







INVESTIGATION OF THE POSSIBLE PROTECTIVE EFFECT OF
Smilax fluminensis STEUD. LEAF IN MICE SUBJECTED TO
OXIDATIVE STRESS BY PARACETAMOL

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Abstract

Paracetamol (PCM) is a drug widely used by the population as an antipyretic and analgesic. If administered in high doses it can cause liver damage, leading to hepatotoxicity. The genus *Smilax*, found in temperate and tropical regions, is traditionally used by the population through the extract of leaves and roots for several conditions, such as in the treatment of syphilis, diabetes, asthma and as a diuretic action. Through this, *Smilax fluminensis* leaf extracts were used to evaluate the protective effect against oxidative stress induced by a high dose of PCM in mice that received the drug and after receiving treatment with crude extract and fractions. Plasma analysis was performed using aspartate aminotransferase (AST), alanine aminotransferase (ALT), glucose, triglycerides and cholesterol, in addition to biochemical techniques such as catalase (CAT), glutathione-S-transferase (GST), reduced glutathione (GSH), ascorbic acid (ASA), substances reactive to thiobarbituric acid (TBARS) and carbonylated proteins (CARBONYL) of liver, brain and kidneys. Fraction 1 of the extract was the most promising, decreasing the plasma levels of AST and ALT, the levels of CAT and GST of the liver, together with GSH and in the renal and brain tissue there was a decrease in carbonylated proteins (PCM + F1 versus PCM). Besides, fraction 1 proved to be hypoglycemic and hypocholesterolemic. It is concluded that fraction 1 of *Smilax fluminensis* leaves has good antioxidant activity in the face of the damage caused by the high dose of paracetamol.

Keywords: Acetaminophen. Free Radicals. Hepatotoxicity. Hypoglycemic Agents. *Smilax fluminensis*.

1. Introduction

Oxidative stress is a result of the imbalance between reactive oxygen species (ROS), nitrogen (ERN) and the antioxidant defense system present in the body (Sahu et al. 2010). When such a balance tends towards an excessive production of these compounds or a deficiency of the antioxidant systems, the oxidative stress condition arises, which is harmful to the cellular components and to the individuals as a whole (Carocho and Ferreira 2013). Through this, several pathologies have been identified such as arthritis, atherosclerosis, diabetes, cataracts, multiple sclerosis, chronic inflammation, brain dysfunction, heart disease, emphysema, aging, and cancer, among others (Phaniendra et al. 2010).

The presence of antioxidants in plants, such as phenolic compounds, flavonoids, tannins and proanthocyanidins can provide protection against various diseases (Gulcin 2012), since they interact with

free radicals before they can react with biological molecules, preventing the occurrence of chain reactions or preventing oxygen activation to highly reactive products (Ratnam et al. 2006).

Paracetamol (PCM) is a medicine with analgesic and antipyretic activity most used by the population (Salwe et al. 2017). The administration of paracetamol is safe when the therapeutic dose is not exceeded, however, it can cause hepatic necrosis, nephrotoxicity, extrahepatic lesions and even death in humans and experimental animals when subjected to overdose (Mohanraj et al. 2013). PCM-induced hepatotoxicity involves the formation of a chemically reactive intermediate, namely N-acetyl-p-benzo-quinone-imine (NAPQI), which is formed by cytochrome P450 enzymes (CYP450s) and normally detoxified through the conjugation of glutathione (Hinson et al. 2010). Thus, paracetamol-induced toxicity (N-acetyl-p-aminophenol, acetaminophen) in rats is one of the experimental models widely used to assess the hepatoprotective activity of plant extracts (Hussain et al. 2014).

Medicinal plants have always been associated with cultural behavior and traditional knowledge. Many studies have shown that medicinal plants contain several bioactive compounds with antioxidant activity, which are responsible for their beneficial effects on health (Dai and Mumper 2010). Through this, there is a great interest in foods of plant origin with antioxidant properties due to their health benefits and the ability to prevent chronic diseases caused by oxidative stress (Desanmi et al. 2009).

