Effects of ginger (Zingiber officinale Roscoe) and garlic (Allium sativum L.) on rats infected with Klebsiella pneumoniae

A Micheal, O Folaranmi, O Ajoke, A Olatunji, A Oluwole, A Adewumi

Abstract
This study investigates the efficacy of ginger (Zingiber officinale), garlic (Allium sativum) and both plants combined in rats infected with Klebsiella pneumoniae. The rats were treated with the plant extracts after symptoms of pneumonia infection were noticed in all the groups; the treatment lasted for seven consecutive days. The average weight gains in the post-administration were: in garlic, ginger, mixture (males), mixture (females) and control were 27.0g, -36.2g, 6.0g, 38.8g and 78.8g respectively. There was a significant reduction in packed cell volume (PCV) in all the groups except in females injected with mixture compared with the control. Also, garlic treated rats showed a significant decrease in total white blood cell (WBC) and neutrophils but an increase in lymphocytes as against to what was observed in the other groups. All the rats in ginger group died with symptoms of pneumonia before seven days after the administration while two rats died in males treated with mixture. No death was however recorded in the other groups.

INTRODUCTION
There has been a great shift from the prescription of antibiotics to the use of medicinal plants. It is estimated that there are 250,000 to 500,000 species of plants on earth (Borris, 1996). A relatively small percentage of these are used as food by both humans and other animal species. It is possible that even more are used for medicinal purposes.

Zingiber officinale Roscoe, commonly called ginger is a perennial plant with narrow, bright green, grass-like leaves and yellowish green flowers with purple markings. Ginger is cultivated in the tropics for its edible rhizome at approximately 10 months of age, with the root stocks serving a variety of purposes, including culinary and medicinal (Grant, 2000). Ginger is among the 20 top-selling herbal supplements in the United States and today, pharmacopeias of a number of different countries list ginger extract for various digestive diseases (Borrelli et al; 2004). The efficacy of ginger is purported to be a result of its aromatic, carminative and absorbent properties (Govindarajan, 1982 a, b). Ginger is a widely used spice and functional food, for centuries ginger has been an important ingredient in Chinese, Ayurvedic and Tibb-Unani herbal medicine. The main constituents of ginger include volatile oil (β-bisabolene, cineol, phellandrene, citral, borned, citronellol, geranialinalool, limonene, zingeberol, zengeberene, camphene), oleoresin (gingerrol, shogoal), phenol (gingerol and zingerone), proteolytic enzymes (zingibain), vitamin B6, vitamin C, and calcium, magnesium, phosphorus, potassium, linoleoc acid (Kikuzaki et al; 1993). Also, the pungency and aroma of ginger are because of the gingerol and volatile oil respectively (Kikuzaki et al; 1994). A recent study (Egwurugwu et al., 2007) observed that ginger had both prophylactic and therapeutic Cadmium detoxification. Ginger has been variously used as anti-diabetic potential (Al-min et al., 2006). Ginger powder 1g daily alleviated clinical nausea of diverse causes including postoperative nausea (Arfeen et al., 1995)

The antibacterial effects of the two plant extracts have been reviewed (Ekwenye & Elegalam; 2005).

The present study is aimed at investigating the therapeutic effect of both plants on Klebsiella pneumoniae and whether the combined extract could have either a synergistic or better say antagonistic properties in rats.

Garlic (Allium sativum, Linn) is a condiment, which for
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several years has been in India, Egypt and China for its medicinal purposes. It has been used for conditions like fever, cough, digestive disorders and respiratory diseases like tuberculosis. Allium sativum has antihelmintic action in vitro against Heterakis gallinae and Ascaridia galli, Heamomonchus contortus, a free- living nematode of Rhabditis sp (Nagaich, 2000, Zafar-iqbal et al, 2001, Chybowski, 1997). Garlic has also been implicated to alleviate some of the risk factors associated with nicotine induced hyperglycemia and hypercholesteremia (Effraim et al., 2000). In the work of Onyeagba et al., 2004, on the antimicrobial effects of garlic, ginger and lime, stated that the crude extract of garlic and ginger applied singly and in combination did not exhibit any in vitro inhibition on Salmonella spp, Escherichia coli, Bacillus spp and Staphylococcus aureus. However, lime has the highest effect on all the test organisms (Onyeagba et al,2004). A similar study ((Ekwenye & Elegalam; 2005) discovered that both ginger and garlic produced marked inhibitory effect on S. typhii and E. coli as representative of enteric microorganisms although this was dependent on the solvents used for the extractions.

Ginger had ameliorative effects on mercury induced hepatotoxicity in rats (Vitalis et al., 2007), mancozeb fungicide induced liver injury in albino rats (Sakr., 2007) and can be used as both as prophylactic and therapeutic detoxificant on Cadmium induced poison (Egwurugwu et al.,2007). Iranloye, 2002 observed a significant increase in RBC, PCV, WBC and total Hb concentration in garlic fed rats. Fresh garlic consumption alone was found to decrease serum lipids, but it can not be used as therapeutic agents in the treatments of hyperlipidemia (Mahmoodi et al., 2006). Tang et al., 1997 showed that treatment with garlic extract improve the activation of natural killer cells T-lymphocytes and stimulates immune functions (Sumiyoshi 1997).

