

Nitrogen Metabolism, Carbohydrates and Sucrose in Young Plants of Paricá (*Schizolobium amazonicum*) Submitted to Different Dosages of Aluminum

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Abstract

The paricá [Schizolobium amazonicum Huber ex Ducke] is a native species of the Amazon region, belonging to the Fabaceae family and is a legume that has great social and economic importance, raw material for the manufacture of wood panels. Heliophilous, with wood of light density and good workability. Amazonian soils are highly weathered and characterized by high acidity, so Al harms the growth and development of sensitive plants, as well as the presence of resistant and/or tolerant plants. Thus, in this work, we sought to study the biochemical metabolism alterations generated in young plants of Schizolobium amazonicum Huber ex Ducke affected at different dosages of AlCl₃. The research was conducted in a greenhouse of the Federal Rural University of Amazonia, using pre-scarified paricá seeds, which were seeded in plastic containers of 3.5 L capacity, with black soil fertilized with NPK 15-15-20. On the 8th day after sowing (DAS), thinning of 5 seedlings was performed for 2, and on day 12 of 2 for 1 seedling. The treatments started after 1 month and 22 days of sowing and the plants were collected 24 days later. A completely randomized experimental design (RED) was used, with 6 treatments (Control, Al 15 mg/L⁻¹, Al 30 mg/L⁻¹, Al 45 mg/L⁻¹, Al 60 mg/L⁻¹ and Al 75 mg/L⁻¹) with 5 replicates each (one plant/pot) totaling 30 experimental units. The AlCl₃ dosages resulted in considerable changes in the biochemical variables evaluated, especially when the 75 mg·L⁻¹ dosage was applied. The considerable reduction in some variables such as nitrate reductase, glycine betaine and total soluble carbohydrates of these compounds to the applied $AlCl_3 \cdot 6H_2O$ dosages, is a way to create resistance mechanisms to try to overcome stress or as a way of showing limitations in the applied dosages. The species was sensitive to the $AlCl_3$ dosages, and resisted only 23 days to the imposed stress. However, it presented defense mechanisms that were able to prolong the physiological activities; since without them, the time of exposure to the stress would possibly be less.

Keywords

Dosages, Aluminum Stress, Schizolobium amazonicum

1. Introduction

The paricá (*Schizolobium amazonicum* Huber ex Ducke) has a rapid growth (cutting age of approximately 7 years), and wood with high prices in the domestic and foreign market, and therefore has been widely cultivated by timber companies in the North and Northeast of Brazil, proving to be a more viable alternative and fast as the eucalyptus, because the paricá has a cylindrical shaft and does not have branches in the first seven meters of the trunk, being a very coveted wood for the production of plywood, and considered the best species for the manufacture of MDF [1].

The species meets market expectations, as well as speed, productivity, profitability and environmental protection, since it is widely used in cultivated forests that replace predatory extraction, and can also be used to recover large areas of degraded pastures. It is characterized as a deciduous tree. The largest ones reached heights of 40 m and 100 cm in diameter at chest height (DAP), measured at 1.30 m from the ground [2]. According to Vidaurre *et al.* [3], of all the forest planted in Brazil today, 76.6% is Eucalyptus and 23.4% is Pinus, ensuring a response to growing world demand for fiber and environmental services.

It is an essentially heliophilic species (it fully realizes its entire life cycle in full sun), which does not tolerate low temperatures. It presents monopodial growth, although in the open, with a straight and clean shaft, due to good natural spillage or self-pruning [4]. Light wood, good workability and moderately dense ($0.30 - 0.62 \text{ g} \cdot \text{cm}^3$) are suitable for the manufacture of linings, toothpick, paper and wood panels. Due to its average density, in the state of Pará, most of the paricá plantation is used for the manufacture of plywood, and its residues and wood tips are used for the manufacture of MDF [5].

In fact, there is still just some researches related to forestry of this species, and there is still little technical information in research institutions. Therefore, the in-depth knowledge of paricá properties in relation to the different ages makes possible its more rational use, avoiding the cutting of trees with very low density, or at too young ages, which could lead to significant losses in the conversion processes besides the low quality of products obtained [3].

Numerous studies on the behavior of the species have been carried out; however, the information about the physiological processes among paricá cultivars is scarce. Thus, it is necessary to evaluate physiological and biophysical aspects to detect the existence of phenotypic variations in the plant, which would answer questions essential for proper management in paricá plantations [6].

