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Protective Effect of Selenium and Curcumin against *Pasteurella multocida* Challenged Hematological and Biochemical Alterations Leading to Hepato-Renal Injury in Rabbit

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Key words
Selenium, Curcumin, *Pasteurella multocida*, Oxidative stress, Hepato-renal injury

ABSTRACT

The current study investigated the role of selenium (Se) and curcumin (Cur) in alleviating the severity of *Pasteurella multocida (P. multocida)* infection associated with hematological and biochemical variations. Twenty rabbits were randomly assigned into four groups (n=5/group). Except group A (Negative control), the rabbits in all groups (B, C and D) were challenged with 0.2 ml *P. multocida* (2× 10^5 colony forming unit (CFU)/ml) via intranasal route. After 24 h of challenge, rabbits were seen depressed and off feed, showing the signs of sneezing, nasal discharge and oral ventilation. Afterwards, group B was kept infected and untreated (Positive control), group C was treated with Cur at a dose 13 g/kg diet and D was treated with Se at the dose 0.5mg/kg diet for 14 days. On days 7th and 14th blood samples were collected for hematology, biochemical hepatic renal markers and indicators of oxidative stress. The challenged rabbits showed increase in leukogram and decrease in erythrogram, total protein, albumin, globulin and total lipid. Moreover, biomarkers of the hepato-renal injury such as aspartate aminotransferase (AST), alanine transaminase (ALT), lactate dehydrogenase (LDH), urea and creatinine were increased in group B than A. The treated groups, after infection causes alteration in such changes in C and D respectively. Infected rabbits showed elevated serum MDA concentration and reduction in glutathione peroxidase and Superoxide dismutase activity in group B than A. The treatment of Cur and Se alleviated the altered findings in group C and D. In conclusion, the treatment of Cur and Se ameliorate *P. multocida* induced oxidative stress and endorsed antioxidant role followed by improving biochemical analysis, hepatic and renal biomarkers. However, Se provided better protection against infection presenting improved anti-bacterial properties than Cur.

INTRODUCTION

Pasteurella multocida, a gram-negative non-motile coccobacillus is an opportunist or secondary pathogen and commonly present in the respiratory tract of animals (Dziva *et al*., 2008). This organism can cause fowl cholera in poultry, hemorrhagic septicemia (HS) in cattle, atrophic rhinitis in swine (Arumugam *et al*., 2011; Guo *et al*., 2012). However, in rabbits pasteurellosis is the disease caused by *P. multocida* (Takashima *et al*., 2001) and is recognized by showing the clinical signs such as respiratory distress, rhinitis, pneumonia, septicemia and abscess. In order to evaluate the pathogenic effect of *P. multocida*, number of studies have been conducted on the effect of *P. multocida* infection in lab as well as domestic animals. Experimentally, successful inoculation of *P. multocida* through different route (Intranasal/ subcutaneous) and different bacterial concentration in turkey (3.3 × 10^8 CFU/

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ml), rabbit (1×10^7 and $2/3\times10^5$ CFU/ml, respectively) (Alam *et al*., 2018; Abdallah *et al*., 2020; El-Sheikh *et al*., 2020) and pigs (3×10^5-10^8 CFU/ml) (Oliveira *et al*., 2015) have showed biochemical alteration in blood and pathological conditions in different parts of the body during necropsy findings. The infection of *P. multocida* in rabbits induce damages to the liver and kidney via showing biochemical changes in hepato-renal biomarkers such as alanine transaminase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), urea and creatinine concentrations (El-Sheikh *et al*., 2020). Furthermore, serum oxidative markers including melondialdehyde (MDA) concentration and glutathione peroxidase (GSH-Px) and superoxide dismuatse (SOD) activities are altered in male rabbits (Alam *et al*., 2018).

