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EFFECT OF *ALLIUM CEPA* AND *ALLIUM SATIVUM* ON SOME IMMUNOLOGICAL CELLS IN RATS.

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Abstract

Extracts of some spices have been reported to play a contributory role in enhancing immune function. We evaluated and compared the effect(s) of single and combined oral administration of fresh aqueous onion (*Allium cepa*) and garlic (*Allium sativum*) extracts at different concentrations on some immunological determinants in rats. CD_4 cells of the rats were estimated using Partec flow cytometric technique, while total and differential white blood cell (WBC) counts were estimated using the Sysmsex® automated haematology analyzing technique. Our findings revealed that, CD4 and total WBC counts were significantly increased ($P \leq 0.05$) in a dose-dependent manner in both onion (250mg/Kg/d: 349±11cell/ul and 2.75±0.15X10^3cell/l; 500mg/Kg/d: 389±10cells/µl and 3.05±0.05 X10^3cell/l; 750mg/Kg/d: 600±11cell/µl and 3.25±0.05X10^3cells/l) and garlic (250mg/Kg/d: 410±10cell/ul and 2.85±0.15X10^3cell/l; 500mg/Kg/d: 494±32cells/µl and 3.30±0.10 X10^3cell/l; 750mg/Kg/d: 684±11cell/µl and 3.55±0.05X10^3cells/l) treated rats when compared to the zero control (200±11cells/µl and 1.55±0.05X10^3cells/l, respectively). Extract of garlic at 750mg/Kg/d had significantly increased the CD4 cells and total white cell count when compared to other concentrations ($P \leq 0.05$). However, no significant effect was observed on these parameters when extracts were combined (250mg/Kg/d: 252±21cell/µl and 1.80±0.10X10^3cells/l; 500mg/Kg/d: 315±21cells/ul and 2.10±0.10X10^3cells/l; 750mg/Kg/d: 368±10cells/µl and 2.35±0.05X10^3cells/l, respectively), the differential WBC count showed a significant increase in the proportion of cell types (lymphocytes, neutophils and monocytes) ($P \leq 0.05$). The results from this study revealed the immune boosting capabilities of *Allium cepa* and *Allium sativum*, but underscored their synergistic activities.

Key words: *Allium cepa* and *Allium sativum*, immunological cells, rats

Introduction

The human immune system has a central role in protecting against various external disease-promoting factors and perhaps against malignant cells. The immune system regulates itself by means of helper and suppressor cells and soluble products. Nutrients and other constituents of spices have the potential to affect almost all aspect of the immune system (Kandil et al, 1987), the relationship between the immune system and nutrients found in spices has been reviewed comprehensively, which shows that spices play a contributory role in enhancing immune function (Kandil et al, 1987).

Among the most effective medicinal spices are garlic and onion. They are both members of the genus *Allium*, belonging to the family Alliaceae (Augusti, 1996). Garlic's high vitamin-C content and antimicrobial properties make it a significant and potent immune-system booster and it is very effective against bacterial, viral, fungal and parasitic infection (Kyo et al., 2001). There is a growing body of evidence that garlic possesses important immune enhancing and anticancer properties (Fleischauer et al., 2000). While most of the work has been conducted on animals or *in vitro*, the human studies that have been conducted are encouraging. According to Stall and Seebeck (1951), preliminary studies in humans, using an alliin standardized garlic powder preparation, have demonstrated positive effects on immunoreactions and phagocytosis. Allicin has a number of beneficial properties, which could act together to enhance the body's response to disease. Kandil et al. (1987) have found that allicin: enhances the activity of phagocytic cells and natural killer cells whilst it inhibits the growth of pathogenic micro-organisms and certain cancer cells. Several garlic components have displayed significant immune enhancing as well as anticancer effects (Abdullah et al., 1989; You, 1989; Dorant et al., 1993).

Like it counterpart, onion has been shown to contain 25 active compounds and is packed with similar therapeutic properties (Chisty et al., 1996). Although thought to be less active than garlic, Several scientific studies have shown that including onion in the diet: stimulate the immune system (Chisty et al., 1996), reduce symptoms associated with diabetes mellitus, inhibit platelet aggregation (i.e involved in thrombosis), prevent inflammatory processes associated with asthma, was associated with a reduced risk of stomach and brain cancer in humans, inhibited platelet-mediated thrombosis (a process leading to heart attacks and strokes), reduces levels of cholesterol, triglycerides, and thromboxanes (substances involved in the development of cardiovascular disease) in the blood, was associated with a reduction in symptoms associated with osteoporosis, inhibit the proliferation of cultured ovarian, breast, and colon cancer cells (Gazzani et al., 1998; Sanderson et al., 1999; Shimura et al., 1999).

