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EXTRACTION OF FLAVONOIDS FROM *JATROPHA CURCAS* L. ROOTS AND EVALUATION OF HYPOGLYCEMIC ACTIVITY

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ABSTRACT

Introduction: *Jatropha curcas L.* (Euphorbiaceae) is a deciduous shrub containing polyphenols with a variety of biological properties used in traditional medicine. The present study focused on the extraction of flavonoids from the plant's roots and the evaluation of their hypoglycemic activity in Wistar rats. **Material and methods**: The flavonoids were obtained following the liquid-liquid extraction method by confronting solvents with increasing polarity. The phenolic compounds were quantified by spectrophotometric assay using gallic acid, quercetin and rutin as reference substances. The evaluation of the hypoglycemic activity was carried out by 02 methods: firstly, in vivo on rats (male and female) whose diabetes was induced using Alloxan, and secondly, in vitro by observing their effect against alpha-amylase and alpha-glucosidase. **Results:** Extraction yields were 8.51% for total dry extract, 2.55% for diethyl ether extract, 2.83% for ethyl acetate extract and 4.05% for n-butanol extract. Spectrophotometric assaying revealed high levels of total phenols (10.3375 mg Eq AG/g dry matter), total flavonoids (9.441 mg Eq Q/g dry matter), flavanols (3.025 mg Eq R / g dry matter), flavones and flavonols (2.853 mg Eq Q / g dry matter). In vivo, at a dose of 100mg/kg, the n-butanol extract had a more pronounced hypoglycemic effect on diabetic rats than glibenclamide on days 4 and 7 of treatment, with a cytoprotective effect on the pancreas and liver. In vitro, only the diethyl ether extract had a remarkable inhibitory effect on alpha-amylase activity compared with alpha-glucosidase. **Conclusion**: Our results show that flavonoid extracts are effective and safe in the oral treatment of diabetes in Wistar rats, justifying the use of *Jatropha curcas L.* in traditional medicine.

KEYWORDS: *Jatropha curcas L.*, extract, Flavonoids and hypoglycemic.

INTRODUCTION

Research into bioactive compounds of plant origin and their therapeutic potential has generated growing interest in the fields of pharmacology and natural medicine. Among these compounds, flavonoids, naturally present in many plants, have attracted attention due to their varied pharmacological properties, including antioxidant, anti-inflammatory and potentially hypoglycemic activity.[1] This is the case of *Jatropha curcas L.*, a deciduous shrub of the Euphorbiaceae family that is widespread in Cameroon's arid and semi-arid zones and is known for its richness in bioactive compounds, such as flavonoids[2], which have attracted particular investigation due to their pharmacological potential, notably in the field of blood sugar regulation.[3] According to the WHO, 80% of the African population rely on traditional medicine for their primary health needs.[4] Much research has focused on combating chronic hyperglycemia, which characterizes diabetes and is both a sign and an aggravating factor in insulin secretion disorders and insulin resistance[5], as well as a major primary risk factor for coronary heart disease.[6] Although chronic hyperglycemia is the common consequence of uncontrolled diabetes, care at the community level is influenced by its etiology[7], the high cost of conventional antidiabetic drugs, and the difficulty of accessibility. All these factors have oriented various research projects towards the development of new

compounds of therapeutic interest from local flora as an alternative in the management of diabetes. In Cameroon, type 2 diabetes is the most prevalent form and has progressed in parallel with sociocultural changes. An estimated 91% of adults affected by the disease have type 2 diabetes.[8] In view of these alarming figures, the World Health Organization has drawn up the WHO Global Diabetes Pact with the aim of accelerating progress in the fight against diabetes and other non-communicable diseases. Despite the measures put in place for the management of diabetes since the advent of Covid-19, we note that advances in research on secondary metabolites (phenolic compounds, flavonoids, etc.) and their potential beneficial effects on health raise some fundamental questions, among which the development of new molecules as potential candidates in the therapeutic arsenal remains a major challenge. With this in mind, the present study aims to evaluate the hypoglycemic activity of flavonoid extracts from the roots of *Jatropha curcas L.*, in order to contribute to the development of research already being carried out on this plant. The main aim of the study was to evaluate the hypoglycemic effect of flavonoid extracts from the roots of *Jatropha curcas L.*, firstly by in vivo methods on Wistar rats whose diabetes had been induced with alloxan, and secondly by in vitro methods on alpha-amylase and alpha-glucosidase activity.

MATERIALS AND METHODS

Plant material

Jatropha curcas L. roots were harvested in central Cameroon and identified at the Cameroon National Herbarium. The harvesting methods used were those of R. Schenll.[9]

Animal material

Rats (Rattus norvegicus) of the Wistar strain aged between 12 weeks (females) and 16 weeks (males) and weighing between 150 and 300 grams.[10]

1. Extraction procedure

After harvesting, *Jatropha curcas L.* roots were sorted, cleaned, dried at room temperature protected from UV rays and ground before being sieved. The powder obtained was subjected to an aqueous extraction to obtain a total dry aqueous extract. This extract was confronted with solvents of increasing polarity (diethyl ether, ethyl acetate and n-butanol) to obtain different flavonoid fractions. Extraction yields were determined according to the following formulas.

Total dry extract

The extraction yield (Rd) of the dry extract, expressed as a percentage, was calculated using the following formula:

$$Rd = \frac{\text{Mass of extract obtained}}{\text{Mass of initial powder}} X\ 100$$

Flavonoid fractions

The flavonoid extraction yield expressed as a percentage was calculated using the formula below.

$$Rd = \frac{\text{Mass fractions obtained}}{\text{Mass of extract obtained}}\ X\ 100$$

2. Quantitative analysis of extracts

Determination of reference compounds such as total phenols and total flavonoids was carried out on the total dry extract.