The infectious effect of *P. multocida* is endemic and therefore is a predisposing source of considerable potential loss. Preventive practices such as improved husbandry and the culling of affected animals may lessen the rate of morbidity and mortality, but requires substantial expenditures. Generally, vaccines are the primary source to target the bacterial infection but under field conditions, they do not provide complete protection (Dabo *et al*., 2008). On the other hand, antibiotic residues in animals affects the animal products such as meat, milk and eggs (Quesada *et al*., 2013), therefore the use of antibiotics is also restricted. Considering these facts, the use of herbal agents can provide the prosperity of multiple sources to deal with drug resistance (Narayanan *et al*., 2011; Potroz and Cho, 2015). Number of researches have elaborated that natural ingredients retain a variety of biological activities, (Abdellatief *et al*., 2017; El-Hawary *et al*., 2019) can efficiently manage diseases and mitigate different toxicities (Galal *et al*., 2016; Gupta and Birdi, 2017; Hassanin *et al*., 2020). *P. multocida* infection treated with different herbal agents such as grape seed (El-Sheikh *et al*., 2020) and allicin (Alam *et al*., 2018) attenuates serum inflammation and oxidative stress (OS) with simultaneous improvement in blood parameters in rabbits.

Selenium (Se) is an essential trace element, certainly required in small amount for a wide range of physiological activities in livestock. The element is an integral component of several enzymes named as seleno-enzymes. GSH-Px is an important seleno-enzyme, which plays a significant role in anti-oxidation system and protects the cell from being damaged by free radicals (Mehdi *et al*., 2013; Saha *et al*., 2016). Free radicals are the by-products of oxygen metabolism and contribute in cellular damage (Samo *et al*., 2018). GSH-Px together with SOD and catalase (CAT) provides first-line antioxidation defense system in animals. Primarily, the enzyme SOD which is normally found in the cytoplasm, converts high reactive radicals such as superoxide ($O_2^{\bullet-}$) into less harmful product i.e H_2O_2. Secondly, GSH-Px and/or CAT splits H_2O_2 into water and molecular oxygen (Zoidis *et al*., 2018). Excessive production of oxidants and insufficient supply of antioxidants develops an imbalance that causes the condition called oxidative stress (OS) (Saha *et al*., 2016; Samo *et al*., 2018). Several studies have demonstrated that Se could ameliorate aflatoxin (AFB_1) induced histopathological lesions in spleen (Wang *et al*., 2013), thymus (Chen *et al*., 2013) and bursa of fibricous (BF) (Chen *et al*., 2014) in broilers, respectively. Furthermore, hepatic injury in mice (*Schistosomamansoni*-induced) (Dkhil *et al*., 2016) and aortic injury in rabbit (High fat diet-induced) (Mehta *et al*., 2013) have also been alleviated by the use of Se. Besides, the synergistic effect of Se with copper affects the blood parameters with ultimate reduction in blood oxidative stress in the buffalo calves introduced with *P. multocida* infection (Mudgal *et al*., 2018).

Curcumin (Cur) is a plant-derived substance isolated from turmeric. Since the herbs contains diverse biological activities and efficiently manage the diseases (Gupta and Birdi, 2017), therefore Cur used as herbal agent mitigate number of diseases. Several studies have confirmed the role of Cur, both *in vivo* and *in vitro* via showing its antioxidant, anti-inflammatory (Lestari and Indrayanto, 2014), anti-mutagenic, antimicrobial and anticancer properties (Vera-Ramirez *et al*., 2013). Furthermore, antibacterial activity of Cur has been demonstrated against Gram positive and Gram negative bacteria by damaging bacterial cell wall or cell membrane (Praditya *et al*., 2019) or by targeting DNA and proteins (Zheng *et al*., 2020). In mice, AFB_1- induced toxicities were reduced by Cur via increasing the activity of antioxidant enzymes such as GSH-Px, SOD and CAT and decreasing lipid peroxidation in serum (Banach *et al*., 2014), liver (Panahi *et al*., 2016) and kidney (Limaye *et al*., 2018), respectively. Additionally, Abdallah *et al*. (2020) demonstrated protective role of Cur on thermally oxidized oil-induced hematological, biochemical and histopathological variations in rabbits. Various reports have given considerable attention to the protective effects of Se and Cur recently. Therefore, considering the beneficial effects of both agents, the scope of current study was designed to evaluate the role of Se and Cur against *P. multocida* induced hematological, biochemical alterations and serum oxidative markers related with hepatic and renal injury markers in rabbit.

MATERIALS AND METHODS

Selection and adaptation of animals

For this study twenty male rabbits at the age of 6-8 weeks, weighing approximelty 1000g ± body weight (BW)

were purchased from local market. The animals were kept at vaccine production unit (VPU) Tandojam, Sindh. Rabbits were reared in separate units and given one week of adaptation to the environment. Common basal diet and water *ad libitum* was offered to animals.

