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Original Article

Effects of partitioned extracts from hydro-ethanolic extract of *Vitellaria paradoxa* on glucose tolerance in rabbits

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ABSTRACT

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia. The majority of people suffering from this disease rushed to the markets in search for medicinal plants for cure, this attitude are justified due to an exorbitant cost of pharmaceutical drugs. For example in this study, partitioned extracts from hydro-ethanolic extract of plant species *Vitellaria paradoxa* administered to rabbits have optimized the antidiabetic activity of the plant. A solvent Partitioning method of hydro-ethanolic extract was used to prepare six (6) extracts concentrated to 400 mg (the hexane aqueous extract, aqueous ethereal extract, dichloromethane extract, the aqueous dichloromethane extract, acetatic extract and aqueous acetatic extract). In addition, these extracts were subjected to a phytochemical screening for chemical compounds identification. For the pharmacological test, an oral injection of each extract to six (6) rabbit's lots as well as distilled water to the control lot (a total number of 42) was used to evaluate the plants' activity on blood glucose. The results of our experiment have shown, on one hand the hypoglycemic effect for aqueous ethereal extract, dichloromethane, aqueous dichloromethane extract and the aqueous acetatic extract. On the other hand an antihyperglycemic effect for acetatic extract (from +49.05 % to -11.32 %) and aqueous acetatic extract (from +14.63 % to 0 %). Of these two extracts, the effect of aqueous acetatic extract on glucose corresponds to the desired glucose tolerance model sought in animals. The aqueous acetatic extract is the most active partitioned extracts showing both hypoglycemic and antihyperglycemic activity.

Keywords: Diabetes -Activity - *Vitellaria paradoxa* - Phytochemical screening

INTRODUCTION

Diabetes mellitus is a disease that is expressed by a set of clinical syndrome as it influences the metabolism of carbohydrates, fats and blood proteins to produce chronic hyperglycemia. It is classified into two (2) types, namely type I diabetes characterized by the massive destruction of β cells resulting in insulinopenia and the type II corresponding to a decrease in insulin secretion and insulin resistance [1]. Many factors that could favor the occurrence of diabetes include obesity, malnutrition [2], a sedentary lifestyle and aging of the population [3]. The complications are nephropathy, atherosclerosis, retinopathy and blindness [4]. Furthermore, the press release concerning the number of people suffering from this disease shows the seriousness in public health. Nearly 382 million people are suffering from diabetes worldwide, incuding more than 5.1 million deaths per year, or 14,000 deaths per day and one death every seven seconds [5]. Compared to HIV where (1.4-1.9 million) people die per year, diabetes is sadly becoming a famous disease [6]. In Ivory Coast, for example, about 1 million Ivorian are suffering from this disease and majority are financially handicapped so therefore cannot afford to pay for their treatment as 70-96 % of their family budget is absorbed by the treatment expenses [7]. Good news, an alternative medicine is available to them: medicinal plants that are easily accessible, at affordable cost and proved efficacy

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[8]. In order to highlight the usefulness of these plants, previous studies on the hydro-ethanolic extract of the plant species *Vitellaria paradoxa* showed antidiabetic activity [9]. Further study, will enhance the antidiabetic activity of this plant extract. The aim of our study was to evaluate the effect of partitioned extract of hydro-ethanolic extract of *Vitellaria paradoxa* on blood glucose.

MATERIAL AND METHODS

Biological test : Barks of *Vitellaria paradoxa* plant were used in this study they were identified at the National floristic Center of the Felix Houphouet-Boigny University. As for experimental animals, they were rabbits scientific name *Oryctolagus cuniculus* from the cross breeds of Cunistar and New Zealanders aged 16 weeks with an average weight of 1.2 kg [10].

For the preparation of plant extracts, 10 g of the powder of the hydro-ethanolic extract were formed and subjected each in 400 mL of solvent-water mixture (v/v): hexane-water, petroleum ether-water, dichloromethane-water, ethyl acetate-water following a partitioning procedure. After decanting, the obtained phases were separated and concentrated using a rotary evaporator to give the following extracts: the hexane aqueous phase (EAH), the ethereal aqueous phase (EAE), the dichloromethane phase (ED) and the aqueous extract dichloromethane (EAD), the acetatic phase (EA) and acetatic aqueous extract (EAA). For the preparation of the solution for each extract, 5 g were dissolved in 12.5 mL of distilled water to obtain a concentration of 400 mg/mL. This solution was autoclaved and intended to be administered at a dose of 400 mg/mL of bw to animals in a state of induced hyperglycaemic by oral route. After the experimental study, each extract was subjected to a phytochemical analysis to identify the compounds present in these extracts.

