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## Evaluation of cardiac biotolerance from total aqueous extract of *Entada mannii* (Fabaceae) at the wistar rat

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### Abstract

For four weeks of treatment, we evaluated the cardiac bio-tolerance of *Entada mannii* (Fabaceae) in rats through enzymatic and metabolic bio-chemical markers. *Entada mannii* is a plant which leaves and barks are used in the treatment of malaria and diabetes in Côte d'Ivoire. The treated rats through oral route with different doses varying from 0 to 800 mg/kg of body weight permitted to determine the serum enzymatic activity of aspartate amino transferase (ASAT), lactate dehydrogenase (LDH), the creatinekinase isoenzyme-MB (CK-MB) as well as the serum concentrations of triglycerides, total cholesterol and HDL-cholesterol.

The statistical analysis of the results shows significantly decrease ( $p < 0.05$ ) in the activity of ASAT and CK-MB as well as a non-significant variation in the activity of HDL. On the other hand, it highlights very significant increases ( $p < 0.01$ ) in the concentrations of triglycerides, total cholesterol and HDL-cholesterol from the end of the third week of treatment. But, the total cholesterol /HDL-cholesterol ration is lower than 4.5 whatever the doses eliminates the risk of cardiovascular accident. In conclusion, the aqueous extract of *Entada mannii* would be well tolerated by the body. However, to minimize eventual risk in human therapeutic, it would be judiciously to reduce dose to 400 mg/Kg and period of treatment to two week.

**Keywords:** *Entada mannii*, Rat, Cardiac bio-tolerance

### Introduction

Health is a priceless good, essential for life. In Africa and particularly in Ivory Coast, face to growing impoverishment and also the high costs of sanitary services, the populations rush towards medical plants for various affections treatment. About 90% of African populations would be concerned by this use. Face to this situation, the World Health Organization (WHO) decided in 1978 to upgrade the traditional pharmacopoeia in order to satisfy the populations in health matters.

The African floristic heritage is enormous. In Ivory Coast, about 50,000 identified plants in which we have 761 species and 1421 medical recipes [1]. However, the question of dosage remains a major challenge in the traditional pharmacopoeia because of the quantity of metabolic elements in preparedness. These molecules could cause damage to some body organs such as the heart during the treatment [2].

*Entada mannii* (Fabaceae) is a plant currently used by the South-East populations of Ivory Coast. It takes part in the treatment of diabetes and that of malaria [3]. The study of the acute toxicity from aqueous extracts of this plant at the wistar rat showed that the DL50 is more than 5000 mg/Kg of corporal weight [4]. Moreover, the phytochemical screening of aqueous extracts revealed the presence of sterols, polyterpenes, polyphenols, flavonoids, alkaloids and saponins [4]. Then, the executed studies have shown a strong antioxiding potential from acetatal and hexane extracts of this plant barks [5]. The dosage specifying the poison, it is necessary to show the limits tolerable by the organism in order to ensure the safety of extracts. It's in this dynamic way that the current study decided to measure the cardiac bio-tolerance from a total aqueous extract of *Entada mannii* by the testing of the seric activity of some enzymes such as Aspartate Aminotransferase (ASAT), Lactate Deshydrogenase (LDH) and Craetinekinase (CK-MB), and concentrations of metabolic elements such as cholesterols (Chol-T and HDL-c) and triglycerides (TG) at the rat.

## Materials and methods

### Preparedness of total aqueous extract of *Entada mannii*

The harvested barks in the area of Agboville (South-East area of Ivory Coast) have been identified in the Floristic National Center of University Felix Houphouët-Boigny (Ivory Coast-Abidjan). They have been washed, cut and dried to sun shed at room temperature for about two (2) months. The dried barks have been grinded with an electric mill of IKAMAG-RCT type. Twenty (20) grams of powder are macerated in 500 mL of distilled water and mixed with a blender. The homogenate we got is then filtered on watman paper 3MM. After the drying of the filtrate we got through a rotary evaporator at 50 °C, we get a brown powder which is used to prepare the total aqueous extract of *Entada mannii*.

