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

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# ABSTRACT

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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. coliat the dose rate of 2.0 x 10<sup>10</sup> CFU without medication, group 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> 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, Hematological parameters, Medicinal plant

# INTRODUCTION

The increasing bacterial conflict to antibiotics has developed a growing APPREHENSION GLOBALLY.<sup>1</sup>With the appearance and growth of microorganisms such as gram negative (E.coli) bacteria which can cause serious infections.<sup>2</sup>There are a number of drugs available to treat gram-positive bacteria, but less in number to treat gram-negative bacteria.<sup>3</sup>In addition, high cost and adverse effects are commonly related to widespread synthetic antibiotics are a chief burning global issue in considering infectious diseases.<sup>4</sup> Infectious diseases pose serious problems to health and they are the main cause of morbidity and mortality worldwide.5 The increasing prevalence of antibiotic-resistant microorganisms, has made it necessary to replace alternative sources of antibiotic products.<sup>6</sup>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.7

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.8 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.<sup>9</sup>*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.<sup>10</sup> *E.coli*, contaminate food and water supplies.<sup>11</sup>

Plants play an important role in human health because they produce a wide array of bioactive molecules which have medicinal values.<sup>12</sup> Despite the efforts in producing a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased.<sup>13</sup> 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*.<sup>14</sup> The oil of this fruit has been reported to possess some nutritive and medicinal potentials.<sup>15</sup>

# **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 genderswas 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, 1<sup>st</sup>group (negative control), 2<sup>nd</sup>group infected with *Escherichia coli* orally at the dose rate of  $2x10^{10}$  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  $2x10^{10}$  CFU and was treated with *Citrus maxima* oil at a dose rate of 1.5ml, 5th group, infected with *E. coli* at the dose rate of  $2x10^{10}$  CFU and was fed with *Citrus maxima* oil at a dose rate of 1.5ml, 5th group, infected with *E. coli* at the dose rate of  $2x10^{10}$  CFU and fed with *Citrus maxima* oil at a dose rate of  $2x10^{10}$  CFU and fed with *Citrus maxima* oil at a dose rate of  $2x10^{10}$  CFU and fed with *Citrus maxima* oil at a dose rate of  $2x10^{10}$  CFU and fed with *Citrus maxima* oil at a dose rate of  $2x10^{10}$  CFU and fed with *Citrus maxima* oil at a dose rate of  $2x10^{10}$  CFU and fed with *Citrus maxima* oil at a dose rate of  $2x10^{10}$  CFU and fed with *Citrus maxima* oil at a dose rate of  $2x10^{10}$  CFU and fed with *Citrus maxima* oil at a dose rate of  $2x10^{10}$  CFU and fed with *Citrus maxima* oil at a dose rate of  $2x10^{10}$  CFU and was fed with *E.coli* at the dose rate of  $2x10^{10}$  CFU and was fed with *E.coli* at the dose rate of  $2x10^{10}$  CFU and was fed with *E.coli* at the dose rate of  $2x10^{10}$  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* ( $2x10^{10}$  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 3<sup>rd,</sup> 4<sup>th</sup> and 5<sup>th</sup> were treated with *Citrus maxima* oil at a dose rate of 1ml, 1.5ml and 2ml, while group 6<sup>th</sup> 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. 1<sup>st</sup> group was kept as negative control, neither infected nor medicated, 2<sup>nd</sup> group served as a positive control which was infected, but not treated 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> group were treated with *Citrus maxima* oil at a dose rate of 1ml, 1.5ml and 2ml and group 6<sup>th</sup> 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).

#### **1**<sup>st</sup>Group (negative control)

About 3ml blood was collected from the animals. The TRBCs of negative control was 5. 73±0. 028x10<sup>6</sup>/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 10<sup>3</sup> /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 10<sup>3</sup>/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  $5.08\pm0.033\times10^{6}/\mu$ l and  $9.81\pm0.13\%$ . Lymphocytes count was  $28.3\pm0.31\%$ . MCH and MCHC level were  $19.7\pm0.41$ Pg and  $30.5\pm0.16$ gm/dl. The MCV, WBCs and Neutrophils count were in the range of  $50.6\pm0.12$ pg,  $5.5\pm0.045\times10^{3}$  /µland  $62.6\pm0.25\%$ . Platelets level was  $528\pm0.11\times10^{3}/\mu$ l and HCT value were  $36.8\pm0.10\%$ .

