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Toxicological Assessment of Methanolic Stem Bark and Leaf Extracts of *Entada africana* Guill. and Perr., Mimosaceae

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Abstract: This study was aimed to assess the possible toxic effects of *Entada africana*, a widely used African medicinal plant. The acute toxicity of the methanolic stem bark and leaf extracts of *Entada africana* Guill. and Perr., (Mimosaceae) was assessed on mice. It revealed an average toxicity with a LD_{50} of 146.7 and 249.9 mg kg^{-1} body weight for stem barks and leaves, respectively. The extracts showed no cytotoxicity against KB and Vero cells. Sub-chronic toxicity was assessed in rabbits, which received orally, daily for a month, a dose corresponding to 10% of the LD_{50}. Compared to the control group this dose caused no significant ($p>0.05$) modification of haematological and biochemical parameters, total cholesterol, urea, creatinine and aspartate amino-transferase (AST). The extracts lowered serum glucose significantly ($p<0.05$) by 52% at first two weeks of treatment. The stem bark and leaf extracts showed temporary decrease ($p<0.05$) of Alamine amino transferase (ALT) by 26.1 and 39.1%, respectively. The stem bark extracts increased triglycerides significantly ($p<0.01$) by 108% at the end of last week of treatment. These investigations seemed to indicate the safety ob sub-chronic oral administration (up to 14.67 and 24.9 mg kg^{-1} body weight) of the methanolic extracts of *Entada africana* in rabbits.

Key words: *Entada africana*, toxicity, LD_{50}, cytotoxicity, haematological and biochemical parameters

INTRODUCTION

According to a World Health Organization (WHO) estimate, 80% of the world population resorts to traditional medicine to satisfy their primary health care needs and African countries consider traditional medicine a priority for health care in their regions (WHO, 2002). The medicinal virtues of plants are long known but their use is not yet free from accidents due to lack of hygiene, to misuse of self-medication or to the toxicity of some plants (Hans-Martin et Bindanda, 1992). Ensuring safety in plant therapy is therefore an important challenge for traditional medicine.

Entada africana is used in traditional medicine for various types of diseases and this use concerns all parts of the plant, such as stem, root and trunk bark, leaves, fruits and plant gum. It is used in Mali as a medicinal plant against malaria (Maiga *et al*., 2005) and an interesting antileishmanial activity has been demonstrated (Ahua *et al*., 2007). Anti-inflammatory, hepato-protective, wound-healing and haemostatic properties have been attributed to this tree (Diallo *et al*., 2001). Hepato-protective activity has been investigated and demonstrated clinically (Douaré, 1991) and *in vitro* (Sanogo *et al*., 1998). *E. africana* is also traditionally used against respiratory tract disorders (Occhiuto *et al*., 1999). In Burkina Faso, the plant is used for the treatment of diabetes, hypertension and diarrhea (Nacoulma-Ouedraogo, 1996). Reports from Senegal refer to decoctions of roots and trunk bark of *E. africana* as an anti-poison remedy and a macerate of the bark against bronchitis and coughing (Kerharo and Adam, 1974). It is also considered an antiseptic and wound-healing agent. Furthermore, roots are also known for their fortifier,

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diuretic, anti-gonococci and anti-syphilitic action. In the areas of northern Nigeria and Ghana, an infusion of leaves or bark is taken as a tonic (Oliver-Bever, 1986). Leaves are also commonly used for their pharmacological properties including haemostatic, antiseptic on wounds, sore and skin-infections, as an emetic administered in case of food-poisoning, as a tonic and stomachic, as an abortifacient, as an antipyretic and for rheumatism (Berhaut, 1975; Burkill, 1995). Moreover, their antioxidant potential has been confirmed by *in vitro* tests (Atawodi, 2004).