The aim of this study is to evaluate and compare the effect(s) of single and combined oral administration of fresh aqueous onion (*Allium cepa*) and garlic (*Allium sativum*) extracts at different concentrations on some immunological determinants in rats.

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Materials and Methods

Study design

This study was carried out at Veterinary Research Institute, Vom, Plateau state. Vom, a quiet rocky village in Plateau State, is situated 1,285 metres above sea level. Largely because of its altitude and constant winds, Vow has a remarkably cool climate. In December and January, the nights may be extremely cold. The wet season extends from late April to middle October. A total of 48 male Wistar rats weighing 100g–170g were purchased from the Small Animal Breeding House, National Veterinary Research Institute, Vom, Plateau state, Nigeria and randomly assigned into 12 groups (n = 4), and treated for 28 days and allowed to recovered for another 28 days. All studies on animal experimentation were conducted in accordance with the Current Animal Care Regulations and Standards approved by the Institute for Laboratory Animal Research (ILAR, 1996).

Collection of plant samples

Cloves of *Allium sativum* (Garlic) and bulbs of *Allium cepa* (Onion) were purchased from the Vegetable market in Benin City, Edo state.

Preparation of crude aqueous extracts

The light scaly leaves on the fresh garlic and onion were peeled using knife and thoroughly washed both in tap and sterile distilled water and then sliced into tiny pieces using knife. About 60 g of the washed garlic and onion were weighed using Mettler weighing balance and homogenised separately in a clean electric blender containing 80ml sterile distilled water according to the method described by Sofowora (1982). These gave 75.0% concentration (using 7.5g/10ml). The homogenates were shaken for 1 hr in a rotary flask and then filtered into separate sterile containers using a funnel containing sterile cotton wood and later with Whatman No. 1 filter paper. The liquid filtrates were transferred into separate sterile MacCartney bottles and stored in the refrigerator after daily administration to the experimental animals.

Preparation of dilution of different extracts

After preparation of the crude extracts as described; additional concentrations (500mg/ml and 250mg/ml) were made from the stock (750mg/ml) with sterile distilled water aseptically.

Housing and feeding

Housing and feeding of the rats were done as described by ILAR (1996). The rats were housed in the Animal House, College of Health Sciences, Igbinedion University Okada separately in well ventilated wire-bottom steel cages under hygienic conditions, with proper aeration at 25 ± 2°C, and a relatively humidity of 45 – 50%. They were fed on pelletisized rat diet (10g/100g body weight) twice daily and tap water *ad libitum*. The rats were allowed to stabilize in the Animal House with standard 12-hour light-dark cycle, for a period of 4 weeks, before the commencement of the study. The animals were weighed weekly during the period of stabilization and net weight gained were noticed and recorded.

Identification of experimental animals

The rats were marked by ear puncturing system for identification purpose.

Experimental pharmacological protocol

The forty-eight (48) male Wistar albino strain rats, weighing 100-170g used in this study were randomly assigned into twelve (12) groups of four rats each (n=4) and treated as Showed in Table 3.1

Table 1: showing the various treatments

Groups	Treatments
G1	Zero Control Group given sterile distilled water daily.
G2	Positive Control Group given Vitamin C in a dose of 20mg/Kg/d
G3	Negative Control Group given Prednisolone in a dose of 1mg/Kg/d.
G4	Treated Group given aqueous extract of onion in a dose of 250mg/kg/d.
G5	Treated Group given aqueous extract of onion in a dose of 500mg/kg/d.
G6	Treated Group given aqueous extract of onion in a dose of 750mg/kg/d.
G7	Treated Group given aqueous extract of garlic in adose of 250mg/kg/d.
G8	Treated Group given aqueous extract of garlic in a dose of 500mg/kg/d.
G9	Treated Group given aqueous extract of garlic in a dose of 750mg/kg/d.
G10	Treated Group given combined aqueous extract of onion and garlic (50:50) in a dose of 250 mg/kg/d.
G11	Treated Group given combined aqueous extract of onion and garlic (50:50) in a dose of 500 mg/kg/d.

