



PHYTOCHEMICAL SCREENING AND IN VIVO EFFECT OF HYDRO-ETHANOLIC EXTRACT OF *TRILEPISIUM MADAGASCARIENSE* D. C. LEEUWENBERG (MORACEAE) LEAVES ON HEMATOLOGIC PARAMETERS IN THE HEALTHY RAT

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ABSTRACT

Plants have an almost limitless ability to synthesize secondary metabolites that possess medicinal properties. The present work deals with the qualitative phytochemical screening and in vivo effect of hydro-ethanolic extract of the leaves of *T. madagascariense*, a medicinal plant widely used against anaemia. Phytochemical screening performed by coloured reactions in tubes revealed the presence of 7 chemical families; ninhydrine reagent test, revealed the presence of amino acids. Extract effect on hematologic parameters realised by blood formulates numeration after oral administration at the doses of 200, 400 and 800 mg/kg show a significant increase of hematologic parameters compare to the control: leucocytes $p < 0.001$ and $p < 0.05$ respectively in males and females; red globules and plates $p < 0.05$ in males and females. These results confirm and encourage the abundant use of this species in traditional medicine against anaemia.

KEYWORDS: *Trilepisium madagascariense*, leaves, hydro-ethanolic extract, phytochemical, hematologic parameters.

INTRODUCTION

Blood cells constituting hematopoietic system play several roles in the human organism, of which the transport of respiratory gases (red globules), defences of organism (leucocytes) and blood coagulation (blood plates). These various cells have allowed margins to achieve their role normally. Unfortunately, several affections such as diabetes¹, malaria, cardiovascular diseases² and dysmenorrhoea³ affect their number, thus creating an imbalance of hematopoietic system, a weakening of immunity and thus several other degenerative pathologies. Former studies realized on plants extracts made it possible to reveal their anti-anaemic potentialities⁴. In Congo-Brazzaville, several plants, among which *T. madagascariense*, are quoted like having anti-anaemic potentialities⁵. The whole results of these studies let believe that anaemic problems due to several pathologies could find an approach of solution by the use of vegetable extracts. Thus, because of the multiple negatives effects of synthetic drugs on one hand and, some difficulties to procure blood products for the transfusion on other hand, we undertook

to realized phytochemical screening and to research the effect of hydro-éthanolique extract of *T. madagascariense* on hematologic parameters of the healthy albino rat.

MATERIALS AND METHOD

Vegetable material

The vegetable material was constituted by the fresh leaves of *T. madagascariense* obtained on the market. The leaves were preciously washed and, dried during 10 days at the temperature of laboratory ($25 \pm 1^\circ\text{C}$) and reduced in powders using a wood mortar.

Preparation of extract

100g of powder was macerated in 1000 ml of hydro-ethanolic solvent (800 ml of ethanol 90/200 ml of distilled water) during 72 h under magnetic agitation. Macerated solution was filtered using Whatman paper N°3 and, the obtained filtrate concentrated with the rotary evaporator and dried with the drying oven at 55°C .

Phytochemical screening

Hydro-ethanolic extract of the leaves of *T. madagascariense* was subjected to the phytochemical screening by the standard methods.^[6] Amino acids presence was revealed thanks to the ninhydrine reagent (300 mg ninhydrine, 100 ml of n-butanol and 3 ml of icy acetic acid). 1 drop of extract was deposited on the filter paper, after absorption, 1 drop of the reagent prepared beforehand was superimposed and the filter paper placed during a few minutes at 100°C in the drying oven. The presence of amino acids was determined by the appearance of a mauve colouring or violet.

Animal material

Male and female rats weighing between 160-200 g were used for hematologic parameters. Animal comes from Faculty of Science and Techniques of Marien Ngouabi University (Brazzaville-Congo). They were fed and maintained under standard lighting conditions (12 hours' light and 12 hours' dark).

Effect on hematologic parameters

In order to research the effect of extract on hematologic parameters, 4 groups of five (5) rats each one was constituted and acclimated for 7 days in the laboratory with free access to water and food before any experimentation. Animals received a daily treatment during 30 days as follow: control group treated with 10 ml/kg of distilled water; the tested groups received 200, 400 and 800 mg/kg of hydro-ethanolic extract. The samples of blood appropriated in EDTA tubes was immediately used to determine the rates of hematologic parameters by classical methods.^[7]

Statistical Analysis

The results expressed affected on average of the standard mistake are submitted to an analysis of the variance to a factor followed of Student-Fischer test. The limit of significativity is fixed at $p < 0.05$.

