**International Journal of Biology, Pharmacy
and Allied Sciences (IJBPAS)**

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

IN-VITRO ASSESSMENT AND COMPARISON OF ANTIOXIDANT EFFECTS OF AZITHROMYCIN WITH ITS SILVER AND GOLD NANO CONJUGATED FORM

ZEHRA D¹, MEMON Z¹, JABEEN A³, ISMAIL K¹, USMAN S², AND AGHA F⁴

¹Department of Pharmacology, Ziauddin University, Karachi

²Department of Research, Ziauddin University, Karachi

³Dr.Panjwani Centre for Molecular Medicine and Drug research, International Centre for
Chemical and Biological Sciences, Karachi

⁴Department of Biochemistry, Liaquat National Hospital and Medical College, Karachi

Authors' contribution: This work was carried out in collaboration of all the authors who read
and approved the final manuscript

Received 29th July 2019; Revised 27th Aug. 2019; Accepted 27th Sept. 2019; Available online 1st Jan. 2020

<https://doi.org/10.31032/IJBPAS/2020/9.1.4930>

ABSTRACT

Oxidative stress is usually associated with the release of reactive oxygen species (ROS) involved in various pathological conditions going on inside the body. Azithromycin is a macrolide antibiotic recommended commonly for the treatment of upper respiratory infections, also acts as an active anti-inflammatory and antioxidant agent so might be beneficial in chronic inflammatory states other than infectious disease. However, at the same time it produces oxidative damages to several organs of the body due to unwanted penetration. The aim of this study is to identify suppressive role of azithromycin on ROS generation in human blood cells via chemiluminescence assay and to compare this effect with its silver and gold conjugated nano form. We have found that azithromycin suppressed ROS generation at the concentration between 250 µg/ml and 500 µg/ml (IC₅₀ =310.8µg/ml). However, the maximum effect was observed with silver nanoparticle (AgNp) conjugated form of azithromycin which showed maximum effect at comparatively lower concentration (between 25µg/ml and 100µg/ml with IC₅₀ value of

34.8µg/ml) while gold nano particle (AuNp) conjugated form remain insignificant for antioxidant activity. Although antioxidative ability is related to azithromycin alone but when it was conjugated to AgNp could lead to higher antioxidant activity in lower concentration. Therefore, the results of our studies support the antioxidant effects of azithromycin enhanced to significant levels when conjugated with silver nanoparticle as compare to azithromycin alone.

Keywords: Azithromycin, antioxidant effects, Gold and silver conjugation

Abbreviations:

ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); AZM: Azithromycin; AgNp: Silver nanoparticle; AP-1: Activator protein 1; AuNp: Gold nanoparticle; CAT: Catalase; CSE: Cigarette smoke extract; DPPH 2,2-diphenyl-1-picrylhydrazyl; FMLP: N-formyl-Methionyl-Leucyl-Phenylalanine; FDA: Food and Drug Administration; GPx: Glutathione peroxidase; GSTs Glutathione S-transferase; GSTT1 Glutathione S-transferase theta-1; GSTM1 Glutathione S-Transferase Mu 1; GRK4 G protein-coupled receptor kinase 4; GSTs Glutathione S-transferase; GSTT1 Glutathione S-transferase theta-1; GSTM1 Glutathione S-Transferase Mu 1; GSH: Glutathione; GSHR: Glutathione reductase; GSHPx Glutathione peroxidase; HBSS+ Hanks Balanced Salt Solution; HDAC2 Histone deacetylase 2; MDA Malondialdehyde (MDA); NF-kB Nuclear factor kappa-light-chain-enhancer of activated B cells; NO: Nitric oxide; Nrf2: Nuclear factor erythroid 2-related factor 2; PAF: Platelet-activating factor; PMA: Phorbol 12-Myristate 13-Acetate; ROS :Reactive oxygen species; SOD: Superoxide dismutase; TAC: Total antioxidant capacity; TEMPO: 2,2,6,6-tetramethylpiperidine-*N*-oxyl; XO: Xanthine oxidase; VEGF: Vascular endothelial growth factor; ZO-1: Zonula occludens-1

INTRODUCTION

Oxidative stress is the damage brought about by disparity between oxidants and antioxidants in favour of oxidants attributed to interruption of normal redox pathways causing cellular damage (**Rahman *et al.*, 2012**). It is not only the disproportion between the production and destruction of reactive oxygen species leading to amplified ROS accumulation but also the overproduction of the reactive nitrogen species, reactive sulfur species, reactive carbonyl species etc. (**Sies, 2015**). Particularly ROS, being act like a double edge sword, is thought to play role not merely in numerous physiological processes, cell signaling, microbial killing, clearance of cells from necrosis and apoptosis from tissue

remodeling but also coupled with different ailments (**Silva *et al.*, 2010**). ROS generates certain compounds and unstable oxygen molecules which have the capability to be converted into free radicals consisting of unpaired electrons that eventually interact with biological polymers such as protein, DNA, lipid and carbohydrate. ROS can be divided into oxygen-centered radicals i.e. superoxide anion (O_2^-), hydroxyl radical (OH), alkoxyl radical (RO) and oxygen-centered nonradicals such as hydrogen peroxide (H_2O_2) and singlet oxygen (1O_2) (**Phaniendra *et al.*, 2015**, **El-Bahr, 2013**). It is produced by number of cytoplasmic enzymes which include xanthine oxidase, lipoxygenase, cyclooxygenase and nitric