Isolation of bacterial strain and growth condition

The master seed of *P. multocida* type A was available in hemorrhagic septicemia (HS) section at VPU. Seed was thawed at room temperature, rehydrated with 1ml BactoTM brain–heart infusion (BHI) medium and incubated at 37^{0}C for 1 h. Rehydrated seed was injected in mice at the dose of 0.2ml subcutaneously. 24 h post infection, postmortem of dead mice was performed and the bacteria was recovered from the heart blood of infected rabbit. The organism was again cultivated on BHI broth and agar and incubated for 24 h at 37 ^{o}C. After assessing the purity of growth through grams staining procedure, the colonies were suspended in sterile saline and the density was adjusted at 2×10^5 colony forming unit (CFU)/ml (Alam *et al*., 2018) before inoculation to the experimental animals.

Preparation of curcumin

Natural turmeric was purchased from the local market. It was air dried and then macerated. Afterward the macerated material was extracted in water by using hot process and the supernatant was concentrated under condensed pressure. The concentrate powder was obtained by spray drying and then fed to animals (Kawasaki *et al*., 2015).

Selenium

Organic selenium in the form of selenium yeast (SY) obtained as by submerged fermenting saccharomyces cerevisiae in a Se rich media (Star Laboratories Pvt. Ltd.) was offered to the rabbits.

Experimental design

After acclimatization research animals were divided into four groups i.e. A, B, C and D (n=5/group). Group A was administered with 0.2 ml sterile saline intranasally and assumed as negative control on day 1st of experiment. On the same day, the rabbits in other groups (B, C and D) were intranasally inoculated with 0.2 ml *P. multocida* (Edrees *et al*., 2017) infection. After 24 h challenge with infection, rabbits were seen depressed and off feed, showing the signs of sneezing, nasal discharge and oral ventilation. Subsequently, group B was kept infected and untreated, assumed as positive control. Group C was treated with Cur at a dose 13 g/kg diet (Azza *et al*., 2011) and group D was treated with Se at the dose 0.5 mg/kg diet (Hu *et al*., 2018). The treatments were continued for 14 days. All the rabbits were monitored throughout the study.

Sample collection

Blood samples were collected from ear vein of rabbits on day 7th and 14th, respectively. For this the rabbit were properly restrained, ear vein was detected carefully and blood was collected in 2 portions. First portion was collected in a tube containing dipotassium salt of EDTA and used for hematological analysis. Simultaneously, second portion was collected in plain tube without anticoagulant. Samples were centrifuged at 3000 rpm for 10 min. Obtained serum was collected and preserved at -20 ^{o}C until analyzed for biochemical and oxidative stress parameters.

Hematological parameters

Erythrocyte number, hemoglobin (Hb) concentration, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), packed cell volume (PCV), total leukocyte count (TLC), and differential leukocyte number were analyzed by using hematology auto-analyzer (Abdallah *et al*., 2020).

Biochemical parameters

Serum total protein (g/dl), albumin (g/dl), globulin (g/dl), and total lipid (mg/dl) assessments were done by Biochemistry System International srl via G. Ferraris, 220 Arezzo-Italia kit methods.

Liver and kidney biomarkers

Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (Tietz, 1976) alkaline phosphatase (ALP) (Belfield and Glodberg, 1971), lactate dehydrogenase (LDH) (Buhl and Jackson, 1978), urea and creatinine were determined according to Vassault *et al*. (1986).

Oxidative stress parameters

Activities of serum glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and the malondialdehyde (MDA) content were analyzed by using commercial assay kits sourced from Nanging Jiancheng Bioengineering Institute (Nanjing China).

Statistical analyses

The data was investigated via statistical program by using SPSS 12.0 (Stata Soft, Tulsa, OK, USA) and calculated by a one-way ANOVA. Results were presented as Mean ± SEM. Differences were considered significant at $P < 0.05$.

RESULTS

During the experiment, rabbits in group B showed an

acute form of disease characterized by severe respiratory distress, conjunctivitis, sneezing. However, the rabbits introduced with the treatment of Cur (C) and Se (D) appeared with less severe clinical signs. At the end of experiment, treated groups (C and D) progressively returned to normal with little to no clinical signs as compared to non-treated infected group B.