Total phenol content

The total phenol content of plant extracts was determined by a method adapted from Singleton and Ross using the Folin-Ciocalteu reagent.[11] The concentrations of total polyphenols contained in the fractions were calculated with reference to the calibration curve obtained using gallic acid as the standard. Results were expressed in mg gallic acid equivalent/g dry matter according to the following formula.

$$T_p = C \times \frac{V \times D}{P}$$

Tp: Total phenol content
C: concentration of polyphenols in gallic acid equivalent deduced from curve
V: extract volume
D: dilution factor
P : weight of dry matter

Total flavonoid content

The method used to estimate flavonoid content was that described by Ordonez.[12] Flavonoid concentrations in fractions were calculated with reference to the calibration curve obtained using quercetin as the standard. Results are expressed in mg reference substance equivalent/g dry matter.

Flavanols content

The method used to estimate flavanol content is that described by Kumaran and Karunakaran.[13] The concentration of flavanols in extracts was calculated by reference to the calibration curve obtained using Rutin as the standard. Results are expressed as mg rutin equivalent/g dry matter.

Flavone and flavonol content

The method used to estimate flavonol levels is that described by (Kosalek et al., 2004).[14] The concentration of flavones and flavonols was calculated with reference to the calibration curve obtained using quercetin as the standard. Results are expressed in mg quercetin equivalent/100 g dry matter.

3. Induction of diabetes

Induction of a persistent hyperglycemic state and insulin resistance was achieved by administration of Alloxan monohydrate at a single or repeated dose of 150mg/kg

body weight intraperitoneally. Rats were weighed, marked for individual identification and placed on a high-fat diet for 21 days prior to the start of the experiment. During this period, 05 days (Day 16) before the start of the experiment, they were kept in separate cages to allow individual acclimatization to laboratory conditions.[15] On Day 22, the animals were deprived of food but not water for 12 hours before administration of Alloxan. Alloxan monohydrate solution was prepared in 0.9% physiological water to a final concentration of 15mg/ml, under aseptic conditions to avoid contamination. The injection volume was 1mL/100g body weight. After injection, the animals were given glucose at 2g/kg body weight to avoid hypoglycemic shock following Alloxan injection. The high-fat diet was maintained. 72 h after intraperitoneal injection of Alloxan in fasting rats, diabetes was detected by measuring blood glucose with a glucometer. Rats with a baseline blood glucose level consistently greater than or equal to 200 mg /dl after 48 to72 hours were considered to have developed persistent hyperglycemia and were included in the experiment.

4. Evaluation of hypoglycemic activity in diabetic rats
Allocation of study batches
Diabetic rats were randomly divided into 06 different experimental batches of 10 rats each (05 males and 05 females)[16] including 03 control batches and 03 batches treated with the different flavonoid extracts at doses of 100mg/kg body weight.
Batch 1: Negative control, consisting of 10 non-diabetic rats receiving only distilled water at 10 mL/kg body weight (BW).
Batch 2: Positive control, consisting of 10 diabetic rats receiving only distilled water at 10 mL/kg body weight (BW).
Batch 3: Reference control, consisting of 10 diabetic rats treated with Glibenclamide at 10 mg/kg body weight (TR).
Batch 4: is a test batch of 10 diabetic rats, treated with diethyl ether flavonoid extract (E-Et2O).
Batch 5: is a test batch of 10 diabetic rats, treated with flavonoid extract with ethyl acetate (E-AcOEt).
Batch 6: a test batch of 10 diabetic rats, treated with flavonoid extract with n-butanol (E-ButOH).

The various substances (distilled water, glibenclamide, diethyl ether fractions, ethyl acetate and n-butanol) were administered via an orogastric tube.[17] During the handling period, the 06 batches were treated three times a day orally with the corresponding substances for each batch.

Variation in body weight and blood glucose levels
The weight of each rat in our different study batches, as well as blood glucose (using a One Touch Ultra strip glucose meter) were measured every 03 days at fixed times for 13 days (D0, D3, D6, D9 and D12), i.e. 05 measurements. The aim of the first blood glucose measurement was to check the onset of diabetes in batches 2, 3, 4, 5 and 6, using the mean baseline blood glucose of batch 1 (Negative Control) before the start of treatment as a normal reference. On day 14, at the same time, the rats were sacrificed, and samples were taken to determine relative organ weights and measure plasma biochemical parameters (glucose, total protein, hepatic transaminases, total lipids, urea and creatinine).

6. Measurement of the percentage inhibition of flavonoid extracts on the activity of some digestive enzymes (alpha-amylase and alpha-glucosidase)
The inhibitory activity of α-amylase was determined following the method of Zengin et al, (2014)[18] with modifications. Each experiment was performed 3 times and inhibition percentages were determined using the formula below.
Inhibition (%) = 1-[(Ac-Ae)-(As-Ab)/(Ac-Ae)]
- ✓ Ac=Absorbance of extracted blanks (all reagents without enzyme solution)
- ✓ Ae=Absorbance of negative control without inhibitor (all reagents without extract)
- ✓ As=Absorbance of reaction containing all reagents
- ✓ Ab=Absorbance of the maximum reaction between starch and iodine (Starch + IKI)

The inhibitory effect of flavonoid extracts was determined using the α-glucosidase enzyme inhibition method as described by Bisht et al. (2013) and Qian et al. (2015) with slight modification according to laboratory conditions.[19;20] The inhibitory activity of flavonoid extracts or acarbose was determined as percentage inhibition, which is expressed using the following formula.
% inhibition = (ΔA Negative Control - ΔA sample / ΔA Negative Control) x100.
ΔA Negative Control : The slope of the tangent of the Negative Control Absorbance.
ΔA sample : The slope of the sample absorbance tangent.[21]

RESULTS
The extraction yield was respectively 2.55% of diethyl ether extract; 2.83% ethyl acetate extract and 4.05% n-butanol extract.