Identified animals were divided into 7 groups of 6 animals each of both sexe. All these animals were acclimated for two weeks and fed daily with 150 g of a conventional pellets supplied by IVOGRAIN® a renown livestock feeds manufacturer.

Regarding their treatment, the animals in group 1 (control lot) received first, 10 ml/kg bw of distilled water orally; secondly, other animals in groups 2-7 respectively were fed with a dose of 400 mg/kg bw of EAH, EAE, ED, EAD, EA and EAA.

Regarding the measurement of blood glucose level, animal were subjected to fasting for a period of 12 hours, their blood were first taken at the ear marginal vein and immediately given distilled water for control rabbits and prepared extracts for other groups (T_{-90}). Subsequently, blood of all animals were collected 90 minutes later (T_0) and were fed immediately with a glucose solution (2 g/kg bw). From that moment T_0, a series of blood sampling were carried out as previously at intervals of 30 minutes for 120 minutes (T_{30}, T_{60}, T_{90} and T_{120}).

The collected blood samples were distributed into tubes containing sodium fluoride and potassium oxalate. They are centrifuged at a speed of 3000 rev/min for 5 minutes. Serum aliquots then were used to measure blood glucose level using glucose-peroxidase method which consisted of 10 µL of serum in 1 mL of reaction solution (15000 U/l glucose oxidase; 1,000 U/l of peroxidase; 92 mmol/L tris buffer pH 7.4; 0.3 mmol/L phenol; 2.6 mmol/L of 4-amino phenazone) at 16-25 °C for 10 minutes. Reading of the optical density was done at the wavelength of λ 500 nm; results obtained were expressed in g/L.

Phytochemical screening: Furthermore, phytochemical analysis was performed in this study to find out the presence of chemical compound like: polyterpenes and sterols, polyphenols, flavonoids, tannins, quinone, alkaloids and saponins. As regards searching for catechin tannins, Stiasny reagent was used, however sodium acetate and ferric chloride are used for the analysis of gallic tannins. Concerning polyphenols, their characterization was made possible in the presence of an alcoholic solution of 2 % ferric chloride. Searching for alkaloids, the ethanol alcohol and reagents of Burchard and Dragendorff was used for their characterization. Sterols and polyterpenes presence in extracts were highlighted with acetic anhydride and concentrated sulfuric acid. The foam index was sed for saponins. As for the flavonoids, dilute hydrochloric acid 2 times, the magnesium turnings and isoamyl alcohol testify to its presence. The diluted hydrochloric acid 1/5, chloroform and ammonia diluted two times were used for the search of quinone.

Statistical method: In addition, the processing of statistical data through the analysis of variance (ANOVA) GRAPH PAD PRISM® software gives blood glucose in g /L (mean ± SD) with an accuracy $P < 0.05$.

RESULTS AND DISCUSSION

Treatment of animals with plant extracts gave results highlighted in the table below (Table I), indicating the antidiabetic activity of different extracts. The values reported in the table above

indicated that the most active extracts were: the acetatic extract and aqueous acetatic extract.

In fact, animals given the aqueous acetatic extract experienced drop in blood glucose 90 minutes after treatment (between 1.23 g/L and 1.16 g/L) and a slight increase 30 minutes (corresponding to T_{30}) after fed (between 1.16 g/L and 1.41 g/L). However, blood glucose subsequently undergoes decrease (between 1.41 g/L and 1.20 g/L), then returns to its base value (from 1.205 g/L and 1.23 g/L) at T_{120}.

Furthermore rabbit given acetatic extract under the same conditions experienced hyperglycemia after 90 minutes of treatment (between 1.06 g/L and 1.21 g/L), and 30 minutes after feeding between 1.21 g/L and 1.58 g/L) followed by a significant drop in blood glucose, with peak value at T_{90} (between 1.58 g/L and 0.95 g/L later dropped to 0.86 g/L). This value is maintained low without returning to its baseline value (0.94 g/L against 1.06 g/L).

However, blood glucose of animals treated with the hexane aqueous extract, ethereal aqueous extract, the extract dichloromethane and the aqueous dichloromethane extract did not undergo any significant decrease till the end of experiment.

The results obtained were used to draw the graph of Figure 1. These graphs illustrate the percentage changes in blood glucose values according to the period of treatment.

