



# The 7th International Electronic Conference on Medicinal Chemistry (ECMC 2021)

01-30 NOVEMBER 2021 | ONLINE

## Antihyperglycemic and insulinomimetic activities of hydro alcoholic extracts of *Anogeissus leiocarpus* (Combretaceae)

Aku Enam MOTTO<sup>1(\*)</sup>, Povi LAWSON-EVI<sup>1</sup>, Kokou TONA<sup>2</sup>, Kwashie EKLUGADEGBEKU<sup>1</sup>

<sup>1</sup>Laboratory of Physiology/Pharmacology. Unit of Pathophysiology, Bioactive Substances and Safety. Faculty of Sciences, University of Lomé. Togo. BP 1515

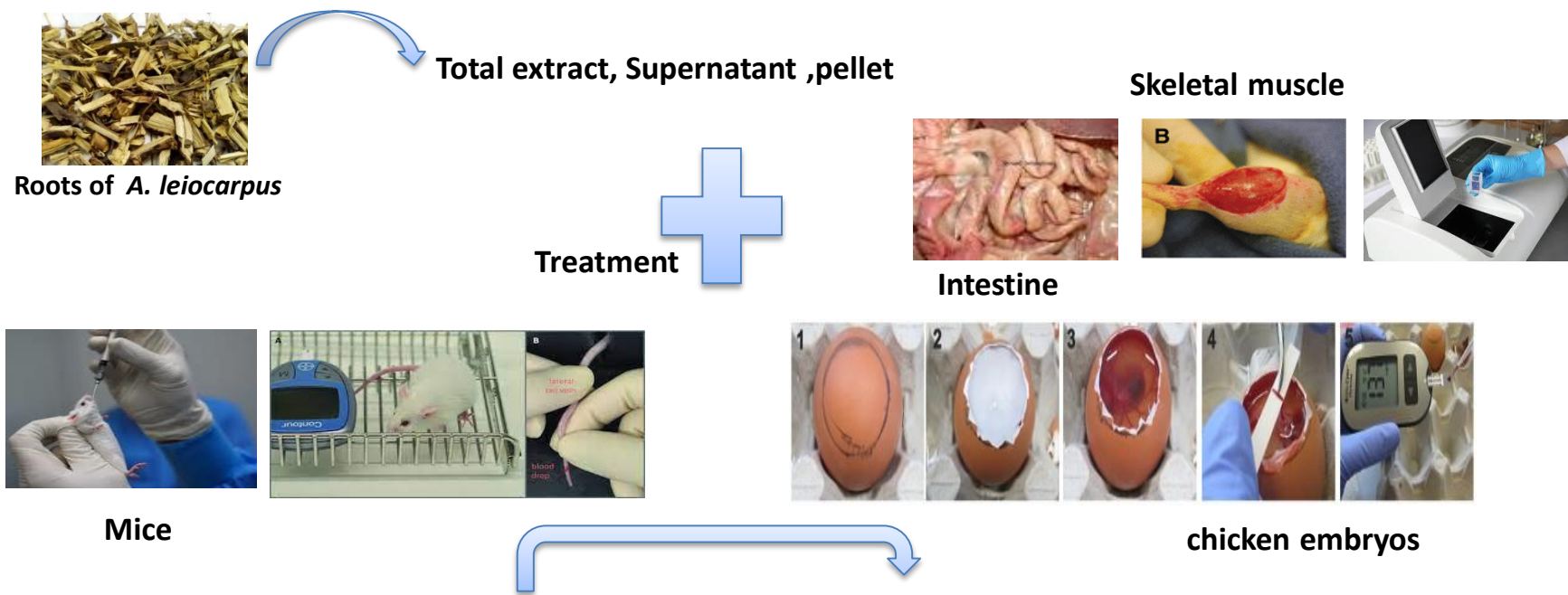
<sup>2</sup> Center of Excellence and Research in Avian Science (CERSA), University of Lomé BP 1515

\* Corresponding author: [nam.motto@gmail.com](mailto:nam.motto@gmail.com)



# Antihyperglycemic and insulinomimetic activities of hydro alcoholic extracts of *Anogeissus leiocarpus* (Combretaceae)

## Graphical Abstract



Extract and supernatant fraction:

- Antihyperglycemic**
- ↑ glucose uptake by muscle**
- ↑ glucose transport in the general circulation**



## Abstract:

Belonging to the family of Combretaceae, *Anogeissus leiocarpus* is a plant used by traditional practitioners to treat people living with diabetes mellitus in Togo (West Africa). The present study aimed to determine the hypoglycemic activity and the probable mechanism of action of the plant. The hydroalcoholic extract and fractions of *A. leiocarpus* roots was evaluated on hyperglycemic mice by oral glucose tolerance test (OGTT) and on normoglycemic mice. The effect of the total extract and fractions was also measured on the absorption of glucose *ex vivo* on intestine and skeletal muscles isolated from rats and *in ovo* on 11-day-old chicken embryos.