The Amazon soils are highly weathered and characterized by high acidity, high aluminum saturation and low nutrient concentration, due to the high leaching rates. The predominant soil units in the region are the Latosols and Argisols, with low-load clays and predominance of kaolinite [7]. Thus, the effects of Al on plants have been the subject of many studies. These studies have shown that Al harms the growth and development of sensitive plants, as well as the existence of resistant and/or tolerant plants [8].

In this sense, information on paricá management should be considered of fundamental importance to support future silvicultural interventions in planting. On the other hand, research and investments in technology must also be integrated with the set of actions that permeate the companies' strategy. The objective of this work was to evaluate the Nitrogen metabolism, as well as the levels of total soluble carbohydrates and sucrose in young paricá plants (*Schizolobium amazonicum* Huber ex Ducke) submitted at different doses of aluminum.

2. Material and Methods

2.1. Plant Material

Were utilized paricá seeds provided by AIMEX (Associação das Indústrias Exportadoras de Madeira do Estado do Pará). They went through the mechanical scarification process with sandpaper 80 mm before the seeding (in order to break the dormancy of the seeds).

2.2. Growing Conditions

The experiment was conducted in a greenhouse belongs to UFRA (Federal Rural University of Amazon—Belém, during the months from April to July 2016, with geographic coordinates of 01°27′21″S, 48°30′16″W and average altitude of 10 m. The climate classification is Af according to Köppen and Geiger with average temperature of 26.8°C, relative humidity of 95% and photoperiod of approximately 12 hours throughout all the year.

The seeds, after scarification and 24 h immersed in water, were seeded in plastic containers with a capacity of 3.5 L, containing black soil previously fertilized with NPK (15-15-20), according to the need of soil fertilization as seen through soil chemical analysis carried out at the Soil Analysis Laboratory at UFRA/Belém (**Table 1**).

Five seeds were placed per pot and 4 days later the germination was observed

RESULT OF SOIL CHEMICAL ANALYSIS												
	Sample	pН	$C_{\rm org}$	O.M	Р	Ν	Κ	Ca	Mg	Al	H + Al	SBTVM
n.lab	Identification	H ₂ O KCl	g∙kg ⁻¹	$g \cdot kg^{-1}$	mg∙dm ⁻³	g⋅kg ⁻¹	cmol _c dm ⁻³				%	
20364	1	4.68 4.23	11.6	20	2.43	0	0.08	5.64	2.73	0.67	7.41	

 Table 1. Result of soil chemical analysis performed at the soil analysis laboratory at UFRA/Belém.

in about 98% of the seeds and soon after the first pair of leaves. Eight days after sowing, thinning of 5 to 2 seedlings was done, and four days later (12 days after sowing) the thinning was done from 2 to 1 seedling, at which stage the first pair of leaves was established and the second pair of leaves emerging. All vessels were irrigated in their field capacity (maximum water retention capacity) daily until the beginning of treatments and weed control was performed throughout all the process.

2.3. Exposure to Aluminum Dosages

After a month and 22 days in acclimatization, the plants received the first application of the aluminum dosages (Day 0) at the concentrations of 15, 30, 45, 60 and 75 mg/L⁻¹. After seven days the second application of the dosages (Day 7), as well as the third (Day 14) and the fourth application (Day 21) occurred. Aluminum was applied in the form of 95% aluminum chloride hexahydrate (AlCl₃·6H₂O), and the pH of the solution was maintained in 4.8 with 0.1 mol·L⁻¹ and NaOH on the solution replacement.

2.4. Collection and Storage of Material

On the 23rd day of treatment or 52 days after sowing (DAS), the plants reacted to the dosages by yellowing the leaves and the defoliation itself. Therefore, the plants were collected after 2 months and 17 days of sowing and 24 days after the beginning of the treatments. The plants were then separated into leaf, stem and root and wrapped in foil and stored in a freezer at -80° C. To determine the biochemical analysis, the material was taken to the forced air ventilation oven at 65°C for 48 h. The dried material was milled until a fine powder was obtained and stored in sealed plastic vials until use in the tests.

