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Evaluation of the glycemic reducing effect of the aqueous extract of *Zea mays* stigmas through oral stimulated hyperglycemia test

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Abstract
Oral glucose-induced hyperglycemia involves having the subject consume 75 grams of glucose, then measuring blood glucose levels 2 and 3 hours later. It allows observation of how the body responds after glucose ingestion. This study aims to evaluate the blood sugar-lowering effect of *Zea mays* stigmas on hyperglycemia. The aqueous extract of stigmas was obtained by decoction, and the toxicity test was conducted orally using mice of the species *Mus musculus*. Secondary metabolites were identified using the colorimetric method, while trace elements were analyzed using the calcination-mineralization method. Hyperglycemia was induced orally in mice of the species *Mus musculus*. The results showed that the extraction yield was 6.2% and that the contents of chromium, potassium, iron, zinc, magnesium, and copper were respectively: 0.39±0.10; 1651±0.84; 1.83±0.32; 61.17±0.43; 577.09±0.75; 157.58±0.68 µg/g of dry extract. Secondary metabolites such as alkaloids, saponins, polyphenols, flavonoids, gallic tannins, catechic tannins, and quinones were found in the said extract. The blood glucose of mice treated with the aqueous extract of *Zea mays* stigmas, at doses of 100 and 200 mg/kg of body weight, did not show any increase compared to the blood glucose of untreated mice. The study shows that the aqueous extract of *Zea mays* stigmas has blood glucose-lowering activity.

Keywords: Hyperglycemia, glucose, stigmas, *Zea mays*, *Mus musculus*

Introduction
Oral glucose tolerance test (OGTT), or oral glucose challenge, involves having the patient consume 75 grams of glucose, then measuring blood sugar at 1 hour, 2 hours, and 3 hours afterward [1]. The OGTT allows observation of how the body responds after ingesting glucose [2]. Postprandial blood sugar does not exceed 1.40 g/L in a healthy individual [3]. Exceeding this value indicates glucose intolerance [1]. Persistent hyperglycemia can lead to diabetes, accompanied by complications affecting the eyes, heart, kidneys, and nerves if it is not diagnosed and managed promptly [4]. This study aims to evaluate the use of *Zea mays* stigmas in managing hyperglycemic episodes, as they are believed to have blood sugar-regulating activity [5]. The specific objectives were, on the one hand, to investigate the secondary metabolites and minerals in the aqueous extract of *Zea mays* stigmas, and, on the other hand, to evaluate the regulatory effect of the aqueous extract of *Zea mays* stigmas on orally induced hyperglycemia in mice of the species *Mus musculus*.

Materials and Methods
Plant material
The plant material consisted of Zea mays stigmas (Figure 1).

Fig 1: Zea mays plant bearing ears topped with stigmas

Animal material
The animal material consisted of mice of the species *Mus musculus* (Figure 2).

Fig 2: Mouse of the species *Mus musculus*

Methods

Preparation of the aqueous extract of *Zea mays* stigmas

The preparation of the aqueous extract of *Zea mays* stigmas was done by decoction [6]. One liter of distilled water was heated in a round-bottomed flask. As soon as the distilled water boiled, 100 grams of stigmas powder (*Zea mays*) were added. The mixture was brought to a boil for 15 to 20 minutes. After cooling, it was filtered twice through absorbent cotton and once with regular filter paper. The collected filtrate was placed in a crystallizer and dried in an oven at 40 °C until completely dry. After drying, the mass that dried at the bottom of the crystallizing dish was scraped and turned into powder, and this powder constituted the aqueous extract of *Zea mays* stigmas. It was weighed to be used in the calculation of the extraction yield.

