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Immunomodulatory Effects of Ethanolic Extract of *Tridax procumbens* on Swiss Alblno Rats Orogastrically Dosed with *Pseudomonas aeruginosa* (NCIB 950)

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Abstract: The immunomodulatory properties of ethanolic leave extract from *Tridax procumbens* was investigated in Swiss albino rat orogastrically dosed with *Pseudomonas aeruginosa*. The animals were divided into six groups of four per group. The first group was given the standard inoculum of *Pseoudomonas aeruginosa* only and the second group was given 8 mL of the standard inoculum of the organism and treated with ethanolic extract of *Tridax procumbens*. The third category was given the ethanolic extract of *Tridax procumbens* only while the last group was given normal saline. The uninfected rats showed a (WBC) values of 4,080 mm^3 which increased to 8,400 mm^3 during infection and later dropped to 3,700 mm^3 after infections was treated with the extract from *Tridax procubens*. The PCV was normal before infection, dropped during infection and increased after infection was treated with extract. The rats infected and treated with *Tridax procumben* showed a WBC, PCV, neutrophil lymphocyte and eosinophil count of 4,100 mm^3, 53, 46, 53 and 1%, before infection; a count of 4,600 mm^3, 29, 50, 50 and 0%, during infection and 3,400 mm^3, 42, 62 and 32%, respectively after infection. The rats given extract only showed a WBC, PCV, neutrophil, lymphocyte and eopsinophil counts of 5, 840 mm^3, 53, 51, 48 and 1%, before infection; a count of 5,400 mm^3, 31, 49 and 51% during infection and 4,000 mm^3, 36, 68 and 30%, respectively after infection. The control rat showed little or no increase in WBC, PCV, neutrophil, lymphocyte and eosinophil count. The urinalysis showed that the rats had pH of 6, Negative to glucose, ketone, nitrite, ascorbic acid, protein, bilirubin, blood and normal urobilinogen for all groups before infection. The control rats showed a pH of 6, negative to glucose, ascorbic acid, ketone, nitrite, protein, bilirubin, blood and normal urobilinogen which showed the same pattern with all groups before infection. The urine microscopy revealed large number of pus cells, casts, crystal and bacterial cells during infection in those infected with the *Pseudomonas aeruginosa* only. While infected - treated rats showed a reduced number of pus cells, casts, crystals and bacterial cells during infection. These results showed that ethanolic extract of *Tridax procumbens* has immunomodulatory properties and it is able to inhibit proliferation of *Pseudomonas aeruginosa*.

Key words: Immunmodulatory properties, ethanolic extracts *Tridax procumbens*, *Pseudomonas aeruginosa* (NCIB 950)

Introduction

The term immunomodulatory means regulation of the immune system by suppression and stimulation of the cells and organs of the immune system. (Bafua and Mishra, 2005). The past decade has witnessed a tremendous resurgence in the interest and use of medicinal plant products, especially in Nigeria. Surveys of plant medicinal usage by the American public have shown an increase from just about 3% of the population in 1991 to over 37% in 1998 (Brevoort, 1998).

The past decade has also witnessed intense interest in nutraceuticals (or functional foods) in which phytochemical constituents can have long-term health promoting or medicinal qualities.

For many of the medicinal plants of current interest, a primary focus of research to date has been in the areas of phytochemistry, pharmacognosy and horticulture. In the areas of phytochemistry, medicinal plants have been characterized for their possible bioactive compounds, which have been separated and subjected to detailed structural analysis.

Tridax procumbens Linn. (Compositae) is common grass found in tropical Southern part of Nigeria, growing primarily during raining season. The extracts of *Tridax procumbens* have been reported to have various pharmacological effects, antimicrobial activity against both gram-positive and gram-negative bacteria and stimulate wound healing (Taddel and Rosas, 2000). Flavones, glycoside, poly saccharide, monosaccharides and asteraceae have been isolated from the leaves of the plant (Ali *et al*., 2001).

