MEDWIN PUBLISHERS
Committed to Create Value for Researchers

Virology & Immunology Journal
ISSN: 2577-4379

Evaluation of the Immunomodulatory Activity of the Methanol-Methylene Chloride Extract of Parkia Biglobosa Jacq Benth. (Fabaceae) Stem Bark in Mice

Peter IE, Nwabunike IA, Onwuka AM*, Mbaoji FN and Obi BC

Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Nigeria Nsukka, Nigeria

***Corresponding author:** Akachukwu Marytheresa Onwuka, Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Nigeria Nsukka, Nigeria, Tel: +2348038357391; Email: akachukwu.onwuka@unn.edu.ng

Research Article

Volume 4 Issue 2

Received Date: July 20, 2020

Published Date: August 14, 2020

DOI: 10.23880/vij-16000246

Abstract

The study was carried out to assess the effect of methanol/methylene chloride extract (MME) of *Parkia biglobosa* stem bark on specific and non-specific immune responses in rodents. Adult Swiss albino mice (20-35 g) of either sex were divided into five groups (n=5) and treated for 10 days. The effect of MME on adaptive immunity was evaluated using neutrophil adhesion test and cyclophosphamide-induced neutropenia while mice lethality test was used to evaluate its effect on non-specific immunity. Results of the neutrophil adhesion test showed a significant ($P<0.05$) increase in neutrophil adhesion at 250 mg/kg. However, there was decrease in neutrophil adhesion at 500mg/kg and 750 mg/kg respectively. The extract also gave no significant protection from cyclophosphamide-induced immunosuppression. Phytochemical analysis revealed the presence of alkaloids, glycosides, saponins, terpenoids, flavonoids and tannins. The oral LD50 of the extract was greater than 5000 mg/kg and considered to be relatively safe. The findings above show that the stem barks extract of the plant enhanced some aspects of specific immunity and therefore to some extent, hold promise for use as an immunostimulatory agent though it may not offer good protection from the effect of cyclophosphamide on the haematopoietic system.

Keywords: Immunomodulatory Activity; Methanol-Methylene Chloride; Saponins; Terpenoids

Introduction

The immune system is a system of biological structures and processes within an organism that protects against diseases by identifying and killing pathogens and tumour cells [1]. Immunomodulation is a therapeutic approach aimed at intervening in the auto-regulating processes of the body defense system. Immunomodulatory drugs alter the response of the immune system by either increasing (immunostimulators) or decreasing (immunosuppressive) the production of serum antibodies [2]. In recent years, there has been increased interest in the search for potential compounds from medicinal plants with immunomodulatory potential [3].

Parkia biglobosa Benth (Family: Fabaceae) commonly known as the African locust bean tree is a perennial, deciduous tree common in West African region. *Parkia biglobosa* is a medicinal plant reported to possess wide range of pharmacological uses that include antidiabetic, anthelmintics, antibacterial, antiplasmodial, analgesic and anti-inflammatory, antihypertensive, antimalarial [4-13]. There is however no documented works on the immunomodulatory potential of the stem bark of this plant hence this study.

Materials and Methods

Drugs

Cyclophosphamide 500 mg/vial (Olphamide®, Kwality Pharmaceuticals Ltd, India) and Levamisole 40 mg tablets (Retrax®, Ecomed Pharmaceuticals Ltd, Nigeria).

Chemicals, Solvents and Reagents

Analytical grades of methanol (Sigma-Aldrich, Germany), dichloromethane (Sigma-Aldrich, Germany), WBC diluting fluid, Leishman's stain, zinc sulphate, barium chloride.

Animals

Adult Swiss albino mice (20-35g) of either sex were used. The animals were obtained from the Laboratory Animal Facility of the Department of Pharmacology and Toxicology, University of Nigeria, Nsukka. Animals were housed in steel cages within the facility under standard conditions and allowed free access to standard pellets and water. Prior to their use, they were allowed two weeks for acclimatization within the work area environment. All animal experiments were in compliance with the National Institute of Health Guide for Care and Use of Laboratory Animal (Pub No. 85-23, revised 1985).