Haematological parameters

Table I shows effect of Se and Cur on *P. multocida* induced hematological changes in rabbits. On days 7th and 14th, as compared to control (A) there was significant ($P < 0.05$) decrease in total RBC count, Hb concentration, MCV, MCHC and PCV % in infected (B). In contrast, treated groups (C and D) showed significant increase ($P < 0.05$) in these parameters as compared to B, respectively. However, the values between C and D were non-significant ($P > 0.05$).

On the other hand WBC, neutrophils and lymphocytes significantly increased ($P < 0.05$) in infected group (B) as compared to control (A) on days 7th and 14th respectively. Conversely, the Cur (C) and Se (D) treated groups showed significant decrease ($P < 0.05$) in such values as compared to group B respectively. However, the difference between C and D was non-significant ($P > 0.05$).

Biochemical parameters

Table II depicts the analysis of some biochemical parameters in rabbit. The levels of total protein, albumin, globulin and total lipid are significantly decreased ($P < 0.05$) in group B (infected, not treated) as compared to A. On the other hand, the groups infected and treated with Cur (C) and Se (D) significantly increased ($P < 0.05$) such values on days 7th and 14th respectively as compared to group B. Moreover, on day 14th the treatment groups (C and D) restored the level of albumin, globulin and total lipid to the level of control (A), showing non-significant difference among the groups ($P > 0.05$).

Table I. Effect of selenium and curcumin on *P. multocida* induced hematological changes in rabbit.

Parameters	Negative control A (n=5)	Positive control B (n=5)	Cur at 13g/kg diet C (n=5)	Se at 0.5 mg/kg diet D (n=5)
Day 7				
Total RBC (× 10^6/µl)	5.33 ± 0.04^a	2.92± 1.33^c	4.01± 2.12^b	5.23± 0.66^a
Hemoglobin (g/dl)	12.28± 1.23^a	7.21± 4.33^c	10.11± 1.21^b	11.07± 3.11^b
MCV (fl)	78.9± 3.24^a	59.2± 0.23^c	70.22± 0.22^b	72.1± 2.04^b
MCHC (g/dl)	38.1± 3.71^a	31.3± 0.01^c	34.24± 2.11^b	35.76± 0.98^b
PCV (%)	38.22± 7.21^a	28.67± 2.43^c	33.3± 2.37^b	33.67± 0.67^b
WBC (× 10^3/ µl)	5.21± 2.41^c	8.19± 2.31^a	7.11± 1.26^b	6.76± 0.94^b
Neutrophil (%)	41.21 ± 0.11^b	48.01± 0.73^a	34.77± 0.37^c	32.21± 0.34^c
Lymphocyte (%)	47.33± 0.22^c	59.21± 2.11^a	54.56± 0.23^b	52.45± 0.21^b
Day 14				
Total RBC (× 10^6/µl)	6.55± 2.11^a	3.92± 4.33^c	5.55± 1.63^b	6.13± 1.67^{ab}
Hemoglobin (g/dl)	13.9± 0.32^a	9.2± 9.21^c	11.03± 3.22^b	11.2± 0.21^b
MCV (fl)	73.4± 7.32^a	56.2± 1.39^d	66.33± 2.11^b	68.3± 0.32^b
MCHC (g/dl)	46.1± 0.12^a	34.3± 5.32^c	39.24± 0.65^b	40.76± 2.22^b
PCV (% age)	41.22± 0.46^a	32.42± 0.78^c	40.52± 3.23^{ab}	42.22± 9.43^{ab}
WBC (× 10^3/ µl)	4.38± 1.09^c	6.19± 1.45^a	5.21± 2.33^b	5.33± 1.90^b
Neutrophil (%)	33.11± 0.21^c	43.17± 1.01^a	39.32± 0.32^b	38.32± 0.54^b
Lymphocyte (%)	63.21± 3.24^b	71.37± 4.11^a	56.12± 0.23^c	57.12± 0.57^c

