**In-Vivo Screening of Citrus Maxima Oil Against, Escherichia Coli Infection in Rabbits**

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**ABSTRACT**

**Introduction:** *Escherichia coli* is a Gram-negative facultative anaerobic bacteria which cause serious damages in animals and humans by causing high morbidity and mortality. The increasing prevalence of antibiotic-resistant microorganisms, has made it necessary to replace alternative sources of antibiotic products. There is a persistent need to recognize new and novel antimicrobial agents that would help in alleviating the problems of emerging resistant bacterial pathogens. Plant derived natural products represent an attractive source of antimicrobial agents since they are natural and affordable, especially in rural societies in poor developing countries.

**Objectives:** The objective of the current research was to explore the antibacterial capabilities of the *Citrus maxima* oil against *Escherichia coli* infection in rabbits. The effect of oil on rabbits' haematological parameters was also assessed.

**Methods:** Rabbits were distributed into six groups, 1st group served as negative control, 2nd group as a positive control, infected with *E. coli* at the dose rate of 2.0 x 10^6 CFU without medication, group 3rd, 4th and 5th were infected with *E. coli* and supplemented with oil at different doses and 6th group was infected with *E. coli* and treated with Moxifloxacin HCL (standard drug) at the dose rate of 7mg/kg. The blood samples of tested animals were collected for the analysis of haematological parameters on different days of the experiments.

**Result:** The results showed a significant increase in WBCs, MCV, HCT and neutrophils during infection. RBCs, MCH, MCHC, haemoglobin, lymphocytes and platelets count decreased during infection. The current study provides a scientific contextual for the significant use of the *Citrus maxima* oil for the treatment of various pathological diseases.

**Conclusion:** The obtained result gives an insight into the antibacterial capabilities of the *Citrus maxima* oil against *Escherichia coli* infection in rabbits. *Citrus maxima* oil exhibited variable, but remarkable antibacterial potential. These oils could be used as a source of antimicrobial agents in pharmaceutical industries.

**Key Words:** Antimicrobial, bacterial pathogens, *Citrus maxima* oil, *Escherichia coli*, Haematological parameters, Medicinal plant

**INTRODUCTION**

The increasing bacterial conflict to antibiotics has developed a growing APPREHENSION GLOBALLY. With the appearance and growth of microorganisms such as gram negative (*E.coli*) bacteria which can cause serious infections. There are a number of drugs available to treat gram-positive bacteria, but less in number to treat gram-negative bacteria. In addition, high cost and adverse effects are commonly related to widespread synthetic antibiotics are a chief burning global issue in considering infectious diseases. Infectious diseases pose serious problems to health and they are the main cause of morbidity and mortality worldwide. The increasing prevalence of antibiotic-resistant microorganisms, has made it necessary to replace alternative sources of antibiotic products. Plant derived natural products represent an attractive source of antimicrobial agents since they are natural and affordable, especially in rural societies in poor developing countries.

The literature survey revealed that peel of *Citrus maxima* fruits is extremely thought to be a universal remedy within the flavouring drugs with various spectra of pharmacologic activity. *Citrus maxima* is the most extensively studied medicinal plant in recent literature. *Citrus maxima* is an edible fruit, its flesh is juicy, soft in texture and wealthy in nutrients.
and is endemic to tropical part of Asia. E. coli is the most commonly found bacterium in the human intestinal tract. Under normal conditions, its presence is conducive to digestive processes. But when present in excess or in virulent form it cause diseases. E. coli, contaminate food and water supplies.

Plants play an important role in human health because they produce a wide array of bioactive molecules which have medicinal values. Despite the efforts in producing a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. The in-vitro anti-bacterial activities of three citrus Plants extracts, Citrus microcarpa, Citrus aurantiu and Citrus maxima against S. aureus but not against E. coli. The oil of this fruit has been reported to possess some nutritive and medicinal potentials.

**MATERIAL AND METHODS**

The main objective of the present study was to investigate the antibacterial activity of Citrus maxima oil against E. coli infection in rabbits. A total of 30 adult Rabbits of both genders was used in the current study as experimental animals.