1. Phenolic compound contents
The contents of total phenols, total flavonoids, flavanols, flavones and flavonols in the total dry extract of *Jatropha curcas L.* roots were determined from the linear regression equations of the curves for each corresponding standard. Each measurement represents the mean (n = 5) ± standard deviation.

Table 1: Phenolic compound contents.

Parameter	Standard	Length wavelength (nm)	Content in mg standard eq/ g dry matter
Total Phenols	Acide gallique (AG)	765	10,3375 ± 0,85
Total Flavonoid	Quercétine (Q)	430	9,441 ± 0,75
Total Flavanols	Rutine (R)	440	3,025 ± 0,15
Total Flavones and flavonols	Quercétine (Q)	415	2,853 ± 0,09

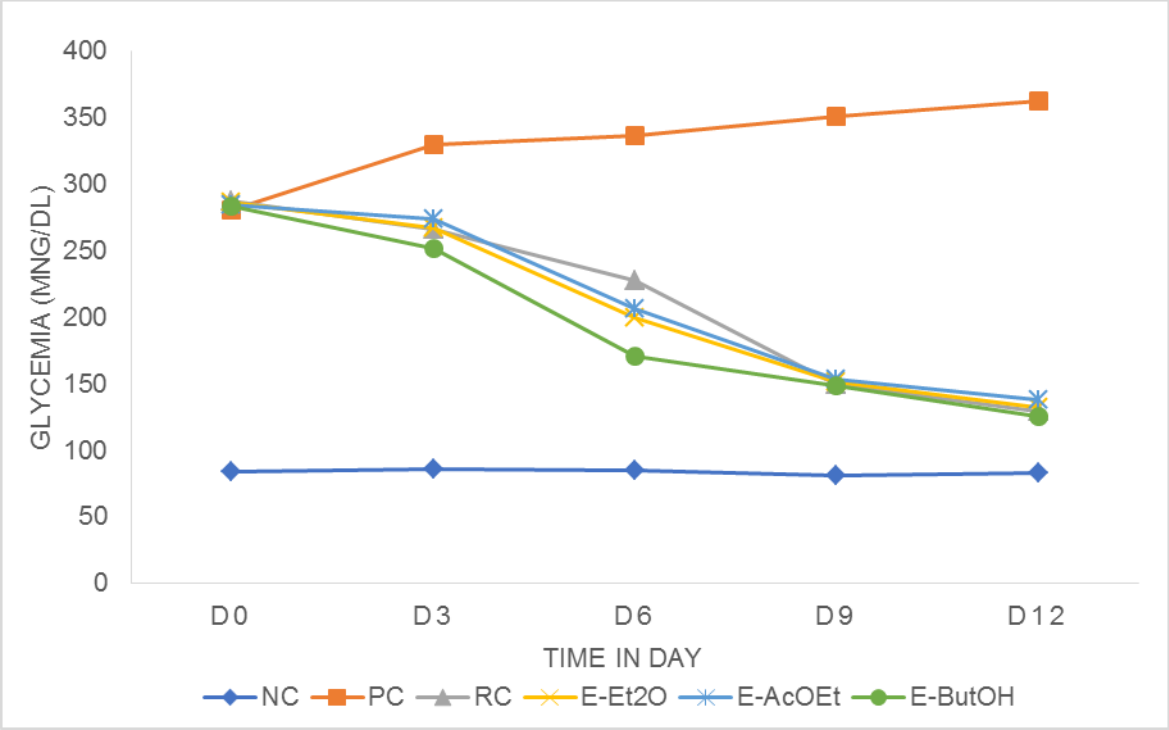
2. Hypoglycemic activity on diabetic rats (Alloxan induction)

The results of blood glucose measurements every 3 days from D0 to D12 are shown in Table 2 and Figure 1 below.

Tableau II : variation in fasting blood glucose before sacrifice in diabetic rats

Batchs		Glycemia (mg/dl)				
		Day 0	Day 3	Day 6	Day 9	Day 12
1. NC	10 ml/kg	83,8 ± 2,89	85,3 ± 2,21	84,4 ± 2 ;71	81,1 ± 3,66	82,7 ± 3,33
2.PC	10ml/kg	280,4 ± 9,61	330 ± 13,35	336,5 ± 14,86	350,6 ± 14,90	362,3 ± 13,38
3.RC	10 mg/kg	287 ± 1,24	266,5 ± 4,79*	227,1 ± 7,50*	149,8 ± 4,56*	129,3 ± 8,79*
4.E-Et2O	100 mg/kg	286,6 ± 5,31	266,6 ± 4,88*	199,9 ± 5,54*#	151,6 ± 2,36*	132,4 ± 5,62*
5.E-AcOEt	100 mg/kg	284 ± 2,90	273,6 ± 4,94*	206,1 ± 6,41*#	153,3 ± 4,85*	137,4 ± 1,57*
6.E-ButOH	100 mg/kg	283,8 ± 2,89	251,3 ± 7,87*#	170,8 ± 7,56*#	148,6 ± 3,59*	125,3 ± 4,85*

Data are presented as mean ± standard deviation (SD) and comparisons are made against the control: * significant difference at $p<0.05$ compared with the positive control (PC) and # significant difference at $p<0.05$ compared with the reference control (RC).



Legendre : D0….D12 : Day 0….Day 12; NC : Negative Control; TP : Positive Control ; TR : Reference Control ; E-Et2o : Extract diethyl ether ; E-AcOET : extract ethyl acetate ; E-ButOH : extract n-butanol.

