INTRODUCTION

Rabbit production is very important to improve the consumption of animal proteins in developing nations (Adeyinka, 2007). The occurrence of diseases is inevitable in any animal production unit and leads to prospective economic losses (Quesada et al., 2013). Pasteurellosis induced by Pasteurella multocida (P. multocida), a virulent and readily transmitted coccobacillus, is one of the rabbit’s most serious bacterial diseases and leads to great financial damages in big production systems worldwide (Takashima et al., 2001). This disease is characterized by multiple clinical symptoms, like respiratory distress, genital disorders, otitis, abscesses and septicaemia, but P. multocida infection may also be asymptomatic (Jaglic et al., 2008). Rabbits could become infected with P. multocida instantly after birth, and the incidence of colonization rises with age to approximately 5 months. Most adult rabbits are thought to have been infected with P. multocida (Palócz et al., 2014).

Abstract

Pasteurellosis is one of the rabbit’s most bacterial severe diseases and leads to considerable financial damages in large production systems worldwide. Antibiotic use in animals may lead to antibiotic residues in animal products, including meat. Therefore, this study was designed to evaluate the potential role of grape seed extract (GSE) in treating Pasteurella multocida infection in rabbits. For this purpose, 45 weaned male New Zealand rabbits were divided into three groups; control, infected and infected-GSE treated. Experimental P. multocida infection in rabbits induced a remarkable decrease in body weight, body weight gain, as well as microcytic hypochromic anaemia, leucocytosis, neutrophilia and lymphocytopenia. Also, a significant increase in the hepatic and renal injury biomarkers, in interleukin-6, total globulin, α, β and γ globulins, as well as a marked reduction in total protein and albumin, were recorded in the P. multocida-infected rabbits. Treatment of infected rabbits with GSE modulated most of these altered parameters. This study endorses the administration of GSE for the treatment of Pasteurellosis in rabbits. Further studies are required to identify the possible additional effects, appropriate doses and duration of the GSE therapy in rabbits Pasteurellosis.

KEYWORDS
GC-MS, grape seed extract, Pasteurella multocida, rabbit
Antibiotic use in animals may lead to antibiotic residues in animal products, including meat, milk, and eggs. The most significant side effect of antibiotic residues is the transfer of antibiotic-resistant bacteria to humans. It is critical to regulate the use of antibiotics in food-producing animals to control the residues (Bacanli & Basaran, 2019). The inappropriate and uncontrolled use of antibiotics in the veterinary sector carries potential microbial resistance hazards that could affect not only the animal production unit but also human health and the environment (Quesada et al., 2013). Taking these facts into account, herbal medicine can give a wealth of attractive ways to fight drug resistance (Narayanan et al., 2011; Potroz & Cho, 2015). Natural ingredients exhibit a variety of biological activities (Abdellatief et al., 2017; El-Sheikh et al., 2019) and can be used efficiently to manage diseases (Galal et al., 2016; Gupta & Birdi, 2017; Hassanin et al., 2020) and to ameliorate different toxicities (Elewa et al., 2019; El-hady & Galal, 2018; Elsheikh et al., 2018; Galal et al., 2019; Mohamed et al., 2020; Osama et al., 2019).

Grape (Vitis vinifera L.) is one of the world’s major fruit crops based on cultivated regions and economic value (Torregrosa et al., 2015). Grape seeds, a by-product of the juice and wine industry, are a rich source of polyphenols (Lin et al., 2014). Grape seed extract (GSE) contains plant flavonoids, potent antioxidants and exert many health-promoting effects (Kar et al., 2006). Resveratrol is a non-flavonoid polyphenol found in grapes; its antioxidant capacity (Abdel-Daim et al., 2019; Bungau et al., 2019; Yeung et al., 2019) exerted neuroprotection (Ibrahim et al., 2018) against several different oxidative insults. GSE has renoprotective (Albrahim & Robert, 2020), cardioprotective (Razmarai et al., 2016) and hepatoprotective (Ali et al., 2015) effects. GSE can be used in broiler chicken diets as an effective natural antioxidant and immunostimulant (Hassan et al., 2016). Also, it has anti-inflammatory effects via reduction in tumour necrosis factor-α and interferon-γ expression and down-regulated NF-κB signalling (Bibi et al., 2016).

Grape seed extract can interfere with the growth of a wide range of gram-negative and Gram-positive bacteria. It inhibits the growth of Aeromonas hydrophila, Bacillus cereus, Enterococcus faecalis, Enterobacter aerogenes, Klebsiella pneumoniae, Escherichia coli, Mycobacterium smegmatis, Pseudomonas aeruginosa, Pseudomonas fluorescens, Salmonella enteritidis, Salmonella typhimurium, Proteus vulgaris, Staphylococcus aureus and Yersinia enterocolitica (Baydar et al., 2004, 2006; Jayaprakasha et al., 2003; Özkan et al., 2004). It also has inhibitory effects against several viruses and fungi. GSE could be a potential source of antimicrobial agents in the food industry and the clinical environment (Memar et al., 2019).

The reduction of antibiotics use in the veterinary sector is of great importance. Therefore, this work was designed to assess the potential effect of GSE in treating P. multocida infection in rabbits. It has been achieved by assessing (a) antibacterial activity of GSE (b) haematological, immunological and biochemical parameters of P. multocida-infected rabbits.

2 | MATERIALS AND METHODS

2.1 | Plant material and extraction

Grape seeds were purchased from a local market, Kasassen, Ismailia, Egypt. Grape seeds were dried in a hot air oven at 40°C till complete dryness and pulverized to a fine powder in a mechanical grinder. The powder was soaked in 70% ethanol (25% w/v) for 72 hr in a dark place (25–30°C) and was stirred three times a day (Badavi et al., 2013). The extract was then filtered through Whatman No. 1 filter paper, and the residue was repeatedly extracted with the same solvent until it was colourless. The liquid extract was collected in a dark brown bottle. The extract was concentrated under reduced pressure in a rotary evaporator (temperature 40°C) till complete dryness. The dried extract was collected, weighed and stored at 4°C until further use. The dried grape seed extract (GSE) extract was used for Gas Chromatography-Mass Spectrometry (GC-MS) analysis. Finally, the extract was reconstituted in 0.5% DMSO to be ready for administration.