RBC, red blood cells; MCV, mean corpuscle volume; MCHC, mean corpuscle hemoglobin concentration; PCV, packed cell volume; WBC, white blood cell. Rabbits were infected with 0.2ml *P. multocida* and treated with Se and Cur at the dose of 0.5mg/kg and 13g/kg feed diet respectively for 14 days. Values are represented as means ± SEM. Means within the same row carrying different superscripts ($^{a, b, c,}$ and ^{d}) are significant at $p < 0.05$.
Group A, negative control, administered with 0.2 ml saline intranasally.
Group B, positive control, administered with 0.2 ml *P. multocida*.
Group C, administered with 0.2 ml *P. multocida* and then treated with Cur at a dose of 13g/kg diet for 14 days.
Group D, administered with 0.2 ml *P. multocida* and then treated with Se at a dose of 0.5mg/kg diet for 14 days.

Table II. Effect of selenium and curcumin on *P. multocida* induced biochemical changes in rabbit.

Parameters	Negative control A (n=5)	Positive control B (n=5)	Cur at 13g/kg diet C (n=5)	Se at 0.5 mg/kg diet D (n=5)
Day 7				
Protein (g/dl)	5.54± 0.40^{a}	2.46± 0.66^{c}	4.54± 0.26^{b}	4.08± 0.51^{b}
Albumin (g/dl)	3.62± 0.39^{a}	2.51± 0.48^{c}	3.24±0.31^{b}	3.19± 0.08^{b}
Globulin (g/dl)	1.75± 0.13^{a}	1.21± 0.05^{b}	1.29± 0.17^{b}	1.89± 0.11^{a}
Lipid (mg/dl)	250.33± 8.92^{a}	168.67±8.56^{c}	198.67± 3.24^{b}	201.33±3.56^{b}
Day 14				
Protein (g/dl)	5.89± 0.23^{a}	3.42± 0.10^{d}	4.74±0.511^{c}	5.211±0.21^{b}
Albumin (g/dl)	3.72±0.71^{a}	2.13±0.19^{b}	3.43±0.46^{a}	3.59±0.19^{a}
Globulin (g/dl)	1.71± 0.06^{a}	1.27±0.05^{c}	1.76±0.03^{a}	1.82±0.04^{a}
Lipid (mg/dl)	260.67±7.02^{a}	201.56±3.71^{b}	249.00±4.58^{a}	255.33±4.13^{a}

Rabbits were infected with 0.2ml *P. multocida* and treated with Se and Cur at the dose of 0.5mg/kg and 13g/kg feed diet respectively for 14 days. Values are represented as means ± SEM. Means within the same row carrying different superscripts ($^{a, b, c}$ and ^{d}) are significant at $p < 0.05$. For details of groups, see Table I.

Table III. Effect of selenium and curcumin on *P. multocida* induced changes in hepatic and renal markers in rabbit.

Parameters	Negative control A (n=5)	Positive control B (n=5)	Cur at 13g/kg diet C (n=5)	Se at 0.5 mg/kg diet D (n=5)
Day 7				
AST	76.11± 7.21^{c}	109.54± 1.52^{a}	93.32± 9.53^{b}	90.21± 1.52^{b}
ALT	40.67± 2.51	76.33± 5.03^{a}	52.667± 3.78^{b}	54.01± 3.07^{b}
ALP	88.00± 4.58^{c}	109.18± 4.35^{a}	98.66± 2.51^{b}	97.67± 8.03^{b}
LDH	99.67± 1.53^{c}	120.03± 2.37^{a}	111.82± 3.21^{b}	108.21± 6.42^{b}
Urea	36.00± 7.22^{c}	64.33± 4.16^{a}	54.87± 4.35^{b}	52.21± 3.58^{b}
Creatinine	1.09± 0.09^{c}	2.48± 0.51^{a}	1.30± 0.04^{b}	1.26± 0.10^{b}
Day 14				
AST	74.022± 7.05^{c}	102.67± 3.21^{a}	97.33± 1.5^{b}	99.67± 5.13^{b}
ALT	47.33± 4.7^{c}	75.03± 8.88^{a}	66.66± 1.15^{b}	64.11± 2.33^{b}
ALP	87.33± 6.35^{c}	113.33± 3.21^{a}	101.33± 1.52^{b}	99.33± 2.51^{b}
LDH	93.23± 6.11^{c}	127.33± 5.68^{a}	115.34± 4.04^{b}	114.33± 5.68^{b}
Urea	30.66± 1.52^{c}	69.01± 2.64^{a}	44.87± 3.01^{b}	42.28± 4.58^{b}
Creatinine	1.15± 0.05^{c}	2.81± 0.14^{a}	1.84± 0.27^{b}	1.18± 0.46^{c}

