Study of liver toxicity and DNA damage due to exposure to the pesticide Mancozeb in an experimental animal model – A pilot model


1Gastroenterology and Hepatology Postgraduate Program, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil
2Laboratory of Genetic Toxicology, Postgraduate Program in Cellular and Molecular Biology Applied to Health (PPGBioSaúde), Universidade Luterana do Brasil (ULBRA), Canoas, RS, Brazil
3Laboratory of Genetic Toxicology, Canoas, RS, Brazil
4Postgraduation Program in Medical Sciences, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil
5Graduate Program in Health Sciences, Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA), Porto Alegre, RS, Brazil

Abstract. – OBJECTIVE: Mancozeb is one of the most widely used Ethylenebisdithiocarbamates fungicides in Brazil. A pilot experimental model was created to evaluate its potential hepatotoxic effect.

MATERIALS AND METHODS: An experimental study was performed with 27 male Wistar rats (3 groups). The Control Group received a saline solution, while Intervention Groups I and II received 250 mg/kg and 500 mg/kg of Mancozeb, respectively, once a week, for 12 weeks. Anthropometric measurements were carried out, and the marker of biological exposure in urine was dosed. Biochemical tests, evaluation micronucleus count, comet and oxidative stress markers assay, and histological assessment of the liver were also performed.

RESULTS: The hepatotoxic effect of Mancozeb was confirmed by anthropometric measurements, genotoxicity, and oxidative stress. Statistically significant results were found when the exposed groups were compared to the control group.

CONCLUSIONS: These results were supported by inflammatory infiltration and balloonization in the treated groups. The experimental model effectively demonstrated the deleterious effect of Mancozeb on the liver.

Key Words: Ethylenebisdithiocarbamates, Mancozeb, Oxidative stress, Genotoxicity, Liver.

Introduction

Brazilian agriculture has developed to such an extent in the last 40 years that the country is set to become one of the world’s largest food suppliers. This sector has played a major role in the Brazilian economy due to high grain production throughout all macro-regions of the country. Indeed, in order to maintain this production, the agricultural sector carries out extensive use of chemical inputs, such as fertilizers and agricultural pesticides, making Brazil one of the main pesticide consumers in the world. Due to the expansive use and adverse events known from the literature, pesticide adoption has had a strong social impact and is considered a challenge to world public health.

Pesticides were developed to avoid pest invasion of crops and to protect the consumer, as a public health aspect. Among the most widely used classes of pesticides there are fungicides, which are appropriate to prevent or eradicate fungal infections in plants or seeds.

Ethylenebisdithiocarbamates (EBDCs) are a group of fungicides that have been widely utilized around the world since the 1940s. Among the EBDCs there are Mancozeb, Maneb, Zineb and Methyran. Manganese Ethylenebis (Mancozeb), according to the literature, is classified as having low toxicity, but has proved to have caused adverse effects in humans. Its toxicity was induced by the activation of free radicals and suppression of antioxidants.

Innes et al (1969) demonstrated that chronic exposure to Mancozeb (18 months) increases the incidence of adenoma and hepatocellular carcino-
Liver toxicity and DNA damage due to Mancozeb

Ahmed et al. (2017) showed evidence of different alterations in the biochemical and hematological parameters. Other authors, such as Yahia et al. (2015), found similar results, mainly involving the transaminases.

Fungicide toxicity is often related to the formation and increase of reactive oxygen species (EROS), resulting in oxidative damaging products and/or changes in the levels of antioxidants and enzymatic systems for eliminating EROS, creating an imbalance called oxidative stress. Exposure to pesticides has been associated with the induction of oxidative stress in multiple systems. Some authors, such as Atamaniuk et al. (2014), focused their research on the evaluation of oxidative stress, which was demonstrated by different enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). Besides the effects of oxidative stress following exposure to Mancozeb, the evaluation of genotoxicity from pesticides triggers chronic effects harmful to humans. These effects begin with cellular damage and potentially cause the development of teratogenesis and cancer.