### Treatment of animals

For this experience, 24 wistar albinos rats was been used. These animals are aged of ten (10) weeks and average about a mass of 178.75 ± 15.3g. they derived from a livestock of Bingerville (South-East Abidjan). Animals are set into four (4) groups of six (6) rats. Among those four groups, three experimental ones are given dosages of the extract that vary from 200 to 800 mg/Kg according to geometrical progression of ratio 2. Rats of group T (witness) are each one given 2 ml of distilled water used to administrate the extract solution. The solutions are used by oral route at the rate of 2 mL/rat. The rats of groups T, 1, 2 and 3 are thus given respectively dosages of 0, 200, 400 and 800 mg/Kg of corporal weight. The work is done everyday, at the same time, between 8 and 10 AM for four weeks. The blood samplings are also done once a week.

### Biochemical analysis of enzymatic parameters

The samplings are done at fasting at the level of the orbital sinus. The sampled blood is put in sterile pipes without anticoagulated. It is then centrifuged at 3000 towers/minutes for ten minutes with centrifuges. Therefore the serums we got are the samples to be analysed.

The reagents of the dosage of the enzymatic and metabolic parameters are formed by a whole of kits ASAT, LDH, CK-MB, Chol-T, HDL-c and TG from a biosystem. The dosages of ASAT, LDH and CK-MB are done with a semi-automaton of biochemistry. The analyses done with a spectrophotometer at 350 nm have permitted to determine the catalytic activity of ASAT by measuring the speed of disappearance of NADH whereas that of LDH is evaluated at 340 nm by measuring the speed of appearance of NADP+H+. For the catalytic of CK-MB, it's evaluated by measuring the speed of appearance of NADP+H+. The concentrations of metabolites are determined by colorimetric method with a spectrophotometer at 500 nm.

### Statistics analysis

The data have been processed thanks to Graph Pad Prism 8.0 software (Microsoft United States). The tests analysis (Anova) has been done according to the test multiple comparison of Tukey for the comparison of the mean values of the biochemical markers in each group. The difference is said to be sharp rise if  $p < 0.05$  and in sharp drop if  $p \geq 0.05$ .

### Results

The results of the seric activities changes of ASAT, LDH and CK-MB so that the concentrations of TG, Chol-T and HDL-c are described in the form of averages ± standard deviation respectively the tables 1-6. Those results are the averages of the five dosages done in each group.

W0 to W4: Week 0, 1, 2, 3, 4 (W0: Before processing).

The mean value of activity of ASAT in the witness group was about 59.33 ± 2.85 UI/L (Table 1) at W0. After four weeks, this value changes from 26 ± 1.73 UI/L (minimum at W2) to 92.5 ± 13.37 UI/L (maximum at W3) about -56.18% (W2) to 55.9% (W3), compared with initial value.

In group 1, the activity of ASAT was about 64.33 ± 7.95 UI/L at W0. During the four weeks, this value increased between 16 ± 1.53 UI/L (minimum at W4) and 63 ± 18.68 UI/L (maximum at W3). These values correspond to the changes from -75.13% (W4) to -2.07% (W3). The percentages of changes saved in group 2 and 3 are respectively from -50.45% (W2) to 11.71% (W3) and -67.82% (W4) to 97.75% (W3). Furthermore, the statistics analysis of result doesn't show any significant influence ( $p > 0.05$ ) of the dosage after the four weeks of processing. However, it denotes an influence of the exposure time with significant drops at W3 (for group 1) and at W4 for the other processed groups.

**Table 1:** The seric activity of ASAT (UI/L) at the processed and non-processed rats during time.

DOSAGES (mg/kg)	GROUP T 0 <sup>a</sup>	GROUP 1 200 <sup>a</sup>	GROUP 2 400 <sup>a</sup>	GROUP 3 800 <sup>a</sup>
W0	59,33 ± 2,85	64,33 ± 7,95	55,50 ± 6,17	48,17 ± 6,02
W1	52,00 ± 4,04	60,50 ± 6,35	47,00 ± 6,10	50,67 ± 3,71
W2	26,00 ± 1,73	50,33 ± 0,88	27,50 ± 1,04	42,75 ± 3,40
W3	92,50 ± 13,37	63,00* ± 18,68	62,00 ± 2,00	95,25 ± 4,03
W4	79,50 ± 9,89	16,00*** ± 1,53	35,00** ± 1,00	15,50*** ± 3,50

