Potential anti-hyperglycemic activity of the polyherbal combination of *Carica papaya* L., *Perseaamericana* Mill., and *Theobroma cacao* L. leaves extract: *in vitro*, *ex vivo*, *in vivo* studies

ABSTRACT

Aims: The present study was to evaluate the anti-hyperglycemic activity of the mixture of *C. papaya* L., *P. americana* Mill. and *T. cacao* L.,and its possible mechanism of action. a recipe traditionally used in Togo-Africa to treat diabetes.
Study design: *in vitro, ex vivo and in vivo*design
Place and Duration of Study: Laboratory of Physiology/Pharmacology, Unit of Pathophysiology, Bioactive Substances and Safety, Faculty of Sciences, University of Lomé. Between April 2022 and December 2022.
Methodology: The hydro-ethanolic extract of the recipe at different concentrations was evaluated *in vivo* on Oral Glucose Tolerance Test (OGTT) on ICR mice, *ex vivo* inhibition of the intestinal glucose absorption and skeletal muscle glucose uptake. The total antioxidant and DPPH free radicals scavenging test were also performed *in vitro*. The phytochemical and physicochemical determinations were done respectively by colorimetric assays and atomic absorption spectrometry.
Results: The results showed the extract of CPT at 200 and 400 mg. kg $^{-1}$ exerted a significant decrease ($p < 0.01$) of the hyperglycaemia by the rate of 46% in a dose-dependent manner after 120 minutes. However, at 400 mg. kg $^{-1}$ the extract of CPT on normo-glycemic mice did not significantly reduce basal blood glucose. *Ex-vivo* and *in-vitro*, the CPT recipe inhibited intestinal glucose absorption and increased peripheral glucose uptake by skeletal muscles by the rate 63% suggesting an extra-pancreatic action of the CPT extract. In addition, the CPT extract scavenged the free radical DPPH in dose dependent manner and had a total antioxidant capacity of 61.37 ± 0.38 µg. Phytochemical and physicochemical test revealed that the extract contained more total phenol, flavonoids, tannin, and polysaccharides and minerals as calcium, magnesium, zinc, potassium and sodium.
Conclusion: These findings support the use of the CPT recipe in traditional medicine to treat diabetes and oxidative stress in Togo.

Keywords: *Carica papaya* L.*, Persea American* Mill.*, Theobroma cacao* L., anti-hyperglycemic, antioxidant, glucose uptake.

1. INTRODUCTION

The international Diabetes federation reports a continued global increase in diabetes prevalence confirming diabetes as major health issue. Today more than half a billion people are living with diabetes worldwide [1]. Diabetes mellitus is a metabolic pathology characterised by a hyperglycaemiawhen the body is not able to produce enough of the insulin or cannot use the produced insulin effectively. Among the different types, diabetes type 2 is the most prevalent[1].
People living with diabetes in long term are exposed to life threatening health complications such as kidney damage, eye disease leading to blindness, nerve damage and cardiovascular diseases [2]And the oxidative stress has been reported to play a pivotal role in the development of these complications[3].
The management of diabetes mellitus remains a big challenge despite the therapeutic advances in the last year, due to their side effects[4]. Moreover, diabetes imposed a serious substantial economic burden on health systems of countries and families. Thus, it is well noted that more than 85 of the

