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Acute oral toxicity and phytochemical study of "Diabenorme" and "Thuquinone" used to treat diabetes

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ABSTRACT

The aim of the study was conducted to search for phytochemicals and evaluate the acute oral toxicity in mice for aqueous extracts of "Diabenorme" and "Thuquinone" used in the treatment of diabetes. "Diabenorme" is a combination of two medicinal plants: *Persea americana* (Lauraceae) and *Anacardium occidentale* (Anacardiaceae) while "Thuquinone" is composed of *Pycnanthus angolensis* (Myristicaceae). Phytochemical analysis was done using standard methods and acute toxicity test (OCDE 423) was performed by a fixed dose procedure consist in administration of three doses of 300, 2000 and 5000 mg/kg body weight of "Diabenorme" and "Thuquinone". Phytochemical analysis showed that the different drugs contained polyphenols, flavonoids, catechin tannins, alkaloids and saponins. Concerning acute toxicity test, no sign of toxicity and mortality were observed during the experiment after limit test of 5000 mg/kg. Thus, there were no significant differences (Fischer test, $P > 0.05$) in the body weights between the control and treated animals. These results show that the aqueous extracts of "Diabenorme" and "Thuquinone" are potentially safe for oral consumption at acute administration up to dose of 5000 mg/kg. Further investigation is needed to evaluate its sub-acute toxicity.

Keywords: Diabenorme, Thuquinone, Phytochemical screening, acute toxicity.

INTRODUCTION

The use of remedies from traditional medicine in sub-Saharan Africa takes a proportion increasingly important (Millogo et al., 2006). According to WHO, nearly 80% of people in developing countries depend on traditional medicine for the primary health care (WHO, 2002). Diabetes is a metabolic disease whose main characteristic is chronic hyperglycemia resulting from defective secretion, insulin action, or both anomalies. In less than a quarter of a century, diabetes

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mellitus has become a public health problem in developing countries. In Côte d'Ivoire, data already indicated a national prevalence of 5.2% (International Diabete Federation, 2013). A study showed an annual increase of 4.2% of diabetic patients followed at the National Institute of Public Health (NIPH) (Oga et al., 2006). The high cost of treatment and lack of access to diabetes medications led diabetics to use plants to care. Although these plants have beneficial effects, but therapeutic doses and toxicity are unknown or poorly known. This toxicity has risks during use. Therefore, it must be evaluated to determine the dose limits. The aim of this study was to assess the acute oral toxicity of "Thuquinone"(TQ) and "Diabenorme"(DB) in mice compared to controls.

MATERIALS AND METHODS

Laboratory materials

Current laboratory glassware and some devices were used during handling. These devices consisted of a precision analytical balance (Sartorius), a rotary evaporator (Heidolph heizbad Rotacool), Pharma test PTZ-S and a freeze dryer (Telstar cryodos-80).

Plant materials

Plants materials was represented by powders of herbals medicines called TQ and DB which were provided by two traditional healers in collaboration with the National Program for the Promotion of Traditional Medicine (NPPTM). TQ is presented in the form of capsules containing exclusively the powdered root of *Pycnanthus angolensis* while DB consisted of a powder mixture of barks and leaves of *Anacardium occidentale* and *Persea Americana*.

Extraction procedure

A mass of 20.0 g of powder of DB were mixed with 1000 ml of distilled water and kept at 100 °C for 20 min. After cooling this solution was decanted and filtered using Whatman number 5 filter paper and then concentrated on a rotary evaporator (HEIDOLPH Hezbad rotacool) at 40 °C to reduce it to 200 ml. the concentrates was freeze-dried and yielded a dry residue of aqueous extract to 0.83 g representing 4.1%.

A mass of 50.0 g of powder of TQ were mixed with 1000 ml of distilled water and kept on a magnetic stirrer for 24 h. The resulting solution underwent the same previous conditions of filtration, concentration. The concentrates was freeze-dried and yielded a dry residue of aqueous extract to 2.3 g representing 4.6%.

Experimental animals

Nulliparous and non pregnant females white mice about 10 weeks with an average weight of 24.7 g (weighting 20 – 32 g) were purchased from the pet shop of Adiopodoumé Pasteur Institute. These animals underwent a minimum of 5 days of acclimatization in the laboratory conditions with free access to water and a standard diet. Before the start of feeding with extracts, the animals were fasted from food for 4 hours and then were weighed to determine the amount of product to be administered. Water was used as vehicle (2 ml/100g body weight). The lack of a national animal ethics committee was one of the limitations of the study.

