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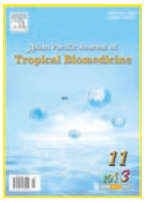

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Assessment of antidiabetic activity and acute toxicity of leaf extracts from *Physalis peruviana* L. in guinea-pig

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PEER REVIEW

Peer reviewer

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Comments

This is an exciting research work in which authors have demonstrated the hypoglycemic activity and acute toxicity of *P. peruviana* leaves in guinea pig. The activity was assessed based on blood glucose lowering effects and biochemical parameters. Leaves demonstrate promising anti-diabetic medicine. The use of leaves should be carefully adjusted because of the possible presence of glycoalkaloids.
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ABSTRACT

Objective: To verify the antidiabetic activity of leaf extracts from *Physalis peruviana* L. popularly used in the Eastern part of the Democratic Republic of the Congo and to point out the possible toxicity.

Method: Aqueous decoctions prepared from dried leaves powder were administrated to guinea pigs at the dose range of 100 mg/kg to 3.2 g/kg of body weight. The hypoglycemic activity was evaluated by glucose tolerance test, loading animals with glucose 4 g/kg and measuring blood glucose concentrations at various times. The effect was compared to the control and glibenclamide as antidiabetic reference drug. Acute toxicity was evaluated by recording mortality rate, changes on blood biomarkers and damage caused to vital organs.

Results: At a dose of 100 mg/kg, the aqueous extract induced a significant reduction of peak concentration at 30 min after glucose loading as compared with control or reference ($P<0.05$). At doses greater than 400 mg, some alterations on blood, kidney and liver markers were observed. Upper 800 mg/kg, mortality was observed with LD_{50} estimated at about 1 280 mg/kg. At the autopsy, vital organs were in haemorrhage and swelling state.

Conclusion: The crude aqueous extracts from the leaves of *Physalis peruviana* L. present hypoglycemic activity in animal model, but at high doses the plant may cause severe intoxication.

KEYWORDS
Physalis peruviana, Leaves, Antidiabetic, Hypoglycemic, Toxicity, Diabetes

1. Introduction

Diabetes is a metabolic disease characterized by a disorder in the regulation of carbohydrate metabolism. It leads to high blood glucose concentration or hyperglycaemia. The predictions made by the WHO indicate that the global growth in the world of the prevalence of diabetic patients, mostly type-2, will reach 439 million people by 2030[1]. Huge progress has been made in pharmacological discovery of new treatments. The cost and availability of modern therapies, however, still make it difficult for a great proportion of African

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populations to fully take benefit of these therapies. The use of antidiabetic plant extracts, thus, will remain a common and unavoidable practice.

Ethnopharmacological surveys conducted in many countries have documented a considerable inventory of plants used to treat diabetes[2,3]. In a survey conducted in the Eastern part of the Democratic Republic of the Congo (DRC) a number of traditional healers pointed out the use of *Physalis peruviana* L. (*P. peruviana*) fruit and leaves for this purpose. *P. peruviana* L.(*physalis*=bladder) is a plant indigenous from South America which has been cultivated in South Africa in the region of the Cape of Good Hope since at least the early 19th century. It is now cultivated in other parts of African regions. Based on size, colour and taste of the fruit, shape of the flower head and the height and size of the plant, *P. peruviana* L. has been classified into ecotypes from different regions or countries. Three types of *P. peruviana* L. are grown in South Africa, Kenya and Colombia, respectively[4]. The fruit, also known as *Mbuma* (Bukavu), Cape gooseberry (South Africa), Peruvian groundcherry, *Pok pok* (Madagascar), *Poha* (Hawaii), *Ras bhari* (India), *Aguaymanto* (Peru), *Uvilla* (Ecuador), *Uchuva* (Colombia), *Harankash* (Egypt), is sweet when ripe, with a characteristic mildly tart flavor, making it ideal for snacks, pies or jams. Medically, *P. peruviana* L. has been used as a medicinal herb to treat cancer, leukemia, malaria, asthma, hepatitis, dermatitis, rheumatism or diabetes. Various studies have been conducted *in vitro* and *in vivo* to get scientific based proofs[5-9].

