ± 3.2	4 ± 1.1
LYMPHO %	52 - 80	Day 0	69 ± 3	68 ± 3	63 ± 12	64 ± 7	-
		ASAA	71 ± 6	73 ± 2	71 ± 3	68 ± 4	65 ± 4
MONO %	0 - 3	Day 0	2 ± 0	1 ± 1	2 ± 2	2 ± 1	-
		ASAA	0 ± 0	1 ± 1	1 ± 1	1 ± 0	1 ± 1
NEUTRO %	19 - 45	Day 0	29 ± 4	31 ± 2	35 ± 11	35 ± 6	-
		ASAA	28 ± 6	27 ± 2	28 ± 2	31 ± 4	35 ± 4
EO %	0 - 1	Day 0	1 ± 1	0 ± 0	0 ± 0	0 ± 0	-
		ASAA	1 ± 0	0 ± 0	0 ± 0	1 ± 1	0 ± 0
BASO %	0 - 1	Day 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	-
		ASAA	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
RBC	5.7 – 8.2	Day 0	7.05 ± 0.08	7.54 ± 0.19	7.68 ± 0.56	7.47 ± 0.29	-
		ASAA	5.65 ± 0.6	5.24 ± 0.6	5.65 ± 0.4	6.82 ± 0.7	7.23 ± 1.93
HGB	11 – 13.6	Day 0	12 ± 0.2	11.9 ± 0.9	12.6 ± 0.6	12.9 ± 0.1	-
		ASAA	12.3 ± 0.9	11.8 ± 0.4	12 ± 0.6	14.4 ± 0.8	13.9 ± 2.3
HCT	33.9 – 41.7	Day 0	36.50 ± 0.7	36.67 ± 2.5	38.67 ± 1.5	39.50 ± 0.7	-
		ASAA	40.8 ± 3.6	36.8 ± 3.6	39.2 ± 3	50 ± 3.7	48.6 ± 10.6
MCV	61 - 71	Day 0	67 ± 1.4	64 ± 1.7	65.3 ± 2.1	68.5 ± 2.1	-
		ASAA	72 ± 1	70 ± 2	69 ± 1	74 ± 2	68 ± 4
MCH	14.9 – 18.1	Day 0	16.9 ± 0.4	15.80 ± 0.9	16.4 ± 0.4	17.30 ± 0.6	-
		ASAA	21.7 ± 0.6	22.8 ± 3.2	21.3 ± 0.6	21.2 ± 0.8	19.5 ± 2.1
MCHC	23.8 – 26.2	Day 0	25.25 ± 0.1	24.57 ± 1	25.13 ± 0.5	25.20 ± 0.1	-
		ASAA	30 ± 0.3	32.4 ± 4.1	30.7 ± 0.9	28.9 ± 0.5	28.8 ± 1.6
PLT	162 - 509	Day 0	410 ± 127	338 ± 32	350 ± 82	236 ± 66	-
		ASAA	352 ± 57	354 ± 70	412 ± 114	260 ± 28	283 ± 8
A/G	1.8 - 4	Day 0	3.2 ± 0.3	2.6 ± 0.5	2.1 ± 0.7	2.7 ± 0.8	-
		ASAA	4.3 ± 1.2	3.2 ± 0.8	3.4 ± 0.3	2.6 ± 0	3.2 ± 0.3
ALT	0 - 112	Day 0	60 ± 21	100 ± 138	74 ± 49	50 ± 12	-
		ASAA	24 ± 14	38 ± 37	47 ± 31	89 ± 37	14.15 ± 1.20
ALB	43.2 – 55.6	Day 0	51 ± 1	45 ± 6	47 ± 3	49 ± 3	-
		ASAA	46 ± 0	45 ± 5	44 ± 2	47 ± 0	54 ± 1