Collection and Preparation of Plant Material

The fresh stem bark of *Parkia biglobosa* was collected in May 2018 from Ede-Oballa in Enugu state. The plant was identified and authenticated at the International Centre for Ethnomedicines and Drug Development (InterCEDD) Nsukka. The plant material was cleaned, sun-dried and reduced to a coarse powder using milling machine.

Extraction of Plant Material

The powdered material (1.57 kg) was extracted by cold maceration in 6 L of a 1:1 mixture of methanol-methylene chloride for 72 hours with intermittent vigorous shaking every six hours. The extract was strained with a muslin cloth and filtered with Whatman No. 1 filter paper. The extract was concentrated in an electric rotary evaporator set at 40^0C to obtain the methanol-Methylene extract (MME).

Phytochemical Analysis of the Extract

The extract was subjected to phytochemical analysis for the identification of constituents using the methods of Harborne [14].

Acute Toxicity Test

The acute toxicity of the methanol-methylene chloride was evaluated using the method described by Lorke [15].

Neutrophil Adhesion Test

Adult Swiss albino mice were divided into five groups of five mice per group. Group 1 and 2 were used as the negative and positive controls with the animals receiving orally 2 ml/kg of the vehicle (distilled water) and Levamisole respectively. Groups 3, 4 and 5 received orally different concentrations of *Parkia biglobosa* extract (250, 500 and 750 mg/kg) respectively for 10 days. On day 11, blood samples were collected from the retro-orbital plexus into heparinized vials and differential leukocyte count (DLC) was determined after fixing the blood smear and staining with the Leishman's stain. After the initial counts, blood samples were incubated with 40 mg/ml of nylon fibres for 15 minutes at 37°C. The incubated blood samples were again analyzed for DLC and TLC (total leukocyte count). The difference in the neutrophil count before and after incubation of blood samples with nylon fibres was determined [16].

Cyclophosphamide Induced Neutropenia

Adult Swiss albino mice were divided into five groups of five mice per group. Group 1 and 2 were used as the negative and positive controls with the animals receiving orally 2 ml/kg of vehicle and 50 mg/kg of Levamisole respectively. Groups 3, 4 and 5 received orally, different concentrations of *Parkia biglobosa* extract (250, 500 and 750 mg/kg) respectively for 10 days. On the 11^{th} day, a neutropenic dose of cyclophosphamide (200 mg/kg, s.c) was administered and this day was labelled as day zero. Blood samples were collected through retro-orbital vein of the mice and the total leukocyte count (TLC) and differential counts (DLC) were performed on day 0 prior to and on day 3 after injection of cyclophosphamide. The TLC and DLC in treated groups were compared with the values of the control group [17].

Non-Specific Immunity Determined by Survival Rate Against Fungal Infection (Mice Lethality Test)

Adult Swiss albino mice were divided into five groups of five mice per group. Group 1 and 2 were used as the negative and positive controls with the animals receiving orally 2 ml/kg of the vehicle and 50 mg/kg of Levamisole respectively. Groups 3, 4 and 5 received orally, different concentrations of *Parkia biglobosa* extract (250, 500 and 750 mg/kg) respectively for 10 days. On the 11^{th} day that is the day of the challenge, all groups were injected with 5 $x10^7$ viable *Candida albicans* cells and observed daily for a period of 10 days and the number of deaths per group recorded [18].

Statistical Analysis

Data obtained was analyzed by one-way ANOVA followed

by Dunnett's multiple comparisons post-hoc test using Graphpad Prism version 5.0. Differences between means were accepted significant at P<0.05. Results were presented as Mean ± SEM (standard error of mean).

Results

Extractive Yield

The extraction process yielded 132.22 g of the methanol/methylene chloride extract (MME) giving a percentage extractive yield of 8.42% w/w.

Phytochemical Constituents of MME

Preliminary phytochemical analysis showed that MME possibly contains tannins, tested positive to saponins, terpenoids, steroids, flavonoids, resins, tannins, carbohydrates, reducing sugar, proteins, glycosides, alkaloids and carbohydrate (Table 1).