#### **3rd Group three (low dose)**

Before infection the RBCs and haemoglobin value were in the range of  $5.70\pm0.17x \ 10^6$  /µl and  $9.45\pm0.057\%$ . The MCH value was  $21.45\pm0.14pg$  and MCHCs value was  $33.03\pm0.081gm/dl$  while MCV was in  $50.76\pm0.072pg$  range. WBCs, lymphocytes and neutrophil count were  $5.71\pm0.036x$  $10^3$  /µl,  $27.05\pm0.26\%$  and  $62.53\pm0.19\%$ . HCT value was  $37.46\pm0.92\%$ , platelets count was  $531.52\pm0.27x \ 10^3$  /µl while RDWC level was  $15.56\pm0.095\%$ .

#### 4<sup>th</sup>Group (medium dose)

RBC and haemoglobin of group 4<sup>th</sup> before infection were  $5.73\pm0.23 \times 10^6/\mu l$  and  $9.52\pm0.27\%$ . Concentration of MCH was  $21.48\pm0.33$ pg while MCHC was  $32.95\pm0.12$ gm/dl. The level of MCV and WBCs were in the range of  $50.74\pm0.12$ pg and  $5.71\pm0.076 \times 10^3/\mu l$ . Lymphocytes, neutrophils and platelets count were  $28.96\pm0.17\%$ ,  $61.47\pm0.44\%$  and  $525.50\pm0.14 \times 10^3/\mu l$  while HCT and RDWC values were in the range of  $37.50\pm0.14\%$ , and  $15.60\pm0.26\%$ .

#### 5<sup>th</sup>Group (high dose)

The RBC count was  $5.76\pm0.074x \ 10^6 \ \mu$ l while haemoglobin value was  $9.75\pm0.38\%$ . The values of MCH, MCHC and MCV were  $21.51\pm0.25$ pg,  $32.95\pm0.25$ gm/dl and  $50.69\pm0.16$ pg. WBCs count was  $5.71\pm0.036x \ 10^3 \ \mu$ l and lymphocytes count was  $28.08\pm0.28\%$  while neutrophil count was  $60.44\pm0.36\%$ . Platelets, HCT and RDWC level were  $530.54\pm0.087x \ 10^3 \ \mu$ l,  $37.53\pm0.15\%$  and  $15.57\pm0.21\%$ .

#### 6<sup>th</sup>Group (standard drug)

The RBCs and haemoglobin values were  $5.78\pm0.77x$  10<sup>6</sup> /µl and  $9.81\pm0.084\%$ . The concentration of MCH was  $21.56\pm0.036$ pg and MCHC was  $33.02\pm0.17$ gm/dl. MCV, WBCs and lymphocytes count were  $50.72\pm0.34$ pg,  $5.73\pm0.045x$  10<sup>3</sup> /µl and 27.03±0.13% while neutrophil count was  $61.52\pm0.27\%$ . Platelets count was  $524.53\pm0.053x$  10<sup>3</sup>/µl, HCT value was  $37.48\pm0.17\%$ .