developing countries populations refer to alternative methods as plants to treat the disease[5]. Nowadays, much attention has been paid to plants extracts as the most promising materials in drug discovery.
water infusion or drinking alcohol maceration of an equivalent proportion of the dry leaves of *Carica papaya* L. (Caricacea), *Perseaamericana* Mill. (Lauracea), and *Theobroma cacao* L. (Malvacea)is used as recipe to treat traditionally diabetes mellitus in Togo.It have been scientifically reported that, *Carica papaya* L., endemic to Mexico with tropical and sub-tropical distribution[6]leaves contain tannin, saponin, alkaloid, flavonoid, and glycoside; while shoots contain various minerals like Ca, Fe, Mg, K, Zn, Mn etc. Enzymes such as papain and chymopapain are present in the unripe fruit with anti-inflammatory and immunomodulatory activities[7].*Perseaamericana*Mill.,cultivated in tropical and subtropical climates is native to central America, seeds oil reveals an antioxidant, anti-inflammatory and nutritional properties[8] moreover, leaves and fruits reveal the amylase inhibitory activity[9].*Theobroma cacao*L., native to Central and South America and some genetic groups are thought to have Amazonian origin; containsPolyphenols and methylxanthines such us theobromine, caffeine, and theophylline present in the cocoa pods and seeds with protective abilities against cardiovascular, neurodegenerative and other metabolic disorders[10].
Regardless previous study and ethnobotanical survey, any scientific studies have not been yet carried out on the mixture of these 3 plants. This study therefore aimed to evaluate the *in vivo* anti-hyperglycemic and antioxidant activity of the polyherbal combination, and to identify its possible mechanism of action.

2. MATERIAL AND METHODS

2.1Animal material

ICR mice weighing 25 ± 5 g were used for *in vivo* OGTT test and Sprague Dawley rats weighing 225 ± 5g, for *in vitro* and *ex vivo* tests. The animals were provided by the Laboratory of the Physiopathology Bioactive Substance and Safety research unit of the Sciences Faculty of the University of Lomé. They were kept in standard environmental conditions (temperature 24–25° C, relative humidity and a 12t/12 h light-dark cycle) and fed with standard rat diet and water ad libitum.

2.2Plant material and extraction

The leaves of *Carica papaya* L., *Perseaamericana* Mill. and *Theobroma cacao* L. were collected from KpéléAkata(Plateaux district of Togo). A voucher specimen of each leaf was authenticated by the Botanical laboratory of the University of Lomé and deposited at the herbarium under following numbers: T0G015890, T0G015891, and T0G015892. The leaves of the plants were dried at room temperature in a safe room free from light for two weeks and then grounded to powder.
To proceed with the extraction, the recipe (600g of a mixture of leaves powder: 200g of *Carica papaya* L.*,* 200g of *Perseaamericana*Mill., and 200g of *Theobroma* cacao L.) was macerated in 6000 ml of a mixture of ethanol/water (50:50, v/v) for 72 h. The macerate was filtered on hydrophilic cotton and then on Wattmanpaper No. n°40 (Ø150 mm) and evaporated to dryness at 45 °C using a rotary evaporator under vacuum (BüchiRotavapor R210, Germany)[11, 12]. The dry extract was collected in a sterile glass vial and placed in a desiccator for 24 h and then stored in the refrigerator at 4-8 °C.

2.3Effect of the extract of CPT recipe on hyperglycemic mice

The Oral Glucose Tolerance Test (OGTT) was performed on mice fasted for 9 hours. Mice were divided into five (05) groups of five (05) mice each. The first was the control group which received 5 $ml.kg^{-1}$ of distilled water. The second, third, and fourth groups respectively treated with 100, 200, and 400 $mg.kg^{-1}$ of CPT recipe. The last one was the reference group which received metformin 100 $mg.kg^{-1}$. CPT recipe and metformin was administeredby gavage 30 minutes before glucose overload. Then, the hyperglycemia was induced by oral administration of $4g.kg^{-1}$ of glucose at a rate of 5 $mL.kg^{-1}$[11].Blood glucose was measured from blood collected from the tail vein of each mouse using an Accu Check Active glucometer over 180 min (0 min before and 30, 60, 120, and 180 min after the administration of the substances).

2.4Effect of the extract of CPT recipe on normoglycemic mice

Fasted mice divided into three (03) groups of five (05) were used. The control group received distilled water ($5mL.kg^{-1}$of body weight). The second group was treated with 400 $mg.kg^{-1}$ of CPT recipeand the

third as reference groups received metformin 100 mg.kg^{-1}. Blood glucose was measured as previously [11].

