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Enhancement of the nonspecific immune system by extracts of *Asparagus africanus* and *Caesalpinia volkensii*

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ABSTRACT

Medicinal plants are continuously being investigated on their ability to enhance the immune functions. In this study, aqueous and hydro-alcoholic extracts of *Asparagus. africanus* root and *Caesalpinia. volkensii* leaves were evaluated on their ability to enhance some immune functions in Swiss mice and New Zealand rabbits. The extracts were administered subcutaneously. The extracts were observed to cause a rise in total WBCs counts as well increase the population of neutrophils in peripheral circulation. The extracts were also observed to decrease the time taken by the rabbit immune system to clear colloidal carbon from its bloodstream. These extracts are therefore effective in improving immune functions in laboratory animals. This may probably explain their use in traditional medicinal practices.

INTRODUCTION

Many medicinal uses of the various parts of the plants from the families Liliaceae and Caesalpinaceae have been reported in traditional folklore medicine. The most frequently cited uses are antibacterial, anti-malarial and anti-helminthic [1-9],[11]. A number of species from two genera namely *Caesalpinia* and *Asparagus* have been investigated. From the root of *Asparagus filicinus*, a folk medicine used in China for treatment of bronchitis, pneumonia and coughs, three saponins have been isolated [7]. A lignan compound with anti-leishmaniasis activity was isolated from *Asparagus racemosa*. The crude methanolic extracts of some members of the genera *Caesalpinia* and *Asparagus* including *A. africanus* were investigated and demonstrated to lack direct anti-microbial activities against test organisms [4]. These results were interesting and the extracts from these plants have been employed in the traditional methods of treatment to combat infections caused by some of the microbes that were under test. Studies by Chhabra and Uiso [4] therefore provided little support to the fact that beneficial effects claimed by patients utilizing these extracts could be due to their abilities to act directly on the pathogens. It is believed that most of these plants extracts act by enhancing the activity of the immune cells. In the present study, extracts of *A. africanus* and *C. volkensii* were evaluated on their ability to enhance the activities of the non specific immune cells *in vivo*. The ability of the extracts to increase the level of white blood cells in circulation and to enhance the phagocytic activities of macrophages in mice was investigated. It is known that these cells are important components of the non-specific immune system and play a leading role as the first line of the body's defense from invading pathogens.

MATERIAL AND METHODS

Colloidal carbon (Edward Gurr Ltd, London, S.W.14) used in the study was kindly donated by Dr. Kabaru of Zoology department, University of Nairobi.

Plant material

Leaves of *C. volkensii* were collected from Nyeri ,Nairobi, Kenya. Roots of *A. africanus* were collected from Limuru, Nairobi, Kenya. The plants were identified in ,Department of Botany, University of Nairobi and voucher specimens were deposited in the department herbarium. The plant materials were dried under shade at temperatures below 30° Celsius and pulverized in a hammer mill fitted with a sieve of 0.5 mm pore size.

Experimental animals:

Adult Swiss mice used in the study were obtained from the animal house, department of Zoology, University of Nairobi and from International Livestock Research Institute (ILRI). These were maintained on mice pelletsl and tap water *ad libitum*

New Zealand rabbits weighing 2.6Kgs to 3.2 Kgs used in the study were obtained from the animal house in the department of Zoology, University of Nairobi and fed on rabbit pellets and tap water *ad libitum*.

Extraction method

The dried powdered materials were extracted using 70% ethanol and water as the extracting solvents. 100g of plant powder was extracted by mixing with 600 ml 70% ethanol. The slurry of solvent and plant powder was stirred and left to stand for 12 hours after which the supernatant was decanted. The residue was then extracted three more times before being discarded. The decanted supernatants were filtered through Whatman ® GF/C glass microfibre filter paper.

For water extracts, 100g of plant powder was boiled for twenty minutes in 800 ml of distilled water. After cooling to room temperature, the supernatants were decanted, centrifuged at 5400× gravity for 10 minutes. The supernatants were then filtered through Whatman ® GF/C glass microfibre filter paper, frozen at -15° Celsius. All extracts were then concentrated to dryness using a Buchii rotary evaporator .

White blood cell counts in mice

Total WBCs counts in tail blood were determined by the method of Baker *et al*, 1996 using a Neubauer haemocytometer. The counts were done in triplicate and the standard error of the counts calculated. The results were calculated as white blood cells per cubic millimeter of blood. Differential white blood cell count was done as described by Tassos [15] .Briefly; thin blood smears were made and stained with Giemsa for 30 minutes. The stain was then flushed off with tap water and the slide left to dry. The cells were then observed under the microscope using oil immersion objective. 100 WBCs were identified, counted and recorded.

