Evaluation of Toxicity, Antihyperglycemic and Hypoglycemic Activities of Mixtures of Extracts of 3 Varieties of Oyster Mushrooms

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ABSTRACT

Our previous studies on the mushrooms showed that a mixture of three varieties of oyster mushrooms had better therapeutic properties than when the mushrooms were studied separately. To overcome the problem of diabetes, it was question here after studying the toxicity of mixtures of extracts (formulations) of 3 varieties of oyster mushrooms, to study the antihyperglycemic and hypoglycemic activity in the short term (over 2 hours of time) of these extracts. After formulating the mushrooms and obtaining the raw extracts, we proceeded to an acute toxicity of these extracts followed by the evaluation of antihyperglycemic and hypoglycemic tests on albino rats of the wistar type. In terms of results, we obtained 4 formulations namely F1, F2, F3 and F4. It appears in terms of toxicity that no signs of intoxication were observed. As for the antihyperglycemic test, 30min after ingestion of the extracts and glucose, all the groups of extracts exhibited had a lower blood glucose level than the positive control with the best extract best extract to limit the increase in postprandial blood glucose being the aqueous extract of formulation 3 (F3aq) with a slight increase in blood glucose of 0.3 g/l against an increase of 0.62g/l in the positive control. As for the hypoglycemic test, 120min after ingestion of glucose and extracts, the extracts showed a good ability to lower blood glucose compared to the positive control, the best extracts being the hydroethanolic extracts of formulations 1 and 3 (F1heth and F3heth) with respectively a decrease in blood glucose of 0.23g/l and 0.22g/l against 0.07g/l in the positive control. It can thus be concluded that formulated mushroom extracts do not show signs of toxicity and that the consumption of a mixture of oyster mushrooms could help in the fight against diabetes.

Introduction

Diabetes is a group of metabolic diseases of various etiology, characterized by chronic hyperglycemia, accompanied by disruption of carbohydrate, lipid and protein metabolisms, resulting from a defect in the secretion of insulin or the action insulin or both of these associated abnormalities. This hyperglycemia causes acute and chronic degenerative metabolic complications [1,2].

The number of people with diabetes continues to increase. It went from 108 million in 1980 to 422 million in 2014. This figure should continue to grow to reach 622 million in 2040 [3]. Although there are anti-diabetic products, these nevertheless have limitations due to their side effects. Hence the interest in finding medicinal plants with anti-diabetic properties with fewer side effects. Indeed, according to the literature, edible mushrooms have therapeutic properties and can be used in several medical fields (antibiotic therapy, oncology, parasitology, cardiology, dermatology, endocrinology, diabetology, gastroenterology, gynecology, hematology, neuropsychiatry, pneumology, oto-rhinolaryngology, traumatology, urology, venereology, etc.) [4].

In this vein, our previous studies on edible mushrooms of the pleurotus genus (mushrooms which are the subject of a myciculture in our country) precisely *pleurotus pulmonarius* and pleurotus floridanus have shown that the aqueous, ethanolic, hydroethanoic and hexane extracts of these mushroom had antihyperglycemic properties and thus may help prevent diabetes [5]. However, the limitation of this work was to find that this property was only observable at high doses. Indeed, for a dose of extract of 400 mg / kg of body weight (BW) administered to rats, no antihyperglycemic activity was observed, but at a dose of 800 mg / kg BW this activity was observed.

In order to make our contribution in the fight against diabetes, we have proposed to increase the efficiency of oyster mushrooms by a formulation or mixture of three varieties of oyster mushrooms namely pleurotus pulmonarius, pleurotus floridanus and pleurotus sajor caju. Indeed our previous work on this mixture of mushrooms showed that when the mushrooms were mixed they had better antioxidant activity than when they were studied separately [6].