2.5Effect of the extract of CPT recipe on skeletal muscle glucose uptake (*In-Vitro* study)

After 24 hours of fastingmale rats, eight (08) groups of 3 tubeswere made, where each of them was containing 250 mg of muscle tissue in the following solutions,they have been dissected after cervical dislocation. The muscles of the femur were exposed and then cut into small pieces of 250 mg and pre-incubated in aerated Erlenmeyer flasks containing 20 mL of Kreb's-Ringer 's Bicarbonate (KRB) buffer at room temperature for 10 min.Before starting the manipulation, the KRB solution was replaced by KRB containing 11.1 mM of glucose (KRB + 11.1 mM glucose = KRB-G: Glucose Medium)[13].

Table 1:*In-Vitro* study of skeletal muscle fragment distribution in incubation solution.

Groups	Tubes content
GM	Tissue + KRB-G (control group)
GM+ Ins	Tissue + KRB-G + Insulin 100 mU. mL^{-1}
GM + CPT 12.5	Tissue + KRB-G + CPT recipe 12.5 mg. mL^{-1}
GM + CPT 12.5 + Ins	Tissue + KRB-G + CPT recipe 12.5 mg. mL^{-1} + Insulin mU. mL^{-1}
GM + CPT 25	Tissue + KRB-G + CPT recipe 25 mg. mL^{-1}
GM + CPT 25 + Ins	Tissue + KRB-G + CPT recipe 25 mg. mL^{-1} + Insulin100 mU.mL^{-1}
GM +Met	Tissue + KRB-G + Metformin 2 mg. mL^{-1}
GM + Met + Ins	Tissue + KRB-G + Metformin 2 mg. mL^{-1} + Insulin 100 mU. mL^{-1}

GM= glucose medium, Met = metformin, Ins = insulin, CPT = CPT recipe.

2.6Effect of the extract of CPT recipe on intestinal glucose absorption (*Ex-Vivo* study)

Rat everted gut sac model was used to study the effect of the CPT recipe on intestinal glucose uptake and glucose transport in *ex-vivo*[14].Intestine segments were inverted and weighted.After cervical dislocation, the fasted rat'sjejunum was removed, cut into small pieces and inverted. The interior of these fragments will then be rinsed with 0.9% NaCl and then placed in an oxygenated Krebs-Henseleit Bicarbonate (KHB, pH=7.4) buffer solution. After ligation of the lower ends, the intestine tissue fragments were filled with 1 mL of KHB **(serosal fluid),**weighed, and then placed in an Erlenmeyerflask containing 10 mL of KHB (mucosal fluid). The medium was oxygenated and incubated at 37°C with continuous shaking[15].Twelves (12) groups of different concentrations of glucose with intestine tissue fragments were made up as followed:

Table 2 : intestine tissue fragment distributionin glucose solution.

Glucose solution (mM.L^{-1})	40	60	80	100
Group 1-4: (Control)	Tissue + KHB	Tissue + KHB	Tissue + KHB	Tissue + KHB
Group 5-8: (Treated with 5 mg. mL^{-1}of CPT recipe)	Tissue + KHB	Tissue + KHB	Tissue + KHB	Tissue + KHB
Group 9-12: (Treated with 10 mg. mL^{-1}of CPT recipe)	Tissue + KHB	Tissue + KHB	Tissue + KHB	Tissue + KHB

After 60 minutes of incubation, the fragments were removed from the gut sac bath, and the serous fluid was drained through a small incision into a test tube. Empty sacs were weighed. Glucose assay have been processed on the serous and mucous fluids by using a GOD-PAP kit.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^{-1} tissue wet weight/hour.

2.7*In vitro* antioxidant assays of the CPT recipe

2.7.1Total antioxidant activity

In acid medium, the extract containing antioxidant compounds reduces molybdenum ion Mo^{+6} to Mo^{+5} and subsequently, the formation of the green phosphate-Mo^{+5} complex[16, 17]. The test was performed by adding 3 mL of reagent (sulfuric acid 0.6 M, sodium phosphate 28 mM, and ammonium molybdate 4 mM) to 0.3ml of CPT extract (1mg.ml^{-1}) prepared in methanol. The mixture was incubated at 95°C for 90 min. After cooling, the absorbance was measured at 695nm against blank. For the blank, the extract was replaced by methanol, and ascorbic acid (31 to 250 µg.ml^{-1}) was used as standard to generate the calibration curve. The total antioxidant capacity was expressed as mg equivalence of ascorbic acid/g of extract.