Search for secondary metabolites in the aqueous extract of *Zea mays* stigmas

The search for secondary metabolites in the aqueous extract of *Zea mays* stigmas was carried out using the colorimetric method [7].

search for Alkaloids

Six (6) mL of the aqueous extract of *Zea mays* stigmas were evaporated to dryness in a porcelain capsule in a sand bath. The residue was taken up in 6 mL of 60° alcohol, and the resulting alcoholic solution was filtered through absorbent cotton and divided into two test tubes. Two (2) drops of Dragendorff's reagent were added to the first tube. The appearance of a precipitate or an orange coloration indicated the presence of alkaloids. In the second tube, two (2) drops of Bouchardat's reagent are added. The appearance of a reddish-brown coloration indicated the presence of alkaloids.

Search for polyphenols

To two (2) mL of plant extract, one drop of 2% aqueous ferric chloride solution was added. The appearance of a bluish-black or greenish coloration to varying degrees indicated the presence of phenolic compounds.

Search for tannins

To five (5) mL of extract, fifteen (15) mL of Stiasny reagent (10 mL of 40% formaldehyde mixed with 5 mL of concentrated HCl) were added. The mixture is kept in a water bath at 80 °C for 30 minutes, then cooled under running water. The observation of large precipitates in the form of flakes characterizes catechic tannins. The solution containing the flakes was filtered, and the filtrate collected was then saturated with sodium acetate. Three (3) drops of 2% ferric chloride were added to the mixture. The appearance of an intense blue-black coloration indicated the presence of gallic tannins.

Search for flavonoids

Two (2) mL of extract were evaporated, and the residue was redissolved in 5 mL of half-diluted hydrochloric alcohol. By adding a few magnesium shavings, heat was released, followed by a pink-orange or violet coloration. The addition of 3 drops of isoamyl alcohol intensified this coloration, confirming the presence of flavonoids. An alcoholic solution of quercetin was used as a control.

Search for polyterpenes and sterols

Five (5) mL of plant extract were dried under a rotary evaporator. The residue was dissolved hot in 1 mL of acetic anhydride and collected in a test tube. A volume of 0.5 mL of concentrated sulfuric acid was poured along the tube. The appearance at the interface of a purple or violet ring, turning blue then green, indicated the presence of polyterpenes and sterols.

Search for quinones

A volume of 2 mL of extract was evaporated to dryness, and then the residue was triturated in 5 mL of hydrochloric acid diluted five times to hydrolyze combined substances. The resulting solution was then brought to a boiling water bath for 30 minutes. After cooling under a stream of cold water, the hydrolysate was extracted with 20 mL of chloroform. The chloroform phase was then collected in a test tube, and 0.5 mL of half-diluted ammonia was added. The appearance of a color ranging from red to violet indicated the presence of quinones.

Search for saponins

Ten (10) mL of plant extract are placed in a test tube. After shaking for a few minutes, the foam height was measured. A persistent foam height greater than 1 cm indicated the presence of saponins.

Research and measurement of trace elements

The research and measurement of trace elements were carried out according to the calcination and mineralization method [8].

Mineralization by calcination

The aqueous extract of *Zea mays* stigmas was dried in an oven at 60 °C, and 0.4 g was weighed using a Sartorius analytic balance (England) in a 30 mL porcelain crucible. This test sample was placed in a Naberthem Germany muffle furnace set to 550 °C for 5 hours. After cooling, 2 mL of 0.5 N hydrochloric acid was added to the obtained ash and then evaporated to dryness on a sand bath. The final residue was filtered into a 100 mL volumetric flask, and distilled water was added to reach the calibration mark. Five (5) mL of the filtrate were taken for the determination of minerals (K, Fe, Zn, Mg, Cu) using the atomic absorption spectrophotometer (AAS 20 type VARIAN, Australia).

Preparation of the Lanthanum reagent

Under a fume hood, 58.65 g of La2O3 was moistened with 50 mL of distilled water, and 250 mL of concentrated hydrochloric acid was added while slowly stirring until the Lanthanum (La2O3) was completely dissolved. Before any measurements, the atomic absorption spectrophotometer was calibrated. To do this, a 100 ppm standard solution was prepared from a commercial 1000 ppm multi-element solution. The preparation was carried out as follows: 2.5 mL of the stock solution (1000 ppm) were introduced into a 25 mL flask and brought up to the mark with concentrated nitric

acid, and this solution was used to prepare the standard ranges.

