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Assessment of antidiabetic activity and acute toxicity of leaf extracts from *Solanum nigrum* L. (Solanaceae) in guinea-pigs

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Abstract

Hypoglycemic and antidiabetic activities and acute toxicity of extracts from leaves of *Solanum nigrum* L., an edible plant used in the Eastern part of the Democratic Republic of the Congo against diabetes, were evaluated on guinea-pigs. The hypoglycemic activity was evaluated by glucose tolerance test by loading animal with glucose and measuring blood concentration at various times. For inducing experimental diabetes mellitus, Alloxan monohydrate was used in normal saline by subcutaneous administration and glibenclamide was used as standard drug. Acute toxicity was evaluated by recording mortality rate, changes on blood biomarkers and damage caused to vital organs. Obtained results showed a significant decrease ($P < 0.05$) in blood glucose levels in guinea-pigs treated with the aqueous extract of *S. nigrum* both in hypoglycemic and antidiabetic tests. The decrease in the group treated with *S. nigrum* aqueous extract was sometimes more pronounced than that of the positive control group, justifying the use of this plant in traditional medicine in the management of diabetes. No death and side effects were observed for doses under 270 mg/kg bw but for doses upper than this value some side effects and death were noticed with 100% of death at the dose of 8640 mg/kg bw. The autopsy analysis in dead guinea-pigs showed the damage caused on vital organs such as kidney, liver; intestines, heart and lungs for intoxicated animals.

Keywords: *Solanum nigrum*, guinea-pig, diabetes, toxicity, hypoglycemic activity, blood biomarkers

1. Introduction

Diabetes is a metabolic disorder associated with hyperglycemia and caused by defect in insulin secretion or insensitivity of target organs to insulin [1-3]. It is a chronic metabolic disease that is listed among life-threatening conditions and projected to grow from the current 0.5% prevalence rate to 9.5%, or 300 million diabetic patients in 2025, among which fifteen million people from sub-Saharan Africa [4, 5].

Despite significant progress in the treatment of this disease, research on new drugs against diabetes continues because many synthetic drugs have shown their limits. Among the proposed solutions, is the use of anti-diabetic herbal medicine. This approach offers to date, an interesting alternative due to the discovery of increasingly growing plant extracts effective in the treatment of type 2 diabetes [6-12].

Solanum nigrum L. or *Solanum americanum* L. is a widely distributed tropical plant used as vegetable and medicinal plant in management of various diseases among which diabetes in Eastern part of the Democratic Republic of the Congo (DRC) [6-11]. It is an annual herb, belonging to solanaceae, a family well known and that has been screened by researchers for medical actions of its species. Studies have been reported in the literature referring to the antifungal, anticancer, anti-oxidant, antipyretic, hepatoprotective, hypolipidemic, anthelmintic, anti-inflammatory, antimicrobial, antihyperlipidemic, anti-tumour and neuropharmacological properties. Solanaceae species are indicated in the treatment of convulsion, skin diseases, rheumatic, gouty joints, etc. [6, 12-17].

However, some solanaceae species contain solanine, a substance known to cause gastrointestinal toxicity (vomiting, diarrhea and abdominal pain) and neurological troubles like headache and hallucination, even death [16].

In our knowledge no studies have been reported on the antidiabetic effect and toxicity of *Solanum nigrum* L in guinea-pigs. Present investigation is therefore designed to determine the hypoglycemic and antidiabetic effects of aqueous extract of this plant species (the locally cultivated plant species) after daily oral administration of dose for twenty-eight days and their possibly toxicity in guinea-pigs in order to optimalise his therapeutic use and security.

2. Material and methods

2.1. 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. These guinea-pigs were kept in the animal boundary of the Faculty of Medicine and Pharmacy, of the Official University of Bukavu. Prepared and used according to the standards required for experiment on laboratory animals [18]. They were fed with natural herbs and kept in housing sun lighted before the experiment (Fig. 1).

