

Full Length Research Paper

Acute toxicity and hypoglycaemic activity of the leaf extracts of *Persea americana* Mill. (Lauraceae) in Wistar rats

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Persea americana Mill. or avocado plant tree is well-known to people from the sub-Saharan part of Africa. Studies carried out earlier reported on the use of leaf extracts of the named plant to cure diabetes and other diseases in the south of Côte d'Ivoire. This study aimed to assess both acute toxicity and hypoglycaemic activity together with performing a comparative study. The acute toxicity was determined using the OECD 423 protocol, followed by the analysis of biochemical indicators, body weight variation and vital organs damage of healthy Wistar rats. The aqueous, ethanol and methanol extracts' hypoglycaemic properties were investigated through the hypoglycaemic activity and oral glucose tolerance test. Thereafter, the phytochemical identification of the molecular compounds' was carried out as well as polyphenols and total flavonoids quantification using Folin-ciocalteu and Neu reagent. The data analysis showed that *P. americana* leaf extracts' are well tolerated in general at a unique dose of 2000 mg/kg. Nevertheless, a slight hepatitis occurrence was observed. Additionally, all extracts exhibited significant anti-hyperglycaemic activity 2 h after glucose administration. Ethanol extract (100 mg/kg) showed a strong activity by depleting the glycaemia rate by 59.6% during 5 h after glucose uptake as compared to glibenclamide at 61.6% followed by methanol extract at 49.2%. The ethanol extract also appeared to be the most provided with phenols and total flavonoids exhibiting respective amounts of $2952.7 \pm 166 \mu\text{g}$ gallic acid equivalent/g and $0.582 \pm 0.012\%$, respectively. The current study showed that both ethanol and methanol extracts displayed a good tolerance and significant anti-hyperglycaemic activity probably due to the presence of polyphenol in the extracts.

Key words: *Persea americana* Mill., avocado, toxicity, glycaemia, diabetes, rat.

INTRODUCTION

According to the World Health Organization (WHO), diabetes mellitus is a chronic disease that occurs when

the pancreas does not produce the required amount of insulin or when the insulin released in the body is not adequately used. As a result, an increase of glucose serum level (hyperglycemia) occurs, bringing about complications such as diabetic neuropathy, retinopathy and cardiovascular diseases (Kumar et al., 2011; WHO, 2015).

Thus, in 2014, the estimated people living with diabetes reached 387 million and the figure is projected to reach 592 million in the coming twenty years (IDF, 2015). To illustrate, the diabetes prevalence in sub-Saharan Africa, which is currently set at 12.1 million, is expected to rise to 23.9 million by 2030 (Keter and Mutiso, 2012). From this perspective, diabetes mellitus constitutes a global health concern. Therefore, the discovery of a sustainable and adequate treatment for this disease appears to be a challenge to scientists. Studies conducted earlier reported on the use of herbal medicines and traditional remedies to cure diabetes. For example, in China and in Africa, traditional medicine accounts respectively for about 40 and 80% of the proposed treatment of the population meeting their health care needs including treatment of diabetes (WHO, 2002, 2013).

Additionally, records show that roughly 800 plants are used worldwide to treat diabetes mellitus (Alarcón et al., 1993). Among them is *Persea americana* mill. The avocado is described as a medium to large tree, 9-20 m high, and belongs to the Tracheophyta division of the Magnoliopsida class in the Lauraceae family, and endemic to Mexico (Central America). *P. americana* can also be found in most sub-tropical and tropical countries like Côte d'Ivoire. The number of species identified in the family is about 500 (Adeboye et al., 1999, Yasir et al., 2010).

Even though previous investigations have reported its biological activities, including anticancer, anti-diarrhea, analgesic and anti-inflammatory properties, little is known about its toxicity and hypoglycemic activity (Adeyemi et al., 2002; Butt et al., 2006; Odo et al., 2014). The study aforementioned showed lower toxicity and hypoglycemic effect. However, the mode of action was not explored in depth.

Therefore, the present study aimed at evaluating the acute toxicity from oral route uptake of three extracts of *P. americana* leaves and the comparative hypoglycemic and anti-hyperglycemic activities in normoglycemic rats.

MATERIALS AND METHODS

Leaves of *P. americana* were collected at dawn before the first sun rays at Adiopodoumé (N 5° 19' 3.49" O 4° 8' 8.66"), a village

located about 10 km from Abidjan (Côte d'Ivoire). The plant specimen was authenticated at the herbarium of the National Floristic Center of the Félix Houphouët Boigny University under the voucher number 8845 deposited at the national herbarium. It is also registered in the Integrated Taxonomic Information System under the number 18154 (ITIS, 2016).