Smilax fluminensis is commonly called "sarsaparilla" or "japicanga", belongs to genus *Smilax*, comprises approximately 370 species that are widely distributed in tropical and temperate zones (Souza and Lorenzi 2008; Judd et al. 2009; Wu et al. 2010; Zhou et al. 2017). It has a vast occurrence in the states of Roraima, Pará, Bahia, Minas Gerais, Rio de Janeiro, São Paulo and states of the Midwest Region, extending to Bolivia, Paraguay and Argentina. *Smilax* species, such as the leaves of *S. excelsa*, are used as food or pharmaceutical materials in many countries, such as in some parts of Turkey (Ozsoy et al. 2008). In addition, the use in folk medicine of plants of this genus is quite common, as is the case of extracts of leaves and roots that are used in the treatment of syphilis, brucellosis, nephritis, gout, rheumatism, skin disorders, asthma, toothache, wounds, tumors, diabetes, dementia and also as a blood purifier and diuretic (Wu et al. 2010; Chen et al. 2011; Wungsintaweekul et al. 2011; Khaligh et al. 2016). Recently, studies performed with different *Smilax* species have demonstrated hypoglycemic and hypotensive activities (Amaro et al. 2014; Romo-Pérez 2019), antioxidant and antifungal potential (Morais et al. 2014; Wang et al. 2019) and cytoprotective effects against oxidative stress in pulmonary cells and in the liver (Rajesh and Perumal 2014).

Petrica and collaborators (2014) identified phytochemicals in extracts obtained from the leaf of *Smilax fluminensis* Steud. (*S. fluminensis*), obtaining as a final result the isolation and elucidation of two compounds containing Quercetin. Quercetin is a flavonoid found in vegetables and fruits, in which it has significant antioxidant and anti-inflammatory effects (Serban et al. 2016). Despite different biological activities and bioactive compounds that are well documented for some *Smilax* species and for not having biological studies with this plant, this is the first work that aimed to investigate the antioxidant action of the crude extract and leaf fractions of *S. fluminensis* using as a model oxidative stress induced by paracetamol in Swiss mice.

2. Material and Methods

Extract preparation

The leaves of the plant were collected in the municipality of Nova Canaã do Norte - MT and an exsiccate of this species was deposited at the Herbarium of the Federal University of Mato Grosso (UFMT), Sinop Campus-MT/ Brazil, under nº 1606, 1607 and 1608. After collection, the leaves were cleaned, dried and crushed. The crushed material was subjected to exhaustive maceration with ethanol (6 L) for one week. After filtration, the crude ethanolic extract (EB) (480 g) was obtained by evaporating the solvent (50 °C) under reduced pressure (700 mmHg). Part of the EB was partitioned with 1.5 L of methanol-water (1:1, V/V) resulting in two extracts, a suspension with chlorophyll (180.0 g) and another ethanolic extract (EE) without chlorophyll (280.0 g). Part of the EE was fractionated in a chromatographic column with silica gel as a stationary phase and elution by polarity gradient producing the fractions: hexane (FH), dichloromethane (FD), ethyl acetate (FAE), methanol (FM), methanol-water (9:1, V/V) (FMA) and methanol/water/acetic acid

(7:3:1, V/V) (FMAA). Part of the EB and the FAE (F1) and FM (F2) fractions, which showed a higher concentration of flavonoids, were used in the experimental model.

Animals and treatment

Male *Swiss* mice (average of 30 g), obtained from the Vivarium of the Federal University of Mato Grosso, Campus in Cuiabá, were kept under conditions with temperature (22 ± 2 °C), light (12 hours light/dark) and humidity control ($51 \pm 2\%$), with water and pellets fed *ad libitum*. The animals remained for ten days under a period of acclimation. Afterwards, the animals received the treatment by gavage, initially being administered paracetamol (inducing liver damage) or filtered water. After three hours, treatment with the extract, fractions or filtered water was started, which lasted for 7 days. The animals were divided according to the following groups: CONTROL (filtered water), PCM (paracetamol 250 mg kg⁻¹ + filtered water), EB (crude extract - 250 mg kg⁻¹), F1 (fraction 1- 250 mg kg⁻¹), F2 (fraction 2 - 250 mg kg⁻¹), PCM + EB, PCM + F1, PCM + F2.

Twenty-four hours after the last dose, the animals were anesthetized intraperitoneally with ketamine 50 mg kg⁻¹, xilazine 2 mg kg⁻¹ and acepromazine 2 mg kg⁻¹. Cardiac puncture was performed to collect blood with heparinized syringes and, after this stage, the animals were euthanized to remove the liver, kidneys and brain, in which they were stored in the ultra-freezer at -80 °C. This study was approved by the Ethics Committee on the Use of Animals (CEUA), UFMT under nº 23108.781869/12-0.