**Grouping of animals**

Rabbits having same weight were kept in the same group, thirty rabbits of both gender, which were randomly divided into six equal groups, 1st group (negative control), 2nd group infected with Escherichia coli orally at the dose rate of 2x10⁶ CFU (served as positive control) which did not receive any drug or essential oil as a treatment, 3rd group, infected with E. coli at the dose rate of 2x10⁶ CFU and was treated with Citrus maxima oil at a dose rate of 1ml, 4th group, infected with E. coli at the dose rate of 2x10⁶ CFU and was fed with Citrus maxima oil at a dose rate of 1ml, 5th group, infected with E. coli at the dose rate of 2x10⁶ CFU and fed with Citrus maxima oil at a dose rate of 2ml, 6th group was injected with E. coli at the dose rate of 2x10⁶ CFU and was fed with standard drug, (Moxifloxacin HCL).

**Chemicals and apparatus**

Citrus maxima oil and Moxifloxacin HCL were purchased from the local market of QissaKhwani Bazar, Peshawar, Pakistan. Equipment used in the current study were Haematological analyser and Weight scale.

**Initiation of medication**

All groups of rabbits received a freshly cultured sample of E. coli (2x10⁶ CFU) orally except the control group. After administration of the bacteria, rabbits were checked for feed intake and other clinical signs. The responses of the rabbits to E. Coli bacteria were identified by the clinical signs like temperature, diarrhoea, weight loss and reduced feed intake.

**Medication of infected rabbits**

After development of clinical signs, group 3rd, 4th and 5th were treated with Citrus maxima oil at a dose rate of 1ml, 1.5ml and 2ml, while group 6th was treated with standard drug, Moxifloxacin HCl. Phenobarbital sodium was used to anesthetize the rabbits for the collection of blood samples. Blood samples (about 3ml) were collected from all rabbits, at day zero, day three, day six and day nine of the experimental work for the analysis of different haematological parameters.

**Statistical analysis**

The data obtained from the study were analysed statistically, using the analysis of variance (ANOVA) and Tukey’s multiple comparison test were used to determine the differences between treatments. The mean and standard deviation (SD) were sorted out of each parameter, using, Graph pad prism software.

**RESULTS**

In the present study, rabbits were divided into different groups. 1st group was kept as negative control, neither infected nor medicated, 2nd group served as a positive control which was infected, but not treated 3rd, 4th and 5th group were treated with Citrus maxima oil at a dose rate of 1ml, 1.5ml and 2ml and group 6th was treated with standard drug (Moxifloxacin).

**Pre infection**

Blood samples was collected on day zero and the results of all the groups have been shown as under: (Table 1).

**1st Group (negative control)**

About 3ml blood was collected from the animals. The TRBCs of negative control was 5.73±0.028 x 10⁶/µl and haemoglobin value was 9.52±0.035. The MCH, MCHC and MCV values were 21.49±0.134 Pg, 33.0±0.848 gm/dl and 50.7±0.070pg. The WBCs count was in the range of 5.72±0.035 x 10³/µl while lymphocytes count was 27±0.494%. The neutrophils, platelets count and HCT value were also in the normal reference range (60.5±0.707%, 530.5±0.035 x 10⁹/µl and 37.5±0.17%).

**2nd group (positive control)**

The same amount of blood was also collected and were analysed for different parameters. The values of the RBCs and haemoglobin were 5.08±0.033 x 10⁶/µl and 9.81±0.13%. Lymphocytes count was 28.3±0.31%. MCH and MCHC level were 19.7±0.41pg and 30.5±0.16gm/dl. The MCV, WBCs and Neutrophils count were in the range of 50.6±0.12pg, 5.5±0.045 x 10³/µl and 62.6±0.25%. Platelets level was 528±0.11x 10⁹/µl and HCT value was 36.8±0.10%.
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3rd Group three (low dose)
Before infection the RBCs and haemoglobin value were in the range of 5.70±0.17x 10^6/µl and 9.45±0.057%. The MCH value was 21.45±0.14pg and MCHCs value was 33.03±0.081gm/dl while MCV was in 50.76±0.072pg range. WBCs, lymphocytes and neutrophil count were 5.71±0.036x 10^3/µl, 27.05±0.26% and 62.53±0.19%. HCT value was 37.46±0.92%, platelets count was 531.52±0.27x 10^3/µl while RDWC level was 15.56±0.095%.

4th Group (medium dose)
RBC and haemoglobin of group 4th before infection were 5.73±0.23x 10^6/µl and 9.52±0.27%. Concentration of MCH was 21.48±0.33pg while MCHC was 32.95±0.12gm/dl. The level of MCV and WBCs were in the range of 50.74±0.12pg and 5.71±0.076x 10^3/µl. Lymphocytes, neutrophils and platelets count were 28.96±0.17%, 61.47±0.44% and 525.50±0.14x 10^3/µl while HCT and RDWC values were in the range of 37.50±0.14%, and 15.60±0.26%.