Extraction method

Fresh leaves of the plant were collected, washed, shade dried, cut into small pieces, and powdered in a Phillips® blender to obtain a weight of approximately 0.5 kg.

Aqueous extract (AE) of the leaves of *P. americana*

Fifty grams (50 g) of *P. americana* leaves powder was boiled in one liter (1 L) of distilled water for 30 min, allowed to cool at room temperature (25°C) and filtered with a Fisherbrand® paper. The decoction was then lyophilized using the Martin Christ® ALPHA 2-4 LDplus, Germany and stored at 2-4°C until use for the bioassays and phytochemical analysis.

Ethanol and methanol extracts (EE, ME)

The dried powder of *P. americana* leaves was macerated three times with absolute ethanol (10%, w/v), Prolabo®, France for 48 h at room temperature and shaken occasionally. The ethanol fraction was pooled after filtration and dried in an oven (Mettler®, Germany) at 40°C. Similar extraction procedure was repeated for methanol extract using absolute methanol (Quimicen®, Spain).

Phytochemical analysis

Phytochemical screening

The phytochemical screening was carried out to investigate the presence of active ingredients of extracts AE, EE and ME. The prospective compounds to be identified were generally alkaloids, flavonoids, tannins, polyphenols derivatives, triterpenoids/steroids and coumarins. These identification are performed by the appropriate extraction solvent. So, alkaloids were isolated through the Dragendorff test (Gidwani et al., 2011), flavonoids identified by the Shinoda test (Vinod et al., 2010), tannins and polyphenols compounds by the FeCl₃ (Acros organic®, Belgium) test (Békro et al., 2007), saponins determined by foam formation and foaming index (Dohou et al., 2003), steroids and tripenoids were revealed by the Liebermann-Bürchard test (Békro et al., 2007), and coumarins were detected using NaOH (Carlo Erba®, France) reagent. The compounds aforementioned were visualized under UV-Visible light ($\lambda=366$ nm) (Rizk et al., 1986).

Determination of total polyphenol compounds

Total polyphenol compounds' content from leaf extracts of *P. americana* were determined using the Folin-Ciocalteu colorimetric method (Singleton and Rossi, 1965). The named method is

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developed as follows:

Primarily, 1 ml of plant extract (0.02 mg/ml) was mixed with 0.5 ml of the reagent. Then, 1.5 ml of Na₂CO₃ (17% w/v; Carlo Erba[®], France) was added after shaking thoroughly with a vortex (Heidoph[®], Germany). This mixture was incubated at 40°C for 10 min in a water bath. The absorbance of the total phenol compounds were read out using a Cecil[®] spectrophotometer (ce1021, UK). Before then, the standard calibration curve was obtained using gallic acid with a concentration range of 1 to 10 µg/ml and the figure recorded were expressed as gallic acid equivalent (GAE) (µg/g) of plant extract.

Determination of total flavonoid compounds

Twenty milligram (20 mg) of each *P. americana* leaves extract were mixed with 10 ml of ethanol (80%, v/v). Then, after 10 min, 50 µl of the Neu reagent mixed with 12 ml of pure methanol were added to 1 ml of the crude extract. In addition, the mixture was shaken using a vortex, and the absorbance was read at a wavelength of 404 nm using the Cecil[®] spectrophotometer. For this experiment, quercetin solution (0.05 mg/ml) was used as positive control and the percentage of total flavonoids was expressed as quercetin equivalent according to the following relationship (Dohou et al., 2003):

$$F(\%) = \frac{0.05 \times A_{ext}}{A_q \times C_{ext}} \times 100$$

F (%): Percentage of total flavonoids in crude extract, A_{ext} : absorbance of crude extract, A_q : absorption of quercetin (standard pure compound), C_{ext} : plant leaves extract concentration (2 mg/ml).

Biological material

Wistar albinos (*Rattus norvegicus* var. *albinus*) bred up to 2-3 months at the Animal Resources Unit of the Institute Pasteur Côte d'Ivoire, and weighing 150-250 g were used for the bioassay. Their living condition could be described as follows: the housing was cross-ventilated at mean temperature of 24-28°C with relative humidity of 60-80% and 12 h/day light. Prior to the experiments, rats were allowed to acclimatize for 2 weeks during which they were fed with standard diet and water *ad libitum* according to the international standards of animal use and care.

Sample preparation

Extracts AE, EE, ME and reference antidiabetic drug (glibenclamide; Daonil[®], Sanofi-Aventis) were prepared in a mixture of water and Tween 80% (2%, v/v) called the vehicle. Then, test samples were taken up orally by force-feeding the animals, which were under mild anaesthesia (lidocaine oral gel; AstraZeneca[®], UK). The negative control group of animals received the vehicle only orally (10 ml/kg, b.w.).

