

Study of Toxicity and Antidiabetic Activity of Ethanolic and Hydroethanolic Extracts of *Pleurotus pulmonarius* and the Aqueous Extract of *Pleurotus floridanus*

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Abstract

Diabetes is a widespread chronic syndrome in the world. Although there are pharmaceutical antihyperglycemic agents, they nevertheless have limits, hence our interest in functional foods. Mushrooms are considered as vegetables though they do not belong to kingdom Plantae. The aim of this work was to evaluate the toxicity and antidiabetic activity of the ethanolic, hydroethanolic extracts of *Pleurotus pulmonarius* (*P. pulmonarius*) and the aqueous extract of *Pleurotus floridanus* (*P. floridanus*) which are two edible species of mushrooms commonly consumed in Cameroon. The various extracts were tested for acute toxicity and their antidiabetic activity was evaluated. Diabetes was induced by intraperitoneal route on adult Wistar-type albino rats using streptozotocin. The extracts were used to treat all animals during 28 days. Results showed that, these extracts revealed no evidence of apparent toxicity. After 28 days of diabetes treatment, a significant difference in body weight was observed between the normal control (NC) and the diabetic control (DC), where NC increases with time and the DC decreases. Both the tests groups and the Reference group showed slight weight loss with the aqueous extract of *P. floridanus* as the best extract ($P < 0.05$). Normal control showed a normal blood glucose level throughout the treatment period, meanwhile blood glucose for DC was > 2 g/L. The aqueous extract of *P. floridanus* and alcoholic extract of *P. pulmonarius* showed the best hypoglycaemic activity with the aqueous extract of *P. floridanus* better than the reference glibenclamide. These results suggest that *P. floridanus* and *P. pulmonarius* have significant hypoglycaemic activity in the treatment of diabetes.

Keywords: Antidiabetic activity; Toxicity; *P. floridanus*; *P. pulmonarius*

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Introduction

Diabetes is a cardiovascular risk associated with chronic hyperglycemia in the blood [1]. In 2013, IDF estimated that 382 million people, or 8.3% of adults, had diabetes worldwide of which 46% are not diagnosed. In Africa, nearly 20 million people have diabetes, thus a prevalence of 4.9% for the region. These estimates are expected to double by 2035 to reach 41.5 million, hence increasing by 109% [2]. In Cameroon, diabetes is spreading with time; according to IDF in 2013, 93 thousand people age 60-79 years have diabetes of which 229 thousand people are not

diagnosed and by 2015 10% of the population suffering from diabetes was diagnosed [3]. Indeed, hyperglycemia increases the production of free radicals that can't be inhibited by the body. This will thus cause oxidative stress in the cell with decreased enzymatic antioxidant defenses resulting in diabetes [4]. Antioxidant supplementation is therefore essential as it helps in inhibiting the harmful action of free radicals on insulin secretion, thus balancing the glycemic index [5]. From literature review, studies on mushroom reveal that they are very good antioxidants and thus prevent from certain metabolic diseases like diabetes [6]. Cameroon is full with a diversified flora of vegetables among

which the mushrooms and precisely oyster mushroom which belongs to kingdom Fungi [7]. But, in the culinary context, oyster mushrooms can be considered as vegetables. They are very good antioxidants but there is little scientific data available in our country concerning the use of oyster mushrooms in diabetes prevention hence in order to contribute in the management of diabetes, our interest was carried on their study.

Research hypothesis

The species of mushrooms *P. pulmonarius* and *P. floridaus* consumed in Cameroon would have a mechanism of action such as the hypoglycemic activity that can be exploited in the treatment of diabetes.

General objective

To evaluate the toxicity and antidiabetic activity of two commonly consumed mushroom species in Cameroon: *P. pulmonarius* and *P. floridaus*.

Materials and Methods

In vitro study: Preparation of experimental plant material

Mushroom harvest: The fresh mushrooms harvested in a myciculture in Pk 21 A suburb of the city of Douala were shade dried in large pans for 3 days and then crushed in a mixer to obtain mushroom powder.

