



RESEARCH COMMUNICATION

Effect of an aqueous extract of *Azadirachta indica* on the immune response in mice

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ABSTRACT

NJIRO, S.M., & KOFI-TSEKPO M.W. 1999. Effect of an aqueous extract of *Azadirachta indica* on the immune response in mice. *Onderstepoort Journal of Veterinary Research*, 66:59–62.

Immunopotentiating agents are useful in enhancing resistance to infections in individuals whose immunocompetence is compromised for one reason or another.

Because of the very wide spectrum of infectious and non-infectious diseases for which preparations from *Azadirachta indica* are said to be efficacious, it was suspected that a general immunopotentiating ability could be part of the mechanisms by which it ameliorates so many disease conditions. Using the haemolytic plaque technique, an aqueous extract of *Azadirachta indica* stem bark was shown to enhance the immune response of BALB/C mice to sheep red blood cells *in vivo*.

Keywords: *Azadirachta indica*, immune response, neem tree

INTRODUCTION

Various parts of the tree *Azadirachta indica* (also known as the neem tree) have been used for many different purposes. Its wood is used for construction, and extracts of it are used as an insecticide to control such pests as mosquito larvae and head lice, and to treat a very wide variety of diseases, both infectious and non-infectious. The tree originates from the Indian subcontinent and is now grown throughout the tropics.

Extracts of it have been proved to have antimicrobial activity against some important fungal pathogens including some in the Genera, *Trichophyton*, *Epidermophyton*, *Microsporum*, *Trichosporum*, *Geotrichum* and *Candida*. With regard to bacterial pathogens, the results have been ambivalent. Whereas *Azadirachta indica* has been shown to be efficacious against important pathogens such as *Staphylococcus aureus* and *Salmonella typhosa*, it has no activity against *Citrobacter*, *Escherichia coli*, *Enterobacter*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aerugi-*

nosa and *Streptococcus faecalis* (National Research Council 1992). Extracts of it have also been proved to be efficacious when used as a treatment for certain protozoal diseases, such as Malaria. The active principle responsible for activity against *Plasmodium falciparum* is genudin (Khalid, Duddeck & Gonzalez-Sierra 1989).

The work reported in this paper was carried out to determine if extracts of *A. indica* possessed a general immunostimulatory effect, which would help to explain why they are efficacious when used as treatment for such a wide cross section of diseases. This general efficacy has been suspected to be due to an enhanced immune response (Van der Nat, Klerx, Van Dijk, de Silva & Labadie 1987). An adaptation of the haemolytic plaque techniques of Jerne & Nordin (1963) and Cunningham & Szenberg (1968) was used to investigate any immunostimulatory effect that an aqueous extract of *A. indica* might possess.

MATERIALS AND METHODS

A. indica extract

This was a lyophilized aqueous extract prepared from the bark of the stem at the Kenya Medical Research

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Accepted for publication 1 February 1999—Editor

Institute (KEMRI). The golden-orange freeze dried powder is stable at room temperature. Separate experiments were also carried out using an aqueous extract of the leaves of the tree.

Sheep red blood cells (SRBC)

Sheep blood was aseptically collected in an equal volume of Alsever's solution and centrifuged at 5 000 revolutions per minute (RPM) for 5 min. The SRBC pellet was washed three times in phosphate buffered saline (PBS), made up to 10 ml using Alsever's solution and stored at 4 °C until needed.

Spleen cells

Spleens were aseptically removed from the test and control mice, minced and the resultant material passed through fine, sterile wire gauze using sterile forceps. The spleen cells were then suspended in the working medium and counted in a haemocytometer after staining with a 1 % Trypan Blue solution.

Working medium

Roswell Park Research Institute (RPMI) 1640 medium was used. It was fortified with L glutamine to a final dilution of 1 %, 100 international units (IU) of penicillin per ml, 100 µg streptomycin per ml, foetal calf serum (FCS) to a final dilution of 5 %, 50 µl of 2 mercaptoethanol (ME) per 100 ml, HEPES buffer to a final dilution of 2 % and 2 µg phytohaemagglutinin (PHA) per ml.