Among the methods to detect damage to DNA, we can cite the micronucleus (MN) test. Toxicity caused by Mancozeb has been reported in various experimental studies, which showed evidence of the suspicion of carcinogenicity in rats and induction of damage to DNA in exposed cells in vitro through oxidative mechanisms. Clinical studies aiming to evaluate the genotoxicity of EBDCs are scarce in the literature, and the histological evaluation of the liver is rarely described. The work by Pirozzi et al. (2016) is one of the few reference studies, in which the degree of liver steatosis associated with exposure to Mancozeb are evaluated, concluding that the fungicide increased the number of intracellular lipid droplets.

Due to the possible damage caused by Manganese Ethylenebis (Mancozeb) following prolonged exposure through contact with the contaminant and also by food residues, we chose to study their hepatotoxic effects in detail in an experimental model.

**Materials and Methods**

An experimental study was performed with 27 male Wistar rats weighing 280 to 300 g. The animals were placed in boxes with 3 rats in each box, on a wood shavings bed, and fed a standard diet and water ad libitum. The rats were maintained on a 12-hour light/dark cycle, at a temperature of 22 ± 1°C. The maximum dose defined for this model was 500 mg/kg, based on the lethal dose of Mancozeb. This dose corresponds to 1/10 of the lethal dose.

**Experimental Design**

The animals were divided randomly into three groups:

- Control Group (CG): 9 rats that received a saline solution (0.9% NaCl) with the same frequency as the other groups during the same period.
- Intervention Group I (MZ1): 9 rats, that received a dose of Mancozeb (Dithane NT) (250 mg/Kg/day) dissolved in a saline solution (0.9% NaCl) with a final volume of 2 ml/Kg administered by gavage, once a week, for 12 weeks.
- Intervention Group II (MZ2): 9 rats that received a dose of Mancozeb (Dithane NT, Dow AgroSciences Industrial Ltda, Jacaré/São Paulo, Brasil) (500 mg/Kg/day) dissolved in a saline solution (0.9% NaCl), with a volume of 2 ml/Kg administered by gavage, once a week, for 12 weeks.

This model proposed to mimic the exposure to Mancozeb to winegrowers in the state of Rio Grande do Sul, Brazil. This is characterized by farmers who apply this fungicide annually, from October to December, with a total of approximately 12 weeks of exposure. This work is a pilot study of a clinical model.

**Anthropometric Measurements and Procedures**

Anthropometric measurements, such as weight, abdominal circumference, and naso-anal length, were taken weekly and at the end of the experiment. Approximately (~2 mL) of urine were collected two days before euthanasia, through metabolic cages, to evaluate the biological indicator of exposure: Ethylenethiourea (ETU).

During the experiment, in week 10, one of the rats in group MZ1 died because of alimentary bronchoaspiration, without any histological change in the liver. After the experiment ended, the animals were anesthetized with isoflurane (Instituto Biochimico Ind. Farm. Ltda. Penedo/Cordovil, Rio de Janeiro, Brazil) at a concentration of 5% diluted in oxygen 100%. After confir-
nformation of the anesthetic level, the animals were exsanguinated by the transcardiac route to collect blood and organs, and some of them were stored under appropriate conditions.

**Biochemical and Hematological Analyses**

The following were analyzed: total bilirubin (TB) and fractions – Direct (DB) and Indirect (IB) – creatinine (colorimetric method), AST and ALT (enzymatic method), and alkaline phosphatase (colorimetric kinetic method) (p-NP - DG KC). Evaluation of blood count and platelets was made using the light absorbance/impedance/flow cytometry and acetylcholinesterase (kinetic enzymatic) methods.

**Genotoxicity**

After collecting peripheral blood and bone marrow from the rat femur, genotoxicity was evaluated using the Micronucleus test, following the protocol of Miller et al.24 (1997) and Comet assay. The first was performed on blood samples that were rubbed and stained with Giemsa, and then the micronuclei present on the slides were counted by two blinded researchers. The Comet assay was evaluated in the blood and liver tissue. In the latter, the tissue was dissected and placed in a buffered solution pH 7.4 (PBS), mixed with agarose 0.75%, and spread on slides, with a later application of electricity for 15 minutes, and neutralized after electrophoresis to be finally analyzed.

**Oxidative Stress**

In serum and liver tissue samples, lipid peroxidation was evaluated using the method of species reactive to thiobarbituric acid (TBARS), followed by the evaluation of Superoxide Dismutase (SOD), Catalase (CAT), Glutathione (GSH) enzymes, and by spectrophotometry, and proteins carbonylated by the Bradford method (1976).

