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RESEARCH ARTICLE

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EVALUATION OF THE ANTIDIABETIC ACTIVITY OF THE EXTRACTS OF VITELLARIA PARADOXA IN ORYCTOLAGUSCUNICULUS RABBIT (LAGOMORPH)

ABSTRACT

Diabetes mellitus is a state of chronic hyperglycemia whose global prevalence is constantly increasing, particularly in Côte d'Ivoire. Currently available drugs encountered more difficulties due to its adverse effect. In other to find lasting solution to this problem, an experimental study was undertaken with the extracts of Vitellaria paradoxa plant to evaluate its antidiabetic activity. Extraction method of bark of plant has enabled us prepare 250 mg/kg/body weight (bw) for both the crude aqueous extract (ETA) and the hydro-ethanolic extract (EHE). For hypoglycemic activity , oral injection of extract into each lots of normoglycemic rabbits (15 in number) was used to select the most active extract, which was selected to evaluate the antihyperglycemic activity of extract with doses of 80, 400 and 800 mg/kg/bw given orally to rabbits in state of temporary hyperglycemia.

The results obtained from these two plant extracts in normoglycemic rabbits showed hypoglycemic effect of ETA and EHA of - 3.57 % and - 15.91 % respectively after 2 hours of treatment. EHE being more active also showed a more antihyperglycemic activity from T_{30} to T_{90}. This activity increases with increasing doses, with a concentration of 800 mg/kg/bw, blood glucose level decreased from 60.87 % to 3.26 % to be stabilized to normal blood sugar level.
In conclusion of these two extracts, hydro- ethanolic extract is the most active. This extract has a double activity hypoglycemic and antihyperglycemic.

Keywords Antihyperglycemic activity, Diabetes, Normoglycemic, Vitellaria paradoxa

1. INTRODUCTION

Diabetes mellitus is an ubiquitous endocrine disease which is characterized by alteration of the homeostasis of carbohydrates, lipids and proteins. Its unbridled growth affects approximately 5 % of the world population [1]; nearly 3.4 million people die from the consequences of this disease [2]. According to WHO projections, the number of deaths will increase and will double by 2030. This will make the disease the seventh leading cause of death worldwide [3]. Moreover, in developing countries nearly 80 % of deaths was recorded [4]. In Côte d' Ivoire, for example, the prevalence of diabetes indicates a rate of 5.7 %, of which 90 % are non-insulin dependent diabetes [5] and the causing factors are the consumption of calorie – rich diet, obesity, physical inactivity and the aging of the population [6]. Chronic hyperglycemia in diabetes mellitus is the cause of many late complications characterized by kidney disease, blindness and the risk of cardiovascular disease [7].

Also available drugs often encounter difficulties in their treatment because of side effects. However, herbal treatment is an alternative in the treatment of this pathophysiology in as much as plants are used to cure diabetes [8]. It is in line with this idea that Pharmacodynamic Biochemical Laboratory conducted study on the plant species Vitellaria paradoxa traditionally
used in northern Côte d'Ivoire for the treatment of diabetes.

Ethnobotanical studies have shown that the leaves of this species are used in the traditional medicine for the treatment of Burili ulcer [9] and its bark in the treatment of malaria, dental pain and neuralgia treatment [10]. In addition, scientific studies have shown antibacterial activity of the extracts of Vitellaria paradoxa [11] and data on the toxicity of this species show a maximum tolerated dose (MTD) and a lethal dose 50 (LD_{50}) to be 800 mg/mL and 820 mg/mL respectively [12, 13].

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The objective of this study is to evaluate the antihyperglycemic and hypoglycemic effect of crude aqueous extracts as well as hydro-ethanolic extracts of this plant which could lead to development of a new anti-diabetics therapy.

2. MATERIALS AND METHODS

2.1. Material

The bark of Vitellaria paradoxa used in this study were identified by National Floristic Center of University of Felix Houphouet -Boigny Abidjan. As for the animals used, they were rabbits of both sexes aged 16 weeks of the race Cunistar and New Zealander with an average mass of 1.2 kg [14].

