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Antiinflammatory Activity of Ethanolic Leaf Extract from *Carica papaya* in Rats Orogastrically Dosed with *Salmonella typhi* and *Staphylococcus aureus*

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Abstract: The anti-inflammatory activity of ethanolic extract of *Carica papaya* was investigated in Swiss Albino rats with induced multiple infections from *Staphylococcus aureus* and *Salmonella typhi*. The first group was given the standard inoculums of *Staphylococcus aureus* only while the second group was given the standard inoculum's of *S. aureus* and treated with ethanol extract of *Carica papaya*. The rats in the third group were given standard inoculums of *Salmonella typhi* only and those in the fourth category were given standard inoculums of *S. typhi* and treated with the extract. The Albino rat in the last group was placed on basal diet and water only to serve as the control. The anti-inflammatory activity of the extract as haematological indices was determined by monitoring the amount, occurrence and distributions of total and differential White Blood Count (WBC), haemoglobin level and Pack Cell Volume count (PCV) before and after infection with the pathogenic bacteria. The rats infected with *Staphylococcus aureus* without extract treatment gave a PCV value of 33%, a WBC of 3650 mm^3, an Hb value of 2.2×10^{12} L^{-1}, a neutrophil count of 60%, lymphocyte 40% and monocytes of 1%. The rats infected with *Staphylococcus aureus* and treated with *C. papaya* showed an increase in PCV count of 36%, the WBC increase to 4050 mm^3, an Hb count 2.4×10^{12} L^{-1} and increase in neutrophil to 74% and reduction in lymphocyte to 24% while the monocytes count was 2%. The rats infected with *S. typhi* only gave a PCV count of 24%, WBC 2050 mm^3, a RBC 1.0×10^{12} L^{-1}, a neutrophil value of 60%, lymphocyte 38% and monocytes count of 2%. The rats infected with *S. typhi* and treated showed an increase in PCV value to 38%, reduction in WBC to 4275 mm^3, an Hb count of 1.9×10^{12} L^{-1}, neutrophil 67%, a lymphocyte count of 30% and Eosiniphils 1%. The control group gave values that were within the acceptable limit. The urinalysis showed that the rats infected with *Staphylococcus aureus* only had a pH of 6, negative to glucose and nitrite, positive to ascorbic acid, ketone, bilirubin, blood (Ca250) and normal urobilinogen with 100 mg mL^{-1} of protein. The group infected with *Staphylococcus aureus* and treated with the extract showed an increase in pH to 7, negative to glucose, ascorbic acid, ketoses and nitrite, 30 mg mL^{-1} of protein, bilirubin, blood (Ca50) and urobilinogen were in the normal range. The animals infected with *Salmonella typhi* only showed a pH of 6, negative to glucose, bilirubin and blood (Ca50) and permissible level of urobilinogen. The rats infected with *Salmonella typhi* and treated with the extract showed reduction in pH to 5, negative to glucose, ketone, nitrite and blood, positive to ascorbic

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acid, protein, bilirubin and normal urobilinogen. The values of all the urinary compositions were normal except blood (Ca250) for the control. The results obtained justify the scientific bases for the use of the plant in ethnomedicine.

Key words: Anti-inflammatory, *Carica papaya*, haematological indices, urinalysis, ethnomedicine

INTRODUCTION

There is an ever growing interest in investigating difference species of plants to identify their potential therapeutic applications. This is due to a tremendous historical legacy in folk medicine use of plants as remedy for treating diseases (Steenkanp, 2003). The use of traditional medicine remain widespread in developing countries while the use of complementary medicine is increasing rapidly in developed countries (Oladunmoye, 2006). Their uses have a comparative advantage over the conventional chemotherapeutic agents for their easy availability, cost effectiveness, accessibility and presumed safely. In the past, scientific studies on plants used in ethnomedicine have led to the discovery of many valuable drugs such as pilocarpine and vincrinstine and others (Akinyemi *et al*., 2003).

Researches on the chemical and biological activities of plants during the past two centuries have yielded compounds for the development of modern drugs (Arivazhagan *et al*., 2000).

Despite little information on the composition and biological activity of many plants substances, there has been little effort developed to the development of chemotherapeutic and prophylactic agents from these plants (Boom, 1989). The use of medicinal plants all over the world predates the introduction of antibiotics and other modern drugs into Africa continent (Akinyemi *et al*., 2003; Oladunmoye, 2006).

Vertebrates are continually exposed to micro organisms and their metabolic products that can cause disease. The immune system is composed of widely distributed cells, tissues and organs that recognize foreign substances and microorganisms and act to destroy them. Immunity refers to the specific defensive response of a host to an invasion by foreign organisms (Weir and Stewart, 1999).