As the results, the total extract and fractions significantly ( $p < 0.0001$ ) reduced hyperglycemia of mice compared to controls, 30 minutes after glucose overload. However, the more pronounced effect was observed with the supernatant fraction at  $100 \text{ mg.kg}^{-1}$ . On normoglycemic mice, the total extract at  $500 \text{ mg.kg}^{-1}$  of the total extract did not lower ( $p > 0.05$ ) basal blood glucose levels compared to controls. In addition, the extract and fractions of *A. leiocarpus* inhibited glucose intestinal absorption *ex vivo* and enhanced the glucose uptake by the muscle in the presence and absence of insulin compared to control group. *In ovo*, *A. leiocarpus* ( $0.03$  and  $0.06 \text{ mg} / \mu\text{L}$ ) induced a significant decrease ( $p < 0.0001$ ) in basal glycemia from the 30th min to 120 min.

In conclusion, the extract of *A. leiocarpus* has a strong antihyperglycemic activity similar to an insulin-mimetic effect.

**Keywords:** *Anogeissus leiocarpus*-antihyperglycemic- *in ovo*- insulinomimetic - *ex vivo*



**The 7th International Electronic Conference on Medicinal Chemistry**

01-30 NOVEMBER 2021 | ONLINE

## Introduction

Diabetes is a chronic disease, with hallmark pathology of hyperglycemia, which is clinically represented by fasting blood glucose greater than 7.0 mM and 2 hours glucose level greater than 11.1 mM after glucose tolerance test.

In diabetics, chronic hyperglycemia, source of oxidative stress remains a very critical condition because of its nature to generate serious complications as retinopathy, nephropathy, neuropathy and cardiovascular disease (Ighodaro, 2018)



**The 7th International Electronic Conference on Medicinal Chemistry**

01-30 NOVEMBER 2021 | ONLINE

## Introduction

Globally, diabetes is the major health issue that has reached alarming levels ( IDF, 2019). For the management of diabetes, Many drugs and interventions are available today to manage diabetes.

Apart from inherent side effects, managing the disease is expensive and cost in developing countries where more than 85% of the populations use medicinal plants in preventive and curative applications.

It is therefore necessary to seek new avenues to manage this major public health problem; hence the growing interest in herbal remedies



**The 7th International Electronic Conference on Medicinal Chemistry**

01-30 NOVEMBER 2021 | ONLINE

## Introduction

*Anogeissus leiocarpus* (Guill and Perr) of the family of Combretaceae, is a tropical plant whose antimicrobial, antiproliferative, antioxidant and hepatoprotective properties (Singh et al .,2016) were reported.

Roots of this plant were also reported by Kpodar et *al* to be used traditionally to treat people living with diabetes in Togo ( Kpodar et al.,2015). However, there is still no scientific report on its antidiabetic property up to now.

Consequently, the present investigation was carried out to evaluate the hypoglycemic activity and the probable mechanism of action of the plant



**The 7th International Electronic Conference on Medicinal Chemistry**

01-30 NOVEMBER 2021 | ONLINE

## Material and methods

### Animals



**ICR mice 25 - 35 g**



**Sprague Dawley Rats  
160-250 g**



**Sasso Eggs**

All the studies were conducted in accordance to the institutional guidelines and ethics of Laboratory of Physiology/Pharmacology of University of Lome-Togo (ref: 001/2012/ CB-FDS-UL).



**The 7th International Electronic Conference on Medicinal Chemistry**

01-30 NOVEMBER 2021 | ONLINE

## Material and methods

### Plant material

Roots of *Anogeissus leiocarpus* were harvested in Tsévié, Zio (TOGO) in the month of July 2018. A voucher specimen was identified and was deposited in the herbarium of the Laboratory of Botany and Plant Ecology under the number TOGO 15483.

Roots of *Anogeissus leiocarpus* were cleaned out with water, cut into small pieces, dried at the Animal Physiology laboratory at 22°C.



**The 7th International Electronic Conference on Medicinal Chemistry**

01-30 NOVEMBER 2021 | ONLINE



## Material and methods

### Extraction

Roots of *A. leiocarpus* (400g) were extracted in water/ethanol (5:5) for 72 hours. The crude extract was filtered on Whatman paper and evaporated in vacuum at 45°C using a rotary evaporator (Buchi R120).

About 30g of hydroethanolic extract obtained was suspended in frozen ethanol 75% within 24 hours. Supernatant was separated from pellet by centrifugation at 2500 trs.min<sup>-1</sup> and evaporated in vacuum at 45°C using a rotary evaporator. Pellet was then concentrated to dryness



## Material and methods

### Effect of total extract of *Anogeissus leiocarpus* and fractions on oral glucose tolerance test

Hyperglycemia was induced in fasted mice (9 hours) by glucose gavage ( $4\text{g}\cdot\text{kg}^{-1}$  bw) (Motto et al.,2020)