2.7.2DPPH*(2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay

In the presence of an antioxidant, the purple colour of free DPPH* radical turns yellow which absorbs at 517 nm. The mixture consisted of 1.5 mL of 100 µmol/LDPPH prepared solution and 0.25 mL of methanolic solution of the extract at different concentrations. The absorbance was determined at 517 nm, after 20 min of incubation. Ascorbic acid at different concentrations served as a standard[18].

2.8Phytochemical and physicochemical analyses of the CPT recipe

2.8.1Phytochemical screening

The screening was performed for detection of phyto-constituents present in the extract of the CPT recipe using standard procedure of[19-21].

2.8.2Total phenols, tannins, flavonoids, polysaccharides content of the CPT extract

2.8.2.1Total phenols and tannins determination

Gallic acid is used as standard at different concentrations (0 to 50 µg.ml^{-1}). Total phenolic compounds contained in the CPT extract were oxidized by the Folin-Ciocalteu which is reduced during the oxidation of the phenols in a mixture of blue oxides of tungstenand molybdenum which can be determined at 735nm.To determine the quantity of tannins, a second dosage was performed after the binding of tannins by Polyvinylpyrrolidone (PVP). The difference between the first and the second dosage corresponded to the total level of tannins according to the method of[11, 22]. The absorbance of the reaction was determined by using the US/VIS Spectrophotometer Wavelength at 735nm.

2.8.2.2Flavonoids content

Flavonoids formed with aluminiumchloride a flavonoid-aluminiumcomplex that absorbs at 415nm. One mL of 2% aluminiumchloride wasadded to 1 mL of the extract. After 10 min of incubation, the absorbance was measured at 415 nm against blank. Rutin at different concentrations (0 to 200 µg/ ml) was used as standard[23].

2.8.2.3Polysaccharides content

According to the method of (24).200 µL of a 5% (w/v) aqueous phenol solution and 1 mL sulphuric were added to 200 µL of the samples (CPT recipe, standard range, and control). The mixture was then incubated at 100 °C for 10 minutes and cooled in darkness for 30 min. Glucose at different concentrations (0–200 µg.ml^{-1}) served as standard. The absorbance was determined at 480 nm using US/VIS Spectrophotometer Wavelength[25].

2.8.3Physicochemical analysis

The minerals quantification was carried out by atomic absorption spectrometry (AAS) according to analysis process standard NF EN 14084 used by the Quality Control Laboratory (LNCQ-ITRA) at Togolese Agronomic Research Institute[26].
Principle: The AAS principle consists of driving the ions into their excited states under a specific, unique wavelength then in the presence of energy quantify them in atom form to quantify the mineral components (Mg, Zn, Fe, Ca, Na, K and Cu…).

Method:The ash represents the mineral load after the loss of the organic load. Ashing procedure was used to fully destroy the organic matter contained within the sample. Analysis was performed following mineralization of samples, 5 grams of the CPT recipe weighed as a test sample were incinerated in a **Naberthem**GmbH® electric muffle furnace at a temperature of approximately 555° ± 15°C for 6 hours until the ash was obtained. After incineration, the ash was cooled in the desiccator and then dissolved in a 1% solution of nitric acid reagent 69% (from Merck®) in a 100 mL volumetric flask topped up with distilled water. Samples in solution were read by 240FSAA/240ZAA Agilente® atomic absorption spectrometry. A **Certipure**multi-element standard solution from Merck of each mineral. The mineral concentration for each element was expressed as $mg.kg^{-1}$.

2.9 Ethical statement

The study was conducted by institutional guidelines and ethics of the Laboratory of Physiopathology bioactive substance and safety, referred as 001/2012/ CB-FDS-UL- Togo.

