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## Biological and Toxicological Study of Aqueous Root Extract from *Mitragyna inermis* (Willd oktze) Rubiaceae

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**Abstract:** We reported the results of biological and toxicological study, realized on *Mitragyna inermis* Willd Oktze, one specie of family of Rubiaceae, well known in traditional medicine in Burkina Faso for his intensification potentialities of resistance against multiple pathologies like infectious and parasitic diseases, adynamia, rheumatic and osteoarthritis diseases. So we have formulated hypothesis of stimulation of organism's defenses for a scientist research. The steeping freeze-dried of the plant's product has been used for different assays: General acute toxicity estimation on Ico mouse (NMRI Han) by intraperitoneal route and orally administration on rabbit from the value of LD50 obtained with the mouse. Biological study: The kinetic interaction between the plant's product chemical group and the evolution of biological elements medium of immunity on the rabbit, has been appreciated. The biological elements include white blood cells, red blood cells, lymphocytes, platelets, total proteins, albumin and globulins. The following results have been obtained about the study: A Lethal Dose (LD50) resulted from maceration (acute general toxicity) at the rate of 800 mg kg<sup>-1</sup> of corporal weight showing a bit toxic product. An interaction between vegetable's extract chemical group and biological elements of rabbit which is expressed by: An increasing (13 to 18%) of total proteins from serum; this increasing was notably after 24 h of administration. Albumin decreasing of 10% in comparison with initial rate, indicated haptens action of plant's extract chemical products.  $\alpha_1$ ,  $\alpha_2$ ,  $\beta$  and  $\gamma$  globulins increasing, respectively 46.8, 14.31 and 26% during the first day of administration of the extract. A lymphocyte increasing of 35% 24 h after administration of the product. This rate is more increased after the second administration; White blood cells are also increasing. These results show an obvious capacity of the plant's macerated extract to stimulate organism natural defenses in relation with antigenous-antibody reaction. This will be an interesting perspective for complementary treatment of pathology like HIV diseases.

**Key words:** *Mitragyna inermis*, traditional medicine, LD50, immune system

### INTRODUCTION

Many African's countries uses traditional medicine for their health needs; According to WHO, 80% of the population uses traditional medicine (WHO, 2003: Aide Mémoire N°939). However the administrated medicines are often unknown concerning their toxicity and biological properties. Plants take up an important place in traditional medicine and scientific research confirmed properties about used plant in African's traditional medicine. Among these plants, *Mitragyna inermis* is knowed for his multiple properties: Gastro-intestinal disturbances, infectious

diseases (Sy *et al.*, 2004), antimalarial activity and the lack of genotoxicity *in vitro* and *in vivo* have. (Monjanel-Mouterde, 2005). The plant is also used for fever decreasing and stimulating properties (Nacoulma, 1996). Moreover the results of an ethnobotanical investigation realized in Ouagadougou (Burkina Faso) concerning the plants used for the reimbursement HIV/AIDS carriers persons have revealed that *Mitragyna inermis* makes up the plants used by medical practitioner. He makes up group plants systematically prescribed to these suspects (Le Pharmacien d'Afrique, 2004).

Pharmacologic properties of plant led us to formulate the hypothesis of stimulation of organism immune system on rabbit, objective of present study.

To reach this purpose, we are going to realize an investigation on *Mitragyna inermis* Willd oktze concerning its effect on stimulation of biological elements, medium of immunity of rabbit. Our previous study showed the chemical principles usefulness of this plant: alkaloids, anthraquinones, sterols and triterpens, saponosids, flavonoids (Ouédraogo, 2003). The study consisted in administrating aqueous extract by orally route on the rabbit and to monitor the progress of biological parameters as white blood cells, red blood cells, lymphocytes, platelets, total proteins and different globulins.

## MATERIALS AND METHODS

The present study was carried out during the year 2004 at Research Institute of health Sciences of Ouagadougou, Burkina Faso.

### Material for study:

- Aqueous extract freeze-dried from *Mitragyna inermis* Willd Oktze root.
- PCS software for data analysis.

### Animals:

- Males rabbit from 1.6 to 2 kg coming from domestic breeding and tabulated at the laboratory before experience.
- Ico Mouse INMRI (I.O.P.S) Han which weighted from 20-30 g coming from Development and Research International Center on Sub Humid Breeding (DRIC SB) sis Bobo Dioulasso (Burkina Faso). Arrived at IRSS/CNRST's installations, growth conditions of animals are fixed:
- Feeding with pellets of the cattle food factory (AFAB) of Bobo-Dioulasso, containing 20% of proteins and water,
- Lighting from 6-18 h to respect the circadian rhythm,
- Continuous conditioned air to keep the temperature of 25°C.