Phytochemical constituents	Relative presence
Alkaloids	+
Carbohydrates	+++
Flavonoids	+++
Glycosides	+++
Reducing sugar	++
Resins	_
Saponins	++
Steroids	_
Tannins	++
Terpenoids	+
Protein	+
Acidic compounds	+

Key: MME = Methanol/methylene chloride extract; ++++ = abundantly present; +++ = present in very high concentration; ++ = present in moderately high concentration; + = present in small concentration; **- =** not present.
Table 1: Phytochemical constituents of MME.

Effect of Extract on Neutrophil Adhesion Test

The percentage neutrophil adhesion in the control group was 44.99±6.54%, in the levamisole- treated group it was 46.18 ± 1.97 whilst for MME-treated group at 250 mg/kg, it was 51.14±5.05% (Table 2). There was a significant ($P < 0.05$) increase in neutrophil count and an increase in neutrophil adhesion at this dose which was higher than that observed with the standard immunostimulatory agent, levamisole (Table 2). However, at 500 and 750 mg/kg doses, there was no increase rather a decrease in neutrophil adhesion compared to the control (Table 2).

Treatment	Dose (Mg/Kg)	TLC (Cell/Mm3) [X]		% Neutrophil [Y]		Neutrophil Index [X Y]		Neutrophil Adhesion (%)
		UnB	FTB	UnB	FTB	UnB	FTB	
Control	2 ml/kg	4880±538	3120±481	31.00±1.89	26.40±1.96	153700±21835	81500±11830	44.99
MME	250	7600±843	4240±756	29.00±2.72	26.20±2.70	217760±26029	106760±18144	51.14*
	500	9240±1224	6140±1071	28.20±3.38	24.80±3.56	267620±64417	145320±25635	38.79
	750	7860±1205	5420±896	22.20±1,28	19.60±1.63	169780±17800	101620±12693	38.95
Levamisole	50	5300±1934	4500±1674	24.00±2.30	15.30±1.76	131866±49564	72866±28923	46.18

* $P < 0.05$ compared to control (One Way ANOVA; LSD post hoc). Values shown are as Mean ± SEM; MME = Methanol/methylene chloride extract. n = 5. UnB = untreated blood, FTB= nylon fiber- treated blood.
Table 2: Effect of MME on neutrophil adhesion.

Effect of Extract on Cyclophosphamide-Induced Immunosuppression

Administration of the methanol/methylene chloride extract did not give a significant increase in the total and differential leucocyte counts after cyclophosphamide administration. The percentage reduction in these parameters as a result of the suppressive effect of cyclophosphamide treatment was lower in the MME-treated groups especially at 750 mg/kg than in both the negative and positive control groups (Table 3).

Treatment	Dose (mg/kg)	TLC (cell/mm3)		% Reduction	% Neutrophil		% Reduction
		Before	After		Before	After	
Control	2 ml/kg	4880±538	1240±248	75	31.00±1.9	16.60±2.0	42
MME	250	7600±843	1300±264	80.1	29.00±2.7	17.70±2.2	35.4
	500	9240±1224	1200±57	85.1	28.20±3.4	16.30±4.4	46.4
	750	7860±1205	2100±623	67.6	22.20±1.3	15.00±2.0	30.3
Levamisole	50	15880±3172	740±163	93.7	21.00±3.8	14.40±2.2	28.7

Values shown are as Mean ± SEM; MME = Methanol/methylene chloride extract; n = 5; TLC=total leucocyte count.
Table 3: Effect of MME on cyclophosphamide induced neutropenia.

However, MME treatment offered no restoration of the suppressed values observed after cyclophosphamide treatment.

Effect of Extract on Non-Specific Immunity Determined by Survival Rate against Fungal Infection (Mice Lethality Test)

From the mice lethality test conducted, no deaths were recorded in all treatment groups. However, exfoliation and death were observed in the negative control. Animals treated with MME and levamisole did not show such exfoliation (Table 4).

Groups	Doses (mg/kg)	Percentage survival rate
Control	2 ml/kg	80
MME	250	100
	500	100
	750	100
Levamisole	50	100

MME = Methanol/methylene chloride extract; n = 5
Table 4: Effect of MME on the non-specific immune system.