2.10Statistical analysis

Results were presented as the mean ± SEM (Standard Error of the Mean) using GraphPad Prism 9.1 software. Analysis of Variance (ANOVA) followed by Dunnett's test was used to compare different groups. Tests are considered significant at $p < 0.05$.

3. RESULTS

3.1 Effect of the hydro-ethanolic extract of CPT recipe on hyperglycemic mice

Glucose overload caused a significant increase (151.85%) of glucose level in mice compared to the controls after 30 minutes.The CPT recipe significantly reduced the hyperglycemia in a dose-dependent manner compared to the hyperglycemic control group. Notably at the dose of 400 $mg.kg^{-1}$ of the recipe, the percentage of reduction was at T30 (35.12%), T60 (41.20%), T120 (46.75%) and T180 with 29.60 % compared to controls (Figure 1A). The metformin used as the standard also reduced significantly the glucose level in mice. This significant reduction was confirmed by the glucose area under the curve (Figure 1 B).

A B

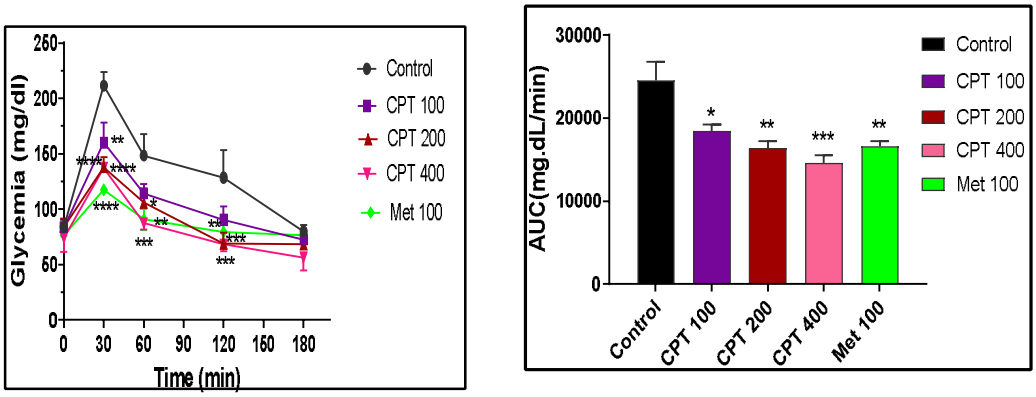

Figure 1: Effect of the CPT extract on hyperglycemia (A) and area under the glycemia curve (B).

Control = Treated with distilled water, CPT 100 = Treated with 100 $mg.kg^{-1}$ of CPT recipe, CPT 200 = Treated with 200 $mg.kg^{-1}$ of CPT recipe, CPT 400 = Treated with 400 $mg.kg^{-1}$ of CPT recipe, Met 100 = Treated with

metformin 100 mg.kg^{-1}. Results are presented as mean ± SEM, *: P<0.05; **: P<0.01; ***: P<0.001. ****: P<0.0001.

3.2 Effect of the hydro-ethanolic extract of CPT recipe on normoglycemic mice

Compared to the control, there was no significant decrease of the basal glucose level in the groups treated with the CPT recipe at the dose of 400 mg.kg^{-1}. The similar effect was noted with Metformin (Figure 2 A). This was confirmed by the area under the curve (Figure 2 B).

A B

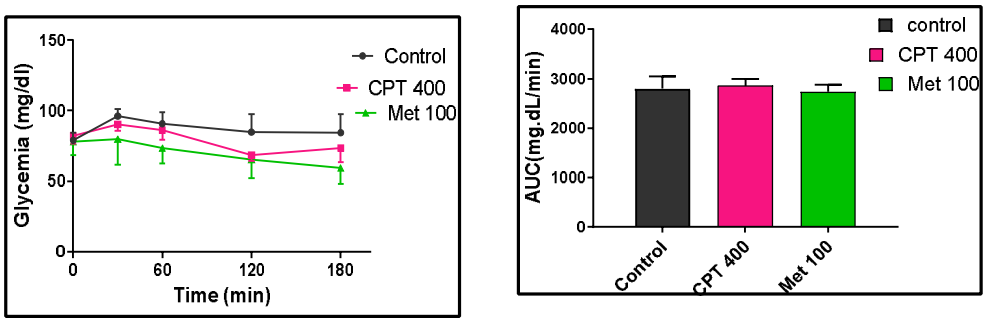

Figure 2: Effect of the CPT recipe on basic glycemia (A) and area under the curve(B)