This work will be discussed, after evaluating the toxicity of extracts from the formulation of oyster mushrooms, to study the short-term antihyperglycemic and hypoglycemic effects of these extracts on rats in which a state of hyperglycemia will be caused. This will particularly concern in vivo:
• To evaluate the toxicity of aqueous and hydroethanolic extracts of oyster mushroom formulations on female albino rats of the Wistar type
• To evaluate the antihyperglycemic effect of these extracts on male albino rats of the Wistar type
• To evaluate the hypoglycemic effect of these extracts on male albino rats of the Wistar type

Material and Method
In vivo studies
Preparation of experimental biological material:
Harvesting and formulation of mushrooms: Fresh oyster mushrooms of the three varieties were harvested in Douala from a mushroom farm in Yassa then dried and crushed to obtain mushroom powder. The different powders obtained from each mushroom were then distributed in the following proportions in order to obtain the mushroom formulations: 1/1/1; 2 / 0.5 / 0.5; 0.5 / 2 / 0.5 and 0.5 / 0.5 / 2.

Preparation of crude mushroom extracts: The aqueous and hydroethanolic extracts of the mushroom formulations were prepared at the Biochemistry Laboratory of the University of Douala. Indeed, the different formulations of powders were each macerated in 1/8 proportions respectively with distilled water and a water / ethanol mixture (1: 1) and then dried in an oven for 4 days at a temperature of 45°C.

Evaluation of the toxicity of the extracts on normal rats
The protocol that was used here is that of the OECD (Organization for Economic Cooperation and Development) [7] from 2008 used to assess the acute toxicity of an extract. In fact, this protocol recommends the administration of a single dose of 2000 mg / kg

of body weight (BW) of substance to a first experimental animal followed by observation of the physiological variations of the animal for 48 hours. If it survives, 04 additional animals are added and given a dose of the substance at 2000 mg / kg of body weight.

A behavioral observation was carried out 3 hours after administration of the extracts. Then hydration and nutrition were carried out daily for 14 days. During this period, signs of toxicity including locomotion, activity, appearance of feces, frequency of urine, sensitivity to noise after shock, appetite, difficulty in breathing, change in coat, as well as the number of deaths were noted.

At the end of the 14 days and after 12 hours of fasting, the rats are weighed, anesthetized with ketamine (50 mg / kg of body weight) then sacrificed. Blood is taken from the tracheal artery. The blood collected is collected in dry EDTA tubes and centrifuged at 3000 rpm for 15 min. The serum obtained made it possible to assay the following biochemical parameters of toxicity: ALT, ASAT, creatinine, protein and urea; these parameters were assayed according to the protocol stated in the commercial kit.

Evaluation of the antihyperglycemic and hypoglycemic activity of the extracts after oral glucose overload of normal rats
This study evaluated the regulatory capacity of extracts of mushroom in the body vis-à-vis carbohydrate overload.

Evaluation of antihyperglycemic activity: This involved force-feeding the Wistar-type rats with a glucose solution at a dose of 2000 mg / kg BW, thirty minutes after administration of the extracts at a dose of 400 mg / kg bw.

For this, forty normal rats were divided into 10 groups of 4 rats and fasted 12 hours before the experiment. They were distributed according to Table 1.

Table 1: Distribution of animals for the antihyperglycemic and hypoglycemic test

Groups	Description
1-GROUP A	Normal rats treated with F1aq + glucose extract
2-GROUP B	Normal rats treated with F1heth + glucose extract
3-GROUP C	Normal rats treated with F2aq + glucose extract
4-GROUP D	Normal rats treated with F2heth + glucose extract
5-GROUP E	Normal rats treated with F3aq + glucose extract
6-GROUP F	Normal rats treated with F3heth + glucose extract
7-GROUP G	Normal rats treated with F4aq + glucose extract
8-GROUP H	Normal rats treated with F4heth + glucose extract
9-GROUP I OR POSITIVE CONTROL	Normal rats treated with distilled water + glucose
10-GROUP J OR NEGATIVE CONTROL	Normal rats treated with distilled water

F1aq, F1heth, F2aq, F2heth, F3aq, F3heth, F4aq and F4heth are respectively the different aqueous and hydroethanolic extracts of formulations 1, 2, 3 and 4

After a light puncture of the distal end of the tail with a lancing device, the blood was collected on blood glucose strips; The first blood sugar (baseline blood sugar) was determined after 12

hours of fasting before the administration of the various products (extracts and glucose). Immediately after basal blood sugar, the extracts were administered by gavage, 30 min before glucose administration. Subsequently, blood glucose was observed for 1 hour at 30 minute intervals in order to observe whether the previously administered extracts would prevent a rise in blood sugar after ingestion of glucose.