5th Group (high dose)
The RBC count was 5.76±0.074x 10^6/µl while haemoglobin value was 9.75±0.38%. The values of MCH, MCHC and MCV were 21.51±0.25pg, 32.95±0.25gm/dl and 50.69±0.16pg. WBCs count was 5.71±0.036x 10^3/µl and lymphocytes count was 28.08±0.087% while neutrophil count was 60.44±0.36%. Platelets, HCT and RDWC level were 530.54±0.087x 10^3/µl, 37.53±0.15% and 15.57±0.21%.

6th Group (standard drug)
The RBCs and haemoglobin values were 5.78±0.77x 10^6/µl and 9.81±0.084%. The concentration of MCH was 21.56±0.036pg and MCHC was 33.02±0.17gm/dl. MCV, WBCs and lymphocytes count were 50.72±0.34pg, 5.73±0.045x 10^3/µl and 27.03±0.13% while neutrophil count was 61.52±0.27%. Platelets count was 524.53±0.053x 10^3/µl, HCT value was 37.48±0.17%.

Table 1: Haematological parameters in different groups of rabbits at day zero

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Negative control group</th>
<th>Positive control group</th>
<th>3rd group</th>
<th>4th group</th>
<th>5th group</th>
<th>6th group</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC (X10^6/µl)</td>
<td>5.73±0.028</td>
<td>5.8±0.033</td>
<td>5.70±0.17</td>
<td>5.73±0.23</td>
<td>5.76±0.074</td>
<td>5.78±0.77</td>
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<tr>
<td>Hb (gm/dl)</td>
<td>9.52±0.035</td>
<td>9.8±0.13</td>
<td>9.45±0.057</td>
<td>9.52±0.27</td>
<td>9.75±0.38</td>
<td>9.8±0.084</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>21.49±0.13</td>
<td>19.7±0.41</td>
<td>21.45±0.14</td>
<td>21.48±0.33</td>
<td>21.5±0.25</td>
<td>21.56±0.03</td>
</tr>
<tr>
<td>MCHC (gm/dl)</td>
<td>33.02±0.848</td>
<td>30.5±0.16</td>
<td>33.03±0.08</td>
<td>32.95±0.12</td>
<td>32.95±0.25</td>
<td>33.02±0.17</td>
</tr>
<tr>
<td>MCV (pg)</td>
<td>50.72±0.070</td>
<td>50.6±0.12</td>
<td>50.76±0.07</td>
<td>50.74±0.12</td>
<td>50.69±0.16</td>
<td>50.72±0.34</td>
</tr>
<tr>
<td>WBC (X10^3/µl)</td>
<td>5.72±0.035</td>
<td>5.5±0.045</td>
<td>5.71±0.025</td>
<td>5.71±0.036</td>
<td>5.71±0.076</td>
<td>5.73±0.045</td>
</tr>
<tr>
<td>L (%)</td>
<td>27.40±0.494</td>
<td>28.3±0.31</td>
<td>27.05±0.26</td>
<td>28.96±0.17</td>
<td>28.08±0.28</td>
<td>27.03±0.13</td>
</tr>
<tr>
<td>N (%)</td>
<td>61.5±0.070</td>
<td>62.6±0.25</td>
<td>62.53±0.19</td>
<td>61.47±0.44</td>
<td>60.44±0.36</td>
<td>61.52±0.27</td>
</tr>
<tr>
<td>PLT (10^3/µl)</td>
<td>530.5±0.03</td>
<td>528±0.11</td>
<td>531.52±0.2</td>
<td>525.50±0.14</td>
<td>530.54±0.087</td>
<td>524.53±0.0</td>
</tr>
<tr>
<td>HCT (%)</td>
<td>37.5±0.17</td>
<td>36.8±0.10</td>
<td>37.46±0.92</td>
<td>37.5±0.14</td>
<td>37.53±0.15</td>
<td>37.48±0.17</td>
</tr>
<tr>
<td>RDWC (%)</td>
<td>15.6±0.10</td>
<td>15.9±0.12</td>
<td>15.56±0.09</td>
<td>15.60±0.26</td>
<td>15.57±0.21</td>
<td>15.63±0.16</td>
</tr>
</tbody>
</table>

During Infection
The rabbits were infected with pathogenic E. coli at the dose rate 2x10^10 CFU, except group 1st (control group). After causing infection, whole blood was collected from all the infected groups for analysis of haematological parameters (Table 2).