Acute toxicity study of extracts

Acute oral toxicity was evaluated by following modified OECD test guidelines (OECD, 2001). Previously, research showed that *P. americana* exhibits low toxicity. Based on that, the "limit test" was used to assess its toxicity level (Figure 1). Thus, the experiment was designed such that, healthy female rats underwent fasting overnight, and they were divided into four groups (n=3) randomly.

Group I received vehicle (10 ml/kg; b.w.) by oral route, was considered as negative control whereas, Groups II, III and IV received 2000 mg/kg, b.w. of AE, EE and ME, respectively. The experiment was repeated 2 weeks later with another group of animals.

After extract administration, the rats were observed continuously every 1h for 4 days. Further observation was carried out every 24 h for 15 days to identify any change such as tremors, convulsions, salivation, diarrhea, lethargy, sleep, coma and decrease of the respiratory rate or any lethality. Thus, after 4 h observation, all rats were allowed to feed themselves and drink water *ad libitum*. Then, the body weight was monitored every four days. From this experiment, the median lethal oral dose (LD₅₀) was determined with respect to the OECD 423 standards fixed by the Globally Harmonized Classification System (GHS) (Figure 1).

At day-15, animals were subjected to fasting overnight, euthanized by cervical dislocation under anaesthesia (Forene[®], Abbott, USA). Blood samples were collected in red topped plastic vials and centrifuged at 1500 g for 10 min. The serum collected out of this step was used for biochemical analysis. A gross necropsy of all animals was also carried out. The heart, liver and kidney were carefully isolated and weighed individually. From the bio-analysis performed, the following parameters were determined: blood urea nitrogen (BUN), creatinine (CREA), aspartate aminotransferase (AST), alanine aminotransferase (ALT), sodium (Na⁺), potassium (K⁺), chloride ions (Cl⁻), using biochemical kits (Hitachi 704R[®]) and electrolyte analyzer (ISE 3000[®]), respectively.

Dose selection

First, a dose of 2000 mg/kg from each extract of the plant was selected for acute toxicity evaluation. Then, both 1/10th and 1/20th of the dose, which do not display any behavioural alterations, were considered for anti-diabetic tests (Oliveira et al., 2008). Additionally, the 1/5th of the dose was also selected for further identical assays.

Acute hypoglycaemic effect on normal rats

The set of healthy males rats was organized by dividing them into 11 groups (n=5 per group), followed by, fasting blood glucose level recording, at an initial time (t=0 h), after the overnight fasting (16 h) and water uptake *ad libitum*. The negative control (Group 1) received by oral route, 10 ml/kg b.w. of vehicle aqueous Tween 80 (2%; v/v), and the positive control group (2) was administered a standard hypoglycaemic drug (glibenclamide) 10 mg/kg b.w. Groups 3 to 11 received AE, EE and ME at 100, 200, 400 mg/kg for each. Then blood was collected from tail-tip after oral administration of test samples followed by a period of observation which lasted ½, 1, 2, 3 and 5 h. Blood glucose levels were recorded using reactive strips (GOD-POD) and a glucometer (Accu-Chek[®] Active, Germany).

Oral glucose tolerance test (OGTT)

Male Wistar normoglycaemic rats were subjected to overnight fasting for 16 h, followed by fasting glycaemia recording before extracts administration, which is called the ½ h glucose administration. Animals were then divided into 11 groups; each of which was made up of five rats (n=5). Group 1 taken as the negative control received the vehicle; Group 2 the positive control was treated with glibenclamide (10 mg/kg, b.w.), and Group 3 to 11 received extracts AE, EE, ME at 100, 200 and 400 mg/kg for each. Each rat was fed 2 g/kg of D(+)-Glucose monohydrate 30 min after