Control = Treated with distilled water, CPT 400 = Treated with 400 mg.kg^{-1} of CPT recipe, Met 100 = Treated with metformin 100 mg.kg^{-1}. Results are presented as mean ± SEM

3.3 Effect of the hydro-ethanolic extract of CPT recipe on the glucose uptake by the skeletal muscle

As depicted in the table 3, in the groups treated with the extract of the CPT recipe, there was a significant increase in the glucose uptake. Notably, the increase of the glucose uptake had been potentialized in the presence of the insulin.

Table 3: Effect ofhydro-ethanolic extract of CPT recipeon glucoseuptake by skeletal muscle.

Groups	T30	T 60	T120	T 180
	Glucose uptake by skeletal muscle in mM g^{-1} of fresh tissue			
GM	1.98±0.28	3.44±0.22	4.08±0.20	11.21±0.30
GM + Ins	3.24±0.12	4.05±0.17	7.61±0.24***	13.85±0.54***
CPT 12.5+MG	2.97±0.05	6,32± 0,23 ***	8,51± 0,20 ***	10,44± 0,24
GM + CPT 12.5 +Ins	3,78± 0,41 **	8,47± 0,05 ***	9,82± 0,13 ***	10,76± 0,16
GM + CPT 25	5,08± 0,90 ***	7,32± 0,64 ***	9,44± 0.65 ***	13,89± 0,02 ***
GM + CPT 25+Ins	8,63± 0,04 ***	12,41± 0,15***	13,94± 0,13 ***	16,44± 0.08***
GM +Met	18,65± 0,06***	23,75± 0,03***	27,15± 0,53 ***	32,22± 0,10***
GM + Met + Ins	21,47± 0,12***	24,24 ± 0,88***	30.22±0.40***	33.21±0.12***

(GM) = glucose medium; (Ins) = insulin; (CPT 12.5) = 12.5 mg.ml^{-1} of CPT recipe, (CPT 25) = 25mg. mL^{-1} of CPT recipe; (Met) = metformin. Results are presented as mean ± SEM. (n=3. **: P<0.01; ***: P<0.001).

3.4 Effect of hydro-ethanolicextract of CPT recipe on the glucose absorption of the intestine.

In presence of 100 mM of glucose, the extract at 5 and 10 mg.ml^{-1} significantly ($p<0.001$) reduced the glucose released compared to the control (Figure 3).

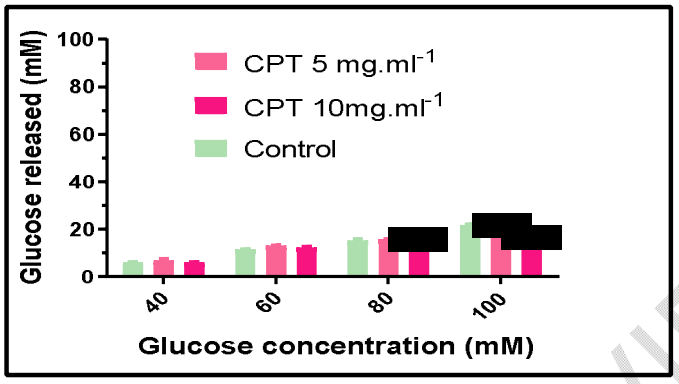

Figure 3: Glucose released into the serous fluid by the intestinal fragments

CPT 5 = 5 mg. mL^{-1} of CPT recipe**,** CPT 10 **=** 10 mg. mL^{-1} of CPT recipe.Results presented as mean **±** SEM, n = 3, ***: P<0.001.

3.5 *In vitro* antioxidant activity ofthe hydro-ethanolic extract of CPT recipe.