Phytochemicals analysis

Phytochemicals tests were performed on the aqueous extract of DB and TQ. Analysis of the phytochemicals was carried out using colorimetric techniques described by Odebiyi and Sofowora (1978). The systematic search of sterols and polyterpènes, catechin tannins and gallic, free anthracene derivatives, flavonoids, polyphenols and quinone substances was made by characteristic reactions. Research sterols and polyterpènes were made by the reaction of Liebermann. The characterization of polyphenols was made by the reaction with ferric chloride to 2%. Flavonoids have been shown by the cyanidin reaction. Catechin tannins and gallic was made by the reaction of ferric chloride and

Stiasny. The free or combined quinone compounds have been demonstrated by Borntraeger reaction. Alkaloids were detected by reacting Dragendorff.

Pharmacotechnical tests

The disintegration tests and uniformity of mass was made to capsules "Thuquinone" according to European Pharmacopoeia (2008). The disintegration test is to determine the ability of six capsules or tablets for disintegration in the liquid medium within a prescribed time by means of suitable equipment. The uniformity of mass is to determine the average weight of the content of 20 capsules. The individual mass of the contents of more than 2 capsules may deviate from the average weight of a higher level than that specified percentage. No mass must deviate by more than double that percentage.

Acute toxicity

The acute toxicity was conducted according to the organization of economic cooperation and development (OCDE) guidelines 423 (OCDE, 2001). In lack of information about a dosage level that can cause signs of toxicity, the choice of moderate initial dose of 300 mg/kg among those recommended was made (Figure 1). The mice were randomly divided into 10 treated group of 3 animals each and 2 control group of 3 animals each. The control group received distilled water while the treated group received aqueous extracts of DB and TQ at doses of 300 mg/kg, 2000 mg/kg and 5000 mg/kg of body weight. The level dose of 5000 mg/kg was a limit assay. Observations were made for general behavioral, neurologic impairment, body weight changes, skin changes, gastrointestinal signs and mortality for a period of 14 days post-treatment. Signs of toxicity and mortality were observed after the administration at the first, second, fourth hour and once daily for 14 days. According to OCDE 423 (2001), the test limit for acute oral toxicity is generally considered to be 5000 mg/kg body weight. If no mortality is observed at this dose level, the lethal dose 50% (LD_{50}) is supposed to be greater than 5000 mg/kg. Thus, the LD_{50} was estimated.

Statistical analysis

The values were expressed as mean ± SD. Statistical analysis between treatment and control groups were performed by Fischer test with the aid of SPSS version 18 software. Fischer test was used to compare the means of the weight. Level of significance was set at P values < 0.05.

RESULTS

Phytochemical screening (Table 1) performed on aqueous extracts revealed the presence of catechin tannins, flavonoids, polyphenols and the absence of gallic tannins, quinone substances, alkaloids, sterols and polyterpenes in both recipes. Anthracene derivatives were only found in "Diabenorme".

Uniformity of mass (Table 2) realized on capsules "Thuquinone" showed no mass variations in excess of 7.5% of the average weight. The capsule disintegration test (Table 3) was also satisfactory. Pharmacotechnical tests conducted were therefore consistent with standards of the European Pharmacopoeia.

The study was made to investigate the acute toxicity of "Thuquinone" and "Diabenorme" in mice. The two recipes of plants were administered at 3 dose levels of 300 ; 2000 and 5000 mg/kg body weight. At single dose of 300 mg/kg (Table 4), the average weights of animals treated with both recipes have evolved in opposite directions. This could mean a weight loss for DB and weight gain for TQ. No significant difference has been noticed between the treated and control group by Fischer's test ($P > 0.05$). During the period of observation of acute toxicity, none of the treated animals succumbed. No sign of toxicity such as tremor, bleeding and coma was raised. Their behavior was rather strengthened compared to the control group. At the end of 14 days, no death and no abnormalities were observed in

treated groups indicating that the lethal dose 50 is higher than 300 mg/kg.

At single dose of 2000 mg/kg (Table 4), no sign of toxicity was noticed. None of the treated animals succumbed although the doses were increased. The average weights are inversely changed compared to 300 mg/kg. No significant difference has been noticed between the treated and control group by Fischer's test ($P > 0.05$).

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Similar observations were made with dose of 5000 mg/kg (Table 4) between the treated and control groups. The oral administration of extracts of TQ and DB to mice at single doses of 5000 mg/kg during two weeks did not induce any side effects indicating that the LD_{50} is higher than 5000 mg/kg.