2nd group (positive control)
The RBCs and haemoglobin count during infection were 3.47±0.042x 10^6/µl and 6.82±0.042%. Neutrophils and Platelets count were 66.4±0.042% and 435±0.042x10^3/µl. The MCH value were20.53±0.042pg and the MCHC were 28.15±0.070gm/dl. The lymphocyte, WBCs and MCV count were 23.6±0.035%, 8.22±0.035x10^3/µl and 59.53±0.028pg. The HCT value was 41.62±0.028%. The level of RDWC was 15.42±0.10% during infection.

3rd Group (low dose)
This group was also infected with E. coli, RBC count was 3.44±0.028x10^6/µl and haemoglobin value was 6.74±0.035%. The MCH and MCHC values were 20.50±0.035pg and 28.17±0.035gm/dl. The MCV, WBCs and lymphocytes count
were 66.56±0.622pg, 8.17±0.014x 10^3/µl and 25.62±0.042% while HCT value was 41.64±0.056%. The level of RDWC was 15.45±0.24%, the neutrophils and platelets count were 65.41±0.414% 491.04±0.21 x 10^3/µl.

4th Group (medium dose)
RBCs and haemoglobin count were 3.48±0.21x 10^6/µl and 6.85±0.14%. The MCH value were 20.49±0.14pg and MCHC were 28.18±0.28 gm/dl. MCV, lymphocytes, WBCs and neutrophils count were 57.52±0.11pg, 24.64±0.49 %, 7.4±0.07x 10^3/µl and HCT value was 42.66±0.056 %. Platelets and neutrophils count were 467.05±0.28x 10^3/µl and 67.41±0.035%. The RDWC level was 15.45±0.24% during infection.

5th Group (high dose)
RBCs count was 3.5±0.014x 10^6/µl while haemoglobin value 6.90±0.14%. The values of MCH and MCHC were 20.52±0.14pg and 28.20±0.28 gm/dl. MCV, WBCs and lymphocytes count were 56.55±0.11pg, 8.33±0.070 x 10^3/µl and 23.65±0.049%. Platelets and neutrophils count were 467.05±0.28x 10^3/µl and 67.41±0.035%. The HCT value was 41.65±0.29 %.

DURING TREATMENT

Total Red Blood Cells (TRBCs)
At day six, the TRBCs count in negative control group was 5.73±0.028x 10^6/µl, the positive control group was 4.54±0.028x 10^6/µl. In groups that were infected with E.coli and treated with C.maxima oil at the dose rate of 1ml/kg, 1.5ml/kg and 2ml/kg, the TRBCs counts were 6.26±0.028x 10^6/µl, 5.40±0.042x 10^6/µl and 4.04±0.028x 10^6/µl. The group which was infected with E.coli and treated with standard drug (Moxifloxacin HCl), the RBCs count was 5.83±0.035x 10^6/µl. A significant difference (P<0.05) was
found in the mean TRBCs counts of control group with the rest of groups (Table 3).

**Haemoglobin (Hb)**
The haemoglobin value in control group and positive control group at day six were 9.52±0.035% and 8.85±0.035%. In groups, that were infected with *E. coli* and treated with *C. maxima* oil at the dose rate of 1ml/kg, 1.5ml/kg and 2ml/kg, the haemoglobin values were 10.12±0.028%, 9.0±0.042% and 8.28±0.035%. The group that was infected with *E. coli* and treated with standard drugs (Moxifloxacin), the haemoglobin value was 9.6±0.035%. A significant difference (P<0.05) was found in the mean Hb value of control group with the rest of groups.

**Mean Corpuscular Haemoglobin (MCH)**
At day 6th, the MCH value of negative control was 21.1±0.134 pg, the positive control value group was 17.8±0.042pg. In groups, that were infected with *E. coli* and treated with *C. maxima* oil at the dose rate of 1ml/kg, 1.5ml/kg and 2ml/kg, the MCH value were 18.51±0.028pg, 19.49±0.042pg and 19.7±0.035pg. That group which was infected with *E. coli* and treated with standard drug (Moxifloxacin), the MCH value was 19.7±0.035pg. A significant difference (P<0.05) was found in the mean MCH value of control group with the rest of groups.