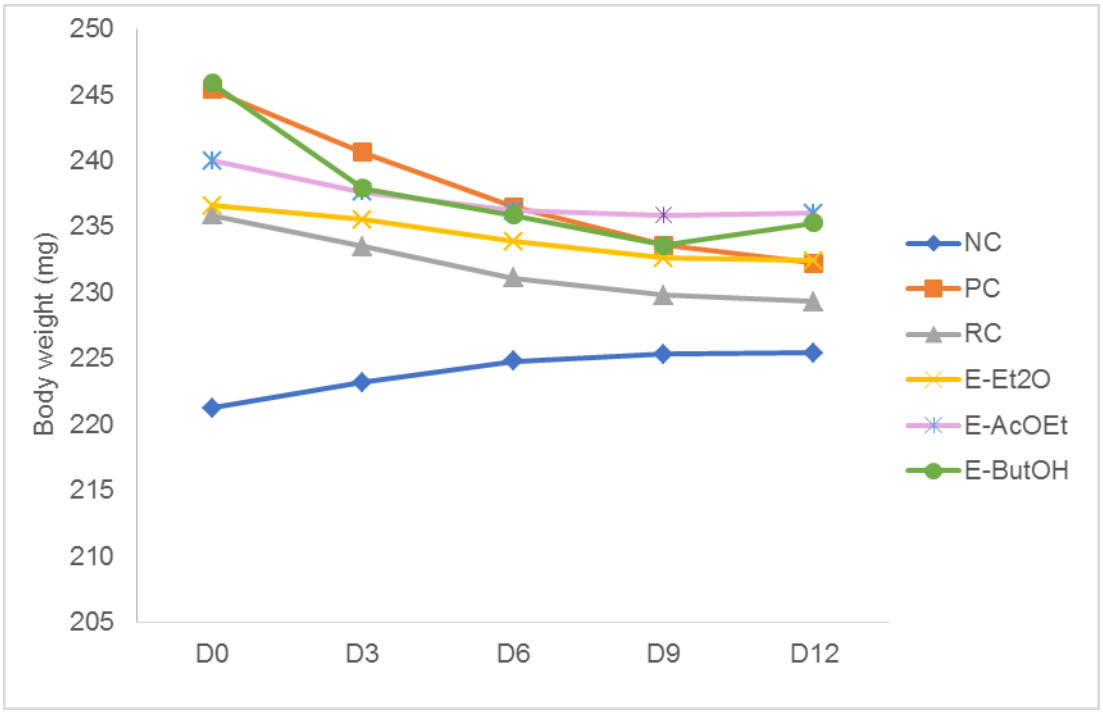
Figure 1: Variation in fasting blood glucose levels in diabetic rats during treatment.

A significant decrease ($p \leq 0.05$) in blood glucose levels was observed for flavonoid extracts with n-butanol at a dose of 100mg more pronounced than glibenclamide at D3 and D6 after the start of treatment.

3. Variation in body weight

Body weights

The 03 extracts at doses of 100mg/kg PC resulted in a slight decrease in body weight in rats from the start of treatment to D9.

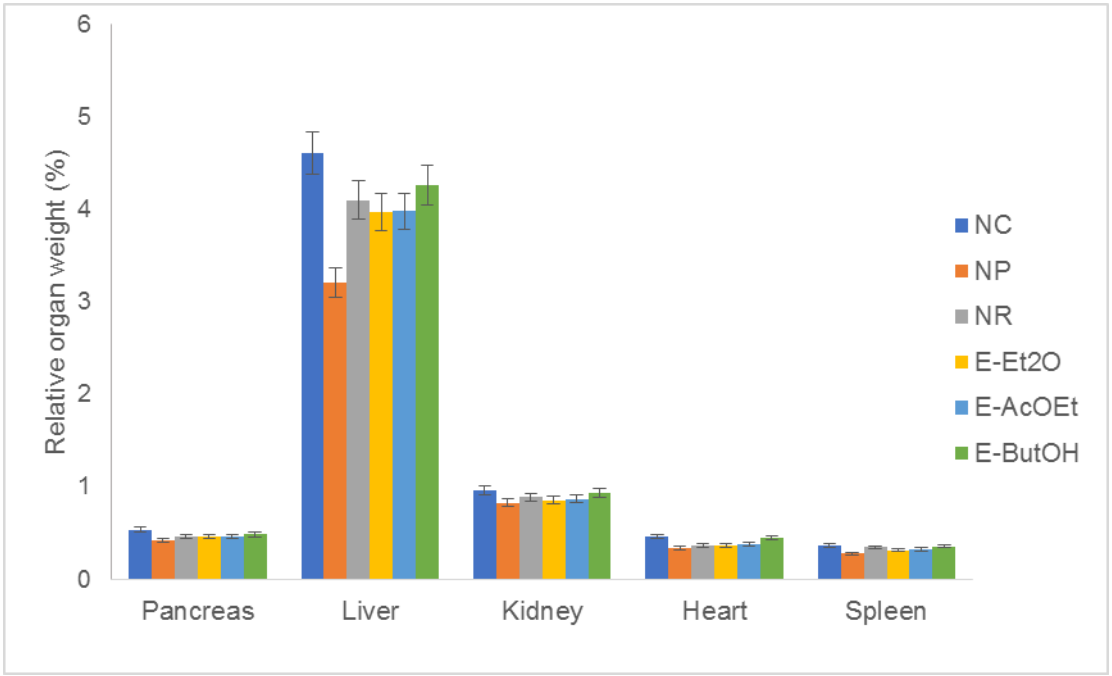


Legendre : D0….D12 : Day 0….Day 12; NC : Negative Control; TP : Positive Control ; TR : Reference Control ; E-Et2o : Extract diethyl ether ; E-AcOET : extract ethyl acetate ; E-ButOH : extract n-butanol.