2.2 | Gas chromatography-mass spectrometry analysis

The GC-MS analysis of GSE was carried out at the National Research Center, El Dokki, Giza, Egypt. The GC-MS analysis was performed using a TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp.), coupled with a THERMO mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). TG-MS fused silica capillary column (30 m x 0.25 mm x 0.1 μm film thickness).

2.3 | Pasteurella multocida strain

Pasteurella multocida strain was obtained from the Animal Health Research Institute (AHRI), El Dokki Giza, Egypt. Colonies were suspended in sterile saline, and the density was adjusted with a final concentration of 1 x 10^7 colony-forming unit (CFU)/ml.

2.4 | The antimicrobial activity of grape seed extract

The antibacterial activity of the GSE was determined by the disc diffusion method (Shrestha et al., 2012). Sterile paper discs (6 mm diameter) were loaded with 20 μl of each concentration of GSE (100, 200 and 500 μg/ml). Overnight cultures of P. multocida strain were diluted with sterile normal saline to give an inoculum size of 1 x 10^7 CFU/ml. The inoculum was evenly spread on the surface of the dried nutrient agar plate by sterile spreader. The plates were incubated at 35°C for 30 min, then ofloxacin disc (5 μg, HiMedia Laboratories, Mumbai, India) and GSE discs were applied aseptically with gentle pressure. The plate was incubated at 37°C for 24 hr. The
degree of sensitivity was determined by measuring the diameter of the inhibition zone around each disc (mm) with a graduated ruler.

2.5 Experimental rabbits and management

A total of 45 recently weaned male New Zealand rabbits, 1 month age, weighing about 600 ± 20 g were obtained from a rabbit farm in Kasaseen, Ismailia, Egypt. They were kept for one week before the commencement of the experiment. They were housed in a double flat galvanized wire, batteries (40 x 50 x 40 cm) and were kept under hygiene and environmental conditions. The rabbits were fed on commercial ration supplied from El-Baraka Company, Kasaseen; Ismailia. The formulation and chemical composition of the basal diet are shown in Table 1. The rabbits were checked three times daily (at 6 a.m., 2 and 10 p.m.) for feed, water and mortality.

2.5.1 Induction of snuffle and experimental design

After 1 week of acclimatization, the rabbits (5 weeks of age) were randomly assigned into three equal groups (each of 15 rabbits). On 1st day of the experiment, group I (control group) intranasally instilled with 0.1 ml sterilized saline. In all other rabbits, sniffles were induced by intranasal instillation of 0.1 ml of P. multocida (1 x 10⁷ CFU) (Jarvinen et al., 1998).

Twenty-four hours later (after the appearance of clinical signs), various treatments were started and continued for five consecutive days.

2.5.2 Sampling

Two blood samples were collected from the ear vein from five rabbits of each group on the 1st and 7th day post-treatment (i.e. 7th and 14th day of the experiment). Frist blood sample was collected in EDTA tube for haematological analysis. The second blood sample was collected in a clean, dry centrifuge tube and was centrifuged at 3,000 rpm for 10 min. The serum was separated and stored at −20°C for further investigations.

2.5.3 Haematological analysis

Blood with EDTA used to determine erythrocytic (RBCs) count, packed cell volume (PCV), haemoglobin concentration (Hb), white blood cell (WBCs) count and the differential leucocytic count (Grindem, 2011).

2.5.4 Biochemical analysis

Commercially available diagnostic kits (bioMérieux, France) were used for colorimetric determination of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) (Reitman & Frankel, 1957), total protein (Doumas et al., 1981), albumin level (Doumas et al., 1972), urea (Chevari & Ebst, 1975), creatinine (Larsen, 1972) and lactate dehydrogenase (LDH) (Buhl & Jackson, 1978) levels. The serum globulin was calculated by subtracting the albumin from the obtained total protein (Bush, 1990).

2.5.5 Immunological studies

Serum protein electrophoresis was used for measuring different globulin fractions. It was done using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) (Smith, 1984) in the Biochemistry Department, Animal Health Research Institute, Ismailia, Egypt. Serum Interleukin-6 (IL-6) level was detected using a specific rabbit ELISA kit (MyBioSource) in following the manufacturer’s procedures.

2.6 Statistical analysis

The obtained data were statistically analysed by a one-way analysis of variance (ANOVA) method followed by Duncan multiple

| TABLE 1 Feed ingredients and chemical composition of the diet |
|-----------------|------------|
| **Ingredients** | **g/Kg** |
| Barley          | 220       |
| Soyabean meal   | 200       |
| Wheat bran      | 150       |
| Clover hay      | 300       |
| Yellow corn     | 70        |
| Molasses        | 30        |
| Calcium carbonate | 5     |
| Di-calcium phosphate | 15    |
| Salt (NaCl)     | 5         |
| Premix          | 3         |
| DL-Methonine    | 2         |
| Chemical composition (as DM basis g/kg Dry Matter) | 897.2 |
| Organic matter  | 934.8     |
| Crude protein   | 174.7     |

Each 3 kilograms of premix contains: Vit. A (12,000,000 IU); Vit. D3 (2,000,000 IU); Vit. E (10,000 mg); Vit. K₂ (2000 mg); Vit. B₁₂ (10 mg); Vit. B₆ (5,000 mg); Biotin (50 mg); Colline Chloride (250,000 mg); Pantothenic acid (10,000 mg); Nicotinic acid (30,000 mg); Iron (30,000 mg); Folic acid (1,000 mg); Zinc (50,000 mg); Manganese (60,000 mg); Copper (10,000 mg); Iodine (1,000 mg); Selenium (100 mg); Cobalt (100 mg); CaCO₃ (3,000 mg).

DE (Kcal/kg) = 4151 - (122*Ash) - (64*Fibre) - (38*Fat) + (23*CP)
range tests to compare the means. Differences were considered significant at \( p < .05 \).

