Lipid Peroxidation and Antioxidant Enzymes Activity of Wistar Rats Experimentally Infected with Leptospira interrogans

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ABSTRACT

Background: Leptospirosis is a zoonotic disease with world-wide distribution, caused by various serovars of Leptospira interrogans and is presumed to be the most widespread zoonosis. Hematological and biochemical changes associated with renal and hepatic pathology are commonly observed in leptospirosis. All leptospires are aerobes and therefore might be expected to generate peroxides during respiration. Enzymatic reduction of \( \text{H}_2\text{O}_2 \) by leptospires has been reported by researchers. The pathogenesis may be related to direct effects of leptospiral compounds or inflammatory response due to oxidative stress. The present investigation was designed to study the lipid peroxidation and the activity of antioxidant enzymes in rats experimentally infected with \( L. \) interrogans.

Materials, Methods & Results: Fifty four male adult rats (Wistar) specific pathogen free, weighing in average 200 grams were used. Rats were divided in nine groups, six animals each group, eight infected groups and one as not infected. Inoculation was performed intraperitoneally (Day 1), using different serovars of \( L. \) interrogans distributed by groups: hardjo (group A), wiffi (group B), grippotyphosa (group C), canicola (group D), icterohaemorrhagiae (group E), bratislava (group F), pomona (group G) and butembo (group H). Group I was composed by not-infected rats, serving as the negative control group. On day 15 PI all animals were anesthetized with isoflurane for blood collection and subsequently decapitated. Liver, spleen, kidney and brain were collected from all animals. Blood was allocated in tube without anticoagulant for serum acquisition to measurement of thiobarbituric acid reactive substances (TBARS). Lipid peroxidation (TBARS levels), superoxide dismutase (SOD), catalase (CAT) and non-protein thiols (NPSH) were measured in the liver, spleen and kidney, and TBARS were also evaluated in serum and brain. Increased lipid peroxidation was observed in all infected groups when compared to not-infected (\( P<0.05 \)); Increase of TBARS levels were verified in serum of groups F and G; brain of groups E and G; liver and spleen of groups A, B and C; and in the kidneys of all infected groups compared to Group I. SOD activity was increased (\( P<0.05 \)) in liver samples of groups A and C; and in the kidney samples of groups A to G. Spleen samples did not show statistical differences to SOD activity. We observed increase in CAT activity (\( P<0.05 \)) in liver samples of groups A, B and G, in spleen samples of groups B and E, and kidney samples of groups A, B, E, F, G and H. NPSH levels increased (\( P<0.05 \)) in spleen of groups B, D, E and G, and kidney samples of groups A, B, C, D and E. Liver samples had no statistical differences between groups in NPSH levels.

Discussion: Based on our results is possible to conclude that the infection by Leptospira interrogans in Wistar rats shows differences between serovars utilized experimentally. The results may suggest that oxidative damage to tissues along with other mechanisms might have taken part in the pathogenesis of leptospirosis and further detailed studies at cellular level are needed to fully understand the pathogenesis and clinical expression of the disease. Kidneys were confirmed as the major host organ to leptospires, resulting in a great stress oxidative response and presenting a high activity of scavenger enzymes, as well as potential generation of oxidative stress to other organs and even to the brain.

Key-words: Leptospira interrogans, lipid peroxidation, oxidative stress, experimental infection.
INTRODUCTION

Leptospirosis is a zoonotic disease with worldwide distribution, caused by various serovars of *Leptospira interrogans* and is presumed to be the most widespread zoonosis [51]. The disease is sustained in nature by maintenance hosts namely infected animals. Human and animals may acquire disease by direct or indirect contact with materials contaminated with excretions of the infected animals such as urine and uterus discharge [40].

The lipid peroxidation is a general mechanism by which free radicals can induce tissue damage and is implicated in several pathological conditions [18]. The reactive oxygen species (ROS) normally are produced in pathologic situations, and counteracted by an intricate antioxidant defense system that includes the enzymatic scavengers superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase. SOD speeds the conversion of superoxide to hydrogen peroxide, whereas CAT and glutathione peroxidase convert hydrogen peroxide to water. Glutathione (GSH), the most abundant nonprotein sulphydryl (NPSH) in most cells, acts as a nucleophilic scavenger of numerous compounds and their metabolites via enzymatic and chemical mechanisms and plays important roles in the protection against oxidative damage caused by ROS [6,12,42]. The balance between ROS production and antioxidant defenses determines the degree of oxidative stress.

