Journal of Applied Biosciences 186: 19733- 19746
ISSN 1997-5902

In vitro antidiabetic potential of two formulated powders of some nutraceutical plants of Cameroon

Bebbe Fadimatou [1*], Doumta Charles Falang [2*], Eyenga Mougnol Jean Sylvain[1], Kuagny Mouafo Blaise Ronald[1], Mbassi Manga Gilbert Ghislain[1]
[1]Department of Biochemistry, University of Yaounde I, Yaounde, Cameroon
[2]Department of Biomedical Sciences, Faculty of Health Sciences (FHS), University of Buea, Buea, Cameroun
Email address: bebbefa@yahoo.fr (Bebbe Fadimatou), cfdoumta@yahoo.fr (Doumta Charles Falang), eyengasylvain5@gmail.com (Eyenga Mougnol Jean Sylvain), kuagnymouaforonaldblaise@yahoo.fr (Kuagny Mouafo Blaise Ronald), mbassigilbert@gmail.com (Mbassi Manga Gilbert Ghislain).
[*]Corresponding author email: bebbefa@yahoo.fr

Submission 31st May 2023. Published online at https://www.m.elewa.org/Journals/ on 31st July 2023. **https://doi.org/10.35759/JABs.187.6**

ABSTRACT

Objective: Many modes of action have been explored in the fight against type 2 diabetes, including the use of drugs. But these drugs, in addition to their relatively high cost, are not without side effects. As alternative to these difficulties, traditional pharmacopoeia use several nutraceutic plants in the treatment of type 2 diabetes, including: *Vernonia amygdalina (Bitter leaf), Tetrapleura tetraptera (Aidon tree), Leptadenia lancifolia* decne (*Sokotoro* in north Cameroun) and Gum Arabic (*Acacia Senegal sap*). The fact that these plants are used for the same treatment means that they contain bioactive contents, which can be different according to plant.

Methodology and Results: For this reason, production of formulated powders (JE1 and JE2) of these plants with good anti-diabetic effect can help in the treatment. To check the efficacy of formulated powders a study of antidiabetic activity of two formulated powders was assessed. Formulation of powders, comparative analyses of bioactive compounds of different formulated powders obtained and antidiabetic activities were done by using classical methods. The results revealed some difference in the phytochemical contents of the both formulated powders. JE1 and JE2 possess strong hypoglycaemic and anti-hyperglycaemic activity but in general JE1 has the better antidiabetic activity compared to JE2. The acute toxicity study reveals that the LD50 is greater than 2000 mg/kg.

Conclusion and Application of results: Indeed the results reveal that JE1 and JE2 have an impact on the control of glycaemia on patient suffering of type 2 diabetes. This explains why *Vernonia amygdalina, Tetrapleura tetraptera, Leptadenia lancifolia* decne and Gum Arabic (*Acacia Senegal sap*) are used in the traditional pharmacopoeia for treatment of type 2 diabetes and hence the consumption of those plants needs to be encouraged.

Key words: Nutraceutical plants, formulated powders, phytochemical contents, antidiabetic activities, treatment of type 2 diabetes.

INTRODUCTION

Type 2 diabetes is a chronic disease that occurs when a person's blood sugar level is high because their body cannot effectively use the insulin it produces. It usually affects people aged 20-79 years and accounts for about 90% of diabetes cases worldwide. Type 2 diabetes is the fifth leading cause of death in the world raising the alarm and classifying it as a public health problem (WHO, 2023). This problem needs a specific management, which consists of lifestyle changes followed by pharmacological treatment including insulin if necessary (Williams, 2019). Many modes of action have been explored to fight against type 2 diabetes including blocking the potassium-dependent ATP pump in pancreatic β-cells (Sulfonylureas-Glipizide); stimulation of Peroxisome Proliferator-Activated Receptor-γ (Thiazolidinediones-Rosiglitazone); stimula tion of adenosine mono-phosphate-activated protein kinase (Biguanides-Metformin) and modulation of Glucagon Like Peptide-1 activity (Incretins-Exematide). These agents act either by stimulating insulin secretion by β-pancreatic cells (sulphonamides), or by decreasing hepatic glucose production (metformin) or the reduction of post prandial blood glucose by inhibiting the activity of intestinal enzymes (α-amylases and α-glucosidases) (Williams, 2019). However, the drugs with its relatively high cost are not without side effects (fatal lactic acidosis, buformin and penformin, nausea, vomiting and diarrhoea (metformin), visual disturbances, upper respiratory infection, sinusitis and weight gain); as a result of this, many of them in the USA/Europe, have limited uses, are not marketed, have almost restricted prescribing and are sometimes even withdrawn from the market (ADA-EASD, 2012). It has been reported that only 3 out of 20 patients are able to buy prescribed drugs in hospitals and only 1 out of every 1000 patients is able to consult a specialist (Kuete & Efferth, 2010). Because of this, there is a rich tradition in the use of herbal medicines for the treatment of several ailments and plans are on the way to integrate traditional medicine in the health care system even though the plans have not been put into action yet (Nkongmeneck *et al.,* 2007). Cameroon however has a rich biodiversity, with ~8,620 plant species (Earth Trends, 2003; Mbatchou, 2004), some of which are commonly used in the treatment of several chronic diseases and are ranged of neglected tropical diseases including malaria, trypanosomiasis, leishmaniasis, diabetes, tuberculosis, etc. (Kuete, 2010). As an alternative to these difficulties, Cameroonians are using nutraceutical foods, which are ordinary foods that have components or ingredients incorporated in them to give a specific medicinal or physiological benefit other than a purely nutritional effect (Effoe *et al*., 2020). The economic production and availability of nutraceutical foods are highly desirable objective to improve the health of people in the country especially that of the poor. Now, the nutraceuticals related research for improving its quality and quantity is an important area for ongoing biotechnological investigations (Bickford *et al*., 2012). Moreover, the Covid 19 pandemic has proven that in Africa and especially in Cameroon, due to the strong ethnobotanical potential, it is possible to overcome many diseases such as type 2 diabetes. In the traditional pharmacopoeia, several nutraceutic plants are used in the treatment of type 2 diabetes, including *Vernonia amygdalina (Bitter leaf), Tetrapleura tetraptera (Aidon tree), Leptadenia lancifolia* decne (*Sokotoro* in north Cameroun) and Gum Arabic (*Acacia Senegal sap*). (Effoe *et al*., 2020). Since nutraceuticals or functional foods can be classified based on their natural sources, pharmacological parameters or according to their chemical constitution. Hence, the combination of those nutraceutical plants would help to improve their efficacies in the treatment of type 2

diabetes. Therefore, the goal of this study target comparative assessment of two combinations of nutraceutic plants on their antidiabetic activities in view of the treatment of type 2 diabetes. To overcome this two formulated powders are produced and are assessed on their phytochemical characterization and antidiabetic activities.