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G12	Treated Group given combined aqueous extract of onion and garlic (50:50) in a dose of 750 mg/kg/d.

The volume of extract (2ml/100g body weight) to be administered to individual rat in each group was calculated, recorded and adjusted daily with changes in body weight throughout the treatment phase (28 days). Oral gavage was employed in the administration of fluids directly into the lower esophagus or stomach of experimental animal using a stainless steel ball tip-feeding needle introduced into the mouth and threaded down the eosophagus as described by ACF (2000).

Weight measurement of experimental animals

Body weights of the animals (control and treated groups) were measured daily pre-treatment, throughout the treatment and recovery period with the aid of Electronic sensitive analytical balance.

Post treatment euthanasia of animals

The animals were placed on eighteen (18) hrs fasting after the last administration and on the 29th day, half of the animals from each group designated as (A_1, B_1, C_1, D_1, E_1, F_1, G_1, H_1, I_1, J_1, K_1 and L_1) were sacrificed by cervical dislocation as described by Ochei and Kolhatkar (2006).

Blood specimen collection

Cardiac blood specimen was taken from each rat by terminal bleeding from the heart. The dead rat was placed on its back on a cork board and strap with two adhesive tapes across the forelegs and hind legs, 40 X 0.8mm needle was inserted in the centre line at the tip of the sternum and pushed forward at an angle of 45^{o}C till it punctured the heart. The needle was advanced until the blood started flowing into the syringe. After collecting 2.5ml cardiac blood specimen, the needle was quickly withdrawn and the blood was transferred into clean container with anticoagulant (EDTA) ready for immunological and haematological investigations.

CD_4 Cell Count

CD_4 cell count was estimated using Partec Cyflow Counter, Germany as described by PCC (2006).

Total and Differential White Blood Cell Counts

Total and differential White blood cell counts were estimated using the Sysmsex® Automated Haematology Analyzer KX-21N, Sysmex Corporation (Kobe-Japan). It employs WBC detector block and WBC/HGB lyse reagent (i.e Stromatolyser-WH) to measure WBC count (i.e both total & differential), using the DC detection method as described by the manufacturer.

Post-recovery euthanasia of animals

To assess whether changes in immunological cell concentrations were reversible following discontinuation of treatment(s), the second half of the animals from each group were left to recover and same procedures as mentioned above were repeated with the remaining half of the animals denoted as (A_2, B_2, C_2, D_2, E_2, F_2, G_2, H_2, I_2, J_2, K_2 and L_2) after twenty-eight (28) days of recovery period (i.e., after total of 56 days).

Statistical analysis

All numerical results were collated from the twelve (12) groups (control and treated). Data are presented as mean±SEM and analysed using one way analysis of variance (ANOVA) and Tukey-Kramer Multiple Comparisons Test using SPSS-18.0. P-values ≤ 0.05 were considered significant.

Results

Our findings revealed that, CD4 and total WBC counts were significantly increased ($P \leq 0.05$) in a dose-dependent manner in both onion (250mg/Kg/d: 349±11cell/ul and 2.75±0.15X10^{3}cell/l; 500mg/Kg/d: 389±10cells/µl and 3.05±0.05 X10^{3}cell/l; 750mg/Kg/d: 600±11cell/µl and 3.25±0.05X10^{3}cells/l) and garlic (250mg/Kg/d: 410±10cell/ul and 2.85±0.15X10^{3}cell/l; 500mg/Kg/d: 494±32cells/µl and 3.30±0.10 X10^{3}cell/l; 750mg/Kg/d: 684±11cell/µl and 3.55±0.05X10^{3}cells/l) treated rats when compared to the zero control (200±11cells/µl and 1.55±0.05X10^{3}cells/l, respectively). Extract of garlic at 750mg/Kg/d had significantly increased the CD4 cells and total white cell count when compared to other concentrations ($P \leq 0.05$). However, no significant effect was observed on these parameters when extracts were combined (250mg/Kg/d: 252±21cell/µl and 1.80±0.10X10^{3}cells/l; 500mg/Kg/d: 315±21cells/µl and 2.10±0.10X10^{3}cells/l; 750mg/Kg/d: 368±10cells/µl and 2.35±0.05X10^{3}cells/l, respectively), the differential WBC count showed a significant increase in the proportion of lymphocytes, neutophils, monocytes ($P \leq 0.05$) (Table 2).

