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IMPROVEMENT BY THE AQUEOUS EXTRACT OF THE LEAVES OF DESMODIUM VELUTINUM WILL DC (FABACEAE) OF BIOLOGICAL PARAMETERS ALTERED BY ETHANOL 30 % IN RATS

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ABSTRACT

Most epidemiological data show that regular alcohol consumption is related to an increase the occurrence of cardiovascular diseases, dysfunction of noble organs and changes in biochemical and hematological parameters. The objective of this work is to evaluate the effects of the aqueous extract of the leaves of *D. velutinum* on the consequences of the subchronic excessive consumption of ethanol 30 % in the wistar rat. Alterations in biological parameters (rat weight, noble organ masses, ALT, ASAT, white blood cells, red blood cells, hemoglobin and platelets) were induced by ethanol overload 30 % daily for eight (8) weeks. The detection of flavonoïds in this extract was made by Thin Layer Chromatography (TLC). At the doses of 250 and 500 mg/kg the aqueous extract improves the biological parameters. This extract contains flavonoïds. Other activities to deepen the effects of the aqueous extract of the leaves of *D. velutinum* on the alterations of the biological parameters induced by the ethanol 30% deserve to be realized.

KEYWORDS: Ethanol 30%, *Desmodium velutinum*, toxicity, biological parameters, flavonoïds.

INTRODUCTION

Ethanol is the alcohol of beverages consumed by man. Alcohol consumption is deeply rooted in most cultures, especially African and European for centuries. Indeed, if it occupies an important place in our daily life and especially during our festive celebrations, the prevention of its harmful consequences for health is nonetheless a public health priority (WHO, 2012, Room et al., 2005). Congolese flora abounds with many medicinal plants used to remedy certain pathologies. Among these plants there is *Desmodium velutinum*, a plant belonging to the family Fabaceae used in traditional Congolese medicine against many no communicable diseases (female infertility, heart and stomach ache and toothache) (Bouquet, 1969). Thus, in the present study, we investigated the effects of the aqueous leaf extract of this plant on the morphological, biochemical and hematological parameters altered by ethanol 30% in the Wistar rat.

MATERIAL AND METHODS

Plant material

The leaves of *D. velutinum*, harvested in Brazzaville were used. The identification of a leaf sample of this plant was made by Systematic Doctor Jean-Marie Moutsambote, Head of the Herbal Service at the Institute of Research of Exact and Natural Sciences (IRENS) of Congo. The leaves were dried out of the sun (28 ± 1ºC) for two (2) weeks. After drying, these leaves were pulverized. The aqueous extract of the leaves of *D. velutinum* was prepared by maceration by immersing 100 g of powder of the leaves in 1000 mL of distilled water for seventy-two (72) hours. The macerate obtained was filtered three (3) times using the hydrophilic cotton and then the filtrate obtained was evaporated using a balloon heater at a temperature of 70 ° C. The powder obtained served as an aqueous extract which was administered to rats at doses of 250 and 500 mg/kg.

Animal material

Female Wistar rats weighing between 150 and 200 g aged 4-5 months were used. These animals were provided by the animal house of the Faculty of Science and Technology of Marien Ngouabi University (Brazzaville, Congo). They where had free access to a standard diet (whose composition is given in Table 1) and water *ad libitum*. Prior to the experiment the animals were acclimated to the laboratory for five (5) days.

Preparation of ethanol 30 %

Ethanol 30% was prepared by diluting 100 mL of ethanol at 90 ° in 206.22 mL of distilled water according to Gay Lussac's table.

Preparation of the Sylimarin solution

The Sylimarine solution was prepared by dissolving 70 mg of sylimarine tablet (Légalon*) in 10 ml of distilled water.

Alteration of biological parameters by ethanol 30 % and treatment of rats with the aqueous extract of the leaves of *D. velutinum*

The biological parameters of the rats were altered by using the slightly modified protocol by Mini and Rajamohan, (2013). For this, five groups of six rats each were constituted and treated concomitantly per os for (8) weeks as follows

Group 1: distilled water (1 mL/100 g/day);

Group 2: ethanol 30 % (2 mL/100 g/day) + distilled water (1 mL/100 g/day);

Group 3: ethanol 30 % (2 mL/100 g/day) + Sylimarin (standard drug, 70 mg/kg/day);

Group 4: ethanol 30% (2 mL/100 g/day) + aqueous extract of *D. velutinum* (250 mg/kg/day);

Group 5: ethanol 30% (2 mL/100 g/day) + aqueous extract of *D. velutinum* (500 mg /kg/day).