MATERIAL AND METHODS

Animals: Four (4) month old weaned male albinos Sprague Dawley rats (Harlan, France) weighing 260± 20g were housed in polycarbonate cages in a controlled environment with a temperature of 25 ±2°C, relative humidity (40–60%), with a 12-h light–dark cycle (12h/12h : 7 – 19 h light and 19 – 7h dark) (Gaíva *et al*., 2003). During an acclimatization period of 1 week, the rats received tap water and a commercial rat diet *ad libitu* (Baba *et al*., 2000). At the end of this period, the rats were weighed and randomly assigned to one group (n = 6 / group) according to the study.

Collection and processing of plant material: The leaves of *Vernonia amydalina* were collected from a field in the Nkolmesseng district of Yaounde V. The fruits of *Tetrapleura tetraptera* and Gum Arabic (*Acacia Senegal sap*) were purchased at the Mfoudi and Briqueterie markets (Yaoundé, Cameroon). *Leptadenia lancifolia* leaves and vines were collected in the Kaele area (Mayo-kani, Far-North Cameroon). The samples were then sent to the Laboratory of Food Science and Metabolism (LabSAM). They were sorted, weighed, put under a stream of water, wrung out and dried in a dehydrator at 45°C until a constant weight was obtained. The dried samples were then crushed and sieved through a 160 micron sieve and the resulting powder was packaged and labelled for analysis.

Formulation and preparation of powders: Formulation was done as shown in table 1. The objective in this constraint was to be able to reach as much as possible in the formulations of the recommended contents of some important molecules in the management of type 2 diabetes. Table 1 presents different formulations ingredients.

Table 1: Different formulations ingredients.

Formulation		**Formulation 1 (JE1) (g/100g)**	**Formulation 2 (JE2) (g/100g)**
Ingredients	*V.amygdalina*	0.41	0
	T.tetraptera	54.29	54
	G. arabic	39.38	39.38
	L. lancifolia	6.18	6.62
Composition	Carbohydrates (g)	52.89	49.90
	Fibres (g)	19.69	19.69
	Vit C (mg)	5.25	5.91
	Mg (mg)	254.66	253.35
	Ca (mg)	196.90	196.90
	Zn (mg)	5.25	5.91

Phytochemical characterisation of formulated powders: Extraction and determination of total phenolic compounds was carried out using the Folin - Ciocalteu reagent as described by Marigo (1973). Flavonoid content was done as described by de Vinson *et al*. (1998). Total tannins were assessed by Ndhlala *et al.* (2007) method.

Phytate content was done base on Olayeye *et al*. (2013) method. Oxalate content was determined by the modified titration method of Aina *et al.* (2012). Saponin content was measured by Koziol (1990) method.

***In vitro* antidiabetic activities of the formulated powders:** It was evaluated through 2 mechanisms: the glucophagic property by the glucose adsorption test and the non-insulin-sensitizing stimulation effects thanks to the absorption of glucose by the yeast. The glucose binding capacity of the powders was determined by the method described by Ou *et al.* (2001). Ability of the powders to act on glucose uptake by yeast was determined by the method of Takuissu *et al.* (2020).

***In vivo* antidiabetic activities of the formulated powders:** *In vivo* antidiabetic activities of the formulated powders were assessed by using glycaemic monitoring and glucose tolerance test. Glycaemic monitoring was done by evaluation of the formulated powders on the glycaemia of normal rats according to Moukette *et al.* (2017) method. Glucose tolerance test of formulated powders was performed by Etame-Loe *et al.* (2018**)** method and glucose determination by using OneTouch glucose meter and Strips.

Acute oral toxicity of the formulated powders: Acute toxicity was considered to investigate the toxic effect of a single dose of product administered as a single administration. The OECD (2001) protocol 423 was used. The amount of combined powder that can be administered to humans was calculated according to the method described by CDER (2005). In fact, the administerable dose in animals was obtained using the LD50 after acute toxicity study.

Statistical Analysis: Results were expressed as means ± standard deviation. For each group, the result obtained was the mean for 6 rats. All results were analysed using a one-way analysis of variance. Duncan's Multiple Range test was performed to evaluate differences between groups. Differences between means were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Evaluation of some secondary metabolites and anti-nutrients: Table 2 shows the contents of secondary metabolites and anti-nutrients assessed in different samples.

Table 2: Secondary metabolite and anti-nutrient contents of formulated powders

Samples	JE1	JE2
Total Polyphenols (mg eq AG/100g DM)	183.88± 0.33^{b}	146.06±0.49^{a}
Flavonoids (mg eq Q/100g DM)	44.49±0.41^{b}	37.62±0.46^{a}
Saponin (mg/100g DM)	4.35±0.22^{b}	3.24±0.28^{a}
Tannin (mg eq leu/100g DM)	42.77±0.69^{b}	10.90±0.11^{a}
Oxalate (mg/100g DM)	4.92±0.098^{b}	2.69±0.13^{a}
Phytate (mg eq AP/100g DM)	7.85±0.16^{b}	5.03±0.28^{a}

GA: gallic acid; Q: quercetin; PA: phytic acid; leu: leucocyanidin; JE1: JE1 powder; JE2: JE2 powder. Values assigned to different letters on the same line are significantly different ($p<0.05$).

