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Impact of Garlic (Allium Sativum) On Wister Rat Infected With Stapylococcus Aureus (Atcc 25923)

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ABSTRACT
Medicinal plants are rich sources of substances which are non-specific immunomodulation of essentially granulocytes, macrophages, natural killer cells and complement functions (Kandil, Abdellah, and Elkadi, 1987). Because of the concerns about the side effect of conventional drugs, the use of natural products as an alternative to conventional treatment in healing and treatment of various diseases has been on the rise in the last few decades (Fong, 2002). This present study was carried out to investigate the immunological impact of garlic in Wistar rats infected with Staphylococcus aureus. In this study, a total of fifteen (15) Male Adult Wistar rats weighing between 200 -250 g were used for this study. The animals were assigned into three (3) groups containing ten (10) rats in each group i.e. Group A: Control Rats plus Staphhylococcus aureus(Staph. Aureus), Group B: Garlic fed Rats plus Staph. aureuswhile Group C: Normal Control Rats. Group B were fed with 0.25g/kg body weight i.e 0.05g (50mg) to 200g body weight of Wister rat and 0.063g (63mg) to 250 body weight of Wister rat daily for the first 7 days of experimental period (Measured cloves were crushed in 1ml distilled water in order to minimize volatile compound loss). A suspension of the organisms to be tested was prepared to equal the turbidity of 0.5 McFarland Standard ($1x10^8$ colony forming unit (CFU) per ml) each. Group A and B were infected with microorganism by Intravenous challenge of 1ml of the suspension of Staphylococcus aureus (ATCC 25923). Animals in Group C were given 1ml of distilled water and serve as negative control. Group A served as positive controls.Group B were continuously fed with 0.25g/kg body weight i.e 0.05g (50mg) to 200g body weight of Wister rat and 0.063g (63mg) to 250 body weight of Wister rat daily for 7 days (Measured cloves were crushed in 1ml distilled water in order to minimize volatile compound loss) after Rats were Intravenously challenged with 1ml of the suspension of Staphylococcus aureus (ATCC 25923). When animals started to show signs of infection (weakness and loss of appetite), animals were anesthetized using Chloroform 24 hours after the last administration of the extracts, and adequate blood was drawn from the rats through the Cardiac route with the aid of Needle and Syringe. 3mls was dispensed into EDTA bottle to prevent coagulation. 2ml of Blood was immediately inoculated into Blood Culture Bottles (Brain Heart Infusion Broth).The phytochemicals of Allium sativum was carried out. Total, differential white blood cell (WBC) counts and packed cell volume were estimated using the Sysmsex® Automated Haematology analyzing technique.Our findings revealed that, total WBC counts, Neutrophil, Monocyte, Eosinophil and Basophile were significantly increased ($P \leq 0.05$) in rats infected with Staphylococcus aureus and treated with garlic (6.14±0.44, 49.02±20.41, 3.88±0.11, 4.72±0.35 & 1.94±0.21) when compared to the Control rats (5.08±0.12, 45.76±0.2, 1.50±0.19, 3.50±0.28, & 0.45±0.22 respectively), while no significant decrease ($P > 0.05$) in Lymphocyte and Packed Cell Volume (49.88±4.45 &41.80±1.79) when compared with the control (53.28±0.31 &43.32±0.89 respectively). The results from this study revealed the immune boosting capabilities of and Allium sativum in fighting infection caused by Staphylococcus aureus.

I. INTRODUCTION

The immune system is involved in the etiology, as well as pathophysiology mechanism of many diseases (Anarthe, Sandhya-Rani & Ganga-Raju, 2014). The human immune system has a central role in protecting against various external

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disease-promoting factors and perhaps against malignant cells. The immune system regulates itself by means of helper and suppressor cells and soluble products (Tatfeng & Samson, 2012).Modulation of immune responses to alleviate various diseases has been of interest for many years (Anarthe et al., 2014). Medicinal plants are rich sources of substances which are non-specific immunomodulation of essentially granulocytes macrophages, natural killer cells and complement functions (Kandil, Abdellah, & Elkadi, 1987). Because of the concerns about the side effect of conventional drugs, the use of natural products as an alternative to conventional treatment in healing and treatment of various diseases has been on the rise in the last few decades (Fong, 2002).

