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Research Paper

Evaluation of the effect of alkaloids of *Mitragyna ciliata* on markers of immunity and some hematological parameters in rabbits

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Abstract

***Mitragyna ciliata* (Rubiaceae) is a plant used in traditional medicine in the treatment of malaria in Côte d'Ivoire. The research for other properties in particular immunostimulatory, of this plant to strengthen malaria treatment is the cause of this study through the evaluation of effect of the total alkaloids on cell and humoral markers of immunity and some hematological parameters in rabbit. This investigation was carried out with 15 rabbits divided into five groups of three rabbits for each group. After treatment, blood samples were taken successively on the third day (D3), the seventh day (D7) and the fourteenth day (D14). The biological parameters such as lymphocytes, neutrophiles, albumin, globulins, red blood cells, platelets, hemoglobin and hematocrit were analyzed. Concerning this study, three major results were obtained. The first element is the fact that the alkaloids of *M. ciliata* not found to have immunostimulatory activity. The second element is the fact that within a homogeneous population of rabbits, the rate of biological parameters is not a fixed value. Finally, the results showed the absence of toxicity of these alkaloids against hematological parameters. *Mitragyna ciliata* remains an important medicinal plant made many pharmacological proprieties, due to the presence of its phytochemical compounds.**

Keywords: *Mitragyna ciliata*, total alkaloids extracts, markers of immunity, hematological parameters, rabbit.

Introduction

Among the most dangerous to humans diseases are infectious diseases that are a major cause of death worldwide according to the World Health Organization (WHO) [1]. The integrity of the organism towards microorganisms is ensured by the mechanical defenses, chemical, and especially by the immune system [2]. The strengthening of this system is one of the concerns of the scientific research. Thus, alongside modern medicine, his investigations relevant to substances extracted from medicinal plants used in traditional medicine. Herbal medicines are an important part of the traditional culture and traditions of African people [3,4]. Many patients from resource poor settings have strong beliefs in the use and efficacy of ethnomedicines [5] on which they are reliant for their health car needs.

Nowadays industrial companies incorporated ingredients from plant origin in their medicines [6]. Approximately, betewen 70-78% of commercial pharmaceuticals included plants [7,8].

According to Gupta and Sharma (2010) [9], renewed interest in traditional pharmacopoeias has meant that researchers are concerned both with biological activities and phytocompounds of medicinal plants. Plants form a great part of the biodiversity in tropical areas such as Africa. Among the species of Africa's flora, *Mitragyna ciliata*(Rubiaceae) was considered.This species is used in traditional medicine in the countries of Gulf of Guinea and has been the subject of several scientific investigations for its many healing properties. These studies have shown that *Mitragyna ciliata* has many pharmacological properties including antimalarial activity [10,11,12], trypanocidal activity [13], anti-inflammatory activity [14], antioxidant activity [15] and cardioprotective activity [16,17].

In addition to these therapeutic properties, it is appropriate to examine whether these molecules also have immunostimulatory activity. This would have the advantage of providing an improved traditional medicine consists of a single molecule, in contrast to the one available on the basis of the works of [10,18]. It would help to reduce the cost in the treatment of malaria and would be a distinct advantage for the population.

This study falls within the overall framework of the policy of revaluation of medicinal plants of the Ivorian pharmacopoeia. The aim of this investigation was to evaluate effect of alkaloids from *Mitragyna ciliata* on markers of immunity and some hematological parameters in rabbit.

Materials and Methods

Plant material

The bark of *Mitragyna ciliata* (Rubiaceae) was used as plant material for this study. It was collected in the village of Koko in the locality of Jacqueville, south-East of Côte d'Ivoire in March 2009. This plant was identified and authenticated by the herbarium of the National Floristic Center, Félix Houphouët-Boigny University of Abidjan, where a voucher specimen was conserved with reference number 8888. Plant material was washed, cut, dried for several weeks in the laboratory and ground to a fine powder using a mechanical grinder (IKAMAG).

Animals

Male rabbits of the species *Oryctolagus cuniculus* (Leporideae), the mean body weight were 1.2 ± 0.2 kg were used for this study. These animals which came from the animal house of the Pasteur Institute of Adiopodoumé (Abidjan, Côte d'Ivoire) were housed in cages in the animal house of the Biosciences Training and Research Unit, at room temperature. They had free access to food (pellets from Ivograins, Côte d'Ivoire) and water. All the experimental procedures were approved by the Ethical Committee of Health Sciences, Félix Houphouët-Boigny University of Abidjan. These guidelines were in accordance with the European Council Legislation 87/607/EEC for the protection of experimental animals.

