Assiut University web-site: www.aun.edu.eg

EFFICACY OF QUERCETIN NANOPARTICLES AS A NEW ANTIVIRAL AGAINST H5N1 INFLUENZA VIRUS REPLICATION

HEMAT S. EL-SAYED [1]; WESAM H. MADY [2]; MOHAMED A. SAIF [2] and WESAM A. TAWFIK [3]

[1] Department of Poultry Diseases, Animal Health Research Institute, Benha Branch, Benha, Egypt

[2] Reference Laboratory for Veterinary Quality Control on Poultry Production, Animal Health Research Institute, Dokki, Giza, Egypt

[3] Naqaa Foundation for Scientific Research, Technology and Development, Giza, Egypt

Received: 6 September 2018; **Accepted:** 21 October 2018

ABSTRACT

The recent growing emergence of H5N1 Avian influenza virus (AIV) mutants calls for the urgent need for new effective antiviral drugs against AIV. Quercetin, is a promising natural candidate for treatment and prevention of AIV infection but unfortunately, low solubility and poor bioavailability of quercetin hinders its use in the medical field. Nanotechnology offers an innovative solution for enhancing quercetin solubility and bioavailability and subsequently boosts its therapeutic effect. In the current study quercetin nanoparticles were prepared by nanoprecipitation technique, quercetin nanoparticles showed a particle size range from 182nm to 240nm at flow rate 10 ml/ min, solvent/antisolvent volume ratio 1:12, stirring speed 1300 rpm and drug concentration 5mg/ml. The determined safe dilution of quercetin nanoparticles 10µg/ml had a significant inhibitory effect on the avian influenza virus H5N1 strain (EPI573317) indicated by reduction of the virus titer and the CPE in MDCK cells. Quercetin nanoparticles could be used as a new promising inhibitor for H5N1 AIV and is expected to be on the top of candidates as antiviral against influenza virus in the near future.

Key words: Antiviral; Quercetin nanoparticles; H5N1; Influenza virus; nanoprecipitation technique; Nanotechnology

INTRODUCTION

Highly pathogenic avian influenza H5N1 virus is one of the most devastating viral infectious diseases affecting poultry causing huge economic losses. Potential health concerns emerged during the last years due to the rapid evolutionary nature of the influenza virus which may result in the emergence of new mutant virulent strain causing global pandemic (Nguyen *et al.,* 2013).

Antiviral agents against influenza are considered one of the most effective strategies to control the virus infection; the current licenced anti-influenza drugs are classified into two categories; neuraminidase inhibitors (oseltamivir and zanamivir) and an M2 ion channel inhibitor (amantadine and rimantadine) (Leonov *et al.,* 2011; Muthuri *et al.,* 2013 and Spanakis *et al.,* 2014). The recent emergence of the amantadine and oseltamivir-resistant H5N1 influenza virus calls for the urgent need to discover new antiviral agents against influenza virus. Natural products represented one of the most promising candidates concerning the anti- influenza drug discovery (Ge *et al.,* 2010; Ha *et al.,* 2014; Ho *et al.,* 2014 and Yang *et al.,* 2014). Quercetin (3,3',4',5,7-pentahydroxyflavone) is a member of flavonoids found abundatley in many plants such as onion, grapes, citrus fruits, and apple (Wiczkowski *et al.,* 2008); tea (Somerset and Johannot 2008); green peppers and tomatoes (Nishimuro *et al.,* 2015). Additionally, it is found in some herbal plants (Manach *et al.,* 2005 and Kunyanga *et al.,* 2011). Quercetin showed an interesting anti-inflenza activity. It acts as HA inhibitor (Kumar *et al.,* 2005; Davis *et al.,* 2008 and Choi *et al.,* 2009). In a recent investigation confirmed that quercetin interact with HA protein, thereby inhibiting viral cell fusion and it successfuly blocked the entry of H5N1 pseudoviruses (Wu *et al.,* 2016). Furthermore, quercetin and its derivatives could inhibit RNA virus infection through blocking viral polymerase (PA, PB1, PB2) (Jassim and Naji 2003 and Gansukh *et al.,* 2016).

Neuraminidase (NA) protein found on the virion particles play an essential role in releasing progeny virions, NA active site has been highly targeted by antiviral therapy. Currently used NA inhibitors Oseltamivir (Tamiflu) and Zanamivir are similar to

Corresponding author: HEMAT S. EL-SAYED
E-mail address: hemat.elsayed@yahoo.com
Present address: Department of Poultry Diseases, Animal Health Research Institute, Benha Branch, Benha, Egypt

sialic acid structure and works competitively by binding the active site of NA resulting in inhibiting the distribution of influenza virion (Collins *et al.*, 2008). The emergence of influenza virus neuraminidase mutant strains urged the scientists to explore and develop new neuraminidase inhibitors. According to the results of molecular docking studies performed by liu *et al.* (2015 and 2016), quercetin showed robust stable binding abilities for neuraminidases from H7N9 A/Anhui/1/2013 and A/Shanghai/1/2013 (oseltamivir-resistant influenza) and H1N1 (A/PR/8/34), respectively. Moreover; quercetin molecule might overcome the oseltamivir resistance caused by R294K mutation for neuraminidase in A/Shanghai/1/2013. Hence, quercetin could be used as the effective lead compounds regarding anti-influenza A.