2.5. Biochemical Evaluations

The biochemical analysis was realized at Laboratório de Estudo da Biodiversidadeem Plantas Superiores (EBPS), located at Federal Rural University of Amazônia (UFRA), Belém, Pará. For this, were determined the contents of Nitrate (NO_3^-) and Free Ammonium (NH_4^+) [9], with adaptations made byLobato and Ferreira [10]; activity of the nitrate reductase enzyme (RNO_3^-) [11]; Total Soluble Aminoacids (TSAA) [12], with adaptations made by Lobato and Ferreira [10]; Total Soluble Proteins (TSP) [13]; Proline [14], with adaptations made by Lobato and Ferreira [10]; Glicine-betayne [15]; Total Soluble Carbohydrates [16]; Sucrose [17].

2.6. Experimental Design and Statistical Analysis

The experimental design was completely randomized, with 6 treatments (Control, Al 15 mg/L, Al 30 mg/L, Al 45 mg/L, Al 60 mg/L and Al 75 mg/L) with 5 repetitions each (one plant/vessel) totalizing 30 experimental units. The 5 replicates of each treatment were submitted to the same type of induction throughout the evaluation period. The data were submitted to statistical analysis, using analysis of variance (ANOVA) and regression analysis (using dosages) in the program Sisvar version 5.4, and the means compared by the Tukey test at the 5% probability level.

3. Results and Discussion

3.1. Content of NO₃⁻ and the Activity of Nitrate Reductase (RNO₃⁻)

It was observed that the application of the 45 mg·L⁻¹ dosage of AlCl₃ promoted in the leaves a decrease of 41.66% in Nitrate concentration, from 0.24 (30 mg·L⁻¹) to 0.14 μ M·g⁻¹ MS (45 mg·L⁻¹) (**Figure 1(a**)). In the roots the decrease was observed from the dosage of 30 mg·L⁻¹, from 0.12 (15 mg·L⁻¹) to 0.07 μ M·g⁻¹ MS (30 mg·L⁻¹) (**Figure 1(b**)). In general, Nitrate was concentrated in larger amounts in the leaves. It was verified that the nitrate reductase showed a continuous decrease in its concentration in the leaves, varying from 1.96 (Control Treatment) to 0.06 NO₂⁻. g MF⁻¹ h⁻¹ (45 mg·L⁻¹ AlCl₃), presenting a slight increase in concentration in the last two treatments (60 and 75 mg·L⁻¹ AlCl₃), (**Figure 1(c)**). In the roots, nitrate reductase concentrations also decreased considerably, ranging from 0.27 (Control Treatment) to 0.07 NO₂⁻. g MF⁻¹ h⁻¹ (15 mg·L⁻¹ AlCl₃), oscillations occurring throughout the treatments, but maintaining values below that obtained in the control treatment (**Figure 1(d**)).

According to Netto [18], NO_3^- is the main nitrogen form absorbed by plants, and its predominance is basically due to the pH of the solution, usually close to neutrality. That is, in an acidic environment due to the presence of aluminum, the NO_3^- tends to decrease. The accumulation of NO_3^- observed in the leaves of the plants under the dosages of 15 and 30 mg·L⁻¹ AlCl₃ (Figure 1(a)) and in the roots at the 15 mg·L⁻¹ dosage (Figure 1(b)) show that in these dosages $NO_3^$ is still capable to carry out their biochemical activities in the plant. In the following dosages, the toxic environment due to the aluminum concentration decreases the NO_3^- entry in the roots and consequently limits the NO_3^- absorption and activity in the plant, resulting in a fall in their contents, as observed in Figure 1(a) and Figure 1(b). Some authors suggest that Al^{3+} causes inhibition of uptake and reduction of the NO_3^- transport system [19].

The levels of nitrate in the roots increased, justified by the decrease in the activity of RNO_3^- (EC 1.6.6.1), which favored the accumulation of nitrate in the



Figure 1. Content of nitrate ((a) leaves; (b) roots) and nitrate reductase ((c) leaves; (d) roots) in young plants of *Schizolobium amazonicum* Huber ex Ducke submitted to the dosages of AlCl₃.

cell's cytosol, compromising the process of reducing NO_3^- to NO_2^- and conversion to NH_4^+ in the plastid.

According to Galangau *et al.* [20], the activity of the RN enzyme in leaves and roots can be induced by the presence of the substrate (NO_3^-). If nitrate occurs a stimulus to the synthesis of this enzyme, whereas an intense stress can reduce its production of 20% to 50%.