Evaluation of hypoglycemic activity: The aim here was to practice the reverse of the antihyperglycemic activity, that is to say to first force-feed the albino rats of the Wistar type with a solution of glucose at a dose of 2000 mg / kg of BW, then thirty minutes later, force-feed them with extracts at a dose of 400 mg / kg BW.

For this, forty normal rats were divided into 10 groups of 4 rats and fasted 12 hours before the experiment. The distribution of the rats was the same as that in Table 1.

Here, just after basal blood sugar, glucose was administered by gavage followed 30 min after gavage with the extracts. Subsequently, blood sugar was observed for 2 hours at 30-minute intervals to see if the extracts would prevent a significant rise in postprandial blood sugar compared to the positive control.

Statistical Analyzes
The data were entered into an Excel spreadsheet (Microsoft Office 2007) and then analyzed with Statview version 5.0 software (SAS Institute, Inc., USA). Data are presented as percent and mean ± standard deviation for qualitative and quantitative variables, respectively. The materiality threshold was set at 5%.

Results (*In vivo*)
Obtaining extracts from formulations
For four proportions formulated, we obtained four formulations namely F1, F2, F3 and F4. Each of these formulations were macerated in two different solvents namely distilled water (hence the aqueous extract which is denoted aq) and the water / ethanol mixture (hence the hydroethanolic extract which is denoted heth) thus making four extracts of formulations that we note: F1aq, F2aq, F3aq, F4aq, F1heth, F2heth, F3heth and F4heth.

Evaluation of the parameters of the toxicity of the extracts
Observation of signs of intoxications: The administration of a dose of 2000 mg / kg of BW of the different extracts on Wistar-type rats allowed us over a period of two weeks to study some signs of toxicity as required by the OECD protocol of 2008, this was shown in Table 2.

From this table, we can say that no particular sign of intoxication was noted either on the behavioral or physical level because the attitudes observed were the same in all the different groups of rats as well in the rats having ingested the extracts than in control rats. It also appears that during this period of toxicity no deaths were recorded.

Also, with regard to the behavior of the rats during the two weeks of observations, Table 3 shows us a weight gain observed in all the different batches of rats, so we can say that for a dose of 2000 mg extract / kg of body weight (BW), the ingested extracts do not prevent female rats from having an appetite for food.

However, other parameters were evaluated in order to be able to confirm the non-toxicity of the extracts, these are the observation of the organs and the dosage of some toxicity parameters.

Observation of organs: No signs of inflammation were observed physically on the organs of the different groups of rats following the sacrifice of the rats after the two weeks of observation for signs of intoxication. In other words, the organs of the different batches of test rats were not different from those of the organs of the negative control batch of rats and therefore the extracts did not damage the organs.

Assay of some biochemical markers of toxicity: Although from the behavioral and physical point of view the animals showed no signs of intoxication, nor with regard to the observation of the organs, the dosage of the biochemical parameters was evaluated in order to ensure good metabolic, hepathic and renal functioning of animals.

Regarding the parameters evaluated here in order to study the toxicity of the extracts at the metabolic level of rats, it appears that the usual values of these parameters in the rat are:
Proteinemia: 60.1 - 79.1g / l
Creatinemia: 36 - 59.2 mg / l
Asat: 65 - 113 IU / l
Alat: 15 - 45 IU / l
Uremia: 38 - 72 mg / dl [8]

Table 3: Variation in the weight of the rats in the different batches during the two weeks of observations of the toxicity of the extracts

Signs of poisoning	F1aq	F1heth	F2aq	F2heth	F3aq	F3heth	F4aq	F4heth	Negative control
Locomotion	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Reduced activity	-	-	-	-	-	-	-	-	-
Feces appearance	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Urine appearance	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Sensitivity to noise	+	+	+	+	+	+	+	+	+
Appetite	+	+	+	+	+	+	+	+	+
Breathing difficulty	-	-	-	-	-	-	-	-	-
coat	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Number of deaths	0	0	0	0	0	0	0	0	0