AST, aspartate aminotransferase, ALT, alanine transaminase, ALP, alkaline phosphatase, LDH, lactate dehydrogenase. Rabbits were infected with 0.2ml *P. multocida* and treated with Se and Cur at the dose of 0.5mg/kg and 13g/kg feed diet respectively for 14 days. Values are represented as means ± SEM. Means within the same row carrying different superscripts ($^{a, b,}$ and ^{c}) are significant at $p < 0.05$. For details of groups, see Table I.

Analysis of liver and kidney function

The activities of serum AST, ALT, ALP, LDH, urea and creatinine shown in Table III, significantly increased (P < 0.05) in infected group (B) as compared to control (A). Conversely, the level of mentioned parameters for liver and kidney function are non-significant between Cur and Se treated (C and D) groups.

Serum oxidative markers

The concentration of serum oxidative markers (Fig. 1) such as MDA was significantly higher and antioxidant enzyme level (GSH-Px and SOD) were significantly

lowered in infected group (B) as compared to control (A), however, the difference between group C and D was observed non-significant (> P 0.05) on day 7th. Nonetheless, on day 14th the decrease in MDA concentration was significantly ($P < 0.05$) lowered by 62.03 % in D as compared to C, and the increase in activities of GSH-Px and SOD were higher ($P < 0.05$) by 45.45 % and 44.64 % in D compared with C.

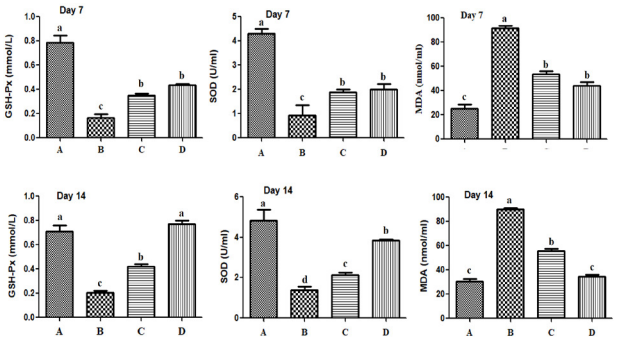

Fig. 1. Effect of selenium and curcumin on *P. multocida* induced changes in serum oxidative markers in rabbit. GSH-Px, glutathione peroxidase; SOD, superoxide dismutase; MDA, melondialdehyde. Rabbits were infected with 0.2ml *P. multocida* and treated with Se and Cur at the dose of 0.5mg/kg and 13g/kg feed diet, respectively for 14 days. Values are represented as means±SEM and [a, b, c and d] different letters exhibits the difference between groups with $P < 0.05$. See Table I for details of groups.