Data are described in average ± SEM, n = 6. The dosages affected with the same letter are not significantly different at the threshold of  $\alpha = 0.05$ . The stars show the differences between the groups of processed animals for each week (\*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ). The works are done compared to the Group T. Group T: Non processed witness, group having got distilled water. Group 1, 2 and 3: Processed groups at respective dosages of 200; 400 and 800 mg/Kg W0 to W4: Week 0, 1, 2, 3, 4 (W0: Before processing).

The mean value of the activity of LDH in the witness group was 266.6 ± 43.2 UI/L (Table 2) at W0. After four weeks, this value varies from 157.6 ± 17.43 UI/L (minimum at W2) to 239 ± 48.85 UI/L (maximum at W3) about -40.89% (W2) to -10.28% (W3), compared to the initial value.

In the group 1, the activity of the LDH was 336.17 ± 53.01 at W0. During processed, this value increased between 197.67 ± 12.13 UI/L (minimum at W4) and 339 ± 48.54 UI/L (maximum at W1). Those values correspond to the changes from -41.2% (W4) to -12.48% (W1).

The percentages of change saved in the groups 2 and 3 are respectively from -42.05% (W4) to -9.21% (W1) and -14.66 (W3) to 12.55% (W1).

Moreover, the statistical analysis of results doesn't show any significant influence ( $p > 0.05$ ) of the dosage nor the exposure time after four weeks of processing.

**Table 2:** Seric activity of LDH (UI/L) at processed and non-processed rats during the time

DOSAGES (mg/kg)	GROUP T 0 <sup>a</sup>	GROUP 1 200 <sup>a</sup>	GROUP 2 400 <sup>a</sup>	GROUP 3 800 <sup>a</sup>
W0	266,60 ± 43,20	336,17 ± 53,01	316,67 ± 60,08	252,33 ± 43,88
W1	176,20 ± 12,98	294,20 ± 33,66	287,50 ± 49,90	284,00 ± 56,32
W2	157,60 ± 17,43	203,33 ± 26,03	280,00 ± 63,51	256,67 ± 42,56
W3	239,20 ± 48,85	283,67 ± 35,38	186,50 ± 0,50	215,33 ± 14,44
W4	233,00 ± 60,01	197,67 ± 12,13	183,50 ± 0,50	241,00 ± 4,00

Data are described in average ± SEM, n = 6. The dosages affected with the same letter are not significantly different at the threshold of  $\alpha=0.05$ . The works are done compared to the Group T. Group T: Non processed witness, group having got distilled water. Group 1, 2 and 3: Processed groups at respective dosages of 200; 400 and 800 mg/Kg

W0 to W4: Week 0, 1, 2, 3, 4 (W0: Before processing).

The mean value of the activity of CK-MB in the witness group was  $158.8 \pm 7.26$  UI/L (Table 3) at W0. After four weeks, this value change from  $98.5 \pm 2.9$  UI/L (minimum at W4) to  $208 \pm 27.09$  UI/L (maximum at W1) about -37.89% (W4) to 31.15% (W1), compared to the initial value.

In group 1, the activity of CK-MB was about  $155.5 \pm 14.37$  UI/L at W0. During four weeks, this value increased between  $163.33 \pm 31.8$  U/L (minimum at W2) and  $220 \pm 30$  UI/L (maximum at W3). Those values correspond to changes from 5.04% (W2) to 41.48% (W3).

The percentages of change saved in the groups 2 and 3 are respectively from -3.7% (W2) to 46.53% (W3) and -10.68% (W1) to 20.55% (W4).

Furthermore, the statistical analysis of results doesn't show any significant influence of dosage after the four weeks of processing. But, it shows a significant drop at W1 for the group 3. However, at W3 and W4, it reveals a significant increase in the groups 1 and 2.

