Research Journal of
Phytochemistry

ISSN 1819-3471

Academic
Journals Inc.

www.academicjournals.com

Research Journal of Phytochemistry 4 (4): 264-269, 2010
ISSN 1819-3471 / DOI: 10.3923/rjphyto.2007.68.73

Comparative Evaluation of the Effects of Leaf Extract from *Spondias mombin* on Rats with Induced Infections from *Bacillus cereus* and *Clostridium sporogenes* *

M.K. Oladunmoye
Department of Microbiology, School of Sciences, Federal University of Technology, P.M.B. 704, Akure, Nigeria

Abstract: Comparative evaluation of the effect of methanolic leaf extract from *Spondias mombin* was investigated in Swiss albino rats with induced multiple infections from *Bacillus subtilis* and *Clostridium sporogenes*. The rats were divided into four groups. The first group was given basal diet only. The second group was orogastrically dosed with standard inoculums of the test organisms. The third group was given standard inoculums of the organisms and treated with methanolic extract of *Spondias mombin*. The fourth group was given 10 mL of the extract only. Haematological analysis revealed an increase in total White Blood Count (WBC) values from 3200 to 3800 mm^3 during infection. The Pack Cell Volume (PCV) was found to be in the normal range before infection but fall bellow the minimum value during active infection and normalcy restored after treatment of the infection with the plant extract. However, the neutrophil count in the infected rat was higher (42%) than in the control (40%). The amount of monocyte and eosinophil was not significantly different before infection and during infectivity whereas there was increase in the amount of lymphocyte in the infection stage which later got reduced after treatment with the extract. The urinalysis showed that the rats had pH of 6, negative to glucose, ketone, nitrite, ascorbic acid, protein, bilirubin, blood and positive urobilinogen for all groups before infection. The control rats showed a pH of 9, negative to glucose, ascorbic acid, ketone, nitrite, protein, bilirubin, blood and normal urobilinogen which showed the same pattern to all groups before infection. The methanolic extract of *Spondias mombin* has effect on the hematological parameters of the rats resulting from possible *in vivo* antimicrobial activity on the pathogens and thus justifies its use in ethnomedicine.

Key words: *Spondias mombin*, haematological indices, urinalysis, ethnomedicine

INTRODUCTION

Spondias mombin is a plant used by Traditional Medical Practitioners in Nigeria for the treatment of various nervous disorders. All residues from extractions are usually dissolved in water and administered as decoction for patients with neuropathy. It was found that the extract abolished the aggressive attacks by rats and reduced swimming time in mice. These effects were found to be most potent with the administration of the ethanol extract (Lydiard, 2003).

A good proportion of the world population particularly those living in developing countries likes Nigeria depend mostly on herbal medicines for their health needs (Oladunmoye, 2006). Medicinal herbs are indispensable part of the traditional medicine practices all over the world due to easy access, low cost and ancestral experience. *Spondias mombin* is a fructiferous tree growing in the rain forest and in the coastal area of Africa. All parts of the tree are medicinally useful (Daniel, 1990). The fruits decoction is drunk as diuretic and febrifuge, the decoction of the bark and the leaves is used as an emetic, antidiarrhoea, dysentery recipe and for haemorrhoids as well as for gonorrhea and leucorrhoea. A tea of the flowers and the leaves is taken to relieve stomachache.

*Originally Published in Research Journal of Phytochemistry, 2007

Offiah and Anyanwu (1989) have reported the abortifacient activity of the aqeous extract.

Spondias mombin has been used in traditional medicine because of its antimicrobial properties of the plant extract which was found to contain a series of 6-alkenyl-salicyli acids. They were isolated from the ethanolic extract of leaves and stems of *Spondias mombin* by a combination of chromatographic methods. The composition of the plant extract contained tannins, anthraquinones, flavonoids, cardiac glycosides and saponins (Clawley, 1999).

Immunomodulators are compounds capable of interacting with the immune system to upregulate or downregulate specific aspects of the host response (Tzianabos, 2000). These immunomodulators can influence iunate 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 of 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 (Paul, 1993).

The use of *Spondias mombin* in ethnomedicine had been well documented (Daniel, 1990; Lydiard, 2003). Nevertheless, there have not been any report on the effect of the leaf extract from this plant on the haematological indices of rat with induced multiple infection from *Bacillus subtilis* and *Clostridium sporogenes*. The present study was designed to evaluate the effect of orogastric administration of methanolic leaf extracts from *Spondias mombin* on some blood parameters in Swiss Albino rats with induced multiple infections from two pathogenic bacteria in order to establish its possible in-vivo antimicrobial activity and thus provide a scientific basis for its use in ethnomedicine.