DISCUSSION

In current study inoculation of *P. multocida* infection to the rabbits caused alteration in hematological finding by showing decrease in erythrogram (total RBCs, hemoglobin, PCV, MCV and MCHC) count. In continuity to our results Edrees *et al*. (2017), Abdelhady and El-Abasy (2015) and Nassar *et al*. (2013) observed decrease in hematological indices in rabbits infected with *P. multocida*. Bacterial infection shows different hemorrhagic lesions in different organs such as liver, lungs, spleen and heart (El-Sheikh *et al*., 2020) that reflects the picture of hemolytic anemia caused by pasteurella endotoxins leading to decreased life span of RBCs. Furthermore, anemia due to bacterial infection results in transport of iron to mononuclear phagocytic system, which makes it available for bacterial consumption hence less available for erythroid precursor cells (Walton, 2013). Further investigation revealed increase in leukogram (WBC, neutrophil, lymphocytes) with *P. multocida* infection in current study. Our results agree with Edrees *et al*. (2017) and Alam *et al*. (2018) who reported significant increase in total leukocyte and neutrophil count with bacterial infection. The first line of defense is provided by innate immune response immediately after any kind of infections, which include neutrophils, lymphocytes, monocytes and eosinophils (Muñoz Carrillo *et al*., 2017). Furthermore, leukocytosis and neutrophilia refer to an inflammatory response most commonly to acute bacterial infection. According to Nassar *et al*. (2013) infection with pasteurella are mostly related with the condition known as leukocytosis as a physiological response from the body to diminish the infection. Therefore, these findings are characterized to the body inflammatory response due to infection and hence change in hematological findings. In contrast, treatment with Se and Cur evoked a significant amelioration in hematological parameters. The role of Se in immune modulation has been studied by various researchers via showing antioxidant and anti-inflammatory role in different animals such as pigs (Pecoraro *et al*., 2022), sheep (Mehdi *et al*., 2013) and cattle (Mehdi and Dufrasne, 2016). Similarly, Cur also has an emerging role in medicine due to its therapeutic properties, which include antioxidant, anticancer, anti-inflammatory, and antimicrobial effects (Hewlings and Kalman, 2017). However, rabbits infected with *P. multocida* later treated with tulathromycin (Edrees *et al*., 2017) and allicin (Alam *et al*., 2018) showed ameliorative hematological status. Cur showed protective effect on blood picture against thermally oxidized oil in rabbits (Abdallah *et al*., 2020). It is stated that supranutritional Se level against *P. multocida* antigen reflects improved blood parameters due to its antioxidant status in calves (Mudgal *et al*., 2018). Therefore, in present results, improved hematological picture by Se and Cur might be due to their antioxidant activity against free radicals generated from administration of *P. multocida* infection.

In blood the concentration of variety of substances such as carbohydrates, protein, lipids, many hormones and enzymes are within very narrow range and tightly regulated, thus plays major role in maintaining homeostasis. The changes in biochemical beyond optimal limits represents the adverse condition. However, investigation and determination of blood biochemical is important in determining the health status of the body. Our study revealed decrease in serum protein, albumin, globulin and lipid level in the rabbits infected with *P. multocida* as compared to control. Similarly, El-Sheikh *et al*. (2020) and Alam *et al*. (2018) revealed detailed changes in different biochemical parameters by showing hypoproteinemia, hypoalbuminemia and hypoglobulinemia respectively. These conditions noted after bacterial infection are attributed to hemorrhages and anorexia that leads to protein catabolism and hence inability of hepatocytes to produce proteins (Lee-Lewandrowski *et al*., 1994; Palócz *et al*.,

2014). On the contrary, rabbits treated with Se and Cur showed improvement in the concentration biochemical findings. Rabbits infected with *P. multocida* later treated with allicin (Alam *et al*., 2018) and grape seed extract (El-Sheikh *et al*., 2020) showed parallel results in the rabbits. According to Dkhil *et al*. (2011) rabbits treated with allicin after infection exhibits reduction in hemorrhage and mitigate hepatic damages. Se and Cur bearing mimicry action like allicin might have improved the appetite and reduction in the harmful effects of bacteria in current study.

Concurrent with elevated biochemical findings we found marked increase in serum liver and kidney biomarkers such as AST, ALT, ALP, LDH, urea and creatinine in *P. multocida* infection as compared to control. Similar effects were recorded by Nassar *et al*. (2011) and Abdelhady and El-Abasy (2015) in the rabbits after *P. multocida* infection. As a consequence of bacterial infection, changes in cell membrane causes cellular permeability which accounts the abnormal release of these markers into serum at high level (Hsueh *et al*., 2021). Furthermore, increased protein catabolism impairs the heart functions and lowers the blood flow to the kidneys (Edrees *et al*., 2017). Contradictory, infected rabbits treated with Se and Cur showed marked decrease in AST, ALT, ALP, LDH, urea and creatinine in disparity to infected group. The influence of *P. multocida* endorse adverse effect of bacteria and its endotoxins on liver and kidney. However, alleviation of liver and kidney injury after treatment authorizes antimicrobial (Hasona *et al*., 2017) and hepato-renal protective role (Khalil, 2018) of Se and Cur in rabbits.

