The purpose of this study was to evaluate the efficacy of γ-oryzanol in acute and long-term mouse experimental models of dyslipidemia. The hypolipoproteinemic activities, to reduce the risk of CVD and to alter hepatic enzymes levels, plasma urea or creatinine concentrations. The results of this study suggest that γ-oryzanol acts as a potential lipid-lowering agent, reducing triglycerides and total cholesterol in dyslipidemia-induced models.

**Objective:** A substantial fraction of the population is intolerant or does not respond well to the recommended treatments for dyslipidemia. The purpose of this study was to evaluate the efficacy of γ-oryzanol treatment in acute and long-term mouse experimental models of dyslipidemia in comparison to Gemfibrozil and Simvastatin.

**Methods:** For the acute dyslipidemia-induced model, dyslipidemia was induced in 40 mice using a single intra-peritoneal administration of Triton WR-1339. For the long-term model, dyslipidemia was induced in 24 mice using a hypercholesterolemic diet over 14 days. Thereafter, animals were divided into different groups of treatment, and orally received treatments with γ-oryzanol (5, 25, 50 mg kg⁻¹), gemfibrozil or simvastatin. For biochemical analysis, glucose, total cholesterol and triglycerides were measured. Body weight and net food intake was registered weekly, and urea, creatinine, AST and ALT levels were evaluated. The data were analyzed by analysis of variance (ANOVA), followed by the Student-Newman-Keuls method and p value of less than 0.05 was considered significant.

**Results:** Only the highest dose of γ-ORZ exhibited significant protective effects. Gamma-oryzanol and Gemfibrozil treatments reduced total cholesterol and triglycerides levels in a similar manner in the acute model. In the second model, γ-ORZ and simvastatin treatments reduced glucose and total cholesterol levels in the same way. In addition, the administration of γ-ORZ did not cause any adverse events, or significantly altered hepatic enzymes levels, plasma urea or creatinine concentrations.

**Conclusion:** The results of this study suggest that gamma-oryzanol acts as a potential lipid-lowering agent, reducing triglycerides and total cholesterol in dyslipidemia-induced models.

**Keywords:** Dyslipidemia, Gamma-oryzanol, Statin, Fibrate, Cholesterol, Triglycerides, Glucose, Lipid-lowering.
1339 (400 mg, kg\(^{-1}\)), as previously reported\([20, 21]\). 48 animals were used. The control group (CTL) received a single intra-peritoneal administration of 0.9% NaCl solution (10 mL, kg\(^{-1}\) ). Thereafter, animals were divided into6 groups (\(n = 8\) each) and orally received the following treatments: Control (vehicle); T1339 (vehicle); GEMF (gemfibrozil100 mg, kg\(^{-1}\)); 5y-ORZ (γ-oryzanol 5 mg, kg\(^{-1}\)); 25y-ORZ (γ-oryzanol, 25 mg, kg\(^{-1}\)); and 50y-ORZ (γ-oryzanol 50 mg, kg\(^{-1}\)). The groups received the treatments three times: 1 hour before Triton WR-1339 administration and 22 and 46 hours after induction. Gamma-oryzanol and gemfibrozil were suspended in 3% (v/v) Tween 80 and then in water. The mice were fasted for 8 h before blood sampling. 24 h and 48 h following the Triton induction, blood samples were collected and centrifuged (15 min, 2,400 x g, RT) to obtain plasma.

For the long-term dyslipidemia-induced model, dyslipidemia was induced using a hypercholesterolemic diet over 14 days, as reported by Wilkon, Nicolson \[22\]. 32 animals were used. Animals were divided into4 groups (\(n = 8\) each) in accordance with their total cholesterol levels and were treated for 2 months as follows: CTL (normal diet + vehicle, p. o.); HCD (hypercholesterolemic diet + vehicle, p. o.), 50y-ORZ (hypercholesterolemic diet+ γ-oryzanol 50 mg, kg\(^{-1}\), p. o.) And SIMV (hypercholesterolemic diet+ simvastatin 20 mg, kg\(^{-1}\), p. o.). The concentrations chosen for γ-ORZ were based on the results observed in the acute model. Gamma-oryzanol and SIMV were suspended in 3% (v/v) Tween 80 in water. HCD controls received the same vehicle. After each month of treatment, the animals were fasted for 8 h, and then blood samples were collected for biochemical analysis.