ALP	19 - 313	Day 0	243 ± 126	161 ± 22	174 ± 60	127 ± 4	-
		ASAA	257 ± 161	106 ± 4	118 ± 62	121 ± 78	120 ± 42
AST	0 - 173	Day 0	58 ± 5	61 ± 15	79 ± 34	138 ± 61	-
		ASAA	76 ± 16	62 ± 26	83 ± 12	301 ± 105	45.5 ± 17.5
BIL-D	0 – 0.64	Day 0	0.9 ± 0.0	0.3 ± 0.3	0.2 ± 0.3	0 ± 0	-
		ASAA	0.4 ± 0.1	0.2 ± 0.2	0.1 ± 0.1	0.3 ± 0.4	0.25 ± 0.07
BIL-T	0.1 – 2.3	Day 0	0.4 ± 0.5	0.1 ± 0.2	0.2 ± 0.2	0.2 ± 0.1	-
		ASAA	1.2 ± 0.4	1.2 ± 0.9	1 ± 0.5	1.2 ± 0	1.7 ± 0.2
CA	1.92 – 3.13	Day 0	2.31 ± 0.07	2.19 ± 0.11	2.37 ± 0.02	2.39 ± 0.19	-
		ASAA	2.31 ± 0.27	2.33 ± 0.40	2.07 ± 0.06	2.24 ± 0.04	2.42 ± 0.04
CHOL	1.79 – 3.50	Day 0	2.35 ± 0.11	2.47 ± 0.76	2.44 ± 0.55	2.81 ± 0.48	-
		ASAA	2.41 ± 0.14	2.96 ± 0.69	2.46 ± 0.82	2.81 0.39±	2.44 ± 0.73
CREA	38 - 70	Day 0	47 ± 1	60 ± 8	64 ± 5	61 ± 4	-
		ASAA	42.5 ± 12	62.7 ± 14	59.7 ± 7.4	51 ± 9.9	69 ± 2
GGT	14 - 244	Day 0	74 ± 30	173 ± 199	95 ± 9	173 ± 109	-
		ASAA	71 ± 30	103 ± 69	125 ± 43	234 ± 126	92 ± 23
GLU	3.32 – 6.55	Day 0	4.14 ± 0.23	3.95 ± 0.56	5.25 ± 0.83	4.03 ± 0.62	-
		ASAA	4.39 ± 0.02	7.34 ± 1.53	4.41 ± 0.79	4.87 ± 0.83	4.59 ± 0.62
PHOS	0.98 – 1.90	Day 0	1.62 ± 0.30	1.18 ± 0.27	1.17 ± 0.67	1.19 ± 0.35	-
		ASAA	1.78 ± 0.16	1.66 ± 0.19	1.49 ± 0.33	1.50 ± 0.14	1.31 ± 0.28
TP	57.5 – 77.1	Day 0	66.5 ± 0.6	63.4 ± 5	71 ± 4.6	67.2 ± 1	-
		ASAA	57.5 ± 2.4	59.7 ± 4.3	57.1 ± 1	65 ± 0.6	70.4 ± 0.6
TRIG	0.33 – 0.71	Day 0	0.560 ± 0.255	0.610 ± 0.125	0.673 ± 0.083	0.570 ± 0.184	-
		ASAA	0.43 ± 0.13	0.59 ± 0.09	0.49 ± 0.19	0.50 ± 0.05	0.48 ± 0.13
UA	0 - 23	Day 0	13 ± 4	12 ± 16	6 ± 7	3 ± 4	-
		ASAA	0 ± 0	0 ± 1	1 ± 1	24 ± 1	15 ± 10
UR	5.53 – 9.18	Day 0	6.1 ± 0.6	5.7 ± 0.3	7.4 ± 1.2	7.2 ± 0.6	-
		ASAA	7.3 ± 0.1	7.2 ± 1	7 ± 0.5	7 ± 1.6	7.4 ± 1