Animals were divided into 9 groups of 7 mice

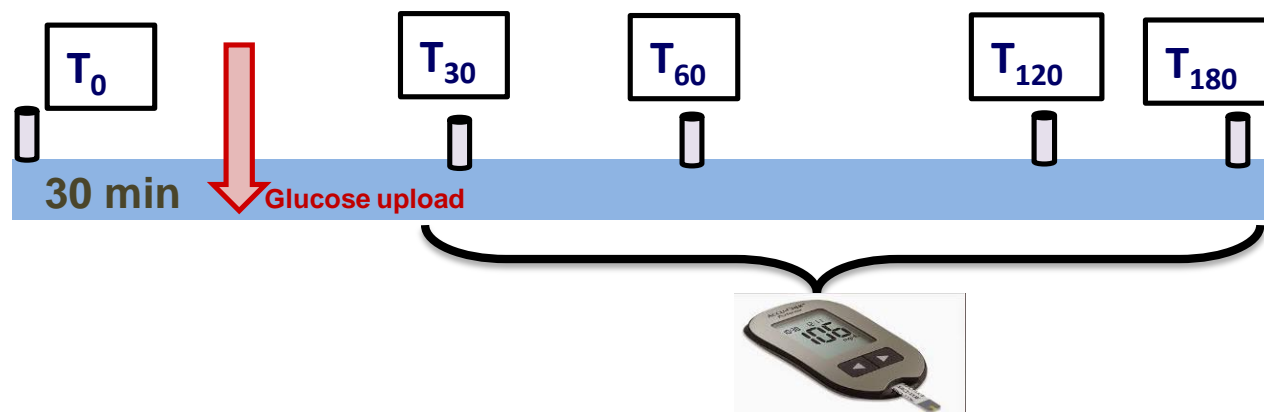
Distilled water control

TE: 125,250,500  $\text{mg}\cdot\text{kg}^{-1}$

Pellet : 50, 100  $\text{mg}\cdot\text{kg}^{-1}$

Supernatant :50, 100  $\text{mg}\cdot\text{kg}^{-1}$

Metformin 100  $\text{mg}\cdot\text{kg}^{-1}$

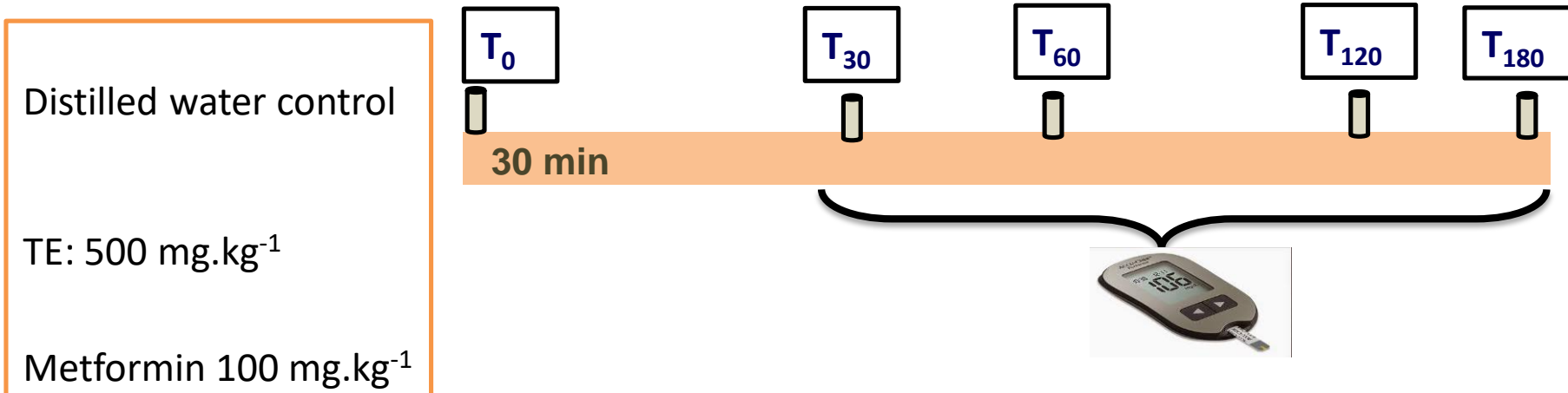


## Material and methods

# Effect of total extract of *Anogeissus leiocarpus* on normoglycemic mice

(Motto et al.,2020)

Animals were divided into 3 groups of 7 mice



## Material and methods

### Effect of total extract on blood glucose level of the chicken embryos

(Lawson-Evi et al.,2021)

Eggs were divided into 6 groups of 10 (after 11 days of incubation)  
Glycemia was measured by the method of Haselgrübler et al. (2018).

