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RESEARCH ARTICLE

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Antihypertensive, Ameliorating Effect on Lipid Profile and Oxidative Stress Markers of Aqueous Extract of *Pleurotus floridanus* in Rats

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ABSTRACT

Context: Hypertension is a silent disease whose prevalence is constantly increasing. Its management is not always controlled. Thus, the objective of this work was to study the antihypertensive effect of the aqueous extract of the edible mushroom *Pleurotus floridanus* on the lipid profile and some markers of oxidative stress in *Wistar* rats.

Methods: Phytochemical screening and the study of toxicity of the aqueous extract of *Pleurotus floridanus* were previously performed. The antihypertensive effect of the aqueous extract of *Pleurotus floridanus* (1000 mg/Kg of BW) was evaluated on Wistar rats, by co-administration of L-NAME (25 mg/Kg of BW) for three weeks. Hemodynamic parameters were measured once a week via the CODA system. Serum parameters such as lipid profile and oxidative stress markers (MDA, catalase, SOD) were evaluated.

Results: The results showed that the aqueous extract of *Pleurotus floridanus* contained tannins, phenols, coumarins, flavonoids, glycosides, steroids and terpenoids and had no particular signs of toxicity. Co-administration of the aqueous extract and L-NAME in the test group resulted, as in the standard (captopril) group, in a moderate increase in systolic, diastolic and mean arterial blood pressure, significantly lower ($P < 0.05$) than that observed in the positive controls. Also, a significant decrease ($P < 0.05$) in heart rate during the last two weeks of treatment was observed in the test group. The administration of the aqueous extract regulated the lipid profile and serum stress markers like the reference drug.

Conclusion: These results demonstrate the antihypertensive potential of the edible mushroom *Pleurotus floridanus*.

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Antihypertensive, *Pleurotus floridanus*, Lipid profile, Stress markers.

Introduction

High blood pressure (HBP) is a medical condition in which the blood pressure in the arteries is consistently above 140/90 mmHg, i.e. systolic blood pressure (SBP) ≥ 140 mmHg and diastolic blood pressure (DBP) ≥ 90 mmHg. It represents a public health problem due to its increasing prevalence and associated cardiovascular diseases. Worldwide, approximately 1.4 billion people suffer from hypertension [1]. In Africa, the prevalence is estimated at 27% and in Cameroon it is about 30.9% [2,3]. Its high prevalence worldwide is linked to the global trend of overweight and obesity, hereditary factors, progressive ageing of the population, sedentary lifestyle, stress, excessive consumption of salt and alcohol, and reduced consumption of fruits and vegetables [4,5]. Hypertension is a silent disease and is estimated to cause one in eight deaths worldwide [2].

Several complications arise from hypertension due to lack of early detection and adequate therapeutic follow-up. Indeed, hypertension is the major risk factor for cardiovascular disease and causes damage to several organs including the heart, liver, kidneys and vessels [2].

Its treatment is based on compliance with hygienic dietary measures (weight loss, reduction of nutritional salt intake, physical activity) and antihypertensive medication [6]. However, treated people do not always have a normalized blood pressure and some drugs often have deleterious side effects [7].

Furthermore, as hypertension is associated with dyslipidemia and oxidative stress, several studies have shown that the use of antioxidants could prevent and fight against hypertension. These are synthesized by the body and could be supplied exogenously via the diet. Hence our interest in researching functional foods, in particular the edible mushroom *Pleurotus floridanus*.

Indeed, recent studies have shown that edible mushrooms

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of the genus Pleurotus, in particular *Pleurotus floridanus*, has interesting antioxidant properties [8] and is rich in bioactive compounds (flavonoids, tannins, phenols, coumarins...) which may prove effective in the prevention and treatment of hypertension. In addition, it has anti-inflammatory, anti-diabetic properties [8,9]. In addition to being a functional food, the edible mushroom *Pleurotus floridanus* has an exceptional nutritional profile due to its high content of protein, carbohydrates, fibers, mineral elements with a high K/Na ratio and its low fat content [10]. Thus, the objective of this work was to study the antihypertensive effect of aqueous extract of *Pleurotus floridanus* on lipid profile and some markers of oxidative stress.

Materials and Methods

Plant material

Fresh *Pleurotus floridanus* edible mushroom was harvested at maturity in the mushroom house of the Department of Biochemistry of the University of Douala. It was dried in an oven (BINDER brand) at 40°C for 48h and ground with a mixer to obtain powders.