Legend: ASAA: After Six ABDALA Administrations.

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Table 6: Non-parametric statistical comparisons for the values of the hematological parameters by experimental groups and samples. Sex: Males.

Kruskal-Wallis test* among the different groups			Wilcoxon Test* Comparison between Final Sampling -Initial sampling			
Parameters	Initial sampling	Final sampling	Placebo	ABDALA Low Dose	ABDALA High Dose	ABDALA Reversion
HGB	0.179	0.236	0.180	0.593	0.285	0.655
RBC	0.837	0.334	0.180	0.109	0.109	0.180
HCT	0.231	0.347	0.655	0.109	0.109	0.180
MCV	0.832	0.242	0.180	0.109	0.102	1.000
MCH	0.856	0.188	0.180	0.109	0.109	0.180
MCHC	0.587	0.130	0.180	0.109	0.109	0.180
PLT	0.757	0.621	0.655	0.285	1.000	0.180
WBC	0.397	0.999	0.180	0.285	0.109	0.180
NEUTRO%	0.910	0.881	0.655	0.655	0.317	0.655
LYMPHO%	1.000	0.880	0.655	1.000	1.000	0.655
MONO%	0.878	0.866	1.000	0.564	0.157	0.655
EO%	0.682	0.969	0.317	1.000	1.000	0.317
BASO%	1.000	1.000	1.000	1.000	1.000	1.000

The significance level is 0.05.

Table 7: Non-parametric statistical comparisons for the values of the hematological parameters by experimental groups and samples. Sex: Females.

Kruskal-Wallis test* among the different groups			Wilcoxon Test* Comparison between Final Sampling -Initial sampling			
Parameters	Initial sampling	Final sampling	Placebo	ABDALA Low Dose	ABDALA High Dose	ABDALA Reversion
HGB	0.251	0.174	0.655	1.000	0.109	0.180
RBC	0.197	0.164	0.180	0.109	0.109	0.180
HCT	0.205	0.162	0.180	1.000	0.593	0.180
MCV	0.153	0.148	0.157	0.109	0.109	0.157
MCH	0.101	0.766	0.180	0.109	0.109	0.180
MCHC	0.868	0.183	0.180	0.102	0.109	0.180
PLT	0.286	0.193	0.180	1.000	0.109	0.655
WBC	0.685	0.168	0.180	0.109	1.000	0.180
NEUTRO%	0.692	0.584	0.655	0.102	0.414	0.180
LYMPHO%	0.902	0.487	0.317	0.109	0.593	0.180
MONO%	0.659	0.334	0.157	0.655	0.593	0.317
EO%	0.487	0.077	0.317	0.317	1.000	0.317
BASO%	1.000	1.000	1.000	1.000	1.000	1.000

The significance level is 0.05.

mulatta (rhesus macaque), *Papio anubis* (baboons) [42], *Macaca nemestrina* (pigtail macaques) [43], *Macaca fascicularis* (cynomolgus macaque), and *Chlorocebus aethiops* (African green monkeys), an element that demonstrated the pertinence of the use of NHP in carrying out safety studies of the ABDALA vaccine.

During the nonclinical development of other vaccine candidates, in rhesus monkeys, no adverse events associated with the product have been detected during therapy or in the follow-up stage. In addition, no differences in body weight, body temperature, electrocardiogram (ECG), ophthalmology, or clinical pathology (hematology, coagulation, and serum biochemical parameters) were observed or detected in animals inoculated with those vaccines [44,45]. In such a sense; the safety of the ABDALA vaccine studied in *Chlorocebus aethiops sabaeus* monkeys revealed a similar and high security profile.

The headway of the animal's body weight constitutes an indicator of animal welfare [46] that must be measured during preclinical safety studies. Slight weight decreases may be explained both by the stress of individual housing and by successive administrations of ketamine that may, in turn, affect food consumption [47]. The stability in the body weight measurements of the animals throughout the study suggests the administration of the ABDALA vaccine does not lead to alterations in health status nor does it evince the presence of toxicity in the inoculated animals. These results are in line with findings reported in the preclinical development of other vaccine candidates in rhesus monkeys [48].

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Table 8: Non-parametric statistical comparisons for the values of the biochemical parameters by experimental groups and samples. Sex: Males.