2.2. Methods

2.3. For the preparation of plant extracts, stem bark was dried in the laboratory away from sunlight for two weeks, and then pulverized in a mechanical grinder. The obtained powder was dissolved in distilled water, for 24 hours decoction. This solution was dewatered in a clean white towel and filtered through hydrophilic sorbent and wattman paper successively. The filtrate was concentrated in a rotary evaporator and dried to give a brown powder which is "the crude aqueous extract" ETA.

Furthermore, 25 g of ETA powder was added to 100 mL of distilled water corresponding to a diluted solution of 250 mg/mL. The solution was sterilized by autoclaved and intended to be administered at a dose of 250 mg/kg/bw. For the preparation of hydro-ethanolic extract (EHE), 35 g of powder was dissolved in ethanol-water solvent mixture in the proportions 70/30; v/v. The mixture was macerated using a magnetic stirrer for 24 hours and then decanted. The supernatant corresponding to the ethanolic phase was subjected under vaccum at 50 °C to obtain the hydro-ethanolic extract 70 %. This extract an hydro- ethanolic solution at a concentration of 250 mg/kg was used. The solution is to be administered under the same conditions as the ETA at the dose of 250 mg/kg/bw in rabbits in a normoglycemic state.

Then, different concentrations of the more active extract were prepared 800 mg/mL, 400 mg/mL and 80 mg/mL respectively, to be administered into rabbits in a state of temporary hyperglycemia. This hyperglycemia state was obtained by subjecting rabbits to treatment to increase the blood sugar level of the subject (rabbits) for a specified time (3h 30) by oral administration of a solution of glucose (2 g/kg/bw). The solution was freshly prepared with 2 g of anhydrous glucose dissolved in 2 mL of distilled water (concentration = 1g/mL). This preparation is to be given to the animals orally using a syringe 8 cm^3 fitted with esophageal catheter [15].

The numbers of identified animals are 35 distributed in different cages. Group A, consisting of 15 rabbits divided into 3 lots of 5 used for normoglycemia study and group B , comprising of 20 rabbits divided into 4 lots of 5 were used for hyperglycemia study. All these animals were acclimated for two weeks and received 150 g daily of a conventional feeds produced by IVOGRAIN ®. With regard to the treatment of animals, (Group A) the recommendations of the Ethnopharmacological symposium was followed [16].

For normoglycemic animals, the treatment was done with the crude aqueous extract and hydro-ethanolic extract. During this evaluation, the animals in the control lot (lot 1) received orally distilled water with a volume of 10 mL/kg/bw, those of lot 2, were administered with crude aqueous extract at a dose of 250 mg/kg/bw, and those of lot 3, received orally hydro-ethanolic extract at a dose of 250 mg/kg/bw. After these treatments, the blood glucose assay was done by taken blood samples from the marginal vein of the ear after fasting for 12 hours, then every 1 hour and that for up till 4 hours after this treatment.

With regard to the treatment of animals in a state of temporary hyperglycemia (Group B), the method is the same as mentioned above.

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Therefore animals in the lot 1 served as a control lot , while those of lots 2, 3 and 4 were administered orally with hydro-ethanolic extract dose 80, 400, and 800 mg/kg/bw respectively.

Concerning blood glucose assay, a first blood sample was taken after fasting for 12-hours from the marginal vein of the ear at 90 minutes before the oral administration of the glucose solution (T_{-90}). Followed by administration of the hydro-ethanolic extract at doses of 80, 400, and 800 mg/kg/bw. Thereafter, a second blood sample was taken, followed by administration of glucose solution at T_0. From this time T_0, a series of blood samples were taken at regular intervals of 30 minutes for 120 minutes (that is at T_{30}, T_{60}, T_{90} and T_{120}). The programming of the hyperglycemic state is performed so that the optimum activity of the hydro-ethanolic extract coincides with the maximum hyperglycemia in animals. Furthermore, blood samples collected were distributed into tubes containing sodium fluoride and potassium oxalate. They were centrifuged at 3000 revs/min for 5 minutes; obtained serums were used for the determination of blood glucose.