Discussion

Modulation of immune responses in an attempt to alleviate diseases has been of keen interest in the recent past years. Immunomodulatory studies involve procedures that can alter the immune system of an organism, hence interfering with immune functions. A resultant enhancement of immune functions indicates the possibility of an immunostimulating potential in the substance being screened which primarily implies stimulation of specific and non-specific systems, i.e. granulocytes, macrophages, complement, certain T-lymphocytes and different effectors substances [19]. In this study, the methanol/methylene chloride stem bark extract (MME) of *Parkia biglobosa* (Fabaceae) was screened for immunomodulatory activity. The extract exhibited some immunostimulatory activity.

Neutrophil adhesion test is a test used for the assessment of immunomodulatory activities of medicinal plants [18]. The neutrophil adhesion to nylon fibres describes the margination of polymorphonuclear lymphocyte in the blood vessels. The extent of recruiting neutrophils as specialized cells playing a key role in the phagocytic function of the innate immune system correlates well with the percentage neutrophil adhesion. These neutrophils are capable of a wide range of responses like chemotaxis and exocytosis [20] preceding the fusion of the phagosomes with lysozyme and the eventual digestion. Interestingly, the extract at 250 mg/kg dose was most effective in stimulating neutrophil adhesion to nylon fibres. An increase in the percentage of neutrophil adhesion, therefore, suggests enhanced neutrophil recruitment and hence stimulation of the function of treated mice phagocytes [21,22] towards site of inflammation.

Cyclophosphamide-induced neutropenia concentrates on the protective effects against cyclophosphamide-induced myelosuppression in the experimental animals [23]. The three doses of MME administrated did not significantly ($P < 0.05$) decrease the observed neutropenic effect of cyclophosphamide suggesting the absence of efficient attenuation of the suppressive effect of the agent on the haematopoietic function of the immune system [24].

The mice lethality test aims at assessing the extent of protection offered by the treatments on non-specific immunity. According to Sudah, et al., [24] mice lethality test is employed to assess the animal's serological response to vaccines. It involves injecting mice with vaccine before administration of bacterial culture and determining death rate [25]. Hence the exfoliation and death observed in the control group could point to the absence of such immunoprotection. Animals receiving MME and levamisole treatment possibly had some level of boost of their non-specific immune potentials hence no death or exfoliation was observed in these treatment groups.

The LD50 of the methanol-methylene chloride of the extract was determined to be greater than 5000 mg/kg and regarded to be safe since it did not cause toxicity up to that dose.

Conclusion

From the findings of this study, it can be concluded that the stem bark of *Parkia biglobosa* possesses immuno-stimulating activity but offers no benefits in the restoration of the immunosuppressive conditions due to cyclophosphamide treatment. Its immunostimulatory potentials could be attributed possibly to the presence of flavonoids, alkaloids, tannins**,** saponin, glycosides and steroids as it is already reported that these naturally occurring phenolic compounds have immunomodulatory activity.

References

1. Beck G, Gail SH (1996) Immunity and the invertebrates. Scientific American 275 (5): 60-66.

2. Avorn J (2011) Learning about the safety of drugs. A half-century of evolution. New England Journal of Medicine 365: 2151-2153.

3. Heroor SS, Beknal AK, Mahurkar N (2013) Immunomodulatory activity of methanolic extracts of fruits and barks of *Ficus glomerata* Roxb. in mice and on human neutrophils. Indian Journal of Pharmacology 45(2): 130-135.

4. Odetola A, Akinloye O, Egunjobi C, Adekunle W, Ayoola A (2006) Possible antidiabetic and antihyperlipidaemic effect of fermented *Parkia biglobosa* (Jacq) extract in alloxan-induced diabetic rats. Clinical and Experimental Pharmacology and Physiology 33(9): 808-812.

5. Soetan KO, Lasisi OT, Agboluaje AK (2011) Comparative assessment of *in-vitro* anthelmintic effects of the aqueous extracts of the seeds and leaves of the African locust bean (*Parkia biglobosa*) on bovine nematode eggs. Journal of Cell and Animal Biology 5(6): 109-112.

6. Ajaiyeoba E0 (2002) Phytochemical and antibacterial properties of *Parkia biglobosa and Parkia bicolor* leaf extracts. African Journal of Biomedical Research 5(3): 125-129.