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Our findings also revealed that, the post recovery mean CD4cell count, total WBC count and differential of these cells showed a significant decreased values when compared to post treatment values ($P \leq 0.05$) (Table 3).

Table 2: Post-treatment mean comparison of the effects of the single and combined administration of onion and garlic extracts on some Immunological determinants.

TREATMENTS	$\bar{X}$±SEM CD_4 CELL COUNT (cell/µL)	$\bar{X}$±SEM TOTAL WBC COUNT (-- X 10^3 cell/µL)	$\bar{X}$±SEM LYM (-- X 10^3 cell/µL)	$\bar{X}$± SEM NEUT (-- X 10^3 cell/µL)	$\bar{X}$± SEM MXD (-- X 10^3 cell/µL)
ZERO CONTROL	200±11	1.55±0.05	0.79±0.09	0.25±0.01	0.06±0.01
POSITIVE CONTROL	579±11	3.45±0.05	2.75±0.05	0.92±0.02	0.28±0.02
NEGATIVE CONTROL	14±2	0.01±0.00	0.07±0.01	0.00±0.00	0.00±0.00
250mg/Kg/d ONION EXTRACT	349±11	2.75±0.15	1.65±0.05	0.61±0.01	0.17±0.04
500mg/Kg/d ONION EXTRACT	389±10	3.05±0.05	2.01±11	0.71±0.02	0.21±0.03
750mg/Kg/d ONION EXTRACT	600±11	3.25±0.05	2.71±0.08	0.95±0.03	0.29±0.03
250mg/Kg/d GARLIC EXTRACT	410±10	2.85±0.15	1.95±0.05	0.61±0.02	0.20±0.02
500mg/Kg/d GARLIC EXTRACT	494±32	3.30±0.10	2.35±0.15	0.82±0.02	0.26±0.02
750mg/Kg/d GARLIC EXTRACT	684±11	3.55±0.05	3.35±0.03	1.25±0.02	0.32±0.02
250mg/Kg/d COMBINED EXTRACTS	252±21	1.80±0.10	1.15±0.10	0.32±0.03	0.12±0.00
500mg/Kg/d COMBINED EXTRACTS	315±21	2.10±0.10	1.50±0.10	0.42±0.05	0.16±0.01
750mg/Kg/d COMBINED EXTRACTS	368±10	2.35±0.05	1.75±0.05	0.53±0.02	0.19±0.04

Keys: Keys: $\bar{X}$: Mean, SEM: Standard Error of Mean, LYM: Lymphocyte, NEUT: Neutophils, EOS: Eosinophils, MXD, Monocytes.

Table 3: Post-recovery mean comparison of the effects of the single and combined administration of onion and garlic extracts on some Immunological determinants.

Keys: $\bar{X}$: Mean, SEM: Standard Error of Mean, LYM: Lymphocyte, NEUT: Neutophils, EOS: Eosinophils, MXD, Monocytes.

TREATMENTS	$\bar{X}$±SEM CD_4 CELL COUNT (cell/µL)	$\bar{X}$±SEM TOTAL WBC COUNT (-- X 10^3 cell/µL)	$\bar{X}$±SEM LYM# (-- X 10^3 cell/µL)	$\bar{X}$± SEM NEUT# (-- X 10^3 cell/µL)	$\bar{X}$± SEM MXD# (-- X 10^3 cell/µL)
ZERO CONTROL	221±11	1.75±0.05	0.85±0.04	0.18±0.02	0.06±0.01
POSITIVE CONTROL	242±11	1.45±0.05	1.15±0.05	0.23±0.02	0.04±0.01
NEGATIVE CONTROL	200±12	2.70±0.20	0.95±0.05	0.30±0.02	0.09±0.01
250mg/Kg/d ONION EXTRACT	243±10	1.55±0.05	1.15±0.05	0.19±0.03	0.05±0.01
500mg/Kg/d ONION EXTRACT	262±11	1.85±0.15	1.25±0.05	0.24±0.02	0.06±0.01
750mg/Kg/d ONION EXTRACT	305±11	1.95±0.15	1.47±0.16	0.43±0.02	0.07±0.02
250mg/Kg/d GARLIC EXTRACT	210±3	1.75±0.05	1.00±0.00	0.21±0.02	0.06±0.01
500mg/Kg/d GARLIC EXTRACT	263±11	2.05±0.05	1.25±0.05	0.27±0.01	0.07±0.01
750mg/Kg/d GARLIC EXTRACT	357±21	2.35±0.05	1.25±0.10	0.59±0.04	0.08±0.01
250mg/Kg/d COMBINED EXTRACTS	190±22	1.45±0.05	0.85±0.05	0.17±0.02	0.04±0.01
500mg/Kg/d COMBINED EXTRACTS	223±7	1.65±0.05	1.05±0.05	0.22±0.01	0.05±0.01
750mg/Kg/d COMBINED EXTRACTS	282±8	2.15±0.05	1.55±0.05	0.32±0.02	0.07±0.00