The principle of the glucose assay is based on the oxidation of glucose in the presence of peroxidase. For this methodology, 10µL of serum was stirred with 1 ml of reaction solution at a temperature of 16-25 °C for 10 minutes. The reading of the optical density was obtained at a wavelength λ of 500 nm and the results are expressed in g/L.

The statistical data are processed using an analysis of variance (ANOVA) of the GRAPH PAD PRISM® software. The results obtained are blood glucose level expressed in g/L (mean ± SD) to a precision P >0.05.

The determination of the percentage change in glucose level is done using average values from the following formula:

$$\% \text{ Variation in glucose level} = \frac{G_t - G_0}{G_0}$$

G_0: glucose values before treatment of rabbits at time t_0.
G_t: glucose values in rabbits treated at time t after administration of different preparations.

3. RESULTS AND DISCUSSION

The data obtained during the treatment of animals in normoglycemic state were shown in Table 1. They allow for comparison of different blood glucose values obtained from total aqueous extracts and hydro-ethanolic extract compared to control.

This evaluation showed that blood glucose was greatly reduced when animals were treated with hydro-ethanolic extract (0.89 g/L to 0.74 g/L) , and a moderate reduction with crude aqueous extract (0.95 g/L to 0.81 g/L) after 2 hours of treatment. The data in Table 1 were used to determine the percentage change in glucose represented by figure 1.

The curves of Figure 1 reflect the percentage change in blood glucose of the crude aqueous, hydro-ethanolic extracts and the control. Thus, curves that get closer to the abscissa indicate a high activity characterized by a drop in blood glucose. Therefore, the curve corresponding to animals treated with hydro-ethanolic extract has a greater hypoglycemic activity (-15.91 %). The result obtained from the treatment of animals in group B are recorded in Table 2 showing the effect of different doses of the hydro-ethanolic extract corresponding to a lower blood glucose.

Before the induction of hyperglycemia (T_{-90} to T_0), the doses of 400 and 800 mg/kg/bw of EHE administered to rabbits induces a

decrease in blood glucose from 0.90 to 0.85 g/L; and from 0.92 to 0.82 g/L respectively, against the 80 mg/kg/bw dose which cause no drop in blood glucose (Table 2). Subsequently, after feeding the animals with glucose solution (T_{30} to T_{90}), blood glucose gradually decreases when animals are treated respectively with doses of 80 mg/kg/bw, 400 mg/kg/bw and 800 mg/kg/bw (Table 2). However, this decrease was temporary, as the blood glucose in animals treated with doses of 400 mg/kg and 800 mg/kg/bw stabilizes around 1 g/L (Table 2) and that of the animals receiving 80 mg/kg/bw reaches a value of 1.58 g/L.

These results were used to determine the percentage change in blood glucose level shown in Figure 2. The curves in Figure 2 represent the percentage change in blood glucose of animals subjected to treatment of different doses of the hydro-ethanolic extract. Thus, curves that approached the abscissa indicate excellent activity characterized by a drop in blood glucose level. Therefore, observation of the curves shows the lowest glucose level before administration of glucose solution (T_{-90} to T_0) in all animals treated , with a significant reduction in the dose of 800 mg/ kg/bw (-10.87 %). However, a peak gradually appears 30 minutes after hyperglycemia respectively at doses of 800 mg/kg/bw (+ 60.87 %); 400 mg/kg/bw (+ 81.11 %) and 80 mg/kg/bw (+ 109.30) followed by an unstable drop in blood glucose which slightly decreases to stabilize around the value of 1 g/L in rabbits treated with doses of 400 mg/kg/bw and 800 mg/kg/bw (1.17 g/L and 0.98 g/L respectively). In particular, at a dose of 80 mg/kg/bw this value reached 1.58 g/L.