Apart from being specifically stimulatory or suppressive, certain agent has been shown to possess activity to normalize or modulate physiological processes and is hence called immunomodulatory agents. The most important areas in which it has not been possible to have any breakthrough is development of adjuvant to be used in vaccination programs or immunosuppressant which can be safely used in organ transplant cases. These basic areas of immunomodulators are currently receiving inadequate attention. A large number of plant products are being investigated for immune response modifying activity. Use of plants as a source of immunomodulators is still in infancy in modern medicine (Bafna and Mishra, 2005).

Some of the plants with known immunomdulatory activity include; *Viscum album, Panax ginseng, Tinospora cardifolia, Asparagus racemosus* etc. Components such as polysaccharides, lectins, proteins and peptide present in plants have been shown to stimulate the immune system (Tzianabos, 2000; Bafna and Mishra, 2005).

In view of the dearth of information in the literature evaluating the immunomodulatory properties of *Tridax procumbens* extracts, the present study was undertaken to investigate the immunomodulatory effect of ethanolic extracts of leaves of *Tridax procumbens* on Albino rats dosed with *Pseudomonas aeruginosa.*

Materials and Methods

Source of Plant Sample, Extraction and Fractioation

The plant *Tridax procumbens* were collected from Ode-Irele, in Irele Local Government area of Ondo State Nigeria and were identified and authenticated by Mr Aduloju of Crop, Soil and Pest Management Department, Federal University of Technology Akure, Ondo State Nigeria.

The leaves of *Tridax procumbens* were air dried and then blended into powdery form. A 60% ethanol was the solvent used for extraction, in which 650 g of the powdered sample was weighed and poured into the solvent and left for 72 h. It was sieved using muslin cloth and concentrated in vacuum using rotary evaporator.

An aliquot of the crude extract was dissolved in 0.1M Tris-HCL buffer (pH 7.02 mL) and applied to a column (5×85 cm) of Sephacryl S-300HR, pre-equilibrated and developed with the same buffer. Fractions showing similar TLC characteristics 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. The eluate obtained was concentrated and lyophilized.

Source of Laboratory Animals and Organisms Used

Swiss albino rats were obtained from Pharmacy Department, Obafemi Awolowo, Ile-Ife. The rats have average weight between 120-250 g. The rats were separated into different sexes. They were fed with standard rodent diet and water *ad libitum*. The typed culture *Pseudomonas aeruginosa* (NCIB 950) was obtained from Microbiology Department of the same institution.

Evaluation of the Immunomodulatory Activity of Extract on the Experimental Animals

Thirty six albino rats were used to assess the effect of the plant extract on the immune system. The rats were divided into 6 groups of six rats per cage each. The first group was given normal saline (Placebo). Four groups were given the standard inoculums of the *Pseudomonas aeruginosa*, One was given booster shot of the standard inoculum 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 extract after 5 days. The last group was given extract only. At the onset of infection and during infection, the weight, haematological test and urinalysis were carried out to assess the lymphocytes produced and damage done to the internal organs. The last analysis was carried out after the rats were killed and checked for histopathological damages.

Haematological Test

For haematological test, blood samples were collected from the rats, which was used for White Blood Cell Count (WBC), Packed Cell Volume (PCV) and differential counts of the white blood cells.

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^{-1} fro 5 min. The supernatant was decanted and the sediment was dropped on the microscopic slide and covered with coverslip which was viewed under the microscope. (Ogwumike, 2002).

Results and Discussion

It was evident that *Tridax procumbens* has high Immunomodulatory activity in albino rat infected with *Pseudomouas aeruginosa* (Table 1). The WBC increased during infection with the pathogenic organism. This might result from production of more white blood cells to engulf the antigen (*Pseudomonas aeruginosa*). T. lymphocyte and other key cells of the immune system are known to activate production of antibody polymrphonuclear granulocte to destroy evading pathogen (Prescott, 1999). The value of the WBC dropped for the animals treated with the plant extract. The reason may be due to the antimicrobial activity of the Tridax extract on the organism which consequently suppress or modulate the immune system from producing antibody (Tzianabos, 2000). The packed cell volume reduce in rats dosed with *pseudomonas aeruginosa* suggesting that the infection is haemolytic The number of neutrophil increase significantly during infection towards point of termination of the albino rat from 50% to 58% to 62% in the infected rat and infected rat treated with the extract respectively. An absolute increase in lymphocytes had been found in bacterial infection (Monica, 2000).