Preparation of crude mushroom extracts: The alcoholic and hydroalcoholic extracts of *Pleurotus pulmonarius* (*P. pulmonarius*) and the aqueous extract of *Pleurotus floridaus* (*P. floridaus*) were prepared in the Biochemistry Laboratory of the University of Douala. For each extract, 100 g dried mushroom powder were macerated in 800 mL of solvent (proportion=1:8) ethanol, water/ethanol (1:1) and distilled water, respectively. The extracts were then filtered with a filter paper and the filtrate obtained was dried in an oven (BINDER mark) for 4 days at 45°C in order to obtain the crude extracts.

In vivo study: Evaluation of the toxicity for the various extracts on rats

Physical and behavioral evaluation: The protocol of the limit test proposed by the OECD in 2008 [8] was used to evaluate the acute toxicity of mushroom extracts. This protocol recommends the administration of a single dose (2000 mg/kg body weight: BW) of extract to a first experimental animal (rodent) followed by observation of the physiological variations for 48 hours. If it survives, 4 other animals are added and are given the extract at 2000 mg/kg BW. The observation of the physiological variations of the animal in this case takes place for two weeks.

Evaluation of the state of organs: At the end of these two weeks of observations, the female rats were then sacrificed in order to better compare their organs to that of the controls negative and certain toxicity markers such as transaminases (ASAT and ALAT) were determined.

Evaluation of the effect of administration the mushroom extracts on body weight and fasting blood glucose of diabetic rats during 28 days of treatment

Experimental induction of diabetes by streptozotocin (STZ):

This study evaluated the long-term hypoglycaemic capacity of mushroom extracts in diabetes induced adult male rats (three months of age). Their weights ranged from 210 g to 250 g. After an overnight fasting (food deprivation for 12 hours with water as libitum), diabetes was induced in rats by intraperitoneal injection of a freshly prepared solution of STZ at 55 mg/kg of body weight, i.e., a volume of 2 mL/kg (dose at which the β -cells are destroyed). The STZ was dissolved in 0.1 M sodium citrate buffer pH 4.5 [9]. Negative control received the same volume of 0.1 M sodium citrate buffer pH 4.5 through intra peritoneal route. After injection, drinking water was replaced with 5% glucose solution for 24 hours in order to overcome STZ-induced hypoglycemia following the destruction of pancreatic β cells and the massive release of insulin. This hypoglycemia can be lethal for rats. [10]. After 48 hours of injection (diabetes development time), diabetes was confirmed in rats by measurement of fasting blood glucose using an SD-type glucose meter codeFree. Only rats with a blood glucose level greater than 2 g/L were considered diabetic and retained for this study.

Evaluation of the hypoglycemic potential of extracts in diabetic rats

Animal treatment: After induction of diabetes, all diabetic and non-diabetic rats were divided into six groups of five rats each and kept under the same conditions. The treatment with mushroom started 24 hours after confirmation of diabetes and was evaluated for 28 days.

Distribution of Animals

- Group I: Healthy controls or negative control (NC) that received daily gavage 5 mL/kg BW distilled water for 28 days.
- Group II: Diabetics control or positive control (PC) which received each day 5 mL/kg BW distilled water by gavage for 28 days.
- Group III: Diabetics treated with the hydroalcoholic extract of *P. pulmonarius* (Phal), which received 400 mg / kg BW of the extract every day by gavage for 28 days.
- Group IV: Diabetics treated with the alcoholic extract of *P. pulmonarius* (Pal) which received 400 mg/kg BW of the extract every day by gavage for 28 days.
- Group V: Diabetics treated with the aqueous extract of *P. floridaus* (Faq), which received 400 mg/kg BW of the extract daily by gavage for 28 days.
- Group VI: Standard which received 0.3 mg/kg BW glibenclamide daily by gavage.

