Studying the Effectiveness of Anise Extract and Alignite Nanoparticles on the Balance of the Microbial Gut Flora and Some Immunological Parameters in the Laboratory Guinea Pigs

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Abstract. The study aims to determine the effect of oral dosage of anise fruit alcoholic extract and Alignite Nanoparticles (AlgNPs) and their interaction with the antibiotics Cefotaxime and Metronidazole concerning the parameters related to the balance of intestine normal flora with some immunological parameters in laboratory guinea pigs induced with diarrhea by E. coli. The results show that the Enterobacteriaceae bacterial colonies counts belonging less than those accounts of lactic acid bacteria in the intestines of laboratory guinea pigs. It is also found that the synergistic action of the nanocomposites with different antibiotics like what: have a better antimicrobial effect than the effect of each individually. There is also a significant increase (p<0.05) in the IgM and IgA values, which are at 1789 and 362 ng/L, respectively compared with their levels in the control group animals which are at 1230 and 184.5 (ng/L), respectively. The value of IgG does not change significantly in the oral dosage of the extract or the AlgNPs. After the recovery status of laboratory animals from infection, the level of IgG increased significantly, which gives evidence of bacterial infection in the infected animals.

1. Introduction
Diarrhea is a multifactorial illness associated with a wide range of pathogens, including parasites, viruses and bacteria. The development in living standards and health conditions have led to a decrease in the incidence of parasites, while the bacteria and viruses remain the main responsible pathogen for the occurrence of acute diarrhea cases in children. The prevalence of a diarrheal pathogen is often closely related to the socio-economic factors of the population. Most cases of diarrheal infections are associated with consumption of food or water contaminated with traces of faces, or transmission from one person to another due to poor personal hygiene [1]. Escherichia coli bacteria are the most common causes of diarrhea, especially among individuals in developing countries. Some survey studies indicate that the bacterial causes of diarrhea vary according to the geographical region. It is found that the bacterial species EPEC, Proteus mirabilis and Salmonella typhimurium are found as causes of diarrhea in Iraq with ratios of 33.6, 13.9. and 12.7%, respectively.

The past decade witnessed the completion of many researches in pursuit of discovering natural compounds with physical and chemical properties compatible with food and used in the treatment of many diseases. Alginate is one of these compounds that have been used as polymers in packaging fruits and vegetables [2] as well as in protection against pathogens from bacteria, fungi and viruses [3]. The mechanism of alginate binding is by being as a crystalline substance that has the ability to...
stabilize, as well as being an emulsifying agent [4]. Therefore, it has been used in the pharmaceutical industry with products that need to be dissolved in hot water before consumption, as in a mixture of vitamins and mixtures for ingredients in the treatment of colds, dizziness and headaches, or as a regulating agent for the delivery of antibiotics and the arrival of pain-relieving medicines to their places of effectiveness [5]. These environment friendly products reduce the amount of non-biodegradable waste, and thus reduce the negative impact of environmental pollution. The use of alginates has contributed to enhancing the absorption states, by being "smart polymers" that have been modified in the form of nanoparticles for use in wound healing, tissue regeneration, and drug delivery to specialized parts of the body, as well as in the treatment of obesity and diabetes [6] and [7]. As for the use of medicinal and aromatic plants, their effectiveness is due to their content of active substances of phenols, conjugates, flavonoids, alkaloids, proteins, tannins, glycosides and spindles [8] [9], where these substances work synergistically to enhance their biological activity [10]. Recently, the demand on the use of anise fruit extracts has increased in the treatment of many diseases, including respiratory infections and cases of diarrhea, due to the stability of its anti-bacterial and anti-oxidant efficacy, as well as its entry into the food, pharmaceutical, perfume and cosmetic industries [11].

From the above mentioned details, the aim of the study is an attempt to determine the effect of oral dosage of anise fruit alcoholic extract and AlgNPs, and the interaction of both with the antibiotic Cefotaxime and Metronidazole on some animal immunity parameters and the normal flora balance in the intestines of laboratory guinea pigs in which diarrhea is induced by *E. coli*.