Table 1: Effect of Ethanolic Extract of *Tridax procumbens* on immunological indices of rat dosed with *Pseudomonas aeruginosa*

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	4,080	52	1	47	52	8,400	38	2	50	48	3,700	40	-	58	40	2
B	4,100	53	1	46	53	4600	29	-	50	50	3,400	42	-	62	32	6
C	5,840	53	1	51	48	5400	31	-	49	51	4,000	36	-	68	30	2
Control	4000	57	1	48	51	5000	22	-	48	52	4,400	30	1	59	49	-

A: Rats infected with *Pseudomonas aeruginosa*, B: Rats infected with *Pseudomonas aeruginosa* at the same time given *Tridax procumbens*, C: Rats given ethanolic extract of *Tridax procumbens* only, WBC: White Blood Count, PCV: Packed Cell Volume, E: Eosinophil, N: Neutrophil, L: Lymphocyte, M: Monocyte

Table 2: Effect of Plant Extract on the Urine Macroscopy

	At infection onset			During infection			At termination			
Test	A	B	C	A	B	C	A	B	C	Control
pH	6	6	6	7	7	7	7	6	6	5
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	-ve
Nitrite	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve
Protein	-ve	-ve	-ve	-ve	-ve	-ve	500	-ve	-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	Ca10	-ve	Ca10	-ve	-ve	-ve

A: Rats infected with *Pseudomonas aeruginosa*, B: Rats infected with *Pseudomonas aeruginosa* plus extract of *Tridax procumbens*, C: Rats given ethanolic extract of Tridax procumbens

Table 3: Effect of ethanolic extract of *Tridax procumbens* as seen in the urine microscopy

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

A: Rats infected with *Pseudomonas aeruginosa*, B:Infected rats treated with ethanolic extract of *Tridax procumbens*, C:Rats given only the ethanolic extract of Tridax procumbens

Table 4: Average body weight of the rats

	Before	During	At termination point
	Weight (g)	Weight (g)	Weight (g)
A	180	175	180
B	190	192	195
C	160	165	168
Control	170	190	198

A: Rats infected with *Pseudomonas aeruginosa*, B: Rats infected with *Pseudomonas aeruginosa* and at the same time treated with ethanolic of *Tridax procumbens*, C: Rats given ethanolic extract of *Tridax procumbens* extract only

The effect of the extract and the *Pseudomonas* on the urine is shown on Table 2. The pH of the urine remains neutral in the infected rats. In a urinary infection, protein and nitrite are often found in the urine (Monica, 2000) and this might account for the observed trent in this study. At infection onset there is presence of nitrite in the urine of infected rat treated with the extract but absent in the urine at the termination. Urinary pathogens, e.g., *E. coli* (commonest cause of UTI), *Proteus* species and *Klebsiella* species, are able to reduce the nitrate normally present in urine to nitrite (Monica, 2000).

The number of protein at termination in rats infected with *Pseudomonas aeruginosa* is 500 which became negative after oral administration of extract.

The infected rats showed 6-8 HPF of pus cells (Table 3). The rat infected with *Pseudomonas aeruginosa* urine showed 4-6 HPF of bacterial cells at termination, indicating less or no resistance of the organism from the albino rat used in the experiment whereas the rats infected with *Pseudomonas aeruginosa* treated with the extract of *Tridax procumbens* shown 0-1 HPF as the control

Finally, the infected rats showed a body weight of 180 g at point of infection, 175 g during infection and 180 g after infection (Table 4).

Investigation revealed that there was reduction of body weight during active infection in those rats infected with *Pseudomonas aeruginosa* probably due to haemolysis. The reduction in PCV might have lower the amount of nutrient the red blood cell can circulate thus lowering the body weight. After administering of the Tridax extract, the increase in body weight may be as a result of elimination of the cause of anaemia in the rat and restoration of the RBC content. Some of the structural component of

the extract may be haemopoetic in agreement with report of Ogwumike (2000). However there was no observed damage to the internal organs of the animals during infectivity and after oral administration of the extract.

Conclusion

Tridax procumbens has stimulatory effect on humoral immunity of the swiss albino rats, stimulated phagocytosis and also offered protection against *Pseudomonas aeruginosa infections.*

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