Preparation of Samples and Standards

Five (5) mL of each sample were taken into a 50 mL flask, and 2 mL of 5% Lanthanum was added before topping up with distilled water to the mark. For the standards, dilutions from standard solutions of each mineral (100 mg/L) were made by completing the various initial volumes to 50 mL with distilled water, in order to obtain an accurate concentration range for each mineral. These calibration solutions are then used for calibrating the flame atomic absorption spectrophotometer. The wavelengths at which potassium (K), iron (Fe), zinc (Zn), magnesium (Mg), and copper (Cu) were read were 766.5 nm; 248.3 nm; 258 nm; 285.2 nm and 324.7 nm, respectively. The results of the optical densities of each mineral made it possible to determine the amounts of minerals (ppm) contained in the aqueous extract. The mineral contents were determined as follows :

$$T = \frac{(C_{ess} - C_{bl})\,V}{P_{ess}} \quad \text{(I) with}$$

C_{ess} : sample concentration (mg/mL)
C_{bl} : white concentration (mg/mL)
P_{ess} : trial run (mg)
V : trial recovery volume (mL)

Induction of hyperglycemia

Thirty (30) mice of the species *Mus musculus* were divided into five (05) groups of six (06) mice each. At time T equal to 0 hour (T0), the blood glucose of each mouse in each group was measured using a glucometer. Then, hyperglycemia was induced by the oral administration of 75 g of glucose [1]. Next, except for the mice in group 1 that received distilled water, hyperglycemia was induced by oral administration of 75 g of glucose [1] to the mice in groups 2, 3, 4, and 5. Except for the mice in group 2, after glucose administration, the mice in groups 3, 4, and 5 immediately underwent treatments to observe the effect of these treatments on blood glucose progression. Thus, the mice in groups 3 and 4 were treated with 100 and 200 g/kg of body weight of the aqueous extract of *Zea mays* stigmas, respectively and those in group 5 were treated with Glymepiride under the brand name AMAREL : an oral antidiabetic belonging to the sulfonylurea family. Once the treatments were completed, the blood glucose levels of the mice in each group were measured again using a glucometer at 1 h, 2 h, and 3 h.

Statistical analysis

The results were expressed as means followed by the standard deviation (SD) of the mean (Mean ± SD). The graphical representation of the data was carried out using the Grap Pad Prism 8.0.2 software. Statistical analysis of the results was carried out using analysis of variance (One Way Anova). Differences between means were determined using Dunnett's multiple comparison test. The significance threshold is set up $p<0.05$ for the expression of the results.

Results

Phytochemical screening revealed the presence of alkaloids, saponins, polyphenols, flavonoids, tannins, and quinones in the aqueous extract of stigmas of *Zea mays*. However, sterols and terpenes were absent from the extract. Table I summarizes the secondary metabolites in the aqueous extract of *Zea mays* stigmas.

Table 1: Phytochemical Screening

Secondary metabolites	Aqueous extract of *Zea mays* stigmas
alkaloids	+
Saponins	+
Polyphenols	+
Flavonoids	+
Tannins	+
Sterols and terpenes	-
Quinones	+

(+) : presence of the metabolite, (-): absence of the metabolite

The measurement of trace elements (Table II) revealed the presence of chromium, potassium, iron, zinc, magnesium, and copper with respective contents of 0.39±0.10; 1651±0.84; 1.83±0.32; 61.17±0.43; 577.09±0.75 and 157.58±0.68 µg/g of dry extract. This measurement showed that potassium was the most abundant trace element in the aqueous extract of *Zea mays* stigmas.

Table 2: Mineral dosage in the aqueous extract of *Zea mays* stigmas

Oligoéléments	contents (µg/g of dry extract)
Chromium	0,39±0,10
Potassium	1651±0,84
Iron	1,83±0,32
Zinc	61,17±0,43
Magnesium	577,09±0,75
Copper	157,58±0,68

Figure 1 shows the different blood glucose levels after oral glucose-induced hyperglycemia in mice of the species *Mus musculus*.