**Plant material:** The vegetable material was collected at 7 km from Ouagadougou (on the road to Fada) in Burkina Faso, at the end of April 1999, (dried season), on the natural site where the plant grew.

The plant material was taxonomically identified by Prof. J. Millogo, a botanist from University of Ouagadougou where specimen of the plant is let.

The vegetable bodies are carefully washed with water and dried in the laboratory, thanks to continuous ventilation, away from sun light and dust. Then, they are crushed finely and kept in closed plastic bags which are also kept far from light and moisture. The vegetable extract used for the study is obtained after maceration.

**Maceration extract:** She is got by blending of 200 g vegetable powder and 1000 mL distilled water under magnetic agitation during 24 h. The macerate is filtered on absorbent cotton and the filtrate is centrifuged to 2000 t/mn during 10 mn.

The freeze-dried is formed at the temperature of -45°C during 72 h with Christ Alpha 1-2. PN 100200.

### Analytical apparatus

#### Electrophoretic system (Laboratoire SEBIA):

- Power supply PN GD61.
- Electrophoresis chamber PN S 60 A.
- Cellogel band from 5.7×14 cm.
- Micro 4 POS applicator.
- Densitometer, Preference/DVS, PN 1500 for integration of protein fraction.
- Spectrophotometer UV. VIS.SF 0188 PN 45.

**Coulter:** PN MDII. (Breillot, 1995). Automatic aspiration hematology instrument for measurement of red blood cells, white blood cells, lymphocytes and platelets.

### Method for study

**Toxicity study:** The mouse choice for LD50 determination is a consensus to appreciate the degree of toxicity of chemical substances.

The study consisted in determining the amount of vegetal extract which leads at least 50% of the experimentation animals to death.

We proceeded to a preliminary test with the same conditions, with 3 batches composed ones 4 mice, giving the following amounts of vegetable extract: 3000, 2500 and 2000 mg kg<sup>-1</sup>.

This preliminary test allowed to determinate doses for the study according to Table 1.

Animals are beforehand deprived from food during 24 h. Then each mouse is weighed. Each batch receives a given dose of vegetable extract. The route of administration is intraperitoneal route.

We used 6 batches of 18 mice and 1 pilot batch. Each animal is identified by a number and a difference mark which is common within each batch.

The method of calculation of LD50 and its trust limits (S) are defined by Miller and Tainter (1944):

$$S = \frac{\text{Lethal dose } 84\% - \text{Lethal dose } 16\%}{2}$$

Table 1: Study doses in order to determine general acute toxicity

Batches	T	1	2	3	4	5	6
Doses (mg kg <sup>-1</sup> )	0	300	500	750	1000	1500	2000

The standard deviation of LD50 is given by the following formula  $2S/2N'$  where N is the total number of animals in batches which death percentages are between 7 and 93%. Graphic curve is realized with experimental data to have LD1 (non toxicity dose), LD50, LD99 (sure toxicity dose).

LD99/LD50 and LD50/LD1 are compared to prove valid experience (same results).

LD99/LD1 is calculated like Security Index (SI) for possible utilization prediction

SI ≤ 5 = bad for potential use; 5 ≤ SI ≤ 10 = acceptable for potential use;  
SI ≥ 10 = good for potential use.

The value of LD50 will allow to find out the level of toxicity of the extract on the toxicity scale suggested by WHO/IPCS (2002), which is an adaptation the scale defined by Hodege and Sterner (1980).

**Biological study:** Analysis methods which were used are validated classical modern methods and often used in medical analysis laboratory: Determination of blood elements with the Coulter principle (Breillot, 1995), proportioning of proteins contained in serum with the method of biuret (copper sulfate in alkaline medium, Biomérieux, 1989), the determination of proteinic fractions with the electrophoresis technique of proteins on acetate (Laboratoire Sebiba).

**Total proteins from serum determination:** The method used to determinate the total proteins is that's giving the biuret colouring (copper sulfate in alkaline medium). The protocol is summarized by following Table 2.

The reading has been realized at 540 nm compare to the blank with spectrophotometer UV-Vis SF 0188 Model 45. (Laboratoire Biomérieux-1989).