2.2. Plant material

The plant shown in Figure 1 was identified in the Laboratory of Ecology and Plants Resource Management, at the Faculty of Sciences and Applied Sciences of the Official University of Bukavu. Fresh leaves of *Solanum nigrum* (L.) were collected in Bukavu town in Eastern part of DRC, dried in the shade and out of direct sunlight, moisture and insects, in the Laboratory of Pharmacognosy and then ground in a mortar before passing the powder through a sieve.



(a)



(b)

Fig 1: *Solanum nigrum* L. (a) and Guinea-pigs naturally herbal fed (b)

2.3. Hypoglycemic test

The antihyperglycemic effect was evaluated with the glucose tolerance test (GTT) as described elsewhere [19]. Healthy animals were randomly assigned to the negative control group (given only 1ml saline/100g BW), the positive control group (given glibenclamide 2.5mg/100g BW) and the test group or *S. nigrum* group (given extract solution 200mg/kg BW). 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 glycemia baseline values (T-30). A

second blood sampling was taken just before the glucose loading (T0). Then, each animal received by force-feeding the solution of glucose 50% (w/v) as 4 g/kg BW. After glucose administration, blood samples were taken at 30, 60, 90, 120, 150 and 180 min respectively. One touch electronic Glucometer (One Touch Ultra®) was used for glucose measurement [19]. Oral glucose tolerance test was addressed in normal animal [19,20].

2.4. Antidiabetic test

Hyperglycemia was induced by injecting alloxan monohydrate at a dose of 120 mg/kg [20-22] subcutaneous. Animals were considered diabetic when the blood glucose level was raised beyond 185 mg/dL of blood. This condition was observed at the end of 6 days after alloxanisation. The animals were segregated into three groups of three guinea-pigs in each. The animals were kept under observation and after 6 days, they were tested for hyperglycemia using glucometer (One Touch Ultra®) and glycemia have been removed at first day (D1), third days (D3), fifth day (D5), seventh day (D7), fourteenth day (D14), twenty-first day (D21) and twenty-eighth (D28). Animals were divided into three groups:

a) Negative Control group: this group was kept as normal control animals without any treatment. It received a saline solution (1mL/ 100 g body weight).

b) Positive control group: This group was taken the solution of Glibenclamide as 2.5 mg/Kg body weight.

c) *S. nigrum* group: This group was taken the extract solution of *Solanum nigrum* equivalent dose of 200 mg/kg body weight.

2.5. Toxicity evaluation

Healthy animals were randomly assigned into five groups and were given the extracts by feeding cannula at the doses of 270 mg, 540 mg, 1080 mg, 2160 mg, 4320 and 8640 body weight. The animals were then observed for 72 hours. Behaviour signs were recorded and the number of dead guinea-pigs in each group was counted to estimate the LD50 graphically by Probit analysis. After intoxication of guinea-pigs, the blood was collected for the determination of biochemical parameters. The 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 Türk's solution and saline solution [22-24]. BUN was measured by Berthelot colorimetric method [22]. The determination of creatinine was made by the method of Jaffe using picric acid and 0.4 mol/L NaOH [24]. 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.

The values of treated groups were compared statistically with the control by Independent Sample t test and one-way ANOVA. The inferences were made from findings at 95% confidence level. Data obtained were presented as mean \pm standard deviation.

3. Results and discussion

3.1. Evaluation of Hypoglycemic effect

Table 1 gives concentration of glucose in blood of animals at different times.