**Histological Analysis**

After laparotomy and extraction of the liver, the liver tissue was stored in formaldehyde at 10% (Formaldehyde solution 10% Sigma-Aldrich. Saint Louis, MO, USA) for 48 hours and placed in paraffin blocks, stained in Hematoxylin and Eosin, to evaluate liver steatosis and Picrosirius Red for fibrosis, categorized in the following patterns:

A: Absence of portal fibrous expansion, perivenular or perisinusoidal fibrosis.
B: Discrete balloonization of perivenular hepatocytes, with occasional foci of inflammatory infiltrates.
C: Discrete balloonization of perivenular hepatocytes.
D: Discrete perivenular inflammatory foci.

**Statistical Analysis**

Normality was evaluated using the Shapiro-Wilk test. The quantitative variables as median, minimum and maximum were described and compared among the groups using non-parametric tests such as Kruskal-Wallis, followed by Dunn-Bonferroni post hoc (for the 3 groups) and Mann-Whitney, when two groups were compared.

Categorical variables were presented as number and percentage. The Exact Fisher’s test was used to compare categorical variables. Associations with $p \leq 0.05$ were considered statistically significant. A statistical analysis was performed using the statistical program SPSS version 20.0 (IBM Corp., Armonk, NY, USA).

**Results**

The findings of this study on anthropometric measurements were demonstrated using the Lee index and Body Mass Index (BMI). There was a statistical significance when the control group was compared to the exposed groups MZ1 and MZ2 ($p = 0.01$).

The medians of weight at the end of the experiment in each group were 527 grams in the Control Group, 485 grams in MZ1, and 479 grams in MZ2, demonstrating a lower weight at the end of the experiment of the exposed groups when compared to the control group (Figure 1).

Abdominal circumference was measured at the end of the experiment, showing evidence of statistical significance in the two groups exposed, MZ1 and MZ2, when compared to the control group, $p = 0.01$, with a median of 23 cm for the control group and 20 cm for the exposed groups.

**Blood Count and Biochemical Parameters**

Among the different blood count parameters, it was possible to detect a significant statistical difference in the platelet count of the exposed groups – (MZ1 $p = 0.003$), and (MZ2 $p = 0.015$) – compared to the control group (CG).
Liver toxicity and DNA damage due to Mancozeb

Table I shows the different measurements of dispersion.

The FA enzyme revealed a significant difference in group MZ2 compared to the control group \( (p = 0.049) \), although no difference was found in comparison to group MZ1.

There was a significant difference between the treated groups MZ1 and MZ2, regarding the Acetylcholinesterase dosage, compared to the control group \( (p = 0.049) \). In evaluating the enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT), no significant difference could be detected \( (p = 0.23 \) and \( p = 0.90) \).

**Biological Marker of Exposure – ETU**

ETU, as a biological marker of exposure, was evaluated and detected in the groups exposed with a median of 219 (ng/mL) in group MZ1, and of 587 (ng/mL) in group MZ2, showing a statistically significant exposure \( (p = 0.05) \) (Table I).

**Genotoxicity**

Genotoxicity was evaluated in different samples: liver tissue, bone marrow blood, and peripheral blood.

**Micronuclei**

In the micronucleus (MNs) count in bone marrow blood and peripheral blood (Figure 2), a statistically significant difference was found \( p \leq 0.05 \), when group MZ2 was compared to the CG (Figure 3). The mean of \( (7.2 \pm 1.1) \) micronuclei was observed in group MZ2, while in the control group (CG), the mean was \( (1.0 \pm 0.5) \). There was no significant statistical difference between groups MZ1 and MZ2.

**Comet Assay**

The Comet Assay evaluation was performed in peripheral blood and liver tissue; there was a significant difference in the evaluation of the liver tissue of the groups exposed \( p \leq 0.05 \) (Supplementary Figure 1).

Table I. Comparative table of the blood count and biochemical parameters.