MATERIALS AND METHODS

Zingiber officinale and Allium sativum: These were purchased from Sango general market, Saki; Nigeria. The ginger was washed and peeled, cut into pieces and sun dried for seven days; the dried ginger was crushed using an electric blender. Also, the garlic was cut into pieces and sun dried for seven days, it was later crushed using an electric blender. The ethanol extraction of the two plants material was prepared as stated elsewhere (Onyeagba et al., 2004). Experimental Rats: twenty-eight rats were reared from parental lines of three female and four male purchased from department of Medicine University College Hospital, Ibadan, Nigeria whose weight ranged from 94 and 176 g between March 2007 and July 2008. The animals were separated into five cages three weeks before the commencement of the study. The animals were allowed to acclimatize, fed twice daily with normal rat chow and distilled water ad libitum. The animals were weighed weekly during the period of acclimatization and net weight gained were noticed and recorded. They were weighed and grouped into five groups randomly. The groupings were: A (Five), B (Six), C (Five), D (Six) and E (Six). The segregation into sexes of the rats in Cage C (males only) and D (females only) was done in order to monitor the effects of mixture of garlic and ginger on Klebsiella pneumoniae with respect to sexes. Cage A was made the control, administered with normal distilled water throughout the course of the experiment. After fourteen day of acclimatization, each rat received 0.5 ml of Klebsiella pneumoniae in 1:10 dilution ratio intra-peritoneally except the control; the bacteria pure clones was obtained from the department of microbiology, University College Hospital; Nigeria and was maintained by sub-culturing the bacteria sample in peptone water for 48 hours before injecting into the rats. The animals were kept under the same condition until symptoms of pneumonia infection were noticed after twelve days. The rats were weighed before the administration of the plant extracts and then weekly for two consecutive weeks. The animals were monitored for general health throughout the experiment.

Administration of test sample: Each animal received an intra-peritoneal equal dose of plant extracts for seven consecutive days in the following manners: Group E Control (Distilled water) group A (garlic only), group B (ginger only), while both group C (males) and D (females) received a mixture of garlic and ginger in 50:50 dilution. Behavioral changes observed was noticed and documented daily.

Collection of blood samples: In order to minimize discomfort to the animal, blood samples was collected from the distal tail region of the rats according to the protocol described by Hoff in 2000.

The rats were warmed prior to bleeding in order to collect the required quantity. About 2mls of blood was collected from each rat into EDTA bottles containing citrate as anti-coagulant for hematological analysis.

RESULTS
Effects of ginger (Zingiber officinale Roscoe) and garlic (Allium sativum L.) on rats infected with Klebsiella pneumoniae

Figure 1
Table 1: Weight distributions pre and post administration period of test samples

<table>
<thead>
<tr>
<th>Group</th>
<th>Pre post</th>
<th>Net</th>
<th>Pre post</th>
<th>Net</th>
<th>Pre post</th>
<th>Net</th>
<th>Pre post</th>
<th>Net</th>
<th>Pre post</th>
<th>Net</th>
<th>Pre post</th>
<th>Net</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (garlic only)</td>
<td>129.0</td>
<td>140.2</td>
<td>10.3</td>
<td>357.1</td>
<td>141.2</td>
<td>12.6</td>
<td>131.2</td>
<td>112.1</td>
<td>-9.1</td>
<td>131.4</td>
<td>131.2</td>
<td>0.2</td>
</tr>
<tr>
<td>B (garlic only)</td>
<td>124.5</td>
<td>131.1</td>
<td>12.6</td>
<td>180.0</td>
<td>166.8</td>
<td>43.0</td>
<td>131.5</td>
<td>131.2</td>
<td>0.3</td>
<td>101.7</td>
<td>158.2</td>
<td>11.7</td>
</tr>
<tr>
<td>C (M.)</td>
<td>118.1</td>
<td>120.7</td>
<td>2.6</td>
<td>252.2</td>
<td>125.2</td>
<td>2.9</td>
<td>141.4</td>
<td>135.6</td>
<td>5.8</td>
<td>127.7</td>
<td>130.2</td>
<td>2.6</td>
</tr>
<tr>
<td>D (M.)</td>
<td>107.3</td>
<td>117.8</td>
<td>10.3</td>
<td>78.0</td>
<td>64.7</td>
<td>11.3</td>
<td>138.0</td>
<td>126.1</td>
<td>19.0</td>
<td>120.5</td>
<td>126.7</td>
<td>6.5</td>
</tr>
<tr>
<td>E (Control)</td>
<td>133.8</td>
<td>126.8</td>
<td>7.2</td>
<td>78.8</td>
<td>78.8</td>
<td>0.0</td>
<td>78.8</td>
<td>78.8</td>
<td>0.0</td>
<td>78.8</td>
<td>78.8</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Legend: * Dead, # died but survived till day 5, M (garlic+ginger), 1= males, 2= females

Figure 2
Table 2: Comparision of the Hematological indices after 30 days post administration