At time T equal to 0 h, the average blood glucose of mice in groups 1, 2, 3, 4, and 5 were respectively 73.67±0.07; 73.00±0.08; 73.67±0.09; 74.33±0.07; and 74.33±0.07 mg/dL. The average blood glucose in each group of mice was essentially equal ($p>0.05$) before the ingestion of 75 g of glucose.

At time T equal to 1 hour, the average blood glucose of mice in the control group (group 1) was 73.67±0.07 mg/dL, which was the same as at T equal to 0 hours. That of the mice given 75 g of glucose and not treated (group 2) was 82.33±1.97 mg/dL. This blood glucose (82.33±1.97 mg/dL) at T equal to 1 hour was significantly higher ($p<0.05$) than the initial value (73.00±0.08 mg/dL) at T equal to 0 hours. For the same time (T equal to 1h), the blood glucose levels of mice in groups 3 and 4 that received 75 g of glucose and were treated with 100 and 200 mg/kg body weight of the aqueous extract of *Zea mays* stigmas, respectively, were 75.67±0.08 and 75.33±0.07 mg/dL. These glucose levels had increased compared to baseline (T equal to 0 h) but remained statistically non-significant ($p>0.05$). The mice in group 5 that received 75 g of glucose and were treated with 150 mg/kg bw of

Glimepiride had an average blood glucose level of 75.33±0.08 mg/dL, which had increased compared to the baseline (time 0 h) but remained statistically non-significant (p>0.05).

At time T equal to 2 hours, the average blood glucose of the control group mice (group 1) remained constant (73.67±0.07 mg/dL). That of the mice that received 75 g of glucose and were not treated (group 2) increased to 101.33±0.15 mg/dL. This blood glucose level (101.33±0.15 mg/dL) at T equal to 2 hours was significantly higher (p<0.05) than that (82.33±1.97 mg/dL) at T equal to 1 hour. The average blood glucose levels of mice from groups 3 and 4, which received 75 g of glucose and were treated with 100 and 200 mg/kg body weight of the aqueous extract of *Zea mays* stigmas, were 82.33±0.14 and 76.33±0.08 mg/dL, respectively, at time T equal to 2 hours. These glucose levels showed an increase compared to those at T equal to 1 hour but remained statistically non-significant (p>0.05). The mice in group 5 that received 75 g of glucose and were treated with 150 mg/kg body weight of Glimepiride had an average blood glucose level of 75.67±0.05 mg/dL. This blood glucose level was higher compared to that at T equal to 1 hour but remained statistically non-significant (p>0.05). At time T equal to 3 hours, the average blood glucose (73.67±0.07 mg/dL) of the control group mice (group 1) remained constant. That of the mice that received 75 g of glucose and were not treated (group 2) rose to 108.00±0.08 mg/dL and was significantly higher (p<0.05) than that (101.33±0.15 mg/dL) at T equal to 2 hours. The blood glucose levels of mice in groups 3 and 4, which received 75 g of glucose and were treated respectively with 100 and 200 mg/kg body weight of the aqueous extract of Zea mays stigmas, were 76.33±0.05 and 74.00±0.06 mg/dL at time T equal to 3 hours. These blood glucose levels had decreased compared to those at T equal to 2 hours but remained statistically non-significant (p>0.05). The mice in group 5 that received 75 g of glucose and were treated with 150 mg/kg body weight of Glimepiride had an average blood glucose level of 73.66±0.05 mg/dL. This blood glucose level was decreased compared to that at T equal to 2 hours but remained statistically non-significant (p>0.05).

From time T equal to 0 h to time T equal to 3 h, the blood glucose levels of the mice that received 75 g of glucose and were treated with aqueous extract of *Zea mays* stigmas at dose of 200 mg/kg bw and 150 mg/kg bw of Glimepiride were almost similar (p>0.05) to those of the control group mice (group 1).