In fact, with regard to FIG. and the usual values of the various biochemical parameters in rats mentioned above, it can be seen that, with regard to the proteinemia, neither the test groups nor the negative control group of rats show a normal level of protein in the blood, this same result was observed with regard to the activity of Asat where no group showed normal activity. On the other hand, we note that in terms of the urea level, almost all the groups presented a normal value of urea, it is only the groups of formulation 2 which presented a high rate of urea. Regarding creatinemia, only three groups do not exhibit a normal level of creatinine, these are the aqueous extracts of formulations 3 and 4 and the hydroethanolic extract of formulation 2. Also, with regard to Alat activity, only two groups exhibit normal activity, these are the aqueous extract of formulation 1 and the hydroethanolic extract of formulation 4.

Regarding the statistical analysis of this toxicity test, it is noted for these 5 biochemical parameters evaluated that there is no significant difference at $P < 0.05$ between the different test groups and the negative control group. However, for certain biochemical parameters, a significant difference is observed between the test groups. In fact, with regard to serum creatinemia, a significant difference is observed between the hydroethanolic extract of formulation 1 and the aqueous extract of formulation 3, as regards proteinemia, a significant difference is observed between the aqueous extract of formulation 1 and the aqueous extract of formulation 3 and as regards uremia, a significant difference is observed between the aqueous extract of formulation 2 and the aqueous extract of formulation 4.

Evaluation of the antihyperglycemic activity of the extracts after oral glucose overload of normal rats or glucose tolerance test

Table 4 shows the evolution of blood glucose for one hour after ingestion of the extracts of formulations in rats followed by gavage with a glucose overload solution. This table presents us in fact 10 tests among which we have 8 tests representing the groups of extracts (F1aq, F2aq, F3aq, F4aq, F1heth, F2 heth, F3 heth and F4 eth) and for the other 2 tests l one represents the positive control group (TP) and the other the negative control group (TN). It can be seen in this table that 30 min after ingestion of the extracts and of the glucose, an increase in blood sugar is observed in all the groups, the TN group having the lowest blood sugar level because the rats in this group ingested only water; With regard to the other groups, we see at T30 that all the groups of extracts show an increase in blood sugar which is less than the increase in blood sugar of the positive control: the extracts thus exhibit an ability to limit the increase of blood sugar after a meal, the best extract being the aqueous extract of formulation 3 (F3aq) where we just observe an increase in blood sugar of 0.3 g / l against an increase of 0.62 g / l in the positive control.

Evaluation of the hypoglycemic activity of the extracts

After administration of a dose of 2000mg / kg BW of glucose followed 30min after gavage of the extracts at a rate of 400mg / kg BW on normal albino rats, we obtained Table 5 below.

Table 4: Evolution of glycemia (in g / l) as a function of time (in min) after administration of a dose of 400 mg / kg of EP from the extracts followed by gavage of glucose at a dose of 2000 mg / kg of CP.

| Time Extracts	T0	T30	T60	Amount of blood sugar increased from T0 to T30
F1aq + Glu	0.85 ± 0.21	1.43 ± 0.07	1.11 ± 0.16	0.58
F1heth + Glu	0.92 ± 0.17	1.34 ± 0.12	1.19 ± 0.05	0.42
F2aq + Glu	0.88 ± 0.14	1.49 ± 0.22	1.18 ± 0.09	0.61
F2heth + Glu	1.1 ± 0.13	1.46 ± 0.09	1.28 ± 0.07	0.36
F3aq + Glu	1 ± 0.09	1.3 ± 0.2	1.04 ± 0.1	0.3
F3heth + Glu	0.93 ± 0.13	1.52 ± 0.12	1.07 ± 0.08	0.57
F4aq + Glu	0.81 ± 0.05	1.35 ± 0.14	0.98 ± 0.13	0.54
F4heth + Glu	0.85 ± 0.1	1.37 ± 0.1	0.95 ± 0.22	0.52
Eau + Glu (PC)	0.85 ± 0.13	1.47 ± 0.15	0.97 ± 0.05	0.62
Eau (NC)	0.71 ± 0.13	0.82 ± 0.11	0.65 ± 0.01	0.11

F1aq, F1heth, F2aq, F2heth, F3aq, F3heth, F4aq and F4heth are respectively the different aqueous and hydroethanolic extracts of formulations 1, 2, 3 and 4, Glu = glucose, PC = positive control and NC = negative control.