3 | RESULTS

3.1 | Chemical compositions of grape seed extract

The hydroalcoholic extraction of 1 kg of grape seed powder yield 28.64 g dry extract. The chemical compositions of GSE are illustrated in Table 2 & Figure 1. Overall, 79 compounds were clarified, constituting 97% of the total ingredients of GSE. Guanosine (20.55%), Glycerin (19.44%), Lycopresen (10.05%), 15-Tetracosenoicacid, methyl ester (6.60%) and Octadecanoicacid, ethyl ester (6.14%) were the main compounds.

3.2 | Antibacterial activity

*P. multocida* showed various degrees of sensitivity to different GSE concentrations. Inhibition zones were 12, 15 and 20 mm for 100, 200 and 500 µg GSE/ml respectively. There was a positive correlation between GSE concentrations and inhibition zones. The highest inhibition was obtained by ofloxacin (35 mm).

3.3 | Clinical symptoms, mortality rate and body weight changes in rabbits in response to *P. multocida* infection and oral administration of GSE

On the second day of the experiment, group II rabbits (infected—non-treated) exhibited inconsistent clinical signs. These signs were mostly depression, off food, sneezing, nasal discharges, rough fur, laboured breathing, and in few cases, there were subcutaneous abscesses and also diarrhoea. However, rabbits treated GSE (group III) revealed less severe clinical signs than did group II. During the experimental period, five rabbits in the infected non-treated group, three rabbits in the infected GSE-treated group died.

Experimental *P. multocida* infection induced a significant (\( p < .05 \)) decrease in the BW and body weight gain (BWG) of the infected group in contrast to the control group on the 7th and 14th day of the experiment. Meanwhile, GSE-treated group showed a significant (\( p < .05 \)) increase in the BW and BWG in contrast to the infected group on the 7th and 14th day of the experiment, as shown in Table 3.

3.4 | Effects of oral administration of GSE on haematological parameters in *P. multocida* experimentally infected rabbits

On the 1st day post-treatment, intranasal inoculation of rabbits with *P. multocida* caused a significant (\( p < .05 \)) reduction in RBC count, PCV, Hb concentration, MCH, MCHC, platelets and lymphocytes and a significant (\( p < .05 \)) elevation in MCV, total WBC count, neutrophil, monocytes and eosinophils in contrast to the control group. While oral administration of infected rabbits with GSE for 5 days significantly (\( p < .05 \)) elevated RBC count, PCV, Hb concentration, MCH, MCHC, platelets and lymphocytes and significantly reduced total leucocyte count, eosinophils and monocytes in contrast to the non-treated-infected group.

On the 7th day post-treatment, a significant (\( p < .05 \)) reduction in RBC count, Hb concentration, PCV, MCH, MCHC, MCV, platelets and neutrophils and a significant (\( p < .05 \)) elevation in total WBC count, lymphocytes, monocytes and eosinophils was recorded in infected non-treated group in contrast to the control group. While oral administration of infected rabbits with GSE for 5 days significantly (\( p < .05 \)) elevation RBC count, Hb concentration, PCV, MCH, MCHC, platelets and neutrophils and significantly decreased total leucocyte count, lymphocytes, eosinophils and monocytes in contrast to the non-treated-infected group (Table 4).

3.5 | Effects of oral administration of GSE on biochemical parameters in *P. multocida* experimentally infected rabbits

On the 1st and 7th day post-treatment, a significant (\( p < .05 \)) elevation in the levels of serum ALT, AST, LDH, urea, creatinine and IL-6 were reported in the infected non-treated group in contrast to the control group. Meanwhile, oral administration of infected rabbits with GSE for 5 days significantly (\( p < .05 \)) reduced the levels of serum ALT, AST, LDH, urea, creatinine and IL-6 in contrast to the infected group (Table 4).

On the 1st day post-treatment, experimental *P. multocida* infection in rabbits induced a significant (\( p < .05 \)) reduction in the levels of serum total protein and albumin and a significant (\( p < .05 \)) elevation in total globulin, \( \alpha \) and \( \beta \) globulins in the infected non-treated group. Meanwhile, oral administration of infected rabbits with GSE for 5 days significantly (\( p < .05 \)) elevated the albumin levels in contrast to infected non-treated group.

On the 7th day post-treatment, a significant (\( p < .05 \)) reduction in the levels of serum total protein and albumin and a significant (\( p < .05 \)) elevation in total globulin, \( \alpha \) and \( \gamma \) globulins was recorded in the infected non-treated group in contrast to the control group. Meanwhile, oral administration of infected rabbits with GSE for 5 days significantly (\( p < .05 \)) elevated the levels of serum total protein, albumin, total globulin, \( \alpha \) and \( \gamma \) globulins in contrast to the infected non-treated group (Table 5).

4 | DISCUSSION

Pasteurellosis induced by *P. multocida*, a virulent and readily transmitted coccobacillus, is one of the rabbit’s most serious bacterial diseases


**TABLE 2** Chemical composition of grape seeds hydroalcoholic extract analysed using GC–MS with relative percentages of components