Kruskal-Wallis test Among the different groups			Wilcoxon Test Comparison between Final Sampling -Initial sampling			
Parameters	Initial sampling	Final sampling	Placebo	ABDALA Low Dose	ABDALA High Dose	ABDALA Reversion
AG	0.369	0.639	0.655	0.593	0.180	0.180
ALAT	0.186	0.067	0.180	0.109	0.109	0.180
ALB	0.320	0.204	0.180	0.109	0.109	0.317
ALP	0.655	0.261	0.180	1.000	0.109	0.180
ASAT	0.197	0.286	0.655	0.593	0.593	0.180
BILD	0.278	0.215	0.317	0.109	1.000	0.655
BILT	0.098	0.653	0.180	0.109	1.000	0.180
CA	0.452	0.414	0.180	0.180	0.593	0.180
CHOL	0.727	0.480	0.655	0.593	0.285	0.180
CREA	0.352	0.448	0.655	1.000	0.109	0.180
GGT	0.710	0.849	0.180	0.593	1.000	0.655
GLUC	0.801	0.133	0.180	0.285	0.109	0.655
PHOS	0.854	0.518	0.180	0.655	0.285	0.655
TP	0.767	0.723	0.180	0.285	0.109	0.180
TRIG	0.164	0.425	0.180	0.109	0.109	0.655
UA	0.056	0.689	0.317	0.317	0.109	0.180
UREA	0.107	0.308	0.655	0.655	0.109	0.655

The significance level is 0.05.

Table 9: Non-parametric statistical comparisons for the values of the biochemical parameters by experimental groups and samples. Sex: Females.

Kruskal-Wallis test Among the different groups			Wilcoxon Test Comparison between Final Sampling -Initial sampling			
Parameters	Initial sampling	Final sampling	Placebo	ABDALA Low Dose	ABDALA High Dose	ABDALA Reversion
AG	0.266	0.259	0.655	0.593	0.180	0.180
ALAT	0.814	0.356	0.180	0.109	0.109	0.180
ALB	0.507	0.372	0.180	0.109	0.109	0.317
ALP	0.195	0.427	0.180	1.000	0.109	0.180
ASAT	0.261	0.154	0.655	0.593	0.593	0.180
BILD	0.290	0.551	0.317	0.109	1.000	0.655
BILT	0.871	0.901	0.180	0.109	1.000	0.180
CA	0.221	0.521	0.180	0.180	0.593	0.180
CHOL	0.710	0.467	0.655	0.593	0.285	0.180
CREA	0.145	0.339	0.655	1.000	0.109	0.180
GGT	0.554	0.381	0.180	0.593	1.000	0.655
GLUC	0.331	0.112	0.180	0.285	0.109	0.655
PHOS	0.710	0.310	0.180	0.655	0.285	0.655
TP	0.239	0.193	0.180	0.285	0.109	0.180
TRIG	0.860	0.561	0.180	0.109	0.109	0.655
UA	0.921	0.099	0.317	0.317	0.109	0.180
UREA	0.152	0.929	0.655	0.655	0.109	0.655

The significance level is 0.05.

Fever is a frequently reported adverse event following immunization [49], therefore, monitoring the results of the body temperature and the temperature in the administration site after the administration of a vaccine is of great relevance. In this sense, the absence of animals with values of body temperature and temperature in the administration site outside the physiological range strongly suggests that the ABDALA vaccine had no influence on the body temperature of animals. Similar findings were reported for other types of vaccines (adenovirus-vector-based vaccine, recombinant

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subunit protein vaccines as API, and nucleoside-modified messenger RNA vaccine) in NHP after administration [50-52], demonstrating that different vaccines are unlikely to cause temperature increases during the development of nonclinical program.

Clinical pathology comprises hematology and serum biochemical analysis. These are extremely powerful tools for both, assessing specific target organ toxicities during the in-life phase and providing correlative information for understanding both disease processes and the relevance of anatomical

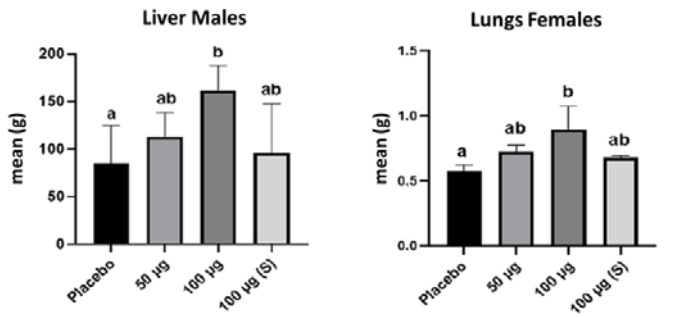

Image 7: Kruskal-Wallis Test´s results for relative weight: liver males (p = 0.0489), lungs females (p = 0.0135). Different letters indicate significant differences, Dunn's test.