Figure 2: variation in body weight of rats during treatment.

Relative organ weights

After sacrificing the rats, it was observed that the 03 flavonoid extracts at doses of 100 mg/kg PC caused a slight reduction in the weight of the pancreas, liver, kidneys, heart and spleen compared with non-diabetic rats. However, a significant reduction in relative liver weight was observed in untreated diabetic rats.



Legendre : NC : Negative Control ; TP : Positive Control ; TR : Reference Control ; E-Et2o : Extract diethyl ether ; E-AcOET : extract ethyl acetate ; E-ButOH : extract n-butanol.

Figure 3: Relative weight of rat organs from the different experimental batches.

4. Effect of Jatropha curcas flavonoid extracts on blood biochemical parameters of diabetic rats

Legendre : NC : Negative Control ; TP : Positive Control ; TR : Reference Control ; E-Et2o : Extract diethyl ether ; E-AcOET : extract ethyl acetate ; E-ButOH : extract n-butanol.

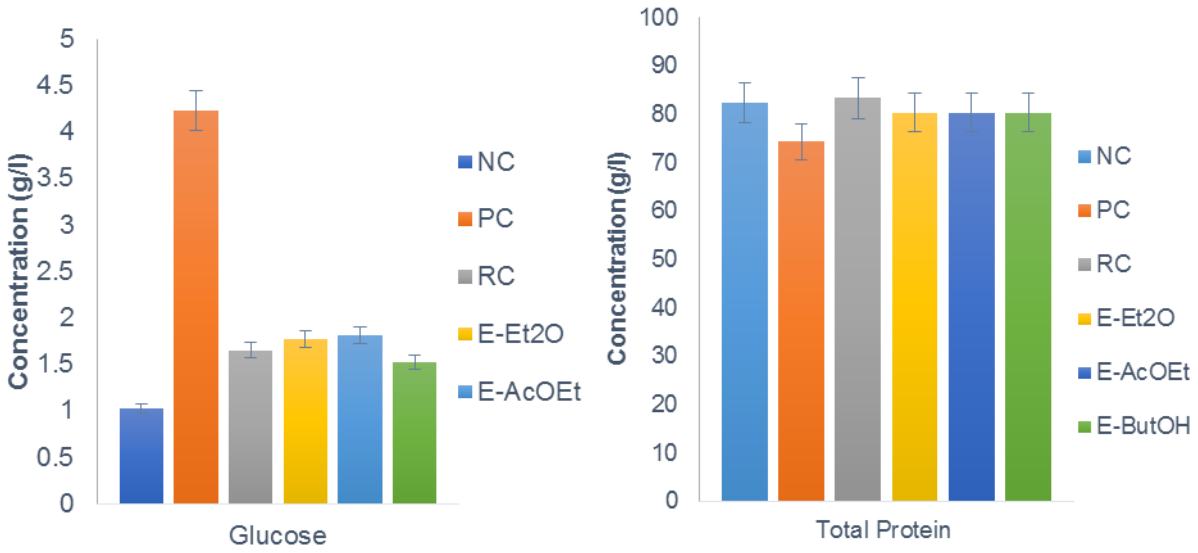


Figure 4: Serum glucose and total protein concentration of rats from different study batches.

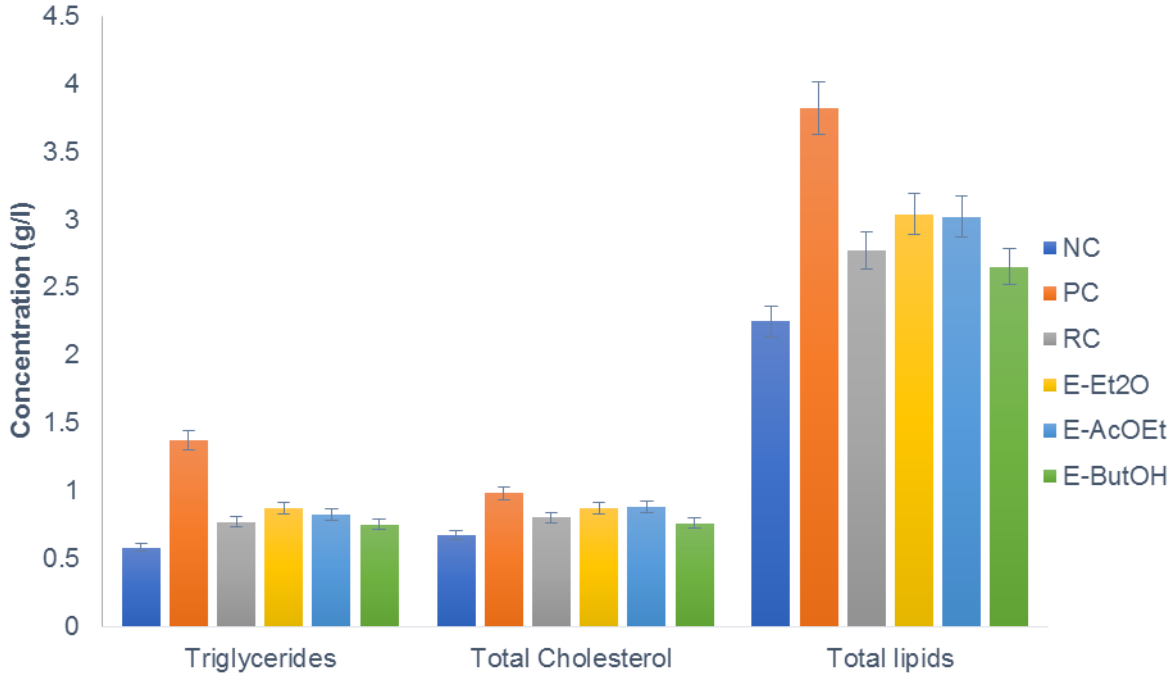


Figure 5: Lipid profile of rats from different study batches.

