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Original Research Article

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

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 were divided into seven groups of six and received each orally a single dose of different drugs for 8 days. Experimental design was as follows : Group I was served as control and received distilled water, group II received Isoprinosine at a dose of 50 mg/kg body.weight (b.w.), group III were 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 treatment, blood was collected in EDTA tubes for the determination of TCD4+ count by flow cytometry and hematological parameters by hemogram

Results: Concerning TCD4+ count, results showed that there is a significant difference (P<0, 05) between control group and all groups of rats treated. Also, there is a significant difference between both extracts. But ethanolic extract at dose (100mg/kg b.w.) showed pronounced activity by TCD4+ increasing in relation with control and all groups treated. Thus, Haematological parameters showed that there is no significant difference (p> 0,05) between the control group and other groups treated. Only, ethanolic extract (100mg/kg b.w.) gave a value of total lymphocytes slightly higher compared to the control group. These observations indicate that lower concentration of ethanolic extract showed much immune response compared to other groups

Conclusion: the present study revealed that extracts of Trichilia emetica possess effective immunomodulatory agents.

Keywords: Immunomodulatory effect, Methylprednisolone, Isoprinosine, Trichilia emetica

1. INTRODUCTION

The immune system is designed to protect the host from invading pathogens and to eliminate diseases. The growing 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 the essential and integral part in complementary and alternative medicine. Herbal drugs are believed 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 analyses play a vital role in 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 purigative 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 complement activating [8]. Phytochemical screening of this plant show the presence of resin, tannin [6], polyphenolic components [9], free alcohol such as kurubasch aldehyde, a sesquiterpenoid [10], alkaloids, cardiac glycosides,

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saponins, flavonoids and tannins [11]. Hence, this study was designed to evaluate the immunomodulatory activity of aqueous and ethanolic extracts of stem bark of *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)Give adequate information to allow the experiment to be reproduced. Already published methods should be mentioned with references. Significant modifications of published methods and new methods should be described in detail. This section will include sub-sections. Tables & figures should be placed inside the text. Tables and figures should be presented as per their appearance in the text. It is suggested that the discussion about the tables and figures should appear in the text before the appearance of the respective tables and figures. No tables or figures should be given without discussion or reference inside the text. Barks of the plant were 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 **Zihiri et Kra**. For the preparation of total aqueous and ethanol extracts 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 is repeated 3 times). The homogenate obtained was first spun in a square of fabric, and then filtered twice successively on cotton wool and once on Whatman filter paper (3 mm). The filtrate was concentrated using a drying oven at 70 °C. The concentrate was 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 of *Trichilia emetica*. Thus, different concentrations were prepared to carry out the experiments

2.2 Animals Preparation

Forty two albinos Wistar healthy rats, weigthing 90 to 110 g were obtained from Animal House. The animals were kept in plastic cages in 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 :

- GroupI (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 groups of animals received orally a single dose daily the differents extracts and reference immunostimulator (Isoprinosine) and reference immunosuppressive(Methylprednisolone).
The dosages of drug administred of different groups were as follows : Group I was served as control and received distilled water, group II received Isoprinosine at a dose of 50 mg/kg b.w., group III were administred Methylprednisolone (15 mg/kg b.w.), group IV and group V received aqueous extract at a doses of 100 and 200 mg/kg b.w. respectively, group VI were administred ethanolic extract at dose100 mg/kg b.w. and VII was administred ethanolic extract at dose 200 mg/kg b.w. Treatments were continued for 8 consecutive days **(Sharma *et al*, 2012).** At the end of treatment (day 8), blood samples were collected in EDTA tubes to prevent blood clotting. The blood is then used the same day for the determination of TCD4+ count by flow cytometry and hematological parameters by hemogram

2.4 Drugs and Chemicals

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

2.5 Laboratory Analysis

At the end of treatment (day 8), blood samples were collected in EDTA tubes to prevent blood clotting. The blood is 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+:

Part of the blood collected in EDTA tubes, was used to determine hemogram on the Sysmex XT-2000i®. As for the other part of the whole blood, it was used to determine the count of CD4+ by the method of Becton Dickinson (BD). Tritest (CD3 FITC/CD4 PE/CD45 par BM) by flow cytometry using the device BD Facscalibur®. Briefly, the method was to pipette 10µl of BD Tritest CD3/CD4/CD45 in the bottom of truCOUNT tubes.Then, 50µl of EDTA anti-coagulated whole blood was added to the bottom of the same tubes that are capped and vortexed gently. The mixture was incubated in the dark chamber for 15 min at room temperature (20-25°C). After incubation, 50 µl of BD FACS lysis solution was added into the tubes. The tubes are vortexed gently and the mixture was incubated again 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.*, (2009) for the determination of TCD4 + and TCD8+ cells in Balb/C Mice blood.