The mechanisms which leptospires uses to cause host tissue damage and disease still not well defined. The pathogenesis may be related to direct toxic effects of leptospiral compounds, causing severe tissue damage due to oxidative stress. The present investigation was designed to study the lipid peroxidation and the activity of antioxidant enzymes in rats experimentally infected with *L. interrogans*.

MATERIALS AND METHODS

Experimental animals

Fifty four male adult rats (Wistar) specific pathogen free, weighing in average 200 grams were obtained from vivarium of Federal University of Santa Maria and utilized in the experiment. The animals were kept in environment with control of temperature and humidity (23°C and 70%, respectively), fed with commercial ration, water *ad libitum* and submitted to a period of 15 days of adaptation. Prior to the infection (day 0) and on the last day of the experiment (day 15) serology was performed (titers from 1:25) in all animals, initially for verification of absence of previous exposure to the agent, and later to substantiate the single infection by each serovar used.

Rats were divided in nine groups, six animals each group, eight infected groups and one as not infected. Inoculation was performed intraperitoneally (Day 1), using different serovars of *L. interrogans* distributed by groups: *L. interrogans* serovar *hardjo* (group A), *L. interrogans* serovar *wolffi* (group B), *L. interrogans* serovar *grippotyphosa* (group C), *L. interrogans* serovar *canicola* (group D), *L. interrogans* serovar *icterohaemorrhagiae* (group E), *L. interrogans* serovar *bratislava* (group F), *L. interrogans* serovar *pomona* (group G) and *L. interrogans* serovar *butembo* (group H). Group I was composed by not-infected rats, serving as the negative control group. All reference strains are maintained in the Leptospirosis Laboratory (Department of Microbiology and Parasitology - Federal University of Santa Maria) in Ellinghausen–McCullough–Johnson–Harris (EMJH).

Infection

The density of leptospiral organisms used to the inoculation was determined by direct counting using a darkfield microscope. Infected groups (group A-H) received $10^8$ leptospires/mL (diluted in EMJH medium), whereas the control group (group I) received only EMJH medium (1 mL).

Estimate of bacteremia

Infection was estimated on day 2, 5, 10 and 15 post-infection (PI) by microscopic examination of slide with urine (at a magnification of 40X). Additionally blood from each animal was collected on the same days and a drop mixed in modified EMJH medium with 5-fluorouracil (300 mg/L) and nalidixic acid (20 mg/L), in order to evaluate the period and percentage of recovery of the each serovar.

Sampling

On day 15 PI, all animals were anesthetized with isoflurane for blood collection and subsequently decapitated with recommended by the ethics committee. Liver, spleen, kidney and brain were collected from all rats. Blood was allocated in tube
Lipid peroxidation

Lipid peroxidation was measured in the serum, brain, liver, spleen and kidney. Lipid peroxidation was determined by TBARS levels according to the method described by Ohkawa et al. [35] and Jentzsch et al. [24]. Results were obtained by spectrophotometry at 535 nm and the expressed in nanomoles of malondialdehyde of milliliter serum or gram tissue (organs).

Superoxide dismutase (SOD)

SOD activity was estimated in the liver, spleen and kidney according to the method described by McCord and Fridovich [31]. Samples were homogenized in 50 mM Tris-HCl, pH 7.5 for 10 min at 1800 g. The dilution 1:80 was made (1000 mg/60 mL), supernatant 1. The buffer for the assay of SOD is always the same (glycine). The results were presented in UI SOD/mg protein.

Catalase (CAT)

CAT activity was measured in the liver, spleen and kidney by the method of Nelson and Kiesow [34] and Aebi [2]. Homogenization was made in 50 mM Tris- HCl pH 7.5 for 10 min at 1800 g, with dilution of 1:60 (1000 mg/ 60 mL). The buffer for the assay of catalase tissue is the trifluoromethyl ketone (TFK) 50 mM pH 7.5. CAT activity was determined in S1 by the decomposition of H$_2$O$_2$ at 240 nm. The enzymatic activity was expressed in moles/mg of protein.