The plant *Carica papaya* belongs to the family Caricaceae. *C. papaya* biologically active compounds are chymopapain and papain which helps to aid digestion. Vitamins and traces of an alkaloid called carpain have also been found in the latex. Apart from natural oils, the seeds of the fruit also contain carbohydrates, carpasemine benzyl senevol and a glycoside (Arivazhagan *et al*., 2000). Other compounds found in the parts of *Carica papaya* are the Nicotine, tannins and Flavones (Atlas, 2004).

Carica papaya can be used as a diuretic, antihelmintic and to treat various disorders. Parts of the plant are also used to combat dyspepsia and other digestive disorders (Akah *et al*., 1997). It also has the ability to inhibit *Candida albcans*. The extracts have a bacteriostatic property against *Staphylococcus aureus*, *Salmonella typhi*, *Bacillus subtilis* and other bacteria. Furthermore, Alpha-D mannosidase and N-acetyl beta D glucosaminidase isolated from the latex acted synergistically to inhibit yeast growth. Its antiinfertility properly is well documented (Chinoy and Padman, 1996; Chinoy *et al*., 1997).

In vitro assessment of the antimicrobial activity of *C. papaya* have been reported (Akah *et al*., 1997); which was linked to the presence of certain biomocules like saponin, tannin, flavonoids and some essential oils (Arivazhagan *et al*., 2000). However, investigations regarding the anti-inflammatory activity of the plant were neglected or if available at all are largely unknown. The lack of scientific investigations in this area prompted the present study which was aimed at assessing the anti-inflammatory potentials of ethanolic leaf extract from *C. papaya* in animals with induce infections with *S. aureus* and *S. typhi* by monitoring certain haematological indices involved in inflammatory responses in animals and thus establish the *in vivo* antimicrobial activity of this plant.

MATERIALS AND METHODS

Plant Sample

Collection, Extraction and Fractionation

The *Carica papaya* plant was collected from the vicinity of Federal University of Technology, Akure in June 2004. It was identified by Dr. M.A. Awodun of Crop, Soil and Pest Management Department, Federal University of Technology, Akure, Nigeria. The leaves of *Carica papaya* were sun dried for six days and then blended with blender into powdery form. A 95% ethanol was the solvent for the extraction. The extract obtained was concentrated in rotary evaporator. 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.

Source of Microorganisms Used and Preparation of Standard Inoculums

The pure isolates of *Staphylococcus aureus* and *Salmonella typhi* used in this study were obtained from Microbiology Department, Obafemi Awolowo University, Ile-Ife, Nigeria. The isolates were maintained in pure culture on agar slants and store in refrigerator prior to use. Standard inoculums were prepared from the broth cultures of the organisms and adjusted to contain approximately 1.0×10^{6} cfu mL^{-1} using Mac- Ferland turbidometry standard.

Source of Laboratory Animals Used

Swiss albino rats were obtained from Pharmacy Department, University of Ibadan, Oyo state. Nigeria. The rats have average body weights between 110 and 200 g. The rats were given animal feeds (Bendel Feeds) and water *ad libitum*. They were housed in standard environmental conditions of temperature and humidity and a 12 h light and 12 h dark cycle. All international ethics and guidelines with respect to animal care in research were carefully followed and adhered to.

Animal Bioassay

The rats were divided into five groups of eight per treatment. Two groups were given the standard inoculums of *Staphylococcus aureus* and *Salmonella typhi* each. Two other groups were each treated with the ethanolic extract of *Carica papaya* after inoculation with the two pathogenic bacteria. The last set of animals was feed with basal diet and water only and serves as the control. Haematological test and urinalysis of the various treatments were carried out before and after infection.

Haematological Tests

Total and differential WBC, PCV and haemoglobin concentration were determined using standard methods with slight modifications. Total White blood count was estimated using the haemocytometer method. Packed cell volume measurements was carried out using the microhaematocrit technique by the use of a microhaematocrit centrifuge and spinning for 5 min at 1000 rev min^{-1} before reading with the haematocrit reader. Hemoglobin levels were measured colorimetrically by the oxyhaemoglobins methods using Reichert's haemoglobinometer while the differential is done by the use of Leishman's stain before viewing under the microscope (Baker *et al*., 2001).

Urinalysis

Urinalysis was carried out by the using standard method to determine blood, urobilinogen, bilirubin, protein, nitrite, ketone, ascorbic acid, glucose and pH in urine (Monica, 2002).

