Changes in the Respiratory System of Rats Exposed to the Herbicide Paraquat

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Abstract: *The use of herbicides is constantly being adopted in developing countries who need to increase productivity in the agricultural sector. The world market for herbicides grew 39% between 2002 and 2011 and predicted a growth of 11% in 2016. The Paraquat® is herbicide with biological activity used worldwide. The present work had as objective to examine the respiratory system of rats exposed to paraquat. Wistarrats, with body weight of 225±25 g were used in this study. The animals were randomly divided into three groups, a group in which it was administered of saline (C), other group were administered paraquat to 10 mg/kg (P10), and a third group was administered paraquat to 30 mg/kg (P30), intraperitoneally. All concentrations used are considered environmentally relevant. The values of the airway resistance (R_N), tissue elastance (H), tissue resistance (G) and hyperresponsiveness of the airway smooth muscles of the animals, were statistically significant. In conclusion, ours results demonstrated the harmful character that exposure to paraquat can cause in the respiratory system of rats. More detailed studies are required that can quantify the level of exposure that the population undergoes when eating some foods, which may present remnants of this pesticide in its composition.*

Keywords: *paraquat, respiratory system, exposure, herbicide.*

1. INTRODUCTION

The world agricultural pesticide industry was transformed by several years of growth. According to the Union of manufacturers of agrochemicals from Brazil, between the years 2000 and 2010, the sale of pesticides in the world grown 96.7%, and in Brazil, this growth was 189.6%. In the period from 2006 to 2011, sales of agrochemicals increased on average 72% and between the main classes of products, sales of fungicides to combat diseases such as soybean rust has tripled, while the insecticide advanced 84% and the herbicides, 44%.(TUIAP, 2012).

Agricultural herbicides are composed of biological activity of extreme importance for the food industry worldwide (Sphefeson et al. 2006). These are among the most common pesticides (Chan et al., 2007) and your use is growing worldwide, mainly in developing countries (Gianessi, 2013). The world market for herbicides grew 39% between 2002 and 2011, and predicted a growth of 11% in 2016. The use of herbicides is constantly being adopted in developing countries need to increase productivity in the agricultural sector (Zhang, 2003).

Studies evidencedthat some herbicides pose a potential risk for humans and other life forms, in addition to harmful effects to the environment (Forget, 1993). The poisoning by herbicides and other chemicals used in agriculture, represents a public health problem throughout the world. More than 20,000 deaths and 2 million hospitalizations occur annually due to poisoning with pesticides and chemicals used for agriculture (Khalighi et al., 2016).Dermal exposure or by spraying, is usually associated with only limited and localized lesions. However, accidental or deliberate ingestion, results in an extremely high mortality rate, and can cause local irritation of the mouth, oropharynx and esophagus and multiple organ failure (cardiac, respiratory, liver and kidney) (Chao et al., 2016).

Among the most widely used agricultural herbicides, paraquat (1.1-dimethyl -4.4'bipyridyl dichloride) (Figure 1), a fast-acting herbicide and low cost (Gunnel et al., 2007) which is commonly applied in cotton, rice, banana, coffee, sugar cane, corn, soybeans and others (NHSA, 2010). The use of paraquat contributes to maintaining a weed flora, because defeat only the growth of the aerial part and does not affect the germination of new seedlings, thus, the vegetation if reset (Martins, 2013).
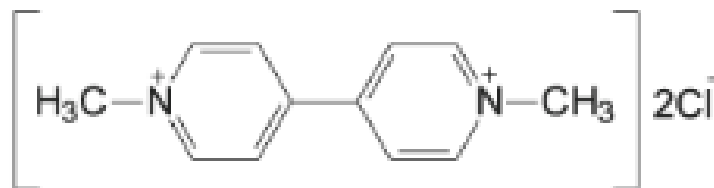

Fig1.*Chemical structure of paraquat*

Paraquat is manufactured by several countries, such as China, USA, Italy and United Kingdom, and in most countries, the raw material is imported and the formulation of the product shall be made on site (Serra et al., 2003). Occupational exposure to paraquat does not entail any danger to health if it is managed safely, however, the number of cases of poisoning is hight, voluntary or accidental (Serra et al., 2003).