| Peak No. | Rt (min) | MF      | MW     | Area % | Identified compounds                                                                 |
|----------|----------|---------|--------|--------|--------------------------------------------------------------------------------------|
| 1        | 6.14     | C8H12   | 108    | 0.42   | 2Methylhept2,6dien4ol                                                                 |
| 2        | 6.29     | C22H28N2O3 | 368   | 0.51   | (1’Benzyli2’oxo1’,2’,3’,4’tetrahydro4’pyridyl)2tertbutyl1aza3oxabicyclo[3.3.0]octan4one |
| 3        | 6.49     | C19H19N7O6 | 441   | 1.08   | Folic Acid                                                                            |
| 4        | 6.66     | C9H17Cl  | 160    | 0.59   | 6Chloro2,3,3trimethyl1hexene                                                              |
| 5        | 6.94     | C18H17NO | 263    | 0.60   | 2,2dimethyl3,5 diphenyl2pyrrole 1oxide                                                 |
| 6        | 7.05     | C27H36O8 | 488    | 0.99   | (22S)21Acetoxy6a,11dihydroxy16a,17propylmethylenedioxypregna1,4diene3,20diene         |
| 7        | 7.12     | C15H24O4 | 268    | 0.55   | Indeno[3a,4b]oxirene2ol,octahydro4amethyl5[(Tetrahydro2Hpyran2yl)oxy],                 |
| 8        | 7.20     | C10H20O3 | 188    | 0.71   | (2R)2Methyl4[(tetrahydro2Hpyran2yl)oxy]butan1ol                                        |
| 9        | 7.26     | C12H19N3O4 | 269 | 0.82   | Pyrimidin2,4dione,1,2,3,4tetrahydro5methyl1[2hydroxymethyl3dimethylaminotetrahydrofur5yl |
| 10       | 7.36     | C16H32N2 | 252    | 0.79   | N[2Cyanocyclopropyl]2methyldecylamine                                                   |
| 11       | 7.46     | C7H12O   | 112    | 0.57   | 1Cyclobutyl cyclopropanol                                                             |
| 12       | 7.61     | C23H46O2 | 354    | 1.49   | Docosanonic acid, methyl ester                                                        |
| 13       | 7.67     | C9H13NO2 | 167    | 0.51   | N(3’Methylbut2’ enoyl)2pyrrolidone                                                    |
| 14       | 7.96     | C11H15N5O3 | 265 | 0.30   | LHistidinamide, 5oxoLpropyl                                                             |
| 15       | 8.25     | C20H36O2 | 308    | 0.60   | 2HPyrrol, tetrahydro2(12penta decynoxy)                                                |
| 16       | 8.73     | C17H30O3 | 282    | 0.44   | Tetrahydropropen Z10dodecenolate                                                      |
| 17       | 8.80     | C22H18   | 282    | 0.26   | 1(oxyl) antracene                                                                     |
| 18       | 9.13     | C20H14N2 | 282    | 1.31   | 1,2Bis(2quinoxylmethylene)                                                            |
| 19       | 9.33     | C26H36O8 | 476    | 0.36   | 1HCyclopropa[3,4][benz[1,2]azeulene5,7b,9,9atetrol                                          |
| 20       | 9.47     | C11H10BrFO2 | 272 | 0.49   | 3Methyl2butenolicacid,2bromo4fluorophenylester                                         |
| 21       | 9.79     | C21H32O3 | 332    | 0.76   | 5,6epoxy3hydroxy Pregnan20one                                                          |
| 22       | 10.00    | C15H18O3 | 246    | 0.38   | 10{ Tetrahydropropen2yloxy} [tricyclo[4.2.1.1(2,5)]deca3,7dien9one                      |
| 23       | 10.27    | C9H14O   | 138    | 0.55   | 3methylenebicyclo[3.2.1]octan1ol                                                       |
| 24       | 10.50    | C26H54   | 366    | 0.26   | 3ethyl[5(2ethylbutyl) Octadecane                                                       |
| 25       | 11.16    | C7H10CI NO | 159 | 0.23   | N,NDimethyl2Hpyran2iminiumchloride                                                     |
| 26       | 11.54    | C19H28O4 | 320    | 0.28   | 2Cyclohexene1carboxylicacid,2(7hydroxy3methyl1,3octaenyl)1,3dimethyl4oxo, meth yl ester |
| 27       | 11.85    | C26H38N4O4 | 470 | 0.29   | Ceanohtine C                                                                          |
| 28       | 12.22    | C22H41NO2 | 351   | 0.22   | 1[8(3octyloxyranyl)1oxooctyl Pyrrolidine                                               |
| 29       | 12.33    | C18H17NO | 263    | 0.28   | 2,2dimethyl 3,5 diphenyl2pyrrole 1oxide                                                |
| 30       | 12.48    | C13H19N5O5 | 325 | 0.40   | 2one,4[Nmethylureido][1]4methylaminocarbonyloxymethyl Pyrimidin                        |
| 31       | 12.89    | C27H38O4S2 | 490  | 0.32   | 2,4aEpoxymethylphenanthrene7methanol,1,1dimethyl2methoxy8(1,3dithiin2ylidene) meth yl1,2,3,4,4a,4b,5,6,7,8,8a,9dodecahydro, acetate                                |
| 32       | 13.18    | C20H24O5 | 344    | 0.26   | 5amethyl[3,8dimethylene2oxodecahydroxirenone][2’,3’:6,7][naphtho[1,2b]furan6yl2methyl2butenoate |
| 33       | 13.26    | C26H34O11 | 522   | 0.29   | 4HCyclopenta[5’6’]benz[1'2':7,8]azuleno[5,6b]oxiren4one,8Bis(acetyloxy)2a][acetyl oxymethyl]1,1a,1b,1c,2a,3a,3a,6a,6b,7,8,8adodecahydro, 3a,6btrihydroxy, 1,1,5,7ete tramethy |
| 34       | 13.53    | C21H25NO2S | 355   | 0.23   | trans2Cyclohexyl[3(2methylphenyl)4phenyl1,2thiazetidine1,1dioxide                           |
| 35       | 13.68    | C22H17N3O2 | 355   | 1.17   | 3[Maleimido2’yl]1methyl[2’1methylindolo2’ylindole                                             |
| 36       | 16.42    | C21H28O3 | 328    | 0.65   | 4,6Androstadien3aol17one,acetate                                                        |
| 37       | 16.55    | C20H28O6 | 364    | 1.06   | Effusan E                                                                             |
| 38       | 18.37    | C26H20Cl2N2 | 430 | 0.74   | 1,3Bis[4chlorobenzy]5,6dihydrobenzoquinazoline                                            |

(Continues)
and leads to significant financial damages in big production systems worldwide (Takashima et al., 2001). The reduction of antibiotics uses in the veterinary sector attracts researchers for seeking natural antibacterial agents. Therefore, this work was designed to assess the potential role of GSE in treating \textit{P. multocida} infection in rabbits.