From results in table 3, it is observed that JE1 formulation showed a higher total polyphenol content (183.88± 0.33 mg eq GA/100g DM) than JE2 (146.06±0.49 mg eq GA/100g DM). Statistical analysis shows a significant difference at the 5% level between these different values. Like vitamin C, total polyphenols help to lower blood sugar levels in diabetic patients as high blood sugar levels can lead to the production of free radicals which when in excess can no longer be neutralised by the body's antioxidants (Grosso, 2014). When regard flavonoids content, JE1 (44.49±0.41 mg eq EQ/100g DM) presents the highest

flavonoid content compared to JE2 (37.62±0.46 mg eq EQ/100g DM). Between the two values a significant difference (p< 5%) is noted. These levels are lower than the recommended daily value of flavonoid, which is 897 mg/day. Just like total polyphenols, flavonoids can neutralise the free radicals produced by excess glucose found in type 2 diabetics (Grosso, 2014). For saponin content, the highest value is obtained with JE1 (4.35±0.22 mg/100g DM) and the lowest with JE2 (3.24±0.28 mg/100g DM). According to the statistical analysis, there is a significant difference (p< 5%) between both values. This difference could be because JE2 does not contain *V. amydalina* thus the reduction of saponin compared to JE1, which has *V. amydalina* in its formulation. Saponins have positive health effects and are only toxic at a dose above 200 mg/Kg (Diwan *et al.*, 2000). They are involved in digestion by increasing the permeability of cell membranes, lowering cholesterol levels by reacting with bile acids to form micelles leading to an acceleration of its metabolism in the liver (Das *et al*., 2012). Saponins act in type 2 diabetics by lowering blood glucose levels and reducing oxidative stress via several mechanisms namely activation of glycogen synthesis, regeneration of insulin action, suppression of gluconeogenesis, suppression of disaccharide activity and modulation of insulin signalling (Ragab *et al.,* 2017). Investigation on tannin content reveals that JE1 has the highest tannin content (42.77±0.69 mg leu eq/100g DM) as compared to JE2 (10.90=±0.11 mg leu eq/100g DM). Statistically, there is a significant difference (p< 5%) between both values. It is well known that tannins inhibit the activities of digestive enzymes and these nutritional effects are related to their interactions with proteins and minerals. However, the levels found in those formulations are largely lower than the dose of tannin considered toxic and which is 150-200 mg/100g DM (Frédéric, 2012). The result of oxalate content of the formulations are 4.92±0.09 mg/100g DM and 2.69±0.13 mg/100g DM respectively for JE1 and JE2. Statistical analysis shows a significant difference at the 5% threshold between these different values. These values are much lower than the daily dose (200 to 500 mg) of oxalate considered as toxic (Ekop *et al*., 2008). The phytate content of the formulations are 7.85±0.16 mg eq AP/100g DM (JE1) and 5.03±0.28 mg eq AP/100g DM (JE2), with a significant difference (p< 5%) between them. High phytate levels are detrimental to health by the fact that they form complexes with minerals leading to a decrease in their solubility and reduce their accessibility in the gut (Priyodip & Balaji, 2019). However, the phytate contents of the various powders formulated are far below the safe dose, which is between 2000 and 500 mg/day (Danso *et al*., 2019). Therefore, they are an antinutrient of interest for the prevention and management of type 2 diabetes insofar as the same phytates reduce the formation of advanced glycation products in type 2 diabetic patients (Sanchis *et al*., 2018).

***In vitro* antidiabetic activities of the formulated powders:** *In vitro* antidiabetic activities of formulated powders were done by evaluation of the capacity of the formulated powders to capture glucose and Non-insulin-sensitising effect (ability of the formulated powders to act on glucose uptake by the yeast).

Capacity of the formulated powders to capture glucose: The study of the capacity of the formulated beverages to bind glucose gave the results presented in figure 1.

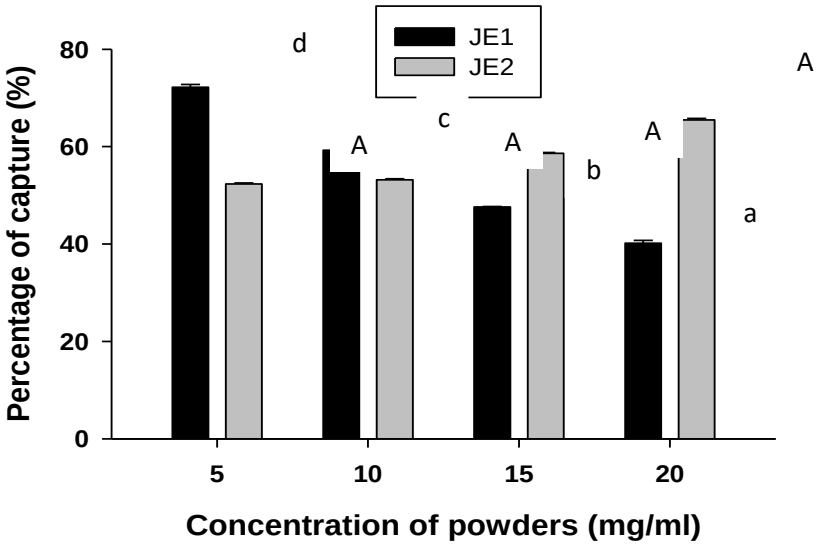

Figure 1: Percentage of glucose fixation by the formulated powders.
JE1 : powder JE1 ; JE2: JE2 powder. Histograms with the same superscript letters and same character are not significantly different ($p<0.05$).

