Journal of Pharmacology and Toxicology

ISSN 1816-496X

Academic Journals Inc.

www.academicjournals.com

Journal of Pharmacology and Toxicology 2 (2): 200-204, 2007
ISSN 1816-496X

The Immunostimulatory Effects of Ethanolic Extract of *Cassia alata* on Immune System of Albino Rats Dosed with *Staphylococcus aureus* (NCIB 8588)

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Abstract: The immunostimulatory effect of the ethanolic extract of *Cassia alata* was tested on Swiss albino rats infected with *Staphylococcus aureus* by evaluating the White Blood Cells (WBC), Packed Cell Volume and total differential WBC count. The rats were in 6 groups of 2 animals per cage. The first group was given the standard inoculum but not treated. The second group was given the standard inoculum and treated with the extract. The third group was given *Cassia alata* ethanolic extract only while the control was given normal saline. Increase in White Blood Count (WBC) from 5000 to 7600 mm^3, decrease in Packed Cell Volume (PCV) from 51 to 21% and increase in neutrophil-lymphocyte ratio indicates active infection in the infected-untreated group. In the group treated with extract there was lower White Blood Count (WBC) of 5000 and 2900 mm^3 before and during infection, respectively. Decrease in neutrophil-lymphocyte ratio indicates suppression of infection/inhibition of proliferation of *Staphylococcus aureus* infection. The group given extract only showed WBC, PCV, Neutrophil and Lymphocyte value of 5200 mm^3, 45, 44 and 55% before infection; 2300 mm^3. 32, 50 and 50% during infection and 3100 mm^3, 32, 72 and 28% after infection, respectively. There is a boosting of the immune system as compared to the control. The result of the urinalysis showed a pH of 5, negative to glucose, Ascorbic acid, Ketone, Nitrite, Bilirubin, Protein and Blood, normal for Urobilinogen for all the groups before infection. The untreated rats showed a pH of 7 positive for nitrite and Bilirubin, negative for other parameters. The infected/untreated rats showed 6-8 pus cells/HPF, 2-4 casts/HPF, 6-8 crystals/HPF and 4-6 bacterial cells/HPF indicating active infection. The result of this study is significant for the development of *Cassia alata* to be utilized as an augment for the current antimicrobial therapy that is becoming less efficacious against *Staphylococcus aureus*.

Key words: Immunomodulatory, *Cassia alata*, lymphocyte, histopathology

INTRODUCTION

Over the past two decades, an expanding body of evidence from epidemiological and laboratory studies have demonstrated that some edible plants as a whole, or their identified ingredients have substantial protective effects (Atawodi, 2005). In recent times, focus on plants research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems (Dahanukar *et al*., 2000). This increasing interest is due to a tremendous historical legacy in folk medicine use of plants as medicine and their easy availability, cost effectiveness and presumed safety (Guerra *et al*., 2003).

Recently, many advances have been made towards understanding host immune responses to infectious diseases (Tzianabos, 2000). Novel cell surface and soluble signaling molecules produced by

cells of the immune system have been discovered that regulate host response to microorganisms. Investigations have focused on discovering compounds that positively or negatively modulates the biologic responses of the immune cells and enhance the host ability to resist microbial infection. (Tzianabos, 2000). Agents that activate host defense mechanisms in the presence of an impaired immune responsiveness can provide supportive therapy to conventional chemotherapy (Dahanukar *et al*., 2000)

A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs (Sofowora, 1993). The traditional use of plant in the treatment of different infection is widely practised in Africa and other developing countries because it is relative cheaper than modern medicine (Pieme *et al*., 2006).

Immunomodulators are compounds capable of interacting with the immune system to up regulate or down regulate specific aspects of the host response (Tzianabos, 2000). These Immunomodulators can influence innate and cell-mediated immunity through interactions with T cells, monocytes, macrophages and polymorphonuclear lymphocytes. This activity depends on a number of factors, including dose, route of administration and timing of administration of the compound in question (Tzianabos, 2000). It may also depend on the mechanism of action or the route or site of activity. The ability to modulate the immune response in an appropriate way can enhance the host's immune response to certain infections (Manosroi *et al*., 2004).