<table>
<thead>
<tr>
<th>Group</th>
<th>A (garlic only)</th>
<th>B (garlic only)</th>
<th>C (M.)</th>
<th>D (M.)</th>
<th>E (control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV</td>
<td>WBC NLE</td>
<td>PCV WBC NLE</td>
<td>PCV WBC NLE</td>
<td>PCV WBC NLE</td>
<td>PCV WBC NLE</td>
</tr>
<tr>
<td>1</td>
<td>20.47 67 32 1</td>
<td>2135.07 24 1</td>
<td>25.86 70 29 1</td>
<td>17.5 560 40 9</td>
<td>29.15 65 37 0</td>
</tr>
<tr>
<td>2</td>
<td>15.28 66 36 1</td>
<td>22.14 71 29 0</td>
<td>21.75 60 30 9</td>
<td>20.12 57 72 0</td>
<td>20.12 57 20 9</td>
</tr>
<tr>
<td>3</td>
<td>25.08 66 32 1</td>
<td>22.15 75 25 0</td>
<td>28.54 70 20 9</td>
<td>28.16 74 25 1</td>
<td>28.16 74 25 0</td>
</tr>
<tr>
<td>4</td>
<td>17.96 70 28 2</td>
<td>** * * * * * * *</td>
<td>35.78 70 29 0</td>
<td>23.15 74 26 0</td>
<td>23.15 74 26 0</td>
</tr>
<tr>
<td>5</td>
<td>14.56 67 33 0</td>
<td>** * * * * * * *</td>
<td>35.86 62 37 1</td>
<td>31.27 74 25 0</td>
<td>31.27 74 25 0</td>
</tr>
<tr>
<td>6</td>
<td>** * * * * * * *</td>
<td>** * * * * * * *</td>
<td>37.12 72 28 0</td>
<td>20.14 57 28 0</td>
<td>20.14 57 28 0</td>
</tr>
</tbody>
</table>

Legend: PCV= packed cell volume (%), WBC= white blood cells (X 1000/mm$^3$), N=Neutrophils (%), L=Lymphocytes (%), E= Eosinophils (%), -= No animal, *= dead

Results of the study are shown in table 1 and 2. The Average Weight Gain (AWG) per group is as follows: garlic (+27.0g), ginger (-36.2g), M1 (6.0g), M2 (38.8g) and Control (78.8g) whereas the total average loss in rats treated with the mixture of garlic and ginger is (44.8g). After the inoculation of Klebsiella, all the rats except the control group developed symptoms of pneumonia after fourteen days, the symptoms presented includes: loss of fur, swollen body, irregular breathing, weight-loss, loss of appetite and in ability to drink water. The plant extracts (ginger and garlic) was administered for seven days; garlic treated group recovered fully on day four, while ginger treated group failed to recover, in fact all the animals in ginger treated group died while two deaths was recorded in male rats treated with mixture of garlic and ginger. No death was however recorded in the control group and female rats treated with mixture of garlic and ginger. We observed a decrease in packed cell volume, total white blood cells counts, neutrophils with an increase in Lymphocytes in rats treated with garlic only. In the rats treated with ginger only, there was a reduction in packed cell volume and lymphocytes but an increase in total white blood cells and neutrophils. We noticed reduced packed cell volume but increased total white cells count, neutrophils and lymphocytes in the male rats treated with mixture whereas there was an increase in packed cell volume, inconsistence total white blood cell count, slight decrease in neutrophils and increased in neutrophils in female rats treated with the mixture when compared to the control group. There was no change in the eosinophil count throughout the course of this study when compared with the control.

DISCUSSION
The virtues of garlic as medicinal plant are known to most cultures of the world, this study further confirmed the earlier works on the efficacy of garlic as therapeutic agent against various ailments. The antibacterial activity of the ethanolic extracts of garlic and ginger against some enteric bacteria was reported; however ginger seems to be ineffective against Klebsiella pneumoniae as observed in our study. Ginger was reported found to cause decrease in body weight, this is however consistent with our study because the highest average weight loss observed in rats treated with ginger only. The exact mechanism of action of both ginger and garlic as proffered therapeutic agents is poorly understood but combining the two in our study could be beneficial; as the relationship seem to be a synergistic one in ameliorating the efficacy of ginger only against Klebsiella infection as observed in our study in female rats treated with the mixture of garlic and ginger.
Whether both garlic and ginger can be used as prophylaxis is left for further studies but both plants have been implicated to have prophylactic uses in both human and rats. Very recently, the root of ginger has been used to stimulate blood flow to the extremities in cases of blood circulation (Vitalis et al, 2007), the hematological parameters monitored in this study sharply disagreed with this. Also, our work failed to concur with the work of Iranloye, 2002 who observed a significant increase in total white blood cells count, neutrophils, monocytes and the lymphocytes in garlic fed rats when compared with the control. Leucocytes are known to increase sharply in the face of infection, as the first line of defense of the body; the factor that led to the reduction in packed cell volume (PCV), white blood cell counts(WBC), and neutrophils noticed in our study in the rats treated with garlic only, still remain unclear.

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References

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