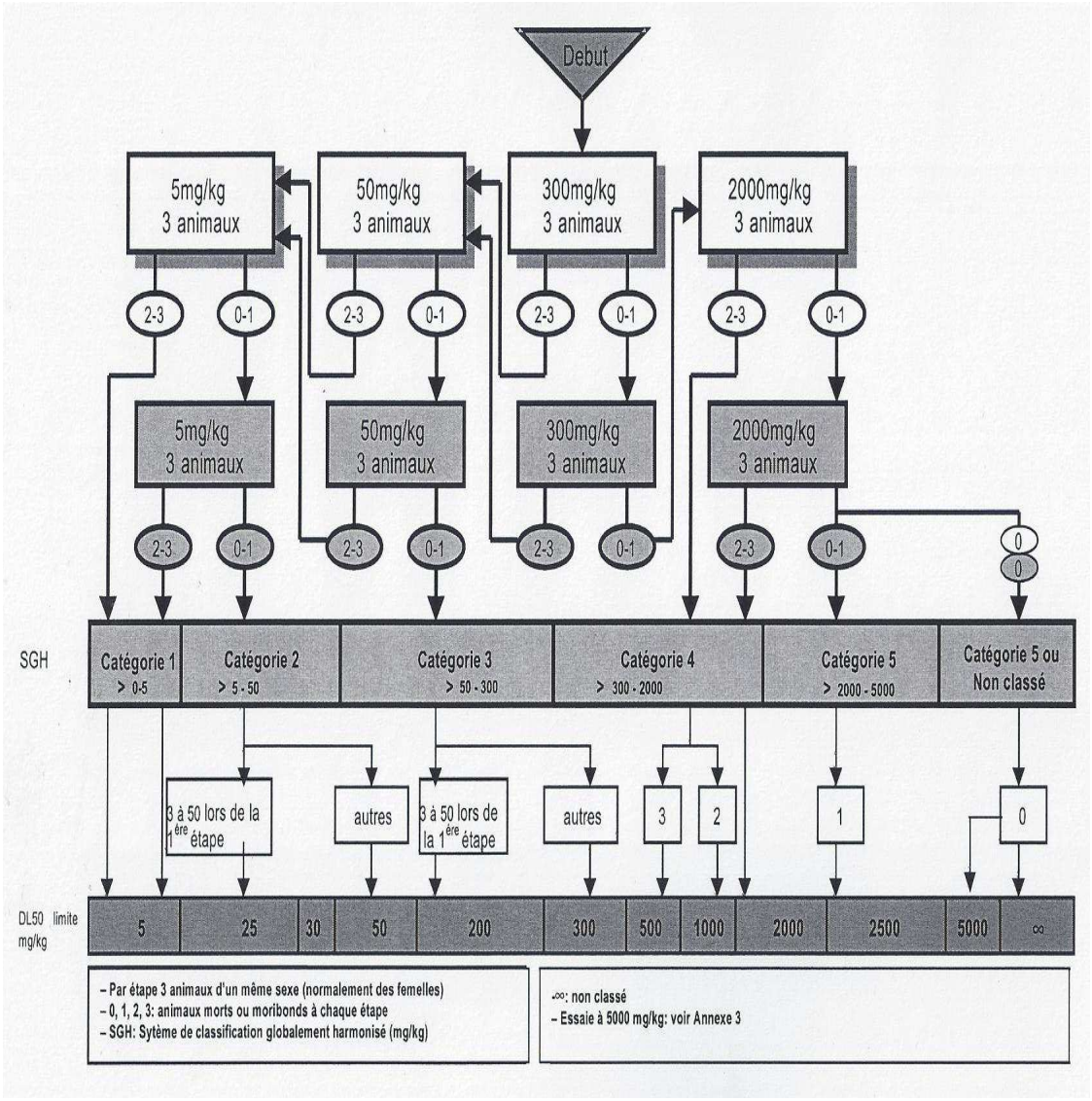

Figure 1: Sequential administration process from a dose of 300 mg/kg (OCDE 423, 2001).

Table 1: Phytochemical screening of aqueous extract of "Thuquinone" and "Diabenorme".

Phytochemicals	**Diabenorme**	**Thuquinone**
Sterols and polyterpenes	-	-
Polyphenols	++	++
Flavonoids	++	++
Tannins catechic	++	+
Tannins gallic	-	-
Quinone	-	-
Alkaloids	+	+
Free anthracenes	+	-
saponins	++	++

+ = Positive (Presence) - = Negative (absence) ++ = Moderately present

Table 2 : Weight of capsule uniformity.