Chemicals

All chemicals used for analyzes meet the quality standards in accordance with international standards. This is hydrochloric acid, methanol, dichloromethane, sodium carbonate and sodium hydroxide purchased from Merck Co. (Darmstadt, Germany). Acetic acid, sodium acetate, magnesium sulfate and poppy red were obtained from Sigma-Aldrich Co. (Steinheim, Germany).

Preparation of methanolic extracts and total alkaloid extracts of *Mitragyna ciliata*

Methanolic extracts (ME)

The preparation of this extract was made in accordance with the method described [19]. One hundred grams (100 g) of *Mitragyna ciliata* powder were extracted in 2000 mL of methanol by maceration for 24 hours using a magnetic stirrer (IKAMAG RTC) at room temperature. The macerate was filtered successively on cotton wool and Whatman filter paper (3 mm). The filtrate was concentrated to dryness under reduced pressure at 38°C with BÜCHI rotavopor R-114. The extracts obtained constitute the methanolic extracts (ME) of *Mitragyna ciliata*.

Total alkaloid extracts (TAE)

The preparation of total alkaloid extracts was made using the method described by [20]. Ten grams (10 g) of methanolic extracts were dissolved in 150 mL of dichloromethane, acidified with 40 mL of hydrochloric acid 0.5 N (pH 2) and 180 mL of distilled water. The solution was mixed and allowed to stand. The organic phase was collected, 40 mL of sodium carbonate 0.5 N (pH 10) and two or five drops of sodium hydroxide were added to the aqueous phase after stirring 150 mL of dichloromethane were added to the solution. The solution was again mixed and the organic layer was collected. This operation was repeated three times. Different organic phases were pooled, dried with anhydrous magnesium sulfate and filtered on Whatman filter paper (3 mm). The filtrate was concentrated under reduced pressure at 38°C using a rotavopor R-114. The resultant extracts constitute the total alkaloid extracts (TAE) of *Mitragyna ciliata*.

Assessment of immunostimulatory activity

Experimental protocol

The assessment of immunostimulatory activity of the total alkaloid extracts (TAE) of *Mitragyna ciliata* was carried out with 15 rabbits according to the method described by [21]. The animals were divided into five experimental groups of three rabbits. The rabbits of group 1 (control) received 1 mL of Mac Ewen (physiological solution). The rabbits of groups 2, 3, 4 and 5 received respectively 10, 20, 25 and 30 mg/kg body weight (bw) of total alkaloid extracts (TAE) dissolved in 1 mL of Mac Ewen. The different solutions were administered via intraperitoneal route at single dose. During 14 days of the experimental phase, blood samples were carried out on animals before treatment, the first day (D1). After treatment, blood samples were taken successively on the third day (D3), the seventh day (D7) and the fourteenth day (D14). The blood was collected with sterile needles microlances.

Biological parameters

The choice of the biological parameters has been made to study of Zirihi et al. 2003 [22]. These include the complete blood count (CBC), total proteins and protein electrophoresis.

Determination of the CBC

The method described by [23] was used for this analysis. Indeed, whole blood collected in tubes with anticoagulant (EDTA) was used to determine mean of Hemoglobin (Hb) concentration, hematocrit, lymphocytes volume, neutrophiles volume, red blood cells (RBC) count and platelet count using a semi-automatic blood cell counter (Sysmex XT - 2000i).

Biochemical parameters

The blood collected into tubes without anticoagulant (dry tubes) was allowed for 30 min at 37°C for coagulation, and centrifuged at 2500 rpm/min for 10 min. The serum obtained was used to achieve the biochemical analyzes (total proteins and protein electrophoresis).The total proteins were measured using an automatic analyzer (180 HumaStar) of biochemistry according to the biuret reaction. The Helena method of serum protein electrophoresis was used for separation and quantification of serum proteins by electrophoresis on cellulose acetate [24].

Statistical Analysis

Data were processed using statistical SPSS package version 7.5 (SPSS Inc., Chicago IL, USA). Analysis of variance (One way ANOVA) was performed and means were separated by Newman-Keuls range test at $P<0.05$. All values are expressed as mean ± standard deviation (SD), n = 3.