Reports carried out in countries using paraquat in the agricultural sector have shown that paraquat poisoning presents a mortality rate between 50% and 90%, representing 13% of all cases fatal (Cha et al., 2014). The Japan has registered significant numbers of deaths arising from exposure to paraquat, more than 1000 per year, whereas in Ireland the mortality reaches 78% and in Costa Rica, 60% (Xarau et al., 2000).

The toxicity of paraquat affects lungs, kidneys, liver, pancreas, heart and nervous system, being the most affected lungs (Naito et al., 1987; Serra et al., 2003). The lungs are the main target organs of paraquat, leading to severe injury and fibrosis, causing epithelial cellular injury due to your action on type I and type II pneumocytes in addition to edema, hemorrhage, infiltration of inflammatory cells and cellular espaços (Rocco et al., 2004; Samson et al., 2006; Choi et al., 2013), however, the oxidative stress plays a crucial role in the pathogenesis of paraquat-induced lung injury (Tian et al., 2014).

In view of the above, and for being the lung, one of the organs most affected by paraquat, the present work had as objective to examine the respiratory system of rats exposed to paraquat (1.1-dimethyl -4.4'bipyridyl dichloride), in an attempt to aggregate knowledge about the real health risks that farmers are submitted by exposure to agricultural products containing paraquat as an active ingredient.

2. MATERIAL AND METHODS

2.1. Animals

This study followed all the regulations in force for the maintenance of good animal welfare. All protocols used were previously approved by the Commission on ethics to the use of animals from the Universidade Estadual do Ceará (Protocol Nº 12237681/28).

Albino rats, Rattus novergicus, Wistar variety, female, with body weight of 225±25 g were used in this study. The animals were randomly divided into three groups. A group in which it was administered 0.2 mL of saline (i.p.), known as Group C (n = 8). In the other group were administered paraquat to 10 mg/kg diluted in saline solution 0.2 mL (i.p.), called P10 Group (n = 8). And a third group was administered paraquat to 30 mg/kg diluted in saline solution 0.2 mL (i.p.), called Group P30 (n = 8). All concentrations used are considered environmentally relevant.

2.2. Experimental Protocol

24 hs after administration of saline solution (C) or paraquat (P10 and P30), the animals were anesthetized with sodium pentobarbital (30 mg/kg, Hypnol® 3%, Syntect, Brazil) intraperitoneally, and undergoing tracheotomy for the insertion of a cannula of 14 gauge (Eastern medikit LTD.) which was secured to the trachea. The cannula was then connected to a ventilator for small animals (Scirec©-flexVent®) computer controlled. The animals were ventilated in basal patterns at a frequency of 90 breaths/min, a tidal volume of 10 mL/kg, with pressure limit of 30 cmH$_2$O positive end-expiratory pressure (PEEP) of 3 cmH$_2$O.

So that the lung function variables were collected from reliable way, it is necessary that the animal is sufficiently sedated and paralyzed so there's no interference of their respiratory muscles. To this end, the vents Pancuronium bromide (0.5 mL/kg, Pancuron®, Cristália, Brazil) was administered intraperitoneally.

Pulmonary function analyses were performed after waited five minutes for the animal accommodation, verification of possible leaks, obstructions, fixes the position of the animal in relation to the fan and the confirmation that the animal was not performing spontaneous inspirations. Initially proceeded to the standardization of mechanical conditions of the respiratory system with two deep inflation (IP) with pressure limit of 30 cmH$_2$O and 6 s duration. To reduce the effect caused by broncoprotetor IP's (Kapsali et al., 2000) the animals were ventilated by a 20 minute period on basal patterns.

Soon after, the impedance of the respiratory system (Z_{rs}) was measured by forced oscillation technique in sequence at intervals of 30 s during 6 minutes. The Z_{rs} wasdetermined by measuring the volume and pressure of the piston in the cylinder while fan 3 disturbances of oscillatory volume were delivered to the airway. These disturbances were carried through overlapped sine waves 13 with changes in amplitude and frequency (1-20.5 Hz). The frequencies were fixed in mutually conditioned values to reduce harmonic distortion that can occur in non-linear systems (HANTOS et al., 1992).