The present study is aimed at evaluating the immunostimulatory potential of ethanolic extract from *Cassia alata* on Swiss albino rat after orogastric dosing with *S. aureus*. *Cassia alata* belong to the family Fabaceae and have been reported to have antimicrobial activity (Pieme *et al*., 2006)

MATERIALS AND METHODS

Source of Plant Sample, Extraction and Fractionation

The leaves of *Cassia alata* collected from the vicinity of Ilupeju Estate Ikire, Osun State, Nigeria. It was identified by Mr Aduloju of Crop, Soil and Pest Management Department Federal University of Technology, Akure. It was air dried for days and then blended with into powdery form. A 60% ethanol was the solvent for extraction. Exactly 450 g of the powdered sample was weighed and 100 mL of the extracting solvent was added to it until it was supersaturated. After 72 h, it was sieved using muslin cloth and concentrated using rotary evaporator from the Central Laboratory, Obafemi Awolowo University, Ile Ife. An aliquot of the crude extract was dissolved in 0.1M Tris-HCl buffer (pH 7.0, 2 mL) and applied on to column (5×85) of Sephacryl S-300 HR, pre equilibrated and developed with the same buffer. Fractions showing similar TLC characteristics are pooled together and concentrated. It was then dissolved in water and applied to a Sephadex G-25, column (1.5×50).The eluate was concentrated and lyophilized.

Source of Laboratory Animal Used

Swiss albino rats were obtained from the Pharmacy Department of Obafemi Awolowo University, Ile Ife. The rats have average weight between 120-250 g. The Swiss albino rats of either sex were separated (2 per group) they were fed with standard laboratory diet and water *ad libitum*.

Source of Microorganism Used

Pure isolate of *Staphylococcus aureus* (NCIB 8588) used for this project was obtained from Microbiology Department, Obafemi Awolowo University, Ile Ife. Osun State, Nigeria. The isolate was maintained in pure culture prior to use.

Standard Innoculum Preparation

The organism was transferred from slant on to plate of nutrient agar and pure colony was picked before inoculating into a nutrient broth, it was incubated at 37°C for 24 h. Serial dilutions was made from the stock solution of broth and diluted serially up to 10^{-5} test tube was dispensed into Petri dish and already prepared molten agar was poured on it. It was allowed to set before incubating at 37°C for 24 h and the colony counted.

Evaluation of the Effect of the Extract on Immunological Indices in Rats

Forty eight albino rats were used to assess the effect of the plant extract on the immune system. The rats were divided into 6 groups of eight rats per cage. The first group was given normal saline (Placebo). Four groups were given the standard inoculums. Out of the 4 groups, one was given booster shot of the standard inoculums after 3 days. Also, 2 of the groups were treated with 250 mg mL^{-1} of the plant extract with one of this two given a booster of the extract after 5 days. The last group was given extract only. At the onset of infection and during infection, the weight, hematological test and urinalysis were carried out to assess the lymphocytes produced and damage done to the internal organs.

The white blood cell count was done using tork's solution and haemocytometer. The packed cell volume was carried out using haematocrit centrifuge before reading through a microhaematocrit reader, while the differential count was carried out using a Leishman's stain and viewing under the microscope.

Urinalysis

The urine macroscopy was carried out using a combi-9 urine test strip which measured the value of pH, glucose, ascorbic acid, ketone, Nitrite, protein, bilirubin, urobilinogen and blood in urine.

The urine microscopy was also carried out by collecting the urine into a centrifuge tube and spinning at 12,000 rev/sec for 5 min. The supernatant was decanted and the sediment was dropped on the microscopic slide and covered with cover slip which was viewed under the microscope. (Ogwumike, 2002).

RESULTS AND DISCUSSION

Table 1 revealed that the ethanolic extract of the leaf of *Cassia alata* has impressive immunostimulatory activity on albino rat dosed with *Staphylococcus aureus*. There was a significant increase in the White Blood Cell (WBC) of the rat dosed with the *Staphylococcus aureus* compared to the control. Also, the the value reduces after the ethanol extract was administer on the rats. This may be that the extract contain several compounds, such as protein, peptides lipopolysaccharides, glycoproteins and lipid derivatives that have immunostimulatory potential on the immune system (Tzianabos, 2000). The increase in total WBC may be as a result of proliferation of phagocytes to engulf the antigen (*Staphylococcus aureus*). It has been reported that microbial infections can through lymphoid lineage produces T-lymphocytes and β-lymphocytes while the myeloid progenitor give rise to mononuclear and polymorphonuclear leucocytes (Weir and Stewart, 1999).