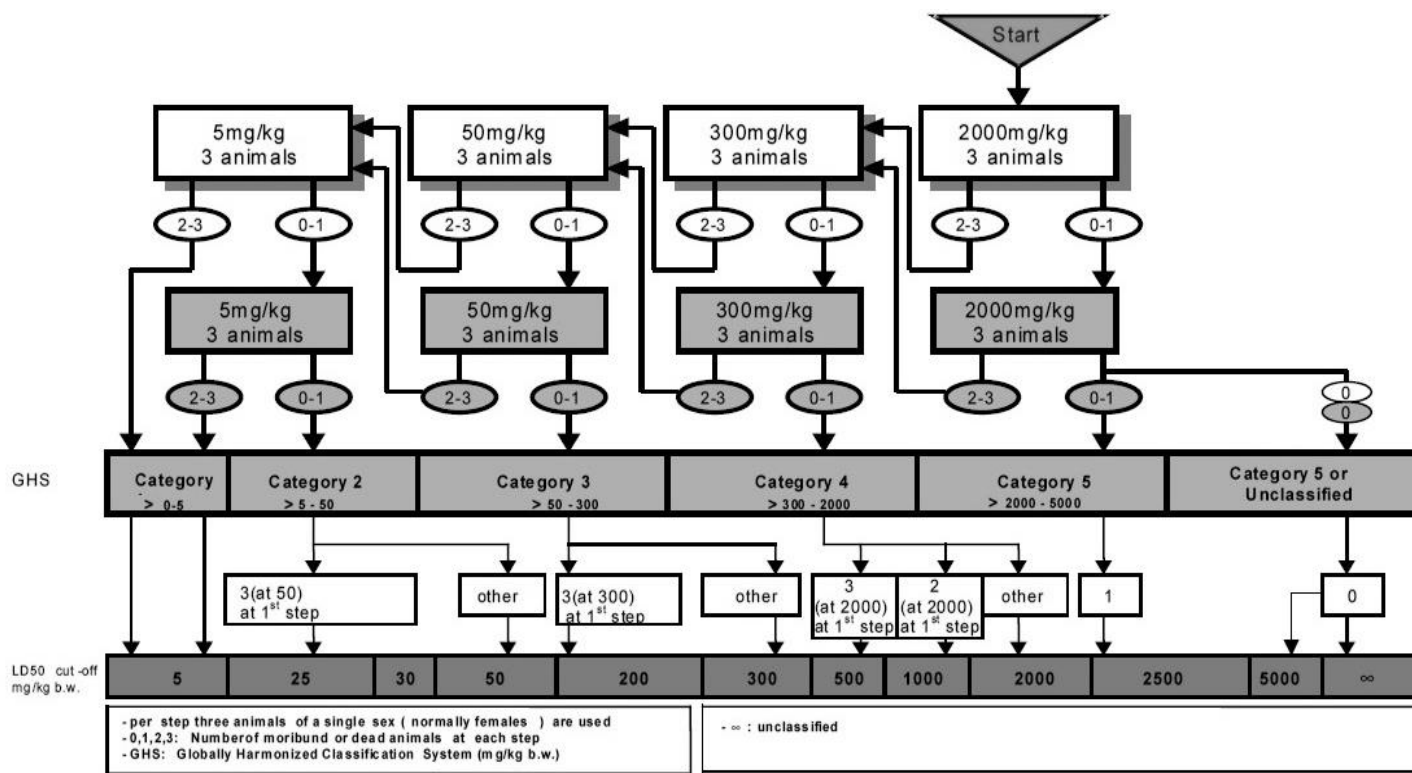


Figure 1. Acute oral toxicity evaluated by following modified OECD test guidelines (OECD, 2001).

administration of extract and was defined as initial time ($t=0$ h). Then, blood was collected from tail-tips at different time periods $-\frac{1}{2}$, 0, $\frac{1}{2}$, 1, 2, 3 and 5 h consecutive to glucose administration. This was followed by glycaemia recording by use of a clinical glucometer (Accu-Chek Active[®], Roche).

Statistical analysis

Results of bioassays were expressed as mean \pm S.E.M using one way ANOVA, followed by the Newman-Keul test (GraphPad Prism, version 5.01) and differences were considered significant at $p \leq 0.05$. The decrease percentage of glycaemia for each group was calculated as follows:

$$\text{Decrease (\%)} = \frac{G_{1/2h} - G_x}{G_{1/2h}} \times 100$$

$G_{1/2h}$: Glycaemia value at $\frac{1}{2}$ h, G_x : glycaemia at x hour; ($x = 1, 2, 3$ and 5).

RESULTS

Phytochemical analysis

Yield of the crude extracts

From the dried plant material, the yield of the crude extract AE was 20.9% (w/w), that of EE was 16.8% and

ME 18.6% (w/w).

Phytochemical screening

The phytochemical screening exhibited saponins, polyphenols, flavonoids, alkaloids, sterols/polyterpenoids and coumarins as the ingredients of the plant extract. Moreover, AE was identified to contain gallic tannins, whereas EE and ME contained catechic tanins. The foaming index for AE, EE and ME was respectively 33, 500 and 250.

Determination of total polyphenol compounds

The total polyphenols compounds identified by the Folin-Ciocalteu colorimetric method displayed absorbance values of 2707.3 ± 155.4 , 2952.7 ± 166.0 and 1873.1 ± 63.5 (GAE) $\mu\text{g/g}$, respectively for AE, EE and ME, using linear regression equation of gallic acid expressed as (GAE) of extract: $y = 0.0138x + 0.0651$, $r^2 = 0.8138$.