Indeed, from this table it emerges that compared to the positive control rats where a rise in blood sugar of 0.17g / l is observed from T0 to T30, the best extracts are the F3heth extract where after ingestion of glucose, instead of observing an increase in blood sugar, we have rather a decrease in glycemia at T30 of 0.06g / l which shows that this extract prevents an increase in postprandial glycemia, then we have the F4heth extract where at T30 we just have an increase in blood sugar of 0.06g / l, then the F1heth extract with at T30 an increase in blood sugar of 0.11g / l and finally the F2heth and F3aq extracts where at T30 we have an increase of 0, 13g / l, the three other remaining extracts, namely F1aq, F2aq and F4aq, at T30 show a rise in blood glucose higher than the possible control. It can thus be seen that from T0 to T30, the best extracts to inhibit the increase in blood sugar after ingestion of a glucose overloaded solution are the hydroethanolic extracts with formulation 3 as the best formulation of all. Thus, 30 min (T30) after ingestion of the extracts and glucose, at $P < 0.05$, a significant difference is observed between the positive control group and the group having ingested the F2aq extract, as well as a significant difference between the positive control group and the negative control group. Likewise, at the end of the experiment (T120), a significant difference was observed between these different groups.

It also emerges from this table that, compared at the start of the experiment or T0 and at the end of the experiment or T120, the glycemia of the positive control rats was initially 0.89 ± 0.14 g / l and at the end of the experiment, it is 0.82 ± 0.08 g / l; we note that the blood sugar is still around 0.8 g / l and for this group we just obtained a slight variation in blood sugar of 0.07 g / l. On the other hand, in the extracts, a considerable difference in blood sugar levels is observed at the end of the experiment, the best extracts being respectively F1heth, F3heth, F4aq, F2heth, F1aq, F3aq and F4heth with a variation in glycemia respectively of 0, 23g / l, 0.22g / l, 0.21g / l, 0.18g / l, 0.15g / l and 0.11g / l for the last two extracts. The F2aq extract being the extract with the lowest activity because at the end of the experiment there is a difference in glycemia of

Table 5: Evolution of glycemia (in g / l) as a function of time (in minutes) after administration of a glucose overload solution at a dose of 2000 mg / kg of EP followed by gavage of the extracts at a dose dose of 400 mg / kg EP.