Creatinine and urea concentrations are used as markers of renal function. One reflects glomerular filtration rate and the other reflects diuresis, dietary nitrogen intake and endogenous protein catabolism. The increase in their concentrations to above-normal levels in the blood suggests renal toxicity. Our 03 extracts at a dose of 100 mg/kg body weight produced a slight increase in these levels compared with the negative control and a non-negligible decrease compared with the positive control.

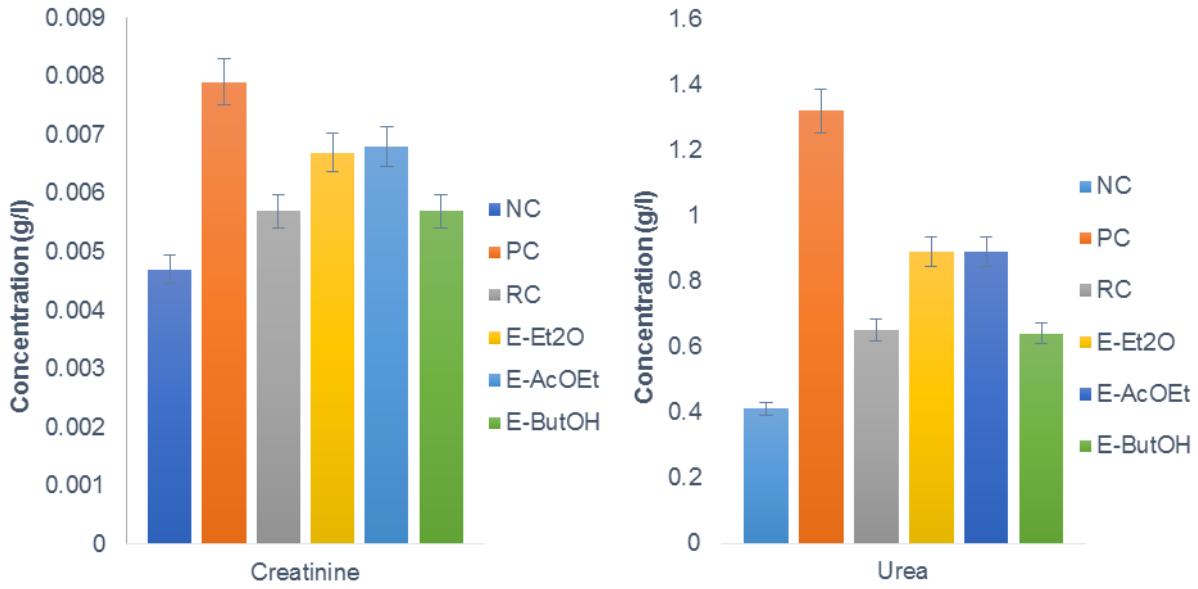
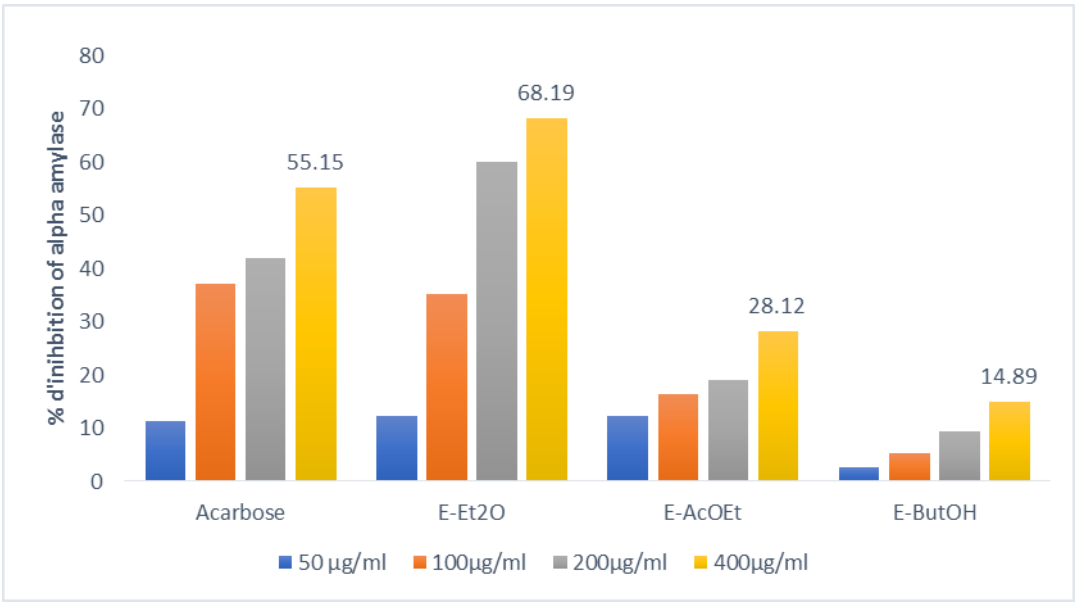


Figure 6: Renal profile of rats from different study batches.

5. Results of Alpha-amylase and Alpha-glucosidase inhibition tests

Alpha-amylase inhibition

After analysis, the inhibition rate decreased with the polarity of the solvents. The diethyl ether extract showed a high inhibition percentage of 68.19±4.01% at the 400 µg/ml concentration. This was slightly higher than that of acarbose (55.15±0.89%) at the same concentration. These observations can be explained by the fact that substances with hydrophobic potential have considerable inhibitory effects than hydrophilic substances, thus explaining their high activity. It can be deduced that flavonoid extracts with diethyl ether show significant inhibition of α-amylase.