**Electrophoresis technique of proteins:** Cellogel bands (cellulose acetate) are immersed in buffer (Tris-Veronal pH 9) during 10-15 mn. After drying and are fixed on fix-bands. The stages are:

- **Migration:** 200 volts during;
- **Coloration:** immersion in flaming-red during 5mn;
- **Discolouration:** bands are submitted to 3-4 baths in acetic acid 5%;
- **Transparency:** dehydration of the bands by pure methanol during 3-4 mn. Immersion in the transparency solution (Methanol pur/acetic Acid/Diacetonalcool) during 1 to 1 mn 30.

Table 2: Determination of total proteins

Parameters	Blank mL	Standard mL	Dosage mL
Serum	-	-	0.1
Reactive 1	-	0.1	-
Work solution	5	5	5

R1 = standard solution: bovine albumin à 100 g L<sup>-1</sup>, R2 = alkaline reactive: tartrate of K (potassium) and Na (sodium) 9 g L<sup>-1</sup>, Hydroxide of Na 0, 2 mole L<sup>-1</sup>, Iodure of K 5 g, Works solution: R2 + R3 (copper sulfate 150 g L<sup>-1</sup>)

After drying at temperature ambient, the reading has been realized by a densitometer "PREFERENCE" (at 530 nm, green filter) which permit to quantify each protein fraction from the determination of total proteins (Laboratoire Sebiba).

**Blood cells numeration:** Blood has been taken by venous puncture on anticoagulant (EDTA rate of 3 mg mL<sup>-1</sup> of blood). Total blood so obtained is introduced in an apparatus model Coulter type MD II which used for medical analysis.

Red blood cells, white blood cells, lymphocytes and platelets numeration are realized thanks to principle impedance variation.

Administrations of the extracts are made by oral route following the LD50 value. The administrated dose is the 8th of the LD50. This dose is chosen because the plant is well known in traditional medicine, regarding to its tolerance. The principle of the dose to use from the value of LD50 is the 1/10th if its toxicity is known and the 1/100th if its toxicity is unknown.

In order to do it, we proceeded to the determination of biological elements at day 0 (before the administration of vegetable extract) on a batch of 4 male rabbits during 2 weeks, respecting standard conditions of the laboratory. A second administration was made on the 7th day following the first administration, with the same dose, in order to appreciate the effects of repeated administrations.

The averages, calculated from the measured values, were used as pilot values, in order to compare with batches of tests.

## RESULTS

**General acute toxicity:** The toxicity of the vegetable extract is shown by different signs for the mouse which can be retained as toxicity element of the extract:

- Tendency to immobilism and regrouping of mice,
- Loss of exploration instinct marked by a regrouping in a corner of the cage,
- Reversible loss of toxicity 2-3 h after the administration of the extract,
- Refusal to feed,
- Animals die after somnolence and general lifelessness.

The LD50 value of the extract was estimated to be  $811 \pm 69$ , 1 mg kg<sup>-1</sup> body i.p. in mice (Fig. 1), with value of

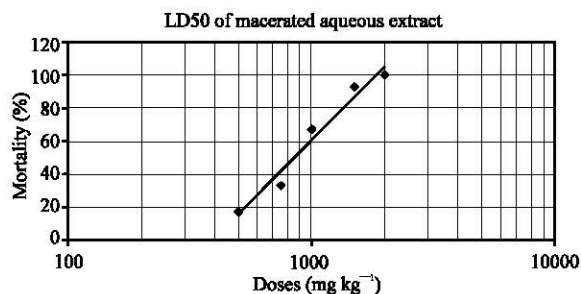


Fig. 1: Representative curve of general acute toxicity from macerated aqueous extract of *Mitragyna inermis* Willd oktze

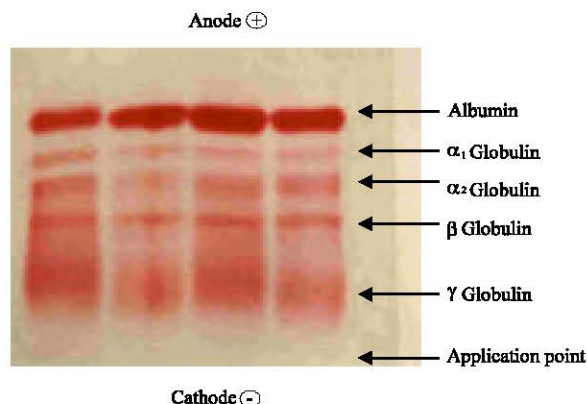


Fig. 2: Rabbits pilot serum (before administration of the extract)

LD1 and LD99, respectively of 305 and 2161 mg kg<sup>-1</sup> of body weight.