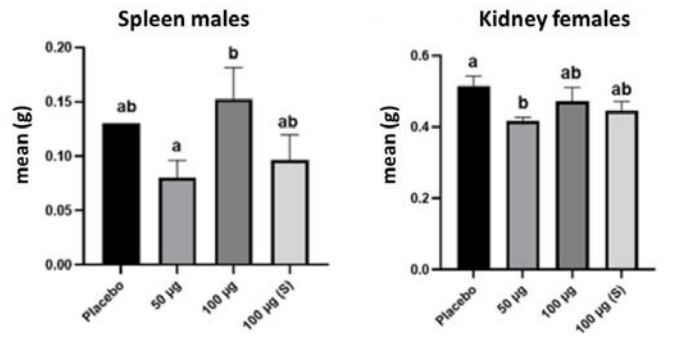

Image 8: Kruskal-Wallis Test´s results for absolute weight: spleen males (p = 0.0028), kidney females (p = 0.0219). Different letters indicate significant differences, Dunn's test.

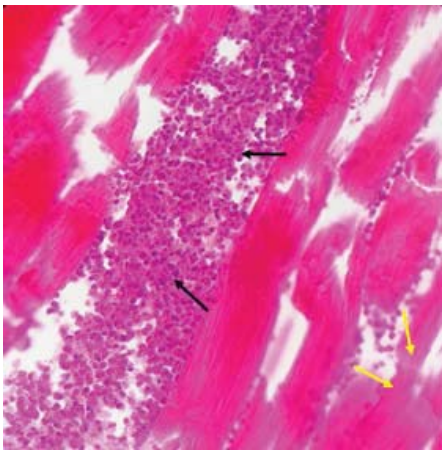

Image 9: Focal chronic inflammatory reaction at the administration site. Black arrows indicate the presence of mononuclear cells and yellow arrows atrophy of muscle fibers. H/E 400x.

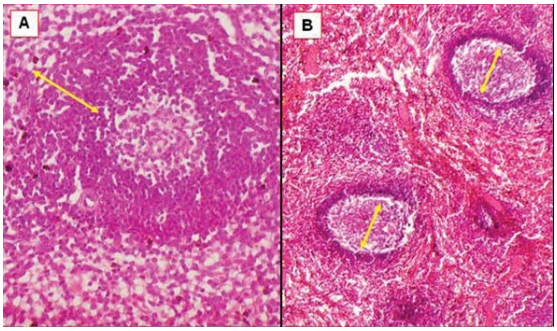

Image 10: A. Hyperplasia and hypertrophy of the white pulp with an increase in follicular lymphoid cells of the spleen (mantle zone) (arrows) (animal Group High Dose). B: Hypertrophy of the germinative centers of the spleen (arrows). H/E 400x-A and 100x-B.

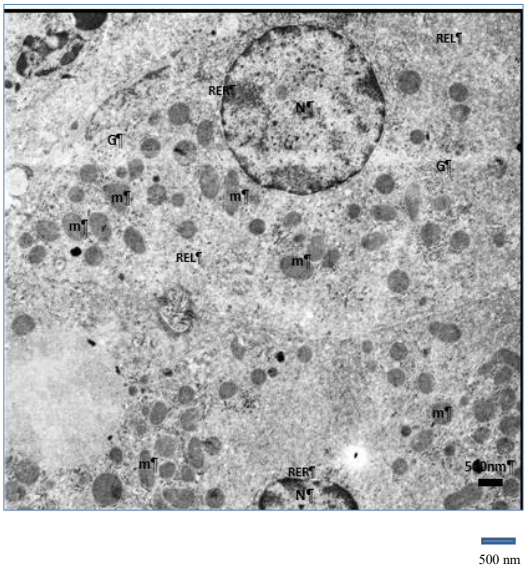

Image 11: Microphotography: Transmission Electron Microscopy.
Liver: Group II: ABDALA 50µg (low dose): Part of three hepatocytes can be seen with their nuclei (N), mitochondria (m), RER, REL, as well as glycogen (G), within normal limits.
Bar=500nm