Table 1: Blood glucose level of animals' groups at different times

Group	Blood glucose level (mg/dL)							
	T-30	T0	T30	T60	T90	T120	T150	T180
Neg. Control	64±3	101±4	294±9	279±14	259±11	233±16	134±19	117±7
Pos. Control	102±9	118±8	188±10	219±11	200±8	185±14	88±5	83±4
<i>S. nigrum</i>	86±4	97±4	255±14	262±14	202±14	153±9	145±10	94±5

Table 1 shows a significant decrease ($P < 0.05$) in blood glucose levels in guinea-pigs treated with the aqueous extract of *Solanum nigrum* L. after 90 minutes of treatment. At T120 the glucose level value of this animal became less than that of the guinea-pigs group treated with the glibenclamide used as positive control. This indicates that *Solanum nigrum* has antihyperglycemic effect and justify the use of this plant in traditional medicine against hyperglycemia. Our results confirm that of Maharama *et al.* [21] on the effect of *Solanum nigrum* leaves in normoglycemic rats. According to these authors, the aqueous extract of *Solanum nigrum* leaves in normoglycemic rats model showed that blood glucose levels decrease significantly ($p < 0.01$) with effect from 6h onwards till the end of 10h. This study showed that the test extract had

dose dependent hypoglycemic effect. The glucose tolerance level of *S. nigrum* leaf extract mediated AgNPs-treated group was also evaluated in diabetic rats by estimating its efficacy in reducing hyperglycemic condition in blood followed an administration of glucose orally, and compared it with standard drug (Glibenclamide 0.5 mg/kg) [25]. At the end of the test, the glycemic value of the experimental group becomes close to that of standard drug group, as it is also observed in our results.

3.2. Evaluation of Antidiabetic effect

Table 2 indicates concentration of glucose in animals' blood from first day (D1) to 28th day (D28)

Table 2: blood glucose level of animals' group at different days

Group	Blood glucose level (mg/dL)						
	D1	D3	D5	D7	D14	D21	D28
Negative control	192±7	185±8	192±4	192±8	-	-	-
Positive control	199±15	199±15	198±14	192±16	141±18	95±2	85±2
<i>S. nigrum</i>	193±14	192±14	191±15	157±18	113±17	96±2	82±2

These results show the death of all animals in the negative control group since the 7th day. It means, no animal in this group has resisted to the alloxanisation and all untreated animals have died. However, no death was recorded during four weeks in all animals treated with the aqueous extract of *Solanum nigrum* and the reference drug (glibenclamide). The blood glucose level showed significant decrease for the following weeks in both glibenclamide (positive control) and *S. nigrum* groups. The decrease in the group treated with *S. nigrum* aqueous extract was more pronounced than that of the positive control group. This indicates that aqueous extract of *Solanum nigrum* prevent induction of diabetes by alloxanisation effect and justify the use of this edible plant in Congolese traditional medicine in the management of diabetes [7, 8, 11].

These results confirm that already obtained by other researchers on other animal models [25-30]. In fact, an experimental study revealed that the methanolic and aqueous extracts from *Solanum nigrum* L. (100 and 200 mg/kg) orally administered produced a significant decrease in the blood glucose level in the model of alloxan-induced diabetes in rats (With tolbutamide as reference drug). The plant extract almost brought down blood glucose level by 50% in diabetic animals [22]. Another work, the effect of ethanolic extract of

Solanum nigrum leaves in alloxan monohydrate induced hyperglycemic rats showed that the extracts reduce blood glucose level significantly in dose dependent manner starting from 2h to the end of 10 h of the study, while standard drug Glibenclamide showed similar effect during the course of experiment [28]. It was also showed that aqueous extracts of leaves and fruits of *S. nigrum* decrease the level of glucose and glycosylated hemoglobin in rats after 30 days, 60 days and 90 days of treatment, while the stem extract has no profound effects [29-31].

The mode of action of alloxan is well documented [29, 31]. It was shown that alloxan damages pancreatic B cells by the liberation of oxygen radicals, with a reduction in antioxidant status. Glibenclamide as sulphonamide derivative is known to act through insulin release from the pancreas. The hypoglycaemic and antidiabetic effects of the *S. nigrum* extract may result from one or many mechanisms. Some chemical groups like flavonoids contained in this plant can prevent diabetes by their antioxidant effect [3, 32].