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

Parameters	Negative control group	positive control group	3 <sup>rd</sup> group	4 <sup>th</sup> group	5 <sup>th</sup> group	6 <sup>th</sup> group
RBC (X10 <sup>6</sup> /µl)	5.73±0.028	5.8±0.033	5.70±0.17	5.73±0.23	5.76±0.074	5.78±0.77
Hb(gm/dl)	9.52±0.035	9.81±0.13	9.45±0.057	9.52±0.27	9.75±0.38	9.81±0.084
MCH (pg)	21.49±0.13	19.7±0.41	21.45±0.14	21.48±0.33	21.51±0.25	21.56±0.03
MCHC (gm/dl)	33.0±0.848	30.5±0.16	33.03±0.08	32.95±0.12	32.95±0.25	33.02±0.17
MCV (pg)	50.7±0.070	50.6±0.12	50.76±0.07	50.74±0.12	50.69±0.16	50.72±0.34
WBC (X10 <sup>3</sup> /µl)	5.72±0.035	5.5±0.045	5.71±0.025	5.71±0.036	5.71±0.076	5.73±0.045
L (%)	27±0.494	28.3±0.31	27.05±0.26	28.96±0.17	28.08±0.28	27.03±0.13
N (%)	61.5±0.707	62.6±0.25	62.53±0.19	61.47±0.44	60.44±0.36	61.52±0.27
PLT (103/µl)	530.5±0.03	528±0.11	531.52±0.2	525.50±0.14	530.54±0.087	524.53±0.0
HCT (%)	37.5±0.17	36.8±0.10	37.46±0.92	37.50±0.14	37.53±0.15	37.48±0.17
RDWC (%)	15.6±0.10	15.9±0.12	15.56±0.09	15.60±0.26	15.57±0.21	15.63±0.16

#### **During Infection**

The rabbits were infected with pathogenic *E. coli* at the dose rate  $2x10^{10}$  CFU, except group 1<sup>st</sup> (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  $3.47\pm0.042 \times 10^6$  /µl and  $6.82\pm0.042\%$ . Neutrophils and Platelets count were  $66.4\pm0.042\%$  and  $435\pm0.042 \times 10^3$ /µl.

The MCH value were20.53 $\pm$ 0.042pg and the MCHC were 28.15 $\pm$ 0.070gm/dl. The lymphocyte, WBCs and MCV count were 23.6 $\pm$ 0.035%, 8.22 $\pm$ 0.035x 10<sup>3</sup>/µl and 59.53 $\pm$ 0.028pg. The HCT value was 41.62 $\pm$ 0.028%. The level of RDWC was 15.42 $\pm$ 0.10% during infection.

# **3rd Group (low dose)**

This group was also infected with *E. coli*, RBC count was  $3.44\pm0.028\times10^{6}$ /µl and haemoglobin value was  $6.74\pm0.035\%$ . The MCH and MCHC values were  $20.50\pm0.035$ pg and  $28.17\pm0.035$ gm/dl. The MCV, WBCS and lymphocytes count

were 66.56±0.622pg, 8.17±0.014x 10<sup>3</sup>/µl and 25.62±0.042% while HCT value was 41.64±0.056%. The level of RDWC was  $15.45\pm0.24\%$ , the neutrophils and platelets count were  $65.41\pm0.414$  %  $491.04\pm0.21x$   $10^{3}/\mu$ l.

# 4<sup>th</sup> Group (medium dose)

RBCs and haemoglobin count were 3.48±0.21x 10<sup>6</sup> /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 count were 57.52±0.11pg, 24.64±0.49 %, 7.4±0.07x 10<sup>3</sup>/µl and HCT value was 42.66±0.056 %. Platelets and neutrophils count were 54.43.8 $\pm$ 0.4, % 444.03 $\pm$ 0.07x 10<sup>3</sup>/µl. The RDWC level was 15.45±0.24% during infection.

The values of MCH and MCHC were 20.52±0.14pg and 28.20±0.28 gm/dl. MCV, WBCs and lymphocytes count were  $56.55\pm0.11$  pg,  $8.33\pm0.070$  x  $10^3$  /µl and 23.65±0.049%. Platelets and neutrophils count were  $467.05\pm0.28 \times 10^{3} / \mu l$  and  $67.41\pm0.035\%$ . The HCT value was 41.65±0.29 %.