Rats infected with *Staphylococcus aureus* and dosed with *Cassia alata* have a polymorphonuclear granulocytes level of 54, 49 and 32 at the onset of infection, during infection and at the termination of the study, respectively. This may be due to a number of factors which include the dose and the route of administration of the extract as well as the ability to modulate the immune system (Tzianabos, 2000) The reduction during infection may be also attributed to their migration into the tissue in response to the infection.

Table 1: Effect of ethanolic extract of *cassia alata* on haematological indices of rats dosed with staphylococcus aureus

	At infection onset					During infection					At termination					
Exptal units	WBC (mm^3)	PCV (%)	E	N	L	WBC (mm^3)	PCV (%)	E	N	L	WBC (mm^3)	PCV (%)	E	N	L	M
A	5000	51	1	45	54	7600	21	-	52	48	3500	33	-	67	33	-
B	5000	50	1	45	54	2900	51	-	49	51	2800	38	5	63	32	-
C	5200	45	1	44	55	2300	32	-	50	50	3100	32	-	72	28	-
Control	4000	57	1	48	51	5000	22	-	48	52	4400	30	1	59	49	-

A: Rats infected with *Staphylococcus aureus*, B: Rats infected with *Staphylococcus aureus* and extract, C: Rats given ethanolic extract of *Cassia alata* only WBC: White Blood Count, N: Neutrophil, L: Lymphocyte, M: Monocyte, PCV: Pack cell volume

Table 2: Effect of plant extract on the urine macroscopy

	At infection onset			During infection			At termination			
Tests	A	B	C	A	B	C	A	B	C	Control
PH	5	5	5	7	7	7	6	5	6	6
Glucose	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Ascorbic acid	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Ketone	-ve	-ve	-ve	++	-ve	-ve	-ve	-ve	-ve	-ve
Nitrite	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve
Protein	-ve	-ve	-ve	30	-ve	+ve	100	30	-ve	-ve
Bilirubin	-ve	-ve	-ve	++	-ve	-ve	+++	-ve	-ve	-ve
Urobilinogen	Norm	Norm	Norm	2	Norm	Norm	Norm	Norm	Norm	Norm
Blood	-ve	-ve	-ve	Ca250	-ve	-ve	Ca10	-ve	-ve	-ve

A: Rats infected with *Staphylococcus aureus*, B: Rats infected with *Staphylococcus aureus* and at the same time treated with ethanolic extract of *Cassia alata*, C: Rats given ethanolic extract of *Cassia alata* only

The immunostimulatory effect of the ethanolic extract of the leaf of *Cassia alata* was observed in the macroscopic and microscopic examination of the urines of the different groups of rats as shown in Table 2 and 3. A protein level of 30 and a negative result for the rats infected with *S. aureus* and rats infected with *Staphylococcus aureus* and dosed with *Cassia alata*, respectively during infection is an indication of the anti-infective capacity of the extract. This anti-infectivity may be due to the immunostimulatory activity of the extract. Proteinuria is an indication of renal impairment as a result of urinary tract infection (Brooks *et al*., 2001). At the termination of the study the protein level in the urine of the two groups of rats was 100 and 30, respectively which is an indication of the suppressive activity on the pathogenesis of *Staphylococcus aureus* on the rats. Macrophages in the capillaries and vascular sinuses in spleen, liver, lung and bone marrow serve a very important role in clearing the blood stream of foreign particulate material such as bacteria. This might account for non observation of histopathological damage after administration of the extract. The leaves and stems bark of *Cassia alata* are used to treat skin diseases, eczema, boils and gastroenteritis (Pieme *et al*., 2006). All other parameters in the urine were normal after dosing with extract. The number of pus cells and bacteria cells from the urine microscopy as shown in Table 3 indicates that *Staphylococcus aureus* is pathogenic and infectious. This is because the pus and bacteria cells for rats orally infected with *Staphylococcus aureus* was in the range of 6-8 and 4-6, respectively while the control have a range of 0-1 and 0-1, respectively. A comparison of the pus cell and bacterial cell level of the groups of rat infected with *Staphylococcus aureus* and those administer with *Staphylococcus aureus* and dosed with the extract showed the anti infectivity of the extract and it may be due to the immunomodulatory activity of the extract The high pus cell during infection suggest that inflammation due to *Staphylococcus* is pyogenic. The administration of the extract lead to reduction in the pus cell. This may results from increase in activity of a number of enzymes leading to the generation of various oxygen and nitrogen intermediates like super oxide anion, hydrogen peroxide, singlet oxygen as well as hydroxyl radical which had been shown to be antimicrobial.