<table>
<thead>
<tr>
<th>Blood Count</th>
<th>Control ( n = 9 )</th>
<th>MZ1 ( n = 8 )</th>
<th>MZ2 ( n = 9 )</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (mg/dL)</td>
<td>17.0 (11.3-17.7)</td>
<td>17.7 (16.3-18.4)</td>
<td>17.4 (16.3-17.7)</td>
<td>0.150</td>
</tr>
<tr>
<td>Platelets ( \times 10^{5} )</td>
<td>9.72 (4.85-11.21)a</td>
<td>11.60 (10.60-12.48)b</td>
<td>11.34 (9.87-12.72)b</td>
<td>0.002</td>
</tr>
<tr>
<td>WBC ( \times 10^{3} )</td>
<td>7.8 (4.7-9.1)</td>
<td>8.25 (6.3-10.1)</td>
<td>7.25 (5.7-9.9)</td>
<td>0.477</td>
</tr>
<tr>
<td>Lymphocytes ( \times 10^{9} )</td>
<td>7.0 (4.3-8.3)</td>
<td>7.3 (5.7-9.0)</td>
<td>6.3 (4.9-7.8)</td>
<td>0.421</td>
</tr>
</tbody>
</table>

**Biochemistry**

<table>
<thead>
<tr>
<th></th>
<th>Control ( n = 9 )</th>
<th>MZ1 ( n = 8 )</th>
<th>MZ2 ( n = 9 )</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT</td>
<td>69 (56-104)</td>
<td>69 (54-123)</td>
<td>56 (42-128)</td>
<td>0.238</td>
</tr>
<tr>
<td>AST</td>
<td>139 (121-204)</td>
<td>135 (112-309)</td>
<td>133 (90-291)</td>
<td>0.908</td>
</tr>
<tr>
<td>Total bilirubin</td>
<td>0.0 (0-0.20)</td>
<td>0.0 (0-0.20)</td>
<td>0.0 (0-0.20)</td>
<td>0.989</td>
</tr>
<tr>
<td>Direct bilirubin</td>
<td>0.08 (0.06-0.96)</td>
<td>0.05 (0.0-0.23)</td>
<td>0.05 (0.04-0.33)</td>
<td>0.042</td>
</tr>
<tr>
<td>Indirect bilirubin</td>
<td>-0.09 (-0.94 – -0.06)b</td>
<td>-0.05(-0.23-0)b</td>
<td>-0.05(-0.31 – -0.04)b</td>
<td>0.010</td>
</tr>
<tr>
<td>Acetylcholinesterase</td>
<td>433 (331-488)</td>
<td>480 (429-521)</td>
<td>497 (404-601)</td>
<td>0.049</td>
</tr>
<tr>
<td>Alkaline Phosphatase</td>
<td>150 (100-174)b</td>
<td>118 (98-169)b</td>
<td>116 (64-133)b</td>
<td>0.049</td>
</tr>
</tbody>
</table>

**ETU**

<table>
<thead>
<tr>
<th>ETU</th>
<th>Control ( n = 9 )</th>
<th>MZ1 ( n = 8 )</th>
<th>MZ2 ( n = 9 )</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>219 (106-1041)</td>
<td>587 (232-1077)</td>
<td>0.059*</td>
<td></td>
</tr>
</tbody>
</table>

Data presented as median (minimum-maximum) and compared using the Kruskal-Wallis test. Different superscript letters represent a statistically different groups. *Mann-Whitney test. WBC: White blood cells; AST: Aspartate Aminotransferase; ALT: Alanine Aminotrasnferase ETU: Ethylenethiourea.
Oxidative Stress

When the statistical analyses of the oxidative stress markers were performed, we found a statistically significant difference in the Superoxide Dismutase (SOD), Catalase (CAT), and Reduced Glutathione (GSH) (Table II).

The results for thiobarbituric acid reactive substances (TBARS) were not statistically significant when the groups were compared ($p > 0.05$).

In the CAT evaluation, there was statistical significance when group MZ2 was compared to the control group ($p = 0.011$).

In the analysis of SOD between groups MZ1 and MZ2, statistical significance was found (Mann Whitney $p = 0.027$).

Reduced Glutathione (GSH) was statistically significant ($p = 0.02$) when groups MZ1 and MZ2 were compared to the CG. No statistical significance was found in any of the groups in evaluating carbonylated proteins ($p > 0.05$).