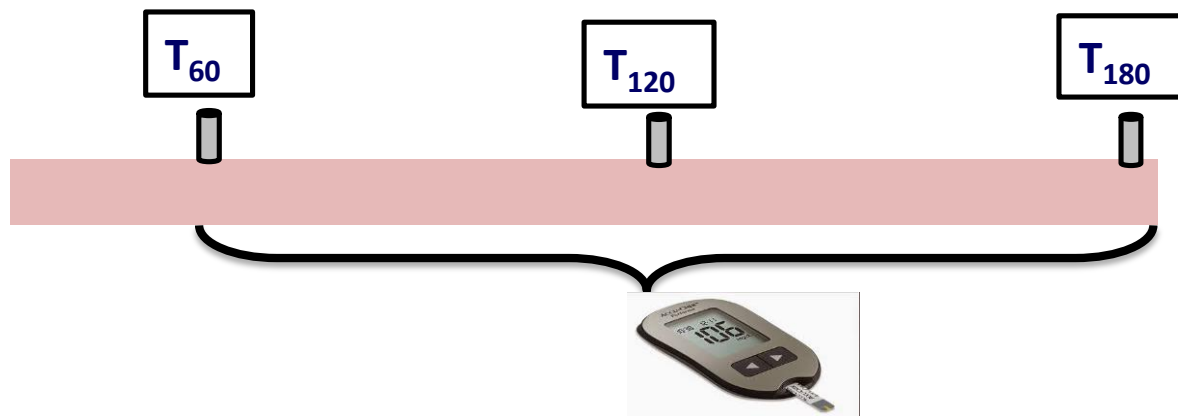
Control

HBSS

TE: 0.03 , 0.06 mg/100  $\mu$ L

Met: 0.01 mg/100  $\mu$ L

Insulin: 3.3UI/mL

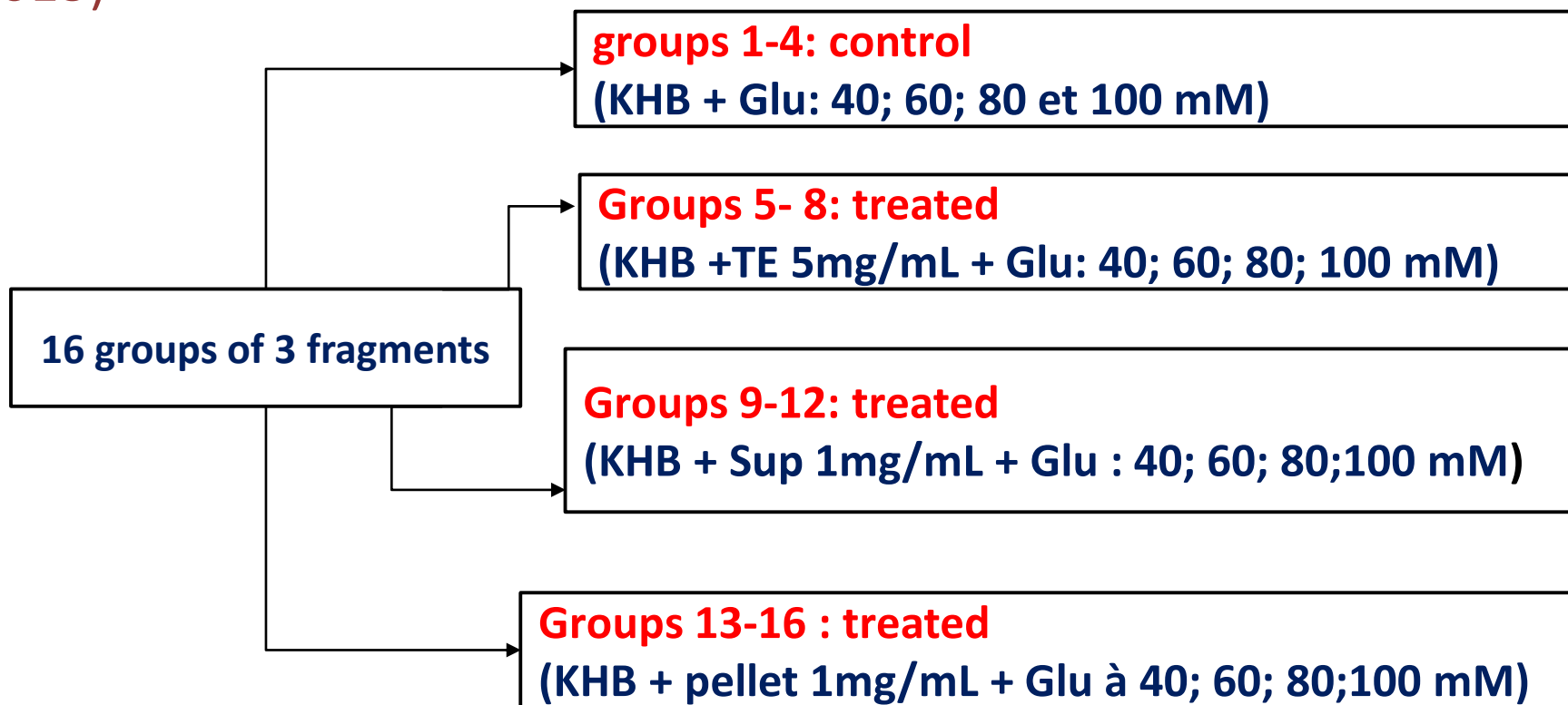


The 7th International Electronic Conference on Medicinal Chemistry

01-30 NOVEMBER 2021 | ONLINE

# Material and methods

Intestinal absorption of total extract and fractions of *A leiocarpus* in rat everted gut sac model (Halmilton and Butt, 2013)



## Material and methods

### Intestinal absorption of total extract and fractions of *Anogeissus leiocarpus* in rat everted gut sac model

After 60 minutes of incubation, the fragments were removed from the gut sac bath, and the serosal fluid was drained through a small incision into a test tube. Empty sacs were weighed. Glucose concentration in the serous and mucous fluids was measured using a GOD- PAP kit (method of Trinder). The standard range was made by glucose. The amount of glucose in the serosal compartment is treated as « release » and was calculated and expressed as mM/g tissue wet weight/h.