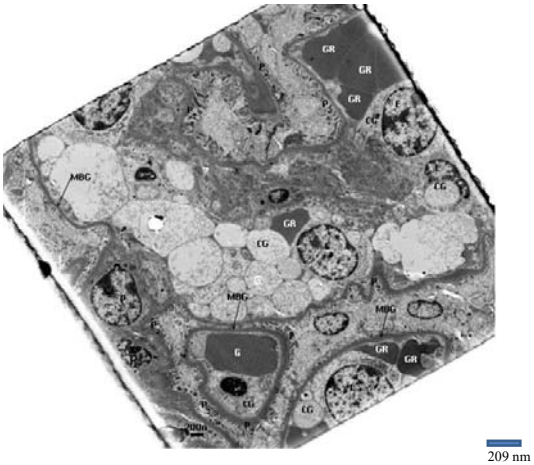

Image 12: Microphotography: Transmission Electron Microscopy.
Renal glomerulus: Group III: ABDALA 100µg (high dose): Part of several cells of the glomerulus can be seen, consisting of the glomerular capillary (CG), and the glomerular basement membrane (GBM) and the nucleus of an endothelial cell (E), and a red blood cell (GR), as well as the podocyte (P), with the feet of secondary podocytes (P2). Ultrastructure within normal limits.
Bar=200nm.

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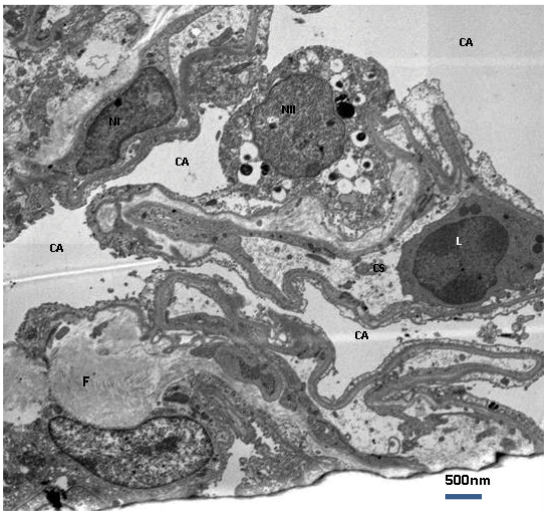

Image 13: Microphotography: Transmission Electron Microscopy.
Lung: Alveolar wall: Group III: ABDALA 100µg (high dose): Part of the alveolar wall is observed with type II (NII) and type I (NI) pneumocytes towards the alveolar cavity (AC), thus like a blood capillary (CS) with a leukocyte (L) inside and a fibroblast (F). Ultrastructure within normal limits.
Bar=500nm.

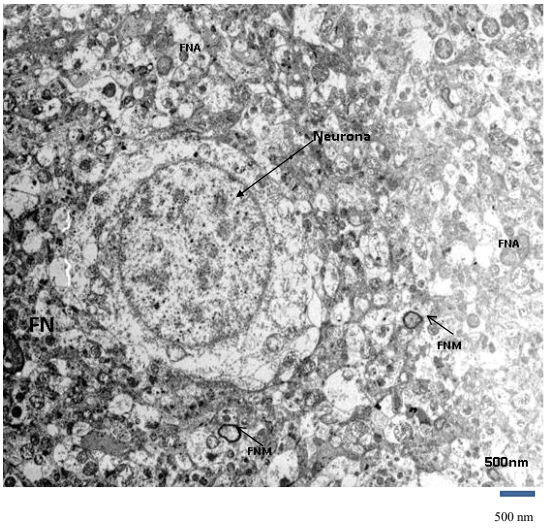

Image 14: Microphotography: Transmission Electron Microscopy.
Cerebral cortex, gray matter: Group III: ABDALA 100µg (high dose): Part of a neuron is seen, surrounded by two myelinated nerve fibers (FMN). A large number of unmyelinated nerve fibers (FNA) are detected in the gray matter. Ultrastructure within normal limits.
Bar=500nm.