MATERIALS AND METHODS

Plant Sample: Source, Extraction and Fractionation

Spondias mombin leaves were collected in April 2006 from Igbara-Odo, Ekiti State Nigeria. Identification and authentication was done by Dr Oduluyi of Department of Crops, Soil and Pest Management, Federal University of Technology, Akure, Nigeria. Comparism was also made with herbarium specimen. The fresh leaves of *Spondias mombin* were collected, dried and extracted with 60% methanol. The extract was dissolved in 0.1 M Tris-HCL buffer (pH 7.0, 5 mL) and applied to a column (5×85 cm) of Sephacryl S-300 HR, pre-equilibrated and developed with the same buffer. Fractions corresponding to the peak were pooled together concentrated and freeze dried. The powder was dissolved in water and applied to a Sephadex G-25, column (1.5×50 cm), then eluted with water and fractions were collected. The eluate obtained was concentrated and lyophilized.

Experimental Animals

Albino rats were obtained from University of Ibadan, Nigeria. They were housed in standard enviroumental conditions, fed with rodent diet (Bendel feeds) and water *ad libitum*. They was acclimatized for few days for the study. The principle of laboratory animal care guidelines, institutional animal ethics and procedures were followed. Sixty rats were used during this investigation.

Microorganisms Used

The pure isolates of *Bacillus subtilis* and *Clostridium sporogenes* were obtained from the Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria. These pathogenic organisms were transferred periodically into fresh nutrient agar slant to maintain their viability till the need arises. The inoculum's size was adjusted to contain approximately 1.0×10^6 cfu mL^{-1} using Mac-Ferland turbidometry standard (Vogel, 2002).

Toxicological Assay

A discrete colony from both organisms was reconstituted by transferring into a fresh nutrient broth for feeding rats. Also 5 g of extract was dissolved into 20 mL of sterile distilled water. Animals of the first group (A) served as control and were given normal saline and basal diet only. The second group (B1) was given 10 mL of broth culture of *Bacillus subtilis* and basal diet. The animals in the third group (Biii) were given the basal diet, standard inoculums of *B. subtilis* and ethanolic extract from *Spondias monbim*. Group four (Ci) was given standard inoculum's of *Clostridium sporogenes*. Group Ciii was dosed orogastrically with the plant extract in addition to the standard inoculum's of *C. sporogenes*. The last group was fed with basal diet ant the plant extract ouly.

Urinalysis

Urine samples of each group of albino rats were collected in universal bottles Test strips of nine parameters (Combi 9) were used for rapid determination of blood, urobilinogen, bilirubin, protein, nitrite, ketones, ascorbic acid, glucose and pH value in urine. Qualitative and quantitative values of these parameters were obtained by comparing the changes on the strips with those on the standard.

Haematological Analysis

The total White Blood Count (WBC) was determined using standard method involving the use of haemocytometer (Monica, 2002). The WBC differential was estimated by staining a thin film of the blood on the slide with Leishmann stain an the amount of neutrophil, basophil, eosinophil, monocyte as a percentage recorded. Pack Cell Volume (PCV) was measured by drawing up to 75×1.5 m capillary tube for ¾ of its length well mixed blood. One end was selected with sealant and place in the haematocrit centrifuge. It was then centrifuge at 12,000 rpm for 5 min. The tube was then placed in the reader and the corresponding reading of the pack cell as a fraction of the total blood recorded. The reading was expressed as a percentage of packed red cells to total volume of blood.

RESULTS AND DISCUSSION

The effects of oral administration of ethanolic leaf extract from *Spondias mombin* on the urine composition before infection and during active infection is shown on Table 1 and 2, respectively. Most of the urinary compositions were affected as a result of the induced infection from the two bacteria species. This was reflected in their values before and during active infection. The most significantly affected parameters are pH, protein and bilirubin. Generally, there was reduction in pH when the rats were dosed with the bacteria and there was no significant change in their values even after the infected rats were treated with the plant extract. The reduction in pH may result from the infection interfering with the metabolic pathways leading to the production of biochemical intermediates or final products possessing different hydrogen ion concentrations. The amount of protein in the urine of the rat before and during infection does not follow a particular pattern. But it was observed that variation exist in the values and also the treatment of the infection with the plant extract also confer an overbearing effect on the quantity of protein detected in the urine. Bilirubin values were higher during infection in rats dosed with the bacteria than those that was not infected. This then suggest that the infection actually involve some vital organs of the rats responsible for metabolism of bilirubin precursor or their chemical intermediates (Vogel, 2002).