LD1 = 305 mg kg<sup>-1</sup>; LD99 = 2161 mg kg<sup>-1</sup>.

$$\frac{LD99}{LD50} = 2.66 \text{ and } \frac{LD50}{LD1} = 2.66; \text{ SI} = \frac{LD99}{LD1} = \frac{2161}{304} = 7.$$

The retained dose for rabbit administration is the 1/10th of LD50 which is around 100 mg kg<sup>-1</sup>.

**Biological study:** The mean value of total protein from serum calculated for 4 rabbits is 70.3 g L<sup>-1</sup>.

Proteins fraction electrophoresis on cellulose acetate are presented in the Fig. 2 (before administration of the extract) and shows 5 proteins zones: Albumin, α<sub>1</sub>, α<sub>2</sub>, β and γ globulin.

The following biological results show:

- At the 4th and 8th days following administration, a whit blood cells (27, 7 to 36), lymphocytes (32, 5 to 67) and platelets (17, 5 to 60) increasing accompanied by a tendency for initial state return. However, no modification for blood cells comparatively to initial rate. (Table 3 and Fig. 3).
- An increasing (13-18 %) for total proteins from serum particularly after 24h of administration and α<sub>1</sub>, α<sub>2</sub> β and γ globulins increasing, respectively 46, 14, 31 and 26 %. During the first day of administration we observe their increasing and their initial state return at the 8th and 9th day (Table 4). However, albumin percentages (10) decreasing observed during the same period are summarised on Table 5 and Fig. 4.

Table 3: Blood cells percentages increasing during days

Days	1	2	3	4	5	6	7	8	9	14
WBC	27.7	35.5	47.5	16.12	19.8	36.6	40.7	85	65	16.39
RBC	-	-	-	-	-	-	-	-	-	-
Lymphocyte	32.5	30.27	33.2	47.1	47.3	67.1	77.2	114.5	88.5	32.68
platelet	17.56	26	50.7	97.9	64.2	60	31.5	12.2	36	8

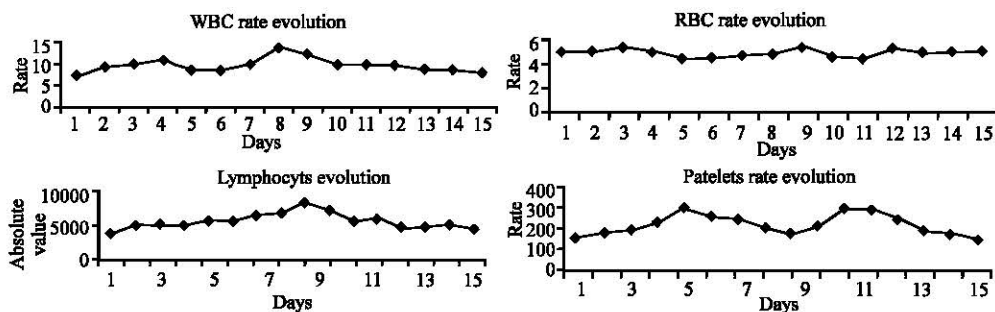


Fig. 3: Batches haematologic parameters evolution curve

Table 4: Biochemical parameters percentages increasing during days

DAYS	1	2	3	4	5	6	7	8	9	14
Total proteins	18.5	5.5	2.9	-	-	1.3	0.93	13.80	3.9	-
$\alpha_1$ globulin	46.8	59.6	78.9	71	58.2	46.8	29.2	46.5	42	-
$\alpha_2$ globulin	0.31	14.9	20	22	20	11	14.9	11	-	8.66
$\beta$ globulin	31.44	32.6	31	45	19.9	29.9	28.3	28.3	37.5	32.9
$\gamma$ globulin	26.6	17.3	-	-	-	-	-	-	-	-