Biochemical analysis: tissue

In the liver tissue, enzymatic antioxidants such as catalase (CAT) were analyzed according to the method of Nelson and Kiesow (1972) and the results expressed as $\mu\text{mol min}^{-1} \text{mg of protein}^{-1}$ and glutathione-S-transferase (GST) by the method described by Habig et al. (1974) and the results expressed in $\mu\text{mol GS-DNB min}^{-1} \text{mg protein}^{-1}$. The dosed non-enzymatic antioxidants were reduced glutathione (GSH) determined according to the method of Sedlack and Lindsay (1968) and the data expressed as $\mu\text{mol GSH mg protein}^{-1}$ and ascorbic acid (ASA) which followed Roe's method (1954) and the results presented in $\mu\text{mol ASA g}^{-1} \text{ tissue}$. The oxidative stress markers evaluated were carbonylated proteins (Carbonyl, protein damage marker) according to Yan et al. (1995) and the results expressed in $\text{nmol carbonyl mg protein}^{-1}$ and substances reactive to thiobarbituric acid (TBARS, marker of lipid damage), which were evaluated according to Buege and Aust (1978), and the data presented in $\text{nmol MDA mg protein}^{-1}$. In addition to liver tissue, CAT and protein damage in renal and brain tissue and the non-enzymatic antioxidant, GSH, in renal tissue were analyzed. The amount of protein in the tissues was determined by the method of Bradford (1976) and compared to a standard curve of bovine albumin.

Biochemical analysis: plasma

The plasma measurements of aspartate aminotransferase (AST), alanine aminotransferase (ALT) activities, as well as the concentration of glucose, triglycerides and cholesterol were performed with commercial kits (Labtest®).

Statistical analysis

Data were analyzed using the mean and standard deviation (SD) according to the unidirectional ANOVA, followed by the *Tukey post hoc* test, considering the differences between the groups when $P < 0.05$.

3. Results and Discussion

After carrying out this first biological study with the extract and fractions of the leaves of *Smilax fluminensis*, in the PCM-induced oxidative stress model in mice, we can observe that at the end of seven days of treatment, EB reduced the oxidative stress markers (TBARS and CARBONYL) in the liver tissue. Both fractions F1 and F2 showed similar results for CARBONYL, depending on the tissue (renal or brain). In addition, we found that F1 answered the objectives of the study, showing good results when assessing

antioxidants in liver tissue (CAT, GST, GSH). Besides, with respect to liver damage markers, EB was toxic as well as F1 and F2, although these have partially reduced the damage caused by PCM. On the other hand, a hypoglycemic effect was also observed for most treatments, reduction of triglycerides by F2 and cholesterol by F1 (Figure 1).

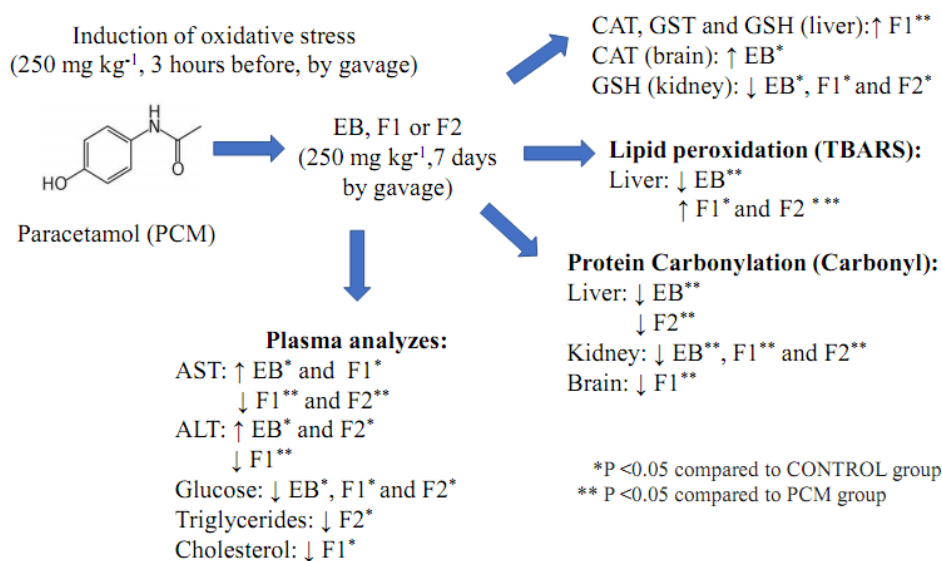


Figure 1. Effect of the EB and F1 and F2 fractions of *Smilax fluminensis*, under oxidative stress induced by PCM in the tissues and plasma.