Before the start of the protocol was obtained necessary dynamic calibration signals to compensate for mechanical ventilator influences in subsequent measurements Z_{rs}. The Z_{rs} was determined by Fourier transform of signals of piston fan volume and pressure of the cylinder as described previously (Hirai et al., 1999). The Z_{rs} was adjusted to constant phase model (Eq. 1 and 2):

$$Z_{rs} = R_N + I(2\pi f)i + \frac{G - Hi}{(2\pi f)^\alpha} \tag{1}$$

with,

$$\alpha = \frac{2}{\pi}\tan^{-1}\left(\frac{H}{G}\right) \tag{2}$$

Where: R_N represents the Newtonian resistance, related central airway resistance, $i = \sqrt{-1}$, f is the frequency (Hz), I is the inertance of the airways, and G and H respectively feature the dissipative and elastic properties of the lung tissue (Hantos et al., 1992).

2.3. Acetylcholine Dose-Responsecurve

For the analysis of the hiperresponsividade of the smooth muscle of the Airways, soon after the analysis of the mechanics of the respiratory system, we collected data on the dose-response curve to acetylcholine (ACh). At the beginning of this protocol, in sequence, the mist of saline solution, followed by nebulization of different concentrations of acetylcholine: 1, 2, 4, 8, 16, 32 and 64 mg/kg.

All atomizations were held over a period of 30 seconds, by means of the ultrasonic nebulizer (Inalasonic® model, NS) coupled to the inspiratory mechanical fan branch. Soon after the nebulization, the impedance of the respiratory system (Z_{rs}) was measured by forced oscillation technique in sequence at intervals of 30 s for 5 minutes.

2.4. Statistical Analysis

Results are presented as mean ± SD, where n represents the number of samples. Data normal distribution and homogeneities of variances were tested with Kolmogorov-Smirnov (with Lilliefors's correction) and Levene median tests, respectively. If both conditions were satisfied, Student's t-test was used. If any condition was refused, Mann-Whitney non-parametric test was used instead. A difference was considered significant if $p < 0.05$.

3. RESULTS

The results concerning the analysis of respiratory impedance (Z_{rs}) are presented in Figure 2. The values of the variables related to airway resistance (R_N), the tissue elastance (H) and (G) of tissue resistance groups P10 and P30 compared to the control group were statistically significant.
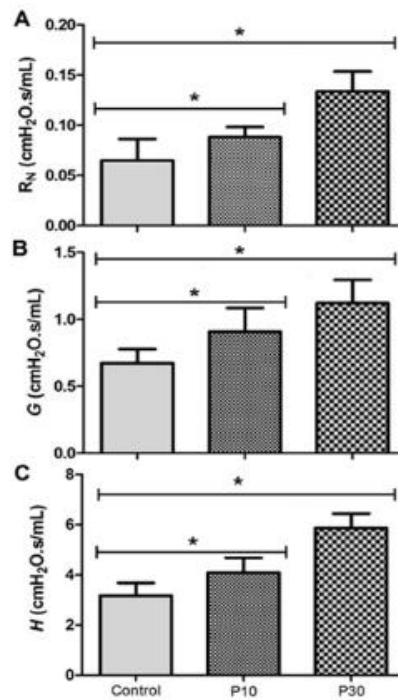
Fig2. *.Airway resistance (R_N) in A, tissue resistance (G) in B, and tissue elastance (H) in C. values are represented by mean ± standard deviation of the mean. *represents statistically significant values compared to the control group (p < 0.05).*

The absolute values, for the calculation of the impedance of the respiratory system (Z_{rs}), for purposes of calculating variables for R_N, G and H, control groups, P10 (10 mg/kg of paraquat) and P30 (30 mg/kg of paraquat), are shown in table 1.