This study evaluated the activity of the aqueous extracts and hydro-ethanolic extract on blood glucose in normoglycemic rabbits. The result from this assessment shows that, crude aqueous extract and hydro- ethanolic extract of the bark of Vitellaria paradoxa induce a gradual drop in blood glucose of -3.57 % and -15.91 % respectively . The EHE is 5 times more active than the ETA (Figure 1). These results also show hypoglycemic activity of these extracts at a dose of 250 mg/kg/bw. The effective dose (250 mg/kg/bw) is within the range of required doses (100 - 1500 mg/kg/bw) in the case of lowering blood glucose in scientifically tested animals [17, 18]. The hypoglycemic activity of the EHE is more active than the total aqueous extract of Moringa oleifera leaves at a dose of 250 mg/kg/bw. Indeed, the EHE of Vitellaria paradoxa induced a significant reduction in blood glucose (-15.91 %) than the most active dose of Moringa oleifera (-14.01 %) after 2 h of treatment [19]. This reveals that the hypoglycemic activity of the hydro- ethanolic extract of Vitellaria paradoxa is 1.13 times more active than that of Moringa oleifera.

Thereafter, rabbits in a state of temporary hyperglycemia after receiving increasing doses of EHE of Vitellaria paradoxa, high blood glucose decreases gradually. This reflects the dose-response effect of EHE (Figure 2). This phenomenon is common to other plant species that have been experimentally studied, like the case of Allium cepa L., Jatropha gossypiifolia, the mixture of (Citrulus colocynthis, Acacia modesta and Polygonum fagopyrum L.) [20, 21, 22]. In contrast, in some species such as Cynodon dactylon [23], increasing doses induce increasingly lowering effect. Moreover, in rabbits treated with 400 mg/kg/bw and 800 mg / kg bw, this decrease is followed by a slight increase that stabilizes around 1 g/L at T_{120} (Table II). Thus this allows us to conclude that the hydro-ethanolic extract has an antihyperglycemic activity. When comparing this activity to that of the crude extract of leaves of Sclerocarya birrea which the percentage change in blood glucose was successively 25.88 %; 7.69 %; and 3.28 % respectively in the 30th; 60th; and 90th minute after feeding. Thus from our result the antihyperglycemic activity of hydro-ethanolic extract of Vitellaria paradoxa is more active than that of this specie [24]. Indeed, this activity varies with time (about 3 times more active at the 60th minute and 90th minute). Thus, the hypoglycemic and antihyperglycemic double activity induced by hydro-ethanolic extract in about 1-7 hours makes it to be classified as potentially antidiabetic plants [25, 26]. This observation is also found in crude acetonic extract of leaves of Vernonia colorata , which has a double activity, hypoglycemic activity in normoglycemic rats and antihyperglycemic in diabetes type 2 induced rats [27, 28, 29].

4. CONCLUSION

The present study shows that the crude aqueous and hydro-ethanolic extracts of the bark of Vitellaria paradoxa induce antiglycemic activity in rabbits at a dose of 250 mg/kg/bw. In addition, hydro-ethanolic extract has an antihyperglycemic activity at doses of 400 mg/kg/bw and 800 mg/kg/bw. This suggests that antidiabetics bioactive substances are present in these extracts with a high concentration in the hydro-ethanolic extract. Further studies on the characterization of active principles will reveal the chemical groups or constituent responsible for the antidiabetic activity.

5. DECLARATION OF CONFLICT OF INTEREST

The authors wish to declare that there is no conflict of interest.

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Glucose level (g/L)					
Groupes	Time (Hours)	Parameters			
		Average	Maximum	Minimum	SD
Control	0	0.82	0.90	0.76	±0.03
	1	0.92	1.13	0.80	±0.08
	2	0.85	0.94	0.80	±0.03
	3	0.90	1.11	0.77	±0.08
	4	0.84	0.88	0.79	±0.02
ETA	0	0.84	0.93	0.77	±0.04
	1	0.95	1.29	0.72	±0.12
	2	0.81	0.88	0.74	±0.03
	3	0.86	1.01	0.70	±0.07
	4	0.85	0.94	0.76	±0.04
	0	0.88	1.00	0.78	±0.05

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EHE	1	0.89	0.91	0.86	±0.02
	2	0.74	0.88	0.60	±0.09
	3	0.76	0.86	0.66	±0.07
	4	0.88	0.91	0.85	±0.02