Evaluation of the effects of the aqueous extract of the leaves of *D. velutinum* on the weight evolution and food and water intake in the rat treated with ethanol 30 %

During the eight (8) weeks of treatment, the body weight of the rats was measured at the end of each week and food and water intake was recorded.

Evaluation of the effects of the aqueous extract of the leaves of *D. velutinum* on the biochemical and hematologic parameters

At the end of treatment, animals asleep with diethyl ether were sacrificed by decapitation for dissection and then the arteriovenous blood was collected rapidly in the dry EDTA tubes respectively for the determination of the biochemical parameters (ALAT, ASAT, TG, HDL Cholesterol, Total Cholesterol, Blood Glucose) and hematologic (white blood cell count, red blood cells, hemoglobin, hematocrits, platelets, mean cell volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, size distribution index of red blood cells and mean volume of polynuclear cells) according to the protocols described by Mboungou Bouesse, (2018).

Evaluation of the effects of the aqueous extract of the leaves of *D. velutinum* on the mass of the liver, spleen, kidneys, heart and left ventricle

After dissection of the rats, the noble organs (liver, spleen, kidneys and heart) were removed and their mass measured using a precision balance. The left ventricle freed of the right ventricle and atria was weighed and the ratio, Heart mass - left ventricle was calculated to determine the left ventricle index (L.V.I).

Highlighting of flavonoids in the aqueous extract of the leaves of *D. velutinum*

0.5 g of the dry extract of *D. velutinum* leaves are dissolved in 1 ml of distilled water. The aqueous extract thus obtained is deposited on the TLC plates using the capillary tubes. The TLC plates were placed in the developing vessels containing a suitable volume of a tertiary mixture of the following solvents: ethyl acetate/formic acid/ water (8: 1: 1). The characteristic tasks were observed in daylight and then in ultraviolet light at 365 nm after revelation of the TLC plates to Neu reagent.

Statistical analysis of the results

All values were expressed as mean ± ESM. These mean and ESMs were calculated using Excel 2010. Analysis of variance followed by Student's test "t" was performed. The significance level was set at $p < 0.05$.

RESULTS

Effects on weight evolution

Figure 1 shows that at the end of treatment, in rats treated with ethanol 30% more distilled water a decrease in weight to 67.22 ± 7.44% ($p < 0.001$) against an increase in 144.36 ± 9.58% in the control rats (distilled water 1 ml / 100g); a decrease weight of 53.43%. In rats treated with ethanol 30% and the aqueous extract of leaf of *D. velutinum* (250 and 500 mg / kg), weights increased to 118.88 ± 11.22 and 150.02 ± 2.82 % respectively versus 67.22 ± 7.44% in rats receiving only ethanol 30% and distilled water (Figure 1). Increased weight is also noted in rats treated with Sylimarin (standard drug, 70 mg / kg).

Effect of aqueous extract of leaves of *D. velutinum* on food and water intake in rats treated with ethanol 30%

In the rats treated in addition to ethanol 30 % with Sylimarin (70 mg/kg) or aqueous extract of leaf of *D. velutinum* (250 and 500 mg / kg), an increase in food consumption was noted compared to the rats treated with ethanol 30% more distilled water (Figure 2). Regarding

water intake (Figure 3), it is noted in rats treated in addition to 30% ethanol with Sylimarin (70 mg/kg) or aqueous leaf extract of *D. velutinum* (250 and 500 mg/kg) an increase in water intake from the 4th week compared to rats treated with ethanol 30% more distilled water.

