Evaluating the Immunomodulatory Potential of the Aqueous Leaf Extract of Sennamimosoides in Wistar Albino Rats


Abstract: Sennamimosoides formerly known as Cassia mimosoides belongs to the family Caesalpinaceae and the genus Senna. The leaf is used in folklore medicine for the treatment of oedema and breastmilk toxicity in neonates. In the present study, the immunomodulatory activity of the aqueous leaf extract of S. mimosoides was evaluated. For the animal model experiment, a total of fifty (50) Wistar albino rats used in delayed type hypersensitivity reaction and humoural antibody titre (twenty five (25) rats for each parameter) were grouped as follows. Rats in group A (control) were administered 0.2 ml of normal saline; rats in groups B, C and D were treated with 50, 250 and 500 mg/kg of the aqueous extract of S. mimosoides respectively while group E rats received 25 mg/kg of levamisol a standard drug. Administration of 50, 100 and 250 mg/kg of the extract resulted in a dose dependent significant (p < 0.05) increase in primary antibody titre with a value of 6, 8, 13, and 2.304 and 2.326 mm for the extract and levamisol respectively. The Humoural antibody (HA) titre and DTH response compare well with that of levamisol a standard immunostimulatory drug at 25 mg/kg. This result shows that the extract has immunostimulatory effect and could be used in boosting immune response.

Keywords: Sennamimosoides, immunostimulatory, HA, DTH, levamisole.

I. Introduction

Immunology is the study of the methods by which the body defends itself from infectious agents and other foreign substances in its environment (Wotherspoon, 2012). An infectious organism that causes a disease is called a pathogen and the individual (person or animal) that is infected by a pathogen is called the host. There is a tremendous variety of cells and molecules capable of specifically recognising and eliminating an apparently limitless variety of foreign invaders, in addition to the recognition and destruction of abnormal cells (Parkin and Cohen, 2001). Once a foreign protein, microorganism (e.g., bacterium, fungus or virus) or abnormal cell is recognised, the immune system enlists the participation of a variety of cells and molecules to mount an appropriate effector response to eliminate or neutralise them (Parkin and Cohen, 2001). Later exposure to the same foreign organism induces a memory response, characterised by a heightened immune reactivity, which serves to eliminate the microbial pathogen, prevent disease and protect against the development of some tumour cells.

Sennamimosoides formerly known as Cassia mimosoides belongs to the family Caesalpinaceae and the genus Senna (Young, 2000). It is known as sensitive senna and its leaves, pods and seeds are edible (Bruce, 2006). Sennamimosoides leaves are used in Nsukka folklore medicine, Ukehe Enugu State, Nigeria precisely to treat oedema in pregnant women and breastmilk toxicity in neonates. Not much research has been done on Sennamimosoides. However, it is known to be a natural lipase inhibitor, i.e. it prevents the absorption of fats from digested food and is therefore used as a slimming agent. It also has a laxative effect i.e. it is used to relieve constipation and support normal body function (Robbins, 2006). The aqueous leaf extract of Sennamimosoides exhibits anti-inflammatory effects by stabilizing membrane, inhibiting phospholipase A₂ activity and prostaglandin synthase activity (Ekwueme et al., 2011).
Levamisole is a synthetic phenylimidazothiazole that is undergoing clinical evaluation as an antineoplastic agent. Although originally used as an antihelminthic drug, oncological interest in this drug stems from early reports demonstrating restorative effects of levamisole on suppressed immune responses, and antitumor activity in animal tumor models (Shah et al., 2011). Levamisole has been shown to improve immunitary defenses and delayed type hypersensitivity in immunodepressed individuals, to restore T helper and T suppressor cell activity in old mice and to evoke in vitro maturation of guinea pig thymocytes (Lai et al., 2002). Its action on macrophage function is well established: in rats, it accelerates clearance of colloidal carbon; in humans, in vivo and in vitro; it increases the metabolic activity of blood monocytes and their affinity for the Fc fragment of IgG. Levamisole does not act directly on antibodies synthesis, but may enhance the responses to T dependent antigens by stimulation of T helper cells, even in normal, non immunosuppressed individuals.

Delayed-type hypersensitivity (DTH) reactions involve a sensitization phase and an effector phase. During the sensitization phase macrophages and dendritic cells secrete IL-12, which induces the development of Th1 cells in regional lymph nodes (Yoon, 2005). A DTH response develops over 48-72 hours when antigen presented by a tissue macrophage in the context of IL-12 and IL-18 secretion activates sensitized Th1 cells in the tissue (Edovitsky et al., 2007). Chemokines (IL-8, MCP-1) and cytokines (IL-2, IFN, MIF and TNFβ) released by the activated Th1 cells attract and activate additional macrophages. Activated macrophages which are the principal effector cell in a DTH response show increased expression of class II MHC molecules, TNF receptors, oxygen radicals, and nitric oxide (Yoon, 2005). These changes enhance the antigen presenting and microbicidal activities of macrophages. Lytic enzymes that leak from activated macrophages cause local tissue destruction. CTL induced by Th1 cells may also participate in tissue destruction.