oxide synthase while mostly the phagocytic ROS produced by polymorphonuclear neutrophils are derived from superoxide, produced by NADPH oxidase present in mitochondria (Bryan *et al.*, 2012) (Mittal *et al.*, 2014). These molecules are critical for host protection against pathogens but if persist for longer duration may play role in the development of various chronic diseases such as diabetes (Nagata and Allergy, 2005), atherosclerosis (Buonocore *et al.*, 2010) and cancer (Sabharwal and Schumacker, 2014). Particularly in carcinogenesis ROS has also got significant importance as excessive accumulation of ROS is involved in promotion of cancer where it was found that these cells have added capability to form more amounts of ROS, thus are more susceptible to oxidative stress interruption than their healthy counterparts (Prasad *et al.*, 2017).

Antioxidant compounds found in diet like retinoid acid, ascorbic acid, tocopherol, carotenoid and other plants derived phenols are renowned for their potential to wrestle with oxidative stress (Lobo *et al.*, 2010). Individually, retinoids showed inhibitory effects on OH⁻, H₂O₂ and O₂⁻ (Mandeville *et al.*, 2010), while tocopherols and tocotrienols induced antioxidant effects by scavenging reactive oxygen species. Likewise ascorbic

acid works via removal of molecular oxygen and last but not the least, carotenoids have most prominent efficiency in quenching of singlet oxygen (Lee *et al.*, 2004). Unfortunately, neither all exogenous antioxidants mentioned above or endogenous antioxidants (glutathione, bilirubin) have generalized proficiency to dilute all types of ROS nor they have such potent free radical scavenging properties that can avert the development of various types of chronic or inflammation mediated disease (Siti *et al.*, 2015).

Moreover, according to multiple clinical trials, these supplements are remained unsuccessful in attenuating the disease progression or increasing the life span of sufferers, particularly in relation to cancerous conditions (Klein *et al.*, 2011). Therefore, researchers are seeking for more potent, efficacious and novel antioxidants that exhibit definite free radical scavenging properties with other well-known mechanisms for their anti-inflammatory or immuno-modulatory actions.

The customary strategy for discoveries of new agents having antioxidant properties require de-novo detection, isolation and confirmation of very original molecules with their active functional groups which is

rather tiresome and costly approach (**Shim and Liu, 2014**).

Therefore, currently new strategy for drug development is in practice which includes driving new indications from compounds having well established efficacies for other health conditions, an approach may be called as drug repositioning that define as unearthing of innovative applications of previously FDA approved drugs, available in market for some other diseases for instance sildenafil and minoxidil (**Juarez et al., 2018**).

Antibiotics for long, are commonly employed group of drugs in the treatment of various infections. Growing body of literature supports that conventional antibiotics have not only bacteriocidal or bacteriostatic activities but also have potential to modulate immunity and alter oxidative balance in the body (**Belenky and Collins, 2011, Yadav et al., 2019**).

In this context, multiple controversies are linked, on one hand many evidences support that tetracyclines (tetracycline HCL, oxytetracycline HCL, minocycline HCL, erythromycin) may inhibit ROS production by neutrophils especially the minocycline HCL which was according to one study suppressed H₂O₂, the most potent oxidant while on the other hand cephalixin,

chloramphenicol, penicillin G, streptomycin had no effect on ROS (**Miyachi et al., 1986**).

Macrolides, the prokaryotic protein synthesis inhibitors, are generally prescribed to treat bacterial infections of lungs, dermis, underlying tissue along with gastro and urogenital tract. Commonly included macrolides are erythromycin, clarithromycin, and roxithromycin having macrocyclic lactone ring in common (**Zimmermann et al., 2018**). Other than antibacterial effects, these drugs are also believed to possess anti-inflammatory activities by suppressing the generation of ROS by neutrophils. The mechanisms involved are membrane stabilization by neutralizing phospholipids such as lysophosphatidylcholine, platelet-activating factor (PAF) etc. (**Kanoh and Rubin, 2010**). They also suppress the nitric oxide, another type of ROS produce from macrophages by interfering with the induction of nitric oxide synthase (**Asano et al., 2003**).

However, azithromycin which is considered the safest among all macrolides have ambiguous role in modulating oxidative stress of the body that is on one side it is reported that AZM enhanced the nuclear translocation of Nrf2 causing increased transcription of antioxidant enzymes (**Cuevas et al., 2016b**), while in one study

done on cystic fibrosis patients displayed no benefit in up regulating the levels of enzymes such as TAC, CAT, SOD or GPx with the use of AZM (Oliveira *et al.*, 2017). Moreover, in another study, azithromycin when combined with paracetamol increased oxidative damage of hepatic cells evident by increased level of MDA (Singh *et al.*, 2016). These findings are unclear and further researches are warranted to overcome this disparity.