2. Materials and Methods

2.1. Preparation of the bacterial suspension

The bacterial suspension is prepared as indicated in which is used in causing the experimental infection of laboratory animals of the guinea pigs, by inoculating 5 ml of the broth medium nourished with a pure isolate of *E. coli* bacteria in a sterile test tube and taking into account the sterilization conditions. Then, incubation is conducted at 37 °C for 24 hours and separating the bacterial cells by centrifugation of the nutrient broth medium after the incubation period has ended at a speed of 1000 cycle / min for 10 minutes. The cells are washed with physiological salt solution by adding 3 ml to the sediment and mixing them well. The centrifugation is repeated, the filtrate is discarded and 5 ml of physiological saline is added to the precipitate. A dilution of 1.5 x 10^-8 cells/ml is prepared to equalize McFarland's standard solution at a concentration of 0.5. The Viable plate count method is also used to control the number of bacterial cells.

2.2. Preparation of anise extract

The fruits of the anise plant are collected from the perfumers in the city of Erbil during the month of April. The samples are sterilized, dried and kept in air-tight polyethylene bags. The aniseed fruits are grinded with an electric grinder and a fine powder is obtained after sifting, kept in sterile packages, and hermetically sealed in moisture-free conditions until the plant extracts are taken. 50 g of the powder is taken in a 500 ml volumetric flask, containing 200 ml of Petroleum ether, the mixture is stirred for 24 hours continuously and then filtered using a boring cloth consisting of eight layers. The extract is centrifuged at 5000 rpm for 10 minutes, the filtrate is collected in a glass beaker and evaporated at room temperature to obtain the extract. The extraction process is repeated a second time using 100 ml of acetone: methanol at a volume of 50:50 with continuous stirring for 24 hours continuously, then filtering the mixture, centrifugation and evaporation using a water bath at a temperature of 60 °C to obtain the extract [11].

2.3. Preparation of the nanoparticles

The nanoparticles of the AlgNPs obtained from the American company NANOSHEL are prepared at a concentration of 30 mg/ml by dissolving in distilled water as stated by [13].
2.4. Initialization of the animals
Thirtysix male guinea pigs, aged 4 months, average weight at 500±22 grams and in good health, are selected and obtained from animal house of the College of Veterinary Medicine-Tikrit University. They are placed in homemade metal cages with one side sliding, the dimensions of each are 75 lengths, 40 widths and 40 cm height. Sawdust is not used to follow their excrement while taking care of the cleanliness of the cages. For the duration of the study, the animals are placed under standardized laboratory conditions in terms of ventilation, temperature, which is 26±3 °C, and a 12-hour light cycle for both lightness and darkness. The method was conducted according the method. It is adopted with some modifications, as nine groups are used, each group includes 4 animals.

2.5. Infection induced to the animals
After starving the animals for 24 hours by removing food from them, oral dosage of E. coli suspension is conducted through the mouth, at an amount of one ml per day, at 1.5x10^8 cells/ml. The health status of the animals is monitored and the changes that occur daily are recorded. The disease symptoms began to appear clearly on the fifth day and appeared more evident on the seventh day, including lethargy, seclusion, lack of movement, lack of appetite for food, as well as cases of diarrhea in animals given E. coli bacteria. A specialized veterinarian is used to ensure that the animals have become ill.