Table 5: Albumin percentages decreasing during days

DAYS	1	2	3	4	5	6	7	8	9	10
% decreasing	10.4	11.3	9.3	8.9	4.8	5.3	3.8	6.2	6	6

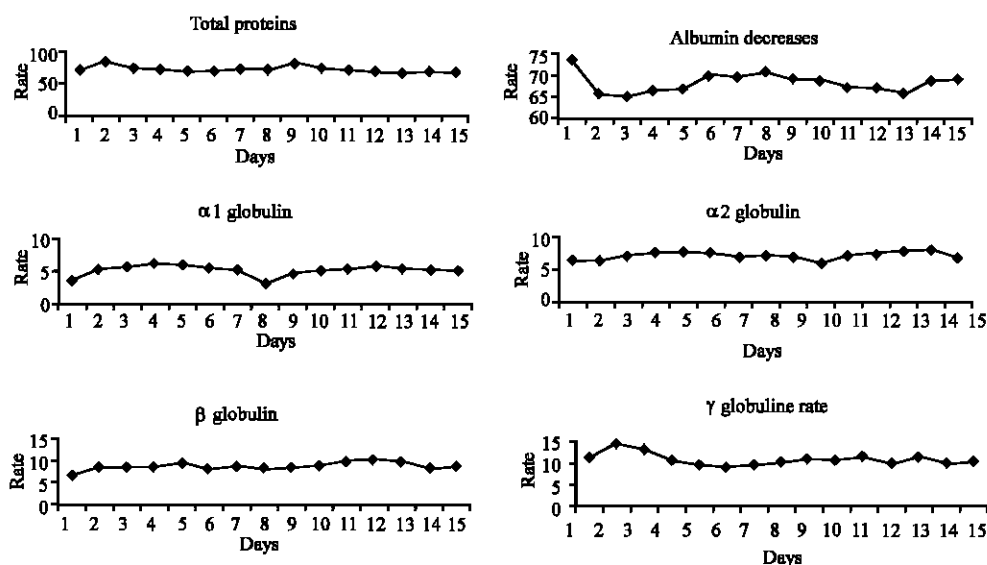


Fig. 4: Batches biochemical parameters evolution curve

## DISCUSSION

**Toxicological interpretation:** The obtained results compared with Lompo *et al.* (1995) study confirm test used performance. LD50 /LD1 (2, 66) and LD99/LD50 (2, 66) rapport is also identical expressing the steadiness of regression line for mortality in relation to dose. Acute toxicity characteristic of tested medicine are showed on below Fig. 1. LD50/LD1 and LD99/LD50 rapport equality confirms the validity of several mortality line according Litchfield and Wilcoxon (1949) method.

LD50 value allows to be placed the plant's extract chemical group with feebly toxic medicine according Hodge and Sterner (1980) toxicity scale. We obtained the atoxic dose at the rate of 305 mg kg<sup>-1</sup> for corporal weight (LD1) and the very toxic dose of 2160 mg kg<sup>-1</sup> for corporal weight (LD99).

LD99/LD1 rapport (around 7) expresses an acceptable Security (Index SI) for medicine use perspective. From the LD50, we can decide the useful dose for human being which is the 10th or 100th of LD50 according to the observed toxicity.

**Biological interpretation:** This study objective consists in establishing the interaction between chemical principles of the plant and biological elements medium of immunity from the rabbit. Orally administration at the rate of 100 mg kg<sup>-1</sup> from corporal weight is used. The administration's impact is observed during the evolution, in addition to a second administration.

- Sensible platelets increasing at day 4 and at Day 12. This phenomenon has a particular interest with homeostasis: formation of platelet boils in presence of wound (bleeding stop).
- No modification for blood cells, no anemia, no haemolysis expressing that orally administration isn't dangerous.
- Total proteins increase is similar to the process which accompanies an antigenic stimulation: active synthesis of protein from clone cellular which is sensible to the antigen, lymphocytes from cell differentiation (Bernard *et al.*, 1990)
- Albumin decreases with a tendency for initial return. This phenomenon is in accordance with the function

that his compound group acts as a carrier protein of antigenic determinants (Charlemagne, 1989).

- $\alpha 1$ ,  $\alpha 2$ ,  $\beta$  and  $\gamma$  globulins. We also observe a new increasing the following day. Globulins decrease during immune deficiencies resulting in either antibody loss (catabolism disturbances), or production lack (anabolism disturbances).

The present study is a preliminary investigation on biological and toxicological of *Mitragyna inermis*. The results obtained gives scientific basis to traditional use.

### CONCLUSIONS

The toxicological and biological results obtained in the current study seem innovative. No similar study has been realized on *Mitragyna inermis Willd Okzte* in literature review we used. LD50 dose are interesting from human security use.

Biological results confirm therapeutic advantages of *Mitragyna inermis Willd Oktze*: Stimulation of biological elements medium for immunity.

Subsequent studies will include acute general toxicity of the aqueous *Mitragyna* extract by orally administration, route used in traditional therapeutic. The study made on totum, a thorough phytochemistry study will permit to aim at chemical group responsible of plant activity.

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