However, being a member of the Solanaceae plant family, *P. peruviana* L. may contain solanine, a substance known to cause gastrointestinal toxicity (vomiting, diarrhea and abdominal pain) and neurological troubles like headache and hallucinations, even death[10]. Given the wide use of this plant, the determination of pharmacological and toxicological properties is worth carrying out to optimize its therapeutic use and safety. This study *in vivo* on animal model in guinea pig has been undertaken to validate the efficacy and safety of using *P. peruviana* L. leaves to treat diabetes.

2. Materials and Methods

2.1. Plant material

Fresh leaves of *P. peruviana* L. were collected in Bukavu town in the Eastern part of DRC. The species was authenticated in the laboratory of Botany at the Faculty of Science and Applied Science of the Université Officielle de Bukavu (UOB).

2.2. Preparation of leaf extract

As in traditional medicine, the extract used was a 10% (w/v) decoction. It was obtained as follows: Fifty grams of powdered leaves and 500 mL of distilled water were mixed in a flask. The mixture is kept boiling for 15 min. After cooling, the extract is filtered through cotton wool and kept in a clean sealed flask. Before the experiment, aliquots of the filtrate were concentrated by evaporation on a hot plate and then placed in oven at 50 °C for 24 h to make a dry extract. Solutions were remade in saline at different concentrations for oral administration.

2.3. Animals

Guinea pigs of both sexes aged 3 to 4 months old and weighing 350 to 450 g were chosen for this experiment to evaluate the hypoglycemic activity and acute toxicity. These guinea pigs were kept in the animal boundary of the Faculty of Medicine and Pharmacy, prepared and used according to the standards required for experiment on laboratory animals (EEC, 1986)[11].

2.4. Hypoglycemic effect test

Healthy animals were randomly assigned to the control and treatment groups, so as to compare the mean body weight values of the groups: the control group, the reference group and the test group. During the experiment, each animal was housed in its own cage. Oral glucose tolerance test on normal guinea pigs was performed using a glucose bolus 4 g/kg body weight delivered with a force-feeding needle[12]. Fourteen hours before experiment (overnight), animals were fasted to enable stable baseline glucose levels to be measured before the oral glucose tolerance test and avoid food interference on the absorption of aqueous extracts of the plant.

Thirty minutes before drug administration blood samples were taken to determine glycaemia baseline values (T_{-30}). Then, the control group received by force-feeding 1 mL of saline per 100 g body weight; the reference group received the solution of glibenclamide as 2.5 mg/kg body weight, and the test group received the extract solution equivalent dose of 100 mg/kg body weight. A second blood sampling was taken just before the glucose loading (T_0). Then, each animal received by force-feeding the solution

of glucose 50% (w/v) as 4 g/kg body weight. After glucose administration, plasma samples were taken passed 30, 60, 90, 120 and 180 min (T_{30}, T_{60}, T_{90}, T_{120} and T_{180}), respectively. One touch electronic Glucometer (One Touch Ultra®) was used for glucose measurement.

2.5. Acute toxicity test

Healthy animals were randomLy assigned into five groups and were given the extracts by feeding cannula at the doses of 200 mg, 400 mg, 800 mg, 1 600 mg and 3 200 mg/kg body weight. The animals were then observed for 96 h. Behaviour signs were recorded and the number of dead guinea pigs in each group was counted to estimate the LD_{50} graphically by Probit analysis.

After intoxication of guinea pigs, we collected blood for the determination of biochemical parameters. Serum was separated and analyzed for creatinine, urea (BUN), and transaminases (AST, ALT), white blood cells (WBC) and red blood cells (RBC) count. WBC and RBC count were determined by hematocytometer method using Türck's solution and saline solution[13,14]. BUN was measured by Berthelot colorimetric method[15]. The determination of creatinine was made by the method of Jaffe using picric acid and 0.4 mol/L NaOH[16]. Transaminases were assayed with Emekyn SGOT (AST) and Emekyn SGPT (ALT) Kits Biovision.

The whole vital organs from dead animals were removed and examined. The macroscopic external features of the selected organs were performed to detect any abnormal signs. One guinea pig from the control untreated group was anesthetized and killed to serve as control.

2.6. Statistical Analysis

All studies mentioned above were done in triplicate except for the LD_{50} study. The LD_{50} was calculated using Probit analysis (SPSS v16). All values were expressed as mean ±standard error of the mean (SEM) and were analysed by One-way analysis of variance (ANOVA) followed by Scheffe post hoc test, and statistically significant findings were considered at P-value<0.05.