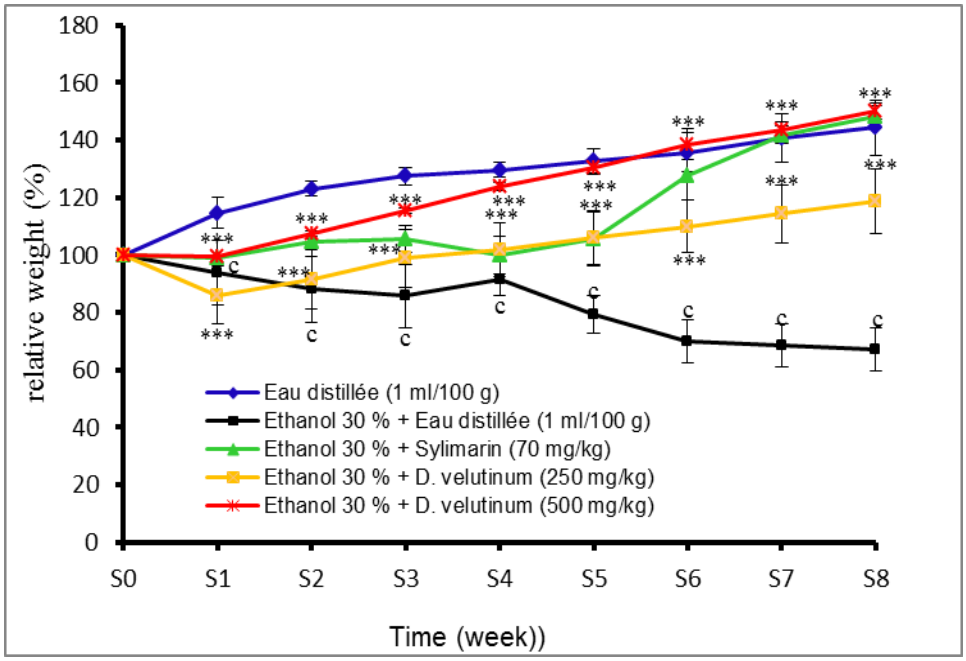

Figure 1: Weight evolution of the rats according to the time. *D. Velutinum* = *Desmodium velutinum*. Every point is a mean ± ESM, with n = 6; c p <0,001 meaningful difference in relation to the witness (distilled water); *** p < 0,001 meaningful difference in relation to the witness (ethanol + distilled water).

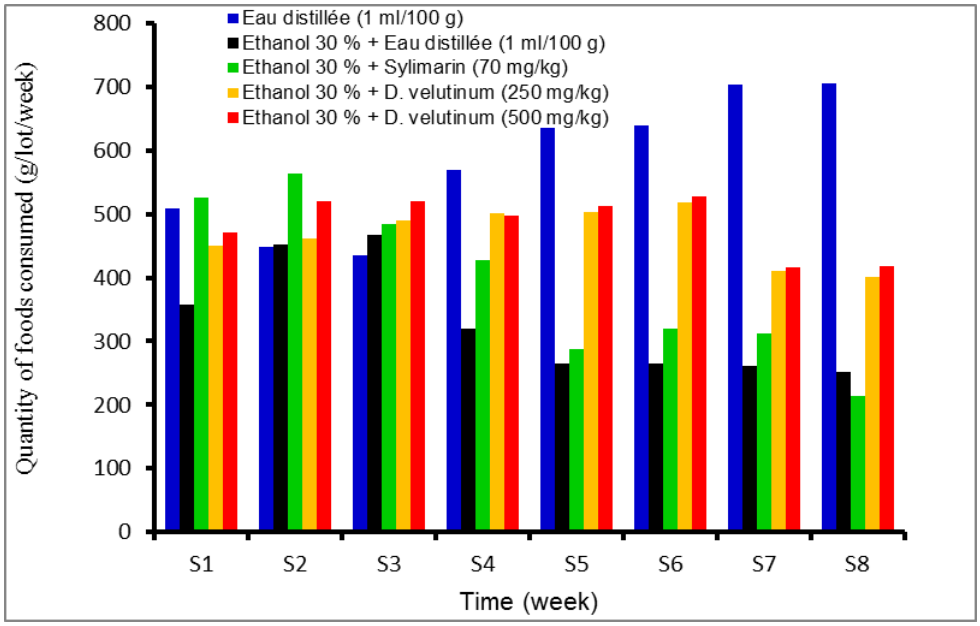

Figure 2: Variation of the food taken in rat in time function. *D. Velutinum* = *Desmodium velutinum*.

