

Immunomodulatory effect of aqueous and ethanolic crude extracts of stem bark from *Trichilia emetica* (MELIACEAE) on rats

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ABSTRACT

Aims: This study was designed to evaluate the effect of aqueous and ethanolic extracts of stem bark from *Trichilia emetica* on levels of some hematological parameters and TCD4⁺ count in rats wistar.

Study design: Forty-two rats have been divided into seven groups of six and each was administered a single oral dose of the samples for 8 days. Experimental design was as follows: Group I served as control and received distilled water, group II received Isoprinosine at a dose of 50 mg/kg body weight (b.w.), group III was administered Methylprednisolone (15 mg/kg b.w), group IV and group V received aqueous extract at a dose of 100 and 200 mg/kg b.w. respectively, group VI and VII were administered ethanolic extract at a doses of 100 and 200 mg/kg b.w. respectively. At the end of the treatment, some blood was collected in EDTA tubes for the determination of TCD4⁺ count by flow cytometry and hematological parameters by hemogram.

Results: Concerning TCD4⁺ count, the results show that there is a significant difference ($P < 0.05$) between the control group and all groups of the treated rats. There is also a significant difference between both extracts. But the ethanolic extract at a dose of 100 mg/kg b.w. showed pronounced activity by TCD4⁺ increasing in relation with control and all the treated groups. Thus, the haematological parameters show that there is no significant difference ($p > 0.05$) between the control group and the other treated groups by aqueous extract and ethanolic extract at a dose of 200 mg/kg b.w. concerning WBC count and total lymphocytes level. However, there is a significant increase ($P < 0.05$) of WBC and total lymphocytes in blood of rats treated by ethanolic extract at a dose of 100 mg/kg b.w. compared with control group.

Conclusion: The present study revealed that both extracts of *Trichilia emetica* possess effective immunomodulatory agents. But lower concentration of ethanolic extract showed much immune response compared to the aqueous extract.

Keywords: Immunomodulatory effect, TCD4⁺, total lymphocytes, Methylprednisolone, Isoprinosine, *Trichilia emetica*.

1. INTRODUCTION

The immune system is designed to protect the host from invading pathogens and to eliminate diseases. Plants possess the ability to produce secondary metabolites like proteins, flavonoids, alkaloids, steroids, phenolic and other substances which are in turn used to restore health and heal many diseases. Due to this, they are essential and integral part in complementary and alternative medicine. Herbal drugs are used to enhance the natural resistance of the body against infection and their immunomodulatory activities have been reported in numerous studies [1]. Environmental pollutants and dietary habits cause

disturbances in immune activities and diet containing micronutrients and antioxidants are known to prevent these alterations [2]. The use of herbs as immunomodulators in the indigenous system of medicines, indeed, can modulate the body's defence mechanism. The following active constituents of plant derivatives such as polysaccharides, lectins, peptides, flavonoids, tannins and saponins have been reported to modulate the immune system in different experimental models [3,4]. Thus, phytochemical analysis play a vital role in the search of new and novel molecules [5].

Traditionally, various parts of *Trichilia emetica* are used for the treatment of a variety of disorders such as liver ailments, hepatic disorders, intestinal worms, to stimulate bronchial secretion. It is also used as remedy for colds, as purgative and against fever. This plant is a source of active phytochemicals and has been extensively used for biological activities which include anti-epileptic, anti-pyretic, general tonic and for bronchial inflammation [6], anti-inflammatory [7] activities and activation of the complement [8]. *Trichilia emetica*'s phytochemical screening showed the presence of resin, tannins [6], polyphenolic components [9], free alcohols such as kurubasch aldehyde, a sesquiterpenoid [10], alkaloids, cardiac glycosides, saponins and flavonoids [11]. Hence, this study was designed to evaluate the immunomodulatory activity of aqueous and ethanolic extracts of stem bark from *Trichilia emetica* on levels of hematological parameters and TCD4⁺ count in the blood of rats wistar.