The development of medicinal plants in the domain of nutrition has unveiled their additional therapeutic potentials (Ramaa, Shirode, Mundada, & Kadam, 2006). In the same era, terms like functional foods, nutraceutical and pharma foods have taken hold of the nutrition market, mainly aiming to provide nutrition and healthy diets (Bar-Chen, Golan, Peri, Ludmer, & Schwartz, 2010). Many plants and their components have been extensively investigated for their health-promoting benefits, including antioxidant, cardio-protective, anti-cancer, antimicrobial and immune-modulatory activities (Huffman, 2003; Miller, Liebowitz & Newby, 2004; Hamlaoui-Gasmi et al., 2012).

Plants of the genus Allium are known for their production of organosulfur compounds, which possess interesting biological and pharmacological properties. Among these, garlic (Allium sativum) is one of the most widely used ones (Salman, Bergman, Bessler, Punsky & Djaldetti, 1999; Reinhart, Talati, White, & Coleman, 2009). Garlic (Allium sativum) is a perennial plant in the family Alliaceae, a member of the same group of plants as the onions (Damir & Davor, 2004). The plant has many local names like Ayo in Igbo, Ayuu in Yoruba, and Tafarnuwa in Hausa. Garlic has attracted particular attention of modern medicine because of its widespread health use around the world, and the cherished belief that it helps in maintaining good health, warding off illnesses and providing more vigour (Olaniyan et al., 2013).

Garlic has been used as medicinal herb since time immemorial in almost every known civilisation (Rivlin, 2001). The exclusive flavor and immune–boosting function of garlic is generally attributed to its rich content of sulphur containing substances, alliin, γ–glutamylcysteine, and their derivatives (Amagase, Petesch, Matsuura, Kasuga and Itakura, 2001; Tsai, Chen, Sheen and Lii, 2012) which are converted into thiosulphinates through enzymatic reactions when raw garlic is processed (Amagase, 2006). Numerous studies determined garlic and its bioactive sulphur compounds to be potent antioxidants by displaying radical–scavenging activity and modulating cellular antioxidant enzyme activity (Tsai et al., 2012).

Garlic has been reported to have immune raising activities that involve promotion of lymphocyte formation, cytokines release, phagocytosis and natural killer cells activity (Kyo et al., 1998). Other properties of garlic are antibacterial and antifungal (Ankri & Mirelman, 1999; Sivam, 2001), antiparasitic, anthelmintic (Worku, Franco & Baldwin, 2009), antiviral, antithrombotic, vasodilatory and anticancer (Agarwal, 1996; Tsai et al., 2012).

Preparations of garlic are mainly liquid (aqueous, oil, or solvent extracts) or solid (dried garlic powder and fresh cataplasm).These extractions can be based on water formulations, oils or by using solvents as alcohols (Amagase et al., 2001). Composition of the extracts depends on the source of the garlic strain, age, storage conditions, and type of processing, and the effects of the extracts are influenced by the method of consumption (Sivam, 2001).

II. MATERIALS AND METHODS

RESEARCH DESIGN: This study is an experimental research study.

DURATION OF STUDY: The study was conducted within a period of six (6) months.

ETHICAL CONSIDERATION: Ethical approval was sought from the Ethical Committee of Ambrose Alli University, Ekpoma, Edo State, Nigeria. Fresh garlic (Allium Sativum) was obtained from Ekpoma market, Edo State, Nigeria. It was botanically identified and authenticated in the Department of the Botany, Ambrose Alli University, Ekpoma.

SAMPLE SIZE: The sample size determined determined in accordance with the internationally accepted principles for laboratory animal use and care as found in Natural Research Council (1996).

INCLUSION CRITERIA

- Apparently healthy Wister Rats were used for the study.
- Weight of the Rats used was within 150-200g.
- Male Wister Rats were used for the study.

EXCLUSION CRITERIA

- Unhealthy Wister Rats was not used for the study.

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- Rats not within the weight range were not used.