Determination of total flavonoid compounds

The spectrophotometric determination of total flavonoid compounds revealed a content of 0.543 ± 0.007 ,

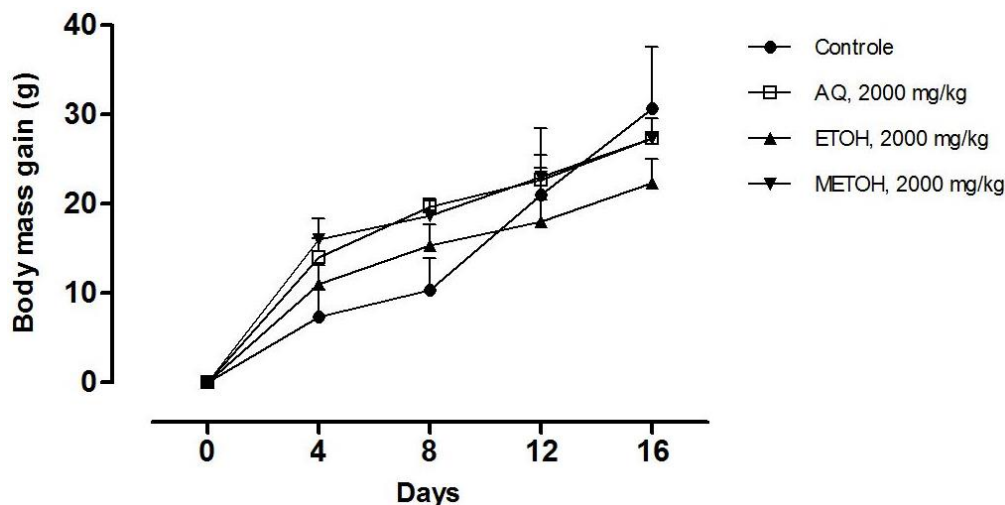


Figure 2. Effect on body mass gain (g) in normal healthy female rats treated with single acute oral dose of 2000 mg/kg AE, EE and ME of *P. americana* leaves. Values indicate mean \pm SEM (n = 3; OECD 423); ANOVA followed by Newman-Keuls, p < 0.05.

0.582 \pm 0.012 and 0.474 \pm 0.007% for AE, EE and ME, respectively.

Acute toxicity determination

In this study, all *P. americana* leaf extracts did not show any toxic effects. Furthermore, neither lethality nor toxic reaction was found at a selected dose of 2000 mg/kg throughout the experiment. Mortality was also not observed when extract were used to treat the rats. With regards to the OEDC guideline, the LD₅₀ values offered by the extract of *P. americana* are higher than 5000 mg/kg. In addition, the gross necropsy test performed on all animals revealed neither hypertrophy, lesion, color change and appearance nor gross pathological impairment of organs at a glance. For further studies, concentrations were fixed at 100 (1/20th), 200 (1/10th) (Oliveira et al., 2008), and 400 mg/kg (1/5th) according to the study protocol.

Changes in body weight

Throughout the 15 days observation of animals, body weight of each within a distinct group was considered for comparison. As a result, no significant difference was noticed between both control and treated groups (Figure 2). Nevertheless, at the end point, body weight gain significantly increased by 27.3% for groups fed AE and ME, and by 22.3% for groups fed with EE.

Organ to body weight ratio

Table 1 shows the organ to body ratio of the different

groups of animals. From the data, there was no significant difference, except the liver to body weight ratio, which was higher in treated rats in comparison with the control group. Another important fact is that, the relative weights of kidney and liver were constant during the experiment.

Biochemical analysis

Table 2 shows the serum content of electrolytic parameters (Na⁺, K⁺, Cl⁻), renal and hepatic function markers (BUN, CREA, AST and ALT) in experimental rat groups as compared to the control. The biochemical parameters of animals treated with AE, EE and ME did not show significant change of level of BUN, CREA, Na⁺, K⁺ and Cl⁻ in the serum even though increase of hepatic function marker levels was noticed in the serum. They appeared to be more pronounced (AST and ALT) for animals groups treated with AE and ME extract (p < 0.01).

Acute hypoglycemic effect

Considering the normoglycemic rats experiment, treatment with *P. americana* extracts at respective concentrations of 100, 200 and 400 mg/kg did not significantly reduce the blood glucose level as compared to that of the control group. Yet, glibenclamide brought about significant reduction of the blood glucose level by 40% (data not shown).

Oral glucose tolerance test (OGTT)

Table 3 shows the blood glucose levels of experimental

Table 1. Organs to body weight ratio of the different groups of animals after 15 days of observation.