Protein oxidation can be an effect caused by high levels of ROS, resulting in protein carbonylation and oxidative damage (Li et al. 2010). Increased levels of hepatic MDA, an important reactive aldehyde that results from the peroxidation of polyunsaturated fatty acids in the cell membrane, are indicative of a causal role of lipid peroxidation resulting from liver damage induced by PCM (Adam et al. 2016). According to Figures 2A and 2B, analyzes of CARBONYL and hepatic TBARS showed an increase in the PCM group and EB was able to restore the levels of these markers at the control level, showing that treatment with this extract was able to inhibit the damage to proteins and lipids and the same occurred with F2 in protein carbonylation, but in lipid peroxidation this fraction didn't present the same effect. The oxidative stress model is already well characterized, as in the work developed by Ajiboye et al. (2017) using the administration of PCM in dose of 300 mg kg⁻¹ in mice, which showed the same protective effect, regarding the reductions in the dosages of TBARS and carbonylated proteins, although they administered the aqueous extract of the leaves of *Phyllanthus muellarianus* (100, 200 and 400 mg kg⁻¹).

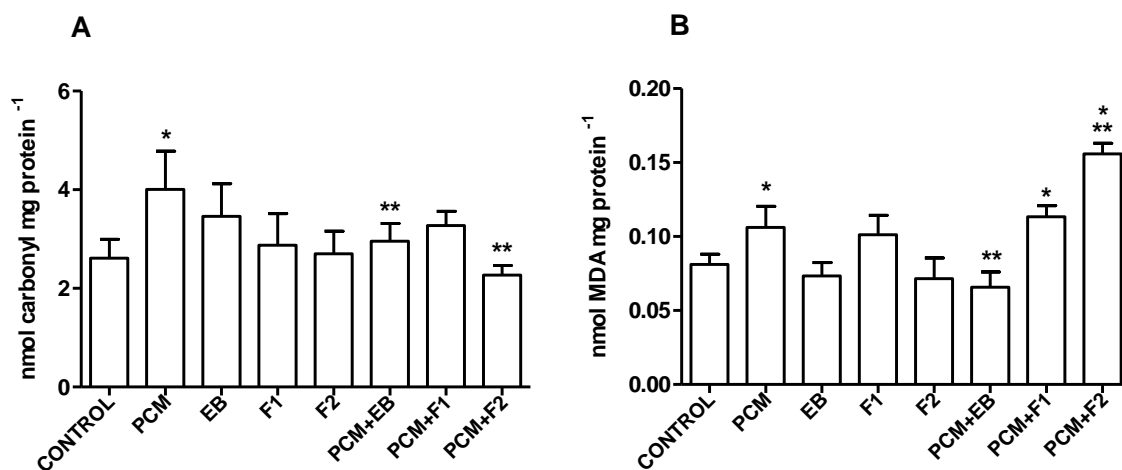


Figure 2. Effect of the EB and F1 and F2 fractions of *Smilax fluminensis*, under oxidative stress induced by PCM in the liver tissue. A – CARBONYL; B – TBARS (n = 8). * P <0.05 compared with the CONTROL group, ** P <0.05 compared with the PCM group. ANOVA followed by Tukey test.

High doses of PCM result in saturation of the metabolism pathways by glucuronidation and sulfation, thus, the PCM metabolism is diverted to the cytochrome P450 system generating the toxic and highly reactive N-acetyl-p-benzoquinone metabolite (Khandelwal et al. 2011). NAPQI depletes glutathione and initiates a covalent bond to cellular proteins leading to disruption of calcium homeostasis, mitochondrial dysfunction and oxidative stress, which can culminate in cell damage and cell death (Hurkadale et al. 2012). Depletion of glutathione causes severe liver damage by increasing the susceptibility of cells to oxidative stress (Hinson et al. 2010).