Oxidative stress (OS) occurs due to extreme level of reactive free radicals species and/ or reduction in the antioxidation mechanisms (Guo *et al*., 2012). Melondialdehyde (MDA) is highly reactive 3-carbon dialdehyde by-product of lipid peroxidation that induces destructive changes resulting in ultimate cell death (Ma *et al*., 2018). The level of MDA found in blood (Sobeková *et al*., 2006) reveals the amount of pro-oxidants and hence identified as an indicator of OS (Sharma *et al*., 2012). In addition, defense mechanism to deal with the oxidative distress comprises antioxidant enzymes. Glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) are considered first-line defense antioxidants that mutually chase free radicals (ROS) and protects different parts of body from cellular OS. SOD catalyzes the conversion of two molecules of superoxide anion (O_2^-) to less reactive hydrogen peroxide (H_2O_2) and molecular oxygen (O_2) through redox reaction, then GSH-Px acts upon H_2O_2 and convert it into water molecule. In addition, GSH-Px provides pivotal prevention against oxidative damages via converting lipid peroxides to their corresponding alcohols and hence inhibiting lipid peroxidation (Ighodaro and Akinloye, 2018).

In present study, MDA concentration in blood was significantly higher in the rabbits infected with *P. multocida*. Accompanied with increase in MDA, the reduction in the level of GSH-Px and SOD confirms the development of oxidative stress with bacterial infection as compared to control. Parallel results were described by Alam *et al*. (2018) in the rabbits reflecting the occurrence of lipid peroxidation and production of reactive oxygen species ROS by bacterial infection (El-Deeb and Elmoslemany, 2016). Conversely, our results indicate that Se and Cur treatment ameliorated OS in infected rabbits represented by elevation of GSH-Px and SOD level and reduction MDA concentration in blood. However, amelioration rate of these results was significant after treatment with Se as compared to Cur on day 14th in current study. The calves with bacterial infection, treated with Se in combination with copper showed higher GSH-Px activity (Mudgal *et al*., 2018). Moreover, the effects of Cur against oxidized oil-induced showed diminution in MDA and raise in CAT activity in rabbits on day 30 and 90, respectively. Allicin bearing antioxidant properties (Alam *et al*., 2018) showed similar results by attenuating the signaling pathways of ROS and stimulate the endogenous antioxidant enzymatic activity (Zhu *et al*., 2020; Shaaban *et al*., 2021). In continuity with these findings, Hepato-renal protection by Se and Cur is probably due to their anti-oxidant capacity against bacterial infection.

Se and Cur improve the systemic markers of oxidative stress by scavenging variety of free radicals, such as ROS and nitrogen species (RNS), respectively (Menon and Sudheer, 2007). Furthermore, they can control the activity of GSH-Px, CAT, and SOD enzymes that vigorously neutralizes free radicals. However, GSH-Px also called, as selenocysteine peroxidase is a Se-containing antioxidant enzyme. The production and secretion of this enzyme is directly related to amount of Se in the tissues, which is often dependent on the level of Se in the diet (Ahmed *et al*., 2016). According to Čobanová *et al*. (2017), the supplementation of 0.56 mg.Se.kg^{-1} dry matter (DM) enhances the concentration of GSH-Px in blood and tissues of sheep. Furthermore, Se insufficiency alters the level of GSH-Px and is the key source to stimulate certain stress signals leading to apoptotic mechanism (Yu *et al*., 2015). However, the better effects with Se treatment on day 14th might be due to its best adaptation and deposition at the dose of 0.5 mg.kg^{-1} against bacterial infection for the synthesis of GSH-Px enzyme as compared to Cur. Furthermore, poor bioavailability of Cur resulting in its poor absorption, rapid metabolism and elimination might results in overlapped effect of Se.

CONCLUSION

Infection with *P. multocida* validate alteration in hematology and blood biochemistry. Moreover, elevated biomarkers of liver and kidney injury in blood endorse infection induced adverse effects followed by OS in rabbits. The protective effect of Se and Cur validated by amelioration of *P. multocida* induced alterations is due to their antibacterial and antioxidant properties. Furthermore, hepato-renal protective effect of Se and Cur reduced harmful effects of the bacteria on liver and kidney. The treatment of Se provided better protection against infection presenting its better anti-bacterial properties as compared to Cur; however, the mechanism behind such property of Se may be examined to elaborate its protective role.

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Ethical approval

The research design was permitted by the Board of Advanced Studies and Research (BASR), Directorate of Advance Research (DAS), Sindh Agriculture University Tandojam under reference number DAS/88 in the year 2023.

Statement of conflicts of interest

The authors have declared no conflict of interest.

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