In the aim to study the *in vitro* anti-diabetic potential, the ability of JE1 and JE2 to bind glucose was observed (Figure 1). It was significantly noted that JE1 and JE2 are amenable to glycosylation reactions even at low doses. The action of JE1 is dominant at 5 and 10 mg/dl and that of JE2 at 15 and 20 mg/dl. However, generally, the highest percentage of binding is that of JE1 (72.21±0.56%) at 5 mg/ml dose. ANOVA analyses show that in JE1 there is significant difference of binding glucose between concentrations of powders used. According to these results, it seems that the binding of glucose in JE1 is in contradiction to the case of JE2. In JE1 the binding decreases with the increase of concentration while in JE2 the binding increases with concentration of powders used. The data processed by the T-test shows that both formulated powders bound glucose. These uptake percentages vary from 40.18±0.56% to 72.21±0.56% for JE1 and from 53.18±0.22% to 65.47±0.32% for JE2. It is evident from these results that both formulated powders are capable to absorb glucose even at low doses. Although each has a maximum absorption at different concentrations JE1 at 5mg/ml and JE2 at 20mg/ml. But nevertheless the best glycosylation activity significant at $p < 0.05$ is that of JE1 (72.21%) at the 5mg/ml dose and which is higher than 43.95(%) obtained by Jose *et al*. (2018) in his study on: Effect Of Combination Of Two Plant Extracts On Diabetes Mellitus; The high contents of phenolic compounds in JE2 and especially JE1 could explain this activity. Indeed, flavonoids, thanks to their hydroxyl group, carry out condensation reactions with glucose molecules to form a glycosyl-flavonoid complex by forming an osidic bond (Li *et al.*, 2014). Knowing that compressed metformin used par excellence in the fight against type 2 diabetes reduces glycosylation of haemoglobin through its ability to capture glucose at that dose (Wémeau *et al*., 2014); seeing therefore the capacity of our JE1 and JE2 powders to capture glucose especially JE1 powder we can say that their effect is comparable to that of metformin. Furthermore, dietary fibre in JE1 (12.84±0.12 g/100 Ms) and JE2 (12.66±0.09 g/100gMS) could also contribute to the complexation of glucose molecules. Somnath *et al.* (2017) showed that, insoluble fibres have this ability

to form complexes with glucose molecules, all of which help to prevent blood glucose related complications in type 2 diabetes.

Non-insulin-sensitising effect: ability of the formulated powders to act on glucose uptake by the yeast: The non-insulin-sensitising study aimed to determine the ability of the formulated powders to promote glucose uptake by yeast, which are non-insulin-dependent cells; the results are shown in Figure 2.

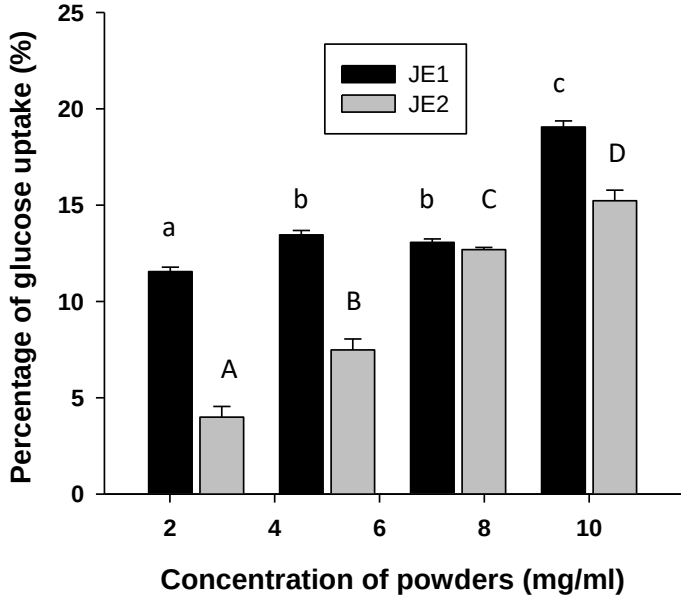

Figure 2: Percentage of glucose uptake by yeast cells as a function of the concentration of the different powders formulated.

JE1: JE1 formulation; JE2: JE2 formulation. Histograms with the same superscript letters and same character are not significantly different ($p<0.05$).

The data processed by the T-test shows that the two formulated powders, at different concentrations resulted in glucose uptake by the yeast cells. Proportionally to the chosen concentrations, the percentages of uptake varied from 11.55 to 19.05% for JE1 and from 3.99 to 15.23% for JE2. Figure 2 shows that the powders have the ability to facilitate the uptake of glucose by yeast even at low doses and in this exercise, it was the JE1 powder that showed significantly higher activity at all concentrations from 2.5 to 10 mg/ml. These results revealed that absorption of glucose increase with the concentration of powder in the both samples. Significant correlation (R= 0.80) is observed between results in JE1 and JE2. The fact that the values obtained with JE1 are higher can be due to the presence of *V. amydalina* in the formulation. In general, observation ANOVA shows that, based on concentration levels of powders, there is significant difference in quantity of glucose uptake. Yeast is a cell that absorbs glucose without the action of insulin, so this gives information about the possible capacity of these powders, especially JE1, to promote the absorption of glucose by our body's receptors such as GULT1, GLUT2 and GLUT3, so their action is not dependent on insulin (Romli, 2016). These biological actions could be due to the presence in the formulated powders of compounds such as: quercetins, β-sitosterols and allyl. Indeed, quercetins and β-sitosterols cause glucose uptake by yeast cells by

stimulation of membrane transporters (Hexose Transporters (HXT1 to HXT17) and Snf3 and Rgt2) which act through a facilitated diffusion phenomenon (Damsud *et al.*, 2017). The binding of glucose to these Snf3/Rgt2 sensors located in the plasma membrane of yeast cells allows the phosphorylation of the two co-receptors (Mth1 and Std1) of Rgt1 (a transcriptional repressor that negatively regulates the activity of HXT genes). This binding exposes Rgt1 to phosphorylation by PKA (Protein Kinase A) allowing an interaction between the central region of Rgt1 and its Zinc finger (Stefano *et al*., 2010). This inhibits the binding of Rgt1 to DNA which is forced to leave the HXT promoter and consequently the synthesis of HTX proteins will be responsible for the passage of glucose into the yeast cell membrane (Dietvorst *et al*., 2010).

In vivo* antidiabetic activities of the formulated powders:In vivo* antidiabetic activities of the formulated powders was assessed by evaluation of hypoglycaemic effect and Oral glucose tolerance test (OGTT).

Test of hypoglycaemic activity in normal rats (Blood glucose monitoring): The hypoglycaemic effect of JE1 and JE2 powders on normal blood glucose levels was evaluated by plotting the blood glucose levels against time as shown in figure 3.