\textit{P. multocida} showed various degrees of sensitivity to different GSE concentrations. There was a positive correlation between GSE concentrations and inhibition zones. The antimicrobial effects of GSE are due to high levels of potent antioxidant polyphenols. The antibacterial effect of GSE, was bactericidal that was obtained by

| Peak No. | Rt (min) | MF                  | MW     | Area % | Identified compounds                                                                 |
|---------|----------|---------------------|--------|--------|--------------------------------------------------------------------------------------|
| 39      | 20.40    | C42H64O2            | 600    | 0.42   | 1,1',2,2'-tetrahydro-1,1-dimethoxy-carotene                                           |
| 40      | 20.61    | C14H23N3O10         | 393    | 0.24   | Pentelic Acid                                                                        |
| 41      | 23.02    | C24H32O8            | 448    | 0.32   | 5H-Cyclopropa[3,4]benz[1,2e]azulen-5-one,9(acetoxy)3-(acetoxy)methyl1,1a,1b,4a,4a,7a,7b,8,9,9,10-decahydro-4a,7b,9,10-trihydroxy-1,1,6,8-tetramethyl |
| 42      | 23.07    | C27H38O8            | 490    | 0.28   | 2-Butenoic acid, 2-methyl, 2(acetoxy)1,1a,2,3,4,6,7,10,11,11-decahydro-7,10-dihydroxy-1,1,3,6,9-pentamethyl-4a,7-epoxy-9-hydroxy-2-cyclopenta[a]cyclopropa[f] cyclododecan-11-ylester7a |
| 43      | 23.14    | C12H20O             | 180    | 0.33   | (+)-5-cyclohexyl-6-hexenal                                                           |
| 44      | 24.90    | C26H44O5            | 436    | 1.60   | Ethyl isosaloxyloleate                                                                |
| 45      | 28.17    | C38H76O2            | 564    | 0.33   | Octadecanoic acid, eicosyl ester                                                     |
| 46      | 29.05    | C15H20O5            | 280    | 0.24   | Tetraneurin Adiol                                                                    |
| 47      | 29.75    | C22H23NO8           | 429    | 0.47   | (6-O methylated flavonols)                                                           |
| 48      | 32.68    | C18H16O7            | 344    | 0.58   | 7,3',4'trimethoxy-Quercetin                                                           |
| 49      | 33.25    | C35H48O3S           | 548    | 0.48   | 9(11)-Dehydroergosterolsotylate                                                      |
| 50      | 33.85    | C36H58O6            | 586    | 0.36   | Hexadecanoic acid, 1,2,5,5a,6,9,10,10-octahydro-5,5-dihydroxy-4-hydroxy-1,1,7,9-tetramethyl-11,12,18,19-tetrahydrocyclopenta[a]cyclopropa[e]cyclododecane6ylester |
| 51      | 35.21    | C20H40O2            | 312    | 6.14   | Octadecanoic acid, ethyl ester                                                       |
| 52      | 35.65    | C21H34O2            | 318    | 0.39   | 17-one, 3-ethyl 3-hydroxy Androstan                                                  |
| 53      | 36.58    | C22H40O7            | 416    | 0.32   | Agaric acid                                                                          |
| 54      | 36.97    | C29H28N2O65         | 532    | 0.42   | 4-[3(3,4-dimethoxyphenyl)]1-oxo11-thiophenyl-1,2,3,4,5,11-hexahydrodibenzo[8,9][1,4] Diazepin11-yl(4-oxobuteric acid |
| 55      | 37.11    | C3H8O3              | 82     | 19.44  | Glycerin                                                                              |
| 56      | 37.37    | C14H16              | 184    | 2.97   | Pentacyclo[7.2.1.0(2,7).0(2,8).0(3,8)]dodecyl2-spiro1-cyclopropane                   |
| 57      | 38.32    | C25H48O2            | 380    | 6.60   | 15-Tetracosenoic acid, methyl ester                                                   |
| 58      | 42.12    | C21H26N2O2          | 338    | 0.32   | 1-acetyloxy-16-methylene Strychine,                                                    |
| 59      | 42.20    | C26H50              | 362    | 3.62   | 1'-dodecylidenebis[4-methyl Cyclohexane,                                               |
| 60      | 42.27    | C42H64O2            | 600    | 0.51   | ..1,1',2,2'-tetrahydro-1,1'-dimethoxy Carotene                                          |
| 61      | 43.70    | C35H46O8            | 594    | 0.40   | 2,4,6-Decatrienoic acid, 1a, 2,5,5a,6,9,10,10-octahydro 5,5-dihydroxy-4-hydroxy-1,1,7,9-tetramethyl-11,12,18,19-tetrahydrocyclopenta[a]cyclopropa[e]cyclododecane6ylester |
| 62      | 43.85    | C10H13N5O5          | 283    | 20.55  | Guanosine                                                                             |
| 63      | 44.10    | C16H32O2            | 256    | 0.89   | Palmitic acid                                                                        |
| 64      | 44.38    | C22H23N             | 301    | 3.42   | 2,6-dimethyl-N(2-methylphenyl)benzyl-L-lysine                                        |
| 65      | 44.99    | C32H54O4            | 502    | 0.25   | 7,8-Epoxylanostan 11, 3-acetoxy                                                      |
| 66      | 46.15    | C35H68O5            | 568    | 0.39   | Hexadecanoic acid, 1-hydroxymethyl-1,2,ethanediyl ester                              |
| 67      | 46.70    | C26H34O11           | 522    | 0.59   | 4H-Cyclopropa[5,6]'benz[1,2'-7,8]azulen-5,6-bis-[acetoxy]2a-[acetoxy]methyl1,1a,1b,1c,2a,3a,3a,6a,6b,7,8,8-adodecahydro-3,3a,6b-1,6,8-tri-hydroxy-1,1,5,7-tetramethyl |
| 68      | 48.30    | C40H66              | 546    | 10.05  | Lycopercen                                                                            |
| 69      | 49.50    | C22H36N6O2          | 416    | 0.31   | 1,2-Bis(1-hydroxyethyl)3,6-diazahomoadamantantyhydrazine9                             |
| 70      | 49.81    | C22H25N07           | 415    | 0.28   | Isochochilifoline                                                                     |