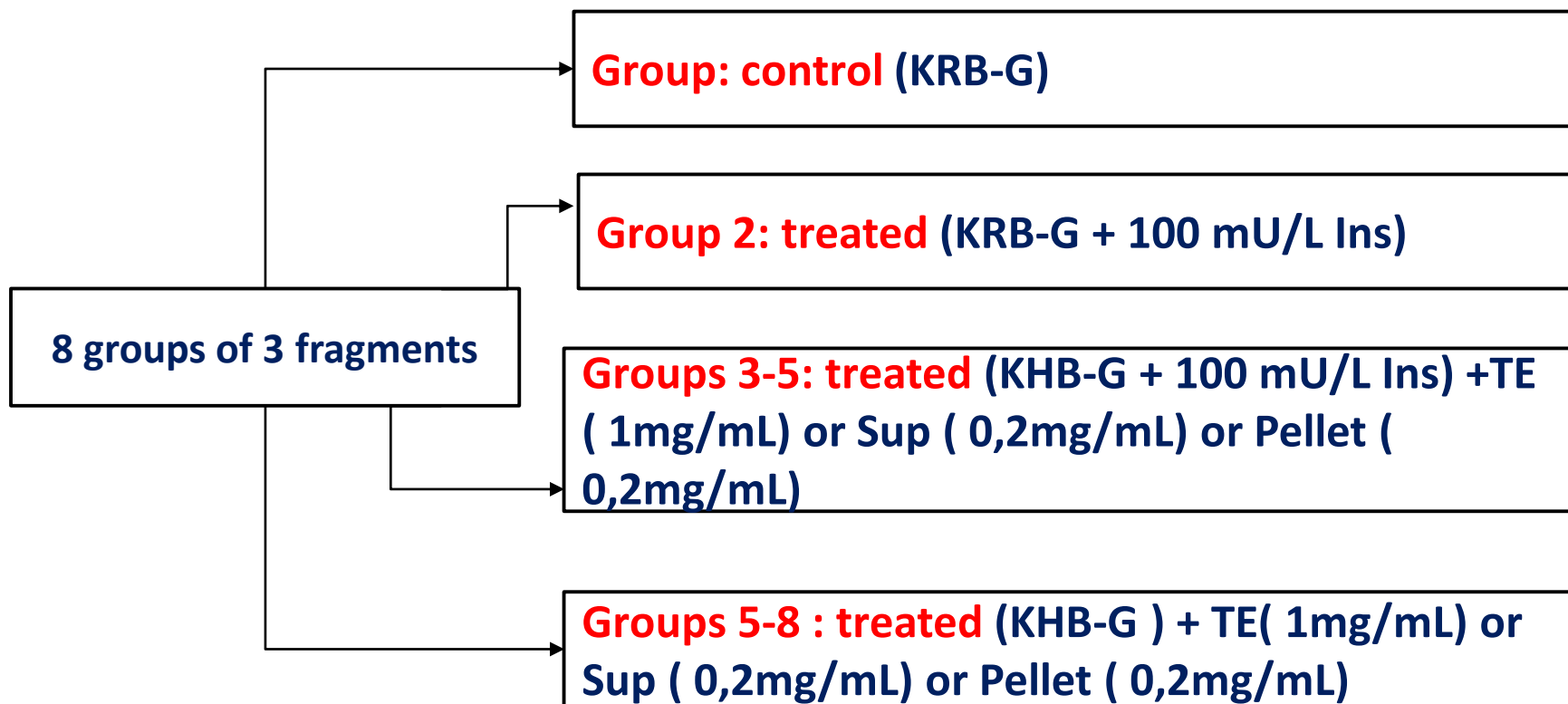


The 7th International Electronic Conference on Medicinal Chemistry

01-30 NOVEMBER 2021 | ONLINE

## Material and methods

Effect of the total extract and fractions on glucose uptake in rat isolated muscle tissue (Lawson-Evi et al.,2021)



## Material and methods

### Effect of the total extract and fractions on uptake glucose in rat isolated muscle tissue

The test tubes were incubated in aerated condition for 3 h. Glucose concentration were measured using a GOD-PAP KIT by the Trinder method of Aliquots of 10  $\mu$ L from each sample from incubation solution at 60, 120 and 180 min

The loss of glucose in the incubation solution is assumed to represent the glucose absorbed by the muscle.

Glucose absorbed (mM / g of fresh tissue)

= (Initial glucose - Final glucose) / 0.25; where 0.25 represents the fresh weight of the muscle tissue.





## Material and methods

### Statistical analysis

Results were expressed as mean  $\pm$  standard error of the mean (SEM). Statistical analysis was performed by two-way analysis of variance (ANOVA) followed by Dunnett's test to evaluate significant differences between groups. The level of significance was set at  $p < 0.05$  and statistical analysis were carried out using Graph Pad Prism 7.0.