Injections of mice

Water and Ethanol extracts for injection in mice were prepared as follow; for water extracts, the test material was dissolved in physiological saline so that the final volume did not exceed 1ml. For ethanol extracts, the test material was first dissolved in 200µl of 70% ethanol. Physiological saline was then added such that the final volume of the solution did not exceed 1ml. For injections, 0.1ml from the solutions prepared were used. In one experiment, a total of 30 Swiss mice were used. These were divided into 6 groups of 5 individual each. Total WBCs was performed for all groups. One group was then designated as dry control and was not manipulated in any way. Another group designated as wet control was injected with 0.1ml solution prepared by mixing 200µl of 70% ethanol and 800µl of physiological saline. The other groups were treated with water and ethanol extracts of *A. africanus* and *C. volkensii*. All injections were given as single doses subcutaneously. On the fourth day, another WBCs counts were determined following which the animals were injected subcutaneously with colloidal carbon. Another WBCs count was done nine days later.

Treatment of the rabbits:

12 New Zealand rabbits were divided into 6 groups of 2 individual each. Two groups served as control. One control group injected with physiological saline alone served as control for the water extract treatment group. The other control group injected with 1ml of a solution prepared by mixing 300µl of 70% ethanol and physiological saline served as control for the ethanol treatment group. Treated groups were injected intraperitoneally at the following dosages, water extracts of *A africanus* (AAH) 1614ppm, *A. africanus* ethanol extract (AAE) 549ppm, *C. volkensii* leaves water (CVH) 16,123ppm and ethanol (CVE) 924ppm.The extracts were administered as single doses.

Carbon clearance test

Colloidal carbon was injected on the fourth day after extract treatment. The rate of clearance of intravenously injected colloidal carbon was then determined according to the method described by Kelly *et al* [10] with modification. Colloidal carbon (16mg/100g of the body weight) suspended in 1ml physiological saline was injected intravenously into the marginal ear vein of rabbits [8]. Blood samples of 0.1 ml in triplicates were then taken from one of the ear vessels not used for injection. This was done approximately every 5 minutes for 30 minutes. The blood samples contained in centrifuge tubes were diluted with 1ml of 1.75N NaoH to hydrolyze proteins. These were then left to stand in a hot water bath at approximately 96° Celsius for 30 minutes. They were then centrifuged at 5400× g for 20 minutes. The supernatants were decanted and discarded. The residue of colloidal carbon was resuspended in physiological saline by thoroughly mixing. The concentration of colloidal carbon was measured as OD at 610nm on a standard spectrophotometer. The disappearance rate was determined from a plot of OD of the concentration as a function of time. The results were analyzed by ANOVA.

RESULTS

18g of dry extracts was isolated from 100g of *A. africanus* roots using water as the extracting solvent while 31g of material was isolated using ethanol as the extracting solvent. It was only possible to extract 6g of dry material from 100g of *C. volkensii* leaves using both water and ethanol as extracting solvents.

The results of water and ethanol extracts of *A. africanus* and *C. volkensii* effect on WBCs counts in mice are presented on Table 1. The results presented are mean counts and their standard error. Results in Table 1 was obtained from the experiment in which the animals were first treated with extracts and later on given colloidal carbon injections with WBCs counts being conducted at various stages before the treatments. From the table, it can be seen that following injections of the animals with water and ethanol extracts of *A. africanus* roots and *C. volkensii* leaves, there was a marked rise in the number of WBCs in circulation. The increase was statistically significant (ANOVA, F2, 10(1) =33.9, $P \leq 0.0001$). There was no significant differences between the extracts in there ability to raise WBCs counts (Bonferroni/ Dunn, $P \geq 0.0033$). When the extracts treated animals were injected with colloidal carbon and WBCs count done nine days later, it was apparent that there was a general decrease in the number of circulating WBCs. This decrease was also found to be statistically significant (ANOVA, F2, 10 (1) =33.9, $P \leq 0.0001$. This decrease in the circulating number of WBCs was within the normal range, not resulting in leucopenia.

Results on the differential WBCs before and after treatment of animals with the various water and ethanol extracts of *A. africanus* roots and *C. volkensii* leaves are represented on table 1 and 2.These results shows that there was an increase in the percentage of neutrophils as compared to the other subpopulations of the WBCs.

The results of the effect of water and ethanol extract of *A. africanus* and *C. volkensii* leaves on the rate of carbon clearance in rabbits is presented in Table 2. Results are presented as the mean of the optical densities and standard error for the various treatment groups. Each bar represents two separate *in vivo* experiments. From the figure, it is apparent that the rate of carbon clearance was significantly improved with time in all groups

Table 1. Differential white blood cell counts in mice before treatment with water and ethanol extracts of *A. africanus* roots and *C. volkensii* leaves.