2.6. Design of the biological experiment
Thirty-six (36) male guinea pig animals are used in the experiment, which are randomly distributed into (9) groups. Each group is placed in a cage independently, and the distribution is conducted as follows: 1) the first group (the control group) was treated with physiological solution at the rate of (1) ml once a day (without infection), 2) the second group (the infected group) treated with the pathogenic dose ID50 of E. coli bacteria at an amount of 1.5x10^8 cells/ml. These are followed by treatment groups that include 3) the third group (plant extract group) the group of animals treated with the pathogenic dose ID50 of E. coli bacteria and treated with plant alcoholic extract at an amount of (1) ml at a rate of twice a day, 4) Group 4 (AlgNPs) are animals treated with ID50 E.coli and treated with AlgNPs particles, 5) Group 5 (Anise extract+AlgNPs particles): Animals treated with ID50 E.coli pathogen and treated with AlgNPs particles and plant extract in a ratio of 1:1, 6) Group Six (Anise extract+anti-Cefotaxime), the group of animals treated with pathogen ID50 of E.coli and treated with anti-Cefotaxime and Plant extract in a ratio of 1:1, 7) Group 7 (Anise extract+anti-Metronidazole), the group of animals treated with the pathogen ID50 of E.coli bacteria treated with the anti-Metronidazole and plant extract in the ratio 1:1, 8) Group 8 (The group of AlgNPs particles+Cefotaxime, animals treated with ID50 of E.coli and treated with nanogenes and anti-Cefotaxime in a ratio of 1:1, 9) Group 9 (AlgNPs particle group+anti-Metronidazole), animals treated with ID50 of E.coli and treated with Particles AlgNPs and metronidazole antagonist in a 1:1 ratio.

The oral dosage to laboratory guinea pigs showed E. coli infection with both AlgNPs particles at a concentration of 30 mg/ml and alcoholic extract of anise fruits at a concentration of 20 mg/ml at a rate of 2 ml for each treatment (1 ml/12 hours). The health status of all animals is monitored and the oral dosage continued until symptoms decreased and completely disappeared. To ensure their safety, the animals are examined by a specialized veterinarian.

2.7. Immunological parameters assays
Blood samples are drawn from the treatments of all laboratory animal groups in two sets of blood collection tubes containing (EDTA), 4 ml of blood was placed in each tube and left until clotting, then centrifuged at 3000 rpm for 15 minutes to obtain the blood serum, which was drawn using a micropipette and then kept at -20°C until the tests for IgA, IgG and IgM immunoassays are performed.

2.8. Estimating the number of normal flora in the intestines
After the end of the experiment, the animals are dissected after being anesthetized using chloroform. The animals are placed in a glass anesthesia box containing a piece of cotton saturated with
chloroform and left for 1-2 minutes. The autopsy is performed under the supervision of a specialized veterinarian. It starts from the top of the neck and goes down to the abdomen. A portion of the small intestine weighing 25 g is taken and placed in 225 gm of normal saline. After stirring, bacterial culture is performed on a MRS medium and a MacConkey agar medium to see the effect of treatments on the microbial balance in the intestines of laboratory animals.

2.8.1. Estimating of the total Coliform bacteria count in the intestine
Roughly 10 g of each part of the animal intestine are collected and preserved using physiological saline in a volume of 90 ml. The necessary dilutions are made up to the fifth dilution, 0.1 ml is withdrawn from the last dilution and spread on MacConkey agar medium and the plates are incubated in an inverted manner at 37°C for 24 hours. After that, colonies are counted on the plate for each sample [15].

2.8.2. Determining the total lactic acid bacteria count
About 0.1 ml of the dilution prepared in the physiological solution in the previous step is taken and spread on the surface of MRS-CaCO3 agar medium and incubated anaerobically at 35 ºC for 24-48 hours. The numbers of cream-shaped colonies that have bright areas around them are calculated [15].

2.9. Statistical analysis
The experiment is carried out according to the Complete Randomized Design, and analysis of variance is conducted using the General Linear Model within the ready-made statistical program (SAS) Statistical Analysis System. 0.05), and using the Duncan Test [15] in the case of significant differences between the different means at the level (0.05).