7. Millogo Kone H, Guissou IP, Nacoulma O, Traore AS (2006) Study of the antibacterial activity of the stem bark and leaf extracts of *Parkia biglobosa* (Jacq.) Benth. on *Staphylococcus aureus.* African Journal of Traditional, Complementary and Alternative Medicines 3(2): 74-78.

8. Udobi CE, Onaolapo JA (2009) Phytochemical analysis and antibacterial evaluation of the leaf stem bark and root of the African locust bean (*Parkia biglobosa*). Journal of Medicinal Plants Research 3(5): 338-344.

9. Abioye EO, Akinpelu DA, Aiyegoro O, Adegboye MF, Oni MO, et al. (2013) Preliminary Phytochemical Screening and Antibacterial Properties of Crude Stem Bark Extracts and Fractions of *Parkia biglobosa* (Jacq.) Molecules 18(7): 8485-8499.

10. Builders M, Wannang N, Aguiyi J (2011) Antiplasmodial activities of *Parkia biglobosa* leaves. *In vivo* and *In vitro* studies. Annals of Biological Research 2(4): 8-20.

11. Kouadio F, Kanko C, Juge M, Grimaud N, Jean A, et al. (2000) Analgesic and anti-inflammatory activities of an extract from *Parkia biglobosa* used in traditional medicine in the Ivory Coast, Phytother Res 14(8): 635-637.

12. Ognatan K, Adi K, Lamboni C, Damorou JM, Aklikokou KA, et al. (2011) Effect of Dietary Intake of Fermented Seeds of *Parkia biglobosa* (Jacq) Benth (African Locust Bean) on Hypertension in Bogou and Goumou-kope Areas of Togo. Tropical Journal of Pharmaceutical Research 10(5).

13. Builders M, Alemika T, Aguiyi J (2014) Antimalarial activity and isolation of phenolic compound from *Parkia biglobosa*. IOSR Journal of Pharmacy and Biological Sciences 9(3): 78-85.

14. Harborne JB (1998) Phytochemical methods: A guide to modern techniques of plant analysis. 3rd (Edn.), London: Chapman & Hall.

15. Lorke D (1983) A new approach to practical acute toxicity testing. Archives of Toxicology 54(4): 275-287.

16. Fulzele SV, Burchandi PM, Kanoje VM, Joshi SB, Dorle AK (2002) Immunostimulant activity of *Asthamangal ghrita* in rats. Indian Journal of Pharmacology 34: 194-197.

17. Thatte UM, Chhabria SN, Karandikar SM, Dahanukar

SA (1987) Protective effects of Indian Medicinal plants against cyclophosphamide neutropenia. Journal of Postgraduate Medicine 33(4): 185-188.

18. Mayank T, Shilpi B, Dixit VK (2007) Immunomodulatory activity of *Chlorophytum borivilianum* Sant. F. Evidence-Based Complementary and Alternative Medicine 4(4): 419-423.

19. Katzung BG (1998) Basic and Clinical Pharmacology. 7th (Edn.), Standford: Connecticut pp: 578-579.

20. Dale MM, Forman JC (1989) Textbook of Immunopharmacology. 2nd (Edn.), Blackwell Scientific Publication, Oxford, UK.

21. Miller LE (1991) Manual of Laboratory Immunology. In: Ludke HR, et al. (Eds.), Lea and Febiger, London.

22. Yogendrasinh BS, Sunita MJ (2010) Immunomodulatory Activity of Ayurvedic Plant *Aparajita* (*Clitoria Ternatea L.*) in male albino rats. The Global Journal of Science Frontier Research 10(3): 2-7.

23. Diwanay S, Chitre D, Patwardhan B (2004) Immunoprotection by botanical drugs in cancer chemotherapy. Journal of Ethnopharmacology 90: 49-55.

24. Sudha P, Asdaq SM, Dhamingi SS, Chandrakala GK (2010) Immunomodulatory activity of methanolic leaf extract of *Moringa oleifera* in animals. Indian Journal of Physiology and Pharmacology 54(2): 133-140.

25. Finco KDL, Galvin JE, Suiter BT, Huether MJ (2001) *Pasteurella multocida* toxin type D serological assay as an alternative to the toxin neutralization lethality test in mice. Biologicals 29(1): 7-10.