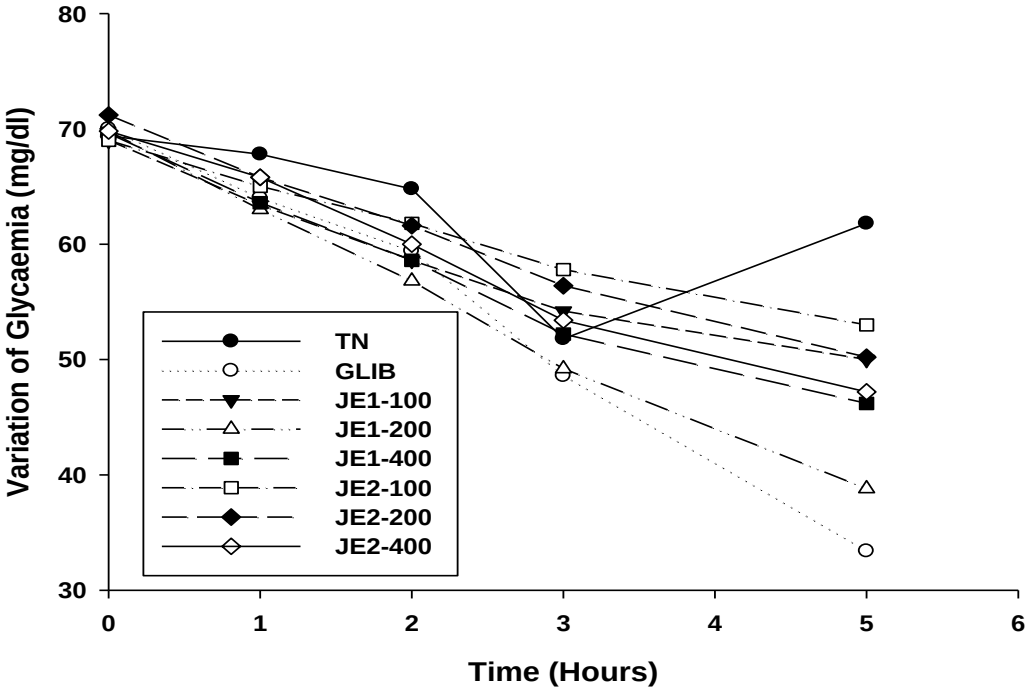

Figure 3: Hypoglycaemic effect of formulated powders**.**

TN= Negative Control, GLIB= Glibenclamide (Positive Control), JE1-100 = JE1 100mg/kg powder, JE1- 200= JE1 200mg/kg powder, JE1- 400 = JE1 400mg/kg powder, JE2-100=JE2 powder 100mg/kg, JE2-200=JE2 powder 200mg/kg, JE2-400=JE2 powder 400mg/kg.

In order to evaluate the hypoglycaemic and anti-hyperglycaemic activity of the powders formulated *in vivo*, the hypoglycaemic activity test in normal rats (glycaemic monitoring) was carried out and the results presented in figure 3. After processing the results by ANOVA test, it can be seen that JE1 powder has the best capacity to stimulate insulin secretion because after 5h, the blood glucose content of normal rats that received JE1 at a dose of 200mg/kg is

38.80 mg/dl. It is observed in normo glycaemic rats that the blood glucose level was significantly decreased in the JE1 200 mg/kg powder group compared to the Negative Control (TN) group (61.80±0.42 mg/dL). This result is lower than 80mg/dl obtained by Leugoue (2013) in similar study on Erigeron floribundus (Kunth) Extracts. In animals treated with Glibenclamide (positive control), this decrease was even more marked (33.40±0.41 mg/dL). This result can be explained by the presence of proanthocyanins and flavonoids, which are known for their ability to raise tissue tolerance to glucose, which would account for the decrease in blood glucose levels, particularly following the increase in glyconeogenesis, the stimulation of the capacity of insulin-secreting beta cells or the entry of glucose into target tissues. Indeed, massive glycogen storage in the liver can occur because of intense glucose internalisation in hepatocytes. The passage of glucose from the blood to peripheral tissues, in this case liver tissues, is likely to be facilitated by various effects of phenolics, including activation of glycogenogenesis, activation of the glucose transporter (GLUT) and potentiation of the insulin secretory effect (Damsud *et al.*, 2017).

Oral glucose tolerance test (OGTT): The anti-hyperglycaemic effect of JE1 and JE2 formulated powders on post prandial hyperglycaemia was assessed by plotting the evolution of blood glucose levels over time. The blood glucose changes obtained after glucose administration are shown in Figure 4.

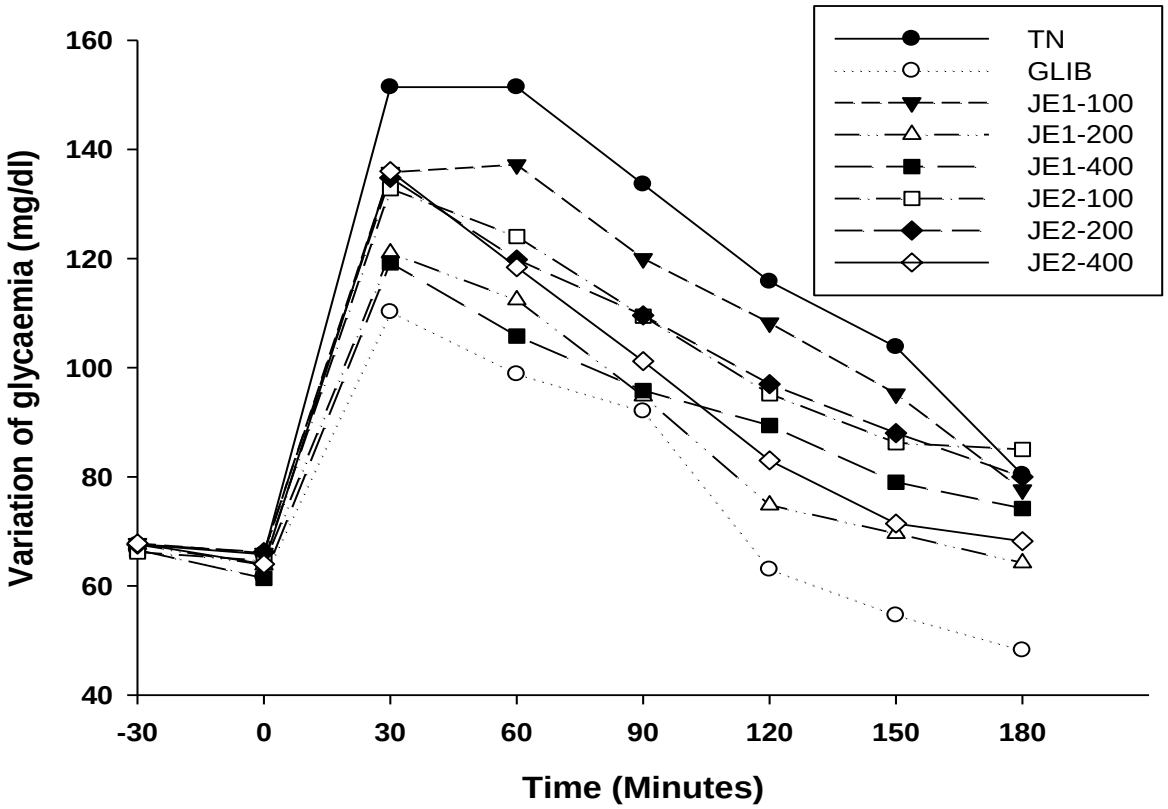

Figure 4: Glucose tolerance test of formulated powders.