The results show that the concentrations of toxic aluminum caused a significant reduction in the activity of RNO_3^- (EC 1.6.6.1), since the first dose (15 mg·L⁻¹), going from 2 NO_2^- g·mf⁻¹·h⁻¹ to 0.06 in leaves and from 0.27 $\text{NO}_2^$ g·mf⁻¹·h⁻¹ to 0.17 in roots. Probably, these results occurred due to the decrease in the transpiratory flow and with this the transport of nitrate (NO_3^-) to the leaves (since this enzyme is highly induced by its substrate), besides the predominance of NH_4^+ in the soil making the form of NO_3^- unavailable to the plants [21]. That is, due to the presence of aluminum, the RN enzyme decreased its activity, causing the nitrate to accumulate in the cytosol of the roots.

 Al^{3+} can directly affect the enzyme RNO_3^- (EC 1.6.6.1) because it causes molybdenum deficiency, necessary for the synthesis and activation of nitrate reductase [22]. This fact explains the decrease in RNO_3^- (EC 1.6.6.1), as well as the competition of aluminum with magnesium, because the lack of magnesium sti-

mulates the phosphorylation of the serine residues, which interact with an inhibitory protein, resulting in the reduction of the activity of the enzyme studied [23].

3.2. Free Ammonium, Total Soluble Aminoacids and Total Soluble Proteins

It was observed that the application of the 15 and 30 mg·L⁻¹ AlCl₃ dosages resulted in a decrease in the free ammonium concentration, with values of 74.68 and 65.36 mmol of NH_4^+/kg of DM, respectively, showing an increase in its concentration from of the 45 mg·L⁻¹ dosage (**Figure 2(a)**). The roots presented similar behavior as the dosages increased, but presented a lower Ammonium concentration than in the leaves (82.48 mmol of NH_4^+/kg of DM in Control leaves and 40.90 in Control roots) (**Figure 2(b**)). That is, difference of more than 50% in the ammonium contents between leaves and roots.

The total soluble amino acids showed, in general, a gradual decrease in the leaves according to the increase of the AlCl₃ dosages, presenting the value of 1.46 $\mu M \cdot g^{-1}$ MS in the control treatment and 0.93 $\mu M \cdot g^{-1}$ MS in the dosage of 75 $mg \cdot L^{-1}$, showing a decrease of 33.97% in their contents (Figure 2(c)). On the other hand, the roots showed a gradual increase of the amino acids contents until the dose of 45 mg·L⁻¹, followed by drop in the last two treatments (Figure 2(d)). The total soluble protein contents in the leaves decreased as the dosages increased, with a value of 25.09 mg/g DM in the control plants and 24.26 mg/g DM in the 75 mg·L⁻¹ dosage, resulting in a decrease of 3.31% (Figure 2(e)). There was a higher content of total soluble proteins in the roots (26.37 mg/g MS -Control treatment and 26.15 mg/g MS - 75 mg·L⁻¹ dosage), with the highest levels observed at 15 and 30 mg/g MS and continuous decrease until the last treatment (Figure 2(f)). The accumulation of ammonium in the leaf tissue in the roots from the dose of 45 mg· L^{-1} probably originates from both direct absorption, nitrate reduction and deamination of nitrogen compounds [24]. This increase in the concentrations of free ammonium probably could have induced other formation routes, through protein breakdown [25]. According to Ferreira et al. [26] another response to this concentration of free ammonium in foliar tissues would be the increase in photorespiratory activity. According to Oliveira Neto [27] the reduction of the photosynthetic activity provides ATP and the formation of reducing power (NADPH, FADH and NADH) for metabolism.

In higher plants, the metabolism of carbohydrates and amino acids is co-regulated [28]. Thus, carbon skeletons from carbohydrate metabolism [29] are required for conversion of nitrogen into amino acids. This may explain the fact that the free amino acids (**Figure 2(c)** and **Figure 2(d)**) decreased simultaneously with those of carbohydrates (**Figure 4(a)** and **Figure 4(b)**) in both leaves and roots. In addition to being able to act on osmotic adjustment [30], these molecules can help in the control of cytosolic pH, detoxification of excess NH_4^+ [31], maintenance of ionic homeostasis, C/N ratio and [32]. These solutes



Figure 2. Content of free ammonium ((a) leaves; (b) roots), Total soluble aminoacids ((c) leaves; (d) roots) and Total soluble proteins ((e) leaves; (f) roots) in young plants of *Schizolobium amazonicum* Huber ex Ducke submitted to dosages of AlCl₃.

can also be a source of usable energy and nitrogen storage during infra-optimal growth periods [33] and help with the removal of free radicals [34]. It has been suggested that increased protein production in plants exposed to Al (15 mg·L⁻¹ dosage in roots, **Figure 2(f)**) may be due to increased expression of genes directly or indirectly related to Al tolerance [35].