When the distance between these graphs from the x-axis is shrinking this explains the stronger the activity of extract is on blood glucose. This is the case for graphs represented in rabbits treated with the aqueous extract of dichloromethane from T_{-90} to T_0, and those stuffed with aqueous acetatic extract and acetatic extract from T_{30} to T_{60}. Careful observation of the curves highlights two important facts.

As for rabbits that received acetatic extract, the evolution of the graph occurs in two aspects. First, an ascending pattern from T_{-90} and T_{30} (from 0 % to + 14.15 % then 49.05 %) indicating an hyperglycaemia state, alternately a descending pattern from T_{30} to T_{90} (from +49.05 % to -10.37 % then -18.86 %) indicating a sustainable antihyperglycemic effect on rabbits and continuous to T_{120} (-11.32 %). On the other hand, in rabbits treated with the dose of the aqueous acetatic extract the curve is in three phases from T_{-90} to T_0 (0 % to -5.69 %) (Descending portion) indicating a hypoglycemic action; alternately an ascending pattern from T_0 to T_{30} (from -5.69 % to 14.63 %) indicating a transient hyperglycemia because first from T_{30} to T_{60} the curve was in almost descending pattern (from 14.63 % to -2.43 %) and from T_{60} to T_{90} substantially ascending (from -2.43 % to -2.00 %) finally constant corresponding to the appearance of normoglycemia (-2.00 % to 0 %) at T_{120}. Meanwhile, the phytochemical test identified chemical groups present in the extracts (Table II).

Previous study has shown that hydro-ethanolic extract is 5 times more active in lowering blood glucose level than the total aqueous extract [9]. Moreover, this extract has an antihyperglycemic effect in dose-response effect. To optimize its effect on blood glucose, it was subjected to partitioning in different solvents. Therefore, the resulting extracts were tested in rabbits in a state of temporary hyperglycemia. At the end of this assessment, it appeared that the aqueous acetatic extract has a hypo and antihyperglycemic effect which brings the blood glucose to its baseline value. This suggests a similar mechanism of action to that of metformin prescribed in the treatment of type II diabetes [11] .On the other hand, the acetatic extract has sustainable antihyperglycemic action similar to that of sulfamides whose mechanism is to promote the secretion of insulin [12].

Furthermore, the phytochemical test showed the presence of identical chemical compounds in the three extracts: aqueous-ethanolic extract,the aqueous acetatic extract and acetatic extract (polyphenols, flavonoids, tannins catechin and alkaloids). The antidiabetic effect of the three extracts is linked to the presence of secondary metabolites such as phenolic compounds (polyphenols, flavonoids and tannins) and alkaloids which act separately or synergistically as demonstrated by Malaisse; and Marles and Farnsworth [13; 8]. These observations are verifiable on the one hand, in the ethyl acetate fraction of *Euonymus alatus* rich in flavonoids and in conjunction with a hypo and antihyperglycemic actions in mice [14]; and secondly, alkaloids involved in pharmaceutical manufacturing Miglitol, an inhibitor antidiabetic gastrointestinal absorption of carbohydrate [15]. However, the absence of chemical compound (polyterpenes and quinone salts) in aqueous acetatic extract (most active extract) would not justify a major role in its activity.

CONCLUSION

The acetatic extract with high antihyperglycemic activity would not be the appropriate model of glucose tolerance for animals. By contrast, the

animals stuffed with the aqueous acetatic extract showed rather a hypo effect and antihyperglycemic which reduces blood glucose to its baseline value. This suggests that the bioactive principles against diabetes are mainly concentrated in acetatic and aqueous acetatic extracts. Further studies will enable us determine the level of concentration of bioactive principles against diabetes.

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Table I: Blood glucose value of rabbits treated with partitioned plant extracts

Concentration of extract (400 mg/kg of bw)	Glycose level en g/L						
		T_{-90}	T_0	T_{30}	T_{60}	T_{90}	T_{120}
Control	mean	0.93	1.60	1.59	1.15	1.02	1.06
	SD	±0.14	±0.27	±0.02	±0.13	±0.12	±0.23
EAH	mean	0.65	1.05	1.58	1.51	1.38	1.11
	SD	±0.22	±0.19	±0.02	±0.19	±0.07	±0.23
EAE	mean	1.07	1.05	1.41	1.37	1.36	1.25
	SD	±0.19	±0.05	±0.34	±0.21	±0.09	±0.07
ED	mean	1.04	0.82	1.69	1.48	1.37	1.30
	SD	±0.03	±0.02	±0.08	±0.02	±0.06	±0.08
EAD	mean	0.95	0.75	1.14	1.20	1.06	1.00
	SD	±0.07	±0.08	±0.06	±0.03	±0.07	±0.08
EA	mean	1.06	1.21	1.58	0.95	0.86	0.94
	SD	±0.27	±0.19	±0.12	±0.09	±0.11	±0.17
EAA	mean	1.23	1.16	1.41	1.20	1.205	1.23
	SD	±0.14	±0.16	±0.51	±0.25	±0.23	±0.14