Abbreviations: Rt, Retention time; MF, Molecular formula; MW, Molecular weight.
a disruption of the bacterial cell wall (Al-Habib et al., 2010). These results were confirmed by the studies of (Filocamo et al., 2015; Shrestha et al., 2012). Sneezing, nasal discharges, laboured breathing are the most common clinical symptoms caused by *P. multocida* infection in rabbits, which is also the main cause of mortality and morbidity. These findings were supported by histopathological findings of the lung that recorded in our previously published paper. In a few cases, subcutaneous abscesses and diarrhoea were recorded in the infected group. The infected group revealed a marked reduction in BW and BWG all over the study period in contrast to the control group. This is due to off food and poor appetite. These results were supported by the studies (Alam et al., 2018; Edrees et al., 2017). Infected groups treated with GSE for five successive days showed milder clinical symptoms, reduction in mortality rate, and significant improvement of BW and BWG compared to the infected non-treated group. These results may be attributed to the antibacterial effect of GSE. The phytogenic additives from plant extracts have been documented to promote higher digestibility of the nutrient, elevate the activity of the digestive enzyme and the secretion of gastric and pancreatic juice, protect the intestinal microvilli and enhance bird performance via antimicrobial effect (Hernandez et al., 2004).

In this study, intranasal inoculation of rabbits with *P. multocida* caused a marked reduction in RBCs count, PCV% and Hb concentration as well as macrocytic hypochromic anaemia (on 1st day post-treatment) and microcytic hypochromic anaemia (on 7th day post-treatment) in contrast to the control group. Reduction of RBCs count may be due to the impact of *P. multocida* endotoxins on RBCs, which leads to a reduction in their life span. Macrocytic hypochromic anaemia, probably due to activated erythropoiesis as a reaction of the bone marrow to the blood loss in the trachea-pulmonary haemorrhage, induced by septicaemia (Feldman et al., 2000). Meanwhile, the most common cause of microcytic hypochromic anaemia observed during infection is decreased iron reserves of the body (Massey, 1992), which may be due to a massive increase in IL-6, which promotes the production and release of hepcidin from the liver, thereby decreasing the iron carrier protein ferroportin, so the access of iron to the circulation is limited (Nemeth et al., 2004). Moving iron to storage in the mononuclear phagocytic system and using iron by bacteria renders it less accessible as an erythroid precursor (Walton, 2013).

The innate response is the first line of defence that occurs immediately in which neutrophils, macrophages, monocytes and eosinophils are involved (Carrillo et al., 2017). *P. multocida* experimental infection in rabbits induced leucocytosis, neutrophilia, monocytosis, eosinophilia and lymphocytopenia. Leucocytes were elevated to overcome infection as the normal reaction of bone marrow to infection leads to an increase in the number of WBCs predominantly polymorphonuclear leucocytes (neutrophil and eosinophil) (Abramson & Melton, 2000). Lymphocytopenia is commonly accompanied by a rise in neutrophil count in various infectious causes (Hawkins et al., 2006; Seebach et al., 1997; Wyllie et al., 2004). Our haematological results were supported by those of (Alam et al., 2018; Edrees et al., 2017). The treatment of *P. multocida*-infected rabbits with GSE improves the haematological status compared to infected non-treated rabbits. Cytokines are multifunctional mediators of the adaptive and innate immune system, stimulating and regulating inflammatory processes, playing a key role in the large network of interacting cells and signalling immune response-related molecules (Eder et al., 2008). IL-6 possesses pro- and anti-inflammatory activities, and it has a broad effect on the immune system cells (Hunter & Jones, 2015). Our results revealed that *P. multocida* experimental infection in rabbits induced significant elevation of serum IL-6. The IL-6 elevation after...
the infection is an inflammatory reaction to control neutrophil and monocyte transition during the inflammatory process (Kaplanski et al., 2003). The treatment of *P. multocida*-infected rabbits with GSE significantly reduced serum IL-6 levels compared to infected non-treated rabbits. This results due to the anti-inflammatory effect of GSE (Bibi et al., 2016; Silvan et al., 2017; Yang et al., 2014).

### TABLE 3  Body weight changes of rabbits in response to *P. multocida* infection and oral administration of GSE

| Parameters       | Groups | 1st | 7th | 14th |
|------------------|--------|-----|-----|------|
| Body weight (g)  | I      | 748.8 ± 10.55 | 875.2 ± 9.6<sup>a</sup> | 999.2 ± 8.45<sup>a</sup> |
|                  | II     | 729.8 ± 2.9    | 706.8 ± 3.45<sup>c</sup> | 786.8 ± 3.9<sup>c</sup>  |
|                  | III    | 733.8 ± 9.6    | 840.2 ± 12<sup>b</sup>   | 947.4 ± 14<sup>b</sup>   |
| Weight gain (g)  | I      | N/A            | 126.4 ± 1.7<sup>b</sup>  | 124 ± 1.7<sup>b</sup>    |
|                  | II     | N/A            | −23 ± 2<sup>c</sup>      | 80 ± 3.53<sup>c</sup>    |
|                  | III    | N/A            | 106.4 ± 5.6<sup>b</sup>  | 107.2 ± 4.35<sup>b</sup> |

Note: Values are represented as the mean ± SE (*n* = 5). Group I (control group) intranasally administered with 0.1 ml sterilized saline; group II (infected—non treated), infected group were orally administered with 0.5% DMSO solution; group III (infected—GSE-treated), infected rabbits were orally administered with GSE 250 mg/kg once daily. Column carrying different superscripts (a, b, c) are significantly different at *p* < .05.