Table1. *Mechanical data of animals exposed to air and paraquat.Values are mean ± SD. * p < 0.05, statistically significant difference.*

Measure	Group	Value	*p*value (*t student*)
Newtonian Resistence (R_N) (cmH$_2$O/mL)	Control	0.064±0.021	
	P10	0.088±0.907	*p*= 0.0292[*]
	P30	0.133±0.019	*p*< 0.0001[*]
Tissue Resistence (G) (cmH$_2$O/mL)	Control	0.67±0.10	
	P10	0.90±0.17	*p*= 0.0064[*]
	P30	1.12±0.17	*p*< 0.0001[*]
Tissue Elastance (H) (cmH$_2$O/mL)	Control	3.17±0.50	
	P10	4.08±0.59	*p* = 0.0068[*]
	P30	5.86±0.57	*p*< 0.0001[*]

The dose-response curve acetylcholine built with the objective of evaluating the hiperreatividade of the smooth muscle of the Airways is presented in Figure 3. The hiperresponsividade Airways is defined as an excessive narrowing of the Airways in response to a variety of chemical and physical stimuli that have little or no effect in healthy individuals (Brusasco et al., 2003).
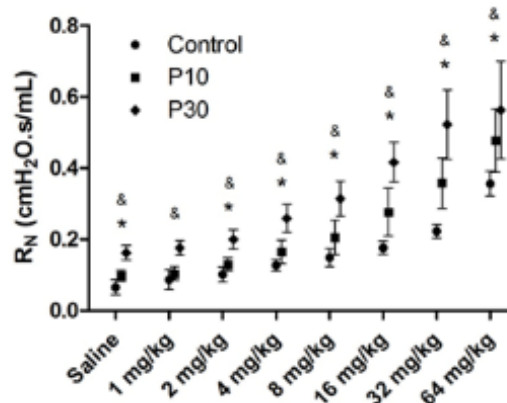

Fig3. *Reactivity of the Airways. Values are mean ± SD. (*) P10 statistically significant difference (p < 0.05) and (&) P30 statistically significant difference.*

4. DISCUSSION

Herbicides control weeds by interfering in how they grow. It is occurs by blocking the germination of seeds or seedlings, preventing the production of essential proteins, carbohydrates or lipids (oils and fats) by plants, or dehydrating their leaves and stems (Powles et al., 2006; Santra, et al., 2008). Paraquat acts in the process of photosynthesis. When solar energy is captured by chlorophyll, it is transferred into a stream of electrons through Photosystem I. Paraquat deflects this flow, which results in the production of free radicals, highly reactive, destroy cell membranes very quickly, pouring the contents manifesting itself as yellowing and dryness. Almost all green plants are affected by paraquat, which makes it a non-selective broad spectrum herbicide (Powles et al., 2006; Santra, et al., 2008).

The pesticide poisoning occurs, generally, by the lack of knowledge that they can penetrate the metabolism by direct consumption of food (fish, molluscs, crustaceans, beef, vegetables, legumes). Other times, the poisoning occurs due to misinformation of their symptoms and on ways of penetration, accumulation and transport of the poison in the human body, about what is the pesticide, and how do you get the product (Castro, 2009).

In relation to ours results, the Newtonian resistance (R_N) has been used as a good estimate of the total airway resistance (Bates, 2009). Thus, it can be assumed that significantly larger values in P10 and P30, exposed to 10 mg/kg and 30 mg/kg, compared to the control group, may represent a greater narrowing or increase in rigidity of airway smooth muscle. Increased airway resistance (R_N) is due to a decrease in the internal diameter of the airway, which can be attributed to the presence of edema, fluid accumulation, broncoconstricção and/or lung volume reduction (Rocco, 2004).

The tissue resistance (G) reflects the viscous dissipation of energy in the pulmonary tissue parameter that is also altered due to distortion of the lung parenchyma, this explains the significant increases in the values of G and H in the presence of paraquat, since there was a relation With the increase in the airway resistance parameter (R_N). Exposure to paraquat generates physiological characteristics of acute lung injury (ALI) (Rocco et al., 2001). Collagen and other elastic components are increased in the ALI process due to a fibroelastosis process initiated early in the first 24 h of lesion evolution (Rocco et al, 2001). Exposure to paraquat can significantly induce the expression of connective tissue growth factor, which results in a process of pulmonary fibrosis (Huang et al., 2015).