3. Results

3.1. Hypoglycemic effect

Table 1 shows blood glucose concentrations measured at different times after 4 g/kg glucose loading. Thirty minutes before starting the experiment, blood samples were collected to obtain the baseline of blood glucose concentration after overnight or 12 h fasting. The values measured (T_{-30}) were not significantly different between the control group given saline solution (113±7) mg/dL, the reference treated with glibenclamide 5 mg/kg (102±19) mg/dL and the test group treated with *Physalis* extract 100 mg/kg (89±21) mg/dL. The baseline values (T_0) taken before glucose loading were (134 ±12) mg/dL for control group, (91±16) mg/dL for reference group, and (80±16) mg/dL for plant extract group. The difference observed between T_{-30} and T_0 values is due to the early hypoglycemic activity of glibenclamide and the extract (Figure 1).

Table 1

Blood glucose concentrations measured at different times (min).

Time	Experiment groups of guinea-pigs			ANOVA	
	Saline (C)	Glibenclamide (R)	*Physalis* extract (E)	T*C	T*R
T_{-30}	113±7	102±19	89±21	NS	NS
T_0	134±12	91±16	80±16	S	NS
T_{30}	600±0	471±44	246±19	S	S
T_{60}	551±55	262±28	214±3	S	S
T_{90}	345±52	190±42	205±3	S	NS
T_{120}	241±35	116±25	149±25	S	NS
T_{180}	164±54	57±16	110±2	S	S

T*C *Physalis vs.* Control; T*R *physalis vs.* Glibenclamide; significant or non significant at P=0.05.

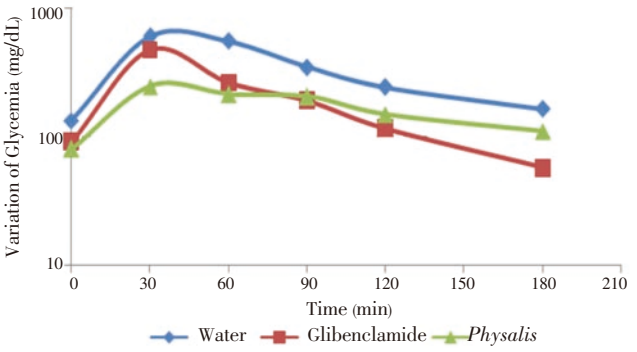

Figure 1. Evolution in Log-normal scale of glycemia (mean values n=3) as function of time.

Reference group received glibenclamide 2.5 mg/kg; Test group received *Physalis* extract 100 mg/kg; Control group 1 mL saline; each animal received glucose solution 4 g/kg.

The blood sugar concentration remains higher from T_{30} to T_{180} compared with both the reference and the *Physalis* groups. *Physalis* extract significantly lowered the peak concentration (P<0.05) compared to both control and reference at the corresponding given doses. The slope of the extract (−0.006), however, is smaller than that of glibenclamide (−0.014).

3.2. Acute toxicity test

Five groups of guinea-pigs, each comprised of 6 animals,

were treated with *Physalis* leaf extract at different doses. No signs of intoxication were noted up to 400 mg/kg dose of the plant extract. At higher (800 mg/kg to 3 200 mg/kg) signs of intoxication were observed including restraint of animals, chills, hesitation, rustling hair, anuria and finally death. Figure 2 shows the experimental data from animals exposed to each of five extract doses. Graphically on probit scale, the equation of the dose-mortality estimated LD_{50}=1 280 mg/kg (95% CL: 927-2 035). Changes in the values of blood biochemical markers are presented in the Table 2. At the dose 100mg/kg used to test the hypoglycaemic activity, the values of WBC, RBC, creatinine and BUN remained in normal ranges for guinea-pigs. From 200 mg/kg dose of the extract, the values of WBC, RBC, creatinine and BUN increased while the values of SGOT and SGPT were decreased. The images of the vital organs are shown in Figure 3. Organs from dead animals are in dark bloody and swollen.