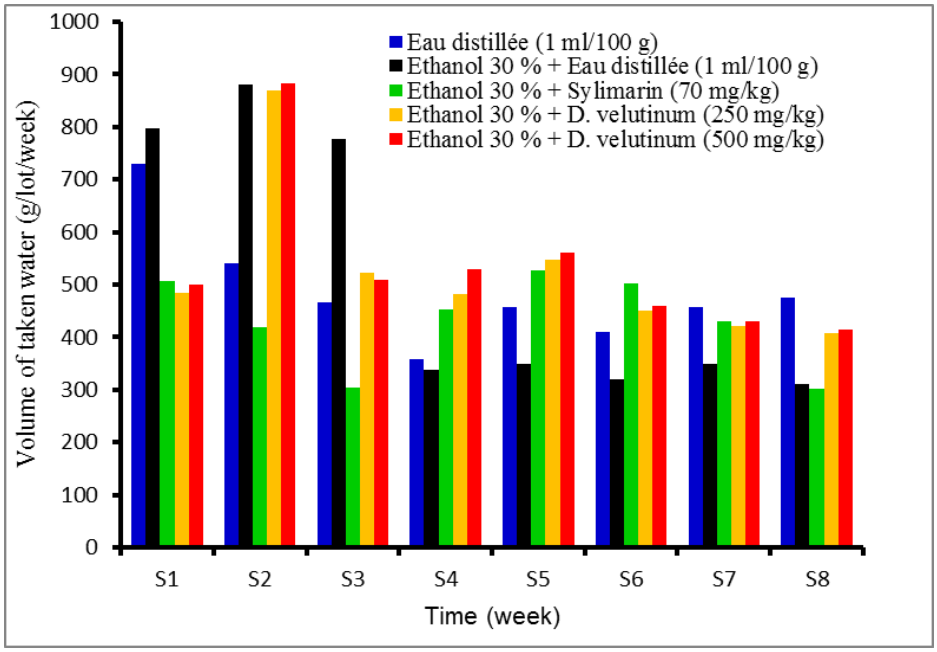

Figure 3: Variation of the water taken in rat in time function. *D. Velutinum* = *Desmodium velutinum*.

Table 1: Food composition.

Foods	**Quantity (kg)**
Flour of soy	2.5
Cornflour	2.5
Flour of wheat	2.5
Flour of fishes smoked	2
Flour of peanut	0.75
Salt	0.01
Oil of palm	50*
Water of faucet	800*

*: ml.

Table 2: Biochemical parameters variation in rat.

Biochemical parameters	**Treatment**				
	WD (1 ml/100g)	**ET 30 % +WD (1 ml/100g)**	**ET 30 % + Syl. (70 mg/kg)**	**ET. 30 % + D.v. (250mg/kg)**	**ET. 30 % + D.v. (500 mg/kg)**
ALAT (UI/l)	5.94 ± 0.90	115.58 ± 13.13 ^{c}	82.07 ± 16.47***	108.07 ± 19.59***	64.46 ± 7.67***
ASAT (UI/l)	18.49 ± 5.68	296.62 ± 18.83 ^{c}	137.45 ± 8.72***	223.50 ± 37.29***	161.81 ± 14.04***
TG (g/l)	4.68 ± 1.24	3.96 ± 0.30^{ns}	2.90 ± 0.11 ^{ns}	2.84 ± 0.68 ^{ns}	3.91 ± 0.54 ^{ns}
HDL-C (UI/l)	326.51 ± 91.68	232.92 ± 42.39^{c}	132.94 ± 51.52***	234.99 ± 23.12***	181.31 ± 20.17***
CT (g/l)	0.40 ± 0.07	0.58 ± 0.04^{ns}	0.64 ± 0.10 ^{ns}	0.60 ± 0.04 ^{ns}	0.46 ± 0.04 ^{ns}
GLY (g/l)	0.73 ± 0.09	0.67 ± 0.05^{ns}	1.30 ± 0.14 ^{ns}	0.73 ± 0.08 ^{ns}	1.45 ± 0.13 ^{ns}

Every value is a mean ± ESM, with n = 6; c p <0,001 meaningful difference in relation to the witness (distilled water); *** p < 0,001 meaningful difference in relation to the witness (ethanol + distilled water); ns: non meaningful difference in relation to the witness (ethanol + distilled water). WD: Water distilled; ET. : Ethanol; Syl.: Sylimarin; *D.v.: Desmodium velutinum*; ALAT: Alanine aminotransférase; ASAT: Aspartate aminotransférase; TG: Triglycéride; HDL: Lipoprotéine of high density; CT: Total cholesterol; GLY: Blood sugar.