Histology

In the histological analysis of liver tissue, findings were compatible with balloonization of the perivenular hepatocytes (A) and discrete perivenular inflammatory foci (B) (Supplementary Figure 2), with a significant difference when the intervention MZ1 and MZ2 groups were compared to the Control Group.

Histological patterns found: (Supplementary Figure 3)

A: Absence of portal fibrous, perivenular or perisinusoidal fibrosis.

B: Discrete Balloonization of perivenular hepatocytes, with occasional foci of inflammatory infiltrate.

### Table II. Oxidative stress.

<table>
<thead>
<tr>
<th></th>
<th>Control n = 9</th>
<th>MZ1 n = 8</th>
<th>MZ2 n = 9</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxidative stress</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TBARS (nmol/mg prot)</td>
<td>0.47 (0.35-1.39)</td>
<td>0.45 (0.30-1.03)</td>
<td>0.58 (0.36-1.04)</td>
<td>0.388</td>
</tr>
<tr>
<td>Superoxide dismutase (SOD) (U SOD/mg prot)</td>
<td>25.4 (4.4-118.7)</td>
<td>35.8 (13.9-63.3)</td>
<td>15.5 (2.6-29.5)</td>
<td>0.086</td>
</tr>
<tr>
<td>Catalase (CAT) (pmoles/min/ mg prot)</td>
<td>2.6 (2.2-2.9)$^a$</td>
<td>2.4 (2.1-3.2)$^r$</td>
<td>2.1 (1.7-2.2)$^b$</td>
<td>0.011</td>
</tr>
<tr>
<td>Reduced glutathione (GSH) (umols/min/mg prot)</td>
<td>0.085 (0.048-0.118)$^{ab}$</td>
<td>0.065 (0.041-0.073)$^a$</td>
<td>0.088 (0.033-0.136)$^b$</td>
<td>0.020</td>
</tr>
<tr>
<td>Carbonylated proteins (nmol carb /mg pro)</td>
<td>2740.7 (1692.2-16996.8)</td>
<td>5370.0 (702.4-17119.7)</td>
<td>4697.1 (938.9-15701.1)</td>
<td>0.728</td>
</tr>
</tbody>
</table>

Date presented as median (minimum-maximum) and compared by the Kruskal-Wallis test. Different superscript letter represent statistically different groups.
Liver toxicity and DNA damage due to Mancozeb

Discussion

Exposure to different agricultural pesticides has become more frequent among the Brazilian population and worldwide. The purpose of this study was to analyze chronic exposure to Mancozeb and its toxic effect on health, mainly in the liver, using an experimental pilot model for a future clinical study.

It was demonstrated that 12-week exposure to Mancozeb led to a delay in weight gain throughout the experiment. There is little literature on measuring or approaching anthropometric measurements in a population exposed to agricultural pesticides. In this work, BMI was compared using the Lee Index, by means of multiple variables, and the result showed a statistically significant difference ($p = 0.01$) in the groups exposed, MZ1 and MZ2, compared to the control group. This result is supported by the difference in the abdominal circumference at the end of the experiment with animals in the groups exposed, which is always smaller after exposure to Mancozeb, compared to the control group.

The evaluation of biochemical and hematological parameters, according to Ahmed et al. (2017), showed results in which hematological damage, expressed in anemia and leukopenia, occurred; these modifications were not detected in this work. The authors also describe alterations in the biochemistry of the liver, such as elevation of ALT, AST, alkaline phosphatase, and acetylcholinesterase activity, among the results observed in rats treated with Mancozeb, at 250 and 500 mg/kg for 4 weeks. Contrary to that study, the findings with statistical significance in the blood count were related to the platelet count in this model, showing an increased number of platelets in the groups exposed (MZ1 and MZ2). Furthermore, there was a drop in the alkaline phosphatase levels in these groups, a finding that was in opposition with the results found in the literature, which are probably related to the nutritional component, clearly seen to be altered after exposure, suggesting malnutrition. Bowling presented studies in which the alkaline phosphatase levels are low and suggested that they are related mainly to bone metabolism or some nutritional disorders.