**Mean Corpuscular Haemoglobin Concentration (MCHC)**
The MCHC value in control group was 33.0±0.848gm/dl, the positive control group was 27.5±0.042gm/dl. In groups, that were infected with *E. coli* and treated with *C. maxima* oil at the dose rate of 1ml/kg, 1.5ml/kg and 2ml/kg, the MCHC values were 30.7±0.028gm/dl, 30.9±0.035gm/dl and 30.20±0.035 gm/dl. The group which was infected with *E. coli* and treated with standard drug (Moxifloxacin HCl), the MCHC value was 30.9±0.035 gm/dl. A significant difference (P<0.05) was found in the mean MCHC value of control group with the rest of groups.

**Mean Corpuscular Volume (MCV)**
In control group at day 6th, the MCV value was 50.7±0.070pg, the positive control group was 66.7±0.035. In groups, that were infected with *E. coli* and treated with olive oil at the dose rate of 1ml/kg, 1.5ml/kg and 2ml/kg, the MCV values were 57.5±0.064pg, 56±0.035pg and 58±0.035pg. That group which was infected with *E. coli* and treated with standard drug (Moxifloxacin), the MCV value was 56.5±0.042 pg. A significant difference (P<0.05) was found in the mean MCV value of control group with the rest of groups.

**White Blood Cells (WBCs)**
The WBCs count in control group was 5.72±0.035x 10^9 /µl, the positive control group was 8.36±0.042x 10^9 /µl. In groups, that were infected with *E. coli* and treated with *C. maxima* oil at the dose rate of 1ml/kg, 1.5ml/kg and 2ml/kg, the RBCs counts were 7.14±0.028x 10^12/µl, 7.05±0.042x 10^12/µl and 7.11±0.035x10^12/µl. The group which was infected with *E. coli* and treated with standard drug (Moxifloxacin HCl), the WBCs count was 7.03±0.028x 10^9/µl. A significant difference (P<0.05) was found in the mean WBCs counts of control group with the rest of groups.

**Lymphocytes (L)**
At day 6th, the lymphocytes count in control group was 28.80±0.494%, the level of positive control group was 21.8±0.042%. In groups, that were infected with *E. coli* and treated with *C. maxima* oil at the dose rate of 1ml/kg, 1.5ml/kg and 2ml/kg, the lymphocytes counts were 26.6±0.028 %, 25.3±0.035% and 26.4±0.042%. That group which was infected with *E. coli* and treated with standard drugs (Moxifloxacin), the lymphocytes count was 24.2±0.035%. A significant difference (P<0.05) was found in the mean lymphocytes counts of control group with the rest of groups.

**Neutrophils (N)**
In control group, the neutrophils count was 62.5±0.707%, the value of positive control group was 69.3±0.035%. In groups, that were infected with *E. coli* and treated with *C. maxima* oil at the dose rate of 1ml/kg, 1.5ml/kg and 2ml/kg, the neutrophils counts were 64.3±0.028%, 65.6±0.035% and 64.3±0.028%. While the group which was infected with *E. coli* and treated with standard drugs (Moxifloxacin HCl), the neutrophils count was 66.7±0.028%. A significant difference of (P<0.05) was found in the mean neutrophils counts of control group with the rest of groups.

**Platelets (PLTs)**
The PLTs count in control group was 530.5±0.035x 10^3/µl, the positive control group was 429±0.035x 10^3/µl. In groups, that were infected with *E. coli* and treated with *C. maxima* oil at the dose rate of 1ml/kg, 1.5ml/kg and 2ml/kg, the PLTs counts were 470±0.071x 10^3/µl, 465±0.035x 10^3/µl and 490±0.035x 10^3/µl. That group which was infected with *E. coli* and treated with standard drugs (Moxifloxacin), the PLTs count was 488±0.035x 10^3/µl. A significant difference (P<0.05) was found in the mean PLTs counts of control group with the rest of groups.

**Haematocrit (HCT)**
At day 6th, the HCT value in control group was 37.5±0.17%, the positive control group was 41.2±0.035%. In groups, that were infected with *E. coli* and treated with *C. maxima* oil at the dose rate of 1ml/kg, 1.5ml/kg and 2ml/kg, the MCHC values were 39.0±0.035%, 40.66±0.042% and 40.63±0.035%. The group which was infected with *E. coli* and treated with standard drugs (Moxifloxacin HCl), the HCT value was 39.5±0.028%.
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A significant difference (P<0.05) was found in the mean HCT values of control group with the rest of groups.