Apart from drug repurposing, a novel nano-sized drug delivery system is commonly employed approach in these days to encounter current changes being affiliated with antibiotic therapy. The expected benefits of nanotechnology are sustained drug release, enhanced solubility, uniform distribution within the tissue and increased cellular internalization hence more targeted drug delivery, reduced dose requirement with decrement in adverse effects (Kalhapure *et al.*, 2015).

In the light of above mentioned literature search this study is designed to evaluate the antioxidant activity of azithromycin on human blood sample in nano-conjugated form with silver and gold to attain maximum benefits by this antibiotic in minimal concentrations.

Moreover, these metallic nanoparticles after being conjugated to different drugs are known to enhance their activities. A study in this regard was also done on gold and silver bimetallic conjugation to upgrade antimicrobial effects of commonly used antibiotics (Fakhri *et al.*, 2017).

METHODOLOGY

Chemiluminescence Assay for the Quantification of Intracellular Reactive Oxygen Species (ROS)

This Study was conducted after getting approval from Independence Ethics Committee, ICCBS (International Center for Chemical and Biological Sciences), University of Karachi. Amongst many methods for detection of in-vitro ROS production and quantification, such as (chemiluminescence probes, fluorescent probes, chromatography methods, fluorescent proteins, spectrophotometric methods) (Zhang *et al.*, 2018), this method is commonly employed as it is economical, less time consuming and gives early and easy interpretation of results (Caldefie-Chézet *et al.*, 2002). Principle of this method is based on the production and quantification of reactive oxygen species being assessed by their capability to catalyze the oxidation of luminol, resulting in emission of light which is recorded by luminometer and can be

measured as relative light unit (RLU) (Liu et al., 1996).

Reagents and Chemicals

Reagents were generously provided by PCMD (Panjwani Center for Molecular Medicine and Drug Research) Karachi. Drugs were purchased from Medchemexpress Co Ltd, United States. Silver and gold Conjugation of azithromycin were kindly supplied by TWC, PCMD, Karachi. We used HBSS+ as media. Serum opsonized zymosan which worked as an activator was used for the production of ROS in blood cells while luminol was added for the detection of amount of ROS produced. Both zymosan and luminol were light sensitive.

Sample collection

The effects of azithromycin and its silver and gold conjugated forms on ROS production were quantified on human blood. For that purpose healthy human venous blood sample was taken voluntarily without any history of smoking, alcohol and/or current use of any sort of medications like antibiotics, vitamins, minerals, special diet or antioxidant. Written informed consent was taken from the participant and complete information regarding the risks, discomfort and benefits was provided. After all aseptic measures being taken, 3 ml blood was drawn from

antecubital vein and immediately transferred to heparinized Vacutainer tube.

Azithromycin, its gold and silver conjugated form were tested at different concentrations (Azithromycin at 25µg/ml, 75µg/ml, 100µg/ml, 125 µg/ml, 250 µg/ml, 500µg/ml, Gold conjugated Azithromycin at 25µg/ml, 75µg/ml, 100µg/ml, 125µg/ml, 250µg/ml and silver conjugated form at 1 µg/ml, 10 µg/ml, 25 µg/ml, 100 µg/ml.

Chemiluminescence assay was carried out to analyze the effect of compounds on ROS production from phagocytes as describe by Mesaik et al (Mesaik et al., 2012). In brief, we carried it out in 96-well plate which was broadly classified as control wells and treated wells, further division was made in control wells as positive and negative. Among treated wells there were three divisions such as azithromycin alone, gold conjugated azithromycin and silver conjugated azithromycin. Both activator and detector were as added in positive control while in negative control only cells and detector were added. In treated wells, 25µL of whole blood (1:50 dilution prepared in sterile HBSS+) was mixed with 25 µL of serially diluted drugs (AZM/conjugated AZM) with different concentrations mentioned earlier. In control wells HBSS+ and blood cells were added containing no drugs. All tests were

performed in 96 well plate and final volume of 100 μL /well was achieved. The mixture was incubated for 5 min in luminoskan chamber at 37°C. After incubation 25 μL serum opsonized zymosan was added in each well for the production of ROS in blood cells. The detection for ROS generation was done by the addition of 25 μL of luminol, which produced luminescence on combining with ROS and used for its quantification. The plate was placed in luminoskan chamber, incubated at 37°C for 50 minutes and results were measured as RLU (relative light units) with peak and total integral values set with repeated scans.

All the experiments were run in triplicate, bar showed number of photons emitted expressed as relative light units and SD was represented

by error bars whereas ** represent P-value of <0.01 and *** represent value P-value of <0.001. Statistical analysis was done by one way ANOVA

RESULTS

In our results, significant inhibition of ROS was observed only in AZM group that is in between concentrations of 250 $\mu\text{g/ml}$ and 500 $\mu\text{g/ml}$ whereas for rest of the concentrations, inhibition was insignificant (**Figure 1**). Moreover inhibition of ROS was found to be highly significant by treatment with silver conjugated azithromycin in between 25 $\mu\text{g/ml}$ and 100 $\mu\text{g/ml}$ (**Figure 2**). While all the concentrations tested for gold conjugated azithromycin were remain unsuccessful in inhibiting ROS production as evident by nonsignificant p-value (**Figure3**).