Preparation of the Extract

The aqueous extract of *Pleurotus floridanus* was prepared in the Biochemistry Laboratory of the University of Douala. For this purpose, 100g of powders were macerated in 800 mL of distilled water (1w:8v) for 48h. The crude extract was then obtained by filtering the macerate and drying the filtrate in an oven (BINDER brand) at 45°C. It was stored in the refrigerator at 4°C for later use.

Experimental Animals

All work was conducted in accordance with the European Directive 2010/63/EU on animal experimentation. Female and male albino rats of *Wistar* strain, 10 to 12 weeks old, weighing between 150 to 200g, were used in this study respectively for the study of toxicity and antihypertensive effect of the mushroom extract. They came from the animal house of the Department of Biochemistry of the University of Douala. They were randomly divided into batches of 5 rats each in polyethylene cages covered with stainless steel mesh. The individuals in each batch were acclimatized for a fortnight before the start of each experiment, under a daily 12/12 light/dark cycle. During this period, the animals had free access to water and food.

Phytochemical Screening of the Aqueous Extract of *Pleurotus floridanus*

The qualitative phytochemical screening allows the presence or absence of secondary metabolites to be identified. A phytochemical analysis was carried out on the aqueous extract of mushrooms in order to determine the presence of phenols, tannins, coumarins, alkaloids, terpenoids, anthocyanins, glycosides and steroids following the protocol of Harbone (1998) [11].

Evaluation of the Toxicity of the Aqueous Extract in Rats

Principles

The protocol for the limit test proposed by the OECD (Organization for Economic Co-operation and Development) in 2008 was used to assess the acute toxicity of the prepared extract [12]. This protocol recommends the administration of a single dose of 2000 mg/kg body weight (BW) of substance to a first experimental animal (rodent) followed by an observation of physiological variations of the animal for 48 hours. If it survives, 04 additional animals are added and receive a dose of substance at 2000 mg/kg BW. The observation of the physiological variations of the animal in this case is carried out for two weeks.

Experimental Protocol

Ten albino *Wistar* rats, divided into two batches of five rats each, were used in this experiment. These two batches received distilled water (1 ml) for the negative control group and the aqueous extract at a single dose of 2000 mg/Kg BW for the test group by gavage through a gastro-esophageal tube. After two weeks of observation of physical signs, the rats were anaesthetized and sacrificed, the serum obtained after centrifugation was used for the analysis of toxicity markers such as transaminases (AST, ALT), creatinine, urea and total protein.

Preventive Effect of Aqueous Extract of *Pleurotus floridanus* on Hypertension in Rats

Experimental protocol

Animals with blood pressure below 140/90 mmHg were used in this experiment. Sixteen albino Wistar rats, randomized into four groups of four rats each, were allocated as follows:

- **Group 1:** negative controls receiving 1 ml of distilled water by gavage for 21 days.
- **Group 2:** positive controls receiving L-NAME at 25 mg/Kg BW and 1 ml of distilled water by gavage for 21 days.
- **Group 3:** test group receiving by gavage and concomitantly the aqueous extract at 1000 mg/Kg BW and L-NAME at 25 mg/Kg BW for 21 days.
- **Group 4:** Standard group receiving captopril 20 mg/Kg BW by gavage and L-NAME 25 mg/Kg BW concomitantly for 21 days.

Hemodynamic parameters (systolic, diastolic and mean blood pressure and heart rate) were measured once a week for three weeks using the CODA System blood pressure monitor (Kent Scientific Co., USA). At the end of the experiment, the rats were anaesthetized and sacrificed; the serum obtained after centrifugation of the blood at 3000 rpm for 10 min was stored in a freezer at -20°C for further analysis.

Biochemical Parameters and Stress Markers

The lipid profile consisted at evaluating of HDL cholesterol, LDL cholesterol, total cholesterol and triglycerides. HDL cholesterol [13], LDL cholesterol [13], total cholesterol [14], triglycerides [15], the antioxidant enzymes catalase (CAT) [16] and superoxide dismutase (SOD) [17] and the lipid peroxidation marker malondialdehyde (MDA) [18] were determined.

Statistical Analysis

All assays were carried out in triplicates and results are expressed as mean ± standard deviation (SD). The data was introduced in an EXCEL spreadsheet (Microsoft Office 2013) and then analyzed with the STATGRAPHICS Centurion XV version 17.1.12 software. Statistical analysis was carried out with one-way analysis of variance (ANOVA) followed by the Fisher LSD *post hoc* test. Values of $p < 0.05$ were considered significant.