### TABLE 4  Effects of oral administration of GSE on haematological parameters in *P. multocida* experimentally infected rabbits

| Days post treatment | Parameters       | Groups |
|---------------------|------------------|--------|
|                     |                  | I      | II    | III   |
| 1st day             | RBCs (10<sup>6</sup>/µl) | 3.25 ± 0.15<sup>a</sup> | 2.45 ± 0.22<sup>c</sup> | 2.6 ± 0.17<sup>b</sup> |
|                     | Hb (g/dl)        | 11.1 ± 0.40<sup>a</sup> | 6.65 ± 0.30<sup>c</sup> | 8.07 ± 0.41<sup>b</sup> |
|                     | PCV (%)          | 23.84 ± 1.79<sup>a</sup> | 19.43 ± 0.001<sup>c</sup> | 20.43 ± 0.05<sup>b</sup> |
|                     | MCV (fl)         | 73.35 ± 0.41<sup>b</sup> | 79.31 ± 0.83<sup>a</sup> | 78.58 ± 2.54<sup>a</sup> |
|                     | MCH (pg)         | 34.15 ± 0.39<sup>a</sup> | 27.14 ± 0.61<sup>c</sup> | 31.04 ± 1.26<sup>b</sup> |
|                     | MCHC (%)         | 46.56 ± 0.39<sup>a</sup> | 34.23 ± 0.61<sup>c</sup> | 39.5 ± 0.6<sup>b</sup>  |
|                     | Platelets        | 182 ± 10<sup>a</sup>   | 94.5 ± 11.6<sup>c</sup>  | 124.33 ± 12.5<sup>b</sup> |
|                     | WBCs (10<sup>3</sup>/µl) | 4.35 ± 0.85<sup>a</sup> | 8.7 ± 0.01<sup>a</sup>   | 7.53 ± 1.72<sup>b</sup> |
|                     | Heterophils (%)  | 41.67 ± 1.7<sup>b</sup> | 50.00 ± 2.1<sup>a</sup>  | 46.00 ± 1.5<sup>a</sup> |
|                     | Lymphocytes (%)  | 50.33 ± 2.3<sup>a</sup> | 36.00 ± 1.0<sup>c</sup>  | 43.00 ± 1.5<sup>b</sup> |
|                     | Monocytes (%)    | 5.00 ± 0.31<sup>c</sup> | 10.00 ± 0.27<sup>a</sup> | 7.21 ± 0.22<sup>b</sup> |
|                     | Eosinophils (%)  | 3.00 ± 0.2<sup>c</sup>  | 4.00 ± 0.3<sup>c</sup>   | 3.79 ± 0.4<sup>b</sup>  |
| 7th day             | RBCs (10<sup>6</sup>/µl) | 2.96 ± 0.3<sup>a</sup>  | 2.56 ± 0.15<sup>c</sup>  | 2.8 ± 0.14<sup>b</sup> |
|                     | Hb (g/dl)        | 9.96 ± 0.44<sup>a</sup> | 7.24 ± 0.79<sup>c</sup>  | 8.28 ± 0.53<sup>b</sup> |
|                     | PCV (%)          | 22.84 ± 1.98<sup>a</sup> | 17.84 ± 1.1<sup>c</sup>  | 20.13 ± 1.36<sup>b</sup> |
|                     | MCV (fl)         | 77.16 ± 2.84<sup>a</sup> | 67.32 ± 1.92<sup>b</sup> | 71.89 ± 2.30<sup>b</sup> |
|                     | MCH (pg)         | 33.65 ± 1.9<sup>a</sup> | 28.28 ± 0.89<sup>c</sup> | 29.57 ± 1.5<sup>b</sup> |
|                     | MCHC (%)         | 43.61 ± 1.9<sup>a</sup> | 40.58 ± 0.89<sup>c</sup> | 41.13 ± 1.78<sup>b</sup> |
|                     | Platelets        | 152.60 ± 2.75<sup>a</sup> | 121.8 ± 3.87<sup>c</sup> | 130 ± 3.70<sup>b</sup> |
|                     | WBCs (10<sup>3</sup>/µl) | 9.62 ± 0.41<sup>a</sup> | 12.1 ± 0.23<sup>a</sup>  | 9.98 ± 0.70<sup>b</sup> |
|                     | Heterophils (%)  | 43 ± 0.5<sup>a</sup>    | 26 ± 0.61<sup>c</sup>    | 36 ± 0.48<sup>b</sup>   |
|                     | Lymphocytes (%)  | 50 ± 1.2<sup>c</sup>    | 60 ± 1.5<sup>a</sup>     | 52 ± 1.35<sup>b</sup>   |
|                     | Monocytes (%)    | 4 ± 0.1<sup>c</sup>     | 8 ± 0.3<sup>c</sup>      | 7 ± 0.70<sup>b</sup>    |
|                     | Eosinophils (%)  | 3 ± 0.3<sup>c</sup>     | 6 ± 0.4<sup>a</sup>      | 5 ± 0.4<sup>b</sup>    |

Note: Values are represented as the mean ± SE (*n* = 5). Group I (control group) intranasally administered with 0.1 ml sterilized saline; group II (infected—non treated), infected group were orally administered with 0.5% DMSO solution; group III (infected—GSE-treated), infected rabbits were orally administered with GSE 250 mg/kg once daily. Row carrying different superscripts (a, b, c) are significantly different at *p* < .05.