TN=Negative Control, GLIB=Glibenclamide (Positive Control), JE1-100 = JE1 100mg/kg powder, JE1- 200 = JE1 200mg/kg powder, JE1- 400 = JE1 400mg/kg powder, JE2-100=JE2 100mg/kg powder, JE2-200=JE2 200mg/kg powder, JE2-400=JE2 400mg/kg powder.

To remove doubts about the capacity of the formulated powders, oral glucose tolerance tests (OGTT) were performed. Figure 4 shows the results of the reduction of postprandial blood glucose in normal rats by JE1 and JE2 powders. It is observed that the blood glucose level decreased significantly and drastically in the JE1 powder 200 groups (64.20±0.76 mg/dL) after 3 h (180 min) compared to the negative control group (TN) (80.40±0.79 mg/dL). This decrease was even more pronounced in the positive control group (48.20±0.61 mg/dL), which thus significantly prevented postprandial hyperglycaemia. These values are lower than 87mg/dl obtained by Leugoue (2013) in similar study done with *Erigeron floribundus* (Kunth) Extracts. These results can be explained by the high contents of antinutrients found in JE1 (**Table 2**) such as: Tannin (42.77 mg eq Leu/100gMs), Saponin (4.35 mg/100gMs), Oxalate (4.92 mg/100gMs) which inhibit a number of digestive enzymes including: α-amylase (glycoproteins containing 478 amino acids divided into 2 globular domains called A (1-380 residues) and B (381 - 478 residues)) ; This action is possible on the one hand thanks to the hydroxyl group that each of these antinutrients possess, which binds to proteins preventing enzymatic action, and on the other hand to the capacity of these compounds to form insoluble complexes with other macromolecules (Elkolli, 2017). This can also be justified by the presence of fibres, which delay the absorption of carbohydrates; all of these elements together thus participate in delaying post-prandial glycaemia (Liévin, 2016).

Acute toxicity: Acute toxicity was assessed by the estimation of percentage change in rat mass (Figure 5).

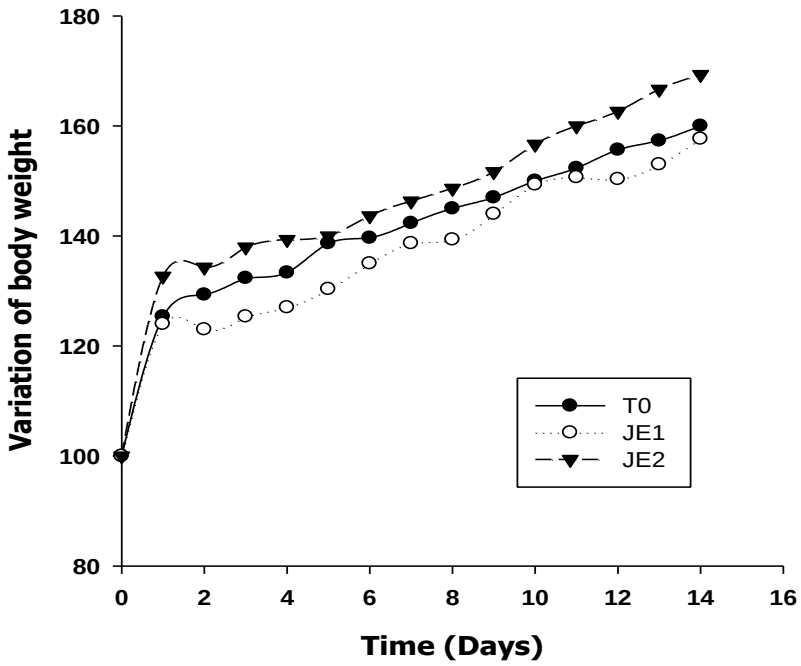

Figure 5: Percentage change in rat mass.
T0= Control, JE1= JE1 powder, JE2= JE2 powder.

No particular signs of acute toxicity (change in coat, motility, tremor, mass, grooming, respiration, sensitivity to noise after metal shock, stool appearance, mobility as well as death) were revealed in rats during 14 days after administration. The LD50 was greater than 2000 mg/kg as no deaths were observed at this dose. According to the Hodge and Sterner

scale, its toxicity index is 4, i.e. low toxicity (Hodge & Sterner, 1980). This suggests a safe use for the formulated powders. These results corroborate those obtained with Fall *et al*. (2011) on the acute and subacute toxicity of aqueous extract of *Aphania senegalensis* (juss. Ex poir.) leaves on wistar rats. They are also similar to those obtained with the wine extract of *Carica papaya* Linn seeds (Etame-Loe et al., 2018). Furthermore, Figure 5 reveals that JE1 powder *leads to* a very low variation in mass compared to JE2 so the effect on the variation in mass, happens to be the highest. In the acute toxicity study where the LD50 was observed, the mass of rats receiving JE1 varied slightly compared to those receiving JE2, yet the energy value of JE1 (242.55±0.14 kcal /100 g DM previously study) is quite high than that is observed with JE2 (258.68±0.08 kcal /100 g DM) which could be explained by the high levels of the anti-nutrients mentioned above which by their multiple actions retard the absorption of the macronutrients contained in JE1. Estimation of the dose of powder to be administered to humans was calculated by using the following parameters:

- Administrative dose: 2500 mg÷kg;
- Animal security factor ÷6;
- Human security factor (≥60kg) ÷ 37;
- Human equivalent dose : 400mg÷kg;
- Human equivalent dose for adult of 60 kg: 24000mg÷kg

A patient could therefore take one sachet of these powders at a dose of 8000mg (8g) in the morning, at midday and in the evening. The dose can be taken before and/or after the meal.