(** shows P-value of <0.01)

Figure 1: Effects of different concentrations (25 $\mu\text{g/ml}$, 75 $\mu\text{g/ml}$, 100 $\mu\text{g/ml}$, 125 $\mu\text{g/ml}$, 250 $\mu\text{g/ml}$, 500 $\mu\text{g/ml}$) of azithromycin on phagocytic oxidative burst

Figure 2: Effects of different concentrations (1 µg/ml, 10µg/ml, 25µg/ml, 100µg/ml) of silver conjugated azithromycin on phagocytic oxidative burst

Figure 3: Effects of different concentrations (25 µg/ml, 75µg/ml, 100µg/ml, 125µg/ml, 250µg/ml) of gold conjugated azithromycin on phagocytic oxidative burst

DISCUSSION

Apart from antimicrobial effects of macrolides, progressive researches signify that these drugs also have potential to combat ROS and thus have a role in reducing inflammation and oxidative stress going on in the body leading to chronic diseases, through various mechanisms. Present research was designed to examine the antioxidant activity of AZM alone and nano conjugated forms with gold and silver.

Our results exhibit efficacy of AZM in reducing ROS production in between the concentration of 250µg/ml and 500µg/ml as evident by decrease light absorption with IC₅₀ of 310.8µg/ml (**Figure 1**).

Role of AZM in reducing inflammation has been extensively studied previously (**Legssyer et al., 2006, Lendermon et al., 2017**) which is closely related to oxidative stress in a cyclical manner like effort to attenuate one condition will ultimately improve the other, i.e. one of the accepted mechanism of reducing inflammation is to neutralize ROS in the same way by decreasing inflammatory cascade, oxidative load of the body can be minimized. From this perspective, various studies support our findings, study done by **Levert et al, 1998**, to observe the effects of azithromycin on neutrophilic ROS generation in which they

demonstrated that the respiratory burst activated by membrane stimulating agents such as FMLP, PMA or *Staphylococcus aureus*, was attenuated by AZM in time dependent manner i.e. 50% inhibition was achieved at minimum concentration (19 µg/ml) when incubated at 4 hours as compared to 2 hours (83 µg/ml) and 15 minutes (586µg/ml). Moreover, IC₅₀ values were lower with *S. aureus* stimulated PMN (272 µg/ml) than with PMA- or FMLP-stimulated neutrophils i.e. 856 and 586 µg/ml respectively (**Levert et al., 1998**). Lately, a study done on cystic fibrosis cell line (IB3-1) using GSTs (GSTT1 and GSTM1) expression as an indicator of oxidative stress condition, azithromycin was found to be effective in reducing the expression of GSTT1 and GSTM1 in IB3-1 cells (**Bergamini et al., 2009**).

Moreover, a study was conducted on ethanol induced animal model of gastric ulcer to evaluate the gastroprotective role of different commonly used drugs including azithromycin. Various parameters of oxidative stress was measured in this study such as CAT, XO, GSH, GSHR, GSHPx and it was observed that amongst different drugs studied, azithromycin exerted significant antioxidant effect (**Popovic et al., 2009**).

As AZM is supposed to be the safest drug, researches are continuously growing to establish its antioxidant potential in various diseased conditions particularly in pulmonary diseases like COPD (Cuevas *et al.*, 2016a), Cystic fibrosis (Cuevas *et al.*, 2016b) etc. where it is commonly prescribed as antimicrobial and anti-inflammatory agent. A study in this regard was conducted on CSE induced A549 cells to evaluate its role, oxidative burden was examined and it was found that azithromycin protected vital functioning of airway epithelial cells and suppressed ROS production by CSE which was evident by decreased alteration in VEGF, ZO-1 and occluding protein expression, otherwise were highly altered in CSE induced control cells with oxidative stress (Chen *et al.*, 2015).

Furthermore, Cuevas *et al.*, 2016a, documented that AZM reduced ROS production in cells via stabilization of cell membrane or increase translocation of Nrf2 to nucleus causing increase synthesis of antioxidant enzymes (Cuevas *et al.*, 2016a).

Other macrolides are also known to cause dose related inhibition of superoxide production by attenuating membrane-destabilization effects of bioactive phospholipids (Dey and Bishayi, 2016). Along with these antioxidant effects,

azithromycin also include downregulation of TNF- α leading to decreased NF-kB and AP-1 activation which are renowned mediators of inflammation (Aghai *et al.*, 2007).

Despite these favorable findings, recent researches are raising question on its safety, and in selected organs it is emerged as hazardous compound where it is associated with cellular damage of hepatic, (Paulose *et al.*, 2016), cardiac (El-Shitany *et al.*, 2016) and reproductive tissues (El-Sayed *et al.*, 2017).

These findings may be dose dependent and attributed to redundant penetration of this drug to various organs causing either reduced actions of antioxidative enzymes like SoD, CAT and GSH Px, or enhanced membrane lipids peroxidation, free radical production during metabolism or mitochondrial dysfunction (Olayinka and Ore, 2014).

For this reason we did conjugate AZM with different inorganic metals such as gold and silver in nano delivery system, the technology to attain better targeting of drugs towards the inflammatory region and to circumvent unwanted consequences (Daraee *et al.*, 2016).

To the best of our literature search this was the first study in which anti-oxidant potential of gold and silver nano conjugated AZM was evaluated and compared.