2. MATERIAL AND METHODS

2.1 Extraction Procedure

The fresh barks of *Trichilia emetica* were collected in February 2014 in the region of Mankono, Northern Côte d'Ivoire. The plant was identified at the National Floristic Centre of Felix Houphouet-Boigny University of Cocody (Abidjan). The barks of this plant have been dried at room temperature for 14 days, ground coarsely in a grinder (IKAMAG RCT®) and then stored for further use. The extracts were prepared according to the method described by ZIRIHI et al. [12]. The preparation of the total aqueous extract and ethanolic extract 70%, 100 g of plant powder were extracted in one liter of distilled water or ethanol-water (70/30, v/v) by maceration using a magnetic agitator (the process was repeated 3 times). The homogenate obtained is filtered twice successively on cotton wool and once on Whatman filter paper (3 mm). The filtrate was concentrated using a rotary evaporator at 60 °C. The concentrate have been evaporated at 50 °C in an oven for 48 hours giving a dry ethanolic and aqueous extract. The powder obtained after drying was dissolved in distilled water to give the aqueous and ethanolic extracts of bark from *Trichilia emetica*. Thus, different concentrations were prepared to carry out the experiments.

2.2 Animals Preparation

Forty-two albinos Wistar healthy rats, weighing 90 to 110 g were obtained from an Animal House. The animals were kept in plastic cages in the environmental conditions. They had free access to standard food pellets and were allowed to drink water *ad libitum*. Animal care and handling conformed to international guidelines. They were randomly divided into seven treatment groups of six rats:

- group I (control – normal untreated)
- group II (Isoprinosine)
- group III (Methylprednisolone)
- group IV and V (Aqueous extract)
- group VI and VII (Ethanolic extract)

2.3 Administration of Drugs

All the groups of animals were administered with a daily single dose of the different extracts orally and reference immunostimulator (isoprinosine) and reference immunosuppressive (methylprednisolone).

The dosages of drug administered to the different groups were as follows: group I served as control and received distilled water, group II received isoprinosine at a dose of 50 mg/kg b.w., group III were administered methylprednisolone (15 mg/kg b.w.), group IV and group V received aqueous extract at doses of 100 and 200 mg/kg b.w. respectively, group VI were administered ethanolic extract at a dose of 100 mg/kg b.w. and VII was administered ethanolic extract at a dose of 200 mg/kg b.w. Treatments have

been done 8 consecutive days [4]. At the end of the treatment (day 8), some blood samples were collected in EDTA tubes to prevent blood clotting.

2.4 Drugs and Chemicals

The reference immunosuppressive used is methylprednisolone sodium succinate[®] at 15 mg/kg body weight (b.w.) from Pharmacia Laboratories[®] (France). As for the reference immune stimulant, we used the isoprinosine[®] at 50 mg/kg (b.w.) provided by Sanofi-Aventis (France).

2.5 Laboratory Analysis

The blood was then used the same day for the determination of TCD4⁺ count by flow cytometry and hematological parameters by hemogram.

2.6. Determination of hematological parameters and TCD4⁺:

The blood samples collected were used for the determination of haematological parameters and for counting TCD4⁺ cells. This count was performed according to the method of Becton Dickinson (BD) Tritest (CD3 FITC/CD4 PE/CD45) by flow cytometry using the device BD FACS calibur[®] [13,14]. The method was to pipette 10 µl of reagent (BD Tritest CD3/CD4/CD45) in the bottom of truCOUNT tubes. Then, 50 µl of blood was added to the bottom of the same tubes which are capped and vortexed gently. The mixture was incubated in the dark chamber for 15 min at room temperature (20-25°C). It was added 10 µl of BD FACS lysis solution into the tubes that are blocked and then vortexed gently. The tubes are incubated against for 15 min in the dark at room temperature (20-25 °C). Samples obtained will be analyzed on the flow cytometer. This method has been used by BHAGWAT et al. [15] for the determination of TCD4⁺ and TCD8⁺ cells in Balb/C Mice blood.