EXPERIMENTAL ANIMALS: A total of fifteen (15) Male Adult Wistar rats weighing between 200 -250 g were obtained for this study. The animals were assigned into five (3) groups containing five (5) rats in each group i.e. Group A: Control Rats plus Staph, Group B: Garlic fed Rats plus Staph, Group C: Normal Control Rats. The animals were housed and bred in the well-ventilated wooden cages with metal wiring. They were allowed to acclimatize for two (2) weeks of acclimatization. The animals would be fed once daily with commercially formulated rat feed and water would be given ad libitum. The research was conducted in accordance with the internationally accepted principles for laboratory animal use and care as found in Natural Research Council (1996).

ALLIUM SATIVUM ADMINISTRATION & MICROORGANISM ADMINISTRATION.: Group A & B were fed with 0.25g/kg body weight i.e 0.05g (50mg) to 200g body weight of Wister rat and 0.063g (63mg) to 250 body weight of Wister rat daily for the first 7 days of experimental period (Measured cloves were crushed in 1ml distilled water in order to minimize volatile compound loss). A suspension of the organisms to be tested was prepared to equal the turbidity of 0.5 McFarland Standard ($1x10^8$ colony forming unit (CFU) per ml) each. Group A and B were infected with microorganism by Intravenous challenge of 1ml of the suspension of Staphylococcus aureus (ATCC 25923). Animals in Group C were given 1ml of distilled water and serve as negative control. Group A served as positive controls.

Group B were continuously fed with 0.25g/kg body weight i.e 0.05g (50mg) to 200g body weight of Wister rat and 0.063g (63mg) to 250 body weight of Wister rat daily for 7 days (Measured cloves were crushed in 1ml distilled water in order to minimize volatile compound loss) after Rats were Intravenously challenged with 1ml of the suspension of Staphylococcus aureus (ATCC 25923).

ANIMAL SACRIFICE AND SAMPLE COLLECTION: When animals show signs of infection (weakness and loss of appetite), animals were anesthetized using Chloroform 24 hours after the last administration of the extracts, and adequate blood was drawn from the rats through the Cardiac route with the aid of Needle and Syringe. 3mls was dispensed into EDTA bottle to prevent coagulation. 2ml of Blood was immediately inoculated into Blood Culture Bottles (Brain Heart Infusion Broth).

ANALYTICAL PROCEDURE: The samples was cultured for infecting microorganism (via Blood Culture) and determination of haematological parameters was carried out. The total and differential White Blood Cell counts and Packed Cell Volume, were determined as described by Dacie & Lewis (1991).

STATISTICAL ANALYSIS: Data obtained was analyzed using SPSS version 20 statistical software package. Results generated was expressed as mean ± SD and a P-value of <0.05 was considered significant. Independent samples t-test (2-tailed) was used to compare means of different parameters between the two groups. The significance difference among the groups was assessed by Analysis of variance (ANOVA).

RESULTS

Table 1 shows Effect of Alium sativum on Staphylococcus aureus in Blood of infected Wister rats. It showed that there was no growth in blood culture of rats infected with Staphylococcus aureus with Allium sativum administered.

Table 2 shows effects of Allium sativum On Wister Rats Infected with Staphylococcus aureus (Staph. aureus).The total White Blood Cell count, Neutrophil, Monocyte, Eosinophil and Basophil of rats infected with Staphylococcus aureus without administration of Allium sativum showed an increase in value of the various mean as compared to the control. While Lymphocyte and Packed Cell volume showed a decrease in value compared to the control.In rats infected with Staphylococcus aureus with administration of Allium sativum there was increase in Total White Blood Cell count, Neutrophil, Monocyte & Eosinophil as against the control. While there was a decrease in Lymphocyte and Packed Cell volume as against the control.

Table 1: Effect of Alium sativum on Staphylococcus aureus in Blood culture

S/N	STAPH. AUREUS ONLY	STAPH AUREUS PLUS GARLIC
1	Growth	No Growth

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2	Growth	No Growth
3	Growth	No Growth
4	Growth	No Growth

Table 4.3: Effects of Allium sativum On Wister Rats Infected with Staphylococcus aureus (Staph. aureus).