The effects of the oral administration of *Spondias monbim* leaf extract on the haematological parameters of the rat with induced infection from *Bacillus cereus* and *Clostridium sporogenes* before infection is presented on Table 3 while Table 4 is the values obtained during active infection. For all the groups, except Biii; there was significant increase in the amount of neutrophil during active infection compared with the corresponding values before infectivity. The observed increase may be

Table 1: Results of urine analysis before infection

Group of rat	A	B1	BIII	CI	C1II	D1
Blood	Ca50	Ca250	Ca5-10	Ca250	Ca5-10	Ca250
Urobilinogen	2	nor	2	Nor	2	2
Bilirubin	+++	+	+	++	+++	++
Protein	100	30	100	100	30	100
Nitrite	-Ve	+	-	N or	+	Nor
Ketones	-Ve	-	-Ve	+	+	Nor
Ascorbic acid	+Ve	-	++	++	+	-
Glucose mg dL^{-1}	-Ve	-	-	50	-	50
pH	9	6	7	6	9	7

A = Animal, feed with basal diet, Bi = Animal, feed with *Bacillus cereus* and basal diet, Biii = Animal, feed with *Bacillus cereus*, extract and basal diet, Ci = Animal, feed with *Clostridium sporogenes* and basal diet, Ciii = Animal, feed with *Clostridiums sporogenes*, extract and basal diet, Di = Animal, feed with basal diet and extract. + = Present; ++ = Present moderately; +++ = Present abundantly; - = Absent

Table 2: Results of urine analysis during infection

Group of rat	A	B1	B111	C_1	C_{III}	D_1
Blood	Ca5-10	Ca250	Ca250	Ca5-10	Ca250	Ca250
Urobilinogen	2	Nor	2	2	2	2
Bilirubin	+++	+++	+++	+++	+++	++
Protein	30	30	500	30	100	100
Nitrite	+	-	-	-	-	-
Ketones	+	-	-	+	+	-
Ascorbicacid	+	-	+	+	+	+
Glucose mg dL^{-1}	-ve	-	-	-	+	-
pH	9	6	8	6	7	7

Key: + = Present, ++ = Presentmoderately, +++ = Presentabundantly,- = Absent

Table 3: Haematological analysis before infection

Group of rat	Neutrophil	Lymphocyte	Monocyte	Eosinophil	Basophil	PCV	WBC
A	40	58	0	0	0	35	$3.8\times10^{9/l}$
Bi	44	56	0	0	0	34	$3.2\times10^{9/l}$
Biii	46	52	0	02	0	27	$4.6\times10^{9/l}$
Ci	34	64	0	02	0	36	$3.5\times10^{9/l}$
Ciii	50	48	0	02	0	36	$5.2\times10^{9/l}$
Di	34	62	0	04	0	37	$3.9\times10^{9/l}$

A = Animal, feed with basal diet, Bi = Animal, feed with *Bacillus cereus* and basal diet, Biii = Animal, feed with *Bacillus cereus*, extract and basal diet, Ci = Animal, feed with *Clostridium sporogenes* and basal diet, Ciii = Animal, feed with *Clostridiums sporogenes*, extract and basal diet, Di = Animal, feed with basal diet and extract

Table 4: Haematological analysis during active infection

Group of rat	Neutrophil	Lymphocyte	Monocyte	Eosinophil	Basophil	PCV	WBC
A	40	58	02	0	0	35	3.8×10^{9}
Bi	62	38	0	0	0	34	$3.810^{9/l}$
Biii	45	55	0	0	0	32	$3.1\times10^{9/l}$
Ci	72	28	0	0	0	34	$3.5\times10^{9/l}$
Ciii	74	26	0	0	0	30	$2.8\times10^{9/l}$
Di	58	42	0	0	0	32	$4.2\times10^{9/l}$

A = Animal, feed with basal diet, Bi = Animal, feed with *Bacillus cereus* and basal diet, Biii = Animal, feed with *Bacillus cereus*, extract and basal diet, Ci = Animal, feed with *Clostridium sporogenes* and basal diet, Ciii = Animal, feed with *Clostridiums sporogenes*, extract and basal diet, Di = Animal, feed with basal diet and extract

due to the ability of the bacteria during infection to elicit certain immune response. This may occur preparatory to intracellular destruction of the pathogens. Most of these immunological products particularly antibodies are found in the serum fraction of the blood (Weir and Stewart, 1999) .This may be one of the reasons for the increase in the number of neutrophil in this investigation. Neutrophil are known to be actively phagocytic (Tortora *et al*., 2002), contain digestive enzymes that degrade ingested pathogens and other foreign materials and thus serve as an important link between iunate and acquired immune mechanism (Prescott *et al*., 2002). Neutrophil has been shown to be able to achieve this

through increase in their activity to produce a number of enzymes that generate various reactive oxygen intermediates like super oxide anion and hydroxyl radical that degrade microorganisms (Weir and Stewart, 1999). This may also account for their increase during the active infection in the induced rat. The values in the infected rat that was treated with the plant extracts was however lowered which is an indication of reduction in the bacterial proliferation in the infected rats partly due to the antimicrobial activity and therapeutic effects of the extracts on the invading bacteria.