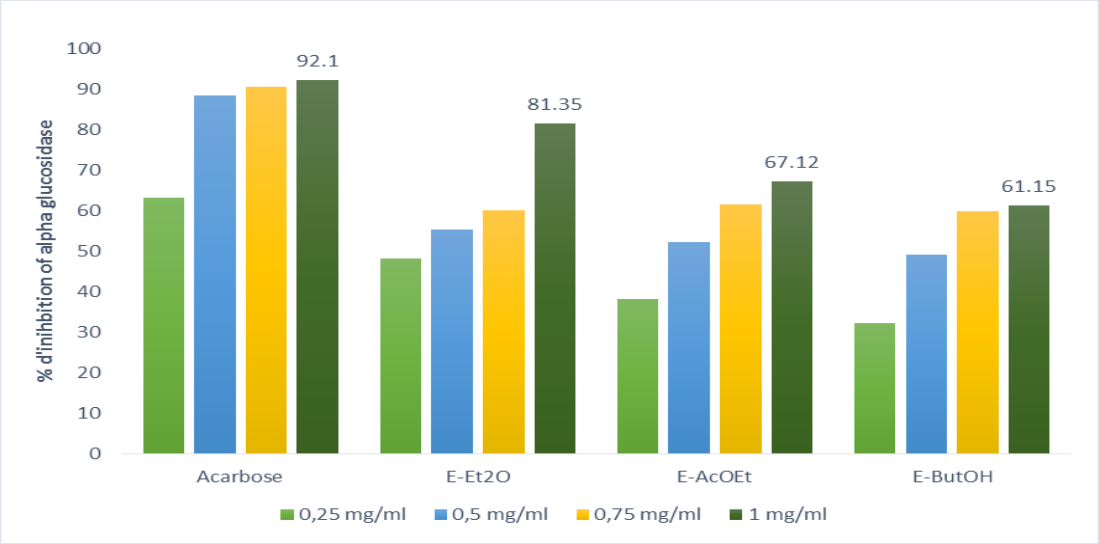


Legendre : Acarbose ; E-Et2o : Extract diethyl ether ; E-AcOET : extract ethyl acetate ; E-ButOH : extract n-butanol.

Figure 7: Inhibition of alpha-amylase activity by flavonoid extracts at different concentrations.

Alpha-glucosidase inhibition

Analysis of the inhibition percentages (%) showed that the rate of inhibition of the extracts increased with concentration. It was more marked for the diethyl ether extract than for the other two extracts. Despite this, the inhibition percentage remained lower than that of acarbose. Consequently, we deduce that the extracts have a significant inhibitory effect on the enzymatic activity of α-glucosidase, but it is no more marked than that of acarbose.



Legendre : Acarbose ; E-Et2o : Extract diethyl ether ; E-AcOET : extract ethyl acetate ; E-ButOH : extract n-butanol.

Figure 8: Inhibition of alpha-glucosidase activity by flavonoid extracts at different concentrations.

DISCUSSION

Flavonoid extracts with n-butanol have a hypoglycemic activity on diabetic rats that is more pronounced than glibenclamide from the 4th day until the 7th day after the start of treatment. This can be explained by the fact that the n-butanol fractions contain a large majority of polar compounds from this family, the literature of which mentions their strong hypoglycemic power.

During the study period, the batches of rats having received our different extracts showed a clear reduction in body weight compared to the negative control batch from D0 to D9. From day 12 we observed a gain in body weight but which remained statistically insignificant ($p \leq 0.05$). This can be explained by the increase in the level of free cholesterol in the blood.

A statistically insignificant reduction in relative weight ($p \leq 0.05$) of the pancreas for the study batches having received our extracts compared to the positive control batch. This suggests that untreated diabetic rats may have developed insulinopenia which would lead to progressive atrophy of the pancreas when untreated.

No liver damage was observed in any of the batches subjected to the extracts studied. This suggests that the extracts may have a cytoprotective effect on the liver. A significant drop in plasma glucose was observed in diabetic rats treated with our 03 extracts, compared to the positive control batch, unlike the protein level which almost did not vary. An increase in triglyceridemia, cholesterolemia and total lipids was observed in diabetic rats having received flavonoid extracts of *Jatropha curcas L.* at a dose of 100 mg/kg body weight. It was very significant for untreated diabetic rats. This increase may be due to a lack of inhibition of triacylglycerol lipase responsible for the release of fatty acids contained in tissues. Thus we can suspect a probable deficiency and/or inaction of insulin which, by increasing the concentration of circulating free fatty acids, increases its β-oxidation, thus producing free cholesterol in diabetic rats.

The diethyl ether extract showed a high inhibition percentage of 81.35±1.25% at the concentration 1mg/ml. This was lower than that exerted by acarbose (92.10±0.74%) at the same concentration. Acarbose is a commercially known α-glucosidase inhibitor, BISHT et al., (2013) note that acarbose has an alpha-glucosidase inhibition potential of 81.9% at a concentration of 4 mg/ml.[19] However, WANG et al. (2010) report a maximum activity of 100% at a concentration of 5 mg/ml.[22]

CONCLUSION

This study showed that flavonoid extracts at doses of 100 mg/kg have a hypoglycemic activity on diabetic rats that is more pronounced than glibenclamide from the 4th to the 7th day of treatment. They improve the lipid balance of rats without causing weight gain and seem to have a cytoprotective effect on the pancreas and liver affected by the toxic effect of alloxan. The quantitative estimation of phenolic compounds showed a high content of total polyphenols (10.3375 mg Eq AG/g DM), total flavonoids (9.441 mg Eq Q/g DM), Flavanols (3.025 mg Eq R/g DM), Flavones and Flavonols (2.853 mg Eq Q / g DM). As for their inhibitory effect on alpha-amylase and alpha glucosidase, we observe that diethyl ether extracts exert a remarkable inhibitory effect on the enzymatic activity of these enzymes.