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 control group and all groups of rats treated. Its show that there is an increase of TCD4+ in blood of rats following treatment with both aqueous and ethanolic extratcs (100 and 200 mg/kg b.w) compared with control group. This increase was more pronounced with the ethanolic extract The discussion should not repeat the results, but provide detailed interpretation of data. This should interpret the significance of the findings of the work. Citations should be given in support of the findings. The results and discussion part can also be described as separate, if appropriate. at dose 100 mg/kg b.w. Both dose of aqueous extract had increased the average count of TCD4+.compared with groups of rats treated with reference immunosuppressive (Methylprednisolone) at dose 15mg/kg b.w and reference immunostimulator (Isoprinosine) at dose 50 mg/kg b.w. We have noted same fact with rats treated by ethanolic extract at dose 100mg/kg b.w. However, ethanolic extract at low dose (100 mg/kg b.w) had significantly increased the average count of TCD4+.compared with aqueous extract but at high dose (200mg/kg bw) ethanolic extract induced a decrease of TCD4+. There is a significant difference between control and groups of rats treated by the molecules reference Isoprinosine (50mg/kg b.w) and Methylprednisolone (15mg/kg b.w). Administration of Isoprinosine (50mg/kg b.w) produced an increase of TCD4+ but administration of Methylprednisolone (15mg/kg b.w).induced a descrease of TCD4+ compared with control group. Also, ethanolic extract at dose 100 mg/kg b.w induced an increase of this cells compared with both reference molecules (Table 1)
Haematological parameters level showed that there is no significant difference (p> 0,05) between the control group and other groups treated by both concentrations (100 and 200mg/kg b.w.) of aqueous extract, ethanolic extract at dose 200mg/kg b.w concerning WBC count. We have noted same fact with rats treated by Isoprinosine (50mg/kg b.w). However, there is a significant difference (P<0,05) between rats treated by ethanolic extract at dose 100mg/kg b.w and group of Methylprednisolone (15mg/kg b.w).Administration of low dose of ethanolic extract produced an increase on WBC count in relation with control group and other groups treated. Also, the same dose of this extract induced a significant increase on total lymphocytes compared with other groups. However, administration of different drugs did not produce any significant change on RBC HCT and HGB.

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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 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. Evaluation of this activity was carried on counting TCD4+ cells and some hematological parameters such as WBC, RBC HCT, HGB and total lymphocytes.

Treatment of rats with ethanolic and aqueous extracts induced proliferative responses of TCD4+ cells, in relation with control levels. 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. Both dose (100 and 200 mg/kg b.w.) of aqueous extract showed weak activity in connection with ethanolic extract. Stimulatory effect of TCD4+ observed with the ethanolic extract leads to saying that this one focuses more active agents capable of inducing stimulation of said cells

Table1: Action of different extracts on TCD4+ count

Doses	TCD4+(cel/µl)	P-Value
Control (distilled water)	425 ± 15,01	> 0.05
Isoprinosine 50mg/kg b.w.	631 ± 9,50	<0.05
Methylprednisolone 15mg/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	HBG (g/dl)	%LYMPHOCYTES	P-Value
Control (distilled water)	15,82±0,06	7,53±0,12	12,90±0,04	57,27 ± 2,87	
Isoprinosine 50mg/kg b.w.	19,07±0,51*	7,52±0,23*	13,18±1,36*	60,03 ± 5,00*	<0.05
Methylprednisolone 15mg/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

Also, there is an increase of hematological parameters(WBC and total lymphocytes) with the ethanolic extract at dose of 100 mg/kg b.w. compared with control levels and others groups treated. The study

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revealed that aqueous and ethanolic extracts of stem bark from *Trichilia emetica* are effective immunomodulatory agents. However, ethanolic extract at low dose showed a higher activity. Thus, this extract potentiated the non specific immune response and this may be attributed to different phytoconstituants.Thus, **Diallo et al.**[8] investigated complement activating effect that found the leaf aqueous extract 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 wound. Whereas, this study showed that aqueous and ethanolic extracts of stem bark from the same plant possess immunomodulatory activity with ethanolic extract that showed a higher activity at low dose. Consequently with the result of this study it can be concluded that the plant holds promise for being used as an immunostimulating agent.

5. CONCLUSION

In conclusion, this study demonstred 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 *Trichilia emetica* extract may stimulate immune response. However the crude ethanolic extract of stem bark from *Trichilia emetica* possesses a good activity at low dose.

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