ISSN- 2394-5125 VOL 7, ISSUE 04, 2020

ESTIMATION OF AG, ZN NANOPARTICLES AND NISIN EFFECTS ON BIOLOGICAL AND IMMUNOLOGICAL PARAMETERS IN MALE RATS

Khalaf N. Ahmed [1] , Karkaz M. Thalij*[2] , Mohammed J. Mohammed [3]

[1,2,3] Food Science Department, College of Agriculture, Tikrit University, IRAQ.
***Corresponding authors email:** kthalij@gmail.com

ABSTRACT
The study aimed to determine the effect of oral dosage of 30% for both AgNPs and ZnNPs alone or combination with 50% of the nisin in some biological and immunological parameters of laboratory male rats after raising them for 21 days. The results showed a significant increased ($p<0.05$) in the Red blood cells counts (RBCs), the packed cell volume (PVC) and the platelets and a significant decreased in the white blood cells counts (WBCs) in all treated groups. The values of IgA, IgE and IgG immune proteins were did not significantly change and appeared at control group at 107.3, 0.38 and 1005 **ng/L**. The values of total protein were not differ significantly while the globulin were significantly increased when treated with AgNPs and ZnNPs alone or with nisin compared with the control group which at 5.56 and 2.23mg/ml respectively, while the albumin values were decreased significantly when compared with control group which at 3.33mg/ml. The conclusion was confirm that orally dosage from AgNPs, ZnNPs or Nisin were didn't has negative effects and was safety to used in antimicrobial used.
Key words: AgNPs ،ZnNPs, Niasin, Biological, Immunological, Rats.

INTRODUCTION

Nanotechnology is the science that involves making materials of atoms size to achieve unique properties, which create effective effects, Where the science of nanotechnology and the technologies emanating from it are wide fields for scientific and technical research, as they use advanced mechanisms and devices. Nano is the one per billion of an objects, and the dimensions of nanomaterials range from 1-100 nanometers. It has related applications fall into most aspects to human life and health, it is part of the specialty of chemistry, physics, molecular biology, applied biology and medical and therapeutic specializations that have made them distinct and different from other technologies and other sciences [1]. Nanoscience is defined as the science concerned with the investigation and characterization of nanomaterials, the study of their physical, chemical and mechanical properties, and knowledge of associated phenomena arising from their small sizes [2]. Nanotechnology is also known as products made from nanomaterials that have general effects, especially in the field of tissue engineering, cancer treatment, sports equipment, solar cells, space materials and cosmetics [3].

Silver nanoparticles (AgNPs) are considered one of the widely marketed nanomaterials as they are used in various applications, it is characterized by multiple positive properties, including high conductivity, chemical stability and high effectiveness as antimicrobial [4]. also, silver particles have the ability to interact with sulfur-containing amino acids inside and outside the cell membrane and this affects the effectiveness of cells [5]. Silver ions can also interact with the thiols group in the amino acids that make up the respiratory chain enzymes of cells [6]. Zinc nanoparticles (ZnNPs) and their oxides are among the materials that have high physical and chemical properties, such as conductivity, surface tension, surface area increase due to the small size of the granules, which has increased the effectiveness of their use in various applications such as electrical and electronic devices, gas sensors, biological and chemical sensors as used in preparations of cosmetic and paint materials [7]. Nisin is one of the most important bacteriocins produced from lactic acid bacteria. It consists of 34 amino acids with a molecular weight of 3354 Dalton and it is characterized by its inhibiting effect towards microorganisms and is widely used in preserving foods, especially milk products [8].

Based on the above, the aimed of this study was to find out the effectiveness of orally dosage from AgNPs or ZnNPs nanoparticles alone or with nisin on some biological and immunological parameters in male laboratory rats after its breeding for 21 days.

MATERIALS AND METHODS

Biological experiment: AgNPs and ZnNPs were synthesized used biological procedure according to [9] and illustrated in our research [10]. While the Nisin compound was obtained from Saco company (Italy).

Intitlilization of laboratory animals: Healthy and disease-free laboratory animals were obtained from the collage of Veterinary Medicine-University of Tikrit with the 30 of adult Albino-Spraque dawly male rats at the age between 8-9 weeks and their weights ranged from 210 to 220 g, Rats were randomly separated into 6 groups. Each group included 5 male rats: T1 control animal group, T2 animals group given only 30% AgNPs, T3

ISSN- 2394-5125 VOL 7, ISSUE 04, 2020

animals group given 30% ZnNPs, T4 animals group given 50% Nisin only, T5 animals group given 30% AgNPs with 50% Nisin, T6 animals group given 30% ZnNPs with 50% Nisin.