Determination of blood glucose and weight: Blood glucose was determined by puncturing the distal tip of the tail using a lancing device. The blood collected on the glucose meter via the test strip allowed us to obtain the blood glucose for the rats. This was measured at one week interval during the 28 days of treatment more precisely on days 0 (24 h after the confirmation test for diabetes), 8, 15, 22 and 29. Glycemia was measured each time after 12 h fasting [10]. Similarly, weight measurement was conducted on a regular basis in fasting rats each week just prior to blood sampling.

Statistical analysis: Data were introduced in a Excel Worksheet (Microsoft Office 2007) then analyzed with the software Statview version 5.0. Data were presented in a percentage and average \pm standard differential for qualitative variables and quantitative respectively. The significance was set at 5%.

Results

Evaluation of toxicity parameters of extracts

Observation of signs of poisoning: Two weeks after receiving various extracts at 2000 mg/kg of body weight of the different extracts, no sign of toxicity was observed (**Table 1**). From **Table 1**, we may say that no particular sign of intoxication, both behaviorally and physically, was observed in the rats given the extract. Also, during this period of toxicity assay, no deaths were recorded. This lack of pathological evidence may be due to the fact that mushrooms of the oyster species are not harmful [11]. However, other parameters such as organ observation and the determination of biochemical enzymes have been evaluated in order to confirm this.

Determination of biochemical enzymes: Though no signs of toxicity were detected from animals' behavior and macroscopic organ observation, the biochemical enzyme assay was evaluated to ensure that there was no organ cytolysis (**Figure 1**). The values of ASAT and ALAT of our samples, compared to the negative control were not significantly different $P > 0.05$. This means that no organ cytolysis has been observed and therefore the heart, liver, muscles, kidneys, brain and pancreas have not been significantly degraded. These results confirm those obtained by Ninkwango in 2007 [11] which showed that fungal mushrooms had no toxicity.

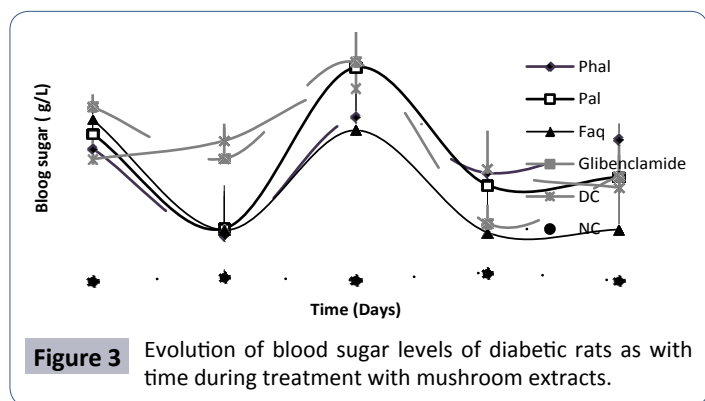
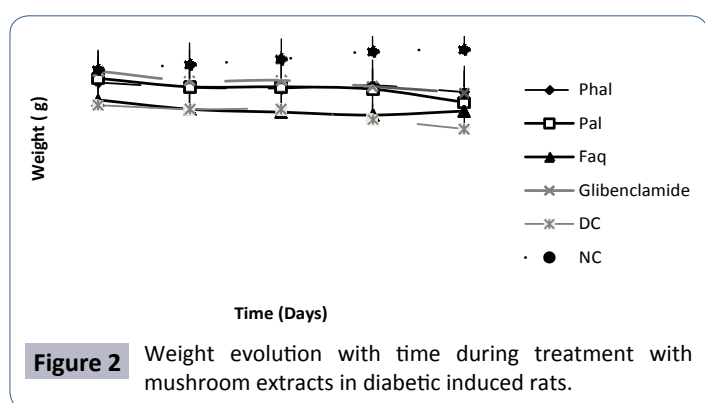
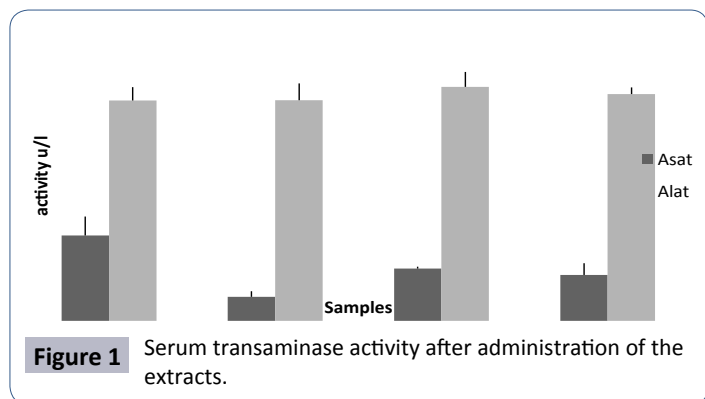
Evaluation of the effect of mushroom extracts on fasting blood glucose of diabetic rats