Time Extracts	T0	T30	T60	T90	T120	Difference in blood sugar variation from T0 to T120
F1aq	0.91 ± 0.05	1.17 ± 0.08	0.98 ± 0.05	0.76 ± 0.05	0.76 ± 0.02	0.15
F1hal	1.04 ± 0.08	1.15 ± 0.09	0.97 ± 0.08	0.85 ± 0.02	0.81 ± 0.05	0.23
F2aq	0.95 ± 0.1	1.26 ± 0.06	0.92 ± 0.03	0.87 ± 0.05	0.91 ± 0.01	0.04
F2hal	1.02 ± 0.13	1.15 ± 0.1	0.91 ± 0.06	0.87 ± 0.039	0.84 ± 0.03	0.18
F3aq	0.98 ± 0.2	1.11 ± 0.07	0.91 ± 0.13	0.86 ± 0.18	0.87 ± 0.12	0.11
F3hal	1.02 ± 0.08	0.96 ± 0.1	0.89 ± 0.02	0.83 ± 0.06	0.8 ± 0.03	0.22
F4aq	0.97 ± 0.14	1.24 ± 0.15	1.13 ± 0.38	0.81 ± 0.19	0.76 ± 0	0.21
F4hal	1 ± 0.1	1.06 ± 0.04	0.96 ± 0.03	0.86 ± 0.02	0.89 ± 0.03	0.11
TP	0.89 ± 0.14	1.06 ± 0.08	0.93 ± 0.12	0.89 ± 0.11	0.82 ± 0.08	0.07
TN	1 ± 0.05	0.89 ± 0.09	0.74 ± 0.13	0.74 ± 0.08	0.67 ± 0.13	0.33
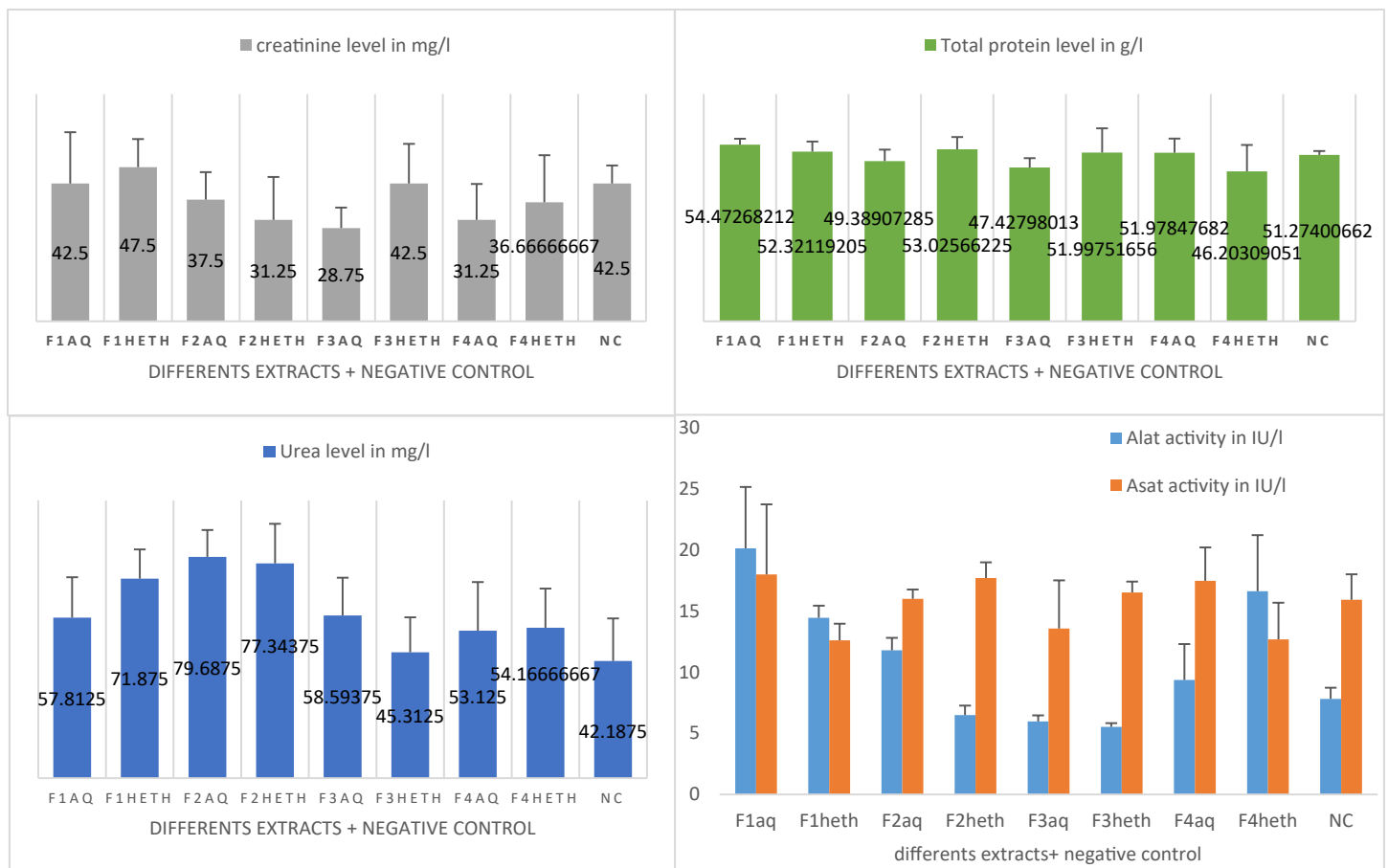
Figure 1: Biochemical parameters of toxicity in rats.