CONCLUSION AND APPLICATION OF RESULTS

From these results it can be observed that both formulated powders, JE1 and JE2 possess strong antidiabetic activities, but in general JE1 has the better activity compared to JE2. all these are made possible thanks to their high contents on bioactive compounds; indeed the results reveal that JE1 and JE2 can have an impact on the control of glycaemia in type 2 diabetes patient. The presence of those bioactive compounds in *Vernonia amygdalina, Tetrapleura tetraptera, Leptadenia lancifolia* decne and Gum Arabic (*Acacia Senegal sap*) can explain their use in the traditional pharmacopoeia for treatment of type 2 diabetes. For this reason, consumption of those plants needs to be encourage.

REFERENCES

ADA-EASD. (2012). Position des experts ADA-EASD sur la prise en charge de l'hyperglycémie chez les patients diabétiques de type 2 : une stratégie centrée sur le patient. p 6.

Aina V., Sambo B., Zakari A., Haruna H., Umar K., & AKinboboye R. (2012). Determination Of Nutrient And Ant-nutritional Content Of *Vitis vinifera* (Grapes Grown In Bomo (Areac)) Zaira. Nigeria. *Food Technology*, 4(6): 225-228.

Baba NH, Ghossoub Z & Habbal Z (2000). Differential effects of dietary oils on plasma lipids, lipids peroxidation and tissue lipoprotein lipase activity in rats. *Nutrition Research*, 2 (8): 1113 - 1123.

Bickford PC, Tan J, Shytle RD, Sanberg CD, El-Badri N, Sanberg PR., (2006). Nutraceuticals synergistically promote proliferation of human stem cells. *Stem Cells Dev.* 15:118-23.

Brower V. (1998). Nutraceuticals: poised for a healthy slice of the healthcare market? *Nat Biotechnol.*, 16: 728-731.

CDER (Center for Drug Evaluation and Research) (2005). Guidance for Industry Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy

Volunteers. *Food and Drug Administration*. 30 p.

Damsud, T., Chanwun, T., & Kaewpiboon, C. (2017). Antidiabetic agents with α-glucosidase inhibition and antioxidant capacity from the shoots of *Clausena cambodiana Guill*. *International Journal of Agricultural Technology***,** 13(4): 449-456.

Danso J., Francis A., Reindorf B., Barimah J. (2019). Effect of drying on the nutrient and anti-nutrient composition of bombax buonopozense sepals. *African Journal of Food Sciences*, 13(1); 21-29

Das T. K., Banerjee D., Chakraborty D., Pakhira M.C., Shrivastava B., & C Kuhad R. (2012). Saponin: Role in Animal system. *Veterinary World*, 5(4): 248-254

Dietvorst J., Karhumaa K., Kielland-Brandt M., & Brandt A. (2010). Amino acid reidues involved in ligand preference of the Snf3 transporter-like sensor in Saccharomyces cerevisiae yeast. *Sensor*, 10(27): 131-138.

Diwan, F. H., Abdel-Hassan, I. A., & Mohammed, S. T. (2000). Effect of saponin on mortality and histopathological changes in mice. In *Eastern Mediterranean Health Journal* (Vol. 6, Issues 2–3, pp. 345–351). https://doi.org/10.26719/2000.6.2-3.345

Earth Trends (2003). Evolution of Cameroon Protected Areas (1995 - 2008). Washington DC: World Resource Institute.

Effoe, S., Gbekley, E. H., Mélila, M., Aban, A., Tchacondo, T., Osseyi, E., Karou, D. S., & Kokou, K.. (2020). Etude ethnobotanique des plantes alimentaires utilisées en médecine traditionnelle dans la région Maritime du Togo. *International Journal of Biological and Chemical Sciences*, *14*(8), 2837–2853. https://doi.org/10.4314/ijbcs.v14i8.15

Ekop, A. S., Obot, I. B., & Ikpatt, E. N. (2008). Anti-nutritional factors and potassium bromate content in bread and flour samples in Uyo Metropolis, Nigeria. *E-Journal of Chemistry*, 5(4), 736–741. https://doi.org/10.1155/2008/530596.

Elizabeth AC. (2002). Over-the-counter products: nonprescription medications, nutraceuticals, and herbal agents. *Clin Obstet Gynecol*., 45(1):89-98.

Elkolli, M. (2017). Cours : Structure Et Activites Des Substances Naturelles : Principes Et Applications. *Ministere de l'Enseignement Supérieur et de La Recherche Scientifique Université Ferhat et Sétif Faculté Des Sciences de La Nature et de La Vie***:** 70. https://fsnv.univ-setif.dz/telecharger/EDT2017/CoursElKolli_Meriem.pdf

Etame-Loe, G., Okalla, C., Ngaba,G.P., & Ekossono, N. (2018). Evaluation Des Activités Hypoglycémiante Et Antihyperglycémiante Des Extraits Combinés Au Vin De Palme Des Ecorces Du Tronc De Musanga Cecropioides Et Des Fruits De Combretum Micranthum Chez Les Rats De La Souche Albinos Wistar. *Journal of Applied Biosciences* **(**126)*: 12717-1272*

Fall, M., Boukandou, M., Fall, A., Cabral, M., Diatta, W., Gueye, P., Faye, M., Bakou, S., Mendes, V., Bassene, E., & Diouf, A. (2011). Toxicité aiguë et subaiguë d'extrait aqueux de feuilles d'*Aphania senegalensis* (Juss. Ex Poir.) Sur des rats Wistar. D*akar Med*., 56(1): 223.

Frédéric, S. (2012). *Determination D ' Une Dose Efficace Et D ' Une Dose Toxique De Tanins*

Gaíva MH, Couto RC, Oyama LM, Couto GEC, Sikveira VLF, Ribeiro EL & Nascimento CMO. (2003). Diets Rich

in Polyunsatured Fatty Acids: Effect on Hepatic Metabolism in Rats. *Nutrition*, 19: 144 - 149.

