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Anti-Inflammatory Activity of Ethanolic Leaf Extract from *Vernonia amygdalina* on the Immune System of Swiss Albino Rats Dosed with *Clostridium sporogenes (NCIB 532)*

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Abstract: The result presented in this research revealed the effect of oral administration of ethanolic leaf extract from *Vernonia amygdalina* as an anti-inflammatory as well as immunoregulatory agent in Swiss albino rats dosed with standard inoculums of *Clostridium sporogenes* (NCIB 532). Forty eight Swiss albino rats were used and divided into six groups. The first group was given normal saline, the second group was given 8 mL of the standard inoculum of the *Clostridium sporogenes* only and the third group was given the standard inoculum at the same time treated with the ethanolic leaf extract from *Vernonia amygdalina*. The other groups were given the booster inoculum of organism and booster shots after 3 and 5 days, respectively. Heamatological tests were carried out to determine the effect of the extract on the total white blood cell counts, packed cell volume, Neutrophil, Eosinophil and lymphocyte of the rats before, during and after infection. The result shows an increase in total WBC in the infected rat but the value reduces after treatment with Vernonia extract. Conversley, the PCV reduces in the in the untreated infected rat but the value became elevated after oral administration of the extract. Neutrophil values reduces during infection but the values became normal after treatment. Also urinalysis was carried out, both macroscopy and microscopy before, during and immediately after the infection in order to evaluate the immunomodulatory effects of the extract on the immune system of the infected rats. The rats were also weighed before, during and after infection, in which there was reduction in their weights during active infection in those that were dosed with the organism due to infection while those that were dosed with the *Clostridium sporogenes* and treated with leaf extract from *Vernonia amygdalina* and those given the extract only, showed an increase in their body weights respectively. This result showed that the ethanolic leaf extract from *Vernonia amygdalina* has immunomodulatory properties and it is able to inhibit proliferation of *Clostridium sporogenes.*

Key words: Anti-inflammatory, immunomodulatory, haematological indices, urinalysis, *Veronia amygdalina*

INTRODUCTION

Most developing countries are endowed with vast resources of medicinal and aromatic plants. Furthermore, these people are precluded from the luxury of access to modern therapy, mainly for economic reasons. The demands of the majority of the people in developing countries for medicinal plants have been met by indiscriminate harvesting of spontaneous flora including those in forests. As a result many plant species have become extinct and some are endangered. The pharmaceutical industry has come to consider traditional medicine as a source for identification of bio-active agents that can be used in preparation of synthetic medicine (Prescott *et al*., 2000; Wink, 1999).

The natural products industry in Europe and United states is equally interested in traditional medicine. In Europe and in America where the phytomedicine industry is thriving, extracts from medicinal plants are sold in a purified form for the treatment and prevention of all kinds of diseases. We are at a stage where traditional medicine is considered more for its capacity to generate other medicine than for its own sake. In many cases research undertakings and the commercial use stemming from that research have always relied on information provided by the local communities that, in many cases, have hardly benefited from the research results (Schulzi *et al*., 1998; Aranya *et al*., 2006).

Modern health care has never been and probably never will be adequately and equitably provided anywhere in Africa, due to financial limitations related to rapid population growth, political instability and poor economic performance, to mention only a few. Hence, the majority of people lack access to health care and even where it is available, the quality is largely below acceptable levels. This situation is further exacerbated by severe financial constraints, the high debt burden, instability, high inflation rates, declining real income and deterioration growth rates (Tyler, 1999).

Vernonia amygdalina is a shrub or small tree of 2-5m with petiolate leaf of about 6mm diameter and elliptic shape. The leaves are green with a characteristics odour and a bitter taste; no seeds are produced and have therefore to be distributed through cutting. It grows under a range of ecological zones in Africa and produces large mass of forage and drought tolerant (Bonsi *et al*., 1995). The bitter taste is due to anti-nutritional factors such as alkaloids, saponins, tannins and glycosides (Ologunde *et al*., 1999). These phytoconstituents have been reported to be responsible for *in-vitro* antimicrobial activity (Aranya *et al*., 2006).

Phytomedicine is the use of plants and plant materials for human and animal medicines. Plants have always been the sources of important medicines since time immemorial and there is the need for better understanding of the biological effects of medicinal plants *in-vivo* before formulation into dosage. These activities involve biological testing of plant extracts and the isolation of their active components and, ultimately, their clinical validations. This focus on well-defined plant extracts which are used for specific illness (Kaufman *et al*., 1999).