**Table 3:** Seric activity of CK-MB (UI/L) at processed and non-processed rats during the time

DOSAGES (mg/kg)	GROUP T 0 <sup>a</sup>	GROUP 1 200 <sup>a</sup>	GROUP 2 400 <sup>a</sup>	GROUP 3 800 <sup>a</sup>
W0	158,60 ± 7,26	155,50 ± 14,37	144,00 ± 16,18	145,17 ± 16,32
W1	208,00 ± 27,09	166,40 ± 18,73	194,00 ± 9,63	129,67* ± 11,86
W2	122,00 ± 15,06	163,33 ± 31,80	138,67 ± 25,73	144,00 ± 22,85
W3	111,20 ± 8,08	220,00*** ± 30,00	211,00** ± 1,00	175,00 ± 12,66
W4	98,50 ± 2,90	183,33** ± 10,40	190,50* ± 0,50	143,00 ± 11,00

Data are described in average ± SEM, n = 6. The dosages affected with the same letter are not significantly different at the threshold of  $\alpha=0.05$ . The stars show the differences between the groups of processed animals for each week (\*  $p<0.05$ ; \*\*  $p<0.01$ ; \*\*\*  $p<0.001$ ). The works are done compared to the Group T. Group T: Non processed witness,

group having got distilled water. Group 1, 2 and 3: Processed groups at respective dosages of 200; 400 and 800 mg/Kg.

W0 to W4: Week 0, 1, 2, 3, 4 (W0: Before processing).

The mean value of the concentration of triglycerides in the witness group was  $0.81 \pm 0.03$  g/L (Table 4) at W0. After four weeks, this value change from  $0.32 \pm 0.05$  g/L (minimum at W4) to  $1.46 \pm 0.3$  g/L (maximum at W2) about -60.19% (W4) to 80.74% (W2), compared to the initial value.

In group 1, the average of triglycerides was about  $0.94 \pm 0.1$  g/L at W0. During four weeks, this value increased between  $0.84 \pm 0.28$  g/L (minimum at W4) and  $1.67 \pm 0.37$  g/L (maximum at W3). Those values correspond to changes from 5.04% (W2) to 41.48% (W3).

The percentages of change saved in the groups 2 and 3 are respectively from 12.57% (W2) to 260% (W4) and 35.45% (W3) to 141.27% (W1).

Furthermore, the statistical analysis of results doesn't show any significant influence of dosage but a very significant influence of the exposure time after the four week of processing.

**Table 4:** Evolution of the seric concentration of triglycerides (g/L) at processed and non-processed rats during the time.

DOSAGES (mg/kg)	GROUP T 0 <sup>a</sup>	GROUP 1 200 <sup>a</sup>	GROUP 2 400 <sup>a</sup>	GROUP 3 800 <sup>a</sup>
W0	0,81 ± 0,03	0,49 ± 0,10	0,58 ± 0,14	0,63 ± 0,18
W1	0,79 ± 0,12	1,09 ± 0,11	1,41 ± 0,16	1,52* ± 0,23
W2	1,46 ± 0,30	1,32 ± 0,14	0,66* ± 0,27	0,87 ± 0,48
W3	0,47 ± 0,13	1,67*** ± 0,37	2,05*** ± 0,15	0,85 ± 0,11
W4	0,32 ± 0,05	0,84 ± 0,28	2,10*** ± 0,30	1,16* ± 0,18

Data are described in average ± SEM, n = 6. The dosages affected with the same letter are not significantly different at the threshold of  $\alpha=0.05$ . The stars show the differences between the groups of processed animals for each week (\*  $p<0.05$ ; \*\*\*  $p<0.001$ ). The works are done compared to the Group T. Group T: Non processed witness, group having got distilled water. Group 1, 2 and 3: Processed groups at respective dosages of 200; 400 and 800 mg/Kg.

W0 to W4: Week 0, 1, 2, 3, 4 (W0: Before processing).

The mean value of concentration of total cholesterol in the witness group was  $0.72 \pm 0.06$  g/L (Table 5) at W0. After four weeks, this value change from  $0.44 \pm 0.12$  g/L (minimum at W2) to  $1.24 \pm 0.13$  g/L (maximum at W1) about -38.5% (W2) to 71.75% (W1), compared to the initial value.