Results

Phytochemical screening

The results of the phytochemical screening show that the aqueous extract of *Pleurotus floridanus* contains phenols, tannins, coumarins, flavonoids, terpenoids, steroids, glycosides, but does not contain alkaloids.

Evaluation of the Toxicity Parameters of the Aqueous Extract in Rats

Observation of signs of intoxication

The observation of the signs of intoxication in rats after a single dose of 2000 mg/Kg BW of the aqueous extract compared to control rats for a fortnight resulted in Table 1 below. The table shows that there were no signs of intoxication, both physical and behavioral, and no deaths.

Study of the Biochemical Parameters of Toxicity of the Extract

After observing the signs of intoxication in the treated rats, which showed no particular signs of damage, the biochemical parameters related to toxicity were evaluated in order to verify these different observations. The results in Table 2 showed that there were no significant differences ($P < 0.05$) between the values of AST, ALT, creatinine, urea and total protein between test and control rats.

Antihypertensive Effect of Aqueous Extract of *Pleurotus floridanus*

Effect on Hemodynamic Parameters: Systolic blood pressure, diastolic blood pressure, mean arterial pressure and heart rate

The evolution of systolic blood pressure, diastolic blood pressure, mean arterial pressure and heart rate of the different groups of treated animals are shown in Figures 1a, 1b, 1c and 1d respectively. Administration of L-NAME resulted in a significant ($P < 0.05$) increase in systolic, diastolic and mean arterial pressure from week 1 to week 3 in the positive control group compared to the negative control group with peak values at week 3 (153.8 ± 4.8; 118.0 ± 4.9 and 129.5 ± 4.8 mmHg versus 118.67 ± 0.58; 74.67 ± 4.24; 88.33 ± 5.26 mmHg). In the test group, as in the standard group, a moderate increase in systolic, diastolic and mean arterial pressure and significantly lower ($P < 0.05$) than in the positive control group was observed from week 1 to week 3. At the 3rd week, these systolic, diastolic and mean blood pressure values were respectively 138.00 ± 4.62; 91.00 ± 3.32; 106.67 ± 3.95 mmHg in the test group, i.e. a percentage reduction of 10.27%; 22.88% and 17.63% compared to the positive control group.

In addition, in the test and standard groups, a significant decrease in heart rate during the last two weeks of treatment was observed with respective percentages of decrease of 5.38% and 4.70% at the 3rd week compared to the negative control group. Also, no significant difference in heart rate was observed between the positive and negative control groups throughout the experiment.

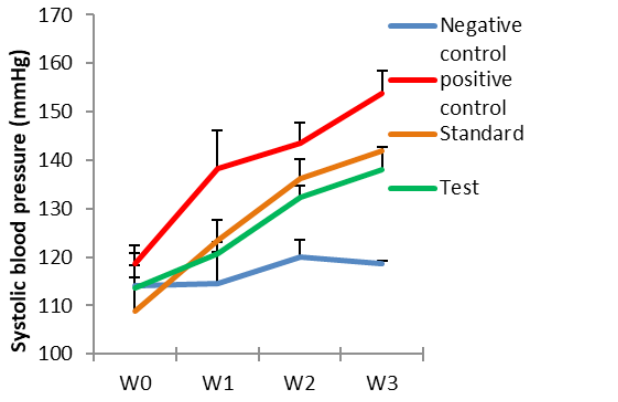

Figure 1a: Antihypertensive effect of aqueous extract of *Pleurotus floridanus* on Systolic blood pressure.

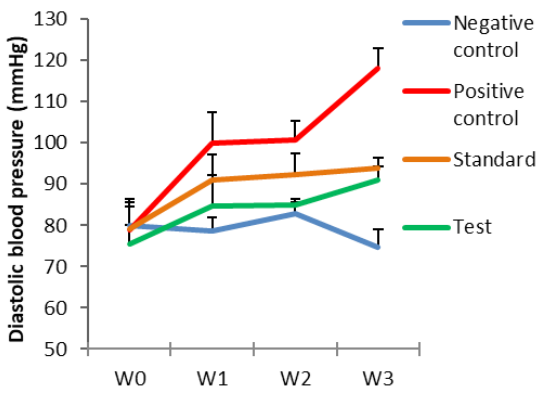

Figure 1b: Antihypertensive effect of aqueous extract of *Pleurotus floridanus* on diastolic blood pressure.