Despite the frequent use of the plant in folk medicine, very few reports can be found concerning its toxicity. Leaves are used for their piscicidal property (Kerharo and Adam, 1974), which suggests some toxicity. However, in toxicity trials on guinea pigs, a dose of 6 mg kg^{-1} body weight was reported not poisonous. The seeds are known to be poisonous (Burkill, 1995) and the roots contain antiproliferative saponins (Cioffi *et al*., 2006). *E. phaseoloides* like *E. africana* is used as fishing poison (Barua *et al*., 1988), the toxicity being attributed by the authors to triterpenoid saponins. Several studies on seeds from three representatives of the *Entada* genus, *E. phaseoloides*, *E. scandens* and *E. glandulosa* revealed their cytotoxic potential against cancer (Liu *et al*., 1972; Dai *et al*., 1991; Graham *et al*., 2000).

On this basis, the objective of the present study was to evaluate the possible toxic effects of *Entada africana* after intraperitoneal administration to mice and oral administration to rabbits. Acute toxicity was assessed and the effects on bone marrow, liver, serum lipid and renal function were investigated through analysing blood biochemical and haematological parameters. The cytotoxicity of the bark and leaves extracts was also tested.

MATERIALS AND METHODS

Collection of plant material: Plant material was collected in Gampéla, 15 km east of Ouagadougou (Burkina Faso) in September 2005. The plant was identified at the department of Biology and Ecology of Research Institute on Health Sciences (IRSS) and a sample (voucher specimen n° AT1) was made available at the herbarium of this Institute.

Preparation of plant extracts: Stem barks and leaves were separately dried under ventilation at room temperature and pulverized into powder form. Stem barks were extracted at room temperature twice sequentially (2×12 h) with methanol. The resulting extract was centrifuged (2000 rpm during 5 min) and concentrated under reduced pressure with a rotavapor (Büchi R 114) before lyophilization. Leaves were defatted with petroleum ether and dried. The extract obtained from the marc was centrifuged and set on activated carbon before concentration and lyophilization.

Experimental animals: Naval Medical Research Institute (NMRI) male and female mice (25-30 g) were obtained from the International Centre for Research-Development of stock farming in Sahelian Zone (CIRDES), Bobo Dioulasso (Burkina Faso). Male and female rabbits weighing on average 1.8 kg were obtained from the monks' stock farming of Koubri (Burkina Faso). Animals were kept in the animal house of the Research Institute in Health Sciences (IRSS/CNRST) at room temperature of 25-30°C and at 45-55% relative humidity for one month, in a 12 h dark and light cycle. They were then fed *ad libitum* with granules (20% of proteins) and tap water.

Determination of acute toxicity: The general acute toxicity was evaluated according to the Lichtfield and Wilcoxon method (Lichtfield and Wilcoxon, 1949), validated at the Institute laboratory. The mice fasted for at least 12 h. Six very homogeneous groups of 6 mice each, from which one untreated control group was selected, were given increasing doses (50-400 mg kg^{-1}) of the extract through an intraperitoneal route. The volume administered was never higher than 0.4 mL (McLeod, 1970). The control group received only the solvent (DMSO 2% in NaCl 0.9%) used for diluting the extracts. Two hours after the different doses administration, the animals were fed and observed for 72 h. The LD_{50} was calculated at the end of 72 h.

Determination of cytotoxicity: The cytotoxicity of leaf and stem bark was tested on KB (Human epidermoid carcinoma) and Vero (African green monkey kidney) cells, according to the method described by Mbatchi *et al*. (2006). The reference used was taxotere.

Haematological and biochemical parameters: Rabbits were divided into three groups of 8, comprised of 4 males and 4 females:

- A control group was administered with 2% DMSO in 0.9% of NaCl;
- Test groups of I and II received 15 and 25 mg kg^{-1} body weight of methanolic stem bark and leaf extracts, respectively.

The doses are 0.1 of the LD_{50} of the extracts and the volume administered ranged from 4.0 to 7.1 mL according to the animals weight (Lu, 1992).