According to our results when AZM conjugated with silver produced significant inhibition of ROS at concentration of 38.4µg/ml as compared to AZM alone which is 310.8µg/ml, whereas when AZM conjugated with gold produce nonsignificant results.

Silver nanoparticles (AgNP) are known to possess huge applications in medical sciences such as drug delivery, gene therapy, antimicrobial, antifungal, antioxidant agents. Therefore, in accordance to our findings study done by Patra *et al* discovered the antioxidant capacity of AgNPs by in vitro assay of NO, DPPH and ABTS radical scavenging potential along with its reducing power. In this study they documented that AgNP exhibited high NO scavenging actions and significant reducing potential while DPPH and ABTS scavenging activities were moderate. Moreover they also reported that antimicrobial effects of antibiotics were enhanced when conjugated with AgNP **(Patra and Baek, 2017)**.

In one more study AgNP were synthesized biologically via plant leaf extract, showed increased scavenging potential of free radicals **(Kharat *et al.*, 2016)**.

In another study the free radical scavenging actions of various antioxidant and their synthesized silver nanoforms, encapsulated

in chitosan, a biocompatible and biodegradable polymer was compared by utilizing different assays including DPPH assay, H₂O₂ and NO scavenging assay They revealed that nano-formulations showed higher antioxidant capacities than their non conjugated basic compounds **(Nayak *et al.*, 2016)**.

Other medicinal values of silver nanoparticles include catalytic activities, and antibacterial effects. Particularly in context of potential antimicrobial activity one study proved that antimicrobial ceftriaxone when conjugated with AgNP showed high and antimicrobial and antioxidant activities but the mechanism remain unclear **(Harshiny *et al.*, 2015)**.

As far as data regarding gold nano formulations of AZM is concerned we could find very little studies which evaluated gold conjugated azithromycin efficacy in terms of antioxidant effects.

However, a study done on the TEMPO conjugated Au NP showed enhanced scavenging activity against overproduction of ROS in human mesenchymal stem cells **(Li *et al.*, 2017)** which is contrary to our findings. Furthermore, the antibacterial activity of gold and silver bimetallic conjugated nanoparticles of doxycycline was assessed under both aerobic and anaerobic

metabolism which indicated that this antimicrobial with metallic conjugation is affiliated with more potent antibacterial activity via suppression of ROS. Further they also documented that activity was more pronounced in aerobic condition as there was more production of ROS when compared to anaerobic conditions (**Fakhri *et al.*, 2017**).

This finding highlights the significance of quantity of ROS generated i.e. greater the ROS production greater would be the inhibitory effects of antioxidants. However further confirmatory researches are warranted in this regard.

Moreover there are numerous studies which prove the efficacy of gold conjugated different anti microbials including macrolides against cancerous cells (**Dreaden *et al.*, 2012**) resistance to chemotherapy (**Dreaden *et al.*, 2014**) and infectious disease (**Emmanuel *et al.*, 2017**), in all these studies authors claimed more encouraging outcomes of nano conjugates over their basic materials.

Like Silver, Gold nanoparticle possessing some therapeutic importance is considered noncytotoxic because of its inert nature but unlike silver it could be toxic and may produce ROS depending upon the surface functional attachments, diameter and shape of nanoparticles which could be one of

reason of dissimilar results. The link between AuNP and oxidative stress is also not clear yet but it is thought to be associated with induction of ROS via damaging DNA, polyunsaturated fatty acids and oxidation of amino acids (**Li *et al.*, 2010**).

Data is yet ambiguous regarding safety concerns of these metallic nano particles, in this context a study was done by **Namasivayam *et al*** on metallic nano particles in conjugation with azithromycin documented no cytotoxicity on blood cells under in-vitro conditions, moreover no morphological aberrations were observed with silver or gold conjugated AZM (**Namasivayam and Samrat, 2016**). This evidence is preliminary and further researches are obligatory to second these results.

CONCLUSION

On the basis of our findings it can be concluded that antioxidant effects of Azithromycin can be enhanced with silver nano conjugation which leads to better results in less concentrations but in case of gold its anti-oxidative capacity is abolished. Nevertheless, these findings are not irrefutable and further in vitro assays with different techniques are requisite before reaching to any conclusion. Additionally, other metallic and nonmetallic nano

approaches should be employed with AZM so that the utmost benefits can be avail from this multipurpose drug with better targeting and least adverse effects.

ACKNOWLEDGEMENT

We are grateful to Hamna Fatima (Lab Assistant Panjwani Center for Molecular Medicine and Drug Research) for her help and valueable input in lab work. We are extremely thankful to Dr.Raza Shah and Zara Aslam (TWC, PCMD, Karachi University) for providing nano conjugated form of drug. We also appreciate Farwa Naqvi (Lab colleague Panjwani Center for Molecular Medicine and Drug Research) and Dr. Faizan Ali Shah Rizvi (Lecturer Ziauddin University) for their guidance in statistical analysis and Dr. Urooj Zafar, Dr. Akhtar Ali and Dr. Nisha Zahid (Lecturers Ziauddin University) for their help and support.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest associated with this manuscript.