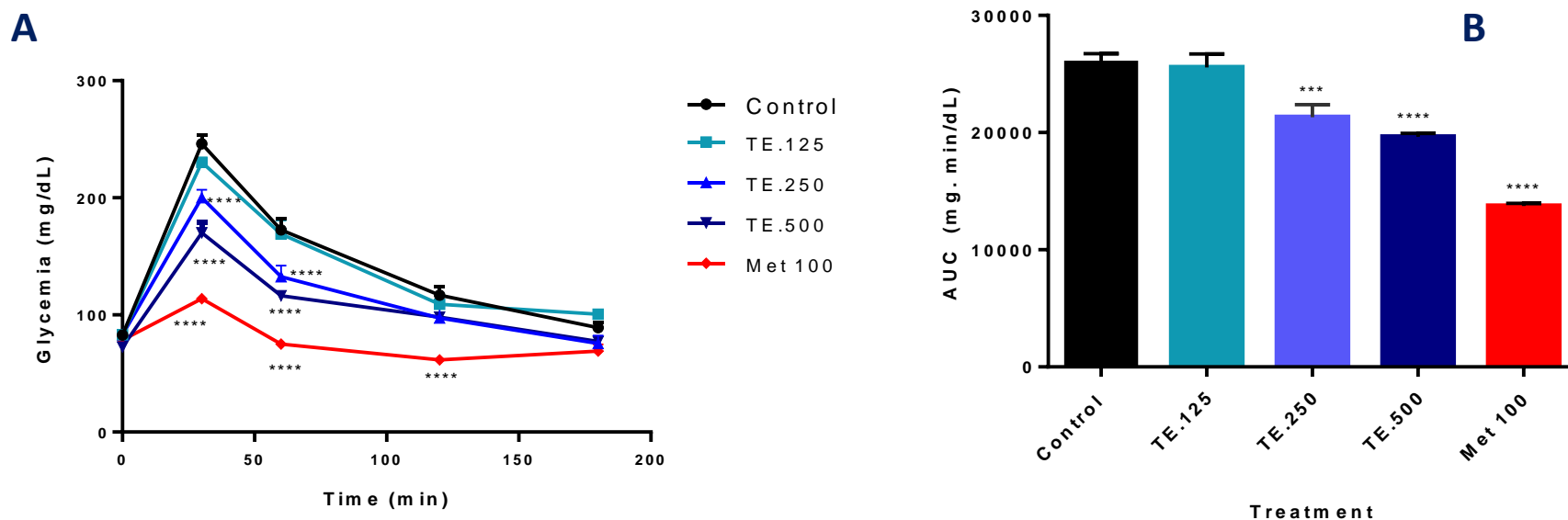


**The 7th International Electronic Conference on Medicinal Chemistry**

01-30 NOVEMBER 2021 | ONLINE

## Results and discussion

### Effect of total extract of *Anogeissus leiocarpus* on OGTT



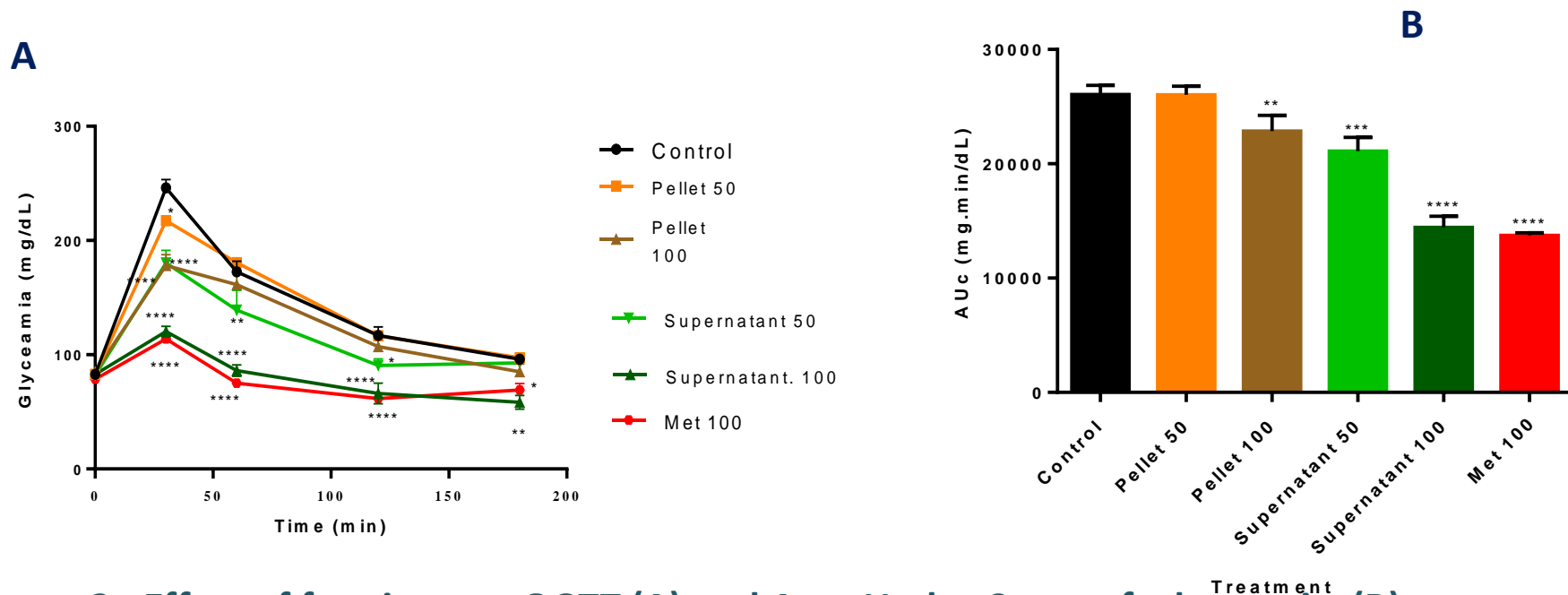
**Figure 1** : Effect of total extract on OGTT (A) and Area Under Curve of glycaemia (B)

M  $\pm$  ESM. \*  $p < 0.05$  ; \*\*  $p < 0.01$  ; \*\*\*  $p < 0.001$  ; \*\*\*\*  $p < 0,0001$  vs controls, N=7