3.3. Acute toxicity test

Table 3 shows the effect of *S. nigrum* aqueous extract at different doses on animals after 96 hours.

Table 3: Effect of aqueous extract of *Solanum nigrum* L. on oral acute test in guinea-pigs after 96 hours.

Group	Number of guinea-pig	Treatment and dose (mg/Kg bw)	Number of guinea-pig died	Percentage of guinea-pig died (in %)
Control	3	0	0	0
Group1	3	270	0	0
Group2	3	540	1	33.3
Group3	3	1080	1	33.3
Group4	3	2160	2	66.6
Group5	3	4320	2	66.6
Group6	3	8640	3	100

Table 4 gives values of biochemical parameters of animals' blood in acute toxicity study

Table 4. Blood biochemical parameters values of guinea-pigs in acute toxicity study in control and groups treated with different doses of *Solanum nigrum* L.

Group	WBC (WBC/mm ³)	RBC (10 ⁹ /L)	Creatinine (mg/dL)	BUN (mg/dL)	SGOT (U/L)	SGPT (U/L)
Control (0mg/kg BW)	2833 ± 181	0.4 ± 0.1	1.0 ± 0.2	28.0 ± 2.9	26.0 ± 6.7	27.3 ± 1.8
G1 (270mg/kg BW)	2333 ± 151	0.3 ± 0.1	3.4 ± 1.8	112.1 ± 17.6	79.5 ± 18.3	49.8 ± 6.2
G2 (540mg/kg BW)	2066 ± 249	0.7 ± 0.2	6.3 ± 0.5	101.0 ± 25.2	80.6 ± 11.9	60.6 ± 8.2
G3 (1080mg/kg BW)	3600 ± 432	0.6 ± 0.3	6.1 ± 2.0	135.8 ± 5.6	135.8 ± 5.6	68.5 ± 47.1
G4 (2160mg/kg BW)	4066 ± 324	0.4 ± 0.1	10.6 ± 4.2	223.0 ± 122.5	95.2 ± 34.6	45.7 ± 6.8
G5 (4320mg/kg /BW)	6266 ± 139	1.1 ± 1.3	18.1 ± 10.6	99.5 ± 7.3	120.7 ± 14.4	93.3 ± 8.2
G6 (8640mg/kg BW)	7833 ± 116	1.1 ± 0.1	14.2 ± 1.2	109.0 ± 3.6	125.0 ± 8.6	108.0 ± 14.9

Table 5 indicates behavioral manifestations of animals in acute toxicity after 72 hours

Table 5. Behavioral manifestations in acute toxicity after 72 hours

Intoxication signs	dose (mg/kg weigh body)						
	0	270	540	1080	2160	4320	8640
Bristly hair	-	-	+	+	+	+	+
Feeding difficult	-	-	+	+	+	+	+
Continous chills	-	-	+	+	+	+	+
Displacement problems	-	-	+	+	+	+	+
Breathing disorders	-	-	+	+	+	+	+
Membrane paralysis	-	-	-	-	-	+	+
Coma	-	-	-	-	-	+	+

Legend: "Positive sign (+), Negative sign (-).

Figures 2a, 2b and 2c show the autopsy of dead guinea-pigs. It can be seen swollen and bloody liver, kidney and heart as compared to untreated animals