Results and Discussion

Cellular and humoral makers of immunity

The results of the various makers of immunity such as lymphocytes and neutrophiles (Table 1), albumin and globulins (Table 2) obtained during immunostimulatory study showed no statistical difference ($P<0.05$) in each case between the results of Mac Ewen (control) and those of different doses of the total alkaloid extracts (TAE) before treatment (D1) and after treatment (D3, D7, D14). It appears that the total alkaloid extracts would not have immunostimulatory activity proved.

Table 1: Concentration of cellular makers of immunity as a function of time for each treatment

Groups	Treatments	Lymphocytes (10^3/µL)				Neutrophiles (10^3/µL)			
		D1	D3	D7	D14	D1	D3	D7	D14
Group 1	1 mL of Mac Ewen	2.03 ± 0.16^{ab}	2.27 ± 0.30^{ab}	1.95 ± 0.24^{ab}	2.12 ± 0.51^{ab}	1.17 ± 0.23^{ab}	1.41 ± 0.18^{ab}	1.34 ± 0.21^{ab}	1.03 ± 0.15^{ab}
Group 2	10 mg/kg bw of TAE	2.25 ± 0.40^{ab}	2.53 ± 0.28^{ab}	2.35 ± 0.20^{ab}	2.09 ± 0.35^{ab}	1.28 ± 0.32^{ab}	1.35 ± 0.27^{ab}	1.2 ± 0.20^{ab}	1.37 ± 0.32^{ab}
Group 3	20 mg/kg bw of TAE	2.62 ± 0.56^{ab}	2.89 ± 0.48^{a}	2.58 ± 0.33^{ab}	2.25 ± 0.19^{ab}	1.43 ± 0.25^{ab}	1.52 ± 0.30^{ab}	1.31 ± 0.17^{ab}	1.50 ± 0.43^{ab}
Group 4	25 mg/kg bw of TAE	2.42 ± 0.37^{ab}	3.2 ± 0.41^{a}	2.85 ± 0.27^{a}	2.63 ± 0.50^{ab}	1.50 ± 012^{ab}	1.65 ± 0.22^{a}	1.40 ± 0.30^{ab}	1.38 ± 0.29^{ab}
Group 5	30 mg/kg bw of TAE	2.77 ± 0.22^{a}	3.5 ± 0.55^{a}	3.29 ± 0.38^{a}	3.07 ± 0.45^{a}	1.73 ± 0.38^{a}	2.13 ± 0.26^{a}	1.55 ± 0.19^{ab}	1.67 ± 0.35^{a}

Values of each parameter are expressed as mean ± SD (n = 3).
Means of each parameter followed by the same letter are not statistically different (P<0.05).
Mac Ewen: Physiological solution, bw: Body weight, TAE: Total alkaloid extracts, D: Day.

Table 2: Concentration of humoral makers of immunity as a function of time for each treatment

Groups	Treatments	Albumin (g/L)				Globulins (g/L)			
		D1	D3	D7	D14	D1	D3	D7	D14
Group 1	1 mL of Mac Ewen	36.83 ± 2.80^{ab}	37.85 ± 2.42^{a}	37.07 ± 3.05^{ab}	36.34 ± 3.20^{ab}	11.06 ± 0.95^{ab}	10.52 ± 1.12^{ab}	10.65 ± 0.83^{ab}	10.82 ± 0.59^{ab}
Group 2	10 mg/kg bw of TAE	37.25 ± 2.45^{ab}	35.75 ± 2.38^{ab}	36.98 ± 2.55^{ab}	38.85 ± 2.78^{a}	10.69 ± 0.75^{ab}	12.02 ± 0.52^{ab}	11.21 ± 1.25^{ab}	10.55 ± 0.87^{ab}
Group 3	20 mg/kg bw of TAE	37.77 ± 2.73^{a}	36.30 ± 2.54^{ab}	37.20 ± 3.12^{ab}	38.52 ± 3.23^{a}	10.58 ± 1.30^{ab}	11.70 ± 1.22^{ab}	10.94 ± 0.77^{ab}	10.22 ± 0.64^{ab}
Group 4	25 mg/kg bw of TAE	37.50 ± 2.45^{a}	34.40 ± 2.36^{ab}	36.80 ± 2.15^{ab}	37.05 ± 2.62^{ab}	10.83 ± 0.73^{ab}	13.44 ± 0.54^{a}	12.12 ± 0.45^{ab}	10.58 ± 0.57^{ab}
Group 5	30 mg/kg bw of TAE	38.25 ± 3.22^{a}	33.9 ± 2.45^{ab}	35.4 ± 2.29^{ab}	38.7 ± 2.63^{a}	10.71 ± 0.67^{ab}	14.75 ± 0.85^{a}	12.95 ± 0.68^{a}	10.15 ± 0.48^{ab}