Animals were placed in plastic cages after brushing their floors with sawdust that was replaced every other day. Animals were fed regularly using ready-made feed according to [11], and were given orally dosage from AgNPs, ZnNPs and Nisin compounds according to the concentrations and sizes proposed in the experiment design.

Parameters assay: Immediately after the end of the experiment period, the animals were left without food for 20 hours, then they were anesthetized by using chloroform. Then the rats were sacrificed from the chest area and drawn blood from the heart directly to perform the necessary tests, as the blood was drawn in two tubes, the first was containing an anticoagulant EDTA to measure the blood and the other was free of it, approximately 5 mL of blood were collected and centrifuged using a centrifuge at 3000 round/ min for 15 minutes to obtain the serum that was kept at -20 ° C until the analyzes were performed as in (Tietz, 2005). From the blood collection tubes containing the anticoagulant (EDTA), the total number of white blood cells (WBCs), red blood cells (RBCs), hemoglobin concentration (Hb), packed cell volume (PCV) and platlates level were estimated as stated in [12] using the Complete blood picture.

Total Protein and albumin were estimated using a number of standard solutions (Kits) supplied by Biolabo (France). The analyzes were carried out by the Shimadzu Spectrophotometer (Japan) and according to the recommended wavelength for each analysis. The concentrations were calculated using the equations according to the company instructions supplied for each crew. globulin was estimated on the basis of the difference between globulin and albumin as stated [12].

The estimation of immunoglobulins IgG, IgA, IgE by using the ELISA Assay technique, according to the instructions of manufacturer's company for the analysis teams as in [12].

Statistical analysis: The data were statistically analyzed through the experimental system within the prepared statistical program [13] and by using the complete randomized design system CRD as the averages were chosen according to the Dancan test [14].

RESULTS AND DISCUSSION

The effect of oral dosage of AgNPs or ZnNPs singly or incombination with Nisin, on blood picture of laboratory rats:

blood picture parameters are among basic tests that give an indication of the physiological and health status of an animal by estimating the total accounts of major blood cells in the blood including red blood curpusular (RBCs), white blood cells (WBCs), platelets (PT) and hemoglobin concentration (Hb). The results in Table (1) showed the effect of oral dosage of AgNPs or ZnNPs alone or mixed with Nisin on the parameters of blood picture of laboratory rats for a period at 21 days. It found that hemoglobin levels in the blood of rats given orally AgNPs at 30% (M2), ZnNPs 30% (M3), Nisin 50% (M4), AgNPs 30% + Nisin 50% (M5) and ZnNPs30%+Nisin 50% (M6) was at 13.4, 12.8, 12.6, 12.1 and 12.0 mg/dl, respectively, Which did not differ significantly at ($p<0.05$) for all treatments compared to the control sample M1 were the Hb level was at 13.0 mg/dl.

Table 1. Effect of oral dosage of AgNPs or ZnNPs with Nisin on blood picture of Male Rats after 21 days.

Treatments	Values of the parameters of the blood picture in rats			
	Hb(mg/dl)	RBCs(×10^6/mm3)	PVC (%)	Platlates (×10^9/L)
M1	13.0a±0.83	5.21a± 0.62	36.6b±1.2	198.0c±4.0
M2	13.4a±0.61	5.10a± 0.43	37.6b±1.7	208.0b±9.5
M3	12.8a±0.46	5.30a± 0.59	36.3b±1.5	222.6a±13.5
M4	12.6a±0.34	5.17a± 0.72	40.6a±0.7	220.3a±7.7
M5	12.1a±0.36	5.12a± 0.48	38.6a±2.4	223.3a±10.0
M6	12.0a±0.49	5.23a± 0.60	39.0a±0.3	222.3a±5.5

- The different letters in each column indicate that significant differences at probability 0.05.
-M1 = Control M2 = AgNps 30% M3 = ZnNps 30% M4 = Nisin 50% M5 = AgNps 30% + Nisin50% M6 = ZnNps 30% + Nisin 50%.