# 6<sup>th</sup> Group (standard drug)

In this group the RBCs and haemoglobin values were 3.51±0.014x 106 /µl and 6.98±0.070%. MCH value was 20.54±0.14pg and MCHC was 28.22±0.28gm/dl. The lymphocyte and MCV count were 21.66±0.084% and 55.2±0.11pg. During infection. Platelets and neutrophils count were  $429.06 \pm 0.28 \times 10^3 / \mu l$  and  $54.44 \pm 0.141 \%$ .

# 5<sup>th</sup> Group (high dose)

RBCs count was 3.5±0.014x 10<sup>6</sup>/µl while haemoglobin value 6.90±0.14%.

Table 2: Haeillatological	parameters in din	erent groups c	of raddits at t	iiira aay	
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Parameters	Negative control group	Positive con- trol group	3rd group	4th group	5th group	6th group
RBC(X10 <sup>6</sup> /µl)	5.73±0.028	3.47±0.042	3.44±0.028	3.48±0.21	3.5±0.014	3.51±0.014
	A	B	B	B	B	B
Hb(gm/dl)	9.52±0.035	6.82±0.042	6.74±0.035	6.85±0.14	6.90±0.14	6.98±0.070
	A	B	C	C	C	C
MCH(pg)	21.49±0.034	20.53±0.042	20.50±0.035	20.49±0.14	20.52±0.14	20.54±0.14
	A	B	C	B	D	B
MCHC(gm/dl)	33±0.048	28.15±0.070	28.17±0.035	28.18±0.28	28.20±0.28	28.22±0.28
	A	B	C	B	C	D
MCV(pg)	50.7±0.070	59.53±0.028	66.56±0.622	57.52±0.11	56.55±0.11	55.52±0.11
	A	B	C	B	B	B
WBC (x10 <sup>3</sup> /µl)	5.72±.035	8.22±0.035	8.17±0.014	7.4±0.07	8.33±0.070	8.16±0.070
	A	B	B	C	B	B
L(%)	27.80±0.094	23.6±0.035	25.62±0.042	24.64±0.49	23.65±0.049	21.66±0.084
	A	B	C	B	D	E
N(%)	61.5±0.707	66 .4±0.042	65±0.014	66.43.8±0.14	67.41±0.035	69.44±0.141
	A	A	A	A	A	A
PLT(10 <sup>3</sup> /µl)	530.5±0.035	435±0.042	491.04±0.21	444.03±0.07	467.05±0.28	434.06±0.28
	A	B	C	D	E	B
HCT(%)	37.5±0.17	41 .62±0.028	41.64±0.056	42.66±0.59	41.60.29	44.65±0.29
	A	B	C	D	E	F
RDWC(%)	15.6±0.10	15.42±0.10	15.44±0.106	15.45±0.24	15.42±0.17	15.46±0.24
	A	B	A	A	A	A

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

# **DURING TREATMENT**

#### **Total Red Blood Cells (TRBCs)**

At day six, the TRBCs count in negative control group was  $5.73\pm0.028 \times 10^6 /\mu$ l, the positive control group was  $4.54\pm0.028 \times 10^{6}$ /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  $10^{6}/\mu$ l, 5.40±0.042x 10<sup>6</sup>/µl and 4.04±0.028x 10<sup>6</sup>/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<sup>6</sup>/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  $9.52\pm0.035\%$  and  $8.85\pm0.035\%$ . 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  $10.12\pm0.028\%$ ,  $9.0\pm0.042\%$  and  $8.28\pm0.035\%$ . The group that was infected with *E.coli* and treated with standard drugs (Moxifloxacin), the haemoglobin value was  $9.6\pm0.035\%$ . 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 6<sup>th</sup>, 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  $18.51\pm0.028pg$ ,  $19.49\pm0.042pg$  and  $19.7\pm0.035pg$ . That group which was infected with *E.coli* and treated with standard drug (Moxifloxacin HCl), the MCH value was  $19.7\pm0.035pg$ . 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  $33.0\pm0.848$ gm/dl, the positive control group was  $27.5\pm0.042$ gm/dl. In groups, that were infected with *E.coli* and treated with *Citrus maxima* oil at the dose rate of 1ml/kg, 1.5ml/kg and 2ml/kg, the MCHC values were  $30.7\pm0.028$ gm/dl,  $30.9\pm0.035$ gm/dl and  $30.20\pm035$  gm/dl. The group which was infected with *E.coli* and treated with standard drug (Moxifloxacin), the MCHC value was  $30.9\pm0.035$  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 6<sup>th</sup>, the MCV value was 50.7 $\pm$ 0.070pg, the positive control group was 66.7 $\pm$ 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 $\pm$ 0.064pg, 56 $\pm$ 0.035pg and 58.4 $\pm$ 0.035pg. That group which was infected with *E.coli* and treated with standard drug (Moxifloxacin), the MCV value was 56.5 $\pm$ 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  $5.72\pm0.035 \times 10^3$  / µl, the positive control group was  $8.36\pm0.042 \times 10^3$  /µl. In