Capsule number	**1-11**	**2-12**	**3-13**	**4-14**	**5-15**	**6-16**	**7-17**	**8-18**	**9-19**	**10-20**
Weight (g)	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.31	0.32
	0.32	0.31	0.31	0.32	0.32	0.32	0.33	0.31	0.31	0.30
Mean ± s.d. (g)	0.31 ± 0.01									
CR7.5%	0.29 - 0.33									

CR: Confidence range at 7.5%. s.d.: Standard deviation.

Table 3 : Disintegrator time of capsules at 37 °C.

Capsule	**Time (min)**	**Reference value**
1	15.09	below 30 min
2	15.34	
3	16.26	
4	16.43	
5	17.12	
6	17.22	

Table 4: Average weight of animals treated versus control.

Dose	Average weight (g)		Weight variation	P value
	Day 1	Day14		
Control (water 2 ml/100 g)	24.9 ± 0.3	26.4 ± 2.2	+1.5	
Diabenorme 300 mg/kg	21 ± 1.3	22.4 ± 1.7	+1.4	0.28*
Thuquinone 300 mg/kg	26.5 ± 1.5	24.9 ± 1.4	-1.7	0.46*
Diabenorme 2000 mg/kg	21.9 ± 1.1	20.6 ± 1.6	-1.3	0.34*
Thuquinone 2000 mg/kg	26.5 ± 1.5	27.2 ± 1.8	+0.7	0.21*
Diabenorme 5000 mg/kg	24.5 ± 1.6	24.2 ± 2.5	-0.3	0.23*
Thuquinone 5000 mg/kg	24.1 ± 1.3	25.5 ± 1.7	+1.4	0.37*

Values are expressed as mean ± s.d. (n = 3) +: weight gain, - : Weight loss
*Not significantly difference from the control ($p > 0.05$), (Fischer's test)
s.d.: Standard deviation

DISCUSSION

The results obtained showed that the objectives of this study were achieved. These results are completed scientific works already done in this area of research on evaluation of the toxicity of phytomedicines used in diabetes. This disease has become a public health problem for our country. This study is a part of the improvement of the management of diabetes. With regard to phytochemical screening, similar chemical compounds such as tannins, saponins, flavonoids, alkaloids and polyphenols have been shown in the works of Arukwe et al. (2012) and Ukwubile (2012). However, secondary metabolites such as saponins, alkaloids, tannins and flavonoids have demonstrated to be responsible for the management of many diseases in the ethno-medicine. Their values in the investigated extracts are appreciable and could add to their properties. Pharmacotechnical tests suggested that the capsules of TQ were produced in accordance with good manufacturing practices. This could ensure the quality of phytomedicines manufactured in Africa. Regarding the acute toxicity, none of the treated animals succumbed although the doses were increased up to 5000 mg/kg. This could mean that LD_{50} is higher than 5 g/kg. According to OCDE 423 (2001), substances that present LD_{50} higher than 5000 mg/kg via oral route may be considered practically non-toxic. These facts led to the conclusion that the test substance is practically non-lethal after an acute exposure. This predicts a security for these products. The test limit for acute oral toxicity is generally considered to be 5000 mg/kg body weight. If no mortality is observed at this dose level, a higher dosage is generally not necessary. These results corroborate those of some authors. The similar result was found with the leaf extracts of *Persea americana* whose LD_{50} was higher than 5 g/kg (Coto et al., 2004). Tedong et al. (2007) have reported no effect of acute toxicity after oral administration of extracts of leaves of *Anacardium occidentale* at doses of 2000 and 6000 mg/kg. However according to these authors, diarrhea and mortality have been recorded at a dose of 16 g/kg. By against Garcia et al. (2005) have reported signs of oral acute toxicity (decreased motor activity, pilo-erection) by freeze-dried fresh leaves extracts of *Anacardium occidentale* at doses of 3000 mg/kg and 5000 mg/kg.

However, the impact of the use of these products for a long time in human organs could not be assessed. Further investigation is needed to evaluate its sub-acute toxicity.

Conclusion

This study assessed the acute toxicity of extracts "Diabenorme" and "Thuquinone" and highlight phytochemicals. However, prolonged use of these drugs could affect the functioning of human organs. It would be wise to extend this study to evaluate the sub-acute toxicity in the interest of public health.

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