Table II : Phytochemical Test

Extracts	Sterols & polyterpenes	Poly phenols	Flavonoids	Tannins		Quinone	Alkaloids	Saponins
				Catechin	Gallic			
ETA	-	+ +	+ +	+ +	-	-	+ + +	+
EHE	+	+ +	+ +	+ +	-	+	+ + +	-
EAH	+	+	+	+	-	-	+	-
EAE	+	+	+	+	-	-	+	-
ED	+	-	-	-	-	+	-	-
EAD	-	+	+	+	-	-	+	-
EA	+	+ +	+ +	+ +	-	+	+ +	-
EAA	-	+ +	+ +	+ +	-	-	+ + +	-

- : absence; ++ : moderate ; + : weak ; +++ : abondance

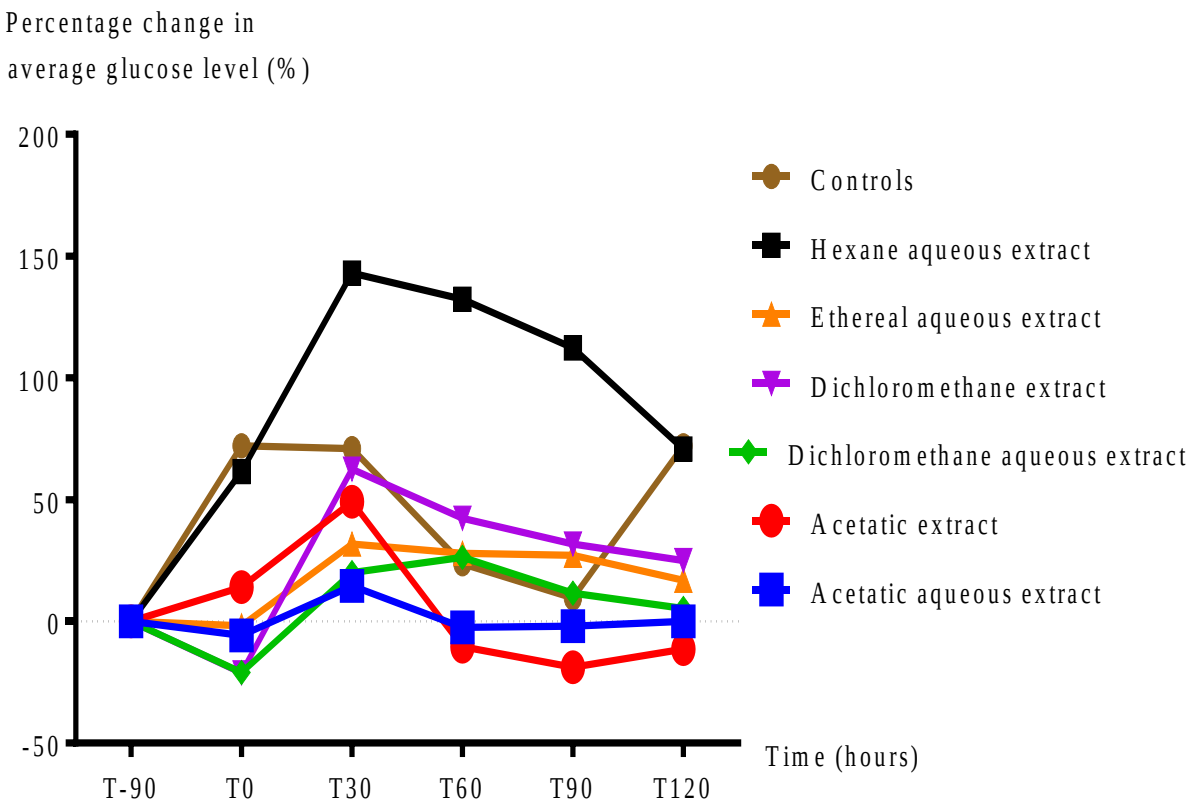

Figure 1: Percentage change in glucose levels of rabbits treated with partitioned extracts

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