groups, that were infected with *E. coli* and treated with *Citrus maxima* oil at the dose rate of 1ml/kg, 1.5ml/kg and 2ml/kg, the RBCs counts were  $7.14\pm0.028 \times 10^3/\mu$ l,  $7.05\pm0.042 \times 10^3/\mu$ l and  $7.11\pm0.035 \times 10^3/\mu$ l. The group which was infected with *E.coli* and treated with standard drug (Moxifloxacin HCl), the WBCs count was  $7.03\pm0.028 \times 10^3/\mu$ l. 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 6<sup>th</sup>, the lymphocytes count in control group was  $28.80\pm0.494\%$ , the level of positive control group was  $21.8\pm0.042\%$ . 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  $26.6\pm0.028\%$ ,  $25.3\pm0.035\%$  and  $26.4\pm0.042\%$ . That group which was infected with *E.coli* and treated with standard drugs (Moxifloxacin HCl), the lymphocytes count was  $24.2\pm0.035\%$ . 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  $62.5\pm0.707\%$ , the value of positive control group was  $69.3\pm0.035\%$ . 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  $64.3\pm0.028\%$ ,  $65.6\pm0.035\%$  and  $64.3\pm0.028\%$ . While the group which was infected with *E.coli* and treated with standard drugs (Moxifloxacin HCl), the neutrophils count was  $66.7\pm0.028\%$ . 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  $530.5\pm0.035 \times 10^3 /\mu l$ , the positive control group was  $429\pm0.035 \times 10^3 /\mu l$ . 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  $470\pm0.071 \times 10^3 /\mu l$ ,  $465\pm0.035 \times 10^3 /\mu l$  and  $490\pm0.035 \times 10^3 /\mu l$ . That group which was infected with *E.coli* and treated with standard drugs (Moxifloxacin), the PLTs count was  $488\pm0.035 \times 10^3 /\mu l$ . 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 6<sup>th</sup>, the HCT value in control group was 37.5 $\pm$ 0.17%, the positive control group was 41.2 $\pm$ 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 $\pm$ 0.035%, 40.66 $\pm$ 0.042% and 40.63 $\pm$ 0.035%. The group which was infected with *E.coli* and treated with standard drugs (Moxifloxacin HCl), the HCT value was 39.5 $\pm$ 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)**

In control group, the RDWC level was  $15.6\pm0.10\%$ , in positive control group was  $15.5\pm0.035\%$ . 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  $16.2\pm0.035\%$ ,  $14.3\pm0.035\%$  and  $14.1\pm0.42\%$ . While the group which was infected with *E.coli* and treated with standard drugs (Moxifloxacin HCl), the RDWC level was  $14.5\pm0.071\%$ . 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