Cunningham chambers

These were made using ordinary microscope glass slides. The lower layer of about ten of these were placed side by side with the long side oriented vertically. Using 6 mm wide, double sided adhesive tape, the glass slides were joined together at three points; across both ends and across their middles. A second glass slide was then placed precisely on top of each of the slides in lower layer. This resulted in pairs of slides one on top of the other, with the strips of adhesive tape joining them across both ends and across the middle. After the tape was cut to separate the pairs, each pair had two half chambers in between the three pieces of sticky tape holding the two slides together (Cunningham and Szenberg 1968).

Dosing protocol

For each assay, 30 BALB/C mice of the same sex, approximately 8 weeks of age and weighing an average of 34 g were divided into three groups of 10 mice each. The *A. indica* bark or leaf extracts to be administered were dissolved in normal saline solution and injected intraperitoneally in volumes of 0,1 ml. Each group of mice was identified by the dosage in mg/kg of the extract that the mice in the group

received. These were 100 mg/kg, 10 mg/kg and 0mg/kg, the latter being the controls which each received 0,1 ml normal saline solution. These doses were repeated daily for 3 d, after which the mice were left for 3 d before exposure to the antigen. All the mice were then each injected with 2×10^8 SRBC intraperitoneally. A mouse from each group was euthanized by cervical dislocation on day 6 following exposure to the antigen and a haemolytic plaque assay carried out. This gave a comparison of the B cell immune response between the two groups and the control group. At times two mice from each group were used and two haemolytic plaque assays carried out on the same day. The remaining mice were kept alive until results of the assays were recorded and were destroyed soon thereafter.

Plaque assay

Spleen cells were prepared as described above from every mouse that was euthanized. A plaquing mixture consisting of 10^4 spleen cells in 150 µl of medium, 25 µl of guinea pig serum (GPS) as complement (diluted 1:1 in medium) and 25 µl of 25 % SRBC suspension in medium (volume to volume) was made up and used to fill each of four Cunningham half chambers by capillary action.

The chambers were then sealed with a mixture of paraffin wax and petroleum jelly and incubated at 37 °C for 1 h, after which the number of haemolytic plaque forming cells (PFC) were counted.

Data analysis

The results of the three different dosage levels for each assay were analysed for significance at $P < 0,05$ using Dunnett's T test (Dunnett 1955).

RESULTS

The results are shown in Fig. 1, which gives mean PFC counts (per million cells) per assay for each dosage level. Assays 1–3 are those of extracts of the stem bark of *A. indica* while assays 4 and 5 are those of the extracts made from the leaves of *A. indica*. In assay 1, both test groups, ie the mice which received 100 mg/kg or 10 mg/kg, gave mean PFC counts that were significantly higher than those of the control group at $P < 0,05$. In assay 2, again both test groups gave mean PFC counts that were significantly higher than those of the control group at $P < 0,05$. In assay 4, the test group with 100 mg/kg, gave mean PFC counts that were significantly higher than those of the control group at $P < 0,05$. It is on the basis of these findings that it is concluded that the aqueous extract of both stem bark and leaves of *A. indica* significantly enhances the immune response of mice against SRBC, a thymus dependent antigen.