3. Results and Discussion
3.1. Effect on the immunoglobulin IgA, IgG and IgM
Determining the levels of immunoglobulin in the body is of great importance because of the possibility of inferring from their levels the dysfunction in the body’s organs as well as the level of the immune status of the organism. The effects of oral dosage of AlgNPs and alcoholic extract of anise fruit on laboratory animals and their interaction with Cefotaxime and Metronidazole on the level of immunoglobulin IgA, IgG and IgM are illustrated in Table (1). The induced infection with E.coli in laboratory Genia pigs has caused a significant increased (p<0.05) in IgM and IgA values at 1789, 362 ng/L, respectively, compared to their levels in control group animals, which are at 1230, 362.5 ng/L, respectively. The value of IgG did not change significantly at the beginning of the oral dosage of the extract or nanoparticles, and there are no significant differences between the control group and the experimentally infected group of E.coli causing diarrhea. After the laboratory animals fully recover from infection, the level of IgG changes and a significant increase in its value is obtained, which is evidence of bacterial infection in infected animals.

The reason for the high level of IgA, IgM, and IgG immunoglobulins can be attributed to the effectiveness of these globulins and their ability to destroy foreign bodies entering the body and getting rid of them through various mechanisms, including analyzing foreign particles or changing their effectiveness. The IgM immunoglobulins, which are characterized by their large size compared to other types, are produced in the blood of the organism 3-7 days after the occurrence of bacterial infections. As for IgG immunoglobulin, it is one of the antibodies that have the ability to cross from the blood serum to the cells due to its small size, and thus it can attach and then cross to the cells or attach to them [17]. For IgA globulin, it is present in the blood by 30% and is locally produced in the urinary and digestive tracts in case of infection, as it works to prevent the adhesion of bacteria to the epithelial cells of the urinary tract and prevents them from penetrating the tissues. Its presence and high concentration in the blood serum coincides with the presence and rise of IgG antibody [18]. The results of this study are identical with [19], who found an increase in the concentration of the three immunoglobulins in infected patients in Baghdad Governorate as a result of bacterial infection. The
results also agree with [20] who found in Najaf Governorate, a significant increase in the concentration of IgA, IgG, and IgM antibodies in patients infected as a result of bacterial infection.

Table 1. The effect of oral dosing of alcoholic anise extract and AlgNPs on IGA, IgG and IgM parameters in animals laboratory infected with E.coli

| Group types | IgM (ng/L) | IgG (ng/L) | IGA (ng/L) |
|-------------|------------|------------|------------|
| C           | 1230c ±0.2 | 1291c ±0.2 | 184.5b ±2.3 |
| E           | 1789a ±0.2 | 1312c ±0.2 | 362.5a ±3.9 |
| PA          | 1380b ±0.4 | 1580b ±0.4 | 190.5b ±6.4 |
| AL          | 1290c ±0.2 | 1585b ±0.2 | 181.5b ±4.9 |
| PA+AL       | 1245c ±0.3 | 1520b ±0.1 | 195.5b ±4.9 |
| PA+V        | 1267c ±0.2 | 1580b ±0.2 | 171.5bc ±0.7 |
| PA+M        | 1190 ±0.3  | 1559b ±0.2 | 175.5b ±2.1 |
| AL+V        | 1321b ±0.4 | 1657a ±0.1 | 182.0 ±4.7  |
| AL+M        | 1288c ±0.2 | 1594b ±0.1 | 193.0b ±2.0 |

Different letters in the same column indicate significant differences at the 0.05 probability level.

C: Control, E: E.coli-infected animals without treatment, PA: E.coli-infected animals treated with alcoholic extract of anise, AL: E.coli-infected animals treated with AlgNPs particles, PA+AL: E.coli-infected animals treated with AlgNPs and treated with PA, PA + C: animals infected with E.coli and treated with PA + Cefotaxime, PA + M: animals infected with E.coli and treated with antibiotics PA + metronidazole, AL + C: animals infected with E. coli and treated with antibiotic + Cefotaxime AL, AL + M: E.coli infected animals treated with antibiotic + Metronidazole AL.