Body weight and organs ratio (%)	Groups			
	Control	AE	EE	ME
Body weight (g)	208 ± 13.50	182 ± 10.50	169 ± 14.50	182 ± 13.35
Ratio heart/b.w.(%)	0.39 ± 0.021	0.40 ± 0.037	0.39 ± 0.032	0.31 ± 0.020
Ratio kidney/b.w.(%)	0.67 ± 0.020	0.57 ± 0.052	0.68 ± 0.023	0.62 ± 0.038
Ratio liver/b.w.(%)	2.80 ± 0.111	3.45 ± 0.098	3.75 ± 0.113	3.53 ± 0.182

Control: Rats not treated; AE: rats treated with 2000 mg/kg of aqueous extract; EE: rats treated with 2000 mg/kg of ethanolic extract; ME: rats treated with 2000 mg/kg of methanolic extract. The values are expressed as mean ± S.E.M. (n = 3/group, OCED 423); ANOVA followed by Newman-Keuls, p < 0.05.

Table 2. Biochemical parameters of rats treated with acute oral dose of 2000 mg/kg of *P. americana* extracts after 15 days of observation.

Biochemical parameters	Groups			
	Control	AE	EE	ME
BUN (mg/dl)	20.0 ± 2.60	20.5 ± 1.70	25.0 ± 3.00	23.0 ± 2.60
CREA (mg/dl)	0.47 ± 0.03	0.50 ± 0.06	0.57 ± 0.03	0.43 ± 0.03
AST (U/L)	151.67 ± 97.834	285.0 ± 53.799**	224.7 ± 48.074	239.33 ± 159.001
ALT (U/L)	46.67 ± 5.457	121.3 ± 11.319	137.7 ± 27.865	185.0 ± 70.117**
AST/ALT	3.25 ± 0.414	2.35 ± 0.398*	1.63 ± 0.023*	1.29 ± 0.161*
Na ⁺ (mM)	139.3 ± 1.202	139.7 ± 0.333	141.0 ± 0.000	138.7 ± 0.882
K ⁺ (mM)	6.9 ± 0.312	7.0 ± 0.136	8.2 ± 0.704	7.0 ± 22.361
Cl ⁻ (mM)	104.3 ± 0.882	104.0 ± 1.155	102.3 ± 0.333	105.7 ± 1.333

BUN: Blood urea nitrogen; CREA: creatinine; AST: aspartate aminotransferase; ALT: alanine aminotransferase; Na⁺: sodium ion; K⁺: potassium ion; Cl⁻: chloride ion. AE: rats treated with 2000 mg/kg of aqueous extract; EE: rats treated with 2000 mg/kg of ethanolic extract; ME: rats treated with, 2000 mg/kg of methanolic extract. The values are expressed as mean ± S.E.M. (n = 3/group, OCED 423). Significantly different from control group values: * P < 0.05; ** P < 0.01.

rats before and after D-glucose (2.0 g/kg, b.w.) administration by oral route. It appeared that glibenclamide induced significant (p < 0.001) reduction of hyperglycaemia within a time period of ½ to 5h. At 5 h, the reduction rate was about 61.5%, whereas no significant decrease was observed in the control group. On the other side, AE induced significant (p < 0.01) glycaemia depletion within ½ h at a single dose of 100, 200 and 400 mg/kg, after D-glucose ingestion as compared to the control group and 100 mg/kg of AE brought the best reduction (5.6%). Furthermore, the EE (100 mg/kg) was responsible for a significant (p < 0.001) depletion in serum glucose level from ½ to 5 h. The reduction rates were 2.5, 36.8 and 59.6% after 1, 2 and 5 h, respectively. With regard to ME, it induced a significant decrease of hyperglycaemia between 1 and 3 h after glucose administration, except for the 200 mg/kg dose.

DISCUSSION

Persea americana Mill. or avocado plant tree is well-known across both the south American and the sub-

Saharan African continent (Adeboye et al., 1999; Ojewole et al., 2007). An ethnobotanical survey carried out in Côte d'Ivoire showed the use of the named plant to treat diabetes (N'guessan et al., 2009). Primarily, the present study led to a phytochemical investigation of three leaf extracts of the plant (aqueous, ethanolic and methanolic). Secondly, the study also compared the acute toxicity of the plant extracts aforementioned as well as their hypoglycaemic and anti-hyperglycaemic activities.

The chemical investigation of the crude extracts of *P. americana* revealed the presence of bioactive compounds, namely alkaloids, flavonoids, tanins, coumarine, sterols/polyterpenoids, saponins and polyphenols. These results were in accordance with those reported previously (Adeboye et al., 1999; Adeyemi et al., 2002). The amount of total flavonoids determined in a hydro-ethanolic leaf extract of *P. americana* by Lima et al. (2012) (0.730 ± 0.005%) was higher than the one obtained in the present study (0.474 - 0.582%). This may be due to the solvent extraction method using the mixture water/ethanol.