Yahia et al., evaluating hepatic biochemical parameters, also found an elevation of the enzymes AST, ALT, alkaline phosphatase, and total bilirubin in a group of rats treated with 500 and 1,000 mg/Kg/day of Mancozeb, for 8 weeks. In this study, there was no statistically significant difference between the transaminases of the groups. Nevertheless, this fact does not invalidate the potential for damage.

The determination of ETU as a marker of exposure to the EBDCs has already been studied and proved by different authors in clinical and experimental models. This experimental work enabled the demonstration of the objective evaluation by detection of ETU as a marker of exposure to Mancozeb. Aprea et al described ETU as a marker with a very rapid elimination kinetic, with maximum excretion within the first 24 hours. This experimental model was dosed several times beyond the 24 hours after the last exposure, and, even so, showed evidence of being a useful tool to evaluate exposure to EBDCs.

On the other hand, Fustinoni et al present results of contamination in the control group, revealing ETU levels in urine. The authors further confirmed the findings of this work, since they excluded the limitations of the external factors that occur in humans. The experimental model developed here enabled the detection of ETU levels in the urine of those exposed and did not show any evidence of ETU in the controls, validating the biological indicator of exposure in this sample.

In evaluating oxidative stress in liver tissue and serum, lipid peroxidation was analyzed by the TBARS technique; we did not observe a significant difference when the animals were exposed to the agent in groups MZ1 and MZ2, respectively. Other experimental models for cirrhosis and cancer, with xenobiotics such as utilizing DEN and CCL4, observed increased lipoperoxidation by TBARS, different from our findings. Other studies have also shown evidence of greater lipoperoxidation in organs such as the kidney, lung and liver of animals that were cirrhotic through CCL4 or ligation of the bile duct. There was also an increase of lipoperoxidation in pictures of colitis through damage to the cellular membranes in an experimental model.

The antioxidant enzyme SOD is considered the first line of defense against the formation of EROS. The decrease of SOD activity in the MZ2 groups could be associated with the increase of TBARS that was consumed in an attempt to
diminish lipoperoxidation, and thus diminish oxidative damage based on the dismutation of the superoxide radical anions and formation of $\text{H}_2\text{O}_2$.

The significant increase of SOD enzyme activity ($p < 0.05$) in the animals of group MZ1 and MZ2 compared to the CG, suggests a protective effect after oxidative damage, which we can, in fact, observe from the lipoperoxidation (TBARS) damage, whose level is equal to those of the control group.

The function of CAT is to act on the $\text{H}_2\text{O}_2$, catalyzing it to water and $\text{O}_2$. In the present study, it can be observed that enzyme activity is diminished in the animals in groups MZ1 and MZ2. These data are in accordance with Schemitt et al., who observed that CAT was diminished in the livers of animals that presented liver damage induced by Thiocetamide.

Increased carbonylation of the liver proteins is associated with oxidative damage provoked by the aggressor agent, Mancozeb. Similar effects were observed with the use of Thioacetamide. In this scenario, the xenobiotic significantly increased the carbonyls, and increased carbonylation of the liver proteins is associated with oxidative damage. On the other hand, using an antioxidant, in this case, melatonin, was linked to a significant decrease. In this study, no evidence of a statistically significant response was found (despite different values among the groups), such as in the findings of Atamianjik et al. in fish. It can be suggested that the antioxidant enzymes acted as scavengers of the free radicals, protecting the cellular membranes and preventing lipoperoxidation and the increased carbonylation of liver proteins. The presence of the carbonyl group, aldehydes, and ketones is the consequence of the oxidative damage caused by agents that attack the cellular membrane.

Aside from the effects of oxidative stress secondary to exposure to Mancozeb, the evaluation of genotoxicity can show chronic effects that are harmful to humans. These effects begin with cellular damage or genotoxic damage and potentially cause the development of teratogenesis and cancer. The genotoxic potential being a primary risk factor for long-term effects. Genotoxicity was evaluated using 2 different methods: Comet assay and Micronucleus count. The methods were compared and analyzed both in liver tissue and in peripheral blood and bone marrow blood. Among the results presented, a significant difference was observed in the analyses of liver tissue of groups MZ1 and MZ2, compared to the control group, and also in the bone marrow blood. It was possible to detect a statistical significance in group MZ2 compared to the control. According to the literature, the genotoxic potential is a risk factor for developing teratogenesis and cancer.