Table 3: Hematological parameter variation in rat.

	Treatment				
Hematological parameters	**WD (1 ml/100 g)**	**ET. 30 % + WD (1 ml/100 g)**	**ET. 30 % + Syl. (70 mg/kg)**	**ET. 30 % + D.v. (250 mg/kg)**	**ET. 30 % + D.v. (500 mg/kg)**
GB (10^3/mm^3)	6.46 ± 0.67	2.82 ± 0.36^b	5.27 ± 1.23**	6.48 ± 0.64***	4.96 ± 1.08*
GR (10^3/mm^3)	7.77 ± 0.34	5.674 ± 0.60^a	5.76 ± 0.95 ^{ns}	6.69 ± 0.92 ^{ns}	5.45 ± 0.56 ^{ns}
HGB (g/dl)	13.80 ± 0.40	11.04 ± 1.04^b	12.05 ± 1.13 ^{ns}	11.98 ± 0.77 ^{ns}	10.76 ± 0.91 ^{ns}
HCT B (%)	48.00 ± 1.72	36.36 ± 2.31^c	39.67 ± 4.03**	47.00 ± 2.64***	34.48 ± 3.06*
PLA (10^3/mm^3)	393.33 ±40.99	230.80 ±118.23^c	594.25 ±138.16***	577.60 ±52.85***	265.00 ±40.66***
VGM B (µm^3)	61.66 ± 0.66	69.40 ± 0.40^c	61.25 ± 1.55 ^{ns}	61.00 ± 0.63 ^{ns}	59.40 ± 0.51 ^{ns}
TGMH B (Pg)	17.80 ± 0.30	19.60 ± 0.31^c	19.30 ± 0.23 ^{ns}	16.58 ± 1.60**	18.54 ± 0.11 ^{ns}
CCMH (g/dl)	28.80 ± 0.26	32.34 ± 0.40^b	31.55 ± 1.11 ^{ns}	30.70 ± 3.09*	31.26 ± 0.23 ^{ns}
IDR (%)	11.96 ± 0.29	13.76 ± 0.30^a	13.67 ± 0.53 ^{ns}	13.52 ± 0.55 ^{ns}	12.96 ± 0.39 ^{ns}
VMP B (µm^3)	5.63 ± 0.18	6.22 ± 0.30^{NS}	6.50 ± 0.42 ^{ns}	6.22 ± 0.31 ^{ns}	7.48 ± 0.68 ^{ns}

Every value is a mean ± ESM, with n = 6; a p < 0.05, b p <0,01, c p < 0.001 meaningful difference in relation to the witness (distilled water); NS: non meaningful difference in relation to the witness (distilled water); *p < 0.05, **p < 0.01, ***p < 0.001 meaningful difference in relation to the witness (ethanol + distilled water); ns: non meaningful difference in relation to the witness (ethanol + distilled water). WD: Water distilled; ET.: Ethanol; Syl.: Sylimarin; *D.v.: Desmodium velutinum*. GB: Globule white; GR: Red globule; HGB: Hemoglobin; HCT: Hematocrite; PLA: Tablet; VGM: Volume globular means; TGMH: Rate corpuscular means in hemoglobin; CCMH: Concentration corpuscular average in hemoglobin; IDR: indication of distribution of the size of the red globule size, middle volume of the polynuclears,

Table 4: Variation of the relative masses of some noble organs of the rats.

Organes (mg/100 g)	Traitements				
	WD (1 ml/100 g)	**ET. 30 % + WD (1 ml/100 g)**	**ET. 30 % + Syl. (70 mg/kg)**	**ET. 30 % + D.v. (250 mg/kg)**	**ET. 30 % + D.v. (500 mg/kg)**
Left Kidney	242.74 ± 12.81	478.93 ± 22.60^{c}	231.53 ± 8.56***	233.33 ± 11.09***	241.46 ± 13.08***
Right Kidney	262.34 ± 10.73	495.81 ± 22.61^{c}	226.70 ± 9.36***	241.75 ± 12.19***	248.61 ± 15.04***
Spleen	186.84 ± 14.21	305.12 ± 11.34^{c}	139.41 ± 2.74***	148.62 ± 5.29***	146.86 ± 13.95***
Liver	2467.89 ±147.55	5427.37 ±99.31^{c}	2967.38 ±204.84***	2652.18 ±47.90***	3517.34 ±230.36***
Heart	324.82 ± 19.84	584.93 ± 14.57^{c}	289.62 ± 13.99***	300.36 ± 10.39***	305.19 ± 21.94***
VG	22.17 ± 7.91	90.85 ± 7.91^{c}	77.59 ± 11.28***	60.62 ± 6.84***	95.05 ± 7.05***
VG (g)/MC(g)	0.06 ± 0.01	0.40 ± 0.21^{a}	0.26 ± 0.03**	0.21 ± 0.02**	0.31 ± 0.01^{ns}