The metabolism associated with glutathione is an important mechanism of cellular protection against agents that generate oxidative stress, eliminating cytotoxic products of lipid peroxidation, in addition, changes in GST activity can cause disturbances in the concentration of GSH (Fernández-Iglesias et al. 2014). Thus, the main function of GST is the cellular defense mechanism against electrophilic xenobiotics and their metabolites (Gopia et al. 2017). Besides, this enzyme also plays a vital role in the detoxification of lipid and electrophilic hydroperoxides toxic by catalyzing their conjugation with GSH, which contributing to the protection of cell integrity (Townsend and Tew 2003). In our studies, GST activity in the liver tissue showed a decrease in the PCM group and the F1 was able to reverse the cellular damage caused by the drug (PCM + F1 versus PCM; Figure 3A).

On the other hand, the GSH plays an important role in the detoxification of NAPQI, because when the therapeutic dose of PCM is within limits, NAPQI is promptly detoxified forming a conjugate with GSH, but when there is an overdose, NAPQI is excessively formed and depleted the content of GSH and, from there, binds to liver cell proteins, causing mitochondrial dysfunction (Radosavljević et al. 2010). Similarly, to GST, F1 of *Smilax fluminensis* promoted an increase in the concentration of GSH, important non-enzymatic antioxidant (PCM + F1 versus PCM; Figure 3B). These two results demonstrate the protective effect of this fraction against damage caused by PCM.

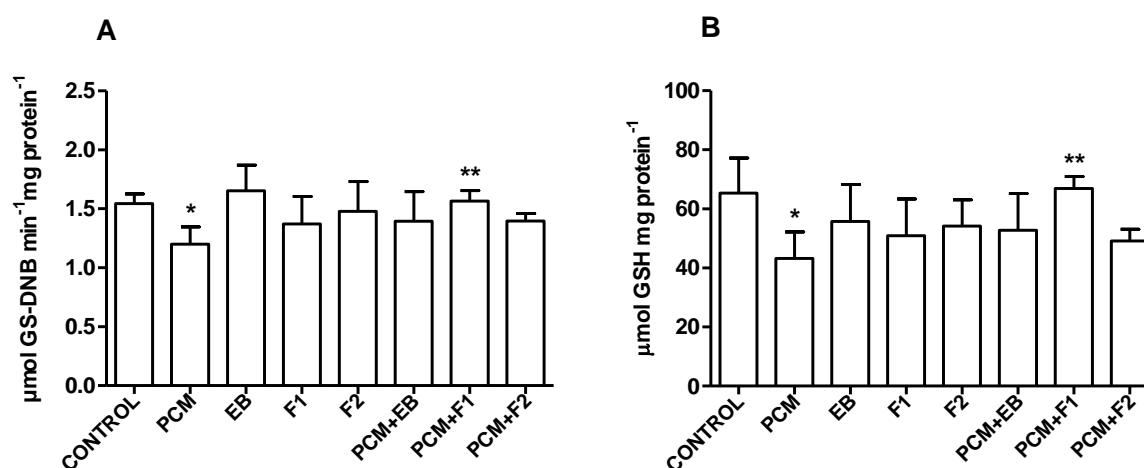


Figure 3. Effect of the EB and F1 and F2 fractions of *Smilax fluminensis*, under oxidative stress induced by PCM in the liver tissue. A – GST; B – GSH (n = 8). * P < 0.05 compared with the CONTROL group, ** P < 0.05 compared with the PCM group. ANOVA followed by Tukey test.

Free radical scavenging enzymes, such as SOD, CAT and GPx, are the first lines of defense against oxidative damage, so the dosage of these antioxidant enzymes is an indirect way to assess the antioxidant status in tissues (Sabir et al. 2012). SOD converts superoxide radicals into hydrogen peroxide and this is toxic to the body, being subsequently metabolized by CAT resulting in water and oxygen. In our study, there was a decrease in hepatic CAT activity in the PCM group and F1 managed to reverse this damage (Figure 4A). In the study by Xia et al. (2010), using a plant from the same family, it was found that CAT activity in the liver of *Wistar* rats that received the ethanolic extract of *Smilax glabra* was restored after the stress caused by lead acetate, which was used as a damage inducer. On the other hand, the non-enzymatic antioxidant ASA did not present any significant difference between the groups (Figure 4B).