The tested products were orally administered daily for a month. Blood samples were collected from the lateral marginal ear vein (Mader, 1997) of rabbits fasted overnight on days 0, 3, 7, 14 and 29 before administration of the drug. The blood samples (2 mL) were divided into two equal portions for dosage of biochemical and haematological parameters. For haematological evaluation, the total blood was collected on EDTA-K_2 anticoagulant in haemolyse tubes. Haematological parameters were evaluated with a COULTER MD II automated haematological analyzer. Biochemical parameters concentrations in serum were measured spectrophotometrically on a COBAS MIRA PLUS. These parameters were glucose (Trinder, 1969), total cholesterol (Allain *et al*., 1974), triglycerides (Fossati and Prencipe, 1982), urea (Gutmann and Bergmeyer, 1974), creatinine (Houot, 1985), aspartate amino transferase and alanine amino transferase (Thefeld *et al*., 1974).

Statistical analysis: Results of the acute toxicity test were analysed with PHARM/PCS 4.2 software using the Lichtfield and Wilcoxon test. For haematological and biochemical parameters, data were expressed as mean±SEM and significant differences between groups were calculated according to Student's t-test using GraphPad Prism version 2.01 for Windows, GraphPad Software, San Diego California USA. Differences were accepted as significant for $p<0.05$.

RESULTS AND DISCUSSION

Preparation of plant extracts: The extraction yield for leaf and stem bark extracts was 8.9 and 20.6%, respectively.

Acute toxicity: Acute toxicity signs noted in the NMRI mouse were the decrease of the exploration instinct marked by a group or individual huddling in a corner of the cage, a bristling of the hair, respiratory problems, a refusal to eat for 24 or 48 h. Death occurred in a position of isolated prostration, of inanition in the middle or corner of the cage. These signs became more prominent as the dose increased. However, the animals seemed to keep their reflexes and there were no visible signs of damage to the locomotor system.

The LD_{50} value obtained for methanolic stem bark and leaf extracts are 146.7 and 249.9 mg kg^{-1} body weight, respectively (Table 1). This places them among drugs of average toxicity according to the Hodge and Sterner scale (Hodge and Sterner, 1943). The regularity of the regression line associated to the lethal effect dependent on the administered dose was confirmed by the identical LD_{99}/LD_{50} and LD_{50}/LD_1 ratios. This confirmed the test validity. The Security Index (SI) expressed by the ratio LD_{99}/LD_1 was average for the barks, with a value of 5.95 but rather weak for the leaves, with a value of 4.64. Therefore, it is probable that the dose evolution was appropriated for the stem barks and it has to be refined for the leaves.

Cytotoxicity: The stem bark and leaf extracts of *E. africana* have absolutely no cytotoxic activity against KB and Vero cells at the concentration of 10 μg mL^{-1}. This supports the traditional use of the plant, demonstrating that if the dose is carefully chosen, a stem bark or leaf extract can be safely prescribed by traditional practitioners.

Haematological parameters: A statistical analysis of these results indicates that both *E. africana* extracts administered orally over a month induce only a slight disturbance of both haematological and biochemical parameters. The stem bark extracts significantly ($p<0.05$) decreased monocyte concentration by 56.14% and the leaves extract significantly ($p<0.05$) increased lymphocytes concentration by 145.5% on the 14th day of treatment. The obtained values (Table 2 and 3) are comparable to those indicated in the relevant literature (Mitruka and Rawley, 1977). The rates of monocytes and lymphocytes returned to normal values before the end of treatment. The disturbances observed on the 2nd week could be explained simply by some animal stress.

Biochemical parameters: The values of the biochemical parameters are shown in Table 4.

- **Glucose metabolism:** Both leaf and stem bark extracts affected the glucose metabolism. Both extracts significantly ($p<0.05$) decreased glucose concentration by 17.04 and 28.6%, respectively on the 7th day of treatment and the leaf extracts decreased concentration by 52.2% on the 14th day of treatment. Such activity could suggest a

Table 1: Acute toxicity of methanolic stem bark and leaf extracts of *E. africana* on the NMRI mouse

Plant parts (mg kg^{-1})	CI limits at 95% Lower	Upper	LD_{50}	LD_{99}	LD_1	LD_{99}/LD_{50}	LD_{50}/LD_1	LD_{99}/LD_1
Stem barks	108.16	198.98	146.70	357.88	60.13	2.44	2.44	5.95
Leaves	192.29	324.81	249.92	538.09	116.07	2.15	2.15	4.64