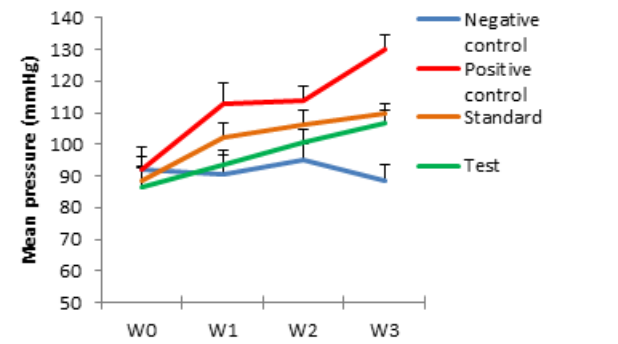

Figure 1c: Antihypertensive effect of aqueous extract of *Pleurotus floridanus* on Mean pressure.

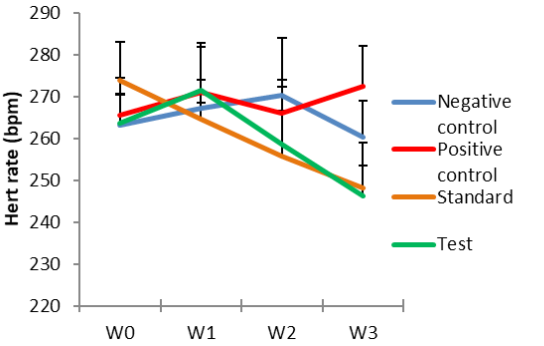

Figure 1d: Antihypertensive effect of aqueous extract of *Pleurotus floridanus* on Heart rate.

Values expressed as mean ± standard deviation (n= 5) in each group. Negative control (only distilled water); Positive control (L-NAME + distilled water); Test group (L-NAME + aqueous extract of *Pleurotus floridanus*); Standard group (L-NAME + captopril). W: weeks.

Table 1: Variation in signs of toxicity.

Signs of intoxication	Negative control	Test group
Locomotion	Normal	Normal
Activity reduced	-	-
Sensitivity to noises	+	+
Difficulty of breathing	-	-
Appetite	+	+
Coat	Normal	Normal
Aspect of urine	Normal	Normal
Stool appearance	Normal	Normal
Number of death	0	0

+: affirmative or yes; - : negative or no; Negative control (only distilled water); Test group (Single dose of aqueous extract of Pleurotus floridanus at a dose of 2000 mg/Kg BW).

Table 2: Evaluation of toxicity markers after acute study.

Groups	ALAT (U/l)	ASAT (U/l)	Creatinine (mg/L)	Urea (g/L)	Total protein (g/L)
Control	16.0 ± 1.4	12.2 ± 0.4	0.18 ± 0.07	0.81 ± 0.07	69.2 ± 3.1
Test	14.8 ± 1.7	13.1 ± 0.9	0.183 ± 0.02	0.83 ± 0.07	69.8 ± 1.9

Values expressed as mean ± standard deviation (n= 4) in each group. Negative control (only distilled water); Test group (Single dose of aqueous extract of Pleurotus floridanus at a dose of 2000 mg/Kg BW).

Table 3: Effect on lipid profile (mg/dL).

Groups	Cholesterol total	Triglycerides	HDL Cholesterol	LDL Cholesterol
Negative control	125.87 ± 3.09^{ac}	127.47 ± 0.96	54.52 ± 1.05^{a}	46.03 ± 2.63^{a}
Positive control	132.65 ± 6.35^{c}	134.72 ± 2.85^{c}	50.15 ± 1.43^{c}	55.55 ± 4.04^{c}
Test group	125.56 ± 4.02^{a}	129.18 ± 1.30^{a}	53.16 ± 0.74^{a}	46.56 ± 4.02^{a}
Standard group	125.84 ± 4.25^{a}	123.88 ± 4.87^{b}	53.11 ± 2.68^{a}	47.17 ± 3.024^{a}

Values expressed as mean ± standard deviation (n= 5) in each group. Negative control (only distilled water); Positive control (L-NAME + distilled water); Test group (L-NAME + aqueous extract of Pleurotus floridanus); Standard group (L-NAME + captopril). a: Significantly different when compared to the positive control group(p<0,05); b : Significantly different when compared to the test group(p<0,05); C: Significantly different when compared to the negative control group (p<0,05).