Discussion and Conclusion

The medical importance of extracts of *A.cepa* and *A.sativum* have been well documented (Kandil et al., 1987; Kyo et al., 2001). Our study further strengthened it by showing that there seemed to be a strong connection between extracts of

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these plants and immune system. Our study revealed a significant increase in the mean CD4 and other immunological cells studied of *A. cepa* and *A. sativum*- treated rats. The post-recovery count of these parameters was also significantly decreased when compared to zero control group values. The appreciable increase in the count of the immunological cellls investigated may be associated with the inherent-immuno-stimulating properties that these extracts possess.

Although there seems to be limited information regarding the effects of these extracts on CD_4 cell count in animal model, in this present study, CD_4 cell count was observed to be significantly increased in all the treated groups in a dose-dependent manner when compared to the zero control, suggesting the immune-stimulating properties of these extracts. This finding was in agreement with earlier works of (Kandil et al., 1987; Abdullah et al., 1989; Lau et al., 1991; Tang et al., 1997), all showed that treatment with garlic extract improve the activation of T-lymphocytes and natural killer cells particularly in Acquired Immunodeficiency Syndrome (AIDS) patients. Sumiyoshi (1997) also reported that garlic extract stimulates immune functions. Cell mediated immune response of T cells and proliferative responses of natural killer (NK) cells to antigens and mitogens have been found to be enhanced by members of the family *Alliacea* (Kandil et al., 1987).

Furthermore, in this study, significant increase total white blood cells count was observed in the treated groups with *A. cepa* and *A sativum* as compared to the zero control, Leucocytes were mostly affected in treated rats; further reaffirming the immune-stimulating properties of these extracts. This finding corroborates with that of Iranloye (2002) who observed a significant increase in total white blood cells count in rats fed with *A. cepa* for 30 days. However, Micheal et al (2009) reported a significant decrease in the immune cells of rats treated with *S. cepa*. Although their work focused on rats challenged with *Klebsiella pneumonia,* it was hoped that the presence of the bacterial cells and treatment of the rats with *S. cepa* would have enhanced the production of these cells. What still remains unclear is how long the infection lasted before the assays were carried out. At higher concentrations, extracts of fresh garlic, were shown to be more effective (as an immune booster) than that of onion; this also support the findings of who reported a higher efficacy of garlic against onion (Kandil et al., 1987; Iranloye 2002). It worthy to note that the effects of these spices on immune response were dose-dependent as higher concentrations yielded higher effect, suggesting that there may be correlation between immune responses and dosage. This work agreed with the findings of Banejere et al.,(2002) on dose-dependent relationship between chronic administration of garlic and induction of endogenous antioxidants in rat heart.

The combine effect of the two extracts as shown in our study was not really beneficial as immune measures were significantly reduced compared to the single outcome of the individual extract. The inherent immune-stimulating property of garlic was observed to be best attained when used alone, and efficacy was observed to have decrease when used in combination with onion. This, therefore, may suggest that there may be a partial antagonistic relationship between the extracts of the fresh garlic and onion. Garlic has been reported to interfere with the action of some herbal remedies in boosting host immunity (Zlotogorski and Littner, 2004).

In conclusion, this study revealed that aside many other medicinal properties, the extracts of fresh *Allium sativum,* and *Allium cepa* and combined have immuno-stimulating properties when administered singly. The extracts of garlic especially at higher concentration showed more immune-stimulating effects than that of the onion.

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