**Red Cell Distribution Width (RDWC)**

In control group, the RDWC level was 15.6±0.10%, in positive control group was 15.5±0.035%. In groups, that were infected with *E. coli* and treated with *C. maxima* oil at the dose rate of 1ml/kg, 1.5ml/kg and 2ml/kg, the RDWC level were 16.2±0.035%, 14.3±0.035% and 14.1±0.42%. While the group which was infected with *E. coli* and treated with standard drugs (Moxifloxacin HCl), the RDWC level was 14.5±0.071%. A significant difference (P<0.05) was found in the mean RDWC level of control group with the rest of groups.

**Table 3: Haematological parameters in different groups of rabbits at sixth day**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Negative control group</th>
<th>Positive control group</th>
<th>3rd group</th>
<th>4th group</th>
<th>5th group</th>
<th>6th group</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC(10⁶/µl)</td>
<td>5.73±0.028 A</td>
<td>4.54±0.028 B</td>
<td>6.26±0.028 C</td>
<td>5.40±0.042 D</td>
<td>4.04±0.028 E</td>
<td>5.83±0.035 F</td>
</tr>
<tr>
<td>HGB(g/dl)</td>
<td>9.52±0.035 A</td>
<td>8.85±0.035 B</td>
<td>10.12±0.028 C</td>
<td>9.02±0.042 A</td>
<td>8.28±0.035 D</td>
<td>9.62±0.035 A</td>
</tr>
<tr>
<td>MCH(Pg)</td>
<td>21.2±0.034 A</td>
<td>17.8±0.042 B</td>
<td>18.5±0.028 C</td>
<td>19.49±0.042 A</td>
<td>19.7±0.035 A</td>
<td>19.7±0.035 A</td>
</tr>
<tr>
<td>MCHC(g/dl)</td>
<td>33.0±0.048 A</td>
<td>27.5±0.042 B</td>
<td>30.7±0.028 C</td>
<td>30.9±0.035 A</td>
<td>30.2±0.035 B</td>
<td>30.9±0.035 B</td>
</tr>
<tr>
<td>MCV(Pg)</td>
<td>50.7±0.070 A</td>
<td>66.7±0.035 B</td>
<td>57.5±0.064 C</td>
<td>56±0.035 A</td>
<td>58.4±0.035 C</td>
<td>56.5±0.042 A</td>
</tr>
<tr>
<td>WBC(10³/µl)</td>
<td>5.72±0.035 A</td>
<td>8.36±0.042 B</td>
<td>7.14±0.028 C</td>
<td>7.05±0.042 C</td>
<td>7.11±0.035 C</td>
<td>7.03±0.028 C</td>
</tr>
<tr>
<td>LYM(%)</td>
<td>28.8±0.094 A</td>
<td>21.8±0.042 B</td>
<td>26.6±0.028 C</td>
<td>25.3±0.035 A</td>
<td>26.4±0.042 D</td>
<td>24.2±0.035 D</td>
</tr>
<tr>
<td>N (%)</td>
<td>62.5±0.707 A</td>
<td>69.3±0.035 B</td>
<td>64.3±0.028 C</td>
<td>65.6±0.035 D</td>
<td>64.3±0.028 D</td>
<td>66.7±0.028 D</td>
</tr>
<tr>
<td>PLT(10³/µl)</td>
<td>530.5±0.035 A</td>
<td>429±0.035 B</td>
<td>470±0.071 C</td>
<td>465±0.035 D</td>
<td>490±0.035 E</td>
<td>488±0.035 F</td>
</tr>
<tr>
<td>HCT (%)</td>
<td>37.5±0.017 A</td>
<td>41.2±0.035 B</td>
<td>39.0±0.035 C</td>
<td>40.66±0.042 D</td>
<td>40.63±0.035 D</td>
<td>39.5±0.028 B</td>
</tr>
<tr>
<td>RDWC (%)</td>
<td>15.6±0.10 A</td>
<td>15.5±0.035 A</td>
<td>16.2±0.035 A</td>
<td>14.3±0.035 A</td>
<td>14.1±0.42 A</td>
<td>14.5±0.071 A</td>
</tr>
</tbody>
</table>

The same alphabets in a row shows no significant difference (P<0.05). Different alphabets in a row shows significant difference (P<0.05).