# Results and discussion

## Effect of fractions of *Anogeissus leiocarpus* on OGTT



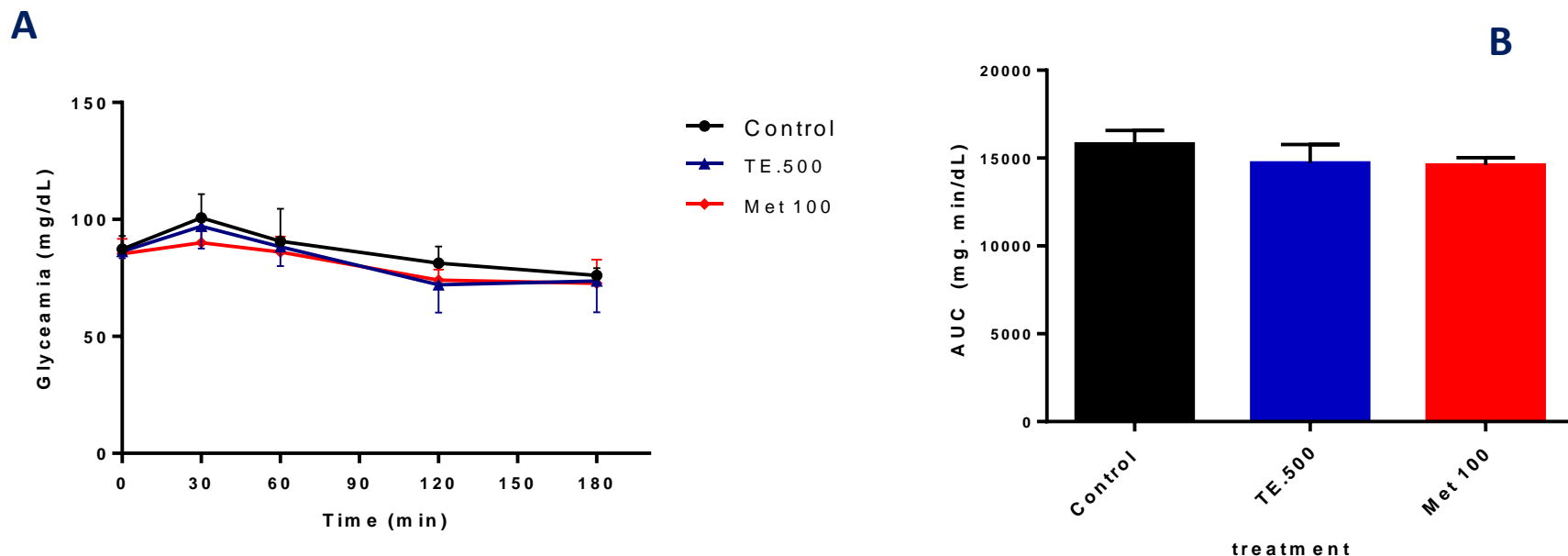
**Figure 2 : Effect of fractions on OGTT (A) and Area Under Curve of glyceamia (B)**

M ± ESM. \*  $p < 0.05$  ; \*\*  $p < 0.01$  ; \*\*\*  $p < 0.001$  ; \*\*\*\*  $p < 0,0001$  vs controls, N=7



## Results and discussion

### Effect of total extract of *Anogeissus leiocarpus* on normoglycemic mice



**Figure 3** : Effect of total extract on basal glycaemia(A) and Area Under Curve of glycaemia (B)

M ± ESM. \*  $p < 0.05$  vs controls, N=7



## Results and discussion

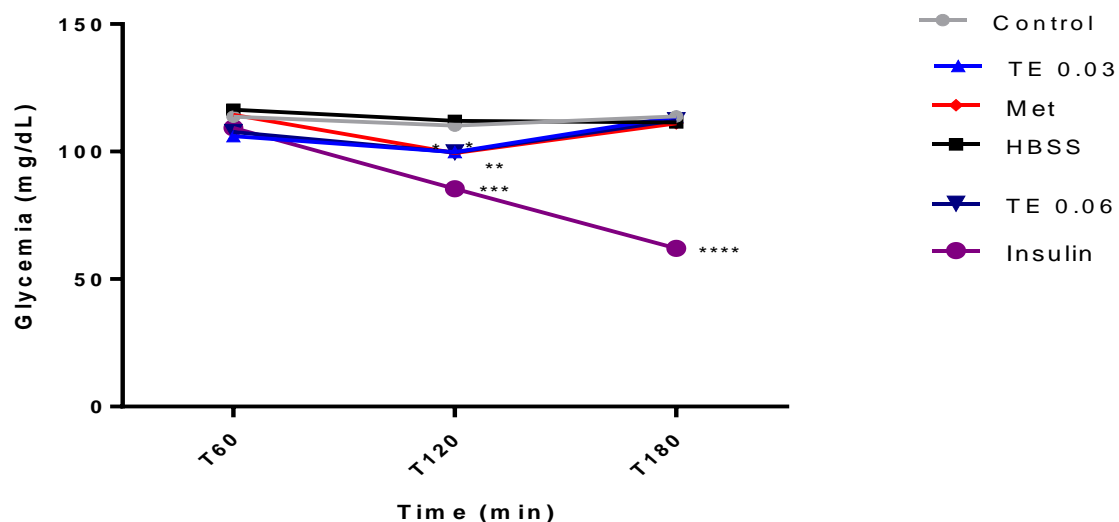
At 30 minutes after glucose overload, the total extract and all fractions (supernatant and pellet) significantly reduced hyperglycemia dose - dependent manner compared to controls. However, this reduction was more pronounced in mice treated with the supernatant fraction at  $100 \text{ mg.kg}^{-1}$ . This was confirmed by the area under the curve.