3.2. Effect on the balance of intestine normal flora of laboratory Genia pigs

The results of bacterial culture of samples taken from the intestines of animals dosed with either AlgNPs or alcoholic extract of anise fruits and the interaction between each of them with the antibiotic Cefotaxime or Metronidazole in Genia pigs induced with diarrhea by E. coli and cultured on MRS and MacConkey agar medium are shown in Table (2). The results show that the coliform bacterial count was increased significantly (p<0.05) in the duodenum, ileum and large intestine of the animal group infected with diarrhea induced with E.coli appeared at 40, 36 and 34 log CFU/g respectively compared with the coliform bacterial counts in the same intestine parts in the control group at Log 11, 20, 17 CFU/gm, respectively. However, the total count of LAB in the duodenum, ileum and large intestine of laboratory animals induced with diarrhea appeared at 7, 9 and 3 log CFU/g respectively compared with counts of LAB in the same parts of intestine in control group of Genia pig’s animals which appeared at 13, 16 and 14 log CFU/g respectively.
Table 2. The effect of oral dosage of alcoholic anise extract and AlgNPs on the balance of normal flora in the intestines of animal’s laboratory infected with *E. coli*

| Treatments | Bacterial type | Bacterial counts (log CFU/g) | Duodenum | Ileum | L.Intestine |
|------------|----------------|-------------------------------|----------|-------|-------------|
| C          | LAB            | 13d                           | 16d      | 14d   |
|            | Coliform       | 11d                           | 20c      | 17d   |
| E          | LAB            | 7e                            | 9e       | 3e    |
|            | Coliform       | 340a                          | 36ab     | 34ab  |
| PA         | LAB            | 11d                           | 15d      | 13d   |
|            | Coliform       | 12d                           | 13d      | 10d   |
| AL         | LAB            | 13d                           | 15d      | 13d   |
|            | Coliform       | 9e                            | 12d      | 14d   |
| PA+AL      | LAB            | 15b                           | 25c      | 17d   |
|            | Coliform       | 7d                            | 11d      | 10d   |
| PA+V       | LAB            | 16d                           | 14d      | 13d   |
|            | Coliform       | 12d                           | 9e       | 11d   |
| PA+M       | LAB            | 15d                           | 13d      | 13d   |
|            | Coliform       | 8e                            | 12d      | 13d   |
| AL+V       | LAB            | 19d                           | 17d      | 15d   |
|            | Coliform       | 12d                           | 14d      | 10d   |
| AL+M       | LAB            | 20c                           | 17d      | 14d   |
|            | Coliform       | 11d                           | 15d      | 12d   |

Different letters in the same column indicate significant differences at the 0.05 probability level.

C: Control, E: *E.coli*-infected animals without treatment, PA: *E.coli*-infected animals treated with alcoholic extract of anise, AL: *E.coli*-infected animals treated with AlgNPs particles, PA+AL: *E.coli*-infected animals treated with AlgNPs + PA, PA + C: animals infected with *E.coli* and treated with PA + Cefotaxime, PA + M: animals infected with *E.coli* and treated with antibiotics PA + metronidazole, AL + C: animals infected with *E. coli* and treated with antibiotic + Cefotaxime AL, AL + M: *E.coli* infected animals treated with antibiotic + Metronidazole AL.

This is significantly higher (p<0.05) than the colony numbers of lactic acid bacteria in the same areas of the intestine which are 13, 16, 14 and CFU/g respectively. Oral dosage of the alcoholic extract of anise fruit as well as AlgNPs nanoparticles cause a significant increase in the numbers of lactic acid bacteria compared to the numbers of coliform bacteria, which shows that the plant extract is effective in improving the body's immunity by inhibiting of bacterial type that causes diarrhea cases by *E. coli* and giving access to allow beneficial bacterial species in growth and proliferation to reach the majority of the gut microbial balance [21]. The same applies to the use of nanoparticles, which are found to be effective in inhibiting the bacterial species that causes diarrhea, which causes an increase in the total numbers of beneficial bacteria that can be safe to use by humans or animals, and this is in agreement with [22].