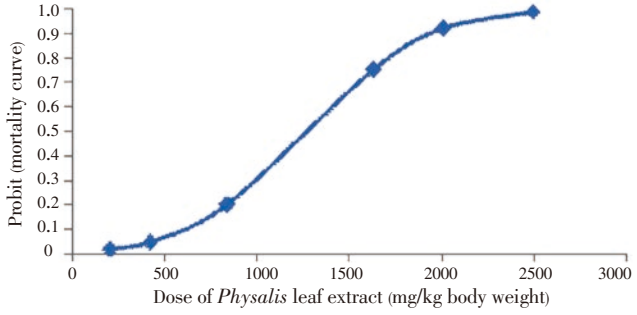

Figure 2. Adjusted probit toxicity curve of leaf extract of *P. peruviana* in guinea-pig LD_{50}=1 280 mg/kg (n=6 animals per dose).

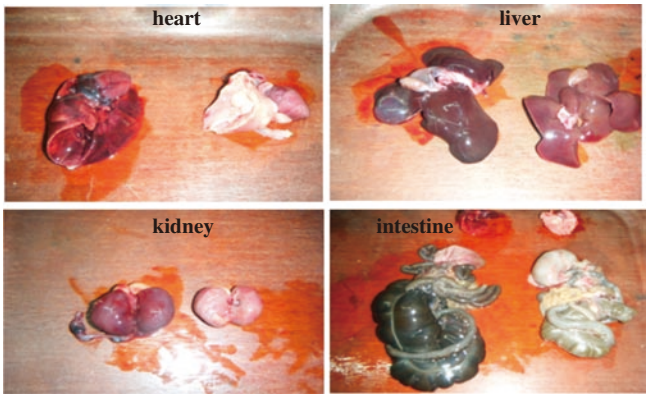

Figure 3. The autopsy of dead guinea pigs showed swollen and bloody heart, liver, kidney and gastro-intestine system as compared to untreated animals (right).

4. Discussion

4.1. Hypoglycemic effect

Many studies reported in the literature are related to the properties of the fruit. In our study we have been interested in the leaves of the plant. The administration of glibenclamide 2.5 mg/kg as reference drug resulted in a rapid decline of the glycaemia after peak concentration obtained 30 min after glucose loading. The administration of 100 mg/kg of leaf extract of *P. peruviana* significantly reduced the glucose peak concentration compared to glibenclamide and control. This finding confirms studies of this kind that have been conducted to evaluate the hypoglycemic activity of the fruit of *Physalis* sp. by different methods *in vitro*[7], in rats[4,7,17] and in human[18]. When comparing the slope of glucose decline after the peak, the reference drug promoted a sustained steeper fall than *Physalis* extract. Glibenclamide as sulphonamide derivative is known to act through insulin release from the pancreas. Herbal medicines for diabetes can be classified into four categories according to their mode of action: drugs acting by modifying glucose utilization, drugs acting by inhibition α-glucosidase the enzyme implicated in hydrolytic cleavage of oligosaccharide in the brush border of small intestine mucosa, drugs acting like insulin, drugs acting on insulin secreting beta cells, and drugs acting by miscellaneous mechanisms[19,20]. The hypoglycaemic effect of the extract may result from one or many of the aforementioned mechanisms.

The main active constituents in the fruit of *P. peruviana* L. are Physalins A, B, D, F and glycosides, which showed multiple activities[21]. This suggests that some of the effects observed in leaf extracts, may be partly due to the action of these pseudo-steroids that can be also present in leaves.

4.2. Toxicity of aqueous extract

No signs of intoxication were noticed with 100 mg/kg dose. Non-observed-adverse-effect-level (NOAEL) was

Table 2

Blood chemistry values of guinea pigs in acute toxicity study in control and groups treated with different doses of *P. peruviana* L.

	WBC ($\times10^3$/mm^3)	RBC ($\times10^8$/mm^3)	Creatinine (mg/dL)	BUN (mg/dL)	SGOT (U/L)	SGPT (U/L)
Normal ranges	7-18	4.5-7.0	0.6-2.2	9.0-31.5	25.3-349.2	0.5-90.8
G1: 0 mg/kg	10.3±0.2	4.8±1.2	0.5±0.1	59.2±2.5	465.9±14.2	109.5±6.2
G2: 400 mg/kg	20.0±0.7	11.8±0.2	2.3±0.2	113.5±1.2	92.1±2.5	97.3±1.2
G3: 800 mg/kg	32.1±0.6	12.1±0.2	3.3±0.2	127.9±2.9	73.2±2.1	75.1±1.3
G4: 1 600 mg/kg	43.3±0.5	12.8±0.1	3.5±0.1	152.6±5.6	57.2±1.5	57.4±1.0
G5: 3 200 mg/kg	35.7±0.2	14.7±0.1	3.6±0.2	135.1±1.9	26.0±1.4	47.7±2.4

Normal values are from Wagner and Manning[24]. All values are means±SEM of three guinea-pigs.