2.7. Statistical Analysis

The statistical differences between mean values were analyzed by the one-way analysis of variance (ANOVA) following Newman-Keuls test (non parametric test suitable for small numbers). It was used to compare mean values of TCD4⁺ count and hematological parameters level. The difference is considered significant between two means if $P < 0.05$.

3. RESULTS

The effects of aqueous and ethanolic extracts of bark of *Trichilia emetica* on TCD4⁺ count are presented in Table 1, while Table 2 shows the effects of the same extracts and reference molecules on haematological parameters of rats wistar in different groups.

The results showed that there is a significant difference ($P < 0.05$) between the control group and all groups of the treated rats concerning TCD4⁺ cells. Indeed, they show that there is an increase of TCD4⁺ in the blood of the rats which followed the treatment with both aqueous and ethanolic extracts (100 and 200 mg/kg b.w.) compared with control group. This increase was more pronounced with the ethanolic extract at a dose of 100 mg/kg b.w. Also, administration of a reference immunostimulator (Isoprinosine 50 mg/kg b.w.) produced an increase of TCD4⁺ but administration of a reference immunosuppressive (methylprednisolone 15 mg/kg b.w.) induced a decrease in relation with control group (Table 1).

Haematological parameters level showed that there is no significant difference ($p > 0.05$) between the control group and the other groups treated by both concentrations (100 and 200 mg/kg b.w.) of aqueous extract and ethanolic extract at a dose of 200 mg/kg b.w. concerning WBC count and total lymphocytes level. We have noted the same fact with rats treated by isoprinosine. However, there is a significant increase ($P < 0.05$) of WBC and the total lymphocytes in the blood of rats treated by ethanolic extract at dose 100 mg/kg b.w. compared with control group. But in the group of Methylprednisolone we noted a decrease of the same cells always in comparison with the control group.

4. DISCUSSION

Trichilia emetica is known for several medicinal uses and has been investigated for different pharmacological properties. Phytochemical studies of this plant revealed the presence of various secondary metabolites such as flavonoids, tannins, saponins that possess immunomodulatory activity. This was the most likely reason of immunomodulatory potential of this plant. In the present study, the effects of *Trichilia*'s extracts on the immune response were assessed by oral extracts administration to rats for 8 days. The evaluation of this activity was carried on counting TCD4⁺ cells and some hematological parameters such as WBC, RBC, HGB and total lymphocytes.

The treatment of rats with the ethanolic and aqueous extracts of stem bark from *Trichilia emetica* induced proliferative responses at the same time in the count of the total lymphocytes and the TCD4⁺ count. Regarding the total lymphocytes, the ethanolic extract at a dose of 100 mg/kg b.w. induced its increase. It is therefore noteworthy that this concentration of ethanolic extract mobilizes the count of total lymphocytes in the peripheral blood. This mobilization is confirmed by comparing action of the ethanolic extract at low dose and those of the reference molecules. Indeed, the administration of a reference immunostimulator isoprinosine induces an increase in the total lymphocytes while this is not the case with a reference immunosuppressive methylprednisolone. These observations enable us to say that the ethanolic extract at a dose of 100 mg/kg b.w. acts as isoprinosine mobilizing the total lymphocytes in the circulating blood. Indeed, LITZMAN et al.[16] showed that the treatment of 43 children suffering from respiratory infection with isoprinosine 50 mg/kg/day three times a week for six months, resulted in an increase in the number of lymphocytes TCD3⁺, TCD4⁺ and TCD8⁺. However, the administration of different drugs did not produce any significant change on RBC and HGB.