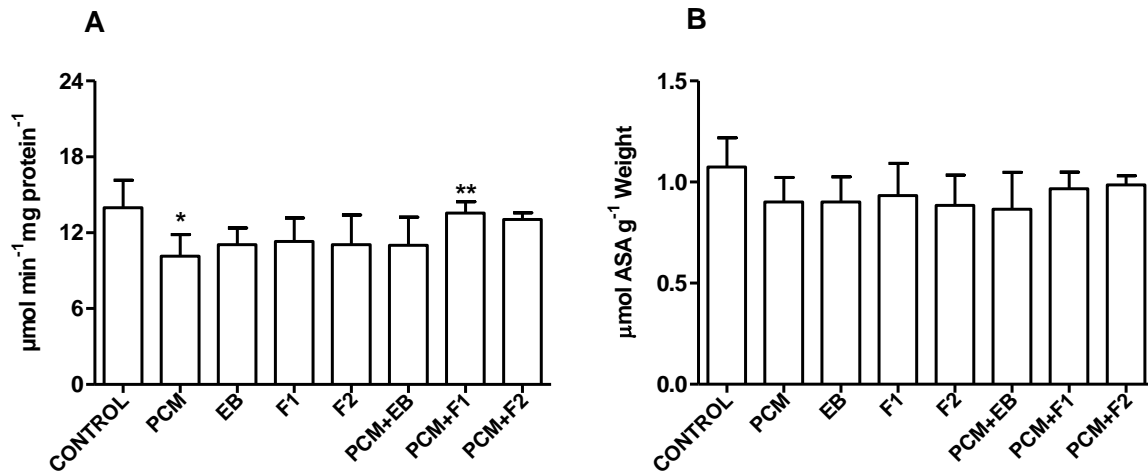


Figure 4. Effect of the EB and F1 and F2 fractions of *Smilax fluminensis*, under oxidative stress induced by PCM in the liver tissue. A – CAT; B – ASA (n = 8). * P < 0.05 compared with the CONTROL group, ** P < 0.05 compared with the PCM group. ANOVA followed by Tukey test.

The serum activities of AST and ALT are indicators of hepatotoxicity induced by acetaminophen (Ajiboye 2015). As a result, the induction of liver damage after a high dose of paracetamol (250 mg kg⁻¹) caused an increase in blood transaminases (AST and ALT; Table 1) in the PCM group, results that agree with the studies by Olaleye and Rocha (2008). However, EB, F1 and F2 showed toxicity, as they increased the activities of AST and ALT. When PCM was administered together with EB, the activity of these enzymes remained high. Although considered toxic by our findings, F1 managed to slightly reduce the increase caused by the drug, showing a significant difference in AST together with F2. Only the F1 fraction was able to decrease the plasma ALT activity increased by PCM (Table 1). In this context, our findings are in agreement with Murali et al. (2012) who evaluated the methanol extract from *Smilax zeylanica* roots and rhizomes and observed a reduction in transaminases using also the experimental PCM model, but with a higher PCM dose (1 g kg⁻¹).

Due to their antioxidant and chelating properties, flavonoids inactivate ROS and thus neutralize the oxidation of LDL in the plasma and promote the reduction of inflammation of the blood vessel endothelium, in addition to acting by decreasing the activity of xanthine oxidase, NADPH oxidase and lipoxygenase. Under these circumstances, its anti-atherosclerotic actions are related to the reduction of inflammation in the blood vessel wall through the inhibition of leukocyte influx (Majewska-Wierzbicka and Czczot 2012; Kozłowska and Szostak-Węgierek 2014).

Added to the antioxidant study, we decided to complement this investigation and evaluated the metabolic parameters in plasma and observed that the F1 fraction had a hypocholesterolemic effect and the F2 fraction promoted hypotriglyceridemia, which leads us to suggest that the presence of the glycosylated quercetin (Petrica et al. 2014) may be inducing this hypolipidemic function. In our studies, except for the alone crude extract group, all the others groups had a hypoglycemic effect when compared to the control group (table 1). According to Rómo-Perez et al. (2019) who investigated and demonstrated that the methanolic extract of roots of *S. moranensis* administered in hyperglycemic rats for a period of 42 days has hypoglycemic properties, corroborating with *S. fluminensis*, of the genus *Smilax*, which in this study presents this property.