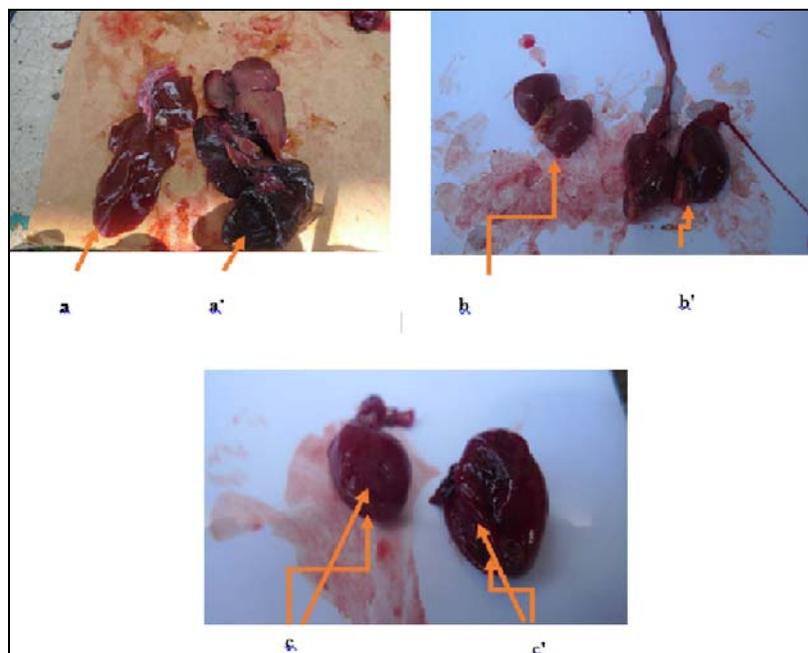
**Fig 2:** The autopsy of dead guinea-pigs. Healthy Liver (a), Intoxicated Liver (a'), Healthy Kidney (b), intoxicated Kidney (b'), Healthy Heart (c) and Intoxicated Heart (c').

Table 3 shows that for doses under 270 mg/Kg bw there is no death of animals and no side effects as confirmed in table 5. For doses upper than this value, some side effects and death began with 100% of death at the dose of 8640 mg/Kg bw. The damage caused on vital organs can be found by the difference between organs of intoxicated and healthy animals (Fig.1). Indeed, the results obtained from the autopsy analysis in dead guinea-pigs showed a swelling and modification of organs such as kidney, liver, intestines, heart and lungs. The presence of clots blood in the heart and coagulated blood in the thorax was observed also.

Despite its beneficial effects *Solanum nigrum* may have some toxic effects. Although *Solanum nigrum* is considered to be an edible plant, its toxicity is mainly due, as for many species in the *Solanaceae* family, to the presence of solanine, a glycoalkaloid causing varying degrees of toxicity in a dose-dependent manner. The symptoms of poisoning in humans due to solanine are reported to include nausea, vomiting, diarrhoea, headache, dizziness, loss of speech, fever, sweating, tachycardia, pupil dilation, blindness, mental confusion, convulsions, coma, and death [33-38]. Some of these effects were noted in guinea-pigs in this study.

An oral median lethal dose of the ethanol extract of *Solanum nigrum* L. was estimated as 3129 mg/kg body weight based on acute toxicity by the oral in mice [6].

Assessment of hematological indices is crucial in evaluating the toxicity of exogenous compounds, including medicinal plants, in the system. It also can be used to explain blood-related functions of plant extracts. This is important in toxicity evaluation because hematological responses to botanicals have higher predictive values for humans when the data are translated from animal studies [39-42].

In this study, blood urea and serum creatinine showed normal levels indicating that the test drug did not interfere with renal function and renal integrity was preserved. In fact, the serum creatinine level is a good indicator of renal function since any elevation of serum levels is associated to a marked failure of nephrons function [43, 44]. Also there was no significant changes in various hematological parameters such as Hb, RBC, WBC, platelets and differential count compared to the control group, which indicates that plant extracts may not be toxic and do not affect circulating red cells hematopoiesis or leucopoiesis [43].

4. Conclusion

This study demonstrated that the aqueous extract of leaves of *Solanum nigrum* L. leaves has anti-hyperglycaemic and antidiabetic effects in the guinea-pigs, comparing to glibenclamide (reference drug). The maximum tolerated dose (MTD) is higher than the dose required having pharmacological effects. However, the plant should be used with caution. While promising, further study is needed for the development of this plant into a pharmaceutical product for the treatment of diabetes.

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6. Ethical approval

All authors hereby declare that 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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