CI = Confidence Interval

Table 2: Effect of the methanolic stem bark and leaf extracts of *E. africana* on white cells and differential values in rabbits

Plant parts	Content in the blood after administration (days)					
(K µL^{-1})	0	3	7	14	29	Ref. value
WBC						
Control	5.23±0.60	4.10±0.54	5.39±0.47	5.49±0.46	5.48±0.53	5-13
Stem barks	4.93±0.59	4.91±0.59	3.93±0.57	6.36±0.72	5.74±0.59	5-13
Leaves	4.79±0.80	3.58±0.70	4.69±0.73	6.77±0.76	6.89±1.05	5-13
Neutro						
Control	1.38±0.42	0.98±0.28	1.99±0.47	1.90±0.32	2.09±0.28	1-4
Stem barks	0.82±0.22	1.79±0.43	0.67±0.34	2.71±0.28	1.80±0.36	1-4
Leaves	0.83±0.26	1.56±0.48	1.47±0.46	1.76±0.22	3.18±0.93	1-4
Eosino						
Control	0.16±0.05	0.19±0.04	0.13±0.05	0.29±0.04	0.24±0.04	< 1.0
Stem barks	0.45±0.15	0.13±0.05	0.22±0.10	0.26±0.05	0.15±0.02	< 1.0
Leaves	0.23±0.07	0.09±0.04	0.20±0.11	0.31±0.08	0.46±0.14	< 1.0
Baso						
Control	0.00±00	0.00±00	0.00±00	0.00±00	0.00±00	< 0.5
Stem barks	0.00±00	0.00±00	0.00±00	0.00±00	0.00±00	< 0.5
Leaves	0.00±00	0.00±00	0.00±00	0.00±00	0.00±00	< 0.5
Lym						
Control	3.41±0.43	1.85±0.22	2.11±0.40	2.70±0.23	2.80±0.33	2.0-8.6
Stem barks	2.55±0.26	2.02±0.49	2.24±0.43	2.96±0.52	2.88±0.62	2.0-8.6
Leaves	2.55±0.48	1.43±0.30	2.27±0.53	3.93±0.56*	2.93±0.29	2.0-8.6
Mono						
Control	0.43±0.11	0.47±0.19	0.56±0.26	0.57±0.13	0.34±0.06	< 0.5
Stem barks	0.88±0.17	0.52±0.21	0.81±0.19	0.25±0.05*	0.19±0.05	< 0.5
Leaves	0.81±0.16	0.60±0.01	0.38±0.15	0.76±0.16	0.32±0.06	< 0.5

Values are mean±SEM, n = 8; Control (DMSO 2% in NaCl 0.9%); Test (15 mg kg^{-1}), *: p<0.05; Ref. Val. = Reference values; WBC = White Blood Cells; Neutro = Neutrophils; Eosino = Eosinophils; Baso = Basophils; Lym = Lymphocytes; Mono = Monocytes

Table 3: Effect of the methanolic stem bark and leaf extracts of *E. africana* on red cells, dependable factors and red cells indices in rabbits

