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Efficacy of Oflaxacin and Grape Seed Extract in the Treatment of *Pasteurella Multocida* Experimentally Infected Rabbits

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LIST OF ABBREVIATIONS

Abbreviation	Expression
ALT	Alanine aminotransferase enzyme
AST	Asparate aminotransferase enzyme
BWG	Body weight gain
B.wt	Body weight
CBC	Complete blood count
DMSO	DiMethyl Sulfoxide
ELISA	Enzyme-linked immunosorbent assay
GC/MS	Gas chromatography/ mass spectro-photometer
GSE	Grape seed extract
H & E	Hematoxylin and Eosin stain
Hb	Hemoglobin
HPLC	High performance liquid chromatog-raphy
HCl	Hydrochloride
IL6	Interlukin 6
LDH	Lactate dehydrogenase enzyme
MCH	Mean corpuscular hemoglobin
MCV	Mean corpuscular volume

Abbreviation	Expression
MCHC	Mean corpuscular hemoglobin concentration
MF	Molecular formula
MW	Molecular weight
PCV	Packed cell volume
PDA detector	Photo Diode array detector
Pt	Post treatment
RT	Retention time
UV detector	Ultraviolet detector
WSS	Working Standard Solution

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SUMMARY

Pasteurellosis is one of rabbit's most serious bacterial diseases and leads to great financial damages in big production systems worldwide. Increased the efficacy of antibacterial drugs with reduction of their residue is of great importance. Therefore, this study was designed to evaluate the potential role of grape seed extract (GSE) in treating of *Pasteurella multocida* infection in rabbits, increasing the efficacy of ofloxacin treatment, and reducing of its side effects.

For this purpose, a total of 75 recently weaned male New Zealand rabbits were used. After one week of acclimatization, the rabbits were randomly assigned into five equal groups. On 1st day of the experiment, group I (control group) intranasally administered 0.1 ml sterlilized saline. In all other rabbits, snuffles were induced by intranasal injection of 0.1 ml of *P. multocida* (1X 10^7 CFU)

Twenty four hour later (after appearance of clinical signs), various drug regimens were initiated that continued for five consecutive days. Experimentally infected rabbits were allocated into group II (infected–non treated), infected group were orally administered 0.5% DMSO solution; group III (infected-ofloxacin-treated), infected rabbits were orally

administered ofloxacin 25 mg/ kg b.wt /twice daily. group IV (infected- GSE-treated), infected rabbits were orally administered GSE 250 mg/ kg b.wt once daily ; group V (infected-co-treated), infected rabbits were simultaneously treated with half dose of ofloxacin and GSE. The final volume of each treatment was adjusted to be 2 ml and administered using plastic syringe connected to rubber stomach tube. The rabbits in all groups were carefully observed throughout the study.

Body weight was determined with an electronic balance on the 1^{st}, 7^{th} and 14^{th} day of experiment.

Two blood samples were collected from ear vein from 5 rabbits of each group on 1^{st} and 7^{th} day post treatment. Frist blood sample was collected in EDTA tube for hematological analysis. Second blood sample was collected in clean dry centrifuge tube and used for serum separation to be used for biochemical investigations, immunological analysis and ofloxacin residue measurement using HPLC. Lung, liver, kidney, heart, spleen and brain specimens were immediately removed after slaughtering of rabbits and preserved in neutral buffered formalin 10% for histopathological finding.

Experimental *P. multocida* infection in rabbits induced significant reduction of body weight, body weight gain as well as microcytic hypochromic anemia, leucocytosis, lymphocytosis and neutropenia. Also, a significant increase in the hepatic and renal injury biomarkers as well as in interleukin (IL-6), total globulin, α, β and γ globulins and as well as a significant decrease in total protein and albumin. Treatment of infected rabbits either with ofloxacin and/or GSE modulated most of these altered parameters.

High performance liquid chromatographic (HPLC) analysis indicated that treatment of infected rabbits concurrently with the half doses of ofloxacin and GSE showed a significant reduction in the ofloxacin residues on the 1^{st} and 7^{th} day post treatment (626.2 ± 11.45 and 217.6 ± 8.15), respectively compared with those of infected- ofloxacin treated rabbits. This study endorses the co-administration of half doses of ofloxacin with GSE for treatment of Pasteurellosis in rabbits and reduction of ofloxacin residues.

The result of histopathological changes of *P.multocida* infected group showed severe peribronchitis and congested blood vessels as well as severe lymphocytic infiltrations and compensatory emphysema in lung. Liver revealed massive

lymphocytic infiltration along with fibrosis around the hepatic area. Diffuse degeneration of hepatocytes and focal necrosis were also observed. Kidneys showed degeneration of renal tubules and focal interstitial aggregations with lymphocytes. **Heart** showed severe congestion of blood vessels and interstitial inflammatory reaction, degeneration and intermuscular edema. **Brain** showed congestion of blood vessels, lymphocytic infiltrations and degeneration of neurons. **Spleen** showed severe congestion and focal depletion of white pulp. Infected rabbits treated with ofloxacin and Infected rabbits treated with GSE showed mild to moderate lesions. Infected rabbits treated with both ofloxacin and GSE showed pronounced improvement in tissues.