Humoral immunity is defined in terms of the B-lymphocytes (B-cells), the antibody producing cells of the immune system. The B-cells are found primarily in the spleen, lymph nodes, Peyer’s patches in the gut, peripheral blood and bone marrow (Nakagawa, 2013). Antibodies function in concert with complement proteins that are produced in the liver and by macrophages to provide protection against bacterial and viral infections (Gupta et al., 2008). Antibodies also help protect man and animals from agents that cause tumours and from some spontaneously occurring tumour cells. Humoral immunity can be further classified with regard to the dependence of antibody production on T lymphocyte help: T-cell dependent and T-cell independent immunities.

Interest in the use of immunostimulants as an alternative to the drugs, chemicals and antibiotics currently being used to control diseases is growing, partially because immunostimulants, in contrast to vaccines, enhance the innate (or non-specific) immune response (Vahedi and Ghodratizadeh, 2011).

The purpose of this study is to evaluate the effect of aqueous leaf extract of Sennamimosoides on humoral response and delayed type hypersensitivity reaction.

II. Materials And Methods

2.1.1 Plant Material

The leaves of (Sennamimosoides) were collected from Ibagwa Roadside, Nsukka in Enugu state of Nigeria, during the months of July and August. The plant characterisation and identification was carried out by a taxonomist Mr P.O. Ugwuozor, in the Department of Botany, University of Nigeria, Nsukka.

2.1.2 Animal Materials

Wistar albino rats and mice of either sex weighing between 130-250 g and 20-30 g respectively were obtained from the Animal House, Faculty of Biological Sciences, University of Nigeria, Nsukka. These animals were given standard feeds (vital) for at least one week after purchase to acclimatize them to their new environment before use.

Aqueous Extraction

A known amount (2000g) of Sennamimosoides leaves was extracted with 8400ml of distilled water using cold maceration. It was then filtered first with calico and subsequently with glass wool and finally Whatmann No.4 filter paper. The filtrate was concentrated by lyophilisation. A brown slurry-like substance was obtained and stored in the refrigerator for further investigation.

Determination of Percentage Yield

The percentage yield of the extract was calculated using the following formula:

\[
\%\text{ yield} = \frac{\text{mass of extract}}{\text{mass of leaves}} \times 100
\]
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Assay of Biological Activity
Acute Toxicity and Lethality:
Investigation on acute toxicity of the extract with estimation of the median lethal dose (LD₅₀) was carried out using Lörke’s method (1983). Thirteen experimental animals (mice with weight range of 20g-30g) were used for the test. In the investigation, three groups of mice containing three mice each were administered 10-, 100- and 1000mg/kg respectively of the aqueous extract intraperitoneally (ip). They were observed closely for 24 hr for lethality or any other behavioural response. Based on the result, further increased doses of 1500-, 2000-, 3000- and 5000 mg/kg were administered ip to four other mice respectively. They were also observed for 24 hr for any death or behavioural changes.

Experimental Design
A total of fifty (50) Wistar albino rats used in Delayed Type Hypersensitivity Reaction and Humoral Antibody titre (twenty five (25) rats for each parameter) were grouped as follows:
Group A: Rats were treated with SRBC and normal saline and it served as control
Group B: Rats were treated with SRBC and 50 mg/kg body weight of extract
Group C: Rats were treated with SRBC and 100 mg/kg body weight of extract
Group D: Rats were treated with SRBC and 250 mg/kg body weight of extract
Group E: Rats were treated with SRBC and 25 mg/kg body weight of levamisol

Immunomodulatory Activity Of Extracts
Preparation of Antigen:
Fresh sheep blood was obtained from the animal farm of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. Sheep red blood cells (SRBCs) were washed three times in continuous volume of pyrogen-free sterile normal saline by centrifugation at 3000×g for 10 min on each occasion. The washed SRBCs were adjusted to a concentration of 10⁹ cells/ml for immunization and challenge.