The results were agreement with that of [15], who found that hemoglobin levels in experimental animals injected with gold and silver nanoparticles did not differ significantly from the control sample. The results also showed that the effect of oral dosage of AgNPs or ZnNPs alone on incombination with Nisin were non differ significantly on RBCs, compared with their values in the control rats group which was at 5.21 x 10^6/ mm. The Packed Cell Volume (PVC) parameter is one of the blood test that indicate the percentage of compressed red blood cells with normal levels between 36-47%. From the results, a significant increase ($p<0.05$) was observed in oral dosage treatments groups of M4, M5 and M6 and became at 40.6, 38.6 and 39%, respectively, compared to the values of the same test in the M1 control sample which was at 36.6%. while the other treatment were not differ significantly. These results were agreed with [16] mentioned by studying the effect of AgNPs on blood

ISSN- 2394-5125 VOL 7, ISSUE 04, 2020

diseases in fish, as it was found that the ratios of PVC in all treatments ranged between 32.5-40%, which showed a significant difference with the control sample.

Blood platelets are one of the main components of blood and their function in the formation of thrombion to prevent bleeding in the blood vessels, and that the occurrence of a decrease or increase the level of these platelets causes platelet dysfunction, so it is an indication of the health of the animal. . The results mentioned in Table (1) that the level of platelets in the blood of rats given AgNPs, ZnNPs, alone or incombination with nisin which M2, M3, M4, M5, M6 did not cause a significant change and became at 208, 222.66, 220.33 , 223.33 and 222.33 $\times 10^{\wedge}9$/L respectively compared to their accounts in the control group M1 whose the account values were at $19.67 \times 10^{\wedge}9$/ L from platelet preparation in the control sample.

The effect of oral dosage of complex AgNPs, ZnNPs, Nisin on white blood cells of rats:

The effect of oral dosage of AgNPs or ZnNPs singly or together with Nisin on the total accouts of WBCs is illustrated in Figure (1). The results showed a significant decrease ($p<0.05$) in the WBCs count values in the treated groups of M2, M3, M4, M5, M6, which became at 9.78, 9.16, 8.46, 6.56 and 7.03 $\times 10^{\wedge}6$/ mm3 compared to their numbers in the control group M1 that appear at 11.59 $\times 10^{\wedge}6$/ mm3, It was also found that the lowest effect was in the treated M6 involving dose of ZnNPs of 30% and Nisin 50%, while the highest effect was in the treated group of M2 which involving oral dosage of AgNPs at 30% compared to other treated groups. The results were agreed with Lee et al.,(2018) that the accounts of WBCs were decreased significantly and became at 6.30 $\times 10^{\wedge}6$/ mm3. Also was agreed with [17] who found significantly decrease in WBCs when exposing to CuO-NPs. WBCs accounts are one of the major blood cells that perform immune functions as part of the immune system and have a biological role in protecting the body from infections caused by bacteria, viruses, and fungi. Usually high numbers of WBCs are associated with microbial infection or the presence of foreign bodies or antigens in the circulatory system, immune system disorders, tension, etc. [18].