All these results justify the use of the *Jatropha curcas L.* plant in traditional medicine in the treatment of diabetes, because it is effective and safe orally in laboratory rats.

CONFLICTS OF INTEREST

None.

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REFERENCES

1. Békro YA., Boua BB., Bi FHT., Ehile EE., Etude ethnobotanique et screening phytochimique de Caesalpinia benthamiana (Baill.) Herend. Et Zarucchi (Caesalpiniaceae). Sciences et Nature, 2007; 4: 217.
2. Adamou Issa1 et al. Effets du type de fertilisation sur la productivité du Jatropha curcas en zone des savanes d'Afrique Centrale cas de la localité de Sanguéré Paul au Nord Cameroun. 2016. Journal of Applied Biosciences, 99: 9433–9440.
3. Wu J, Liu Y, Tang L, Zhang F and Chen F (2011) A study on structural features in early flower development of Jatropha curcas L. and the classification of its inflorescences. African Journal of Agriculture Research, 6: 275-284.
4. Organisation Mondiale de la Santé. Réglementation des médicaments à base de plantes. La situation du monde. Genève, 1998.
5. Organisation mondiale de la santé. Rapport mondiale sur le diabète. Première édition. Genève. OMS 2016. [consulté le 13 novembre 2023]. 88 p. Disponible:http:// www.who.int/diabetes/global-report/fr/
6. E.M. Grundy. Les maladies cardiovasculaires, 2004; 7-16.
7. Azzi R. Contribution à l'étude des plantes médicinales utilisées dans le traitement traditionnel du diabète sucré dans l'Ouest algérien : enquête ethno-pharmacologique; analyse pharmaco-toxicologique de Figuier (Ficus carica) et de coloquinte (Citrullus colocynthis) chez le rat wistar. [Thèse de doctorat]. Béchar: Université Abou Bekr Belkaid, 2013; p.5].
8. Fédération internationale du diabète. Atlas du diabète. 10e édition. Bruxelles. FID.2021. https://diabetesatlas.org/
9. R. Shnell., Techniques d'herborisation et de conservation des plantes dans les pays tropicaux, Journal d'agriculture traditionnelle et de botanique appliquée Année, 1960; 7-1-3 pp.1-48).
10. Barker T.H., Howartha G.S. et Whittaker A.L. (2016) The effects of metabolic cage housing and sex on cognitive bias expression in rats. Applied Animal Behaviour Science, 177: 70-76.
11. Singleton V.L, Ortofer R, Lamuela-Raventos R.M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin Ciocalteu reagent. Methods in Enzymology. Orlando. Academic Press, 1999; 152-178.
12. Ordonez A, Gomez J, Vattuone M, Isla M. Antioxydant activities of Sechiumedule (Jacq.) Swartz extracts. Food chemistry, 2003; 97: 452-458.
13. Karunakaran R., et Kumaran A. "In vitro antioxidant activities of phenolic phytochemicals from five phyllanthus species from india. Lwt, 2007; 81: 321-326.
14. Kosalec I., Bakmaz M., Pepeljnjak S. and Vladimir-Knez EICS. Quantitative analysis of the flavonoids in raw propolis from northern Croatia. Acta Pharm, 2004; 54: 65-72.
15. OCDE, 2001. Guideline for testing of chemicals. Guideance N° 423.
16. Chichonyi Kalungia A., Mataka M., Kaonga P., Gono Bwalya A., Prashar L., Munkombwe D. 2018. Opuntia stricta Cladode Extract Reduces Blood Glucose Levels in Alloxan-induced Diabetic Mice. International Journal of Diabetes Research, 7(1): 1-11.
17. Jouad H., Lemhadri A., Maghrani M., Burcelin R., Eddouks M. 2003. Hawthorn evokes a potent antihyperglycemic capacity in streptozotocin induced diabetic rat. Journal of Herbal Pharmacotherapy, 3: 19-23.
18. Zengin, G., Sarikurkcu, C., Aktumsek, A., Ceylan, R., & Ceylan, O. (2014). A comprehensive study on phytochemical characterization of Haplophyllum myrtifolium Boiss. endemic to Turkey et its inhibitory potential against key enzymes involved in Alzheimer, skin diseases et type II diabetes. Industrial Crops et Products, 53: 244-251.
19. BISHT S., KANT R et KUMAR V., 2013. α-d-Glucosidase inhibitory activity of polysaccharide isolated from Acacia tortilis gum exudate. International Journal of Biological Macromolecules, 59: 214–220.
20. QIAN J-Y., BAI Y-Y., TANG J., CHEN W., 2015. Antioxidation and a-glucosidase inhibitory activities of barley polysaccharides modified with sulfation, LWT- Food Science and Technology, 64: 104-111.
21. SULISTIYANI, MEGA S., YOANA P., 2016. Inhibition of α-glucosidase activity by ethanolic extract of Melia azedarach L. leaves, IOP Publishing, Series: Earth and Environmental Science, 31: 012025.
22. WANG Y., YANG Z et WEI X., 2010. Sugar compositions, α-glucosidase inhibitory and amylase inhibitory activities of polysaccharides from leaves and flowers of Camellia sinensis obtained by different extraction methods. International Journal of Biological Macromolecules, 47: 534–539.