Values of each parameter are expressed as mean ± SD (n = 3).
Means of each parameter followed by the same letter are not statistically different (P<0.05).
Mac Ewen: Physiological solution, bw: Body weight, TAE: Total alkaloid extracts, D: Day.

Indeed, for a phenomenon immunostimulatory we witness generally to the increase in concentration of these markers of immunity, as shown by the results of the work of [18] using the proteins from *Mitragyna ciliata*. Similarly, [21] showed immunostimulatory activity *in vivo* in rabbits by a fraction of glycoproteins isolated from *Aerva lanata*. It is the same for [25] have highlighted the immunogenic action. Knowing that protein structure is much more complex than that of alkaloids therefore a

molecular weight of proteins higher than that of alkaloids, one could easily understand that the immunostimulatory power of total alkaloids of *Mitragyna ciliata* was not prevented.

However, our results show that alkaloids from *Mitragyna ciliata* show no toxicity to markers of immunity. In the case of a presence of toxicity which is characterized by the decrease in the concentration of the marker of immunity, resulting from the destruction of the immune cells is described as immune suppression. Phenomenon demonstrated by the work of [26] called reversible immune suppression, liver and kidney markers, and phosphate metabolism in healthy rabbits. Thus, these alkaloids of *Mitragyna ciliata* can be used as carrier in the therapeutic treatment of malaria without being toxic to immune cells in the body. Indeed, [10] showed the antiplasmodial activities of alkaloids from this plant. Similarly, antiplasmodial activity of steroidal alkaloids from *Funtumia elastic* was demonstrated by [20]. Moreover, the works of several authors have shown that *M*. *ciliata* has many pharmacological properties. Indeed, the results of the works [12] revealed an antimalarial activity of the methanolic extracts of this plant. The anti-inflammatory [14] and trypanocidal activities [13] have been put in evidence from studies with extracts of *Mitragyna ciliata*.

Finally, the results show that the makers of immunity such as lymphocytes, neutrophiles, albumin and globulins in the first day (D1) range without being significantly different (P<0.05). These results showed that in a single homogeneous population of rabbits, these biological parameters is not a fixed value, but varies to some margin consistent with normal life rabbits. These results are in according to with those of [21,26,27,28].

Hematological parameters

Table 3: Concentration of some hematological parameters as a function of time for each treatment

Groups	Treatments	Red blood cells (10^6/µL)				Platelets (10^6/µL)			
		D1	D3	D7	D14	D1	D3	D7	D14
Group 1	1 mL of Mac Ewen	8.22 ± 0.27^a	7.95 ± 0.32^a	8.03 ± 0.40^a	8.12 ± 0.54^a	1073± 50.93^a	1109 ± 45.36^a	1084 ± 43.73^a	1052 ± 82.35^a
Group 2	10 mg/kg bw of TAE	8.12 ± 0.18^a	7.65 ± 0.23^a	7.79 ± 0.35^a	7.93 ± 0.40^a	995 ± 35.54^a	1172 ± 70.87^a	1141 ± 65.12^a	1096 ± 53.70^a
Group 3	20 mg/kg bw of TAE	7.82 ± 0.26^a	7.27 ± 0.34^a	7.50 ± 0.42^a	7.65 ± 0.28^a	1007 ± 44.64^a	1158 ± 51.25^a	1183 ± 53.89^a	1092 ± 60.47^a
Group 4	25 mg/kg bw of TAE	7.64 ± 0.37^a	7.15 ± 0.25^a	7.28 ± 0.30^a	7.52 ± 0.20^a	1167 ± 42.38^a	1190 ± 38.63^a	1125 ± 39.50^a	1089 ± 45.95^a
Group 5	30 mg/kg bw of TAE	8.26 ± 0.34^a	7.55 ± 0.19^a	7.84 ± 0.23^a	8.03 ± 0.31^a	1077 ± 39.41^a	1163 ± 50.34^a	1095 ± 47.29^a	1148 ± 43.77^a

Values of each parameter are expressed as mean ± SD (n = 3).
Means of each parameter followed by the same letter are not statistically different (P<0.05).
Mac Ewen: Physiological solution, bw: Body weight, TAE: Total alkaloid extracts, D: Day.