From the acute toxicity evaluation performed according to the self-modified OCDE 423 protocol, on healthy

Table 3. Effect of *P. americana* extracts on fasting blood glucose level (mg/dl) after oral load of D-glucose (2.0 g/kg) in normoglycemic rats.

Groups	Dose mg/kg	Blood glucose concentration (mg/dl) ± S.E.M (% Decrease)						
		-½ h (Initial)	0 h	½ h	1 h	2 h	3 h	5 h
Control	-	63.8 ± 2.267	81.4 ± 2.064	140.8 ± 4.974	128.0 ± 5.206(9.1)	92.0 ± 8.361(34.7)	76.0 ± 2.408(46)	59.6 ± 3.203(57.7)
Glibenclamide	10	58.2 ± 3.056	58.0 ± 1.844***	101.0 ± 3.688**	69.8 ± 2.035***(30.9)	43.4 ± 2.482***(57)	38.8 ± 2.035***(61.6)	38.8 ± 2.596** (61.6)
Aqueous extract (AE)	100	62.6 ± 3.341	74.8 ± 2.354	118.8 ± 3.056**	112.2 ± 1.200*(5.6)	81.6 ± 3.516(31.3)	69.4 ± 0.980(41.6)	60.6 ± 2.482(49.0)
	200	63.4 ± 3.265	72.2 ± 2.800*	117.2 ± 5.886*	114.8 ± 2.871(2.0)	96.6 ± 4.771(17.6)	72.0 ± 3.098(38.6)	63.4 ± 2.315(45.9)
	400	55.6 ± 2.462	80.8 ± 2.083	119.4 ± 5.381**	122.2 ± 5.911(-2.3)	92.6 ± 9.563(22.4)	79.6 ± 7.922(33.3)	69.8 ± 7.513(41.5)
Ethanol extract (EE)	100	64.8 ± 6.807	68.8 ± 3.513*	105.4 ± 10.948**	102.8 ± 5.463*(2.5)	66.6 ± 4.118**(36.8)	50.8 ± 2.083***(51.8)	42.6 ± 1.691***(59.6)
	200	59.0 ± 2.408	75.0 ± 2.302	124.0 ± 4.290	125.8 ± 5.774(-1.5)	97.8 ± 6.522(21.1)	79.2 ± 3.513(36.1)	68.4 ± 4.250(44.8)
	400	59.2 ± 5.314	78.4 ± 4.675	78.4 ± 4.675*	114.2 ± 5.911(-5.0)	105.6 ± 2.542(2.9)	86.4 ± 5.988(26.6)	74.2 ± 2.478(31.8)
Methanol extract (ME)	100	60.8 ± 4.684	71.8 ± 4.554	104 ± 9.891**	82.8 ± 4.883*** (21.0)	67.0 ± 5.701*(36.1)	58.6 ± 6.713*(44.1)	53.2 ± 7.677(49.2)
	200	64.2 ± 3.826	75.2 ± 2.354	114.0 ± 6.641*	94.4 ± 5.689*** (17.2)	76.0 ± 2.588(33.3)	72.2 ± 3.555(36.7)	63.2 ± 3.891(44.6)
	400	58.0 ± 2.966	70.2 ± 1.655*	122.8 ± 2.289*	92.2 ± 3.121*** (24.9)	74.8 ± 5.398(39.1)	56.6 ± 4.377*(53.9)	46.0 ± 3.701(62.5)

The values are expressed as mean ± S.E.M. (n = 5/group). Statistically different from control (ANOVA followed by Newman-Keuls, p < 0.05); *P < 0.05; **P < 0.01; *** P < 0.001; Values in parenthesis indicate the percentage of decrease calculated from the corresponding ½ h glycaemia value in each group.

female rats, the leaf extracts of *P. americana* were found to be well tolerated by animals at a unique oral route dose of 2000 mg/kg. Neither mortality nor compartmental trouble was observed. The protocol states that *P. americana* is part of the category 5 of the Global Harmonized classification System (GHS) with a LD₅₀ value greater or equal to 5000 mg/kg (LD₅₀ ≥ 5000 mg/kg), hence, non-toxic. Then, the obtained figures in this study best fit within the latter limit. Furthermore, gaining around 27g in weight by rat was observed after extracts uptake (Figure 2), even though there was no significant difference with the control group. The macroscopic autopsy of the vital organs (liver, heart and kidney) did not show any injury or color change, even though the ratio of organs to body weight index exhibited a slight increase of the

treated rat's liver weight. This, according to literature, suggests that the leaf extracts used for the experiments did not show significant toxicity for the liver; consequently would do not exhibit any functional side effect on kidneys (Odo et al., 2014).