It was observed an increase in the protein content in the roots submitted to 15 and 30 mg·L⁻¹ of AlCl₃, followed by decrease in the contents in the last



Figure 3. Contents of proline ((a) leaves; (b) roots) and glycine bethayne ((c) leaves; (d) roots) in young plants of *Schizolobium amazonicum* Huber ex Ducke submitted to dosages of AlCl₃.

treatments. Thus, the proteins were related to the sensitivity characters in the studied variety. Additionally, a relationship between the variations in soluble and amino acid contents indicating a cause-effect relationship of protein bio-synthesis or proteolysis was not observed in this study.

3.3. Proline and Glycine Betaine

The concentration of proline in the leaves increased until the dosage of 30 mg·L⁻¹, after which there was a decrease in the dosages of 45 and 60 mg·L⁻¹, followed by a dose increase of 75 mg·L⁻¹, with a value of 5.93 μ M·g⁻¹ DM (**Figure 3(a)**). In the roots, the proline concentration was lower than in the leaves, showing first increase in the contents at 30, 45 and 60 mg·L⁻¹, followed by a decrease in concentration at 75 mg·L⁻¹, with a value of 1.32 μ M·g⁻¹ DM (**Figure 3(b)**). It was observed that the application of the 15 mg·L⁻¹ dosage promoted a considerable decrease in the glycine-betaine concentration (2.84 mg/g DM) when compared to the concentration in the control treatment, with a value of 5.70 mg/g DM, signaling a decrease of approximately 50% (**Figure 3(c)**). The glycine-betaine content in the roots showed higher values than in the leaves, with 7.34 mg/g MS in the control treatment and 6.33 mg/g MS in the dose of 75 mg·L⁻¹ of AlCl₃, a decrease of 13.76% in its contents (**Figure 3(d)**).

Proline content tends to accumulate in plant cells subjected to stress, with the objective of being used as energy after the end of stress, with the redistribution of nitrogen and carbon, for the recovery of physiological activities in the plant. It also plays an essential role in the stabilization of proteins and cell membranes in plant cells in the presence of high levels of osmolytes (FAROOQ *et al.*, 2009) [36].

The highest proline concentration in leaves and roots (30 mg·L⁻¹) is possibly derived from biosynthesis and its accumulation in the vacuole or cytosol, maintaining water balance and preserving the cellular integrity of proteins, enzymes and membranes for the continuity of vital activities and, constituents of one of the adaptive strategies of the plants to the multiple effects caused by the stresses, considering that it is an osmoprotective amino acid [37].

Thus, the increase of proline levels in leaves and roots of 15 and 30 mg·L⁻¹ can activate several cellular functions: carbon reserve and nitrogen used in growth for restoration after stress, osmotic adjustment, membrane protein stabilizer, detoxification excess ammonia and free radical scavengers. This result corroborates the hypothesis that amino acid biosynthesis was affected by lower Al-induced carbohydrate availability [38].

According to Silveira *et al.* [39], there is evidence that the biosynthesis of this amino acid could also be associated with the regulation of the cytosolic pH or mediation of the increase of the NADP+/NADPH ratio, influencing the carbon flux due to the oxidative pathway of pentose phosphate.

The level of proline in the roots showed less activity at the dose of 75 mg·L⁻¹, which corroborates with the hypothesis that the amino acid biosynthesis was affected by the lower availability of carbohydrates (**Figure 4(d**)) induced by Al.

The synthesis of glycine-bethayne is due to the probable formation of amino acids through the degradation of the proteins, possibly of the photorespiration and processes of desaminações [40].