The tissue resistance (G) and the tissue elastance (H) are related to the intrinsic properties of the fabric. There are several hypotheses to explain its alterations, one of which is due to the modification in the properties of alveolar surfactant and/or loss of lung capacity due to the presence of liquid on the alveolar surface (Gregory et al., 1991, Rocco et al., 2003). Another pathway is through the influence that the narrowing of the airways, resulting in what could be called a distortion of the lung parenchyma with the closure of small airways. In the first 24 h, fluid accumulation occurs in the interstitium and in the air spaces of the lung, leading to hypoxemia, decreased pulmonary compliance and increased respiratory work (Rocco et al., 2003).

Bronchoconstrictors have been widely used in research of animals with lung inflammation because of the notion that the primary problem in asthma is the excessive shortening of smooth muscle airway, whether due to an exacerbation of smooth muscle response capacity itself (Fredberg et al., 1997), or by an increase in airway wall stiffness and thickness (Moreno et al., 1996; Wagers et al., 2004). There are several ways in which the lung can become hyperresponsive to bronchoprovocation, these include mechanisms by which the effect of smoothness of airway smooth muscle is amplified through geometric effects (Bates et al., 2007) and the mechanisms involving the Increased muscle shortening (Ding et al., 1987).

Measurement of airway responsiveness, with alterations in some airway pathologies, can be accomplished through the use of agonists that cause bronchoconstriction. It is well known that the action that a bronchoconstrictor causes on the R_N variable, since it causes a contraction of the smooth muscle of the airways, which in turn leads to a narrowing of the airway lumen, thus increasing resistance to airflow, so that when the increase is excessive it is said that the smooth muscle is hyperresponsive (Bates, 2009). Rocco and collaborators (2003), after administration of 10 mg/kg (P10) and 25 mg/kg (P25) of paraquat, found an increase in polymorphonuclear cells in rat lungs in both groups when compared to the respective control. In addition, a significant increase of the inflammatory mediators in the P25 group could be observed when compared to the group P10.

In the present study, the results showed a hyperresponsiveness of the airway smooth muscles of the animals of groups P10 and P30, in relation to the control group (Figure 3), confirming the effect of paraquat in the two dosages used. Several factors need to be taken into account in order to achieve precision on the factor that contributes to the excessive narrowing of the airways in situations of previous lung disease such as asthma. An excessive expression of myosin light chain kinase may be responsible for excessive narrowing of the airways as well as a change in length and integrity in the myosin or actin filaments or also a change in the composition of myosin chain isoforms in asthmatics (Xuefei et al., 2002).

Inflammatory mediators typically present in asthma may trigger abnormal narrowing of the airways, even when the tracheal smooth muscle is perfectly normal (Iipma et al., 2015). This fact may explain the excessive increases in airway resistance in the P10 and P30 groups containing paraquat, since the lung injury induced by this herbicide is characterized as a potentially fatal disease of respiratory failure due to an inflammatory response of the lungs (Tyagi et al., 2016).

5. CONCLUSION

The need to produce food for more than 7 billion people worldwide is one of the greatest challenges today. In the current model of production, the use of technology and agrochemicals are necessary to maintain adequate productivity and volume of production, where the use of agrochemicals involves biossocial, political, economic and socio-environmental aspects. However, due to the environmental and health problems generated by the uncontrolled use of pesticides, the use of other forms of production should be encouraged, with the reduction or absence of agrochemicals, such as organic and agroecological production. Public policies that encourage the growth of these alternative production methods are needed to value more sustainable forms of production. In conclusion, the results demonstrated the harmful character that exposure to paraquat can cause in the respiratory system of rats.The present work demonstrates the importance of the professional and adequate management of certain agricultural herbicides currently used to avoid further health complications and to reinforce the discussion about the conscious use of agrochemicals in the world food industry. More detailed studies are required that can quantify the level of exposure that the population undergoes when eating some foods, which may present remnants of this pesticide in its composition. Such an initiative can help to clarify the problem inherent in the unconscious ingestion of pesticides.

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