Grosso, G., Stepaniak, U., Topor-Madry, R., Szafraniec, K., & Pajak, A. (2014). Estimated dietary intake and major food sources of polyphenols in the Polish arm of the HAPIEE study. *Nutrition*, *30*(11–12), 1398–1403.

Hodge, A. & Sterner, J. (1980). In études de toxicité : quelques données fondamentales (A. K. Done). *Tempo Médicale Afrique*, 7: 18.

Jose, D. N., Aleykutty,A., & Harindran,J.. (2018). Effect Of Combination Of Two Plant Extracts On Diabetes Mellitus. *International Journal of Pharmacy and Pharmaceutical Sciences.:* 1-4.

Koziol, M. (1990). Afrosimetric estimation of threshold saponin concentration for bitterness in quinoa (*Chenopodium quinoa Willd*). *Journal of the Science of Food and Agriculture*, 54(2): 211- 219.

Kuete V, Efferth T. (2010). Cameroonian medicinal plants: pharmacology and derived natural products. *Front Pharmacol*, 1:123.

Leugoue, Y. S. (2013). Evaluation des Propriétés Antidiabétiques et antioxydantes Des Extraits *D'Erigeron floribundus* (Kunth). Université de yaoundé I Département De Biochimie,**:**1-55.

Li Y., Saxena D., Chen Z., Liu G., Abrams, W., & Phela J. (2014). Infection and microbial diversity in saliva. *Journal of Clinical Microbiology*, 52(5): 1400-1411.

Liévin, S**. (**2016). Syndrome métabolique et compléments alimentaires. *Universite De Rouen*,**:** 62–71.

Marigo, G. (1973). Méthode de fractionnement de d'estimation des composes phénoliques chez les végétaux. *Analysis*. 106 – 110.

Mbatchou GPT. (2004). Plant diversity in Central African rain forest: Implications for biodiversity and conservation in Cameroon. Wageningen University, Department of Plant Sciences: PhD thesis 77p.

Moukette M., Vicky, A.., & Nya C., (2017) .Antioxidant and Synergistic Antidiabetic Activities of a Three-Plant Preparation Used in Cameroon Folk Medicine. *Hindawi International Scholarly Research Notices* Volume 2017, Article ID 9501675, 7 pages https://doi.org/10.1155/2017/9501675.

Ndhlala., Kasiyamhuru., Mupure., Chitindingu., Benhura., & Muchuweti. (2007). Phenolic composition of Flacourtia indica, Opuntia megacantha and sclerocarya birrea. *Food Chemistry*, 103: 82-87.

Nkongmeneck BA, Mapongmetsem PM, Pinta YV, Nkuinkeu R, Tsabang N, Fongnzossie E, Kemeuze V, Jiofack T, Johnson M, Asaha S, Sakwe C, Mboufack C. (2007). Etat des lieux des plantes médicinales importantes à conserver et des jardins des plantes médicinales à promouvoir. Geneva: Rapport CEN/OMS/MEM; 2007.

OCDE**. (**2001). Guidance for testing of chemicals. Acute Oral Toxicity- Acute Toxic Class Method, **(**17): 423-427.

Olayeye., Owolabi., Adesina., & oIsiaka. (2013). Chemical composition of red and white cocoyam (Colocasia esculenta) leaves. *International journal. Science Research*, 11: 121-125

Ou, S., Kwok, K., Li, Y., & Fu, L. (2001). In vitro study of possible role of dietary fiber in lowering postprandial serum glucose. *Journal of Agriculture and Food Chemistry*, **(**49): 1026-1029.

Priyodip P; Balaji S. (2019). An in vitro chicken gut model for the assessment of phytase producing bacteria, *3*

biotech. (9):294. Online 2019 Jul 8 dol: 10.1007/s13205-019-1825-2.

Ragab R; Evans J.G; Adriano Battilani; Domenico Solimando. (2017). Towards accurate estimation of crop water requirement without the crop coefficient Kc : New approach using modern technologies: Estimation Of Crop Water Requirement. *Irrigation and Drainage*, 66(4):469-477

Romli, H. (2016). Prise En Charge Et Traitement Du Diabete De Type 2. *These de Doctorat En Pharmacie,***:** 41.

Sanchis P.; Rivera, R; Berga F; Fortuny, R; Adrover, M; Costa-Bauza A.; Grasses F.; Masmiquel L. (2018). Phytate decreases formation of advanced glycation end-products in patients with type ii diabetes: randomized crossover trial. *Sci. Rep.*, 8, 9619

Somnath D., Mangesh A., Dheeraj S., Ganesh H., & Tejashri S. (2017). In-vitro hypoglycemic effects of unripe and ripe fruits of Musa sapientum. *The Brazilian Journal of Pharmaceutical Sciences***,** 53(4) : 1-4.

Stefano B., Paola C., Lilia A., & Marco V. (2010). Glucose signaling-mediated coordination of cell growth and cell cycle in Saccharomyces cerevisiae. *Sensors,* 10: 6195-6240.

Takuisssu, N, G., Ngondi, J., & Oben Julius E. (2020). Antioxidant and Glucose Lowering Effects of Hydroethanolic Extract of Baillonella toxisperma Pulp, *Journal of Food Research, Vol. 9, No. 2;: 1-12.*

Vinson, J., Yong Hao., Xeuhui Su., & Ligia Zubik. (1998). Phenol antioxidant qauntity and quality in foods: vegetables. *Journal of Agricultural and Food Chemestry*. 46:3630-3634.

Wémeau. J., Vialettes. B., Schlienger. J. (2014). Le diabète type 2, In: Endocrinologie, diabète, métabolisme et nutrition pour le praticien, ed.Elsevier Mansson, *Issy-les-Moulineaux cedex***:** 227-233.

Whitman M., (2001). Understanding the perceived need for complementary and alternative nutraceuticals: lifestyle issues. *Clin J Oncol Nurs*., 5:190-194.

Williams, R. (2019). L'atlas Du Diabète De La Fid 9ème Édition. In Atlas du diabète de la FID: Vol. 9ème édition. 2019.

World Health Organization. (2023). Diabetes., 5 April 2023 (https://www.who.int/news-room/fact-sheets/detail/diabetes) Consulted 19 Jully 2023.