Table 3: Effect of ethanolic extract of *cassia alata* in the urine microscopy

Exptal units	Pus cell/HPF	Cast/HPF	Crystal/HPF	Bacterial cell/HPF
A	6-8	2-4	6-8	4-6
B	0-1	2-4	0-1	2-4
C	0-1	0-1	0-1	0-1
Control	0-1	0-1	0-1	0-1

A: Rats infected with *Staphylococcus aureus*, B: Rats infected with *Staphylococcus aureus* and at the same time treated with ethanolic extract of *Cassia alata*, C: Rats given ethanolic extract of *Cassia alata* only

Table 4: Average body weight of the rats during the bioassay

	Before	During	At termination
Exptal units	Weight (g)	Weight (g)	Weight (g)
A	175	150	182
B	170	165	160
C	140	150	170
Control	170	190	198

Rats infected with *Staphylococcus aureus*, B: Rats infected with *Staphylococcus aureus* treated with *Cassia alata* extract, C: Rats given ethanolic extract of *Cassia alata* only

It was observed from Table 4 that the body weight of the rat was significantly lowered during active infection in those rats infected with *Staphylococcus aureus* due to the diseased state of the rats, while those rats dosed with the extract of *Cassia alata* only showed an increased body weight. This is due to the healthy state of the rats as a result of the impressive immunomodulatory activity of *Cassia alata* which is a trend observed in the control rats too. This increase in weight can be attributed to elevated level of PCV (Table 1) in treated rats that permit transportation of enough nutrients by the red blood cell.

CONCLUSIONS

The ethanolic extract of *Cassia alata* had an immunomodulatory effect on the infected rats; which may be due to the presence of several compounds such as proteins, peptides, lipopolysaccharides, glycoproteins and lipid derivatives that have potent effects on the host immune system (Tzianabos, 2000).

Ethanolic extract of *Cassia alata* have the potential to intefere with the process of immune activation either by inhibiting or stimulating antibody production depending on its concentration.

REFERENCES

Atawodi, S.E. 2005. Antioxidant potential of African medicinal plant. Afri. J. Bioitech., 4: 128-133.

Brooks, G. F., J.S. Buteland S.A. morse, 2001. Medical Microbiology. 22nd Edn. Lange Medical Books/McGraw-Hill, New York ISBN: 0-8385-6298-1, pp: 609-610.

Dahanukar, R.A., N.N. Kulkarni and Rege, 2000. Pharma of medicinal plants and Natural products. Indian J. Pharmacol., 32: 81-118.

Guerra, R.N.M., L.M.S. Pereiraand R.S.G. Olea, 2003. Immunomodulatory properties of *Alternanthera tenella Colla* aqueous extracts in mice. Brazilian J. Med. and Biol. Res., 36: 1-7.

Manosroi, A. A. Saraphanchotiwitthaya and J. Manosroi, 2004. Immunomodulatory activities of fractions from hot aqeous extract of wood from *Clausena excavate*. Ffitoterapia, 75: 302-308.

Ogwumike, O.O, 2002. Haemopoietic effect of Aqeous extract of the leaf sheath of sorghum bicolor in albino rats. Afr. J. Biomed. Res., 5: 69-71.

Peime, C.A; V.M. Panlap and F.X. Etoa, 2006. Evaluation of acute and subacute extracts of leaves of *Cassia alata* (*cearalpinaceea*). Afr. J. Biotechnol., 5: 283-289.

Sofowora, A, 1993. Medicinal plants and traditional Medicine in Africa. 2nd Edn. Spectrum Books Limited, Ibadan.

Tzianabos, A.O, 2000. Polysaccharide munomodulators as therapeutic agents: Structural aspects and biologic functions. Clini. Microbiol. Rev., 13: 523-533.

Weir, D.M. and J. Stewart, 1999. Immunology. Churchill Livingstone. 8th Edn., pp: 362.