The main function of glycine bethayne in leaves (**Figure 3(c)**) is to protect the membranes of thylakoids in order to maintain photochemical efficiency in photosynthesis, as observed in **Figure 4(c)** in all $AlCl_3$ dosages, the attempt of photosynthetic protection. However, this defense mechanism was not enough to conserve the photosynthetic pigment contents, which decreased as the $AlCl_3$ dosages increased.

The greater accumulation of glycine betaine in the root system (**Figure 4(d)**) indicates that this is the first organ affected by aluminum in the plant. Thus, this accumulation occurred to protect plant metabolism since glycine bethayne can act as the compatible osmolyte and maintain the balance of water between the plant cell and the environment, stabilizing the macromolecules [41].

3.4. Total Soluble Carbohydrates and Sucrose

The total soluble carbohydrates content in the leaves ranged from 43.63 (Control)



Figure 4. Contents of total soluble carbohydrates ((a) leaves; (b) roots) and Sucrose ((c) leaves; (d) roots) in young plants of *Schizolobium amazonicum* Huber ex Ducke submitted to dosages of AlCl₃.

to 27.24 mg/g DM (75 mg·L⁻¹), decreasing 37.60% (**Figure 4(c)**). In the roots the concentration was lower than in the leaves, with values from 26.15 (Control) to 20.38 mg/g DM (75 mg·L⁻¹), a decrease of 22% (**Figure 4(d)**).

The concentration of sucrose in the leaves presented values ranging from 4.91 mg/g DM in the control treatment to 9.01 mg/g DM in the dosage of 75 mg·L⁻¹, showing an increase in the concentrations from the dose of 45 mg·L⁻¹ (**Figure 4(c)**), an increase of 83.50% in its contents. While in the roots the variation in sucrose concentration was much less expressive, presenting values of 2.79 mg/g DM in the control treatment at 3.95 mg/g DM at the dose of 75 mg·L⁻¹ (**Figure 4(d)**), with a 41.60% increase.

Carbohydrates are considered an important category of compatible solutes, which are accumulated in response to various stresses [42]. Thus, according to Tabuchi *et al.* [43], the accumulation of carbohydrates in leaves and roots up to the dose of 60 mg·L⁻¹ may contribute to the reduction of the osmotic potential and lower inhibition of root growth in Al tolerant plants. the carbohydrate concentrations decreased by 37.60% in the leaves and 26.15% in the roots, probably due to the low photosynthetic activity, considering the values presented by the photosynthetic pigments in the dose of 75 mg·L⁻¹.

In a situation of stress, the metabolism of carbohydrates is altered, and there is

often the conversion of other sugars to sucrose [44]. In most plants, sucrose is the main sugar exported from the synthesis sites (leaves) to the consuming regions (stem, vegetative buds, roots and reproductive organs) where it will be used for growth and/or storage. The hexoses released from the hydrolysis of sucrose can be used in anabolic or catabolic processes and also to the supply of reducing sugars, being much used for the process of osmotic adjustment [45].

Sucrose content in paricá leaves increased from the dose of 45 mg·L⁻¹ (Figure 4(c)) and also in the roots from the 15 mg·L⁻¹ dosage (Figure 4(d)). This behavior is due to the biosynthesis of sucrose, and is probably promoted by the greater activity of the sucrose phosphate synthase enzyme that acts on the photosynthetic cell located in the cytosol, with the function of protecting the integrity of membranes and proteins under stress conditions [46]. However, Lee *et al* [43] argue that the accumulation of soluble carbohydrates occurring during aluminum stress is more a function of starch hydrolysis (Figure 4(a)), through the enzyme α and β -amylase in sugars. These sugars can be broken and then transported to the various drains in the form of sucrose.

4. Conclusions

1) Biochemical variables indicate that paricá (*Schizolobium amazonicum* Huber ex Ducke) shows sensitivity to aluminum toxicity in the time and in the dosages studied.

2) The dosages of aluminum chloride hexahydrate 95% (AlCl₃· $6H_2O$) applied for 23 days resulted in considerable changes in the biochemical variables evaluated, especially when the dose of 75 mg·L⁻¹ was applied.

3) For the specific conditions of this work, the *Schizolobium amazonicum* Huber ex Ducke species was sensitive to the AlCl₃ dosages, and only resisted the stress imposed for 23 days. However, it presented defense mechanisms that were able to prolong the physiological activities; since without them, the time of exposure to the stress would possibly be less.

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