Parameters	Negative control group	Positive control group	3 <sup>rd</sup> group	4 <sup>th</sup> group	5 <sup>th</sup> group	6 <sup>th</sup> group
RBC(x10 <sup>6</sup> / <sup>µl</sup>	5.73±0.028	4.54±0.028	6.26±0.028	5.40±0.042	4.04±0.028	5.83±0.035
	A	B	C	D	E	F
HGB(g/dl)	9.52±0.035	8.85±0.035	10.12±0.028	9.0±0.042	8.28±0.035	9.6±0.035
	A	B	C	A	D	A
MCH(Pg)	21.1±0.034	17.8±0.042	18.51±0.028	19.49±0.042	19.7±0.035	19.7±0.035
	A	B	B	C	D	D
MCHC(g/dl)	33.0±0.048	27.5±0.042	30.7±0.028	30.9±0.035	30.20.035	30.9±0.035
	A	B	B	B	B	B
MCV(Pg)	50.7±0.070	66.7±0.035	57.5±0.064	56±0.035	58.4±0.035	56.5±0.042
	A	B	C	D	C	D
WBC(x10 <sup>3</sup> / <sup>µl</sup> )	5.72±0.035	8.36±0.042	7.14±0.028	7.05±0.042	7.11±0.035	7.03±0.028
	A	B	C	C	C	C
LYM(%)	28.80±0.094	21.8±0.042	26.6±0.028	25.3±0.035	26.4±0.042	24.2±0.035
	A	B	C	C	D	E
N (%)	62.5±0.707	69.3±0.035	64.3±0.028	65.6±0.035	64.3±0.028	66.7±0.028
	A	B	C	C	D	D
PLT(103/µl)	530.5±0.035	429±0.035	470±0.071	465±0.035	490±0.035	488±0.035
	A	B	C	D	E	F
HCT (%)	37.5±0.017	41.2±0.035	39.0±0.035	40.66±0.042	40.63.2±0.035	39.5±0.028
	A	B	C	D	D	E
RDWC (%)	15.6±0.10	15.5±0.035	16.2±0.035	14.3±0.035	14.1±0.42	14.5±0.071
	A	A	A	A	A	A

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  $5.73\pm0.028 \times 10^6$  /µl, and in positive control group, it was  $3.47\pm0.042 \times 10^6$  / µl. Groups infected with *Citrus maxima* oil at the dose rate of 1ml/kg, 1.5ml/kg and 2ml/kg, the RBCs counts were  $6.25\pm0.028 \times 10^6$  /µl,  $5.06\pm0.035 \times 10^6$  /µl and  $5.52\pm0.028 \times 10^6$  /µ. The group that was infected with *E. coli* and treated with standard drugs (Moxifloxacin), the RBCs count was  $5.54\pm0.035 \times 10^6$  /µl. A significant difference (P<0.05) was found in the mean RBCs counts of control group with the rest of groups.

# Haemoglobin (Hb)

It was observed in control group, that the Hb value was  $9.52\pm0.29\%$ , the value of positive control group was