PARAMETERS	CONTROLS Mean±SD N=5	STAPH. ONLY Mean±SD N=5	STAPH. PLUS GARLIC Mean±SD N=5	F-VALUE	P-VALUE
WBC ($x10^9$ /l)	5.08±0.12	6.04±0.19	6.14±0.44	16.957	0.000
NEUT (%)	45.76±0.24	48.74±0.17	49.02±20.41	283.784	0.000
LYM (%)	53.28±0.31	52.72±0.54	49.88±4.45	2.469	0.126
MONO (%)	1.50±0.19	3.64±0.24	3.88±0.11	245.276	0.000
EOSINOPHIL	3.50±0.28	4.32±0.38	4.72±0.35	16.625	0.000
BASOPHIL	0.45±0.22	2.22±0.41	1.94±0.21	52.647	0.000
PCV (%)	43.32±0.89	34.80±3.27	41.80±1.79	21.347	0.000

Data are presented as mean ± standard error of mean
P-value< 0.05 is significant

III. DISCUSSION

This present study was carried out to investigate the immunological impact of garlic in Wistar rats infected with pathogenic organisms(Staphylococcus aureus). The antimicrobial activities of extracts of garlic have long been linked to the presence of these bioactive compounds. These bioactive compounds are known to work synergistically to produce various effects on the human and animal subjects (Amagase, 2006).

From the present study, the antibacterial evaluation of aqueous extracts of garlic revealed a significant antibacterial potency against the test organism. There was growth inhibition of Staph aureus in blood of rat treated with aqueous extract of garlic when compared with non-treated rats. The growth inhibition produced by the garlic extracts against the test organisms indicated the potency of the active principle in them. Drugs present in plants are known as active principle and these active principles are divided chemically into a number of chemical classes including glycosides, alkaloids, volatile oils, steroids flavonoids, resins and sterols. Most of these active principles have measurable antibacterial activities against microorganisms. The outcome of our investigations agreed with the works of Tatfeng &Samson, (2012) and Olaniyan et al., (2013). From the present study, there was increase in total white blood cell count in rats treated with aqueous extract of garlic when compared with the control groups (normal control and untreated infected rats). While the increase was significant ($p<0.05$) between the rat treated with garlic and normal control group, the increase was not significant between the treated and untreated group. This result agreed with the works of Adebolu, Adeoye& Oyetayo, (2011), Olaniyan et

al., (2013) &Tende, Eze, Muhammad and Daikwo, (2014). Garlic containing substances has been reported to have antibiotic effects and antibiotics should enable the proliferation of circulating white blood cells considering that white blood cells function to protect the body from teratogens according to Augusti, (1996), this was confirmed by the significant increase in the Total white blood cell count in the animal treated with garlic juice extract in this study.

The administration of aqueous extract of garlic to infected rats caused an increase in the neutrophil and monocyte count. This observation agrees with the report of Bjarnsholt et al. (2005) & Adebolu et al., (2011) that innate immunity of mice increased when fed with garlic. The increase in the count of these two cell types in rats fed with the extract showed that garlic can also boost the innate immunity of rats. Neutrophils provide protection against a variety of intracellular organisms by both phagocytic and none phagocytic mechanisms while monocytes replenish resident macrophages and dendritic cells which migrate quickly to sites of infection in the tissues and ingest invading bacteria (Adebolu et al., 2011).

Also from the present study, there was a significant reduction in the packed cell volume of infected rats treated with aqueous extract of garlic when compared with control rats. This result agreed with the works of Banerjee and Maulic, (2002), Adebolu et al., (2011) and Olaniyan et al., (2013) who had also in their various study recorded reduction in the packed cell volume of garlic treated rats. According to Banerjee and Maulic, prolong feeding with high levels of raw garlic in rats may result to anemia, weight loss and failure of the rat to grow. They also reported that garlic contain toxic components that may damage red blood cells and provoke haemolytic anaemia accompanied by Heinz bodies in erythrocytes of animals such as cattle, water buffalos, sheep, horses, dogs and cats. Osagie & Eka, (1998) also submitted that saponins, a steroid or triterpenoid glycosides characterised by their bitter or astringent taste, foaming properties has haemolytic effect on red blood cells.

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