For many of the medicinal plants including *Veronia amygdalina,* current interest is primarily focused on phytochemistry; pharmacognosy, horticulture as well as characterization, separation and subjection of their possible bioactive compounds to detailed structural analysis (Brinskin, 2000). Nevertheless, there have not been information and data relating to immunoregulatory potential and gross pathological damage as a result of induced bacterial infection to vital organs of experimental animals. The present investigation is fashioned out to assess the anti-inflammatory and immunostimulatory property of *Vernonia amygdalina* extract in of rats orogastrically dosed with *Clostridium sporogenes* and examine possible pathological damage to the vital organs as a result of treating the infection with the extract arising from its toxicity.

MATERIALS AND METHODS

Plant sample: Collection, extraction and fractionation: Fresh leaves of *Vernonia amygdalina* were collected from Igbokoda in Ilaje local government area of Ondo State Nigeria and were identified by Mr. S.O. Aduloju of Crop, Soil and Pest Management Department, Federal University of Technology, Akure, Nigeria.

The dried leaves were extracted, using 60% ethanol and concentrated using rotary evaporator. The extract was dissolved in 0.1M 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 microorganism used: Pure isolate of *Clostrodium sporogenes* was obtained from Microbiology Department, Obafemi Awolowo University, Ile-Ife. Nigeria. The isolate was maintained in pure culture prior to day of use.

Source of laboratory animals (Albino rats): The albino rats (Swiss) were obtained from Pharmacy Department, Obafemi Awolowo University, Ile-Ife. The average weight of the rat is between 120-250 g. The rats are of different sexes which were separated accordingly. And were housed in standard environmental conditions and fed with standard rodent diet and water *ad libitum.*

***In-vivo* immunoassay:** The rats were divided into 6 groups of eight each in a cage. The first group was given normal saline (placebo). Four groups were given standard inoculum. Out of the 4 groups, one was given booster shot of the standard inoculum after 3 days. Also, 2 of the group 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 groups were given the extract only. At the onset of infection and during infection, the weight, Heamatological 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 according to international ethics and standard to check for gross histopathological damages.

Heamatological test: Blood samples were collected from the tails of 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 heamatocytometer. The packed cell volume was carried out using heamatocrit 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, Nitrate, 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} 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 (Ogwunmike, 2002).

RESULTS AND DISCUSSION

The result obtained from the blood analysis as an immunologic index is on Table 1. The table shows White Blood Count WBC (total and differential), Packed Cell Volume (PCV) on the animals infected with organisms, the ones treated and the one given only extract at infection, during infection and the termination of the investigation. There was increase in total WBC during the active infection stage and reduction in its values after treatment with extract suggesting an *in-vivo* antimicrobial activity against the pathogens. The increase during infection may arise from proliferation of lymphocytes required for phygocytosis of the foreign agent (Jawetz *et al*., 2004). The immuno-modulatory effect of the plant could be seen from many indices gotten from the result. Also, the packed cell volume reduced in rats infected with *Clostridium sporogenes* suggesting possible haemolytic activity. It has been reported that anaemia as a result of haemolysis has been associated with most microbial infections (Tortora *et al*., 2002). There PCV value became elevated after the infections was treated with the Vernonia extract. This might be that the extract had successfully inhibited the growth of the microbe involved in the haemolytic process thus increasing the oxyhaemoglobin values of the red blood cell.

The number of neutrophil reduces significantly during infection. This may be as a result of migration of neutrophil into tissue to destroy the invading pathogen. Similar report was observed by Oladunmoye (2006) while investigating the immunostimulatory activity of extract from *Ocimum gratissimum* in albino rat orogastrically dosed with *E.coli* observed a reduction in neutrophil level during infection which later increased after treatment with the extract. An absolute increase in neutrophil can be found in most bacterial infection (Ryan *et al*., 2004).

Examination of the effect of plant extract on the urine macroscopy showed that the pH of the urine remained neutral in the infected rats but the rats given *Vernonia amygdalina* ethanolic leaf extract only has an acidic pH (Table 2). In a urinary infection, protein and nitrite are often found in the urine (Brock and Madigan, 2000). Urinary pathogens, e.g. *E.coli* (commonest cause of UTI), *Proteus* sp. and *Klebsiella* sp. are able to reduce the nitrate normally present in urine to nitrite (Jawetz *et al*., 2004).

Protenuria is found in most bacteria urinary tract infections (Monica, 2000). The number of protein at termination in rats infected with *Clostridium sporogenes* is 100 while the protein of rats infected with *Clostridium sporogenes* treated with extracts was negative during active infection due to infection and immunomodulatory effects of the extract.