RESULTS

Phytochemical screening

Table 1 presents phytochemical screening of hydro-ethanolic extract of the leaves of *T. madagascariense*. The qualitative analysis by colored reactions in tube method permitted to revealed the presence of 7 chemical families: flavonoids, glycosides cardiotonics followed by alkaloids, saponins, tanins, steroids and terpenoids. One notes the presence of amino acids and the absence of coumarins and anthraquinons.

Effect on hematologic parameters

Tables 1 and 2 presents the effect of the hydro-ethanolic extract on the hematologic parameters in male and female rat at the doses of 200, 400 and 800 mg/kg. The obtained results show that at the studied doses, hydro-ethanolic extract induces a significant increase of hematologic parameters in the animals of the two sexes compared to the control having received distilled water: white blood corpuscles ($*p < 0.05$ and $**p < 0.01$ in females, $**p < 0.001$ in males), red blood corpuscles and plates ($*p < 0.05$). However, it is noticed that at the dose of 200 mg/kg the extract does not induce significant increase of rate of red blood corpuscles in males' rats. With these doses, the extract induces the increase in the hematologic parameters, by respecting the normal margins of the hematologic constants.

Table 1: Phytochemical screening of hydro-ethanolic extract of the leaves of *T. madagascariense*.

Chemical family	Hydro-ethanolic extract
Alcaloids	+
Saponin	+
Amins acids	+
Flavonoids	++
Anthraquinons	-
Coumarins	-
Tanins	+
Cardiotonic Glycosids	++
Steroids	+
Terpenoids	+

Table 2: Effect of Hydro-ethanolic extract of *T. madagascariense* leaves on hematologic parameters in male rat.

Hematologic parameters	Norms	Control (Distilled water)	Hydro-ethanolic extract		
			200 mg/kg	400 mg/kg	800 mg/kg
White globules ($10^3/\text{mm}^3$)	3.00-25.00	5.20 ± 0.29	12.10 ± 1.73 ***	15.20 ± 1.12 ***	16.90 ± 0.98 ***
Lymphocyt (%)	65.00-85.00	60.00 ± 1.11	68.80 ± 2.12	74.40 ± 1.34	74.20 ± 1.06
Monocyt (%)	0-5.00	13.20 ± 0.88	2.50 ± 0.75	3.90 ± 0.95	4.70 ± 1.88
Granulocyt (%)	9.00-40.00	23.80 ± 2.12	28.70 ± 1.99	21.90 ± 1.72	21.10 ± 2.65
Red globules ($10^6/\text{ml}$)	7.20-9.60	7.41 ± 1.23	7.67 ± 2.02	8.74 ± 1.98 *	8.58 ± 1.54 *
Hemoglobin (g/dl)	12.00-17.50	10.67 ± 0.45	10.10 ± 0.13	12.40 ± 1.45	14.50 ± 2.67
Hematocrit (%)	39.00-53.00	36.40 ± 2.16	40.50 ± 1.89	43.70 ± 1.05	48.75 ± 2.00
VCM (μm^3)	- // -	51.00 ± 0.67	52.00 ± 0.88	54.00 ± 1.32	51.00 ± 1.55
RCMH (g/dl)	-//-	21.90 ± 2.43	21.50 ± 1.98	21.70 ± 1.05	20.70 ± 2.43
CCMH (g/l)	-//-	43.00 ± 2.09	41.10 ± 2.05	40.50 ± 1.66	40.20 ± 2.89
IDRBC	-//-	18.90 ± 1.98	18.00 ± 2.00	19.90 ± 1.75	18.70 ± 2.21
Plates ($10^3/\text{mm}^3$)	320.00-1200.000	730.00 ± 0.75	933.00 ± 1.11 *	860.00 ± 0.67 *	1061.00 ± 1.78 **

Values are mean \pm SEM, $*p < 0.05$; $**p < 0.01$; $***p < 0.001$; significative difference in comparison with control; $n = 5$.

Table 3: Effect of Hydro-ethanolic extract of *T. madagascariense* leaves on hematologic parameters in female rat.