Non-protein thiols (NPSH)

NPSH concentration in supernatants was determined as an indicator of reduced glutathione (GSH) following the colorimetric procedure of Ellman [13]. NPSH concentration was quantified by comparison with a GSH standard curve. An homogenization in 50 mM Tris-HCl, pH 7.5 for 10 min at 1800 g was made, and a dilution of 1:10 carried out (1000 mg/10 mL), obtaining a supernatant 1 (S1). Other 1:1 dilution (2 mL/2 mL) was performed with the supernatant and 10% TCA to precipitate proteins. The enzymatic activity was expressed in imol/g tissue.

Statistical analysis

The packaged SPSS program for windows version 10.0.1 (SPSS, Chicago, IL, USA) was used for statistical analysis. Data were expressed as mean ± standard deviation. Differences between groups were determined by means of a Student t-test. Significance level was set at $P < 0.05$.

RESULTS

Course of infection and clinical changes

The behaviors of animals were evaluated daily. In the first and second days post-infection it did not show changes in its behavior starting on the third day decreasing the feed and water intake. It behaviors remained approximately until the seventh day PI when the rats apparently returned to normal intake. Alterations mentioned above were not observed in the control group. Serological tests performed at day 0 did not show any titration in all groups, and on day 15 PI all infected groups showed titers ranging from 1:200 to 1:800 only to the respective serovar inoculated on day 1. Only from the fifth day PI was possible to observe the presence of leptospires in the urine in all infected groups, and it remained until the 15th day. Urine from control group did not show the presence of the spirochete. Blood samples collected on days 2 and 5 PI showed growth of leptospires in all infected groups after six weeks of culture, while blood samples of days 10 and 15 from the same groups did not show leptospires growth. Group I did not present any growth on the same period.

TBARS levels

TBARS analyses were carried out in samples of brains, serum, liver, spleen and kidney (Table 1). It was observed increase in lipid peroxidation of infected groups compared to not-infected ($P < 0.05$). In serum it was a verified in groups F and G; in the brain of the groups E and G; in the liver and spleen of groups A, B and C. In the kidneys TBARS levels increased in all infected groups compared to Group I.

SOD activity

SOD analyses were carried out in samples of liver, spleen and kidney (Table 2). It was observed increase in SOD activity of infected groups when compared to not-infected ($P < 0.05$). Statistical
Table 1. Media and standard deviation of the TBARS levels in serum (Mmol MDA/mL), brain, liver, spleen and kidney (Mmol MDA/g tissue) in experimentally infection in rats with *Leptospira interrogans*.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum*</th>
<th>Brain*</th>
<th>Liver*</th>
<th>Spleen*</th>
<th>Kidney*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: L. hardjo</td>
<td>15.3± (±1.1)</td>
<td>3.34± (±0.53)</td>
<td>2.87± (±0.31)</td>
<td>1.26± (±0.12)</td>
<td>6.78± (±1.23)</td>
</tr>
<tr>
<td>B: L. wolffi</td>
<td>15.5± (±1.2)</td>
<td>2.80± (±0.40)</td>
<td>2.38± (±0.59)</td>
<td>1.31± (±0.19)</td>
<td>6.11± (±0.99)</td>
</tr>
<tr>
<td>C: L. grippotyphosa</td>
<td>16.3± (±0.8)</td>
<td>2.83± (±0.67)</td>
<td>2.45± (±0.52)</td>
<td>1.15± (±0.09)</td>
<td>5.75± (±1.1)</td>
</tr>
<tr>
<td>D: L. canicola</td>
<td>14.6± (±0.7)</td>
<td>2.51± (±0.35)</td>
<td>2.3± (±0.23)</td>
<td>0.93± (±0.08)</td>
<td>6.60± (±1.19)</td>
</tr>
<tr>
<td>E: L. icterohaemorrhagiae</td>
<td>14.6± (±0.9)</td>
<td>4.22± (±0.61)</td>
<td>1.97± (±0.24)</td>
<td>0.92± (±0.11)</td>
<td>6.20± (±1.14)</td>
</tr>
<tr>
<td>F: L. bratislava</td>
<td>16.9± (±0.8)</td>
<td>4.77± (±0.45)</td>
<td>2.07± (±0.24)</td>
<td>0.96± (±0.08)</td>
<td>6.30± (±1.21)</td>
</tr>
<tr>
<td>G: L. butembo</td>
<td>18.5± (±1.1)</td>
<td>2.52± (±0.34)</td>
<td>1.95± (±0.50)</td>
<td>0.99± (±0.14)</td>
<td>5.40± (±0.89)</td>
</tr>
<tr>
<td>H: L. pomona</td>
<td>14.5± (±1.2)</td>
<td>2.42± (±0.29)</td>
<td>1.86± (±0.39)</td>
<td>0.83± (±0.11)</td>
<td>2.36± (±0.34)</td>
</tr>
<tr>
<td>I: Not-infected</td>
<td>14.0± (±0.6)</td>
<td>2.63± (±0.39)</td>
<td>1.78± (±0.21)</td>
<td>0.81± (±0.07)</td>
<td>1.55± (±0.19)</td>
</tr>
</tbody>
</table>