Evaluation of the weight evolution during the 28 days treatment of the diabetes with mushrooms extracts: **Figure 2** shows that there is an increase in weight for healthy rats or normal control (NC) time, from 250.3 ± 21.70 g to 272.3 ± 21.36 g (thus, an increase of 22 g) at the end of the 28 days. All the other groups, with the exception of the Faq extract, show a slight decrease in weight over time. Except for the DC, there was no significant difference ($p < 0.05$) between the various tests groups and the NC. There was a significant difference in the untreated diabetic control, also referred to as DC, in the third weeks (22 days) and in the fourth weeks (29 days). On day 22, there was a significant difference of 195.775 ± 17.49 g in the DC compared to 270.25 ± 20.02 g in the NC (a difference of 74.475 g). Also, on day 29, there was a significant difference of 185.125 ± 14.06 g in the DC compared 272.3 ± 21.36 g in the NC (A difference of 87.175 g). The NC curve thus appears to be the most significant curve over time, with the DC curve showing a lower weight over time and the control curve representing the batch of diabetic rats treated with the reference, it shows a slight decrease over time, as do extracts, but it does have a greater weight than extracts over time. With, the curve of the extract Pal being merged with the curve of the extract Phal. As for DC, our results are in accordance with those reported by Pari and Latha [12] who found that in male rats of Wistar albino strain, injection of STZ caused a significant weight loss within three weeks. They indicated that these conditions are due to the excessive loss of tissue proteins. Similarly, Sathishsekar and Subramanian [13] and Taleb-Senoucia and et al. [14] also reported similar results and suggest that body weight loss in the untreated diabetic group can be explained by lipids and structural proteins catabolism due to lack of hydrated carbons that are used as energy source. For the Phal and pal extracts, although they do not show a significant decrease for $p < 0.05$, the slight decrease observed could also be due to a partial catabolism of lipids and structural proteins. Thus, these extracts do not exhibit a high activity, such as the Faq extract, where weight increase is observed after the third weeks. The strong activity of the Faq extract to limit weight loss better than the other extracts could be due to the fact that it would exhibit a

Table 1 Variation of the signs of toxicity depending on extracts.

Sign of poisoning	Faq	Phal	Pal	Negative control
Locomotion	Normal	Normal	Normal	Normal
Activity reduced	-	-	-	-
Stool appearance	Not significant	Not significant	Not significant	Not significant
Aspect of urine	Normal	Normal	Normal	Normal
Sensitivity to noises	+	+	+	+
Appetite	+	+	+	+
Difficulty of breathing	-	-	-	-
Coat	Normal	Normal	Normal	Normal
Number of death	0	0	0	0

+: Affirmative or yes; -: Negative or Not.



better antioxidant activity than the other extracts. The ability of our extracts to protect diabetic rats from the massive loss of body weight could thus be due to their ability to limit the catabolism of lipids and proteins [15-17] and thus their ability to reverse gluconeogenesis and control protein loss [18,19].