	Content in the blood after administration (days)					
Plant parts	0	3	7	14	29	Ref. value
RBC (M µL^{-1})						
Control	5.36±0.23	4.76±0.31	4.27±0.34	4.49±0.16	4.66±0.13	3.8-7.9
Stem barks	5.03±0.37	4.51±0.50	3.86±0.25	4.54±0.21	4.29±0.09	3.8-7.9
Leaves	5.12±0.34	4.53±0.48	3.86±0.25	4.67±0.46	4.85±0.22	3.8-7.9
Hb (g dL^{-1})						
Control	12.41±0.44	10.80±0.60	10.66±0.32	10.19±0.29	10.45±0.28	9.4-17.4
Stem barks	11.40±0.62	10.59±0.71	9.52±0.48	10.50±0.44	9.82±0.20	9.4-17.4
Leaves	11.58±0.60	10.62±0.98	10.51±0.89	10.74±0.39	10.61±0.31	9.4-17.4
PCV (mL %)						
Control	37.04±1.48	34.12±1.60	32.20±0.94	31.64±1.06	32.98±0.83	33-50
Stem barks	34.69±2.18	32.76±2.55	28.28±1.67	32.01±1.48	30.68±0.55	33-50
Leaves	36.19±1.85	32.62±2.83	33.06±2.79	34.23±1.12	34.63±0.99	33-50
MCV (fL)						
Control	69.13±0.44	68.86±0.59	70.63±0.71	70.50±0.63	70.88±0.67	50-75
Stem barks	69.38±1.02	68.88±1.13	71.00±1.07	70.57±1.31	71.67±0.61	50-75
Leaves	71.50±2.04	70.43±0.87	71.13±1.62	70.86±1.30	72.13±1.16	50-75
MCH (pg)						
Control	23.20±0.26	22.81±0.30	23.40±0.26	22.76±0.27	22.41±0.29	18-24
Stem barks	22.94±0.51	22.46±0.57	24.04±0.31	23.19±0.47	22.98±0.46	18-24
Leaves	22.86±0.55	24.07±0.59	22.93±0.45	22.23±0.34	22.13±0.33	18-24
MCHC (g dL^{-1})						
Control	33.55±0.26	33.16±0.26	33.18±0.16	32.23±0.21	31.75±0.35	22.0-38.7
Stem barks	32.99±0.44	32.68±0.40	33.77±0.34	32.83±0.28	32.02±0.49	22.0-38.7
Leaves	32.00±0.29*	32.82±0.41	32.29±0.59	31.39±0.20	30.70±0.25	22.0-38.7
Platelet (K µL^{-1})						
Control	206.91±14.52	164.05±23.85	136.60±8.80	156.30±20.48	161.32±17.05	120-650
Stem barks	175.33±10.91	156.37±19.85	171.83±17.44	123.13±18.43	158.00±22.65	120-650
Leaves	200.69±16.28	145.32±42.87	178.28±29.32	167.08±20.83	134.80±16.48	120-650

Values are mean±SEM, n = 8; Control (DMSO 2% in NaCl 0.9%); Test (15 mg kg^{-1}); *: p<0.05. Ref. Val. = Reference values; RBC = Red Blood Cells; Hb = Hemoglobin; PCV = Packed Cell Volume; MCV = Mean Corpuscular Volume; MCH = Mean Corpuscular Hemoglobin; MCHC = Mean Corpuscular Hemoglobin Concentration

Table 4: Effect of the methanolic stem bark and leaf extracts of *E. africana* on blood biochemical parameters in rabbits

	Content in the blood after administration (days)					
Plant parts	0	3	7	14	29	Ref. Value
Glucose (mM)						
Control	6.88±0.75	6.04±0.72	8.45±0.13	8.92±0.33	7.24±0.22	4.2-8.9
Stem barks	8.72±0.58	5.66±0.48	7.01±0.60*	7.77±0.44	6.88±0.27	4.2-8.9
Leaves	8.18±0.41	7.19±0.36	6.03±0.95*	4.26±0.60***	7.06±0.57	4.2-8.9
Cholesterol (mM)						
Control	1.27±0.98	1.98±0.43	1.26±0.30	1.56±0.23	2.96±0.53	0.1-2.00
Stem barks	1.10±0.32	2.08±0.25	1.33±0.13	2.14±0.32	3.80±0.44	0.1-2.00
Leaves	1.07±0.30	1.70±0.17	0.87±0.08	1.03±0.15	3.20±0.54	0.1-2.00
Triglycerides (mM)						
Control	0.77±0.15	0.55±0.09	0.88±0.12	0.79±0.11	0.80±0.09	1.4-1.76
Stem barks	1.26±0.19	0.85±0.11	0.83±0.19	1.39±0.28	1.67±0.29**	1.4-1.76
Leaves	1.10±0.15	0.71±0.07	0.75±0.08	0.66±0.10	0.98±0.09	1.4-1.76
Creatinine (µM)						
Control	191.70±7.49	87.33±9.57	107.40±5.98	97.25±6.40	147.10±6.18	53-124
Stem barks	185.00±7.43	76.33±7.91	93.17±9.35	101.60±4.33	153.70±4.47	53-124
Leaves	209.10±7.43	89.17±4.74	91.13±8.69	38.13±11.84***	149.60±6.77	53-124
Urea (mM)						
Control	8.37±0.58	7.45±0.68	8.20±0.62	8.72±0.56	11.24±0.69	9.1-25.5
Stem barks	8.46±0.69	7.97±0.75	7.92±0.91	8.04±0.89	11.78±0.69	9.1-25.5
Leaves	9.49±0.79	7.60±0.56	7.61±0.70	5.46±0.83**	12.77±0.78	9.1-25.5
AST (IU L^{-1})						
Control	55.14±12.35	49.67±8.26	28.00±4.31	42.88±3.50	68.13±9.91	10-98
Stem barks	43.00±11.30	36.00±5.65	24.60±3.80	53.86±11.75	72.50±16.64	10-98
Leaves	54.57±18.43	36.71±3.50	28.00±5.13	41.88±9.06	98.00±23.88	10-98
ALT (IU L^{-1})						
Control	106.92±9.60	107.50±14.56	83.38±7.08	84.13±6.91	127.10±7.32	25-65
Stem barks	81.43±16.60	92.00±15.38	60.00±11.91	82.14±18.50	93.80±11.52*	25-65
Leaves	97.43±23.98	130.50±18.23	84.00±14.59	51.25±9.34*	164.30±40.34	25-65