findings [53]. The hematological parameters are considered useful tools for evaluating the nutritional and health status of animals, as well as for understanding the possible changes produced by external agents and diseases. These tests are more revealing than behavioral observation and less traumatic than biopsy and surgery. Factors such as age, sex, diet, maintenance conditions, stress, pregnancy, and anesthesia; among others, can affect the parameters assessed [54].

In this respect, Pfizer/BioNTech vaccine (messenger RNA vaccine) led, in rats, to a decrease in the total count of erythrocytes, haemoglobin, haematocrit, and reticulocytes and to an increase of neutrophils, eosinophils, and basophils [55]. Concerning a vaccine containing a recombinant protein as API, Banihashemi *et al.*, [50] reported no significant changes in the analysis of hematological parameters, electrolyte biochemical blood, liver function tests[[, coagulation tests, and renal function evaluation tests in BALB/c mice, Syrian hamsters, guinea pigs, or New Zealand White Rabbits.

Similar outcomes were found in this study after six administrations of the ABDALA vaccine: absence of alterations in the hematology and serum biochemical parameters, which supports the Nonclinical safety of the ABDALA vaccine and of recombinant subunit protein vaccines in general.

After the administration of the inactivated vaccine, no side effects were observed in the animals post-immunization [56]. In the case of the ABDALA vaccine, the chronic focal inflammatory reaction described at the administration site of the treated animals was associated with the adjuvant (aluminum hydroxide) present in the formulation. This agent enhances the immune response against antigens, and also causes inflammatory reactions that indirectly strengthen the immune response [57-59].

With respect to the thymus atrophies, these may be linked to a degenerative physiological process that begins when animals reach puberty and culminates with death [60,61]. This process depends fundamentally upon the hormonal secretions occurring at this stage. Hence, the thymus atrophies detected can be most likely associated with the developmental events taking place and not with a possible action of the ABDALA vaccine. Von Tresckow et al. [62] reported a case of thymic hyperplasia associated with mRNA SARS-CoV-2 vaccination in humans, which demonstrates the safety of ABDALA and protein subunit vaccines in general.

Conversely, the hyperplasia and hypertrophy of the spleen white pulp seem to be directly associated with the administration of the ABDALA vaccine and represent an increase in the activity of the APC and the proliferative activity of the antibody-forming splenic lymphoid cells, which shows increased activity of the immune system [59]. During the nonclinical development of the Moderna anti COVID-19 vaccine, (a vaccine based on messenger RNA) in rats, splenic alterations and/or splenic toxicity were consistently observed. These alterations ranged from splenomegaly (significant weight increases were frequently observed throughout all tested groups), decreased cellularity of the periarteriolar lymphoid sheath, increased cellularity of macrophages (e.g. in red pulp), neutrophilic infiltration in the red pulp, single cell necrosis of lymphocytes in the spleen (periarteriolar sheath), and increased extramedullary haematopoiesis [63]. Pfizer/BioNTech vaccine also provoked enlargement of spleen and inguinal lymph nodes in rats [55]. On this, the ABDALA administration (in the doses and frequency described in the present study) was associated with an increase in the activity and function of the spleen. Nevertheless, these modifications are not indicative of damage to the organ, and at a nonclinical level, evidence supports that it can be regarded as safer than other vaccines.

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Conclusion

In sum, the present study provides evidence of the lack of behavioral, anatomical, and functional alterations linked to a possible toxic effect of the ABDALA vaccine in *Chlorocebus aethiops sabaeus* monkeys. Hence, it strongly suggests that the ABDALA vaccine administered intramuscularly in a spectrum of doses and with a frequency much higher than that administered in humans is a safe product in *Chlorocebus aethiops sabaeus* monkeys.

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