Table 4: Effect on some oxidative stress markers.

Groups	Superoxide Dismutase (µmol SOD/min/mg of proteins)	Catalase (µmol H_2O_2/min/mg of proteins)	Malondialdehyde (µM)
Negative control	45.024 ± 2.760 ^{a}	50.117 ± 2.005 ^{a}	6.461 ± 0.358^{a}
Positive control	24.244 ± 2.585 ^{c}	40.110 ± 0.222 ^{c}	7.368 ± 0.073^{c}
Test group	41.277 ± 1.342 ^{a}	45.852 ± 1.423 ^{b}	6.462 ± 0.053 ^{a}
Standard group	43.242 ± 4.214 ^{a}	45.350 ± 1.423 ^{b}	6.384 ± 0.147 ^{a}

Values expressed as mean ± standard deviation (n= 5) in each group. Negative control (only distilled water); Positive control (L-NAME + distilled water); Test group (L-NAME + aqueous extract of Pleurotus floridanus); Standard group (L-NAME + captopril). a: Significantly different when compared to the positive control group(p<0,05); b : Significantly different when compared to the negative and positive control groups(p<0,05); C: Significantly different when compared to the negative control group (p<0,05).

Effect of aqueous extract of *Pleurotus floridanus* on some serum biochemical parameters

Effect on Lipid Profile

The evaluation of the lipid profile (HDL-cholesterol, LDL-cholesterol, triglycerides and total cholesterol) was presented in table 3. Indeed, the administration of L-NAME in the positive control group resulted in a non-significant increase in total cholesterol, a significant increase (P < 0.05) in LDL cholesterol and triglyceride levels and a significant decrease at P < 0.05 in HDL cholesterol levels compared to the negative control group In the test and standard groups, there was a significant increase in HDL cholesterol levels and a significant decrease in total cholesterol, LDL cholesterol and triglyceride levels compared to the positive control group. Also, no significant differences in the above parameters were observed between the test and negative control groups and between the standard and negative control groups.

Effect on some serum stress markers

The determination of stress markers in serum was presented in table 4. It appears that the administration of L-NAME resulted in a significant increase in serum MDA concentration and a significant decrease at P < 0.05 in Catalase and SOD activity in the positive control group compared to the negative control group. Treatment with aqueous extract resulted in a significant decrease (P < 0.05) in serum MDA concentrations compared to the positive control group, but no significant difference compared to the negative control group. Regarding catalase and SOD activity, a significant increase in catalase and SOD activity was observed in the test group compared to the positive control group. On the other hand, no significant difference was observed between the test and standard groups regarding the aforementioned stress markers.

Discussion

Phytochemical analysis of the aqueous extract of *Pleurotus floridanus* shows that it consists of several secondary metabolites

in particular coumarins, phenols, tannins, flavonoids, terpenoids, steroids, and glycosides. These are generally soluble in polar solvents [19]. In this study, administration of the aqueous extract at a single dose of 2000 mg/Kg showed no signs of toxicity either macroscopically or biochemically. This result corroborates the one obtained by Etoundi and al. in 2017, which also showed that this extract was not toxic [8].

The evaluation of the preventive effect of the aqueous extract of *Pleurotus floridanus* on arterial hypertension was tested following the L-NAME model. Indeed, L-NAME-induced hypertension is a model that mimics hypertension in humans and is very suitable to study the cardiovascular effects of new agents [20]. In this study, oral administration of L-NAME to normotensive rats was associated with a significant increase in systolic blood pressure, diastolic blood pressure, and mean arterial pressure. This result was obtained by numerous researchers including Kojom and al. in 2019 [21]. Indeed, L-NAME, a structural analog of L-Arginine binds competitively to endothelial NOS. This leads to a reduction in NO/cGMP activity, activation of the renin-angiotensin-aldosterone system (RAAS), and an increase in sympathetic activity leading to an increase in peripheral vascular resistance, resulting in an increase in blood pressure [22]. Regarding heart rate, no significant difference was observed between the positive and negative control groups. This means that the administration of L-NAME alone does not alter the heart rate. This result corroborates those obtained by Fortepiani and al. in 2002 and Kojom and al. in 2019 [21,23].