Assessment of blood glucose changes during the 28 days of treatment of diabetes with mushroom extracts: In order to better evaluate the evolution of blood glucose during the treatment period, it should be noted that at the beginning of experimental period, there was no significant difference in blood glucose for all groups. Also, after induction of chronic hyperglycaemia, precisely at time 0, with the exception of the normal control (NC), there was no significant difference between the other batches of rats. Thus, the analysis in **Figure 3** below present six curves showing the evolution of blood glucose levels in controls and trials during the treatment of diabetes. This figure shows that the NC groups have the lowest values for blood glucose throughout the experimental

period, ranging from 0.70 ± 0.05 to 0.71 ± 0.05 g/L, which is normal because in fasting healthy subjects, blood glucose according to the standard should be between 0.7 and 1.1 g/L. From the figure, the mushroom extracts show a better hypoglycemic activity than standard during the first 2 weeks and this effect continued to be observed for the next two weeks (3rd and 4th) only with the Faq extract. However, there was a significant decrease in blood glucose levels in weeks 1 and 3 in rats receiving the extracts and standard group. On the other hand, the DC showed a significant increase in blood glucose levels during the first two weeks of treatment 2.8 ± 0.39 to 4.03 ± 0.54 g/L. From the third weeks, there is a decrease in blood glucose up to the fourth weeks. **Figure 3** shows that at the end of the 28 days of treatments, the Faq, Pal extracts and the reference glibenclamide, showed hypoglycemic activity with glycemia variation being respectively from 3.49 ± 0.32 to 1.59 ± 0.67 g/L, 3.24 ± 0.24 to 2.5 ± 0.55 g/L and 3.77 ± 0.22 to 2.5 ± 0.68 g/L. The Phal extract, on the other hand, at the end of the 28 days shows a slight rise in blood glucose from 2.99 ± 0.72 to 3.15 ± 0.27 g/L. The Faq extract is thus the best hypoglycemic extract. The hypoglycaemic activity of the Faq and Pal extracts could be explained by the fact that these extracts could act in the same way as some oral antidiabetics by restoring the damage caused by streptozotocin. Indeed, the hypoglycemic effect observed in these extracts could be due to the fact that the latter inhibited the release of the reactive species of oxygen caused by streptozotocin. This led to a closure of the K^+ /ATP channels followed by membrane depolarization of the beta cells with stimulation of the Ca^{2+} inflow, which represents the first key step for insulin secretion [12]. Thus, the hypoglycemic effect of the Faq and Pal extracts can be assimilated to both the organic and the inorganic constituents. It is important to note that the inorganic constituents that medicinal plants contain sometimes play an important role in improving their medicinal properties, including hypoglycaemic activity [10]. Bhaskar and et al. [19], who studied the hypoglycemic effect of aqueous mucuna pruriens extract (200 mg/kg) in rats rendered diabetic by STZ, reported that a number of essential minerals such as Na, K, Ca, Zn, Mg, Fe, Cu and Mn may be associated with an insulin release mechanism and its activity. Since the floridanus species is richer in minerals than the pulmonarius species according to Bhaskar et al. [19], this may explain why the Faq extract exhibits the best hypoglycaemic activity.

Conclusion

At the end of our study which consisted in evaluating the toxicity and antidiabetic activity of two species of mushroom commonly consumed in Cameroon, namely *P. pulmonarius* and *P. floridanus*, it was found that the alcoholic and hydroalcoholic extracts of *P. pulmonarius* and the aqueous extract of *P. floridanus* revealed no significant signs of toxicity. The antidiabetic test carried out after injection of the streptozotocin shows that the Faq and Pal extracts exhibit good antidiabetic activity with the Faq extract as the best hypoglycemic extract.

With regard to the above results, we can say that the extracts of *P. pulmonarius* and *P. floridanus* exhibit antidiabetic activity. This suggests the use of these fungi as functional food. We thus

envisage for a future research to carry out a phytochemical screening of these extracts in order to know the compounds which would be at the origin of the antidiabetic activity of these extracts.

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