 $6.82\pm0.039\%$ . Groups infected with *C.maxima* oil at the dose rate of 1ml/kg, 1.5ml/kg and 2ml/kg, the Hb value were  $10.11\pm0.028\%$ ,  $8.16\pm0.035\%$  and  $8.86\pm0.035\%$ . That group which was infected with *E.coli* and treated with standard drug (Moxifloxacin), the haemoglobin value was  $8.9\pm0.035\%$ . 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  $21.1\pm0.13$ pg and  $17.1\pm0.042$ pg. Groups infected with *C.maxima* oil at the dose rate of 1ml/kg, 1.5ml/kg and 2ml/kg, the MCH values were  $21.1\pm0.028$ pg,  $20.49\pm0.042$ pg and  $20.52\pm0.035$ pg. The group that was infected with *E.coli*and treated with standard drugs (Moxifloxacin), the MCH value was  $20.54\pm0.035$ pg. 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  $33.0\pm0.8$ gm/dl, and in positive control group, it was  $28.5\pm0.028$ gm/dl. Groups infected with *Citrus maxima* oil at the dose rate of 1ml/kg, 1.5ml/kg and 2ml/kg, the MCHC values were  $32.6\pm0.035$ gm/dl,  $31\pm0.035$ gm/dl and  $31.3\pm0.672$ gm/dl. The group that was infected with *E. coli* and treated with standard drugs (Moxifloxacin), the MCHC value was  $31.8\pm0.035$ gm/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  $50.7\pm0.7$  pg and  $67.2\pm0.035$ pg. Groups infected with *Citrus maxima* oil at the dose rate of 1ml/kg, 1.5ml/kg and 2ml/kg, the MCV values were  $55.8\pm0.028$ pg,  $52.3\pm0.035$ pg and  $55\pm0.035$ pg. That group which was infected with *E. coli* and treated with standard drugs (Moxifloxacin), the MCV value was  $53.9\pm0.042$ pg. 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  $5.7\pm0.03 \times 10^3/\mu$ l, and in positive control group, it was  $8.41\pm0.042 \times 10^3/\mu$ l. Groups infected with *C.maxima* oil at the dose rate of 1ml/kg, 1.5ml/ kg and 2ml/kg, the RBCs counts were  $6.51\pm0.028 \times 10^3/\mu$ l,  $5.82\pm0.042 \times 10^3/\mu$ l and  $5.96\pm0.035 \times 10^3/\mu$ l. The group that was infected with *E.coli* and treated with standard drugs (Moxifloxacin), the WBCs count was  $6.02\pm0.028 \times 10^3/\mu$ l. 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  $28.0\pm0.49\%$ , and in positive control group, it was  $19.6\pm0.042\%$ . Groups infected with *Citrus maxima* oil at the dose rate of 1ml/kg, 1.5ml/kg and 2ml/kg, the lymphocytes counts were  $27.3\pm0.028\%$ ,  $27\pm0.035\%$  and  $26.8\pm0.042\%$ . That group which was infected with *E. coli* and treated with standard drugs (Moxifloxacin), the lymphocytes count was  $28.6\pm0.035\%$ . 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  $62.5\pm0.72\%$  and  $70.4\pm0.035\%$ . Groups infected with *C.maxima* oil at the dose rate of 1ml/kg, 1.5ml/

kg and 2ml/kg, the neutrophils counts were  $63.6\pm0.028\%$ ,  $61.51\pm0.035\%$  and  $62.6\pm0.028\%$ . The group that was infected with *E.coli* and treated with standard drug (Moxifloxacin), the neutrophils count was  $61.8\pm0.028\%$ . 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  $530\pm2.0 \times 10^3$ /µl, and in positive control group, it was  $395\pm0.028 \times 10^3$ /µl. Groups infected with *C.maxima* oil at the dose rate of 1ml/kg, 1.5ml/ kg and 2ml/kg, the PLTs counts were  $495\pm0.042 \times 10^3$ /µl,  $520\pm0.035 \times 10^3$ /µl and  $515\pm0.035 \times 10^3$ /µl. That the group which was infected with *E.coli* and treated with standard drugs (Moxifloxacin), the PLTs count was  $517\pm0.035 \times 10^3$ / µl. A significant difference (P<0.05) was found in the mean PLTs counts of control group with the rest of groups.

#### **Haematocrit (HCT)**

The HCT value in control group was  $37.5\pm0.17\%$ , and in positive control group, it was  $45.4\pm0.035\%$ . Groups infected with *C.maxima* oil at the dose rate of 1ml/kg, 1.5ml/kg and 2ml/kg, the HCT values were  $40.5\pm0.035\%$ ,  $38\pm0.042\%$  and  $39.8\pm0.035\%$ . The group that was infected with *E.coli* and treated with standard drugs (Moxifloxacin), the HCT value was  $39.3\pm0.028\%$ . 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  $15.6\pm0.10\%$  and  $14.35\pm0.035\%$ . Groups infected with *Citrus maxima* oil at the dose rate of 1ml/kg, 1.5ml/kg and 2ml/kg, the RDWC level were  $15\pm0.035\%$ ,  $14.6\pm0.035\%$  and  $14.0\pm0.035\%$ . The group that was infected with *E.coli* and treated with standard drugs (Moxifloxacin), the RDWC level was  $13.4\pm0.035\%$ . 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.

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