RESULTS AND DISCUSSION

The total and differential White Blood Counts (WBC), Packed Cell Volume (PCV) as well as the Hemoglobin concentration (Hb) were found to differ in animals that were administered with *C. papaya* extract after inoculation with the pathogenic bacteria and those that were not treated (Table 1). The ability of the extract to alter the distribution and occurrence of lymphocyte, neutrophil, eosiniphils, basophil and monocytes suggest the potentiality of the extract acting as an immunostimulant. The rats infected with *S. aureus* and not treated with extract (A) showed a lower WBC than the treated (B) and the control group (E). This is contrary to the expectation as more white cells were suppose to be produced during infection in readiness for phagocytosis. The unexpected result might be explain in terms of the physiology of *S. aureus* that are known to produce coagulase enzyme (Jawetz *et al*., 2004). The enzyme helps in blood clotting by converting soluble fibrin in the blood to insoluble fibrinogen. This might have form a protective coat around the cell of the bacterium and thus protects it from phagocytosis. However neutrophil count was higher and lymphocyte reduced in the animals that were infected but treated with extracts than the infected but without orally treated with the plant extract. The reduction may result from possible migration into tissue in response to the infection from the pathogenic organism. Neutrophil is the most abundant circulating granulocyte and their granules contain numerous microbicidal molecules and when a chemotactic factor is produced as a result of infection or injury, in an extracellular site, these cells enter the tissues Weir and Stewart, 1999). However the trend was reversed with lymphocytes that remain higher in untreated animals (A and C) than treated after infection with the pathogens (B and D). This can be explain in terms of lymphocyte remaining the only freely circulating granulocyte after migration of neutrophil into tissue during inflammation. The lower values in the treated animals are probably due to the *in vivo* antibacterial activity of the extract against the pathogen. This antagonistic property can be linked to the presence of certain biomolecules of pharmacological importance in the plant. Phenolic derivatives had been known to be potent antimicrobial agents (Oladunmoye, 2006). The data relating the amount of the various types of granulocytes obtained from rats with salmonella infection followed similar pattern with those of *S. aureus*. The same reason adduced to the former observations may also be responsible for these findings.

The haemoglobin level and PCV were higher in infected but treated groups of rats (Band D) than those infected without oral administration of extract from *Carica papaya* (A, C and E). Acute inflammation from most pathogenic microorganisms results in haemolysis which is manifested in lower haemoglobin level and PCV (Kumarnsit *et al*., 2006). The higher values of these haematological indices in rats treated with the extract after infection from the pathogens can be due to their inability to cause haemolysis resulting from the anti-inflammatory potentials inherent in the *C. papaya* extract.

The values showed that the protein level was abnormally high during infection with *S. aureus* but became normal after the infectivity stage (Table 2). The amount of blood was equally high. This might be that during infection with these organisms, there was inflammation of the excretory organs and

Table 1: Effect of ethanolic leaf extract of *Carica papaya* on some haematological parameters

Parameters	A	B	C	D	E
WBC (mm^3)	3650.0	4050.0	2050.0	4275.0	3850.0
Hb	2.2	2.4	1.0	1.9	1.3
PCV (%)	33.0	36.0	24.0	38.0	30.0
Neutrophil (%)	60.0	74.0	60.0	67.0	65.0
Lymphocyte (%)	40.0	24.0	38.0	30.0	34.0
Monocytes (%)	1.0	2.0	2.0	-	-
Eosiniphils (%)	-	-	-	1.0	1.0

A: Rats infected with Staphylococcus aureus; B: Rats infected with *Staphylococcus aureus* and treated with ethanolic extract of *Carica papaya*.; C:Rats infected with *Salmonella typhi*; D: Rats infected with *Salmonella typhi* and treated with ethanolic extract of *Carica papaya*; E: Control

Table 2: Effect of ethanolic extract of *Carica papaya* on the urine of rats during and after infection

Parameters	A	B	C	D	E
pH	6	7	6	5	6
Glucose	-	-	-	-	-
Ascorbic acid	+	-	+	+	-
Ketone	+	-	-	-	-
Nitrite	-	-	-	-	-
Protein	100	30	-	30	30
Bilirubin	++	+++	+	+	-
Urobilinogen	Norm	Norm	Norm	Norm	-
Blood	Ca250	Ca50	Ca50	Ca50	Ca250

A: Rats infected with *Staphylococcus aureus*; B: Rats infected with *Staphylococcus aureus* and treated with ethanolic extract of *Carica papaya*.; C: Rats infected with *Salmonella typhi*; D: Rats infected with *Salmonella typhi* and treated with ethanolic extract of *Carica papaya*; E: Control. +: Present, ++: Present moderately, +++: Present abundantly, - : Absent

this became manifested in the urinary composition. The values however became normal after treatment of the infected rats with the extract suggesting a possible killing of the pathogens and thus their inability to cause inflammation in the excretory organs. This may also be as a result of presence of phytoreactants in the plant (Trease and Evans, 2005).

RECOMMENDATIONS AND CONCLUSIONS

This research clearly show that extract of *C. papaya* has anti-inflammatory activity in rats orogastrically dosed with *S. aureus* and *S. typhi* as revealed by the distribution, occurrence and patterns of certain haematological indices produce during inflammatory process. This activity was also confirmed by the variation in composition of rats' urine during infection and after the infectios was treated with the plant extract. These findings thus provide scientific bases for the use of *C. papaya* in ethnomedicine.

Further research is necessary to establish the actual constituent(s) responsible for the anti-inflammatory activity. There is also the need for toxicological studies of the extract for possible histopathological damages to the vital organs using enzyme markers.

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