Abbreviations: RBCs, Red blood cells; PCV, Packed cell volume; Hb, Haemoglobin; MCV, Mean corpuscular volume; MCHC, Mean corpuscular haemoglobin concentration; WBCs, White blood cells.
TABLE 5  Effects of oral administration of GSE on biochemical parameters in P. multocida experimentally infected rabbits

| Days post treatment | Parameters | Groups |
|---------------------|-----------|-------|
|                     | I         | II    | III   |
| 1st day             |           |       |       |
| AST (U/L)           | 62.9 ± 2.14c | 144.46 ± 5.60a | 137.68 ± 7.06b |
| ALT (U/L)           | 30.02 ± 3.55c | 45.6 ± 3.20a | 43.50 ± 4.73b |
| Urea (mg/dl)        | 29.42 ± 1.09c | 72.74 ± 6.22a | 46.08 ± 2.26b |
| Creatinine (mg/dl)  | 0.63 ± 0.05c | 1.01 ± 0.02a | 0.95 ± 0.03b |
| LDH (U/L)           | 148 ± 10.43c | 602.32 ± 24.49a | 509.16 ± 27.14b |
| Total protein (g/dl)| 8 ± 0.12a | 6.89 ± 0.17b | 7.09 ± 0.14b |
| Albumin (g/dl)      | 5.88 ± 0.17a | 2.77 ± 0.14c | 3.04 ± 0.24b |
| Globulin (g/dl)     | 2.12 ± 0.20 b | 4.12 ± 0.34a | 4.05 ± 0.20 a |
| α globulin (g/dl)   | 0.72 ± 0.04b | 1.79 ± 0.03a | 1.62 ± 0.04a |
| B globulin (g/dl)   | 0.69 ± 0.03b | 1.63 ± 0.11a | 1.66 ± 0.07a |
| γ globulin (g/dl)   | 0.71 ± 0.04a | 0.7 ± 0.05a | 0.77 ± 0.03a |
| IL-6 (pg/ml)        | 0.29 ± 0.03c | 2.13 ± 0.22a | 1.21 ± 0.11b |
| 7th day             |           |       |       |
| AST (U/L)           | 53.06 ± 4.12c | 92.16 ± 3.36a | 74.62 ± 4.48b |
| ALT (U/L)           | 28.63 ± 0.43c | 42.46 ± 0.82a | 38.35 ± 0.89b |
| Urea (mg/dl)        | 35.74 ± 1.54c | 44.04 ± 0.30a | 40.48 ± 1.46b |
| Creatinine (mg/dl)  | 0.70 ± 0.03c | 0.82 ± 0.03a | 0.78 ± 0.02b |
| LDH (U/L)           | 126.63 ± 6.38e | 421.57 ± 7.04a | 205.25 ± 9.90b |
| Total protein (g/dl)| 8.21 ± 0.24a | 4.43 ± 0.33c | 7.54 ± 0.36b |
| Albumin (g/dl)      | 6.40 ± 0.38a | 2.11 ± 0.13c | 3.95 ± 0.26c |
| Globulin (g/dl)     | 1.81 ± 0.12c | 2.32 ± 0.12b | 3.59 ± 0.33b |
| α globulin (g/dl)   | 0.38 ± 0.09c | 1.44 ± 0.07b | 1.62 ± 0.05a |
| B globulin (g/dl)   | 0.72 ± 0.04a | 0.68 ± 0.05a | 0.67 ± 0.02a |
| γ globulin (g/dl)   | 0.71 ± 0.04c | 1.20 ± 0.02b | 1.30 ± 0.07a |
| IL-6 (pg/ml)        | 0.36 ± 0.03c | 2.14 ± 0.05a | 1.65 ± 0.04b |

Note: Values are represented as the mean ± SE (n = 5). Group I (control group) intranasally administered with 0.1 ml sterilized saline; group II (infected—non-treated), infected group were orally administered with 0.5% DMSO solution; group III (infected—GSE-treated), infected rabbits were orally administered with GSE 250 mg/kg once daily. Row carrying different superscripts (a, b, c) are significantly different at p < .05.

Abbreviations: ALT, Alanine Aminotransferase; AST, Aspartate transaminase; LDH, Lactate dehydrogenase; IL-6, Interleukin-6.

Hypoproteinaemia, hypoalbuminaemia and hyperglobulinaemia were noted in P. multocida-infected group all over the experimental period in contrast to the control group, which may be attributed to off food, poor appetite and the inability of the liver to synthesize proteins. Hyperglobulinaemia may be one of the immune system responses against P. multocida infection. Meanwhile, oral administration of infected rabbits with GSE for five days significantly (p < .05) increased the levels of serum total protein, albumin, total globulin, α and γ globulins in contrast to the infected non-treated group. These results may be attributed to improving the appetite of rabbits, reduction in the damaging effects of bacteria on the liver, and immunomodulating outcomes of the testing agent.

A marked elevation in the serum levels of ALT, AST, LDH, urea and creatinine was recorded in P. multocida-infected group all over the experimental period compared with the control group, which could be due to the worse effect of micro-organism or its toxin on the liver and kidney. However, infected rabbits treated with GSE revealed a significant decrease in serum ALT, AST, LDH, urea and creatinine levels in contrast to the infected-non-treated group. These findings were supported by histopathological findings of liver and kidney that recorded in our previously published paper (El-Sheikh et al., 2020). These results confirmed the antibacterial activity, hepatorenal protective effect of GSE that reduced the harmful effects of bacteria on the liver and kidney. These results may be attributed to the antioxidant activity of GSE or its active constituents (Abdel-Daim et al., 2019; Albrahim & Robert, 2020; Ali et al., 2015). The hepato-renal protective effect and antibacterial activity of GSE were supported by those of (Hasona et al., 2017; Khalil, 2018).

5 | CONCLUSION

Pasteurella multocida infection remains a major public health problem in rabbits. It leads to high economic loss due to disturbance in all body parameters, including growth performance, haematological, biochemical and immunological parameters. Our study demonstrated...
that GSE has a potential therapeutic role in treating Pasteurellosis in rabbits. This study endorses the administration of GSE as a useful therapeutic tool demonstrating the potential for enhancement of Pasteurellosis treatment in rabbits. Further studies are required to identify the possible additional effects, appropriate doses and duration of the GSE therapy in rabbits Pasteurellosis.

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The authors declare that they have no conflict of interests.

AUTHOR CONTRIBUTION
Sawsan El-Sheikh: Conceptualization; Data curation; Formal analysis; Supervision. Fatma M. Youssef: Investigation; Methodology. Gaber Batiha: Visualization; Writing-original draft. Ashraf Albrakati: Haidi I. Mohamed: Investigation; Methodology; Validation. Gaber Batiha: Supervision. Fatma M. Youssef: Investigation; Methodology. Sawsan El-Sheikh: Conceptualization; Data curation; Formal analysis; Writing-review & editing.

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