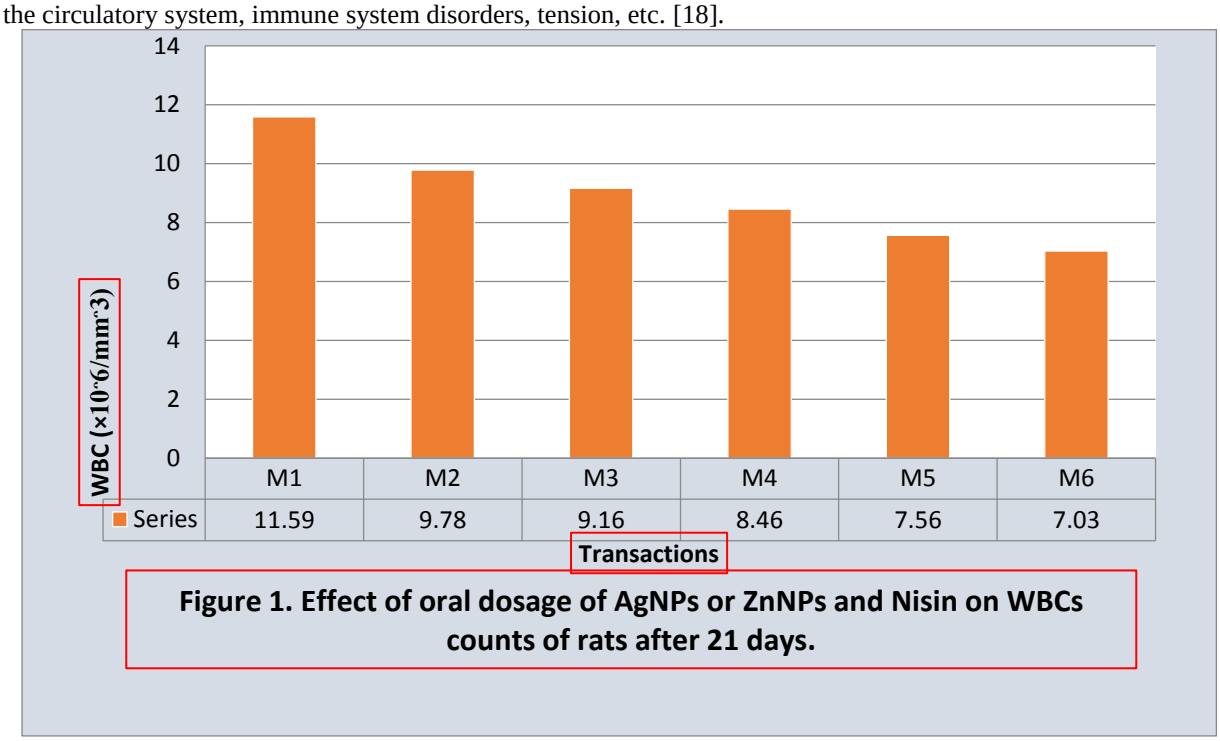

Figure 1. Effect of oral dosage of AgNPs or ZnNPs and Nisin on WBCs counts of rats after 21 days.

M1 = Control M2 = AgNps 30% M3 = ZnNps 30% M4 = Nisin 50% M5 = AgNps 30% + Nisin50% M6 = ZnNps 30% + Nisin 50%.

The effect of oral dosage of AgNPs, ZnNPs, or Nisin on protein values in the male rats.

Table (2) shows the effect of oral dosage of each AgNPs, ZnNPs alone or incombination with nisin, on the value of serum protein parameters in rats raised for 21 days. The results found that the total protein was increased significantly ($P<0.05$) in all treated groups M2, M3, M4, M5, M6 that appeared at 5.86, 5.9, 5.7, 5.76 and 5.93 mg/ ml on respectively compared to its value in the control group M1, which was at 5.59 mg/ ml, the highest value was shown in the M6 treatment, while the M4 treatment was the lowest value in the total protein level. The results also indicated that the level of albumin were significantly decreased in the treated groups M2, M3, M4, M5, M6 that at 2.63, 2.83, 2.83, 2.96 and 2.73 mg/ ml, respectively, compared to the M1 control sample in which the albumin level reached at 3.33 mg/ ml. The treated M5 was characterized by the highest albumin content, and in contrast, the treated M2 recorded the lowest value. The globulin protein values was significantly decreased in the orally treated M2, M3, M4, M5 and M6 which appeared at 3.23, 3.07, 2.87, 2.8 and 3.2 mg/ ml, compared with the value in control group M1 which was at 2.23 mg/ ml. The results were agreed with what [19] who found about the effect of Nisin given to rats at a concentration of 50 mg/ kg per day.

ISSN- 2394-5125 VOL 7, ISSUE 04, 2020

Blood proteins are considered as one of the most important components of serum that perform important functions in the body as they are the building unit for many organs, hormones and enzymes, as they are necessary in enhancing the immunity and health of the body. The total blood serum protein test can be used in the ability to know the amount of Albumin and Globulin proteins, Albumin helps regulate osmotic pressure in the plasma to prevent fluid leakage from blood vessels. Globulin includes enzymes and antibodies from immunoglobulins.

Table 2. Effect of oral dosage of AgNPs, ZnNPs or Nisin on protein values in male rats after 21 days.

