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Positive Effects of Aqueous and Ethanolic Extracts of Stem Bark from *Trichilia emetica* (MELIACEAE) on Cellular Immunity Markers in Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Authors DAP, YHF and DSR designed the study, wrote the protocol and supervised the work. Authors DAP and DSR carried out all laboratories work and performed the statistical analysis. Author DGB managed the analyses of the study. Author DAP wrote the first draft of the manuscript. Authors NJD and DAJ managed the literature searches and edited the manuscript. All authors read and approved the final manuscript.

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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 the cellular immunity markers ($TCD4^+$ count, Lymphocytes, WBC, RBC) in rats wistar.

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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 (100 and 200 mg/kg b.w.) 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* have positive effects on cellular immunity markers such as $TCD4^+$, total lymphocytes, WBC, RBC. However, lower concentration of ethanolic extract showed much positive effects compared to the aqueous extract.
The results of this preliminary study could be used to explore the spleenocyte proliferation and the analysis of spleen cells in order to see the real immunomodultory activity.

Keywords: Cellular immunity markers; 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 plays 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 desorders, 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 effects of aqueous and ethanolic extracts of stem bark from *Trichilia emetica* on the cellular immunity markers in the blood of rats wistar.

2. MATERIALS AND METHODS

2.1 Materials

The plant material is constituted by the fresh barks of *Trichilia emetica*, collected in February 2014 in the region of Mankono, Northern Côte

d'Ivoire. The plant was identified at the National Floristic Centre of Félix 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.

Albinos Wistar healthy rats obtained from an Animal House are used for this study. 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.

2.2 Methods

2.2.1 Preparation of the extract

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℃. The concentrate have been evaporated at 50℃ 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.

2.2.2 Evaluation of extracts effects on cellular immunity markers in rats

Forty-two albinos Wistar healthy rats, weighing 90 to 110 g were used. They were randomly divided into seven treatment groups of six rats: 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 group 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. The blood was then used the same day for the determination of $TCD4^+$ count by flow cytometry and hematological parameters by hemogram.

2.2.2.1 Determination of cellular immunity makers

Part of the blood collected was used to determine some haematological parameters on the Sysmex XT-2000i®. As for the other part of blood, it was used 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℃). It was added 500 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℃). 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.

2.2.3 Drugs and chemicals

The reference immunosuppressive used is methylprednisolone sodium scuccinate® 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).

2.3 Statistical Analysis

The data were analyzed by the one-way analysis (ANOVA) following Newman-Keuls test (no parametric test suitable for small numbers). The mean value is accompanied by the standard error of the mean (Mean±SEM). 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 immunosupressive (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 reponse 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, WBC 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 immunosupressive 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 HBG.

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

Table 1. 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

Table 2. Action of different extracts on haematological parameters

Doses	WBC(10^3) cel/µl	RBC(10^6) cel/µl	HBG (g/dl)	% Lymphocyte	P-value
Control (distilled water)	15.82±0.06	7.53±0.12	12.90±0.04	57.27±2.87	> 0.05
Isoprinosine 50 mg/kg b.w.	19.07±0.51	7.52±0.23	13.18±1.36	60.03±5.00	<0.05
Methylprednisolone 15 mg/kg b.w.	10.8±0.99	6.68±0.60	11.78±1.18	25.50±2.00	<0.05
Aqueous extract 100 mg/kg b.w.	14.20±2.38	7.39±0.71	12.74±1.21	50.80 ±2.20	<0.05
Aqueous extract 200 mg/kg b.w.	19.10±1.28	7.91±0.09	13.30±0.10	64.50± 4.00	<0.05
Ethanolic extract 100 mg/kg b.w.	24.07±2.57	7.85±0.54	13.97±0.81	78.83±7.63	<0.05
Ethanolic extract 200 mg/kg b.w.	19.73±2.3	7.75±0.31	13.37±0.87	61.80±6.10	<0.05

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 µg/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 demonstered 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. The present study revealed that both extracts of *Trichilia emetica* have positive effects on cellular immunity markers such as TCD4$^+$, total lymphocytes, WBC, RBC. However, lower concentration of ethanolic extract showed much positive effects compared to the aqueous extract. The results of this preliminary study could be used to explore the spleenocyte proliferation and the analysis of spleen cells in order to see real immunomodultory activity.

ETHICAL APPROVAL

The experimental procedures and protocols used in this study were approved by the Ethical Committee of Health Sciences, University Félix Houphouet-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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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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