There are studies in the literature which presents mechanisms to glycosylated quercetin absorption (Murota and Terao 2003) and about the microbial catabolism process on the deglycosylation of quercetin glycosides (Serra et al. 2012; Murota et al. 2018). Bahadoran et al. (2013) explain that one of the main effects of this process is to decrease intestinal assimilation of carbohydrates in the diet, regulation of enzymes involved in glucose metabolism, alteration of β cell function, insulin activity and stimulation of insulin secretion, besides of the hypoglycemic effect. Therefore, we suggest that the hypolipidemic effect also observed in this work can be correlated to hypoglycemic effect presented by involvement of deglycosylation of quercetin glycosides.

Table 1. Effect of the EB and F1 and F2 fractions of *Smilax fluminensis*, under oxidative stress induced by PCM in plasma.

Grupos	AST	ALT	Glucose	Triglycerides	Cholesterol
CONTROL	75.6 ± 19.6	27.0 ± 5.3	314.6 ± 37.5	133.5 ± 26.2	63.4 ± 10.6
PCM	330.2 ± 37.3*	45.5 ± 3.9*	269.9 ± 40.6	128.4 ± 27.1	60.0 ± 7.1
EB	170.7 ± 29.7*	81.3 ± 11.3*	274.9 ± 61.8	115.4 ± 21.8	64.2 ± 14.6
F1	155.7 ± 34.3*	24.3 ± 3.3	227.5 ± 29.3*	110.5 ± 23.9	45.4 ± 10.8*
F2	98.4 ± 21.7	41.3 ± 6.4*	225.8 ± 21.7*	87.3 ± 18.9*	48.3 ± 10.7
PCM + EB	288.5 ± 59.1*	41.0 ± 7.5*	247.3 ± 39.7*	120.0 ± 27.3	54.5 ± 9.1
PCM + F1	164.5 ± 21.8**	29.6 ± 5.8**	189.4 ± 37.9*	104.8 ± 24.1	50.0 ± 6.2
PCM + F2	107.6 ± 20.5**	37.1 ± 8.9	229.0 ± 36.2*	90.1 ± 16.4*	60.6 ± 11.7

The results are expressed as mean ± SD; n = 7-8 animals. * P <0.05 compared with the CONTROL group, ** P <0.05 compared with the PCM group. ANOVA followed by Tukey test.

Drug-related nephrotoxicity is due to the role of the kidneys in the clearance of toxic substances (Shahrbaf and Assadi 2015). Elevated levels of toxic liver metabolites, arising from high PCM intake, mainly NAPQI, quickly deplete GSH and covalently modify cellular proteins, leading to the generation of ROS and the depletion of ATP, this results in mitochondrial, hepatocyte and renal damage (Hinson et al. 2010; Hodgman, Garrard 2012). In the renal tissue, there was no significant difference between the groups regarding the CAT activity and for the GSH levels, there was a decrease in the PCM and F1 groups compared to the CONTROL group (table 2). The oxidative stress marker, CARBONYL, showed an increase in the positive control group (PCM) and the extract and fractions were able to reverse the protein damage caused by the drug (PCM + EB, PCM + F1 and PCM + F2 versus PCM) (Table 2). Studies realized by Pereira et al. (2018), who used the same experimental model of oxidative stress induction (dosage of 250 mg kg⁻¹ PCM), showed the same pattern of kidney damage from the drug to GSH and CARBONYL. Some studies have shown that high doses of PCM can cause acute and chronic renal failure, and the mechanism involved included deficits in the antioxidant defense mechanisms and lipid peroxidation in the renal tissue (Ghosh et al. 2010). However, in our study, the crude extract or fractions could contribute positively to the improvement of the CARBONYL results in this tissue, in face of the damage caused by the PCM. On the other hand, Wang et al. (2019) showed that a flavonoid-rich fraction from rhizomes of *S. glabra* Roxb. ameliorates renal oxidative stress on uric acid nephropathy rats, restoring CAT activity and GSH levels besides reducing TBARS levels (lipid damage biomarker). Differently, in this study we investigate a protein damage biomarker and all treatments reduced this parameter although other parameters didn't present similar results to the *S. glabra* in this tissue.