REFERENCES

- [1] Aghai, Z. H., Kode, A., Saslow, J. G., Nakhla, T., Farhath, S., Stahl, G. E., Eydelman, R., Strande, L., Leone, P. & Rahman, I. J. P. R. 2007. Azithromycin Suppresses Activation Of Nuclear Factor-Kappa B And Synthesis Of Pro-Inflammatory Cytokines In Tracheal Aspirate Cells From Premature Infants. 62, 483.
- [2] Asano, K., Kamakazu, K., Hisamitsu, T. & Suzaki, H. J. A. O.-L. 2003. Suppressive Activity Of Macrolide Antibiotics On Nitric Oxide Production From Nasal Polyp Fibroblasts In Vitro. 123, 1064-1069.
- [3] Belenky, P. & Collins, J. J. J. S. 2011. Antioxidant Strategies To Tolerate Antibiotics. 334, 915-916.
- [4] Bergamini, G., Cigana, C., Sorio, C., Peruta, M. D., Pompella, A., Corti, A., Huaux, F. A., Leal, T., Assael, B. M., Melotti, P. J. A. J. O. R. C. & Biology, M. 2009. Effects Of Azithromycin On Glutathione S-Transferases In Cystic Fibrosis Airway Cells. 41, 199-206.
- [5] Bryan, N., Ahswini, H., Smart, N., Bayon, Y., Wohler, S. & Hunt, J. A. J. E. C. M. 2012. Reactive Oxygen Species (Ros)—A Family Of Fate Deciding Molecules Pivotal In Constructive Inflammation And Wound Healing. 24, E65.
- [6] Caldefie-Chézet, F., Walrand, S., Moinard, C., Tridon, A., Chassagne, J. & Vasson, M.-P. J. C. C. A. 2002. Is The Neutrophil Reactive Oxygen Species Production Measured By

- Luminol And Lucigenin Chemiluminescence Intra Or Extracellular? Comparison With Dcfh-Da Flow Cytometry And Cytochrome C Reduction. 319, 9-17.
- [7] Chen, M., Yang, T., Meng, X. & Sun, T. J. M. M. R. 2015. Azithromycin Attenuates Cigarette Smoke Extract-Induced Oxidative Stress Injury In Human Alveolar Epithelial Cells. 11, 3414-3422.
- [8] Cuevas, S., Yang, Y., Armando, I. & Jose, P. A. 2016a. Mechanisms Involved In The Antioxidant Properties Of Azithromycin In Lung Epithelial Cells.
- [9] Cuevas, S., Yang, Y., Armando, I. & Jose, P. A. J. T. F. J. 2016b. Mechanisms Involved In The Antioxidant Properties Of Azithromycin In Lung Epithelial Cells Stimulated With Cigarette Smoke Extract. 30, 982.2-982.2.
- [10] Daraee, H., Eatemadi, A., Abbasi, E., Fekri Aval, S., Kouhi, M., Akbarzadeh, A. J. A. C., Nanomedicine, & Biotechnology 2016. Application Of Gold Nanoparticles In Biomedical And Drug Delivery. 44, 410-422.
- [11] Dey, S. & Bishayi, B. J. J. O. I. 2016. Riboflavin Along With Antibiotics Balances Reactive Oxygen Species And Inflammatory Cytokines And Controls Staphylococcus Aureus Infection By Boosting Murine Macrophage Function And Regulates Inflammation. 13, 36.
- [12] Dreaden, E. C., Mwakwari, S. C., Austin, L. A., Kieffer, M. J., Oyelere, A. K. & El-Sayed, M. A. J. S. 2012. Small Molecule-Gold Nanorod Conjugates Selectively Target And Induce Macrophage Cytotoxicity Towards Breast Cancer Cells. 8, 2819-2822.
- [13] Dreaden, E. C., Raji, I. O., Austin, L. A., Fathi, S., Mwakwari, S. C., Humphries Iv, W. H., Kang, B., Oyelere, A. K. & El-Sayed, M. A. J. S. 2014. P-Glycoprotein-Dependent Trafficking Of Nanoparticle-Drug Conjugates. 10, 1719-1723.
- [14] El-Bahr, S. M. J. S. I. 2013. Biochemistry Of Free Radicals And Oxidative Stress. 1, 111-117.
- [15] El-Sayed, M., Kandiel, M. & Ebied, D. 2017. Changes In Reproductive Organs, Semen Characteristics And Intra-Testicular Oxidative Stress In