In our conditions, the extract at the  $500 \text{ mg.kg}^{-1}$  of the total extract in normoglycemic mice did not significantly decrease basal blood glucose levels compared to controls. As metformin, the extract would possess an antihyperglycemic activity, suggesting an extra-pancreatic mechanism of action.



## Results and discussion

### Effect of total extract on blood glucose level of the chicken embryos



**Figure 4 : Effect of total extract on glycaemia of chicken embryos**

M  $\pm$  ESM. \*  $p < 0,05$  ; \*\*  $p < 0,01$  ; \*\*\*  $p < 0,001$ ; \*\*\*\*  $p < 0,0001$  vs control, N=10



## Results and discussion

On day 11, serum insulin level in the chicken embryos is practically nonexistent. However, the embryo at this stage is sensitive to the effect of insulin because it has insulin receptors (De Pablo et al., 1991).

The results showed that the extract has decreased significantly the basal hyperglycemia of chicken embryos.

This could be explained by the fact that the extract as well as the metformin have transiently lowered the basal glycemia of the embryo, before the glucagon intervention. In addition, the extract may have allowed the expression of glucose transporters on the surface of cell membranes allowing the glucose uptake by the tissue (Salemi et al., 2016).



## Results and discussion

### Intestinal absorption of total extract and fractions of *Anogeissus leiocarpus* in rat everted gut sac model

**Table 1:** Effect of total extract and fractions of *Anogeissus leiocarpus* on the release of the varying concentrations of glucose by everted gut sacs

Glucose concentration (mM)	Released Glucose (mM.g <sup>-1</sup> tissue wet weight.h <sup>-1</sup> )			
	Control	Total Extract (5mg/ml)	Pellet (1mg/ml)	Supernatant (1mg/ml)
0.4	0.23 ± 0.01	0.24 ± 0.02	0.41 ± 0.04	0.36 ± 0.08
0.6	1.38 ± 0.01	0.92 ± 0.14**	0.69 ± 0.03***	0.53 ± 0.10***
0.8	1.52 ± 0.01	1.08 ± 0.13*	1.06 ± 0.11**	1.05 ± 0.02**
1	1.72 ± 0.02	1.39 ± 0.12	1.35 ± 0.18*	1.14 ± 0.18***





## Results and discussion

Severe postprandial hyperglycaemia is avoided if intestinal glucose absorption is inhibited.

*Ex vivo*, the significant reduction of released glucose in the reverse intestine observed, showed that the total extract and supernatant fraction would regulate glucose 's transport to the general circulation via GLUT 2 transporters

This effect was more potent with supernatant fraction than the total extract



**The 7th International Electronic Conference on Medicinal Chemistry**

01-30 NOVEMBER 2021 | ONLINE

## Results and discussion

### Effect of the total extract and fractions on glucose uptake in rat isolated muscle tissue

**Table 2: Effect of total extract and fractions on glucose absorption in skeletal muscle**

Groups	Absorbed Glucose (mM/g de tissu frais)		
	60 min	120 min	180 min
Glucosed Medium (GM)	3,6 ± 0,46	6,08 ± 0,71	10 ± 0,24
GM + Insulin (100mU/L)	8,6 ± 0,11	12,96 ± 0,43	17,02 ± 0,12
MGM + TE (1mg/mL)	<b>15,08 ± 0,24*</b>	<b>20,96 ± 0,87**</b>	<b>22,00 ± 0,11**</b>
GM + TE (1mg/mL) + Insulin (100mU/L)	<b>17,28 ± 0,10**</b>	<b>23,36 ± 0,29***</b>	<b>24,41 ± 0,29**</b>
GM + Supernatant (0.2mg/mL)	7,88 ± 0,17	10,08 ± 0,23	18,8 ± 0,83
GM + Supernatant (0.2mg/mL) + Ins (100mU/L)	12,27 ± 0,29	11,36 ± 0,17	<b>19,8 ± 0,17*</b>
GM + pellet (0.2mg/mL)	5,48 ± 0,24	7,88 ± 0,17	14,26 ± 0,17
GM + pellet (0.2mg/mL) + Ins (100mU/L)	0 ± 0	11,08 ± 0,24	14,56 ± 0,06



## Results and discussion

An increase of glucose uptake by the muscle was observed in groups treated with total extract and supernatant fraction. This effect was more pronounced in the presence of insulin.

This suggests that the extract would firstly improve the sensitivity of the muscle to the action of insulin by stimulating the glucose capture pathways and mechanisms (St-Amand, 2015).

In addition, it would mobilize the glucose transporters on the surface of the cell membrane in the absence of insulin through insulino-mimetic activity.



**The 7th International Electronic Conference on Medicinal Chemistry**

01-30 NOVEMBER 2021 | ONLINE

## Conclusion

The study has revealed the antihyperglycemic activity of the total hydroalcoholic extract, fractions of *A. leiocarpus* roots comparable to metformin

*In ovo* and *ex vivo* tests confirmed the extra pancreatic activity of the plant by enhancing the glucose uptake, and regulating glucose transport into the general circulation by the intestine

This justifies the traditional use of *Anogeissus leiocarpus* in the treatment of diabetes type 2.



The 7th International Electronic Conference on Medicinal Chemistry

01-30 NOVEMBER 2021 | ONLINE