Means followed by the same letters within the same column are not significantly different from each other at Tukey test (at 5% probability). *Mmol MDA/mL; *Mmol MDA/g tissue.

Table 2. Media and standard deviation of the SOD activity in liver, spleen and kidney (UI SOD/mg protein) in experimentally infection in rats with *Leptospira interrogans*.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Liver*</th>
<th>Spleen*</th>
<th>Kidney*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: L. hardjo</td>
<td>124.7± (±12.1)</td>
<td>21.82± (±3.5)</td>
<td>48.5± (±5.6)</td>
</tr>
<tr>
<td>B: L. wolffi</td>
<td>101.6± (±11.3)</td>
<td>18.5± (±2.8)</td>
<td>44.7± (±7.2)</td>
</tr>
<tr>
<td>C: L. grippotyphosa</td>
<td>123.3± (±14.4)</td>
<td>20.0± (±4.1)</td>
<td>47.4± (±6.6)</td>
</tr>
<tr>
<td>D: L. canicola</td>
<td>105.7± (±10.1)</td>
<td>29.8± (±3.2)</td>
<td>46.3± (±4.8)</td>
</tr>
<tr>
<td>E: L. icterohaemorrhagiae</td>
<td>111.5± (±9.1)</td>
<td>28.6± (±2.8)</td>
<td>51.3± (±5.2)</td>
</tr>
<tr>
<td>F: L. bratislava</td>
<td>90.3± (±10.8)</td>
<td>22.9± (±3.9)</td>
<td>44± (±6.5)</td>
</tr>
<tr>
<td>G: L. butembo</td>
<td>91.1± (±8.1)</td>
<td>25.1± (±2.5)</td>
<td>45.1± (±6.2)</td>
</tr>
<tr>
<td>H: L. pomona</td>
<td>93.9± (±9.6)</td>
<td>23.2± (±3.6)</td>
<td>40± (±5.8)</td>
</tr>
<tr>
<td>I: Not-infected</td>
<td>97.4± (±8.7)</td>
<td>28.4± (±2.2)</td>
<td>38.9± (±4.7)</td>
</tr>
</tbody>
</table>

Means followed by the same letters within the same column are not significantly different from each other at Tukey test (at 5% probability). *UI SOD/mg protein.

Table 3. Media and standard deviation of the CAT activity in liver, spleen and kidney (x10⁻⁹ moles/mg protein) in experimentally infection in rats with *Leptospira interrogans*.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Liver*</th>
<th>Spleen*</th>
<th>Kidney*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: L. hardjo</td>
<td>16.7± (±1.8)</td>
<td>0.40± (±0.14)</td>
<td>2.7± (±0.66)</td>
</tr>
<tr>
<td>B: L. wolffi</td>
<td>12.4± (±1.14)</td>
<td>0.48± (±0.10)</td>
<td>2.3± (±0.47)</td>
</tr>
<tr>
<td>C: L. grippotyphosa</td>
<td>6.9± (±0.9)</td>
<td>0.39± (±0.09)</td>
<td>2.1± (±0.55)</td>
</tr>
<tr>
<td>D: L. canicola</td>
<td>8.9± (±1.3)</td>
<td>0.45± (±0.16)</td>
<td>2.09± (±0.42)</td>
</tr>
<tr>
<td>E: L. icterohaemorrhagiae</td>
<td>7.3± (±1.12)</td>
<td>0.55± (±0.12)</td>
<td>2.25± (±0.64)</td>
</tr>
<tr>
<td>F: L. bratislava</td>
<td>7.7± (±0.8)</td>
<td>0.38± (±0.08)</td>
<td>2.34± (±0.54)</td>
</tr>
<tr>
<td>G: L. butembo</td>
<td>13.2± (±1.5)</td>
<td>0.44± (±0.11)</td>
<td>2.32± (±0.36)</td>
</tr>
<tr>
<td>H: L. pomona</td>
<td>9.0± (±0.98)</td>
<td>0.46± (±0.09)</td>
<td>2.25± (±0.35)</td>
</tr>
<tr>
<td>I: Not-infected</td>
<td>6.7± (±0.45)</td>
<td>0.35± (±0.08)</td>
<td>1.5± (±0.24)</td>
</tr>
</tbody>
</table>