- Adult Male Rats Caused By Azithromycin.
- [16] El-Shitany, N. A., El-Desoky, K. J. O. M. & Longevity, C. 2016. Protective Effects Of Carvedilol And Vitamin C Against Azithromycin-Induced Cardiotoxicity In Rats Via Decreasing Ros, Il1-B, And Tnf-A Production And Inhibiting Nf-Kb And Caspase-3 Expression. 2016.
- [17] Emmanuel, R., Saravanan, M., Ovais, M., Padmavathy, S., Shinwari, Z. K. & Prakash, P. J. M. P. 2017. Antimicrobial Efficacy Of Drug Blended Biosynthesized Colloidal Gold Nanoparticles From Justicia Glauca Against Oral Pathogens: A Nanoantibiotic Approach. 113, 295-302.
- [18] Fakhri, A., Tahami, S., Naji, M. J. J. O. P. & Biology, P. B. 2017. Synthesis And Characterization Of Core-Shell Bimetallic Nanoparticles For Synergistic Antimicrobial Effect Studies In Combination With Doxycycline On Burn Specific Pathogens. 169, 21-26.
- [19] Harshiny, M., Matheswaran, M., Arthanareeswaran, G., Kumaran, S., Rajasree, S. J. E. & Safety, E. 2015. Enhancement Of Antibacterial Properties Of Silver Nanoparticles–Ceftriaxone Conjugate Through Mukia Maderaspatana Leaf Extract Mediated Synthesis. 121, 135-141.
- [20] Juarez, M., Schcolnik-Cabrera, A. & Dueñas-Gonzalez, A. J. A. J. O. C. R. 2018. The Multitargeted Drug Ivermectin: From An Antiparasitic Agent To A Repositioned Cancer Drug. 8, 317.
- [21] Kalhapure, R. S., Suleman, N., Mocktar, C., Seedat, N. & Govender, T. J. J. O. P. S. 2015. Nanoengineered Drug Delivery Systems For Enhancing Antibiotic Therapy. 104, 872-905.
- [22] Kanoh, S. & Rubin, B. K. J. C. M. R. 2010. Mechanisms Of Action And Clinical Application Of Macrolides As Immunomodulatory Medications. 23, 590-615.
- [23] Kharat, S. N., Mendhulkar, V. D. J. M. S. & C, E. 2016. Synthesis, Characterization And Studies On Antioxidant Activity Of Silver Nanoparticles Using Elephantopus Scaber Leaf Extract. 62, 719-724.
- [24] Klein, E. A., Thompson, I. M., Tangen, C. M., Crowley, J. J., Lucia, M. S., Goodman, P. J.,

- Minasian, L. M., Ford, L. G., Parnes, H. L. & Gaziano, J. M. J. J. 2011. Vitamin E And The Risk Of Prostate Cancer: The Selenium And Vitamin E Cancer Prevention Trial (Select). 306, 1549-1556.
- [25] Lee, J., Koo, N., Min, D. B. J. C. R. I. F. S. & Safety, F. 2004. Reactive Oxygen Species, Aging, And Antioxidative Nutraceuticals. 3, 21-33.
- [26] Legssyer, R., Huaux, F., Lebacq, J., Delos, M., Marbaix, E., Lebecque, P., Lison, D., Scholte, B. J., Wallemacq, P. & Leal, T. J. R. R. 2006. Azithromycin Reduces Spontaneous And Induced Inflammation In Δf508 Cystic Fibrosis Mice. 7, 134.
- [27] Lendermon, E. A., Coon, T. A., Bednash, J. S., Weathington, N. M., Medyer, J. F. & Mallampalli, R. K. J. R. R. 2017. Azithromycin Decreases Nalp3 Mrna Stability In Monocytes To Limit Inflammasome-Dependent Inflammation. 18, 131.
- [28] Levert, H., Gressier, B., Moutard, I., Brunet, C., Dine, T., Luyckx, M., Cazin, M. & Cazin, J. J. I. 1998. Azithromycin Impact On Neutrophil Oxidative Metabolism Depends On Exposure Time. 22, 191-201.
- [29] Li, J., Zhang, J., Chen, Y., Kawazoe, N., Chen, G. J. A. A. M. & Interfaces 2017. Tempo-Conjugated Gold Nanoparticles For Reactive Oxygen Species Scavenging And Regulation Of Stem Cell Differentiation. 9, 35683-35692.
- [30] Li, J. J., Hartono, D., Ong, C.-N., Bay, B.-H. & Yung, L.-Y. L. J. B. 2010. Autophagy And Oxidative Stress Associated With Gold Nanoparticles. 31, 5996-6003.
- [31] Liu, L., Dahlgren, C., Elwing, H. & Lundqvist, H. J. J. O. I. M. 1996. A Simple Chemiluminescence Assay For The Determination Of Reactive Oxygen Species Produced By Human Neutrophils. 192, 173-178.
- [32] Lobo, V., Patil, A., Phatak, A. & Chandra, N. J. P. R. 2010. Free Radicals, Antioxidants And Functional Foods: Impact On Human Health. 4, 118.
- [33] Mandeville, J., N'soukpoé-Kossi, C., Neault, J., Tajmir-Riahi, H. J. B. & Biology, C. 2010. Structural Analysis Of Dna Interaction With

