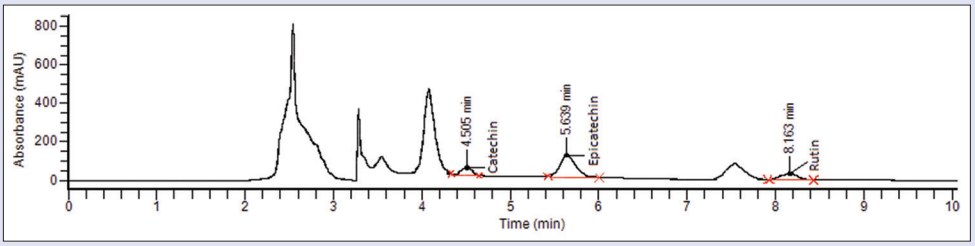

Figure 1: Chromatogram of *Drimys brasiliensis* extract (10 mg/mL) for detection of monomers of condensed tannins and flavonoids

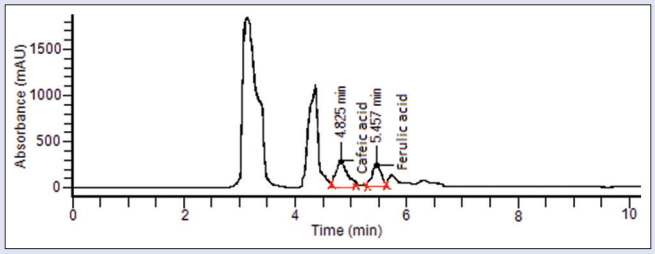

Figure 2: Chromatogram of *Drimys brasiliensis* extract (10 mg/mL) for detection of phenolic acids

weighed. The weights of the organs were normalized to the mass of the rat on the day of sacrifice. Protein quantification in the organs was performed by the Lowry method.[20]

Biochemical analysis

Measurements of total cholesterol, High-density lipoprotein (HDL)-cholesterol, triglycerides, and glucose in plasma were performed using 350 BTS BioSystem® (Spain) dosing equipment using enzymatic kits (Labtest®, Minas Gerais, Brazil) according to manufacturer's recommendations. The blood of the animals was sent to the Clinical Laboratory School of the University of Passo Fundo for hematological analysis using the impedance method (Diagon®, D-Cell 60, EU).

Statistical analysis

The results were submitted for descriptive and inferential statistical analysis. The pattern of distribution of data was analyzed by Kolmogorov–Smirnov test. The results were subjected to the comparison of means by analysis of variance (One-Way ANOVA for parametric data) followed by Dunn's *post hoc* test, with a significance level of $P < 0.05$. The results of body weight gain and food intake were evaluated through the analysis of two-way ANOVA for repeated measures. Data were expressed as a mean ± standard deviation.

RESULTS AND DISCUSSION

In vitro assay

HPLC analysis of *D. brasiliensis* extract (10 mg/mL) detected some interesting substances. The amounts of catechin, epicatechin, and rutin were calculated from the integrated peak areas of the extract, as 134.20 μg/mL (1.34%), 348.26 μg/mL (3.48%), and 85.98 μg/mL (0.86%), respectively [Figure 1]. Caffeic and ferulic acids were found in the extract at concentrations of 45.36 μg/mL (0.45%) and 83.67 μg/mL (0.84%), respectively [Figure 2].

The anti-inflammatory effect of thirty flavonoids was evaluated, and among them, rutin and jaceosidina exhibited the best anti-inflammatory effect.[21] Rutin also exerts antithrombotic action by eliminating free radicals that cause platelet aggregation.[22] Flavonoids have antioxidant activity owing to their redox properties, which can neutralize free radicals.

To the best of our knowledge, this is the first study to report the presence of rutin and monomer precursors of condensed tannins (catechin and epicatechin) in *D. brasiliensis*. In *Drimys* genus, some flavonoids were detected, but rutin, tannins, and phenolic acids have not been described thus far.[4] These compounds could be responsible for its pharmacological activity. Drimane sesquiterpenes[23] were isolated from the aerial parts of *D. brasiliensis*, and polygodial drimane sesquiterpenes, drimanial compounds, 1 β (p methoxy cinnamyl) polygodial, epifuegina, acids, and fatty alcohols were isolated from the bark.[6] Furthermore, the flavonoids, i.e., astilbin, quercitrin, isoastilbin, and neoastilbin were found in *D. brasiliensis*.[24] Other works describe the volatile compound composition of *D. brasiliensis* Miers and *D. angustifolia* Miers.[25]

D. brasiliensis extract presented strong antioxidant potential by reducing DPPH radicals. The perceptual reduction, with the higher concentration of the extract (500 μg/mL), reached 63% [Figure 3a]. The potential antioxidant effect of *D. brasiliensis* extract was converted into Vitamin C (VCEAC), demonstrating the great antioxidant potential of the extract [Figure 3b]. It is well known that volatile oils isolated from *D. angustifolia* and *D. brasiliensis* do not present antioxidant activity.[26] However, for the first time, the antioxidant properties of *D. brasiliensis* bark extract have been described.