Table1: Action of different extracts on TCD4⁺ count

Doses	TCD4 ⁺ (cel/μl)	P-Value
Control (distilled water)	425 ± 15.01	> 0.05
Isoprinosine 50 mg/kg b.w.	631 ± 9.50	<0.05
Methylprednisolone 15 mg/kg b.w.	349 ± 9.53	<0.05
Aqueous extract 100 mg/kg b.w.	860 ± 20.00	<0.05
Aqueous extract 200 mg/kg b.w.	880 ± 13.75	<0.05
Ethanolic extract 100 mg/kg b.w.	1030 ± 13.23	<0.05
Ethanolic extract 200 mg/kg b.w.	500 ± 10.00	<0.05

Table2: Action of different extracts on haematological parameters

Doses	WBC(10 ³) cel/μl	RBC(10 ⁶) cel/μl	HGB (g/dl)	%LYMPHOCYTES
Control (distilled water)	15.82±0.06	7.53±0.12	12.90±0.04	57.27 ± 2.87
Isoprinosine 50 mg/kg b.w.	19.07±0.51*	7.52±0.23*	13.18±1.36*	60.03 ± 5.00*
Methylprednisolone 15 mg/kg b.w.	10.8±0.99**	6.68±0.60*	11.78±1.18*	25.50 ± 2.00**
Aqueous extract 100 mg/kg b.w.	14.20±2.38*	7.39±0.71*	12.74±1.21*	50.80 ± 2.20*
Aqueous extract 200 mg/kg b.w.	19.10±1.28*	7.91±0.09*	13.30±0.10*	64.50 ± 4.00*
Ethanolic extract 100 mg/kg b.w.	24.07±2.57**	7.85±0.54*	13.97±0.81*	78.83 ± 7.63**
Ethanolic extract 200 mg/kg b.w.	19.73±2.3*	7.75±0.31*	13.37±0.87*	61.80 ± 6.10*

All the values are mean $\pm$ SD, n=6. *P> 0.05 when compared with the control group and **p<0.05 when compared with the control group.

Concerning TCD4⁺ cells, the results show that both extracts induced an increase in their count. However the highest count was induced by ethanolic extract at a dose of 100 mg/kg b.w. Indeed, the ethanolic extract was found to be most effective at low dose whereas, high dose (200 mg/kg b.w.) of the same extract was moderately effective in modulating TCD4⁺ cells. This shows that the ethanolic extract at high dose causes a decrease in TCD4⁺ and acts as methylprednisolone. This is consistent with the studies of FRANCIOTTA et al. [17] that showed that the administration of high doses of methylprednisolone would be proapoptotic for TCD4⁺ and would entail an immediate decline in the count of TCD4⁺ within three (3) days after administration in the peripheral blood of sclerotic patients [18].

Also, the increase of the total lymphocytes and TCD4⁺ in this experiment confirms the studies of NICOARA et al.[19] that showed that the isoprinosine was responsible for the proliferation of total lymphocytes and their maturation. In fact, TCD4⁺ cells represent 59% of the total lymphocytes which in turn represent 78.5% of total lymphocytes in female rats of Wistar strain [20]. That said, our study revealed that the aqueous and ethanolic extracts of stem bark from *Trichilia emetica* are effective immunomodulatory agents. However, the ethanolic extract at low dose showed a higher activity. Thus, this extract at low dose potentiated the non-specific immune response and this may be attributed to different phytoconstituants. DIALLO et al. [8] investigated complement activating effect that found the leaf aqueous extract from the same plant to have an effect on the complement system with an IC50 of 45 μ g/ml which may be related to the healing of burns and wounds. However, this study showed that the aqueous and ethanolic extracts of stem bark from the same plant possess immunomodulatory activity with ethanolic extract, which showed a higher activity at low dose.

5. CONCLUSION

In conclusion, this study demonstrated that oral administration of crude extracts from *Trichilia emetica* affects various aspects of the immune system, including the effects on the composition TCD4⁺ and hematological parameters. These findings demonstrate that the *Trichilia emetica* extract may stimulate immune response. Consequently, it can be concluded that the plant holds promise for being used as an immunostimulating agent. However, the crude ethanolic extract of stem bark from *Trichilia emetica* possesses a good activity at low dose.

ETHICAL APPROVAL

The experimental procedures and protocols used in this study were approved by the Ethical Committee of Health Sciences, University Félix Houphouët-Boigny. These guidelines were in accordance with the European Council Legislation 87/607/EEC for the protection of experimental animals. All efforts were made to minimize animal suffering and reduce the number of animals used.

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