The values of the lymphocytes however reduces during active infection but the number increases after the infected rats were administered with the plant extract (Table 3 and 4). The explanation for this may be that lymphocyte which make up about 20% of the white blood cell are able to kill certain tumor and microorganisms infected cell (natural killing) and destroy cell coated with immunoglobulin (antibody dependent cell-mediated cytotoxicity). During this killing of foreign materials, some of the lymphocytes themselves will be eliminated (self destruction) during the process (Paul, 1993; Tortora *et al*., 2002).

The values obtained for basophils (0%) basophils (0-4%) before and during active infection were in the normal range. This can be explained in terms of the nature of the pathogenic organisms involved been bacteria and not parasite. It has been shown that eosiniphils which are polymorphonuclear leucocytes contains array of enzymes and toxic molecules that are active against parasitic worms like helminths. These large parasites can not be internalized by phagocytes and must therefore be killed extracellularly. The Bacillus and Clostridium used in this investigation are bacteria and not parasite and hence this may be responsible in the conservation of the values of these haematological parameters (Dhawanm and Dhawanm, 2003).

There was significant increase in Pack Cell Volume (PCV) of Biii group of animals before and after infection and reduction in Ci, Ciii and Di. The reduction may be due to possible haemolytic activity of the extract and the infection due to the organisms. Some plants extracts with proven antimicrobial activities in ethnomedicine has been shown to be haemolytic (Oladunmoye, 2006). Generally, the total WBC was found to reduce during infection suggesting that the infection is leucopaenia and not leucocytosis.

CONCLUSION AND RECOMMENDATION

The extract of the plant was found to significantly affect the haematological parameters of the animals after orogastric dosing with the two pathogenic bacteria. Urinary components were equally altered. This then suggest that the plant extract contain biomolecules of pharmacological importance and thus justifies its uses in traditional medicine.

Further research into structural elucidation of the pythoreactants in the extracts and histopathological studies of the vital organs is necessary for possible formulation into dosage and clinical trials.

ACKNOWLEDGMENT

The technical assistance of Mr. Erinle of the University health centre is appreciated for the haematological analysis.

REFERENCES

Clawley, J.N., 1999. Behavioral phenotyping of transgenic and knockout mice, experimental design and evaluation of general health, sensory function, motor abilities and specific behavioral test. Brain Res., 835: 18-26.

Daniel, K.A., 1990. Useful Plant of Ghana, Intermediate Tech. Pub. The Royal Botanic Garden, pp: 654.

Dhawanm, K. and S. Dhawan, 2003. Attenuation of benzodiazepine dependence in mice by a trisubstituted benzoflavone moiety of *Passiflora incavnata* Linneaus: A non-habit forming anxiolytic. J. Pharm. Sci. 6:215-222.

Lydiard, R.B., 2003. The role of GABA in anxiety disorders. J. Clin. Psychiatry, 64 (Sppl 3): 21-27.

Monica, C., 2002. District Laboratory Practice in Tropical Conntries: Part 2, Low Price Edition Cambridge University United Kingdom, pp: 20.

Offiah, V.N. and I.I. Anyanwu, 1989. Abortifacient activity of an aqeous extract of *Spondias mombin* leaves. J. Ethnopharmacol., 26: 317-320.

Oladunmoye, M.K., 2006. Effect of concentration on the rate of killing of some organisms and haemolytic activity of two varieties of *Acalypha wilkesiana*. Res. J. Microbiol., 1: 453-457.

Paul, W.E., 1993. Fundamental Immnnology. 3rd Edn. Raven Press, New York, pp: 701.

Prescott, L.M., J.P. Harley and D.A. Klein, 2002. Microbiology. 4th Edn. McGraw-Hill Inc., pp: 978.

Tortora, J.G., R.B. Funke and C.L. Case, 2002. Microbiology, an Introduction. 7th Edn., Benjamin Cumming Publishers, pp: 555.

Tzianabos, A.O., 2000. Polysaccharide Immnnomodu as Therapeutic Agents: Structural Aspects and Biologic Functions. Clin. Microbiol. Rev., 13: 523-533.

Vogel, H.G., 2002. Drug Discovery and Evaluation: Pharmacological Assays. Springer New York, pp: 401.

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