Every value is a mean ± ESM, with n = 6; a $p < 0.05$, c $p < 0.001$ meaningful difference in relation to the witness (distilled water); **$p < 0.01$, *** $p < 0.001$ meaningful difference in relation to the witness (ethanol + distilled water); ns: non meaningful difference in relation to the witness (ethanol + distilled water). WD: Water distilled; ET.: Ethanol; Syl. : Sylimarin; *D.v.*: *Desmodium velutinum*; VG: Left ventricle; MC: Mass of the heart

Table 5: Report frontal of the aqueous extract of the leaves of *Desmodium velutinum*.

Revealing: Neu	**System of éluant: AcOET / HCOOH / H_2O**				**Compounds supposed**
Extract : aqueous	**Coloration**		**Repport frontal**		
Chromatogramme 1	Blue		0.17		Flavonoïde
	Blue		0.31		
	Blue		0.47		
	Blue		0.7		
Chromatogramme 2	Blue		0.15		Flavonoïde
	Blue		0.27		
Chromatogramme 3	E. aq 1	E. aq 2	E. aq 1	E. aq 2	Flavonoïde
	Blue	Blue	0.16	0.17	
	Blue	Blue	0.41	0.24	
	Blue	Orange	0.47	0.82	
	Blue	Blue	0.82	0.84	

E .aq: aqueous extract.

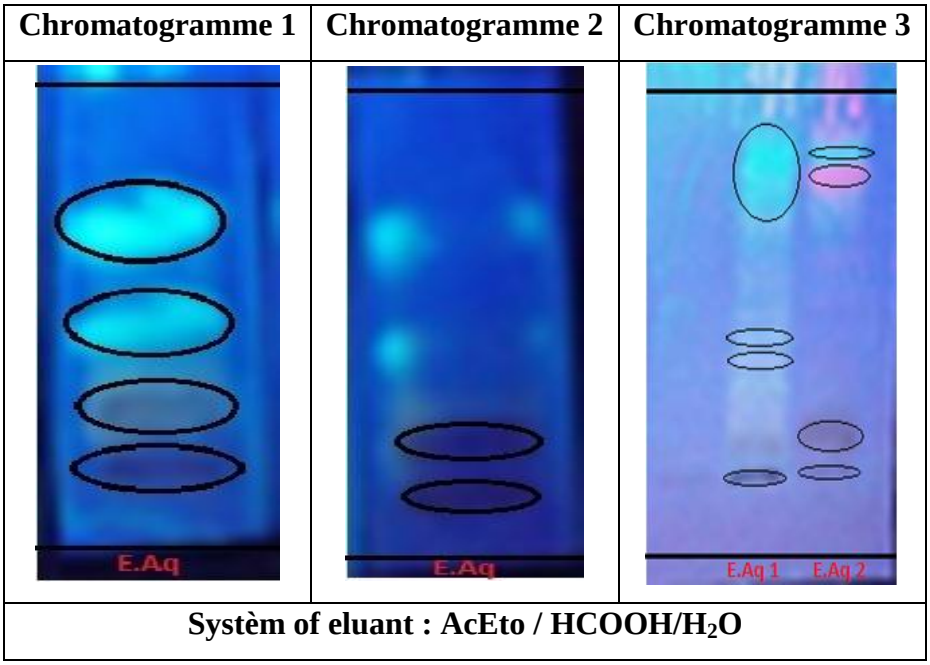

Figure 4: Chromatogram of the extract aqueous of the leaves of *Desmodium velutinum*.