Hematologic parameters	Norms	Control (distiled water)	Extrait hydro-ethanolic		
			200 mg/kg	400 mg/kg	800 mg/kg
Whites globules ($10^3/\text{mm}^3$)	3.00-25.00	5.20 \pm 2.20	6.20 \pm 1.93 *	7.70 \pm 1.29 *	9.04 \pm 2.57 **
Lymphocyt (%)	65.00-85.00	64.25 \pm 1.45	69.07 \pm 1.22	75.70 \pm 1.79	75.01 \pm 1.06
Monocyt (%)	0-5.00	15.05 \pm 0.97	7.68 \pm 1.11	3.78 \pm 1.43	5.69 \pm 0.63
Granulocyt (%)	9.00-40.00	20.70 \pm 1.17	23.25 \pm 2.00	20.52 \pm 1.33	19.30 \pm 0.52
Red Globules ($10^6/\text{ml}$)	7.20-9.60	5.38 \pm 0.75	7.93 \pm 1.87 *	7.58 \pm 2.13 *	7.14 \pm 0.96 *
Hemoglobin (g/dl)	12.00-17.50	10.50 \pm 3.04	9.10 \pm 2.06	12.50 \pm 1.78	14.20 \pm 2.75
Hematocrit (%)	39.00-53.00	32.20 \pm 1.44	41.50 \pm 1.35	48.70 \pm 1.79	46.20 \pm 1.23
VCM (μm^3)	-/-	55.00 \pm 0.63	55.00 \pm 0.94	51.00 \pm 1.66	51.00 \pm 2.08
RCMH(g/dl)	-/-	25.20 \pm 1.86	23.10 \pm 1.81	20.70 \pm 2.31	19.80 \pm 0.55
CM H (g/l)	-/-	45.50 \pm 2.11	42.20 \pm 1.49	40.20 \pm 1.62	39 ;10 \pm 1.79
IDRBC	-/-	17.60 \pm 1.33	19.90 \pm 1.40	19.70 \pm 2.23	19.10 \pm 1.48
Plates ($10^3/\text{mm}^3$)	320.00-1200.00	570.00 \pm 1.75	675.00 \pm 2.21 *	770.00 \pm 1.41 *	890.00 \pm 1.33**

Values are mean \pm SEM, * $p < 0.05$; ** $p < 0.01$; significative différence in comparaisn with control, n = 5.

RCMH : Rate of Corpuscular Mean in Hmoglobin,

VCM : Corpuscular Volum Mean,

CCMH : Concentration of Corpuscular Mean in Hemoglobin

IDRBC : Index of Distribution of Red blood corpuscles.

DISCUSSION AND CONCLUSION

This study was initiated to research phytochemical compounds and in vivo effect of hydro-ethanolic extract of *T. madagascariense* on hematologic parameters in the healthy rat. Vegetable extracts indeed are the seat of bioactive substances with interesting therapeutic potentialities. Their use currently play an essential role because they take an active part in the basic needs for health in many countries in the process of development. *T. madagascariense* is a specie widely used in Congo-Brazzaville by rural populations against anaemia. If it is allowed in Congolese traditional medicine that this specie is endowed anti-anaemic potentialities, it should equipped by chemical compounds responsible of this effect. The results of phytochemical analysis revealed that hydro-ethanolic extract of the leaves of *Trilepisium madagascariense* contain seven (7) chemical compounds with a prevalence of flavonoids and cardiotoxic glycosids. One notes the presence of tanins, steroids, terpenoids and acid amino. Indeed, several studies indicate that polyphenols of vegetable extracts would stimulate hematopoietic system function and the production of monocytes.^[8,9,10] Another study revealed that amino acids increase the blood rate of glutathione.^[11] These hypothesis suggest that the extract of *T. madagascariense* could have an effect on hematopoietic system function and would probably explain his traditional use against anaemia. Thus, the daily administration during 30 days of hydro-ethanolic extract (200, 400 and 800 mg/kg) of the leaves of *T. madagascariense* to the healthy rats, showed a significant increases of the rates of white blood corpuscles ($p < 0.001$), red blood corpuscles ($p < 0.05$) and plates ($p < 0.01$); confirming rural populations suppositions and a possible effect of vegetable extract on hematopoietic system. Similar results had already been observed with other studies with various extracts of

Ocimum basilicum;^[12] *Parkia biglobosa* and *Gongronema latifolium*;^[13] *Acalypha wilkesiana*;^[14] *Pseudocedrela kotschy*.^[15] However they are in disagreement with those obtained with *Prosopis cineraria* which showed a reduction of hematologic parameters.^[16] The obtained results permitted to justify the use of the leaves of *T. madagascariense* in the treatment of anaemia and microbial infections^[5] (Ahombo et al, 2012). Thus of this fact this plant could be associated with others in the traditional treatment of diseases which provoke anaemias, such as malaria, dysmenorrhoeas, diabetes.^[17,1] The results of this study showed that hydro-ethanolic extract of the leaves *T. madagascariense* is rich in secondary metabolites endowed of pharmacological activities. Its presents, a simulative effect on hematologic parameters in the healthy rat Wistar. This plant does not have a toxic effect on the system hematopoietic, which would explain its abundant use in traditional medicine. It could be used for the development of an improved traditional drug to fight against anaemia.

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