Legend: F1, F2, F3, and F4 are respectively the different formulations 1, 2, 3 and 4; aq and heth are respectively the different aqueous and hydroethanolic extracts of which F1aq corresponds to the aqueous extract of formulation 1, F1heth corresponds to the hydroethanolic extract of formulation 1 and so on, NC being the negative control.

0.04 g / l: lower than that of the positive control which is 0.07 g / l. The best formulations being formulations 1 and 3.

Discussions

Postprandial hyperglycemia is manifested by excessive glycemic excursions after meals which remain limited in healthy subjects, but the degradation of postprandial glycemic control is a first step towards glucose intolerance which increases the risk of later developing type 2 diabetes. One of the important therapeutic approaches to prevent postprandial hyperglycemia is the slowing of digestion and intestinal absorption of dietary carbohydrates after a meal by inhibiting digestive enzymes [9,10]. But antidiabetic drugs usually have side effects: decreased effectiveness over time, ineffectiveness against long-term diabetic complications, and low

cost-effectiveness [11]. Therefore, the discovery and development of new anti-diabetic drugs is even more than necessary. Indeed, plants are an inexhaustible source of new substances with therapeutic potential, hence the use of their extracts as a common practice in traditional medicine. The majority of current drugs are of plant origin (extracts) or are made from their model by a chemical synthesis of the active ingredients [12]. As part of the fight against diabetes, this part of the work was in question to highlight the hypoglycemic and antihyperglycemic potential of the formulations of oyster mushrooms. Hyperglycemia can be induced either temporarily from a glucose solution or permanently by contact with agents such as alloxan or streptozotocin [13,14]. In this part of the study, it was a temporary induction by a solution of glucose.

Indeed, for this study, before demonstrating the hypoglycemic and antihyperglycemic activity of the extracts of mushroom formulated on wistar rats, we first carried out a toxicity test of the various extracts.

As for the toxicity test, it appears that no real signs of toxicity were found over time on the physical, behavioral and physiological levels of the animals.

In fact, during the two weeks of observation of the toxicity of the extracts, weight gain was observed in all the different batches of rats as well as in the negative control. This could be explained by the fact that for a dose of extract at 2000 mg / kg of BW administered to the rats, the rats show a normal physiological adaptation response to this dose [15], which could still be explained. by the fact that the extracts do not cause a loss of appetite in the rats. Likewise, with regard to the biological parameters studied, it is noted that there is no significant difference between the negative control group and the different groups of extracts, which could further confirm the fact that the extracts do not present any toxicity.

Indeed, Alat and Asat are markers of hepatic toxicity and their elevated serum levels indicate hepatocyte damage [16], but since a significant difference was not observed between the negative control group and the test group, it can be said that for a dose of extract administered at 2000 mg / kg bw, the extracts did not disturb the serum levels of these transaminases although the threshold values were not reached. Indeed the fact that the negative control group does not present the threshold value like the test groups could be explained by the fact that it is not the ingestion of the extract which would be at the origin of this but another parameter which could be specific to all the different groups of rats, either the habitat, or the diet or others. Also, the serum concentrations of urea, protein and creatinine which are markers of impaired renal function [17] did not vary significantly in the presence of the extracts. The administration of the extracts did not significantly modify the serum values of all the biochemical parameters of the markers of kidney, heart and liver damage compared to the rats of the negative control group, which suggests that the extracts have not affected these vital organs.

Regarding the antihyperglycemic test, it appears that in view of the positive control where we have at T30 an increase in blood sugar of 0.62 g / l, our various extracts have a capacity to limit the increase in blood sugar because they present at T30 an increase in blood glucose lower than that of the positive control. This ability of extracts to limit the rise in blood sugar may be due to their antioxidant potential. Because according to Pyo et al. in 2004 [18], antioxidants may destroy free radicals and improve insulin secretion. In fact, antioxidants can act by various mechanisms, in particular allowing better absorption of glucose in peripheral tissues, allowing stimulation of the secretion of insulin by β cells of the pancreas, promoting a decrease in the glycation of circulating proteins, in particular l glycated hemoglobin, a marker of the long-term glycemic state of type 2 diabetes (Pari and Latha, 2002) [19]. This ability of extracts to limit the increase in blood sugar could also be explained according to Mangano et al. in 2010 [20] by the fact that polyphenols and phenolic acid are useful in the prevention of type 2 diabetes by causing reduction in intestinal glucose absorption, which thus reduces blood sugar spikes after meals. So, the ability of the extracts to limit the blood sugar peak could be due to the phenolic compounds they contain because, according to our previous studies [6], we were able to show that the formulated mushroom extracts were rich in phenolic compounds.