Means followed by the same letters within the same column are not significantly different from each other at Tukey test (at 5% probability). *moles/mg protein.
differences were found in liver samples of groups A and C. Spleen samples don’t showed statistical differences to SOD activity and in the kidney samples it was found Groups A-G with statistical differences.

**CAT activity**

CAT analyses were carried out in samples of liver, spleen and kidney (Table 3). We observed increase in CAT activity of infected groups when compared to not-infected ($P < 0.05$). Liver samples showed statistical differences in groups A, B and G. Spleen samples showed statistical differences to the groups B and E, and kidney samples it was found Groups A, B, E, F, G and H with statistical differences.

**NPSH levels**

NPSH analyses were carried out in samples of liver, spleen and kidney (Table 4). It was observed increase in NPSH levels of infected groups compared to not-infected ($P < 0.05$). Liver samples had no statistical differences between groups. Spleen samples showed statistical differences in the groups B and E, and kidney samples it was found Groups A, B, E, F, G and H with statistical differences.

**DISCUSSION**

The clinical signs observed that usually accompany bacterial infections including a decrease in feed intake and disturbances of carbohydrate, protein, and fat metabolism [11,30]. This could explain the decrease feed and water intake observed, and also the apathetic behavior of infected animals in the first days PI. Researchers in an experimental infection observed a slight elevation in body temperature in guinea pigs in the first hours following the injection of the leptospiral infected tissue suspension and a marked hyperthermia at the end of the first day which continued to rise for the next two days [22]. Other signs that accompanied fever were anorexia, prostration and oliguria, the same clinical signs observed in our study.

Estimation of lipid peroxidation is complicated due the large number of potential peroxidation products and by the reactivity of these metabolites, thus the most common technique for measuring lipid peroxide involves the use of TBARS. The TBARS rise may be considered as a sign of the oxidation of cellular compounds [43]. In the present study, elevated TBARS levels were demonstrated in brain, serum, liver, spleen and kidney, varying according to the leptospires serovar.

Central nervous system involvement in people most commonly manifests as aseptic meningitis [37]. Leptospires enter the cerebrospinal fluid (CSF) in the early septicemic phase of the illness. The meningeal signs often appear in the second week of illness, when the leptospires are being cleared from the CSF and antigen-antibody complex-induced inflammation may be responsible for the symptoms [29,37]. The serotype *L. icterohaemorrhagiae* has been observed in most of the sickest patients, who

Table 4. Media and standard deviation of the NPSH levels in liver, spleen and kidney (µmol/g tissue) in experimentally infection in rats with *Leptospira interrogans*.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Liver*</th>
<th>Spleen*</th>
<th>Kidney*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: L. hardjo</td>
<td>1.4±0.24</td>
<td>1.82±0.21</td>
<td>1.34±0.11</td>
</tr>
<tr>
<td>B: L. wolffi</td>
<td>1.76±0.21</td>
<td>2.1±0.23</td>
<td>1.13±0.14</td>
</tr>
<tr>
<td>C: L. grippotyphosa</td>
<td>1.66±0.14</td>
<td>2.0±0.12</td>
<td>1.12±0.13</td>
</tr>
<tr>
<td>D: L. canicola</td>
<td>1.59±0.19</td>
<td>2.1±0.18</td>
<td>1.55±0.18</td>
</tr>
<tr>
<td>E: L. icterohaemorrhagiae</td>
<td>1.67±0.22</td>
<td>2.1±0.17</td>
<td>1.78±0.21</td>
</tr>
<tr>
<td>F: L. bratislava</td>
<td>1.5±0.22</td>
<td>2.07±0.21</td>
<td>0.21±0.06</td>
</tr>
<tr>
<td>G: L. butembo</td>
<td>1.7±0.18</td>
<td>2.28±0.24</td>
<td>0.43±0.09</td>
</tr>
<tr>
<td>H: L. pomona</td>
<td>1.73±0.13</td>
<td>2.0±0.22</td>
<td>0.22±0.07</td>
</tr>
<tr>
<td>I: Not-infected</td>
<td>1.71±0.19</td>
<td>1.95±0.20</td>
<td>0.21±0.07</td>
</tr>
</tbody>
</table>