As shown in the table 4, the extract scavenged the DPPH° radical and possessed a strong total antioxidant capacity in dose dependent manner.

Table 4: ***In vitro* antioxidant activity of hydro-ethanolic extract of CPT recipe.**

	Ascorbic acid	**CPT recipe**
IC $_{50}$ of DPPH test	35.62 ± 0.36 µg. mL^{-1}	367.46 ± 0.40 µg. mL^{-1}
Total antioxidant	-	61.37 ± 0.38 mg/ g AA

n=3, AA: ascorbic acid.

3.6 Phytochemical analysis ofthehydro-ethanolic extract of CPT recipe.

The phytochemical screening of the CPT recipe revealed the presence of saponin, flavonoids, condensed tannins, alkaloids, phenolic compounds, and triterpenes compounds. In addition, the extract contains total phenols, tannins, flavonoids, and polysaccharides as mentioned in table 5.

Table 5: **Content of phenolic compounds and polysaccharides in thehydro-ethanolic extract of CPT recipe.**

Compounds	Contents
Total phenols(µg EqAG / mg of extract**)**	**20.69 ± 0.86**
Tannins (µg EqAG / mg of extract)	**11.97±0.95**
Flavonoids (µg EqR / mg of extract)	**90.77±2.88**
Polysaccharides (µg EqG /mg of extract)	**122.85±8.88**

Results are presented as mean ± SEM.n = 3,EqAG = Gallicacid equivalence; EqR = rutin equivalence; EqG = glucose equivalence.

3.7 Physicochemical analysis of the hydro-ethanolic extract of CPT recipe.

As depicted in the table 6, the extract has a higher percentage of ash than the dry leaves, which makes a clear difference in the concentrations of (Ca, Cu, Mg, K, Na and Zn), which are more concentrated in the extract than in the dry leaves. However, only the iron content is more concentrated in the dry leaves than in the extract.

Table 6: **Physicochemical parameters of hydro-ethanolic extract of CPT recipe**

Physicochemical parameters		
	Dry leaves	**Hydroalcoholic extract**
Humidity (%)	10.25	9.14
Ash content (%)	7.06	12.32
Minerals ($mg.kg^{-1}$)		
Calcium (Ca)	1964.50	3902.00
Cuivre (Cu)	3.18	6.03
Iron	46.47	12.39
Magnesium (Mg)	1338.55	2793.80
Potassium (K)	7203.41	30521.21
Sodium (Na)	1295.70	6894.41
Zinc (Zn)	8.62	24.74

n=3

4. DISCUSSION

The abnormal regulation of the blood glucose level leading to hyperglycaemia remainsa pivotal factor in the development of diabetes mellitus**.** The objective of this study was to evaluate the *in vivo* anti-hyperglycemic and antioxidant activity of the polyherbal combination of *C. papaya*, *P. americana*, and *T. cacao,* and to identify its possible mechanism of action.

In this study, OGTT was undertaken to measure the effect of the polyherbal combination on the hyperglycemia in mice. A significant increase of the blood glucose level of the mice has been noticed after the glucose overload. This rise of the glycemia reached a peak at 30 minutes, which returned to the basal glucose level after two hours. This phenomenon explained the fact that, after

glucose uptake, it first increases the blood glucose level; before being used for organism need or being stored in the liver and muscles under the direct action of insulin[27].Compared to the control group, the administration of the CPT extract at different concentrations significantly reduced the glucose level of the mice in dose dependent manner.The reduction of hyperglycemia was more pronounced with the CPT at 400 $mg.kg^{-1}$. This was confirmed by the area under the curve, which measures the blood level glucose per unit time. The same effect was revealed in the metformin treated group.

As metformin, 400 $mg.kg^{-1}$ of CTP extract administered to the normo-glycemic mice did not significantly decrease the basal blood glucose level compared to controls. Henceforth, the CPT extract processed as an anti-hyperglycemic extract and confer to it an extra-pancreatic mechanism of action. Many studies revealed that metformin has various mechanisms of action. Metformin improves insulin sensitivity by increasing peripheral glucose utilization, decreasing hepatic output and reduced intestine glucose absorption[28]. The main action of metformin appears in the liver mitochondria via activation of adenosine-5'- monophosphate-activated protein kinase[29], inhibitsthe hepatic glucose production and glucose 6-phosphatase gene expression[30], then leading to the reduction of the hyperglycemia.