According to Ghanem et al. (2016), there is the fact that acute liver failure occurs due to high ingestion of paracetamol and there is the secondary development of brain changes caused by the drug, with paracetamol crossing the blood-brain barrier and being distributed throughout the central nervous system (Courad et al. 2001; Kumpulainen et al. 2007). Through this, we investigated the brain tissue that showed an increase only in the activity of the CAT enzyme in the EB group, and the increased protein damage caused by the PCM was reversed by F1 (Table 2).

Table 2. Effect of the EB and F1 and F2 fractions of *Smilax fluminensis*, under oxidative stress induced by PCM in renal and brain tissues.

	Kidney Tissue			Brain Tissue	
	CAT	GSH	Carbonyl	CAT	Carbonyl
CONTROL	18.72 ± 2.76	36.51 ± 8.38	4.39 ± 0.96	1.44 ± 0.31	4.52 ± 0.77
PCM	21.52 ± 2.88	22.27 ± 5.02*	6.54 ± 1.32*	1.44 ± 0.29	6.29 ± 1.02*
EB	18.54 ± 2.29	27.75 ± 5.79	4.24 ± 0.99	2.49 ± 0.52*	5.33 ± 1.04
F1	15.61 ± 1.87	25.02 ± 5.99*	4.26 ± 0.94	1.53 ± 0.36	5.29 ± 1.31
F2	18.21 ± 3.34	32.60 ± 8.03	3.92 ± 0.91	1.59 ± 0.37	4.10 ± 0.82
PCM + EB	17.73 ± 3.68	21.33 ± 4.81*	3.39 ± 0.79**	1.83 ± 0.27	4.73 ± 1.15
PCM + F1	20.05 ± 2.91	25.77 ± 5.81*	3.32 ± 0.68**	1.20 ± 0.28	3.93 ± 0.85**
PCM + F2	18.64 ± 3.84	23.38 ± 3.22*	4.33 ± 0.91**	1.22 ± 0.28	4.97 ± 1.15

The results are expressed as mean ± SD; n = 8 animals. * P <0.05 compared with the CONTROL group, ** P <0.05 compared with the PCM group. ANOVA followed by Tukey test.

Studies by Posadas et al. (2010), found that PCM in the doses used (above 1 mM *in vitro* and 250 mg kg⁻¹ *in vivo*), are neurotoxic to rat cortical neurons, thus, we can suggest that in this studied model, the EB and F1 were beneficial for this tissue. Therefore, according to Anand-David et al. (2016), flavonoids exert beneficial effects on the vascular system, which can lead to changes in cerebrovascular blood flow, being able to change neuronal morphology and can cause neurogenesis and angiogenesis, besides those also have the potential to protect neurons against neurotoxin-induced lesions. So, this first study suggest the involvement of these important molecules on the effects observed in mice treated with PCM.

4. Conclusions

In this first study we identified that F1 reverses the effects caused by PCM in the enzymatic antioxidants CAT and GST and the non-enzymatic antioxidant GSH in the hepatic tissue and CARBONYL reduction in brain and kidney. F2 reverses protein damage in liver and kidney and EB presents the same effect in these tissues, besides reduces TBARS in the liver. In plasma biochemical analyses, EB was hepatotoxic, increasing ALT and AST activities, while F1 and F2 reduce the damage caused by PCM. The most of treatments presents hypoglycemic action and F1 being hypocholesterolemic and F2 reduces triglycerides. Therefore, it is possible that the good antioxidant activity which the extracts show may be due to the flavonoid compounds, in this specific case, glycosylated quercetins which were identified by our group of the study. However, more studies will be necessary to explore the biological potential of *Smilax fluminensis*, in order to have more knowledge about the possible benefits of this plant.

Authors' Contributions: DA CUNHA, A.P.S.: conception and design, acquisition of data, analysis and interpretation of data, drafting the article; FERREIRA, L.S.: analysis and interpretation of data; PASUCH, A.J.: analysis and interpretation of data; PETRICA, E.E.A.: acquisition of data, analysis and interpretation of data; SINHORIN, A.P.: acquisition of data, analysis and interpretation of data, and critical review of important intellectual content; SINHORIN, V.D.G.: conception and design, analysis and interpretation of data, and critical review of important intellectual content. All authors have read and approved the final version of the manuscript.

Conflicts of Interest: The authors declare no conflicts of interest.

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