Effects of aqueous extract of leaves of *D. velutinum* on biochemical parameters

In rats receiving ethanol 30% plus aqueous extract of *D. velutinum* leaves, serum of ALT and ASAT levels were reduced compared to are reduced compared those rats treated with ethanol 30% plus distilled water (Table 2). For triglycerides, total cholesterol, and blood glucose levels, there were no significant changes in rat treated with ethanol 30 % plus aqueous extract of leaves of *D. velutinum* leaves (250 and 500 mg/kg) as in rats treated with ethanol 30 % plus distilled water.

Effects of aqueous leaves extract of *D. velutinum* on hematological parameters in rats treated with ethanol 30 %

In rats treated with ethanol 30 % plus distilled water, ethanol caused a significant decrease in white blood cell (RBC), red blood cell (RBC) and hemoglobin (HGB) levels. These rates increased from 6.46 ± 0.67 10^3 / mm^3 to 2.82 ± 0.36 (GB) $p < 0.01$; 7.77 ± 0.34 to 5.674 ± 0.60 10^6 / mm^3 (GR) $p < 0.05$; 13.80 ± 0.40 to 11.04 ± 1.04 g / dl (HGB) $p < 0.01$ compared to the control group (Table 3); are relative decreases of 56.34; 27.02 and 20 %. In rats receiving ethanol 30% plus aqueous extracts of leaves of *D. velutinum* (250 and 500 mg / kg), these serum white blood cell (RBC), RBCs (RBC) and hemoglobin (HGB) are elevated relative to those of rats treated with ethanol 30 % (Table 3); with increases of 129.78; 18.16 and 8.69 %.

Effects of the aqueous extract of the leaves of *D. velutinum* on the mass of the noble organs in rats treated with ethanol 30 %

Table 4 shows that in the rats treated with the aqueous extract of the leaves of *D. velutinum* (250 and 500 mg / kg) in addition to the ethanol 30 %, there is a significant decrease of the masses of the noble organs as well as ratio of left ventricle-Heart mass versus rats treated with ethanol 30 % plus distilled water.

Presence of flavonoïds in the aqueous extracts of the leaves of *D. velutinum*

Orange and blue fluorescence spots observed at 365 nm on the aqueous extract, are attributable to flavonoïds (chromatogram 1, 2 and 3 of Figure 4). Table 5 shows the different frontal and color ratios on each chromatogram.

DISCUSSION

The objective of the present study was to evaluate the effects of the aqueous leaf extract of *D. velutinum* on the morphological, biochemical and haematological parameters altered by ethanol 30% in the wistar rat. The results obtained show a weight increase in the rats treated with ethanol plus the aqueous extract (250 and 500 mg / kg) compared to the control group (ethanol 30% + distilled water) in which it is noted a decrease of weight. This extract is therefore opposed to the decrease of weight caused by ethanol 30% in the rat. In the present study, an increase in food consumption was observed in rats treated with ethanol 30 % plus aqueous extract at both doses compared to the control group (ethanol 30% + distilled water). This result suggests that this extract would increase appetite which according to Lieber, (2000) is inhibited by chronic high-dose consumption of alcohol and consequently leads to weight loss. It is therefore possible that this effect of the aqueous extract of *D. velutinum* partly explains the weight gain observed in the rats treated in addition to ethanol at 30% with the extract at the two doses studied. The important water intake in the rats treated with ethanol plus aqueous extract of leaves of *D. velutinum* (250 and 500 mg / kg) compared to the control group (ethanol 30% plus distilled water) could be explained by the fact that ethanol develops a diuretic action which after dehydration leads to an intense thirst that this extract could stimulate. In addition, there was a significant decrease in the mass of noble organs (kidneys, spleen, liver and heart) in rats treated with ethanol 30 % plus this extract (250 and 500 mg/ kg) compared to the control group (ethanol 30 % plus distilled water). This extract would protect against the harmful effects of alcohol on noble organs. It is known that the hypertrophy of organs (kidneys, spleen, liver and heart) following the consumption of

ethanol is at the origin of certain pathologies (Verdecchia et al., 1998; Thulstrup et al., 1999). The special protection of the liver and heart suggests that this extract has hepatoprotective and cardio-protective effects. To test this hypothesis, the effects of this extract on ALT and ASAT, two biochemical markers of liver and muscle damage including the heart, were evaluated in the rat. The results show that this extract is significantly opposite to the increase of the two markers induced by ethanol 30 %, such as Sylimarin (standard drug, 70 mg/kg). Similarly, the results of the present study show that this extract opposes the increase in left ventricular mass and left ventricular hypertrophy induced by 30% ethanol. This result confirms the probable cardio-protective effects of this extract and the installation of cardiovascular risk factors (Devereux et al., 1997). Many authors have shown the hepatoprotective and cardio-protective effects of extracts from other medicinal plants (Sharma, 2001; Suchalatha and Shyamala Devi, 2003; Bouagnon et al., 2015; Preeti Biswas, 2017).