Thus, nanoparticles can be used in cases of protection against intestinal diseases through the mechanism of inhibition of pathogenic bacterial species, and this has been proven by [23] in which there is the absence of any signs of toxicity in adult mice after exposure to nanoparticles. It also protects against foodborne diseases. [24] has indicated the potential to improve the behavior and cognitive impairment in behaviorally problematic mice by regulating ionic balance and physiological functions of neurons. In addition, the fruits of the anise plant contain a number of effective chemical compounds that have antimicrobial, immunostimulant, antioxidant and anti-inflammatory activity, making it widely used in the medical and therapeutic fields. The results show that the synergistic action of the nanocomposites with different antibiotics has better antimicrobial effects than the nanocomposites or the antibiotics alone [25]. It is found that the effect of using anti-amoxicillin
against the pathogenic bacterial species of *E. coli* is greater when loaded with nanoparticles compared to it when used separately. The overall treatment intake and side effects can be significantly reduced by combining antibiotics with nanoparticles [26]. Drug delivery to target areas aims at reducing side effects of medications as well as lowering the therapeutic dose.

This is also proved in a study conducted in the synergy between nanoparticles and antibiotics on the effect on gram-negative bacteria. It is found that the effect is on the lipopolysaccharide layer present in the wall of *E. coli* bacteria, which is observed through transmission electron microscopy. It is also found that the bacterial cells are greatly reduced by nanoparticles and antibiotics, and this explains what happens in the current results regarding the increase in the number of lactic acid bacteria compared to the intestinal bacteria.

4. Conclusion

The use of the plant extract of the fruits of anise and AlgNPs nanomaterial led to the elimination of *E. coli* bacteria that cause diarrhea, in addition to an increase in the immunoproteins and an increase in the number of lactic acid bacteria compared with the intestinal bacteria in the intestinal tract.

References

[1] Kotloff KL, Blackwelder WC, Nasrin D, Farag TH, Panchalingam S, Wu Y, Sow SO, and Sur D 2013, Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. Lancet. 20;382(9888):209-22.

[2] Gundewadi, G, Rudra S, Sarkar D, and Singh D 2018, Nanoemulsion basedalginate organic coating for shelf life extension of okra. *Food Packag and Shelf Life* 18, 1–12.

[3] Arroyo, B, Bezerra A, Oliveira L, Arroyo S, De Melo E, and Santos A 2020, Antimicrobial active edible coating of alginate and chitosan add nanoparticles applied in guavas (*Psidium guajava* L.). *Food Chemistry* 309,125566.

[4] Jiang, Y, Yu G, Zhou Y, Liu Y, Feng Y, and Li J 2020 a, Eects of sodium alginate on microstructural and properties of bacterial cellulose nanocrystal stabilized emulsions. *Colloids Surf.*

[5] Martau, GA, Mihai M, and Vodnar D 2019, The use of chitosan, alginate, and pectin in the biomedical and food sector—Biocompatibility, bioadhesiveness, and biodegradability. *Polymers* 11, 1837.

[6] Mantha, S, Pillai S, Khayambasi P, Upadhyay A, Zhang Y, Tao O, Pham H, and Tran S 2019, Smart hydrogels in tissue engineering and regenerative medicine. *Materials* 12, 3323.

[7] Getachew, A, Jacobsen C, and Holdt S 2020, Emerging technologies for the extraction of marine phenolics: Opportunities and Challenges. *Marine Drugs* 18, 389.

[8] Cowan, MM 1999, Plant products as antimicrobial agents. *Clinical Microbiology Reviews* 12, 564–582.

[9] Alassi, BS, and Ahmed AA 2020, Effect of adding of the Milk Thistle (SILYBUM MARIANUM) seed powder in the traits of biochemical blood of the quail. *Plant Archives* 20(1), 962-964.