The histopathological findings in this work will contribute to knowledge regarding liver damage. It should be highlighted that there is very little literature that discusses the histopathological evaluation of the liver after exposure to Mancozeb. In this study, the first of its kind in Brazil, the evaluation was performed in all the rat livers. The findings of this study were balloonization and discrete perivenular inflammatory infiltrate in the groups exposed, without developing into a severe lesion. There was no fibrosis in any of the samples, nor any other alterations suggesting evolution to advanced liver disease, probably due to the time of exposure. In the literature, the study by Innes et al. showed evidence of an increased incidence of adenoma and hepatocarcinoma in rats treated for 18 months, with a time of exposure 6 times greater than in this study.

This information is useful, however, in the present study, the main reason why the time of 12 weeks was chosen was to mimic real life in a pilot model, considering that the workers are exposed to the product (Mancozeb) for approximately 2 to 3 months, during the cultivation period, after which they stop and only resume their activities a long time later. In no case is the exposure continuous for longer than 6 months. This was based on the duration of the life of a rat under animal research laboratory conditions. These animals live an average of 18 months (547.5 days), and when this period is converted into years of life, 12 weeks (84 days) correspond to approximately 11 years of life, a reasonable time length when considering chronic exposure.

**Limitations**

The main limitation of the study was having to perform the exposure to Mancozeb by gavage, and not by inhalation since gavage is the only method approved by the Research Ethics Committee. As described in the objectives of this work, the idea was to mimic real life, however, following the guidelines and normativity in force in the animal experimentation unit, the use of exhaustion hoods for exposure by inhalation, in order to protect the research team was not approved.

This study was carried out during the COVID-19 pandemic, a circumstance that conditioned its development and also became a limi-
Liver toxicity and DNA damage due to Mancozeb

Conclusions

The results confirmed the efficacy of the experimental model to induce hepatotoxicity. In the animals treated, Mancozeb could alter aspects ranging from anthropometric measurements to liver histology.

After developing an experimental model mimicking the reality encountered in the country in terms of agriculture and grain production, with the consequent use of chemicals such as pesticides, specifically Mancozeb, it was concluded that this pesticide is prejudicial to health, especially to DNA; this was demonstrated by means of blood tissue from the bone marrow and liver tissue of the rats studied.

The study described is a pilot model, the beginning of a large line of research related to chronic exposure to agricultural pesticides, continuing the clinical model that seeks to evaluate the effect of Mancozeb on viticulture.

Conflict of Interest

None of the authors have competed for financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding

No funding.

Authors’ Contribution

N.D Suarez Uribe, M.F. Pezzini and D. Joveleviths designed and coordinated the study; J. Dall Agnol, N. Marroni, S. Benitez, D. Benedetti, J. da Silva, C.T. Cerski, E. Dallegrave, S. Macedo performed the experiments, acquired and analyzed data; N.D Suarez Uribe, M.F Pezzini and D. Joveleviths interpreted the data; N.D Suarez Uribe, M.F Pezzini wrote the manuscript; all authors approved the final version of the article.

ORCID ID

Nelson David Suarez Uribe: 0000-0003-4774-2311
Marina Ferri Pezzini: 0000-0003 0475 6326
Juliana Dall Agnol: 0000-0003-0353-7009
Norma Marroni: 0000-0001-7856-7953
Sandyelle Benitez: 0000-0003-2662-5116
Danieli Benedetti: 0000-0002-3343-2999
Juliana Da Silva: 0000-0002-1089-6766
Carlos Thadeu Cerski: 0000 0003 0673 5916
Eliane Dallegrave: 0000-0001-6586-2080
Sandra Macedo: 0000-0001 9379 701X
Sarah Carobini Werner de Souza Eller Franco de Oliveira: 0000-0003-2200-6959
Dvora Joveleviths: 0000 0002 0741 0235.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics Approval

This study was submitted to and approved by the Medical Ethics Committee of Hospital de Clínicas de Porto Alegre, HCPA, under number 2019-0647.

Informed Consent

Not applicable.

References


Liver toxicity and DNA damage due to Mancozeb