Group of animals	Populations of white blood cells (%)				
	Neutrophils	lymphocytes	Eosinophils	Monocytes	Basophils
Control	50.6 ± 2.07	36.6 ± 2.19	5.8 ± 0.45	5.4 ± 0.89	1.6 ± 0.89
CVH	51 ± 3.39	37.2 ± 1.92	5.2 ± 0.84	5 ± 1.22	1.6 ± 1.14
AAE	53.4 ± 3.50	35.4 ± 2.88	5.4 ± 0.89	5 ± 1.22	0.8 ± 0.84
CVE	51 ± 3.4	37.4 ± 3.21	5.4 ± 0.54	5 ± 1.22	1 ± 1

Table 1. Differential white blood cell counts in mice before treatment with water and ethanol extracts of *A. africanus* roots and *C. volkensii* leaves.

Group of animals	Population of white blood cell.				
	Neutrophils	Lymphocytes	Eosinophils	Monocytes	Basophils
Control	54.2 ± 3.96	33 ± 3.08	5.2 ± 1.09	5.8 ± 0.45	1.8 ± 1.30
CVH	91.8 ± 2.28	4.6±1.67	1.4 ± 1.14	1.6 ± 0.89	0.6 ± 0.89
AAH	88.4 ± 5.46	5.4 ± 2.61	2 ± 1.22	3.4 ± 1.82	0.8 ± 0.84
AAE	91.6 ± 2.41	5.2 ± 2.59	1.2 ± 0.84	2 ± 1.22	0.2 ± 0.45
CVE	86.8 ± 3.4	5.8 ± 1.64	2.8 ± 1.92	3.6 ± 1.52	1 ± 1

(ANOVA, F5, 25 (1) = 2262.9, P≤ 0.00001). Treatment with the extracts had the effect of enhancing the rate of carbon clearance from the bloodstream of the rabbits. This enhancement was found to be statistically significant (ANOVA, F5, 25 (1) =154.6, P≤ 0.00001). Five minutes after giving the colloidal carbon injection, clearance from the blood was low for all groups of animals but by thirty minutes, most of the colloidal carbon had been cleared. This was more so for the extract treated group in comparison to control groups.

DISCUSSION

The present findings shows that extract of *A. africanus* roots and *C. volkensii* leaves are effective in raising WBCs counts. This may provide an insight into possible mechanism of their actions. It could be possible that the extracts have a way of stimulating the body's natural defense by enhancing the efficiency of white blood cells especially the macrophages and neutrophils. These cells are the body's first defense against invading bacteria and other pathogens. They destroy the invading organisms before the body produces antibodies against the foreign particles. It is possible that the extracts contain substances that could be working by mimicking bacterial attack. It is known that when a bacterium comes into contact with a white blood cell, it stimulates an antenna-like molecule found on the cell's surface known as the B glucan receptor. This in turn triggers a series of biochemical messages which increase the antibacterial activity of the WBCs, at the site of infection as well as attracting more macrophages and neutrophils to the area and in some instances increasing the number of WBCs [19]. Whether the plant extracts contain immunogens that could elicit the above kind of reaction or it contains immunostimulators that would enhance production of WBCs by the spleen, bone marrow, lymphoid tissues or thymus is a matter of further research. Indeed Bray *et al* (2) have suggested that most plant extracts do not exert their activities via direct action on the pathogens. They are known to act by modulating the immune function. A number of plants extracts have been reported to posses immunostimulating activities. *A. racemosa* was reported to significantly inhibit ochratoxin induced suppression of chemotactic activity of macrophages and also enhance production of the cytokine interleukin-1 (IL-1) and tumor necrosis factor alpha (TNF-α) by macrophages in mice [6].These molecules are known to be immunoregulators [5], [13]. A number of compounds have also been shown to be capable of increasing WBCs in circulation [9], [12] and enhancing phagocytosis [17-19].

The carbon clearance test measured the ability of the plant extracts to stimulate the peritoneal macrophages. It can be seen that following treatment of rabbits with single doses of the plant extracts, there was enhancement of clearance of intravenously injected colloidal carbon from the bloodstream of the rabbits. All the extract treated rabbits showed rapid clearance of colloidal carbon as compared to the control groups. This was a sure indication that there was definite stimulation of the peritoneal macrophages and probably other phagocytic cells. These results are not surprising as a number of substances have been reported to enhance the rate of clearance of intravenously injected colloidal carbon from the bloodstream of laboratory rodents when they are administered prior to the test. These substances include calcium ions [1] glucan, zymosans and bacterial endotoxin [3], [10]. A number of compounds have been shown to enhance the phagocytic activity of phagocytes both *in vivo* and *in vitro*. These include polysaccharides and saponins isolated from plants [17-19]. It is possible that the extracts from these plant materials contained similar substances. These given intraperitoneally elicited stimulation of the peritoneal macrophages. It is therefore possible to speculate on the mode of action of these plant extracts. The compounds present in them are capable of stimulating phagocytic cells of body such as the Kupffer cells of the liver and splenic macrophages. Such stimulation would often be accompanied by an increase in the rapidity with which colloids are cleared from the bloodstream [3]. This is easy to understand when the number of phagocytic cells is increased or it could be possible that the phagocytes may have increased surface activity which would be manifested by more or longer processes.