Delayed Type Hypersensitivity (DTH) Reaction
Delayed type hypersensitivity (DTH) reaction was investigated using the method of Navedet al. (2005). DTH reaction was induced in rats using SRBCs as antigen. Animals were sensitized by subcutaneous injection of 0.02 ml of 10⁹ cells/ml SRBCs (day 0) in the plantar region of right hind foot paw and challenged on day 5 by subcutaneous injection of the same amount of antigen into the left hind paw. The oedema produced by antigenic challenge in the left hind paw was measured as the difference in the paw thickness before challenge; 24 hr and 48 hr after the challenge. The paw thickness was measured with a pocket-sized screw guage (Navedet al., 2005). The extracts were administered three days prior to sensitization and continued daily till the challenge.

Humoral Antibody (HA) Synthesis
Rats were immunized by an intraperitoneal injection (i.p) of 0.1ml of 10⁹ SRBC ml⁻¹ on day 0 and challenged by similar ip injection of the same amount of 0.1 ml on day 5. Primary antibody titre was determined on day 5 (before the challenge) and secondary titre on day 10 (Sharma et al., 1996) by haemagglutination technique (Nelson and Mildenhall, 1967). The aqueous extract (50, 100 and 250 mgKg⁻¹) was administered 3 days prior to immunization and continued daily for 5 days after challenge. Blood samples were obtained by retro-orbital puncture and collected in a test tube and allowed to clot. For each sample, a 25µL serum was obtained after centrifugation and serially diluted two-fold in 96-U well microtitre plates using pyrogen-free sterile normal saline as control. The diluted sera were challenged with 25µL of 1% (v/v) SRBC in the plates and then incubated at 37°C for 1 hr. The highest dilution giving rise to visible haemagglutination was taken and antibody titre expressed in graded manner, the minimum dilution (1/2) being ranked as 1 (calculated as Log₂ of the dilution factor). The mean ranks of different treatment groups were compared for statistical significance.

III. Results
Extract of Sennamimosoides
Dried leaves of Sennamimosoides 2000g which was subjected to cold aqueous extraction yielded 35.3%. The extract which was brown and slurry-like was used in all biological activity determination.

LD₅₀
In the investigation, there was no lethality or behavioural change in the three groups of mice that received 10, 100 and 1000 mg/kg of the extract. Based on this result, further increased doses of 1500, 2000, 3000 and 5000 mg/kg of the extract were administered to four other groups respectively. Those that received 3000 and 5000 mg/kg showed weakness and drowsiness. No death occurred within 24 hr of administration.
Table 3: The Median Lethal Dose of Aqueous Extract of the Leaf Extract of S.mimosoides

<table>
<thead>
<tr>
<th>Phases</th>
<th>Dosages mg/kg body weight</th>
<th>Mortality</th>
<th>Behavioural Changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase I</td>
<td></td>
<td></td>
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<tr>
<td>Group 1</td>
<td>10</td>
<td>0/3</td>
<td>Nil</td>
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<tr>
<td>Group 2</td>
<td>100</td>
<td>0/3</td>
<td>Nil</td>
</tr>
<tr>
<td>Group 3</td>
<td>1000</td>
<td>0/3</td>
<td>Nil</td>
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<tr>
<td>Phase II</td>
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<tr>
<td>Group 1</td>
<td>1500</td>
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<tr>
<td>Group 2</td>
<td>2000</td>
<td>0/3</td>
<td>Nil</td>
</tr>
<tr>
<td>Group 3</td>
<td>3000</td>
<td>0/3</td>
<td>Nil</td>
</tr>
<tr>
<td>Group 4</td>
<td>5000</td>
<td>0/3</td>
<td>Weakness and drowsiness</td>
</tr>
</tbody>
</table>

Effect of Extract on Humoural Immunity

Fig. 1 below reveals the effect of the extract on humoural immune response which was assessed using humoural antibody titre. The result indicates that there was augmentation of the humoural immune response to SRBCs by the extract and levamisol which was evidenced by the increase in the antibody titre. It also reveals that as the number of pretreatment days increased, the primary and secondary antibody increased in the rat. Both the primary and secondary antibody titre induced a significant rise (p < 0.05) in the antibody titre when compared to the control A. Administration of the extract produced dose dependent increase in humoural antibody titre. It is also obvious from the figure that the effect of 250mg/kg of extract was more than that of levamisol solution.

Effect of extract on Cell Mediated Immunity

Fig. 2 shows the induction of DTH reaction induced in the hind foot paw of rats using SRBC and DTH response checked by increased footpad thickness using vernier calliper. The challenged rat elicited a significant increase (p < 0.05) in thickness of footpad 24 hr and 48 hr after challenge. Administration of 50, 100 and 250 mg/kg of extract produced a dose and time dependent increase in footpad swelling of the rats.