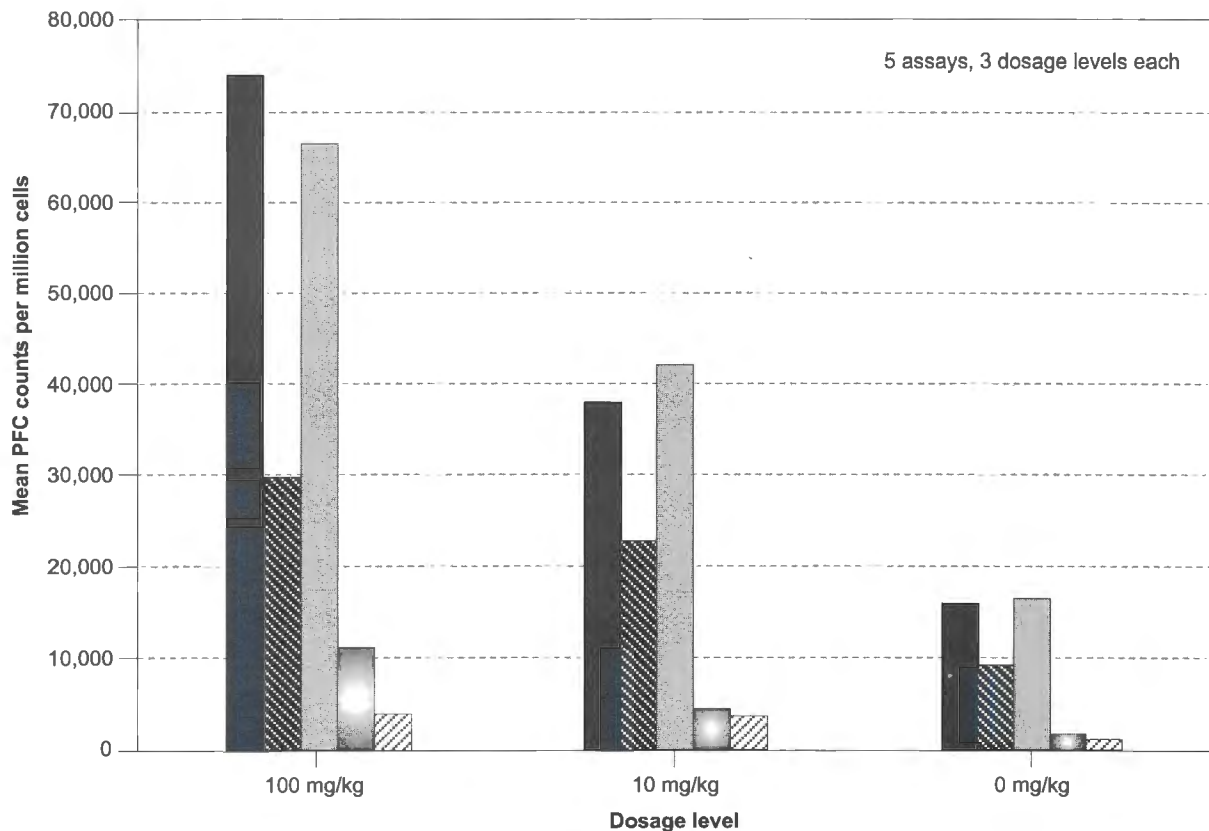


FIG. 1 Mean PFC counts (per million cells) per assay for each dosage level

DISCUSSION

Immunopotentiating agents are useful in enhancing resistance to infections in individuals whose immunocompetence is compromised for one reason or another. One such agent is Levamisole (De Cree & Symoens 1979; Reyer, Stockl & Thalhammer 1979). A number of infections and infestations in Veterinary Medicine are characterized by a suppression of the immune response. These include Canine Distemper, Canine Parvovirus Enteritis, *Demodex canis* infestation, Feline Panleukopaenia and Bovine Virus Diarrhoea (Slauson & Cooper 1982). Research into the identification of more immunopotentiating agents is important as it would eventually provide new tools for better management of such diseases.

The results of this experiment show that *in vivo* exposure of mice to aqueous extracts of *A. indica* stem bark leads to a dose dependent increase in the number of plaque forming cells formed against SRBC, using the haemolytic plaque technique. This means that the *A. indica* extract causes a stimulation or a potentiation of the immune response. This supports the work done by others who found that *A. indica* extract enhances at least one aspect of the immune response (Van der Nat *et al.* 1987; Ray, Banerjee & Sen 1996). In one study, an aqueous extract of the stem bark was found to produce a dose

dependent increase in the production of Migration Inhibition Factor (MIF) *in vitro*. *In vivo*, this would lead to the localization of macrophages and monocytes, which in turn favours an enhancement of the immune response (Van der Nat *et al.* 1987). In another study, a stem bark extract was found to increase both IgM and IgG levels. It also inhibited macrophage migration. This led the authors to conclude that extracts of the tree enhanced both humoral and cell mediated immunity (Ray *et al.* 1996).

This stimulation of the immune response would account, at least in part, for the wide variety of diseases that preparations from this tree are reputed to ameliorate. As Fagoonee (1987) has so aptly stated, "The spectrum of diseases that can be cured by this plant is so wide that it is known as "the village doctor."

ACKNOWLEDGEMENT

This work was funded through a grant from the Deans' Committee, University of Nairobi.

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