**DAY 9 RESULT**

**Total Red Blood Cells (TRBCs)**

In control group, that the RBCs count was 5.73±0.028 x 10⁶ /µl, and in positive control group, it was 3.47±0.042x 10⁶ /µl. Groups infected with *C. maxima* oil at the dose rate of 1ml/kg, 1.5ml/kg and 2ml/kg, the RBCs counts were 6.25±0.028x 10⁶/µl, 5.06±0.035x 10⁶/µl and 5.52±0.028x 10⁶/µl. The group that was infected with *E. coli* and treated with standard drugs (Moxifloxacin), the MCHM value was 8.9±0.035%. A significant difference (P<0.05) was found in the mean TRBCs counts of control group with the rest of groups.

**Haemoglobin (Hb)**

It was observed in control group, that the Hb value was 9.52±0.29%, the value of positive control group was 6.82±0.039%. Groups infected with *C. maxima* oil at the dose rate of 1ml/kg, 1.5ml/kg and 2ml/kg, the Hb value were 10.11±0.028%, 8.16±0.035% and 8.86±0.035%. That group which was infected with *E. coli* and treated with standard drug (Moxifloxacin), the haemoglobin value was 8.9±0.035%. A significant difference (P<0.05) was found in the mean Hb values of control group with the rest of groups.

**Mean Corpuscular Haemoglobin (MCH)**

At day 9, the MCH value in control group and positive control group were 21.1±0.13pg and 17.1±0.042pg. Groups infected with *C. maxima* oil at the dose rate of 1ml/kg, 1.5ml/kg and 2ml/kg, the MCH values were 21.1±0.028pg, 20.49±0.042pg and 20.52±0.035pg. The group that was infected with *E.coli* and treated with standard drugs (Moxifloxacin), the MCH value was 20.54±0.035pg. A significant
difference (P<0.05) was found in the mean MCH values of control group with the rest of groups.

**Mean Corpuscular Haemoglobin Concentration (MCHC)**

MCHC value in control group was 33.0±0.8gm/dl, and in positive control group, it was 28.5±0.028gm/dl. Groups infected with Citrus maxima oil at the dose rate of 1ml/kg, 1.5ml/kg and 2ml/kg, the MCHC values were 32.6±0.035gm/dl, 31±0.035gm/dl and 31.3±0.672gm/dl. The group that was infected with E. coli and treated with standard drugs (Moxifloxacin), the MCHC value was 31.8±0.035gm/dl. A significant difference (P<0.05) was found in the mean MCHC values of control group with the rest of groups.

**Mean Corpuscular volume (MCV)**
The MCV value in control group and positive control group was 50.7±0.7 pg and 67.2±0.035pg. Groups infected with Citrus maxima oil at the dose rate of 1ml/kg, 1.5ml/kg and 2ml/kg, the MCV values were 55.8±0.028pg, 52.3±0.035pg and 55±0.035pg. That group which was infected with E. coli and treated with standard drugs (Moxifloxacin), the MCV value was 53.9±0.042pg. A significant difference (P<0.05) was found in the mean MCV values of control group with the rest of groups.

**White Blood Cells (WBCs)**
In control group, the WBCs count was 5.7±0.03x 10^9/µl, and in positive control group, it was 8.4±0.042x 10^9/µl. Groups infected with C.maxima oil at the dose rate of 1ml/kg, 1.5ml/kg and 2ml/kg, the RBCs counts were 6.5±0.028x 10^9/µl, 5.82±0.042x 10^9/µl and 5.96±0.035x 10^9/µl. The group that was infected with E.coli and treated with standard drugs (Moxifloxacin), the WBCs count was 6.02±0.028x 10^9/µl. A significant difference (P<0.05) was found in the mean WBCs counts of control group with the rest of groups.

**Lymphocytes (L)**
It was observed in control group, that the lymphocytes count was 28.0±0.49%, and in positive control group, it was 19.6±0.042%. Groups infected with Citrus maxima oil at the dose rate of 1ml/kg, 1.5ml/kg and 2ml/kg, the lymphocytes counts were 27.3±0.028%, 27±0.035% and 26.8±0.0 42%. That group which was infected with E.coli and treated with standard drugs (Moxifloxacin), the lymphocytes count was 28.6±0.035%. A significant difference (P<0.05) was found in the mean lymphocytes counts of control group with the rest of groups.