In vivo assay

To determine the doses of *D. brasiliensis* extract for the *in vivo* study, doses of 250 and 500 mg/kg were initially administered by gavage to rats (6 animals per group). However, the 500 mg/kg dose caused the death of three animals; hence, this dose was reduced to 100 mg/kg. The dose of 500 mg/kg/day orally was defined as the lethal dose (LD_{50}). The doses of 100 and 250 mg/kg/day were used for this study. Clinically, two rats of GDbP 250 had epistaxis, difficulty breathing, and diarrhea. The group treated with 100 mg/kg/day showed no clinical signs of toxicity. No statistically significant differences in the hematological parameters were observed between the groups.

The acute toxicity of essential oils of *D. brasiliensis* leaf at doses of 175, 550, and 1000 mg/kg has been previously assessed.[26] No deaths were observed, but signs of toxicity such as reduced locomotor activity, ptosis, exophthalmoses, urination, diarrhea, salivation, tremors, increased respiratory rate, and squirming were apparent at all doses. The toxicity of the ethanolic extract of the *D. angustifolia* bark was investigated, and deaths were observed in the groups treated with 3500 mg/kg (two animals of ten) and 5250 mg/kg (four animals of ten),[9] and a decrease in the spleen and liver weight was observed. No deaths occurred with the leaf extract. The differences between the LD_{50} values in the different studies can be explained by the differences in the polarity of the extracts. Essential oils present a chemical composition that is rich in lipids with no polar compounds (cyclocolorenone, terpinen-4-ol, and myristicin),[26]

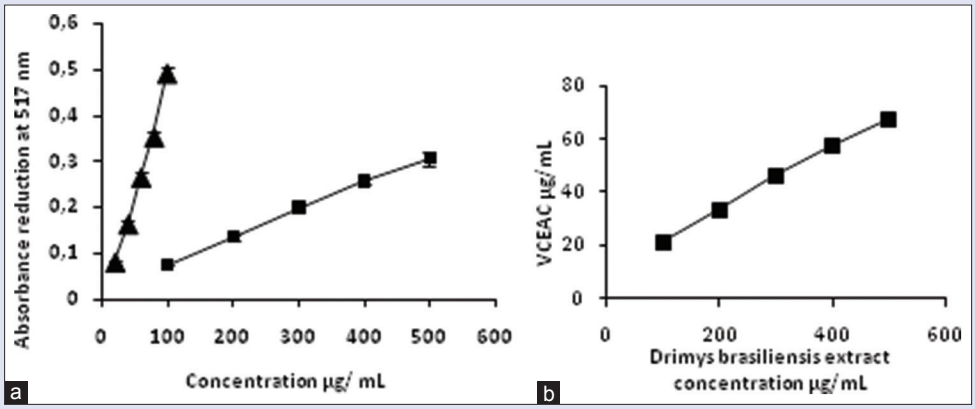

Figure 3: (a) Relationship between ▲ Vitamin C (y = 0.005x – 0.032, *r* = 0.9912) or/*Drimys brasiliensis* extract (y = 0.0006x + 0.0184, *r* = 0.9979) and absorbance reduction of 1,1-diphenyl-2-picrylhydrazyl radicals at 30 min. Vertical bar: Standard deviation. (b) Conversion of antioxidant potential of/*Drimys brasiliensis* extract into Vitamin C equivalent antioxidant capacity by 1,1-diphenyl-2-picrylhydrazyl assay