In group 1, the average of total cholesterol was about  $0.52 \pm 0.08$  g/L at W0. During four weeks, this value increased between  $0.62 \pm 0.19$  g/L (minimum at W2) and  $1.98 \pm 0.35$  g/L (maximum at W3). Those values correspond to changes from 21.04% (W2) to 283.82% (W3).

The percentages of change saved in the groups 2 and 3 are respectively from 42.86% (W2) to 162.13% (W4) and 0.32% (W2) to 272.24% (W3).

Furthermore, the statistical analysis of results doesn't show any significant influence of dosage after four weeks of processing. However, it shows an influence highly significant of the exposure time at W3 and W4 for the whole of the processed groups in the sense of increasing.

**Table 5:** Evolution of the seric concentration of total cholesterol (g/L) at processed and non-processed rats during the time.

DOSAGES (mg/kg)	GROUP T 0 <sup>a</sup>	GROUP 1 200 <sup>a</sup>	GROUP 2 400 <sup>a</sup>	GROUP 3 800 <sup>a</sup>
W0	0,72 ± 0,06	0,52 ± 0,08	0,50 ± 0,05	0,53 ± 0,06
W1	1,24 ± 0,12	0,81 ± 0,08	0,82 ± 0,13	0,73 ± 0,05
W2	0,44 ± 0,15	0,62 ± 0,19	0,72 ± 0,06	0,53 ± 0,09
W3	0,78 ± 0,23	1,98*** ± 0,35	1,23 ± 0,08	1,97*** ± 0,45
W4	0,46 ± 0,23	1,71*** ± 0,05	1,32** ± 0,09	1,55*** ± 0,05

Data are described in average ± SEM, n = 6. The dosages affected with the same letter are not significantly different at the threshold of  $\alpha=0.05$ . The stars show the differences between the groups of processed animals for each week (\*\*  $p<0.01$ ; \*\*\*  $p<0.001$ ). The works are done compared to the Group T. Group T: Non processed witness, group having got distilled water. Group 1, 2 and 3: Processed groups at respective dosages of 200; 400 and 800 mg/Kg.

W0 to W4: Week 0, 1, 2, 3, 4 (W0: Before processing).

The mean value of concentration of cholesterol HDL in the witness group was  $0.22 \pm 0.03$  g/L (Table 6) at W0. After four weeks, this value varies from  $0.26 \pm 0.04$  g/L (minimum at W1) to  $0.36 \pm 0.13$  g/L (maximum at W3) about 18.18% (W1) to 62.73% (W3), compared to the initial value.

In group 1, the average of total cholesterol was about  $0.17 \pm 0.01$  g/L at W0. During four weeks, this value increased between  $0.2 \pm 0.01$  g/L (minimum at W1) and  $1.05 \pm 0.42$  g/L (maximum at W4). Those values correspond to changes from 13.38% (W1) to 507.69% (W4).

The percentages of change saved in the groups 2 and 3 are respectively from 30.3% (W3) to 59.69% (W2) and -18.11% (W2) to 577.95% (W4).

Furthermore, the statistical analysis of results doesn't show any significant influence of dosage but denotes an influence highly significant of the exposure time at W4 for groups 1 and 3 during the processing.

**Table 6:** Evolution of the seric concentration of HDL cholesterol (g/L) at processed and non-processed rats during the time.

DOSAGES (mg/kg)	GROUP T 0 <sup>a</sup>	GROUP 1 200 <sup>a</sup>	GROUP 2 400 <sup>a</sup>	GROUP 3 800 <sup>a</sup>
W0	0,22 ± 0,02	0,17 ± 0,01	0,17 ± 0,02	0,21 ± 0,03
W1	0,26 ± 0,04	0,20 ± 0,01	0,25 ± 0,04	0,27 ± 0,05
W2	0,27 ± 0,09	0,21 ± 0,02	0,26 ± 0,03	0,17 ± 0,01
W3	0,36 ± 0,13	0,23 ± 0,03	0,22 ± 0,02	0,20 ± 0,06
W4	0,32 ± 0,05	1,05*** ± 0,42	0,23 ± 0,04	1,44*** ± 0,34

Data are described in average ± SEM, n = 6. The dosages affected with the same letter are not significantly different at the threshold of  $\alpha=0.05$ . The stars show the differences between the groups of processed animals for each week (\*\*  $p<0.01$ ; \*\*\*  $p<0.001$ ). The works are done compared to the Group T. Group T: Non processed witness, group having got distilled

water. Group 1, 2 and 3: Processed groups at respective dosages of 200; 400 and 800 mg/Kg.