Treated	Average values for protein components in rats (mg/ ml)		
	Total protein	Albumin	Globulin
M1	5.56a±0.06	3.33a±0.20	2.23b±0.09
M2	5.86a±0.17	2.63b±0.25	3.23a±0.31
M3	5.9a±0.34	2.83b±0.37	3.07a±0.11
M4	5.7a±0.30	2.83b±0.12	2.87a±0.25
M5	5.76a±0.14	2.96b±0.10	2.8a±0.15
M6	5.93a±0.12	2.73b±0.03	3.2a±0.31

- The different letters on column indicate that a significant differences at probability0.05.
-M1 = Control M2 = AgNps 30% M3 = ZnNps 30% M4 = Nisin 50% M5 = AgNps 30% + Nisin50% M6 = ZnNps 30% + Nisin 50%.

The effect of oral dosage of AgNPs, ZnNPs, or Nisin on IgA, IgE and IgG values in the male rats.

The effect of oral dosage of AgNPs, ZnNPs, alone or with Nisin on the IgA, IgE and IgG in blood of male rats were illustrated in table (3). The results showed that oral dosage from all treatments were not caused significant change ($p<0.05$) in IgA, IgE and IgG in male rats. The values of IgA in treatments M2, M3, M4, M5, M6 are at 108, 107, 109, 110 and 109 ng/L respectively compared for the same parameters in control group T1 which appeared at 107 ng/L. Also the values of both immunoglobulins IgE and IgG were not significantly different since their value in the control animals group which was at 0.38 and 1005 ng/L respectively. The results were agreed with [20] who that found not change in immune system parameters when approved the laboratory rats with metal nanoparticules. These results were also agreed with [21], which confirmed our results in didn't change the immune parameters when orally doage with AgNPs to male rats. Immunoglobulins are proteins that have a fundamental role in the body's immunity against the pathogens of bacteria and fungi and foreign substances entering it, The lack of significant difference in the groups treated with oral dosage of the complexes under study indicates that they do not cause an increase in the immune response of the immune system of laboratory animals in any of these types of globulin, which confirms the safety of their use and has no pathological effect [22].

Table 3. Effect of Oral dosage of AgNPs, ZnNPs, Nisin on Immunological values of Rats after 21 days.

Treatments	Immunoglobulin values for rats		
	IgA (ng/L)	IgE (ng/L)	IgG (ng/L)
M1	107.3a±6.4	0.38a±0.06	1005a±6.60
M2	108.8a±3.7	0.33a±0.18	1013a±15.7
M3	107.6a±7.5	0.39a±0.08	1015.3a±14.9
M4	109.6a±5.8	0.35a±0.005	1015.3a±15.2
M5	110.7b±8.8	0.48a±0.05	1007.3a±17.0
M6	109.1a±3.1	0.65a±0.04	1009a±12.6

- Different letters in each column indicate that significant differences at probability 0.05.
-M1 = Control M2 = AgNps 30% M3 = ZnNps 30% M4 = Nisin 50% M5 = AgNps 30% + Nisin50% M6 = ZnNps 30% + Nisin 50%.

Conclusions: The conclusion of oral dosage from AgNPs or ZnNPs singly or in combination with Nisin were didn't significantly change at most Blood picture, serum protein and immunoglobulins parameters that confirms the safety of their use its and has non negative side effects.

ISSN- 2394-5125 VOL 7, ISSUE 04, 2020

Acknowledgement: Our appreciation to the staff of Food Science department in College of Agriculture, Tikrit University for funding supported, as well as to laboratory animals house staff in the College of Veternary Medcine in Tikrit University in provide and breeding of animals which helping to successful search.

Author's Contributions: Karkaz M. Thalij, suggested the experiments procedures and supervision for this research work, and prepared the manuscript setting. Kalaf N. Ahmad, participated in successively all the experiments peocedures Also, the preparing of draft manuscript and he complete the statistical analyses. Mahmood J. Mohammed were performed in helped to supported in certain aspects of this research.

Ethics: The authors declare their concern for any of the ethical issues that may arise after the publication of this manuscript.

REFERENCES

1.Sondi, I. and Salopek-Sondi, B. (2004). Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. Journal of colloid and interface science, 275(1): 177-182.

2.Gopinath, V. and Velusamy. P. (2013). Extracellular biosynthesis of silver nanoparticles using Bacillus sp. GP-23 and evaluation of their antifungal activity towards Fusarium oxysporum. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 106: 170-174.

3.Bachhav, R. M. and Deore, S. N. (2015). A Review on Nanomaterials. International Journal of Science and Research (IJSR), 4(9): 1451-1457.