Means followed by the same letters within the same column are not significantly different from each other at Tukey test (at 5% probability). *µmol/g tissue.
had renal or liver failure [20,39,48], while meningeal forms of the disease seem to be more frequently associated with the serotype *L. grippotyphosa* [39], *L. canicola* [20,21,39] or *L. pomona* [37]. Our study found increase of TBARS levels in the samples of brain in groups infected with *L. icterohaemorrhagiae* and *L. bratislava*, bringing new facts that could be better studied and, may related to meningeal forms of leptospirosis, since those studies mentioned above, associated meningeal forms of the disease to serotype *L. grippotyphosa, L. canicola* and *L. pomona.*

TBARS levels in our study, indicated by MDA concentration, was higher in the infected groups F and G representing infection with *L. bratislava* and *L. butembo*, respectively. MDA is the most abundant aldehyde produced during lipid peroxidation, and its assay is often used as a marker for oxidative stress in several diseases [28], including Alzheimer’s disease, atherosclerosis and diabetes [47]. Samples of liver and spleen showed an increase of TBARS levels in the same groups, A, B and C, being representative of infection by *L. Hardjo, L. Wolfii* and *L. Grippotyphosa*, respectively. In liver disease, free radicals have been implicated in the inflammatory process, and increased lipid peroxidation has been found [16]. In acutely infected animal models, liver pathology seems to be related to large numbers of leptospires and associated cytotoxic factors in tissues [5]. Researchers observed spleens of leptospirotic hamsters and found progressive changes in vasculature and congestion of the splenic sinusoids with hemorrhagic areas scattered throughout the red pulp [33]. In an observation of 33 fatal cases of human leptospirosis found large zones of hemorrhage which resulted from disruption of splenic vasculature, with Seven cases showed gross splenomegaly and the histopathology showed congestion with scattered hemorrhagic areas [7]. These observations are reporting damage to the liver and spleen and may be able to justify our finding of oxidative stress in these organs, since free radicals usually are implicated in the inflammatory process, tissue damage, causing increased lipid peroxidation.

As known, kidney is an important target organ in leptospiral infection [44,45]. Clinically, renal involvement in leptospirosis occurs in 16-40% of cases and is unique because of the atypical presentation of polyuria, hypokalemia, and sodium and potassium wasting [1,3,50]. As expected, kidney samples showed statistical differences for all infected groups when compared to the control group. These TBARS levels increased in all infected groups may be explained or justified due to the possibly interstitial nephritis as a direct result of the presence of *Leptospira* spp. In tissue, like suggested by Ajay et al. [3] in their review, and may be related to direct toxic effects of leptospiral compounds on renal transporters and microcirculation and to indirect effects of the pro-inflammatory response, with severe tissue damage [8,46].

Superoxide dismutase, an oxidase catalyzing the dismutation of $O_2^−$ into hydrogen peroxide and $O_2$, is thought to be essential for the protection of cells against ROS and is used experimentally and clinically as an antioxidant drug [6,12,42]. In the present study, it was demonstrated elevated SOD levels in liver, spleen and kidney, varying according to the *Leptospira*’s serovar. The liver is one of the tissues that showing high rate of ROS, which has an effect on hepatic SOD activities [49]. Statistical differences were found in groups A and C represented for *L. hardjo* and *L. grippotyphosa*, respectively. Activated phagocytic cells are involved in antibacterial defense but also produce tissue injury associated with production of ROS [9,26]. Researchers reported that activated phagocytic cells produce ROS and cause hepatic injury in gram (-) bacteremia [38]. Authors reported that hepatic SOD activity increased on two days in infected guinea pigs after the injection *L. interrogans* [25], and this was followed by a 20% decrease (from 3 to 14 days) resulting in levels comparable to healthy guinea pigs. Our study was carried out in 15 days, and this period could contributed to mostly groups infected had no statistical differences, since SOD increased only in animals infected with serotype *L. hardjo* and *L. grippotyphosa*.