### Table 1: Diet Formulations

<table>
<thead>
<tr>
<th>Diet component</th>
<th>Control Diet</th>
<th>Hypercholesterolemic Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>22 ± 5</td>
<td>19 ± 1</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>57 ± 6</td>
<td>51 ± 3</td>
</tr>
<tr>
<td>Fat</td>
<td>4 ± 1</td>
<td>13 ± 3</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0 ± 0</td>
<td>11 ± 3</td>
</tr>
<tr>
<td>Vitamin, mineral, other</td>
<td>9.5 ± 6</td>
<td>8.5 ± 0</td>
</tr>
<tr>
<td>Humidity</td>
<td>7.5 ± 0</td>
<td>6.4 ± 0</td>
</tr>
</tbody>
</table>

For biochemical analysis, glucose, total cholesterol and triacylglycerols were measured using commercially enzymatic kitsGlicose GOD, CoolesterolLiquiform and TriglicerídeosLiquiform (LabtestDiagnóstica, Brazil). In addition to these, body weight, net food intake was registered weekly and urea, creatinine, AST and ALT levels were measured by commercial kitsUreia CE, CK-NAC Liquiform, AST/GOT Liquiform and ALT/GPT Liquiform (LabtestDiagnóstica, Brazil), respectively.

### Statistical analysis

The results are presented as the mean ± standard error of the mean (SEM). The data were analyzed by analysis of variance (ANOVA), followed by the Student-Newman-Keuls method. \(P\) value of less than 0.05 was considered significant.

### RESULTS

Intra-peritoneal injection of Triton WR-1339 clearly induced dyslipidemia, characterized by an increase in blood glucose, total cholesterol (TC) and triacylglycerols (TG) 24 h after induction, there was a statistically significant increase in blood glucose (70 %), TC (310 %) and TG (4925 %) (Figure 1A) in the T1339 group compared to control.

Among doses of γ-ORZ tested (5, 25 and 50 mg, kg\(^{-1}\)), only the highest dose exhibited significant protective effects. Mice treated with 50 mg γ-ORZ showed a statistically significant (25 %) reduction in blood glucose levels when compared to the T1339 group, whereas there was no change in the GEMF group. Both 50 γ-ORZ and GEMF significantly reduced the TC and TG in comparison to the T1339 group (Figure 1B and 1C). Even at 48 h after induction, the T1339 group showed higher levels of GLU, TC and TG when compared to the CTL group. In addition, the 50y-ORZ group exhibited significantly reduced levels of all the parameters, approaching levels similar to those measured in the CTL group. Fig. 1

After the first month of the hypercholesterolemic diet-induced dyslipidemia model, the HCD group showed a statistically significant increase in glucose levels (20 %) compared to animals that received a normal diet (CTL). HCD efficiently increased cholesterol levels in all groups (47 %) compared to the CTL group. However, somewhat unexpected was the observation that triglyceride levels were reduced in the HCD group compared to CTL.

The 50y-ORZ-treated animals showed a statistically significant reduction of 22 % in cholesterol levels when compared to the HCD mice, reaching levels similar to those in the CTL mice. In addition, the hypercholesterolemic diet nearly doubled (188 %) cholesterolemia in the HCD group. SIMV effectively reduced the total cholesterol (14 %) compared to the HCD group. Mice receiving the 50y-ORZ treatment exhibited a greater reduction in total cholesterol of around 30%
triacylglycerolemia, dyslipidemia was induced with Triton WR1339. Triacylglycerol-rich lipoproteins by lipoprotein lipase (LPL). This nonionic detergent acts to prevent the catabolism of triacylglycerols. A reduced glucose and total cholesterol levels in a similar manner.

To evaluate the acute effects of γ-ORZ on cholesterol and triacylglycerolemia, dyslipidemia was induced with Triton WR1339. Nonionic detergent acts to prevent the catabolism of triacylglycerol-rich lipoproteins by lipoprotein lipase (LPL). This nonionic detergent acts to prevent the catabolism of triacylglycerols. A reduced glucose and total cholesterol levels in a similar manner.

There were no statistically significant differences in final body weights among the groups. No side effects were observed in any of the groups. The plasma urea levels were significantly higher in the HCD mice when compared to the CTL. Nevertheless, this increase in urea levels was reversed by the γ-ORZ and SIMV treatments. No significant changes were observed in the levels of AST, ALT or creatinine in the studied groups (data not shown).

DISCUSSION
The study presented here sought to evaluate the efficacy of γ-ORZ as a lipid-lowering drug, and compared it to existing drugs commonly used to treat dyslipidemia. Results presented here clearly show that γ-ORZ efficiently reduced blood triglycerides level and total cholesterol in dyslipidemia models.

The results of this study suggest that 50 mg·kg⁻¹ γ-oryzanol dose (50γ-ORZ) simvastatin (SIMV). Each value represents the mean ± SEM. The groups that statistically differed from CTL and from HCD were marked as (*) and (§), respectively.

CONCLUSION
The results of this study suggest that γ-ORZ may be valuable for designing future pharmacologically active agents to tackle dyslipidemia.

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CONFLICT OF INTERESTS
There is no conflict of interest in this paper.

REFERENCES