Table 1: glucose values in rabbits of group A

ETA : Crude aqueous extract
EHE : Crude hydro-ethanolic extract

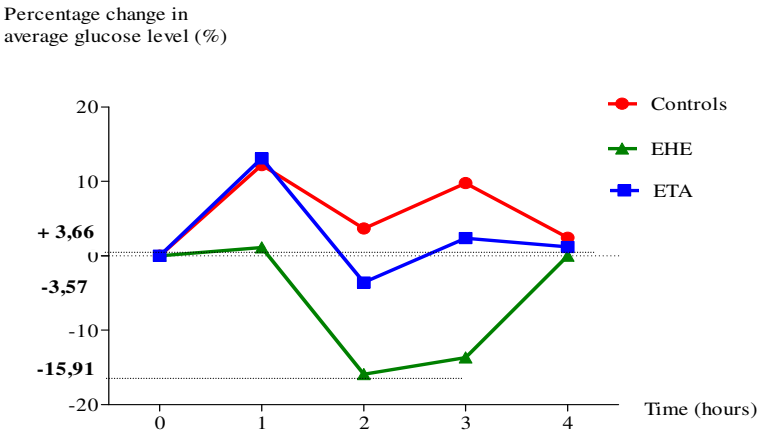

Figure 1: Percentage change in glucose level of the vitellaria paradoxa extract

Concentration EHE (mg/kg/bw)	Glycemia (g/L)						
		Times					
	Parameters	T_{-90}	T_0	T_{30}	T_{60}	T_{90}	T_{120}
[0]	average	0.93	0.98	1.86	1.92	1.90	1.95
	maximum	1.12	1.16	2.73	2.96	2.80	2.02
	minimum	0.77	0.80	0.99	0.88	1.00	1.88
	SD	±0.06	±0.06	±0.26	±0.47	±0.27	±0.03

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[80]	average	0.86	0.88	1.80	1.50	1.48	1.58
	maximale	1.00	1.06	2.60	2.00	1.78	1.88
	minimale	0,75	0,70	1,00	1,00	1,18	1,28
	SD	±0.08	±0.12	±0.50	±0.38	±0.20	±0.20
[400]	average	0.90	0.85	1.63	1.11	1.09	1.17
	maximale	1.20	1.10	2.36	1.22	1.28	1.54
	minimale	0.52	0.60	0.90	1.00	0.90	0.80
	SD	±0.18	±0.14	±0.39	±0.07	±0.10	±0.21
[800]	average	0.92	0.82	1.48	0.95	0.92	0.98
	maximale	1.06	0.92	2.01	1.02	1.03	1.14
	minimale	0.78	0.72	0.95	0.88	0.81	0.82
	SD	±0.06	±0.04	±0.22	±0.03	±0.03	±0.05

Table 2 : Values of glucose level of rabbits in Group B in a state of temporary hyperglycemia

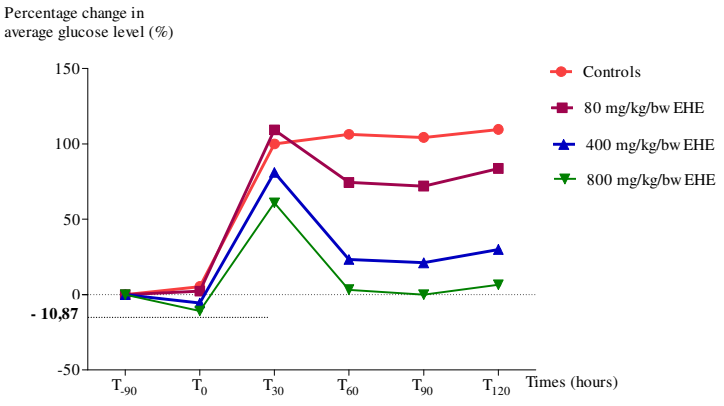

Figure 2: Percentage change in blood glucose based on doses of hydro-ethanolic extract of vitellaria paradoxa

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