Table 1: Effect of plant extract on haematological indices of rat

	Before infection					During infection					After infection					
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	2.520	51	-	58	49	8.800	24	2	48	50	2.800	23	-	40	60	-
B	2.540	54	-	43	57	2.000	30	1	40	51	3.100	34	-	68	32	-
C	2.740	55	-	43	57	2.400	33	-	41	60	2.600	35	-	60	36	4
Control	4.000	57	1	48	51	5.000	22	-	48	52	4.400	30	1	59	40	-

Key: A: Rats infected with *Clostridium sporogenes*, B: Rats infected with *Clostridium sporogenes* given ethanolic leaf extract from *Vernonia amygdalina* C: Rats given ethanolic leaf extract from *Vernonia amygdalina* 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)

	Before			During			After			
	A	B	C	A	B	C	A	B	C	Control
pH	6	6	6	7	7	6	7	6	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	-ve
Nitrite	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve
Protein	-ve	-ve	-ve	-ve	-ve	-ve	100	-ve	-ve	-ve
Bilirubin	-ve	-ve	-ve	++	-ve	-ve	++	-ve	-ve	-ve
Urobilinogen	Norm	Norm	Norm	Norm	Norm	Norm	Norm	Norm	Norm	Norm
Blood	-ve	-ve	-ve	Ca250	Ca10	Ca50	Ca10	-ve	-ve	-ve

Key: A: Rats infected with *Clostrdium sporogenes*, B: Rats infected with *Clostrodium sporogenes* given ethanolic leaf extract from *Vernonia amygdalina*

Table 3: Effect of ethanolic extract of *Vernonia amygdalina* as seen in the urine microscopy

	PUS cell/HPF	Cast/HPF	Crystal/HPF	Bacteria cell/HPF
A	6-8	2-4	2-4	4-6
B	2-8	0-1	1	2-4
C	0-1	0-1	0-1	0-1
Control	0-1	0-1	0-1	0-1

Key: A: Rats infected with *Clostridium sporogenes*, B: Infected rats treated with ethanolic leaf extract from *Vernonia amygdalina*, C: Rats given ethanolic extract leaf extract from *Vernonia amygdalina* alone

Table 4: Average body weights of rats during the course of experiment

	Weight (g)		
	Before	During	After
A	220	200	215
B	150	145	147
C	172	175	180
Control	170	190	198

Key: A: Rats infected with *Clostridium sporogenes*, B: Rats infected with *Clostridium sporogenes* and treated with ethanolic extract of *Vernonia amygdalina*, C: Rats given ethanolic extract leaf from *Vernonia amygdalina* only

The effect of ethanolic leaf extract from *Vernonia amygdalina* as seen in the urine microscopy of the experimental rats at termination, the infected rats showed 6-8 HPF of pus cells due to active infection is shown on Table 3. The infected rats treated with ethanolic extract of *Vernonia amygdalina* showed 2-4 HPF due to suppression of infection by the extract used (Prescott *et al*., 2000). The rats infected with *Clostridium sporogenes* shown 2-4 HPF of cast in the urine compares to 0-1 HPF in rat infected with *Clostridium sporogens* treated with the extract. The infected rat also shown 2-4HPF of crystals compare to none HPF of crystal seen in the urine of infected rat treated with the extract, crystals are not expected in urine from normal individuals and the presence signifies renal abnormalities (Monica, 2000). However, there was no observed pathological damage to any of the vital organs.

The effect of the leaf extract from *Vernonia amygdalina* was observed on the body weight (Table 4). The infected rat showed a body weight of 220 g before infection and 215 g after infection. The treated rats showed an average weight of 145 and 160 g after infection.

Those given extract alone showed body weight of 172 g at the onset of the infection, 175 g during infection and 180 g after infection while the control group showed increase in the body weight from 170, 190 and 198 g before, during and at termination point respectively, due to no infection in control rats as well as immunomodulatory effects in those given extract only. The gain in body weight may be related to higher value of the PCV permitting large amount of nutrient to be circulated into the body system. The reduction in body weight during active infection may be due to suppression of immune system and possible haemolytic activity resulting in lowering of PCV leading to low oxyhamoglobin necessary for nutrient uptake.

CONCLUSION

The results presented in this investigation showed that the ethanolic leaf extract from *Vernonia amygdalina* has anti-inflammatory and immunomodulatory properties as seen in the humoral and cell mediated response of the treated albino rats. *Vernonia amygdalina* leaf extract has antimicrobial activity by inhibiting proliferation and thus lowers the total White Blood Count (WBC) which is involved in phygocytosis.

RECOMMENDATION

The use of *Amygdalina vernonia* in ethnomedicine should be encouraged owing to its immune enhancement potentials but with caution because of likely toxicity. Further research should be carried out to identify the particular phytoreactant that stimulate the immune system in order to eliminate the toxic moiety.

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