At 24 hr after challenge, it is evident that the extract doses of 50, 100 and 250 mg/kg and 25 mg/kg body weight of levamisol significantly boosted (p < 0.05) DTH reactions observed as 1.412, 1.504, 1.816 and 1.827 mm difference respectively in thickness of footpad before challenge and 24 hr after challenge. In group A serving as the control, there was a non-insignificant increase (p < 0.05) with a difference of 0.614 mm in the thickness of footpad before challenge and 24 hr after challenge.

Fig. 1: Effect of S. mimosoides on primary and secondary antibody titre.

The values are expressed as (Mean ± SD) n=5 per group p < 0.05
Group A = Rats treated with SRBC and normal saline and it served as the control
Group B = Rats treated with SRBC and 50 mg/kg body weight of extract
Group C = Rats treated with SRBC and 100 mg/kg body weight of extract
Group D = Rats treated with SRBC and 250 mg/kg body weight of extract
Group E = Rats treated with SRBC and 25 mg/kg body weight of levamisol
At 48 hr after challenge, Fig. 2 also shows an additional increase in footpad swelling of rats in groups B, C, D and E observed as 1.908, 1.918, 2.304 and 2.326 mm respectively. This reveals that at 48 hr after challenge, the extract and levamisol still had stimulatory effect on T lymphocyte and accessory cell types required for expression of DTH reaction.

Fig. 2: Effect of S. mimosoides treatment on cell mediated immune response by DTH induced footpad oedema.

The values are expressed as (Mean ± SD) n=5 per group p < 0.05.
Group A = Rats treated with SRBC and normal saline and it served as control
Group B = Rats treated with SRBC and 50 mg/kg body weight of extract
Group C = Rats treated with SRBC and 100 mg/kg body weight of extract
Group D = Rats treated with SRBC and 250 mg/kg body weight of extract
Group E = Rats treated with SRBC and 25 mg/kg body weight of levamisol

IV. Discussion

Immunomodulation is a procedure which can alter the immune system of an organism by interfering with its functions; if it results in an enhancement of immune reactions it is named as an immunostimulative drug which primarily implies stimulation of specific and non specific system, i.e. granulocytes, macrophages, complement, certain T-lymphocytes and different effector substances. Immuno-suppression implies mainly to reduce resistance against infections and stress and it may occur on account of environmental or chemotherapeutic factor (Weir et al., 2011). The results obtained in the present study indicate that aqueous extract of Senna is a potent immunostimulant, stimulating specific and non-specific immune mechanisms.

To evaluate the effect of aqueous extract of Senna on humoural response, its influence was tested on sheep erythrocyte specific Ab titre in rats. From the results obtained in the present study and shown in Fig. 1 above, there was increase in humoural immune response to SRBCs as evidenced by increase in antibody titre. This indicates that aqueous extract of Senna is a potent immunostimulant, with the ability to stimulate specific and non-specific immune mechanisms. This suggests that the extract might have the ability of enhancing the interaction of B cells with the antigen and subsequent proliferation and differentiation of B-lymphocytes into antibody-secreting plasma cells (Sumenet al., 2004). The antibody produced by the activated plasma cell then inactivates the antigen by complement fixation where proteins attach to antigen surface and causes holes to form leading to cell lysis; by neutralization where the Ab binds to specific site preventing antigen attachment; by agglutination which involves clumping together of the Ag inactivating them; by precipitation whereby insolubility is forced and the antigen settles out of solution (Debebe, 2004).

Moreover, the extract might be able to activate lymphoid dendritic cell which stimulate lower amounts of IL-12 and higher amounts of IFN-α which primarily elicit Th2 development. Activated Th2 cell stimulate Ab production by secreting IL-4 which proliferates B-cell to plasma cell. Activated Th2 also releases cytokines such as IL-2, IL-4 and IL-10 which activates mast cells leading to the production of serotonin and histamine, mediators of inflammatory response (Vega and Corbi, 2006).
The ability of the extract to induce primary Ab titre shows that it may stimulate IgM which is the major Ab present in the body during primary immune response. IgM is effective in activating the complement components which facilitate the phagocytosis of pathogens by macrophages (Parkin and Cohen, 2001). Furthermore, the result also showed that the extract significantly increased (p < 0.05) secondary Ab titre suggesting that on re-exposure to the same Ag, the extract was able to accelerate secondary response and enhance the production of IgG. IgG and IgM are the major immunoglobulins which are involved in the complement activation, opsonization, neutralization of toxins (Selman, 2004; Gupta et al., 2008). The stimulation of immunoglobulin by the extract suggests that it activates IgA and lactoferrin the major immunoprotein present in breastmilk and which confers immunity to the neonate.