400 mg/kg body weight. At higher (800 mg/kg to 3 200 mg/kg) signs of intoxication were observed including restraint of animals, tremors, hesitation, hair rustling, anuria and finally death. The magnitude of oral LD_{50} (1 280 mg/kg body weight) is about three times greater than NOAEL.

According to our results, doses higher than 100 mg/kg induc higher values of WBC, RBC, creatinine and BUN; and lower values of SGOT and SGPT. The increase in blood cells may be caused by loss of intravascular fluids. The increase of urea could be explained by increased degradation of protein compounds, but also by impaired renal function while changes in transaminases witness liver disturbances. Arun and Asha[9] demonstrated the antihepatotoxicity of *Physalis* ripe fruit against acute hepatotoxicity induced by carbon tetrachloride and concluded that the extract was found to be devoid of any conspicuous acute toxicity in rats. Ahmed and Kamal[22] also demonstrated the possible protection against nephrotoxicity caused by carbon tetrachloride. In our study using leaf extracts, the feature of vital organs at autopsy showed haemorrhage.

Even though the nature of toxic components were not determined, *P. peruviana* (L.), as member of Solanaceae family, contains some amount of solanine a toxic substance belonging to poisons called glycoalkaloids that are commonly found in the nightshade family[23]. Common effects of solanine poisoning in man can be stomach cramps, nausea, throwing up, diarrhea, irregular heartbeat, dizziness, and headaches. More severe effects can be dilated pupils, fever, hallucination, loss of sensation, paralysis, jaundice, hypothermia and death. It has been suggested that the lowest dose of solanine to cause nausea in an adult is 25 mg and a 400 mg dose of solanine to be potentially fatal for an adult. Rabbits given 20 mg/kg *ip* generally died within 24 h[10].

This study focused on the evaluation of the hypoglycemic activity and acute toxicity of aqueous extracts of leaves of *P. peruviana* L. in guinea pigs. Oral administration of 100 mg/kg of a 10 % (w/v) decoction revealed hypoglycaemic properties of *P. peruviana* L. leaves in guinea pigs. The maximum tolerated dose (MTD) is higher than the dose required having pharmacological effects. However, like unripe fruit which has been recognized toxic, the use of leaves should also be carefully adjusted or discouraged.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Diabetes mellitus is a metabolic disorder in which a person has abnormally high blood sugar concentration. This is due to the fact that the body does not produce enough insulin. It is therefore necessary to use plant extracts for stimulating insulin secretion or to reduce glucose level in blood.

Research frontiers

The present research work depicts in vivo anti-diabetic and acute toxicity activity of aqueous extract of *P. peruviana* in guinea pig assessed by measuring blood glucose and estimating mortality rate, changes on blood biomarkers and damage caused to vital organs.

Related reports

There are related reports but not on the leaves of this medicinal plant. The only related work is on fruits. This report is on the anti-diabetic activity of the leaves of *P. peruviana*.

Innovations and breakthroughs

P. peruviana fruits are widely used in the eastern part of DRC to treat diabetes. In the present study, authors have demonstrated for the first time the hypoglycemic activity of the leaves of *P. peruviana* and vital organs damage caused at high dose in guinea-pig model.

Applications

From the literature survey it has been found that *P. peruviana* is toxic to humans. This study support and suggest the use of the leaves instead of the fruits of this plant as blood glucose lowering at a low dose.

Peer review

This is an exciting research work in which authors have demonstrated the hypoglycemic activity and acute

toxicity of *P. peruviana* leaves in guinea pig. The activity was assessed based on blood glucose lowering effects and biochemical parameters. Leaves demonstrate promising anti-diabetic medicine. The use of leaves should be carefully adjusted because of the possible presence of glycoalkaloids.

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