4.Kaler, A.; Patel, N. and Banerjee, U. C. (2010). Green synthesis of silver nanoparticles. Curr Res Inf Pharm Sci, 11(4): 68-71.

5.Yuan, Z.; Li, J.; Cui, L.; Xu, B.; Zhang, H. and Yu, C. P. (2013). Interaction of silver nanoparticles with pure nitrifying bacteria. Chemosphere, 90(4): 1404-1411.

6.Maurer, L. L. and Meyer, J. N. (2016). A systematic review of evidence for silver nanoparticle-induced mitochondrial toxicity. Environmental Science: Nano, 3(2): 311-322.

7.Vaseem, M.; Umar, A. and Hahn, Y. B. (2010). ZnO nanoparticles: growth, properties, and applications. Metal oxide nanostructures and their applications, 5: 1-36.

8.Gharsallaoui, A.; Oulahal, N.; Joly, C. and Degraeve, P. (2016). Nisin as a food preservative: part 1: physicochemical properties, antimicrobial activity, and main uses. Critical reviews in food science and nutrition, 56(8): 1262-1274.

9.Ibrahem, E. J., Thalij, K. M., Saleh, M. K., & Badawy, A. S. (2017). Biosynthesis of zinc oxide nanoparticles and assay of antibacterial activity. Am J Biochem Biotechnol, 13, 63-69.

10.Ahmed, K.N., Thalij, K.M., Mohammrd M.J. (2020). Evaluation of Bacterial Inhibition from ZnO and Ag Nanoparticles that Synthesized by Aspergillus niger. Am J Biochem Biotechnol, (Under published).

11.NRC, National Research Council (1995).Nutrient Requirements of Laboratory Animals 4th ed. National Academy Press; Washington; DC.

12.Tietz, Y. (2005). Clinical Biochemistry; 6 th ed.; McGraw-Hill; New York. 825.

13.SAS, (2012). Statistical Analysis System, User's Guide. Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA.

14.Duncun, O. D., & Duncun, B. (1955). A methodological analysis of segregation Index. American Sociological Review, 20, 210-217.

15.Lee, J. H.; Gulumian, M.; Faustman, E. M.; Workman, T., Jeon, K. and Yu, I. J. (2018). Blood biochemical and hematological study after subacute intravenous injection of gold and silver nanoparticles and coadministered gold and silver nanoparticles of similar sizes. BioMed research international., Volume 2018, Article ID 8460910:1-10.

16. Imani, M. , Halimi, M. Khara, H. (2015). Effects of silver nanoparticles (AgNPs) on hematological parameters of rainbow trout, Oncorhynchus mykiss. Comp Clin Pathol 24:491–495.

17.Kaviani, E. F.; Naeemi, A. S. and Salehzadeh, A. (2019). Influence of Copper Oxide Nanoparticle on Hematology and Plasma Biochemistry of Caspian Trout (Salmo trutta caspius), Following Acute and Chronic Exposure. Pollution, 5(1): 225-234.

18.Yunus, Y. M.; Narowi, M. and Lai, H. I. (2017). Morphological Features Analysis in Pathogenic Dengue Infection as an Alternative Screening Method. International Journal of Academic Research in Business and Social Sciences, 7 (2): 2222-6990.

19. Gupta, S.M., Aranha, C.C., Reddy, K.V. (2008). Evaluation of developmental toxicity of microbicide Nisin in rats. Food Chem Toxicol. 46 (2): 598-603.

20. Kulak, E., Sembratowicz, I.; Stępniowska, A. and Ognik, K. (2018). The effect of dosage of silver nanoparticles on the immune status of chickens. Annals of animal science. 18(2): 401-416.

21.Abd-Elhakeem, M. A.; Badawy, I. and Raafat, A. (2016). Efficacy of Silver Nanoparticles as Antimicrobial Agent against Salmonella Infection and Accompanied Biochemical, Immunological and Histopathological Changes in Rats.12:112-117.

ISSN- 2394-5125 VOL 7, ISSUE 04, 2020

22.Supraja, N., Prasad, T. N. V. K. V., Krishna, T. G., & David, E. (2016). Synthesis, characterization, and evaluation of the antimicrobial efficacy of *Boswellia ovalifoliolata* stem bark-extract-mediated zinc oxide nanoparticles. Applied Nanoscience, 6(4): 581-590.