The augmentation of the humoral responsiveness to SRBC in rats as consequence of both pre and post immunization treatment with levamisol corresponds with the report of Lai et al., (2002) who reported levamisol improved immunitory defences by its interferon-like activity and by restoring Th cell activity; evoking maturation of thermocytes; increasing the metabolic activity of blood monocytes and their affinity for the Fc fragment of IgG.

Cell-mediated immunity (CMI) involves effector mechanisms carried out by T lymphocytes and their products (lymphokines). DTH is a Type IV hypersensitivity reaction that develops when antigen activates sensitized TDTH cells (Kunstfeld et al., 2004). DTH response was checked by increased footpad thickness using vernier caliper. The animals treated with levamisol and extracts showed a significant (p < 0.05) change in DTH response as compared to control animals. As can be evident from (Fig. 2), a significant (P < 0.05) increase in DTH response was observed at the doses: 50, 100 and 250 mg/kg dose of Senna extract and levamisol 25 mg/kg. Therefore, increase in DTH reaction in rats in response to T cell dependent antigen revealed the stimulatory effect of aqueous extract of Senna on T cells.

The result shown above suggests that during the first stage of the DTH response known as sensitization stage, the extract might have activated Th1 cells and also expanded clonally APCs with class II MHC (Kumar and Sharma, 2010). Then the protein of the antigen (in this case SRBC) will be presented at the external wall of the APC in a changed MHC class II, to chemotactically attract naive proT-lymphocyte known as Th0. The Ag-peptide will be taken from the MHC of the APC and bound to T-cell receptor activating the Th0 cell converting it to Th1 cell. Th1 cell then commences the effector phase of the DTH response.

Moreover, the activated Th1 which may have been enhanced by the extract, proliferates and releases cytokines (IL-2, IL-12, IL-18, IFN-γ, TNF-α) which increases vascular permeability, induces vasodilation, activates and accumulates macrophages and NK cells and all these promotes increased phagocytic activity and increased concentration of lytic enzymes (Pichler et al., 2004; Gordon and Taylor, 2005).

The probable stimulation of Th1 cell by the extract also suggest that the NK cell which was subsequently stimulated used a protein named perforin to destroy the SRBC by inculating the protein into the antigen (in this case SRBC) cell membrane. This perforin molecule then assembles in the membrane to form a pore, through which other molecules can flow into the target (Yoon and Jun, 2005). Moreover, it also produces IFN which are powerful activators of macrophages.

Treatment with the aqueous extract of Senna enhanced DTH reaction, which is reflected from the increased footpad thickness compared to control group suggesting heightened infiltration of macrophages to the inflammatory site (Baba et al., 2006). The probable mechanism of action of the extract might be by activating myeloid dendritic cells which are known to also produce a large amount of IL-12 and preferentially induce Th1 development. The extract might be immunogenic by activating complement which recruit and stimulate cellular elements of the immune system, such as histamine, basophils, macrophages, within 24-72 hr initiating inflammatory reactions and phagocytosis of pathogens which lead to increase in the rat paw oedema as depicted in Fig 2 above (Edovitsky et al., 2007). Increase in the DTH response indicates that drug has a stimulatory effect on lymphocytes and necessary cell types required for the expression reaction. As shown in fig. 2 above, DTH response, which is direct co-related to cell-mediated immunity, was significantly increased (p < 0.05) with aqueous extract of Senna extract as compared to untreated control. There are some plants like Aesculus indica (Chakraborthy, 2009), Argyreiaspeciosa (Gokhale et al., 2003) which are reported for acting on delayed type of hypersensitivity. The increase in rat paw oedema of rats treated with levamisol as shown in the Fig above corresponds with former reports of Yadav et al., (2011) showing the stimulatory effect of levamisol on lymphocytes and other cell types. Lai et al., (2002) reported that levamisol improved DTH in immunodepressed individuals to restore Th and Ts cell activity and also increases their activity.

V. Conclusion

Delayed type hypersensitivity reaction also stimulated by S.m. significantly indicates that the extract could stimulate the haemopoetic system. The mechanism of action could be unfolded only after detailed investigations whereby the extract modulates the immune system however; the extract contains compounds which had immunomodulatory activity. CMI responses are critical to defence against infectious organisms,
infection of foreign grafts, tumor immunity and delayed-type hypersensitivity reactions. Therefore, increase in DTH reaction in rat in response to T cell dependent antigen revealed the stimulatory effect by pretreatment of levamisole on T cells (Table 2). DTH is a part of the process of graft rejection, tumour immunity, and, most important, immunity to many intracellular infectious microorganisms, especially those causing chronic diseases.

Reference