[10] Chen, N Chang CC, Ng CC, Wang CY, Shyu YT, and Chang TL 2008, Antioxidant and antimicrobial activity of Zingiberaceae plants in Taiwan. *Plant Foods for Human Nutrition* 63 (1), 15-20.

[11] Tirapelli, CR, Andrade, De Cassano AO, De Souza FA, Ambrosio SR,Costa FB, and Da Oliveria AM 2007, Antispasmodic and relaxant effects of the hydroalcoholic extract of pimpinella anisum (*Apiaceae*) on rat anococcygeous smooth muscle. *Journal of Ethnopharmacology* 110 (1), 23-29.

[12] Naseem, HK 2019, Effectiveness of some nanoparticles with plant extracts on storage of soft cheese. Agriculture-Tikrit University.

[13] Ahmed, KN, Thalij KM, and Mohammed MJ 2020b, Evaluation of some food poisoning bacterial inhibition from zno and ag nanoparticles that synthesized by aspergillus niger. *IOP Conference Series: Materials Science and Engineering* 1058 012080: 1-9.
[14] Harrigan, WF, and McCance MF 1979, Laporatory method in food and dairy microbiology. Academic press. London.
[15] Duncun, OD, and Duncun B 1995, A methodological analysis of segregation Index. American Sociological Review 20, 210-217.
[16] Nikolaeva, MA, Kulakov VI, and Ter-Avanesov GV 1993, Detection of antisperm antibodies on the surface of living spermatozoa using flow cytometry:prelimin ary study. Fertility and Sterility 59, 639-644.
[17] Helen, C, Mansel H, Siraj M, and Neil S 2006, Essential of clinical immunology.5thed..Blackwell.
[18] Khalf, ZS 2010, Identification of Some Bacterial Pathogens Associated With Male Infertility and Detection of Microdeletion in AZF Genes on Human Y Chromosome Using. PCR Technology. Thesis, College Of Science, Baghda University.
[19] Al-Sakr, RJH 2009, Study of some immunological and physiological aspects of patients with asthenozoospermia. PhD thesis, College of Science, University of Kufa.Catholic - Beirut - Lebanon.
[20] Ahmed, KN, Thalij KM, and Mohammed MJ 2020a, The Role of Ag, Zn nanoparticles and Nisin in Biological and Immunological Parameters in Male Rats. Journal of Critical Reviews 7 (14), 3839-3844.
[21] Roselli, M, Finamore A, Garaguso I, Britti MS, and Mengheri EI 2003, Zinc oxide protects cultured enterocytes from the damage induced by Escherichia coli. The Journal of Nutrition 133(12), 4077-4082.
[22] Amara, S, Ben-Slama I, Mrad I, Rihane N, Jeljeli M, El-Mir L, Ben-Rhouma K, Rachidi W, Sève M, Abdelmelek H, and Sakly M 2014, Acute exposure to zinc oxide nanoparticles does not affect the cognitive capacity and neurotransmitters levels in adult rats. Nanotoxicology 8(1), 208-215.
[23] Yongling, X, Yiyi W, Tao Z, Guogang R, and ZhuoY 2012, Effects of nanoparticle zinc oxide on spatial cognition and synaptic plasticity in mice with depressive-like behaviors. Journal of Biomedical Science 19, 14.
[24] Li, M, Zhu L, and Lin D 2012, Toxicity of organic nanoparticles to Escherichia coli: mechanism and the influence of medium components. Environmental Science and Technology 45(5), 1977–83.
[25] Chen, N, Chang CC, Ng CC, Wang CY, Shyu YT, and Chang TL 2013, Antioxidant and antimicrobial activity of Zingiberaceae plants in Taiwan. Plant Foods for Human Nutrition 63(1), 15-20.
[26] Roy, A, Ameena P, Anil K, and Ambika PMVN 2016, Effect of nano-titanium dioxide with different antibiotics against methicillin-resistant Staphylococcus aureus. Journal of Biomaterials and Nanobiotechnology 1, 37-41.