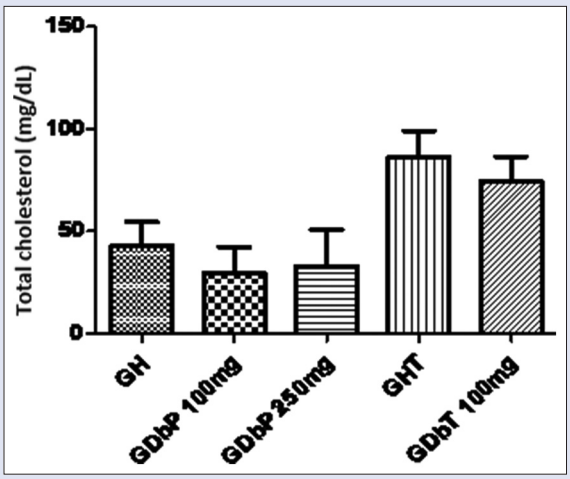

Figure 4: Comparative analysis of the total cholesterol (mg/dL) of the rats treated for 20 days with the extract, concomitantly fed a hypercaloric diet (prevention assay) or after 20 days of the administration of the diet (treatment assay). Results were expressed by mean ± standard deviation

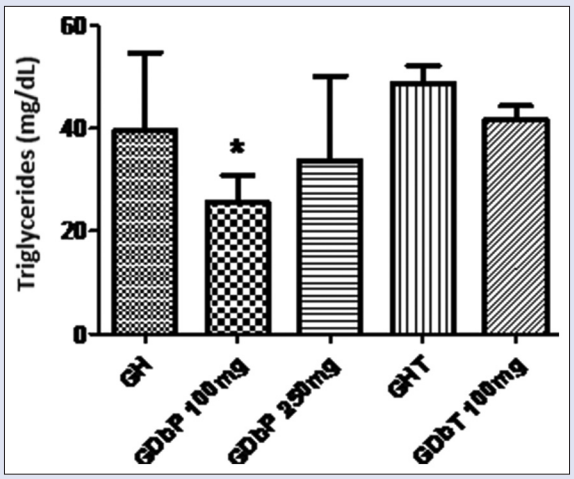

Figure 5: Comparative analysis of the triglyceride levels (mg/dL) in rats treated for 20 days with the extract, concomitantly fed a hypercaloric diet (prevention assay) or after 20 days of administration of the diet (treatment assay). Results were expressed by mean ± standard deviation. *Statistical difference related to GH (Hypercholesterolemic control group)

whereas we used polar compounds in this study. Hence, *D. brasiliensis* can be considered more potent than *D. angustifolia*.

Statistical changes in food consumption, relative body weight gain, relative organ masses, and protein levels were not observed in the organs between groups. Interestingly, the plant extract was able to reduce cholesterol [Figure 4] and triglycerides [Figure 5] both in the prevention and treatment assay, with a significant effect observed at a dose of 100 mg/kg/day. In the treatment assay (GDbT 100 mg and GHT), the total cholesterol levels were the highest compared to the prevention assay owing to the fact that these groups received a hypercaloric diet for 40 days while the other groups were fed a hypercaloric diet for 20 days. No significant differences between groups were observed in HDL cholesterol and blood glucose levels. Considering the absence of significant changes in food consumption, which could result in lower hypercaloric intake, and consequently improvement of lipid profile, we speculate that anti-lipid activity is directly related to the antioxidant activity of *D. brasiliensis* extract as proven using the DPPH test. The antioxidant activity is directly related to the chemical composition of the extract where monomers of condensed tannins, flavonoid, and phenolic acids were detected. A linear relationship was not observed between hypocholesterolemic activity and dose since other unidentified mechanism could be involved. Thus, this is the first study to report that *D. brasiliensis* extract has the ability to reduce triglycerides and total cholesterol, thereby aiding to control this disease.

Other studies have related the presence of phenolic compounds to an improvement in the plasma antioxidant capacity. The diet consisting of Jaffa grapefruit (100 g/kg) for 4 weeks improve the plasma lipid levels and increase the plasma antioxidant activity.[27] Furthermore, the hypocholesterolemic and antioxidant activities of durian, mangosteen, and snake fruit (50 g/kg) in rats were evaluated, and no difference among the tropical fruits was observed.[28] In this way, the dose used in our work is lower than that used in other works, and the results were significant.

CONCLUSIONS

For the first time, we have proven that *D. brasiliensis* Miers extract has antioxidant activity and reduces triglycerides *in vivo*. Mechanisms responsible for this pharmacological effect possibly are related to the

scavenging of free radicals as demonstrated *in vitro*. Doses used can be considered safe since animals did not present any clinical signs of toxicity during treatment. The chemical composition (monomers of condensed tannins, flavonoids, and phenolic acids) was found to be directly related to its antioxidant activity. Consequently, the importance of ethnopharmacology for the development of drugs has once more been proven. *D. brasiliensis* shows incredible potential to be used as a nutraceutical and to develop new drugs owing to its antioxidant and anti-lipid properties.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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