At this point in time, we can only make intelligent guess at what really happens *in vivo* when the extracts are administered. The elucidation of the exact mechanism is a subject of further research. Also the extent to which polymorphonuclear neutrophils contributes to this enhancement is also a subject of further research as it is believed that most colloidal particles are cleared from circulation by the cells of the macrophage population located in the liver and spleen [3].

CONCLUSIONS

The present study has revealed that the extracts of *A. africanus* and *C. volkensii* have the capacity to stimulate the cells of nonspecific immune system and this may well explain their therapeutic effectiveness in the traditional medicinal practices where they are used in the treatment of infectious diseases.

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REFERENCES

1. Bolis, L; Luly, P and Braun W.) Enhancement of phagocytic activity by elevated Ca^2 levels and its relation to antibody formation. Proceedings of the Society for experimental Biology and Medicine.1971 89:577-579.
2. Bray, D.H., Warhurst, D.C., Connolly, J.D., O'Neill, M.J. and Phillipson, J.D. Plants as sources of Antimalarial drugs.Part 7. Activity of some species of Meliaceae plants and their constituent limonoids. Phytother. Res. 1990:41(1):29-35.
3. Carr, I.A. The macrophage: A Review of ultra structure and function. 1973 Academic
4. Chhabra, S.C. and Uiso, F.C. Antibacterial activity of herbs used in traditional medicine in Eastern Tanzania for treating gastrointestinal diseases. International Journal of BioChemiPhysics 1995:4 (1 and 2):24-28.
5. Curtis, B.M., Widerner, M.B., DeRoos, P. and Qwarnstrom, E.E. IL-1 and its receptor are translocated to the nucleus. Journal of Immunology 1990: 144(4):1295-1303
6. Dhuley, J.N. Effects of some Indian herbs on macrophage functions in ochratoxin A treated mice. Journal of Ethnopharmacology 1997:58 (1): 15-20.
7. Ding Y, Yang CR Yao Xue Xue Bao. 1990: 25(7):509-14. Chinese.
8. Gordon. L.E, Cooper, D.B and Miller, C.P. (1955) Clearance of Bacteria from the blood of irradiated rabbits. Proceedings of the Society for experimental Biology and Medicine.1955: 89: 577-579.
9. Iwu, M.M. Handbook of African medicinal plants. CRC Press Inc, USA.
10. Kelly,L.S, Dobson, E.L, Finney, C.R and Hirsch, J.D (1960) Proliferation of the reticuloendothelial system in the liver. American Journal of Physiology. 1960:198(5):1134-1138
11. Kokwaro, J.O. Medicinal plants of East Africa. African Literature Bureau, Nairobi, Kenya.1976
12. Peigen, X. and Keji, C. Recent Advances in Clinical Studies of Chinese Medicinal Herbs: Clinical Trials of Chinese Herbs in a Number of Chronic Conditions. Phytother. Res. 1988:2 (2):5562.
13. Roitt, I., Brostoff, J. and Male, D. Immunology. Barcelona, Spain: Times Mirror International Publisher Limited press. New York 1988
14. Tampion, J. Dangerous plants. David and Charles Publishers Ltd, Canada.1977
15. Tassos, P. F Experimental physiology and biology. Educators Publishing Services, Inc. Cambridge Massachusetts.1967: Pp 43-46.
16. Valigra, L. Engineering the future of antibiotics. New Scientist 1994: p. 25-27.
17. Varljen, J., Liptaka, A. and Wagner, H. Structural analysis of Rhamno- arabinogalactan and Arabinogalactans with immunostimulating activity from Calendula officinalis. Phytother Res. 1989:28 (9): 2379-2383.
18. Vollmar Angelika, Schafer, W. and Wagner, H. Immunologically active polysaccharides of Eupatorium cannabinum and Eupatorium perfoliatum. Phytochemistry 1986:25: 377-381.
19. Wagner, H., Ludwig, C., Grotjahn, L. and Khan, S.Y. Biologically active saponins from Dodonae viscosa. Phytochemistry 1987:26 (3): 697-701.