- Retinol And Retinoic Acid. 88, 469-477.
- [34] Mesaik, M. A., Jabeen, A., Halim, S. A., Begum, A., Khalid, A. S., Asif, M., Fatima, B., Ul-Haq, Z., Choudhary, M. I. J. C. B. & Design, D. 2012. In Silico And In Vitro Immunomodulatory Studies On Compounds Of Lindelofia Stylosa. 79, 290-299.
- [35] Mittal, M., Siddiqui, M. R., Tran, K., Reddy, S. P., Malik, A. B. J. A. & Signaling, R. 2014. Reactive Oxygen Species In Inflammation And Tissue Injury. 20, 1126-1167.
- [36] Miyachi, Y., Yoshioka, A., Imamura, S. & Niwa, Y. J. J. O. I. D. 1986. Effect Of Antibiotics On The Generation Of Reactive Oxygen Species. 86, 449-453.
- [37] Nagata, M. J. C. D. T.-I. & Allergy 2005. Inflammatory Cells And Oxygen Radicals. 4, 503-504.
- [38] Namasivayam, S. K. R. & Samrat, K. J. D. P. L. 2016. Cytotoxicity Of Chitosan Stabilized Silver And Gold Nanoparticles Loaded Azithromycin And Ofloxacin Nanodrug Conjugate Agnp-Az, Of And Aunp-Az, Of) Against Blood Cells. 8, 421-424.
- [39] Nayak, D., Minz, A. P., Ashe, S., Rauta, P. R., Kumari, M., Chopra, P., Nayak, B. J. J. O. C. & Science, I. 2016. Synergistic Combination Of Antioxidants, Silver Nanoparticles And Chitosan In A Nanoparticle Based Formulation: Characterization And Cytotoxic Effect On Mcf-7 Breast Cancer Cell Lines. 470, 142-152.
- [40] Olayinka, E. T. & Ore, A. J. B. J. O. P. R. 2014. Influence Of Azithromycin Treatment On Hepatic Lipid Peroxidation And Antioxidant Defence Systems Of Rats. 4, 240-256.
- [41] Olveira, C., Padilla, A., Dorado, A., Contreras, V., Garcia-Fuentes, E., Rubio-Martin, E., Porras, N., Doña, E., Carmona, A. & Olveira, G. J. T. E. J. O. M. 2017. Inflammation And Oxidation Biomarkers In Patients With Cystic Fibrosis: The Influence Of Azithromycin. 49, 118.
- [42] Patra, J. K. & Baek, K.-H. J. F. I. M. 2017. Antibacterial Activity And Synergistic Antibacterial Potential Of Biosynthesized Silver Nanoparticles Against Foodborne Pathogenic Bacteria Along With Its

- Anticandidal And Antioxidant Effects. 8, 167.
- [43] Paulose, P., Juliet, S., Samraj, S., Nair, S., Chandrasekhar, L., George, A., Narayanan, G., John, K. & Ravindran, R. J. A. A. V. S. 2016. Ameliorative Effect Of Vitamin E On Azithromycin Induced Biochemical And Histological Changes In Liver Tissue Of Rats. 4, 85-91.
- [44] Phaniendra, A., Jestadi, D. B. & Periyasamy, L. J. I. J. O. C. B. 2015. Free Radicals: Properties, Sources, Targets, And Their Implication In Various Diseases. 30, 11-26.
- [45] Popovic, M., Janicijevic-Hudomal, S., Kaurinovic, B., Rasic, J. & Trivic, S. J. M. 2009. Antioxidant Effects Of Some Drugs On Ethanol-Induced Ulcers. 14, 816-826.
- [46] Prasad, S., Gupta, S. C. & Tyagi, A. K. J. C. L. 2017. Reactive Oxygen Species (Ros) And Cancer: Role Of Antioxidative Nutraceuticals. 387, 95-105.
- [47] Rahman, T., Hosen, I., Islam, M. T., Shekhar, H. U. J. A. I. B. & Biotechnology 2012. Oxidative Stress And Human Health. 3, 997.
- [48] Sabharwal, S. S. & Schumacker, P. T. J. N. R. C. 2014. Mitochondrial Ros In Cancer: Initiators, Amplifiers Or An Achilles' Heel? 14, 709-721.
- [49] Shim, J. S. & Liu, J. O. J. I. J. O. B. S. 2014. Recent Advances In Drug Repositioning For The Discovery Of New Anticancer Drugs. 10, 654.
- [50] Sies, H. J. R. B. 2015. Oxidative Stress: A Concept In Redox Biology And Medicine. 4, 180-183.
- [51] Silva, F., Marques, A. & Chaveiro, A. J. T. O. V. S. J. 2010. Reactive Oxygen Species: A Double-Edged Sword In Reproduction. 4.
- [52] Singh, H., Prakash, A., Kalia, A., Majeed, A. B. A. J. J. O. T. & Medicine, C. 2016. Synergistic Hepatoprotective Potential Of Ethanolic Extract Of Solanum Xanthocarpum And Juniperus Communis Against Paracetamol And Azithromycin Induced Liver Injury In Rats. 6, 370-376.
- [53] Siti, H. N., Kamisah, Y. & Kamsiah, J. J. V. P. 2015. The Role Of Oxidative Stress, Antioxidants And Vascular Inflammation In Cardiovascular Disease (A Review). 71, 40-56.

- [54] Yadav, V., Talwar, P. J. B. & Pharmacotherapy 2019. Repositioning Of Fluoroquinolones From Antibiotic To Anti-Cancer Agents: An Underestimated Truth. 111, 934-946.
- [55] Zhang, Y., Dai, M. & Yuan, Z. J. A. M. 2018. Methods For The Detection Of Reactive Oxygen Species. 10, 4625-4638.
- [56] Zimmermann, P., Ziesenitz, V. C., Curtis, N. & Ritz, N. J. F. I. I. 2018. The Immunomodulatory Effects Of Macrolides—A Systematic Review Of The Underlying Mechanisms. 9, 302.