On the basis of the morphologic and lipid peroxidation studies, the renal cortex appears to be a major site for protection by free radical scavengers. SOD, on the other hand, has a molecular weight of 32,600 and is rapidly taken up by the kidney, where it has been localized to the cortex [23,31]. All infected groups had increase of SOD levels excepting group H that represents serotype *L. pomona.* Oxygen free
radicals could theoretically produce damage in renal arteriolar endothelial cells, glomerular mesangial cells, and renal tubular epithelial cells [2,36]. Leptospirosis infection can cause renal insufficiency and failure is the result of tubular damage associated with colonization and replication of the organisms in renal tubular epithelial cells [15]. Acute impairment of renal function also may result from decreased glomerular filtration and hypoxia caused by kidney swelling that impairs renal blood perfusion [17]. Thus, the increase of levels of SOD suggests an attempt to kidneys protection, seeking for the decrease of free radicals produced by the injuries.

The burden of ROS production is largely counteracted by an intricate antioxidant. Catalase converts hydrogen peroxide to water. Researchers identified the enzyme catalase in several pathogenic serovars of *Leptospira* spp. [14,41]. CAT was studied in liver, spleen and kidney and levels of catalase rised was found in all organs, especially at kidneys. Liver samples showed a small variation in level of CAT, showing only group A (*L. hardjo*) with statistical difference. Thus SOD, when dismutes superoxide radical, form the reactive oxygen species the hydrogen peroxide (*H₂O₂*) and this is a substrate for the enzyme catalase (CAT), our findings in the liver samples were expected, since high levels of SOD was only found in groups A and C. According to Langston and Heuter [27] *L. grippotyphosa* typically are associated with minimal liver involvement, what can explain the absence of elevations in CAT levels. Levels of CAT in spleen samples were elevated in groups B and E, or infected with *L. wolffi* and *L. icterohaemorrhiae* respectively. Authors observed spleens of leptospirotic hamsters and found progressive changes in vasculature and congestion of the splenic sinusoids with hemorrhagic areas scattered throughout the red pulp [33]. The severity of the lesions increased with longer periods of leptospirosis infection [33], and this might explain our findings which corresponding in only two groups with CAT levels elevated (Group B and E), since our study was carried out in 15 days, that could be considerate a short time or an acute phases.

Catalase levels also had variations in the kidney samples to mostly groups. Excepting groups C (*L. grippotyphosa*) and D (*L. canicola*) all other groups showed statistical differences when compared to the control group. Leptospirosis infection can cause renal insufficiency and failure is the result of tubular damage associated with colonization and replication of the organisms in renal tubular epithelial cells [15]. Acute impairment of renal function also may result from decreased glomerular filtration and hypoxia caused by kidney swelling that impairs renal blood perfusion [17]. Again, these findings could be justify the increase of CAT in kidney samples, suggest an attempt to protect this organ, seeking with this increase an attempt to decrease the free radicals produced by the injuries caused by infection.

NPSH content varied significantly according to sampling collected and analyzed. The highest mean values were observed in samples of kidney in groups A, B, C, D and E, and samples of spleen in groups B, D, E and G. Intracellular nonprotein sulphhydryl groups protect cells against the cytotoxic effects of endogenous or exogenous electrophiles [19]. Leptospirosis is considered a toxin-mediated disease leading to lipid peroxidation as lipopolysaccharide of its membrane plays role in the cytotoxicity [4,29,50], and elevated level of NPSH may suggest the production of free radicals and of lipid peroxidation.

Based upon our results is possible to conclude that the infection by *Leptospira interrogans* in Wistar rats shows differences between serovars utilized experimentally. The results may suggest that oxidative damage to tissues along with other mechanisms might have taken part in the pathogenesis of leptospirosis and further detailed studies at cellular level are needed to fully understand the pathogenesis and clinical expression of the disease. It was confirmed that kidneys are the major host organ to leptospires, resulting in a great stress oxidative response and presenting a high activity of scavenger enzymes, as well as potential generation of oxidative stress to other organs and even to the brain.

**Ethical Approval.** The procedure was approved by the Animal Welfare Committee of Federal University de Santa Maria (UFSM), number 23081.015724/2009-47.

**Declaration of interest.** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.
REFERENCES