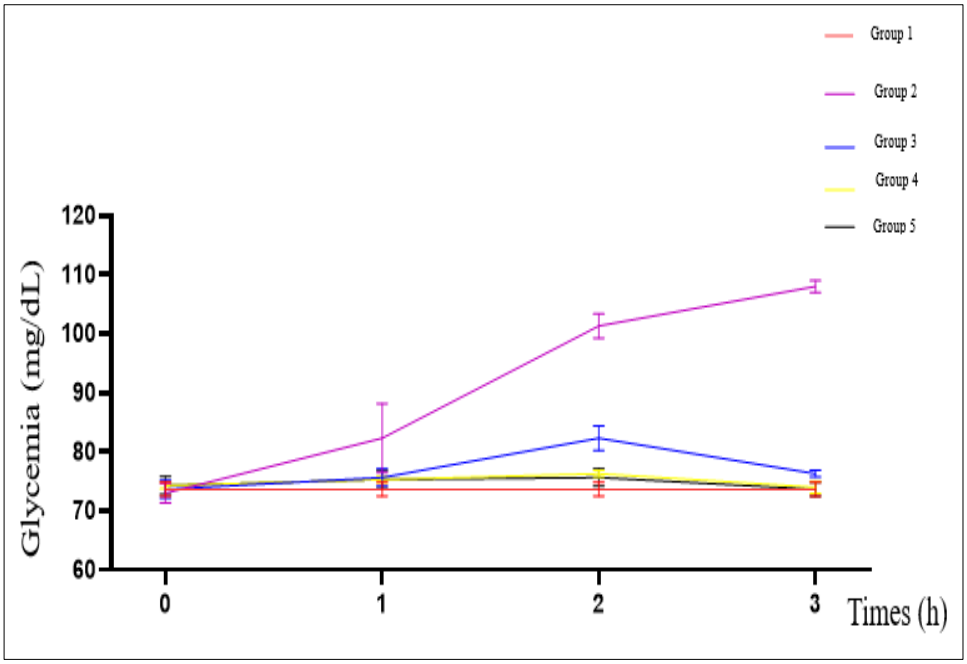

Fig 3: Evolution of glycemia in mice (*Mus musculus*) after OGTT

Discussion

The presence of alkaloids and tannins in the aqueous extract of *Zea mays* stigmas by decoction contradicts the results of Hassani (2023) [9] who did not find these secondary metabolites in the aqueous extract of *Zea mays* stigmas by infusion. The decoction used in this study and the infusion used by Hassani (2023) [9] could be the basis for this difference in secondary metabolites, as the decoction at 100 °C for 15 to 20 minutes causes cell disruption by facilitating solvent penetration and molecule solubilization [10]. The abundance of potassium in the aqueous extract of *Zea mays* stigmas is consistent with the result of Duru (2020) [11] who found a significant amount of potassium in the *Zea mays* hull. This also supports the fact that potassium is the most abundant element in plants [12].

The increase in blood glucose after a meal is due to the sugars contained in foods that tend to raise blood glucose: this is referred to as postprandial blood glucose [13]. The absence of a blood glucose rise in rats treated with the aqueous extract of *Zea mays* stigmas could be explained by the presence of secondary metabolites such as flavonoids. Indeed, flavonoids activate the metabolic pathways involved in glucose transport into cells. The absence of increased blood sugar in rats treated with aqueous extract of *Zea mays* stigmas could be explained by the presence of minerals such as zinc and chromium. Indeed, chromium facilitates insulin action and regulates blood sugar [14]. Zinc affects the structure of insulin and its binding to the insulin receptor [14].

Conclusion

At the end of this study, it appears that the aqueous extract of *Zea mays* stigmas reduces blood sugar levels in mice fed with 75 grams of glucose. The dose of 200 mg/kg body weight of the aqueous extract of *Zea mays* stigmas mimics the action of

Glimepiride, an oral antidiabetic belonging to the sulfonylurea family, which lowers blood sugar levels.

Déclarations

Conflict of Interest

The authors declare no competing interests.

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