Values are mean±SEM, n = 8; Control (DMSO 2% in NaCl 0.9%); Test (15 mg kg^{-1}); *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$; Ref. Val. = Reference values; AST = Aspartate amino transferase; ALT = Alanine amino transferase

hypoglycaemic effect of the plant, but the extract effect kept the rabbit's glycaemia values within the biological norms (4.2-8.9 mM). Thus, the traditional use of *E. africana* in the diabetes treatment (Nacoulma-Ouedraogo, 1996) cannot be validated by our kind of study. Complementary studies would be necessary.

- **Lipid metabolism:** The extracts do not seem to have a significant effect on the lipid metabolism. Cholesterolemia is not affected but the stem bark extracts were found to significantly ($p<0.01$) increase triglycerides concentration by about 108.7% during the last week of treatment. This value is still within the biological norms (1.4-1.76 mM).
- **Kidney exploration:** The leaf extracts showed significant decrease of creatinine ($p<0.001$) and urea ($p<0.01$) by 61.0 and 51.4% respectively at the end of the second week of treatment. This effect is only temporary, since the values were returned to a normal concentration at the end of the last week of treatment. These results do not suggest a kidney failure, because such a failure would rather increase urea and creatinine concentration in blood (Ovuru *et al*., 2004).
- **Hepatic exploration:** The elevation of ALT in the treated groups is not due to the treatment, since the values are as high in the control group. It does not clearly point to a liver injury, since AST values are normal and the ALT value was not greater than 3 times the upper limit of normal, which is (in human beings) the marker of a liver injury (Holt and Ju, 2006). From the results, stem bark and leaf extracts were found to decrease ($p<0.05$) ALT by 26.1% and 39.1% respectively. The decrease in transaminase level due to the treatment shows that the extracts do not cause any cytolysis but may have hepatoprotective effect (Diallo *et al*., 2005; Sanogo *et al*., 1998). The hepatoprotective activity of *E. africana* was attributed to the saponins and tanins of the plant (Sanogo *et al*., 1998).

This study has clearly demonstrated an average toxicity of stem bark and leaf extracts of *E. africana* at 15.0 and 25.0 mg kg^{-1}, respectively. From the haematological and biochemical results, we conclude that *E. africana* stem bark and leaf extracts may have slight beneficial effect on serum glucose and this justifies further investigations. An extended treatment up to a month may

have some side effects on lipid metabolism. The use of this plant in traditional medicine may pose few risks since there was no cytotoxicity against KB and Vero cells as vital functions such as bone marrow, kidney or liver were not negatively affected by a daily administration of one month.

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