However, this effectiveness of the extracts at this dose of 400mg / kg BW is believed to be due to the fact that the mushrooms were initially formulated before the extracts were obtained. Because indeed, according to the study made by Etoundi et al. in 2018 [5] which focused on the antihyperglycemic activity of rats after administration of extracts of mushroom of Pleurotus pulmonarius and Pleurotus floridanus at a dose of 400 mg / kg bw followed by force-feeding with a glucose overload solution, it emerged from this work that the mushroom studied separately did not exhibit antihyperglycemic activity at a dose of 400 mg / kg of BW (but rather at 800 mg / kg of BW): at T30 all the extracts exhibited a blood glucose level higher than that of the the positive control.

On the other hand, we see here that when the mushrooms were formulated at the start, we observe the opposite effect, all the extracts have a lower blood sugar level than the positive control and therefore exhibit an antihyperglycemic activity at 400 mg / kg of BW hence, we can appreciate here the usefulness of the formulation. This is the reason why for this antihyperglycemic test we did not observe the evolution of the glycemia after 2 hours of time but only after 1 hour because it is the behavior of the glycemia at T30 that interested us. So as to know if when the mushrooms were originally formulated they could have antihyperglycemic activity at a dose of 400mg / kg BW.

We also note from this test that at T60 the glycemia of all the rats had decreased, this could be explained by the good functioning of an organism which, 1 hour after ingestion of a food, transforms this food into nutrient and precisely into glucose. which is then transported from the blood to the various target organs where it is used for the various metabolic reactions of the body.

Likewise, with regard to the hypoglycemic test, it is found that the extracts have a good ability to limit the increase in blood sugar. Because indeed, at T30, the extracts F3heth, F4heth, F1heth, F2heth and F3aq have a glycemia lower than the TP. Also, in view of the blood glucose levels at T120, it can be seen that the extracts with the exception of F2aq have a good hypoglycemic potential because after 2 hours after ingestion of a saturated glucose solution, the blood glucose levels of the rats having ingested the extracts had not only returned to normal but also was considerably lower than the starting blood sugar unlike the possible control rats where the blood sugar just returned to normal after 2 hours of time; This being the case, for untreated diabetic subjects, at the end of 2 hours after consumption of a meal, the blood sugar will simply be returned to the starting blood sugar level (before consumption of the meal) which is a higher than normal blood sugar level (because fasting diabetic blood sugar is greater than 1.26g/l). On the other hand, for diabetic subjects treated or having consumed the mushrooms, their blood sugar could return to normal after 2 hours after a meal, which could help them to regulate their blood sugar levels while thus avoiding the complications associated with diabetes.

The hypoglycemia and reduction in hyperglycemia observed in rats treated with the formulated mushroom extracts could then be explained by a stimulation of the secretion of insulin by the pancreas [21] and / or, probably, by an increased peripheral glucose use in the presence of the extracts [22].

Conclusion
At the end of this work, which consisted in evaluating the toxicity of the extracts and studying the short-term effect of these extracts on antihyperglycemic activity and hypoglycaemic activity in normal rats, it emerges that:
- The formulated mushroom extracts do not show signs of toxicity
- At a dose of 400 mg / kg BW, the mushroom extracts inhibit the increase in postprandial blood sugar with the aqueous extract of formulation 3 as the best antihyperglycemic extract.
- At a dose of 400 mg / kg BW, the mushroom extracts significantly reduce the increase in postprandial blood sugar with the hydroethanolic extract of formulation 3 as the best hypoglycemic extract.

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