To elucidate the possible mechanism of action of the polyherbal combination, the evaluation of the CPT extract on the glucose uptake by the skeletal muscle and on the intestinal glucose absorption inrat everted gut sac model had been carried out.The results showed that the CPT extract exhibited an insulin-mimicking action when tested on skeletal muscle, by promoting the uptake of glucose of the muscle. The skeletal muscle is the major site of insulin stimulated glucose uptake which plays a crucial role in the maintenance of normal glucose homeostasis in postprandial state[31]. With the stimulation of the insulin, glucose transport is accelerated by translocating the glut 4 transporters from an intracellular pool out to the T-tubule sarcolemmamembrane[32, 33].

Moreover, theCPT extract significantly reduced the release of glucose in the everted intestine.It reported that intestinal glucose absorption is mediated by Sodium-glucose cotransporter-1 (SGLT1) located on the apical membrane of the intestine. Glucose is transported through the apical membrane of enterocytes by GLUT1 and then through the basilar membrane into the blood by GLUT2[34, 35]. Therefore, the inhibition of the glucose absorption of the intestine by the CPT can be related to the intestine glucose transporters inhibition which mediated the glucose transport into the blood.

In the long term, the hyperglycemiapromotes the accumulation of the reactive oxygen species (ROS). This inducesthe oxidative stress, which causes the cellular damages leading to disabling and life-threatening health complications such as kidney damage, nerve damage, cardiovasculardisease, eye disease[36]. For this reason, *in vitro* antioxidant capacity of CPT extract was carried out. Our results highlight that the CPT extract scavenged the DPPH*free radical in dose dependent manner and possessed a strong total antioxidant activity. The antioxidant activity of the polyherbal combination could help to reduce the risk of diabetes-related complications.

Additionally, the preliminary phytochemical study of the polyherbal combination had revealed the presence of Saponins, phenolic compounds, flavonoids, tannins, alkaloids. Quantitively, the CPT extract is richer in phenolic compounds and polysaccharides. The antioxidant activity of the CPT extract could be attributed to the presence of phenolic compounds which are recognised their ability to be hydrogen donators, singlet oxygen quenchers and reducing agents.They also maintain the homeostasis of the glucose by enhancing the glucose uptake, as well as promoting the formation of glycogen and enhancing the translocation of GLUT4 glucose transporter[37].In improving glucose tolerance, terpenoid and polysaccharides are known to protect pancreatic β cell and promote the response of the body to the insulin[38, 39].

It is well established that the supplementation of micronutrients has positive influence on glycemic control and deficiencies in these minerals can contribute to insulin resistance[40]. In our study, the atomic absorption spectrometry analysis revealed that Ca, Cu, Mg, K, Na and Zn, are more concentrated in the CPT extract. It scientifically reported that, Magnesium facilitates insulin action and Zinc is vital for insulin storage and secretion[41].Therefore, the CPT extract contains phytochemical compounds and micronutrient which may act alone or in synergy to fight against the hyperglycemiaand the oxidative stress**.**

5. CONCLUSION

This present study is the first to report on the synergistic action of the hydroethanolicextract of *C. papaya*, *P. Americana*, and *T. cacao.* The polyherbal combination exhibited a notable

antihyperglycemic activity and antioxidant activities through several mechanisms of action. The mixture of the three plants inhibited the absorption of glucose in the intestine and enhanced the glucose uptake by the skeletal muscle *ex vivo*. This justifies the traditional use of the mixture of *Carica papaya* L., *Perseaamericana* Mill., and *Theobroma cacao* L. leaves in the treatment of diabetes type 2. todiscover new drugs, it is necessary to conduct more in-depth analysis of the polyherbal chemical constituents and more preclinical tests.

ETHICAL APPROVAL

Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee"

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