Statistical analysis of the results of the various hematological parameters such as red blood cells and platelets (table 3), Hemoglobin and Hematocrit (table 4) of immunostimulatory study revealed no statistical difference (P<0.05) in each case between the results of Mac Ewen (control) and those of different doses of the total alkaloid extracts (TAE) before treatment (D1) and after treatment (D3, D7, D14).

Table 4: Values of hemoglobin and of hematocrit as a function of time for each treatment

Groups	Treatments	Hemoglobin (g/dL)				Hematocrit (%)			
		D1	D3	D7	D14	D1	D3	D7	D14
Group 1	1 mL of Mac Ewen	12.30 ± 0.52^{a}	12.05 ± 0.45^{a}	12.13 ± 0.93^{a}	12.24 ± 0.72^{a}	38.42 ± 2.34^{a}	38.80 ± 2.12^{a}	38.65 ± 2.42^{a}	38.53 ± 1.85^{a}
Group 2	10 mg/kg bw of TAE	12.15 ± 0.64^{a}	11.67 ± 0.58^{a}	11.79 ± 0.74^{a}	11.92 ± 0.87^{a}	37.77 ± 3.05^{a}	34.62 ± 2.85^{ab}	35.43 ± 2.73^{ab}	37.27 ± 2.54^{ab}
Group 3	20 mg/kg bw of TAE	12.22 ± 0.70^{a}	11.97 ± 0.62^{a}	12.25 ± 0.49^{a}	12.32 ± 0.38^{a}	37.23 ± 2.92^{ab}	35.54 ± 2.27^{ab}	36.95 ± 2.35^{ab}	38.48 ± 2.48^{a}
Group 4	25 mg/kg bw of TAE	11.87 ± 0.86^{a}	11.48 ± 0.75^{a}	11.72 ± 0.57^{a}	12.05 0. 62^{a}	38.19 ± 2.67^{a}	34.03 ± 1.88^{ab}	35.82 ± 2.46^{ab}	37.59 ± 2.39^{a}
Group 5	30 mg/kg bw of TAE	12.54 ± 0.47^{a}	11.97 ± 0.73^{a}	12.3 ± 0.44^{a}	12.73 ± 0.55^{a}	38.25 ± 2.78^{a}	33.12 ± 2.60^{ab}	35.47 ± 2.89^{ab}	37.79 ± 2.57^{a}

Values of each parameter are expressed as mean ± SD (n = 3).
Means of each parameter followed by the same letter are not statistically different (P<0.05).
Mac Ewen: Physiological solution, bw: Body weight, TAE: Total alkaloid extracts, D: Day.

These results show the absence of toxicity of the total alkaloids of *Mitragyna ciliata* against hematological parameters. They are in agreement with those obtained by [29]. However, a presence of toxicity leads to a decrease in the values of some of these hematological parameters as shown by several authors [30,31]. Our results allow us to say that the toxic action of these alkaloids is mainly directed to the plasmodium parasite that causes malaria. Finally, as in the case of markers of immunity, the results show that the hematological parameters such as red blood cells, platelets, Hemoglobin and Hematocrit in the first day (D1) range without being significantly different (P<0.05). These results showed that in a single homogeneous population of rabbits, these biological parameters is not a fixed value, but varies to some margin consistent with normal life rabbits. These results are in according to with those of several authors [21,26,27].

Conclusion

This study of the total alkaloids of *Mitragyna ciliata* revealed three main results. These alkaloids whose anti malarial activity has been demonstrated several times have not expressed under our experimental conditions immunostimulatory activity proved. The lack of toxicity of these compounds towards the blood cells at the doses administered. Finally, these results showed that in a single homogeneous population of rabbits, these biological parameters is not a fixed value, but varies to some margin consistent with normal life rabbits. *Mitragyna ciliata* is an important medicinal plant made of the many pharmacological properties, due to the presence of its phytochemical constituents What justifies its high use in traditional medicine in the countries of the Gulf of Guinea in general and in Côte d'Ivoire in particular.

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