Administration of the aqueous extract in the test group decreased recorded hypertension by decreasing blood pressure and heart rate compared with the positive control group. This could be due to the fact that the aqueous extract would have had an opposite effect to that of L-NAME by lifting the inhibition of vasorelaxation caused by L-NAME or by stimulation of NO synthase or by inhibition of one of the enzymes of the renin-angiotensin-aldosterone system. In addition, phytochemical analysis shows that this extract contains tannins, flavonoids, and coumarins, which are thought to have a vasorelaxant effect that lowers blood pressure [24,25]. Captopril, the reference drug, attenuated hypertension observed by reducing blood pressure and heart rate. It was reported to act by increasing NO synthase and vasodilatory prostaglandin activity and inhibiting the conversion of angiotensin I to angiotensin II, thereby preventing sympathetic system stimulation, vasoconstriction, and aldosterone and vasopressin release [26,27].

HTA is usually associated with dyslipidemia [28]. In this study, induction with L-NAME resulted in a significant increase in LDL cholesterol and triglycerides, a non-significant increase in total cholesterol, and a significant decrease in HDL cholesterol. Treatment with aqueous extract and captopril regulated these lipid parameters. Indeed, NO plays an important role in lipid metabolism and its inhibition creates a lipid disorder [28,29]. It inhibits the oxidation of LDL cholesterol [30] and induces activation of hepatic sterol regulatory element binding protein (SREBP)-2, a transcriptional factor required for cholesterol metabolism and LDL cholesterol expression, leading to improved cholesterol uptake in liver cells. This plays an important role in the normalization of blood cholesterol. In addition, L-NAME-induced hypertension is associated with oxidative stress. Most of the pathophysiological mechanisms associated with oxidative stress are para clinically reflected by a significant decrease in HDL giving way to bad cholesterol [31]. Reducing blood lipids is an effective method to prevent and treat cardiovascular disease [32]. These observations suggest that the aqueous extract and captopril would exhibit these effects through their ability to increase NO availability in rats. In addition, the Pleurotus species stands out as a potential regulator of cholesterol metabolism. These fungi are able to synthesize lovastatin, a highly cholesterol-lowering statin that inhibits HMG-CoA reductase, a key enzyme in the regulation of cholesterol biosynthesis in the liver by reversible competition with the substrate for the enzyme's active site [33]. Lovastatin is thought to increase eNOS expression and NO production as LDL cholesterol receptor activity. In addition, some Pleurotus beta-glucans are able to bind to bile acids, reducing micelle formation and cholesterol absorption [34-36]. This effect on blood lipids may suggest its beneficial effect against lipid peroxidation and even more so against oxidative stress [37].

Oxidative stress is one of the main causes of endothelial dysfunction accompanying hypertension. Growing evidence suggests a link between NO deficiency and the development of oxidative stress [38]. NO inhibition by L-NAME will stimulate the catalytic action of lipo-oxygenases (LOX), cyclooxygenases (COX), and NADPH oxidase. The latter, which oxidize fatty acids, are identified as a potential source of ROS [39,40]. Also, excess O^{2-} interacts with NO to form peroxynitrite (ONOO-) which exerts a detrimental effect on vascular function [40]. Consistent with this, our results showed that administration of L-NAME in the positive control group resulted in a significant decrease in antioxidant enzymes (SOD and CAT) and a significant increase in MDA, the most important marker of lipid peroxidation.

The effect of the aqueous extract compared to captopril was evaluated and the results obtained showed that the pre-administered aqueous extract and captopril regulated the biological markers of stress evaluated through an increase in their concentration for catalase and SOD and a decrease for MDA. Such effects reflect the antioxidant capacity of the extract due to its richness in phenolic compounds. The phytochemical screening revealed its content of flavonoids and phenols, powerful antioxidants. Also, this extract would contain statins that would prevent the activation of NADPH oxidase and decrease the production of oxygenated free radicals [33,34]. In addition, the angiotensin II inhibitory effect of captopril would also play an important role in improving oxidative status because when activated, angiotensin II has the effect of producing ROS [41].

Conclusion

In conclusion, we investigated the antihypertensive effect of the edible mushroom *Pleurotus floridanus* on the lipid profile and some serum stress markers. From this work, it was found that the aqueous extract of *Pleurotus floridanus* prevented a sharp rise in systolic blood pressure, diastolic blood pressure, means arterial pressure and lowered the heart rate. Also, this mushroom extract restored the lipid profile and stress markers

(SOD, catalase, MDA). These results show that the aqueous extract of *Pleurotus floridanus* constitutes an alternative in the prevention of arterial hypertension and its associated metabolic disorders.

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