**Neutrophils (N)**
At day 9, the neutrophils count in control group and positive control group were 62.5±0.72% and 70.4±0.035%. Groups infected with C.maxima oil at the dose rate of 1ml/kg, 1.5ml/kg and 2ml/kg, the neutrophils counts were 63.6±0.028%, 61.51±0.035% and 62.6±0.028%. The group that was infected with E.coli and treated with standard drug (Moxifloxacin), the neutrophils count was 61.8±0.028%. A significant difference (P<0.05) was found in the mean neutrophils counts of control group with the rest of groups.

**Platelets (PLTs)**
In control group, the PLTs count was 530±2.0x 10^9/µl, and in positive control group, it was 395±0.028x 10^9/µl. Groups infected with C.maxima oil at the dose rate of 1ml/kg, 1.5ml/kg and 2ml/kg, the PLTs counts were 495±0.042x 10^9/µl, 520±0.035x 10^9/µl and 515±0.035x 10^9/µl. That the group which was infected with E.coli and treated with standard drugs (Moxifloxacin), the PLTs count was 517±0.035x 10^9/µl. A significant difference (P<0.05) was found in the mean PLTs counts of control group with the rest of groups.

**Haematocrit (HCT)**
The HCT value in control group was 37.5±0.17%, and in positive control group, it was 45.4±0.035%. Groups infected with C.maxima oil at the dose rate of 1ml/kg, 1.5ml/kg and 2ml/kg, the HCT values were 40.5±0.035%, 38±0.042% and 39.8±0.035%. The group that was infected with E.coli and treated with standard drugs (Moxifloxacin), the HCT value was 39.3±0.028%. A significant difference (P<0.05) was found in the mean HCT values of control group with the rest of groups.

**Red Cell Distribution Width (RDWC)**
At day 9, the RDWC level in control group and positive control group were 15.6±0.10% and 14.35±0.035%. Groups infected with Citrus maxima oil at the dose rate of 1ml/kg, 1.5ml/kg and 2ml/kg, the RDWC level were 15±0.035%, 14.6±0.035% and 14.0±0.035%. The group that was infected with E.coli and treated with standard drugs (Moxifloxacin), the RDWC level was 13.4±0.035%. A significant difference (P<0.05) was found in the mean RDWC level of control group with the rest of groups.

**DISCUSSION**
In the current study, the effect of E. coli was evident in all infected groups. Body weight was significantly decreased, where there was a significant increase in the counts of white blood cells (WBCs) and neutrophils and the lymphocytes level was decreased which may be due to the activation of the animal’s defence mechanism and the immune system. The haematocrit (HCT) and MCV level increased, haemoglobin (HGB)and RBCs counts decreased which may be due to increase in the rate of breakdown of the red cells.
Furthermore, the result of the study also indicates that during treatment of the tested animal with *Citrus maxima* oil the haemoglobin concentrations increased suggesting that the selected oils could assist in protecting the body against *E. coli* infection. Essential oils have great potential against bacteria, due to their antioxidant and antimicrobial properties. There is no reported data on the effect of *Citrus maxima* oil in rabbits against *E. coli* infection. This is the first time that these oil has been used against *E. coli* and showed better effect on the haematological parameters of rabbits.

**CONCLUSION**

On the basis of the obtained results, it may be concluded that:

1. Rabbits infected with *E. coli* have hematologic alterations on RBCs, WBCs, HCT, MCV, haemoglobin, lymphocytes, platelets, and neutrophils.
2. Oil of *Citrus maxima* has its own chemical composition, which may be correlated with its antibacterial activities.
3. *Citrus maxima* oil showed better antimicrobial activity against the *E. Coli* infection in rabbits
4. Oil of *Citrus maxima* can be used as an alternative antibacterial medicine.

**RECOMMENDATION**

The present study recommends additional in-vivo studies and clinical trials to develop novel antimicrobial agents in this era of antimicrobial resistance.

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**CONFLICT OF INTEREST**

The authors declare that they have no potential competing interest.

**AUTHORS’ CONTRIBUTION**

Nisar Ahmad and Ashfaq Ahmad performed the experimental work. Muhammad Bilal and Farooq Akbar Khan took part in the care of the animals and the collection of blood samples. Muhammad Shahab Khan supervised the entire project and wrote the manuscript. All authors read and approved the manuscript for submission.

**ETHICAL CLEARANCE**

The current study was approved by the Ethical Committee, Department of Zoology, University of Malakand, KPK Pakistan.

**REFERENCES**