## Discussion

CK-MB, ASAT, Cholesterols (total and HDL) are enzymes and metabolites which activities and concentrations permit to qualify the heart vitality [6]. For the LDH, its changes inform on the extent of cardiac tissue necrosis [7, 8, 9]. The mean values of different parameters saved before the processing whatever the group (witness and processed) don't show a significant difference and correspond to the standard values at the rat [10, 11].

The results of statistical analysis show a decrease highly significant ( $p<0.001$ ) of the seric activity of ASAT in the processed groups at the end of processing (W4) compared to the witness. The cardiac damages are generally linked with an increase of the ASAT activity [12, 13]. A plant like *Entada mannii* which extracts diminishes the ASAT activity would therefore be tolerated by heart.

Concerning the LDH, the results of statistical analysis don't show any significant influence of the dosage ( $p>0.05$ ) nor the exposure time after the four weeks of processing. The increase of the LDH activity calling for a cardiac tissue necrosis [8], it is clear that the aqueous extract of *Entada mannii* wouldn't favour the degradation of the cardiac tissue.

About the CK-MB, the results of statistical analysis highlight a significant decrease ( $p<0.05$ ) of the seric activity at the processed animals to the highest dosage (800 mg/Kg) at W1 so that a significant increase at W3 and W4 to 200 and 400 mg/Kg dosages. Generally speaking, the increase of the seric activity of CK-MB is linked with the damages of the cardiac tissues [8]. The reliability of this enzyme in the detection of the coronary thrombosis is so precise because of its sensitivity and its cardiospecificity [7, 10, 14]. This decrease of the CK-MB activity to the highest dosage would explain that the aqueous extract of *Entada mannii* wouldn't have a negative effect on the heart. Nevertheless, a reducing of the processing time to two weeks would permit to avoid possible adverse reactions.

During this current study, the seric concentrations of triglycerides knew significant increases at W3 and W4. This hypertriglyceridemia could favour the formation atheromatous plaque that increase the cardiovascular and thrombotic risks [15, 16]. However, these values are less than the double values of reference at the rats [17]. Those changes in the increasing sense could therefore be considered without particular damages for the cardiac tissues.

For the HDL cholesterol, the 200 and 800 mg/Kg dosages of corporal weight has led to a significant increase of this lipoprotein at W4. In the same way, the concentrations of total cholesterol has increased significantly at W3 and W4 for the whole of processed groups. The increase of the cholesterolemia can lead hepatitis, cardiac and renal damages [18]. In this case, the ratio Total cholesterol/HDL-cholesterol is used to guess a cardiovascular risk when it's more than 4.5 [19]. In our study, this ratio is about 3.01; 4.1 and 2.3 respectively for 200, 400 and 800 mg/Kg dosages. Seeing that, the ratio Total cholesterol/ HDL-cholesterol is less than 4.5, it's mean that the use of the aqueous extract of *Entada mannii* to dosages till 800 mg/Kg couldn't cause damages on the heart.

## Conclusion

From our study we notice that the use of aqueous extract of *Entada mannii* to dosages between 200 and 800 mg/Kg of corporal weight at the rat leads to a significant decrease

( $p < 0.05$ ) of the ASAT and CK-MB (to the highest dosage) so that a non-significant change ( $p > 0.05$ ) of LDH. These reductions testify that this extract would be well-tolerated by the heart. Concerning the concentrations of triglyceride, cholesterol-HDL and total cholesterol, they increased significantly as a function of time. However, the ratio total cholesterol/ HDL-cholesterol less than 4.5 for the whole of dosage averts all the risks cardiovascular accidents.

Moreover, the current study recommend a reduction of the dosage (at 400 mg/Kg) and that of the processing duration (to two weeks instead of four) in order to avoid possible risks in human therapeutic.

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