Similarly, the aqueous extract of the leaves of *D. velutinum* opposes the decrease in the level of certain hematological parameters (white blood cells, red blood cells, hemoglobin and platelets) caused by the subchronic administration of ethanol 30%. This extract therefore corrects these aggregations: leukopenia, erythropenia, hyperanemia and thrombocytopenia, synonymous with infection caused by ethanol 30 % (Benistant and Rubin, 1990). The presence of flavonoïds in the aqueous leaf extract of *D. velutinum* is thought to be responsible for the improvement of biological parameters altered by 30% ethanol. Indeed, flavonoïds are known for their antioxidant and anti-radical, hepatoprotective and cardio-protective effects (Bruneton, 2003).

CONCLUSION

The aqueous extract of the leaves of *D. velutinum* improves at the doses of 250 and 500 mg / kg the biological parameters altered by ethanol at 30%. This extract has hepatoprotective and cardio-protective effects. Flavonoïds, known to protect many biological parameters, present in the leaves of this plant are partly responsible for these effects. A study of the effects of the aqueous leaf extract of *D. velutinum* on ethanol-induced oxidative stress parameters in rats is interesting.

REFERENCES

1. Benistant C., Rubin R. Ethanol inhibits thrombin-induced secretion by human platelets at a site distinct from phospholipase C or protein kinase C. Biochem J., 1990; 269: 489-497.

2. Bouagnon R., Yeo D., Kouassi K., Beugre K., Djaman J.A., et Nguessan J.D. Hepatoprotective Effect of Aqueous Extract of *Lippia multiflora* Leaves against Ethanol-induced Toxicity in Wistar Rats. European Journal of Medicinal Plants, 2015; 7(3): 146-155.
3. Bouquet A. Féticheurs et médecines traditionnelles du Congo (Brazzaville), O.R.S.T. O.M. Paris, 1969; 188.
4. Bruneton. J. Pharmacognosie phytochimie plantes médecinales, 3ème édition, Tec et Doc, Paris, 2003; 1120.
5. Devereux R. Measurement of left ventricular mass: methodology and expertise. J hypertens, 1997; 15: 801–809.
6. Lieber C.S. Alcohol: Its metabolism and interaction with nutrients, Annu Rev Nutr., 2000; 20: 395-430.
7. OMS. Alcohol in the European Union: consumption,harm and policy approaches. (p. 161). Copenhague: Bureau régional d'Europe, 2012.
8. Preeti Biswas, Neelanchal Trivedi, Bhuvnesh Kumar Singh et Jha K.K. Evaluation of hepatoprotective activity of ethanolic root extract of "*Vaccaria pyramidata*" against CCl_4-induced hepatotoxicity in wistar rats. European Journal of Pharmaceutical and Medical Research, 2017; 4(9): 532: 538.
9. Room R., Babor T., Rehm J. Alcohol and public health. Lancet, 2005; 365(948): 519-530.
10. Sharmam Kishore K., Gupta S.K., Joshi S., et Arya D.S. Cardioprotective potential of *Ocimum sanctum* in isoproterenol induced myocardial infarction in rats. Journal of Experimental Biology, 2001; 225: 75-81.
11. Suchalatha S., et Shyamala Devi C.S. Protective effect of *Terminalia chebula* against experimental myocardial injury induced by isoproterenol. Journal of Experimental Biology, 2003; 219: 35-40.
12. Thulstrup A., Sorensen. H., Steffensen F., Vilstrup H., Lauritzen T., Changes inliver-derived enzymes and self-reported alcohol consumption. A 1-year follow-upstudy in Denmark. Scand J Gastroenterol, 1999; 34: 189-193.
13. Verdecchia P. Prognostic significance of serial changes in left ventricular mass in essential hypertension. Circulation, 1998; 97: 48–54.