The biochemical analyses carried out however revealed a significant increase (p < 0.01) in the amount of either AST or ALT, respectively for group AE and ME as compared to the control group. In this study, the ratio AST/ALT, which fell respectively at 2.35 and 1.29 for groups EE and ME, indicates an abnormality as to the hepatic function of the liver; thus, confirming the outcomes of the study performed (Ezejiofor et al., 2013). On the other side, these results displayed the leaves of *P. americana* to be of lesser toxicity than that of

the ripe fruit's seeds extracts. The latter parameter falls within the range of 1200.75 and 1767 mg/kg for the mice investigated (Padilla-Camberos et al., 2013; Pahua-Ramos et al., 2012). All in all, it could be stated that the unique dose of 2000 mg/kg affects in a short term, the liver's metabolism rather than that of the kidney, as confirmed by the biochemical factors (urea, creatinine and blood ionogram). However, chronic toxicity survey needs to be carried out by applying the unique dose of 2000 mg/kg to ascertain its tolerance in the long term. This could be done by assessment of the liver's synthesis function (albumin and triglycerids), cell integrity (transaminase), hepato-biliary channels permeability (alkalin phosphate and bilirubine) and its malignancy (prothrombine time period)

because the treatment of diabetes last for life. All in all, the fact that anti-hyperglyceamic effective doses are far from the LD₅₀ is encouraging.

The present study confirms the hypoglyceamic and antihyperglyceamic effect of the glibenclamide, which are mostly encountered with normal glyceamic rats. These effects are more pronounced than the extracts' doses administered in the course of our experiment. Previous investigation as related to hypoglyceamic and anti hyperglyceamic activity on normoglyceamic rats showed that if *P. americana* did not have any effect on basic glyceamia (fasting glyceamia), it would have significantly stopped the provoked hyperglyceamia. In fact, aqueous, ethanol and methanol extracts of *P. americana* exhibited an anti-hyperglyceamic activity of variable intensity and life span.

On the contrary, the three doses (100, 200 and 400 mg/kg) of the AE prevented prematurely postprandial or provoked hyperglyceamia after one hour of glucose administration. This suggests that the extracts were responsible for inhibited intestinal absorption of glucose. Consequently, the peak of hyperglyceamia was decreased in comparison with the control and glibenclamide. This phenomenon suggests that the AE could contain bioactive molecules capable of inhibiting α -amylase and α -glycosidase. Nevertheless, these results need further investigations such as absorption and inhibition mechanisms of glucose.

The EE and ME also extended the anti-hyperglyceamic activity at a low dose of 100 mg/kg after 3 to 5 h of glucose administration. Therefore, this activity seems not to be dose-dependent and could be correlated with the polyphenol's content and especially with that of flavonoids. EE having significant content of polyphenols and total flavonoids ($2952.7 \pm 166 \mu\text{gGAE/g}$ and 0.582%, respectively), as compared to the ME ($1873.1 \pm 63.5 \mu\text{gGAE/g}$ and 0.474%), brought about long lasting anti-hyperglyceamic activity. In fact, the inhibition of intestinal absorption of glucose has already been demonstrated earlier on (Tadera et al., 2006), and seems to be similar to that of hypoglyceamic sulfamids like glibenclamide.

However, extension of the anti-hyperglyceamic activity requires other mechanisms that involve the liver, the pancreas and measles. Mastery of the mechanisms mentioned, led to further studies that identified polyphenols as glucose metabolism regulators; using several mechanisms such as, protection and recovery of β cells' integrity, stimulation of insulin secretion and increase of glucose uptake by cells (Solayman et al., 2016; Vinayagam et al., 2016). Studies carried out previously, displayed an important anti-oxidizing role of polyphenols. Especially, flavonoids exhibited a protecting effect against toxicity and oxydating stress. On the other hand, saponines also demonstrated an anti-hyperglyceamic effect due to inhibition of glycogenesis (Elekofehinti, 2015; Ezejiofor et al., 2013).

Finally, flavonoids, tannins and saponins also displayed

anti-hyperglyceamic properties through inhibition of the sodium-glucose transporter 1 (S-Glut 1) according to Tiwari and Rao (2002).

Conclusion

The findings of the present study demonstrated the anti-hyperglyceamic potential of the leaf extracts (AE, EE and ME) of *P. americana* along with their lower toxicity. Consequently, it constitutes justification of the named plant as a medicinal herb. Additionally, diabetes mellitus is a chronic disease, further pharmacological investigations over a long period of time are needed to better assess plant's toxicity and elucidate mode of action.

Conflict of interests

The authors have not declared any conflict of interests.

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