Although quercetin possesses antiviral activity (Zakaryan *et al.*, 2017) and other pharmacological effects such as being an antioxidant (Robaszkiewicz *et al.*, 2007), antibacterial (Li and Xu 2008), anticancer (Dajas 2012) and antiproliferative (Delgado *et al.*, 2014); there was a major obstacle facing its application in pharmaceutical field due to its low water solubility and poor bioavailability (Ratnam *et al.*, 2006 and Cai *et al.*, 2013). Nanotechnology offers an innovative solution regarding improving water solubility and bioavailability of quercetin (Al-Jameel and Abd El-Rahman, 2017) which in turn provides better bioavailability and higher efficiency of quercetin used in pharmaceuticals and subsequently in near future quercetin nanoparticles will become the best choice concerning therapeutic agents for disease treatment and prevention. Therefore the present study aims to prepare quercetin nanoparticles and determine its antiviral efficacy aganist H5N1 AIV.

MATERIALS AND METHOD

In the current study, Quercetin (3, 5, 7, 3', 4'-pentahydroxyflavone) was purchased from Sigma-Aldrich, Egypt and was used as received (purity ≥ 95% (HPLC)). All reagents used were of technical grade. Absolute ethanol (99.5 %) was obtained from El Howamdia Company, Egypt.

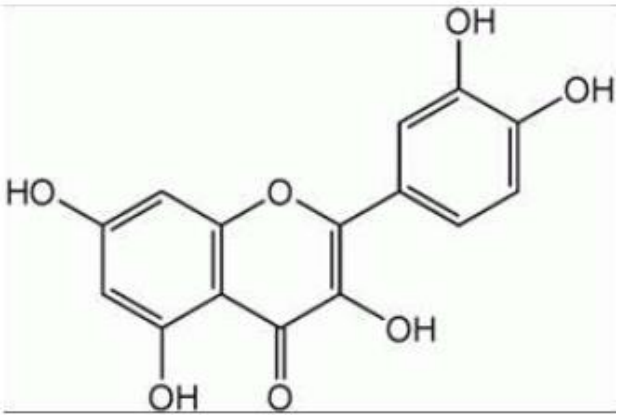

Fig. 1: Chemical structure of Quercetin (3, 5, 7, 3', 4'-pentahydroxyflavone)

1. Preparation of quercetin nanoparticles (QUENPS)

Quercetin nanoparticles were prepared by the nano-precipitation technique (Kakran *et al.*, 2012). quercetin was dissolved in ethanol at concentration of 5mg/ml. The drug solution was quickly injected at a fixed flow rate (10ml/min) in to the anti-solvent (deionized water) of definite volume under magnetic stirring (1300 rpm). The volume ratio was (1:12). The quercetin nanoparticles were filtered and air dried.

2. Characterization of quercetin nanoparticles (QUENPS)

Quercetin nanoparticles morphology was observed using a SEM (Quanta 3D FEG/ FEI) with 20 kV, a collector bias of 300 V. The powder samples were spread on a SEM stub and sputtered with gold before the SEM observations.

3. Selection of Avian influenza virus strain

The selected avian influenza virus strain was A/chicken/Egypt/1575S NLQP/2015 of 10^7/ml EID50 of Accession Number EPI573317 (EpiFlu), obtained from chicken, the strain was provided to Reference Laboratory for veterinary quality control on poultry production (RLQP).

4. Virus propagation on cell culture

Confluent monolayer of Madin-Darby Canine Kidney (MDCK) adherent cell line was grown in T-25 tissue culture flasks, cells were obtained from the Germany Type Culture Collection (Lonza, Germany). The growth media was discarded and washing the cells was carried 3 times with PBS. 200 µl of the AI virus was inoculated and the inoculum was allowed to be adsorbed for 30 minutes in CO2 incubator at 37ºC and 5% CO2, then 5 ml of maintenance media (DMEM with 5% Fetal bovine serum and 1% penstrept) were added and the inoculated cell was

incubated in CO2 incubator at 37ºC and 5% CO2 for 3 days with daily observation for cytopathogenic effect (CPE). The cells were harvested at 3rd day and then reverse transcriptase PCR was performed for H5N1 AIV confirmation.

5. Virus titration

Madin-Darby Canine Kidney (MDCK) cells were seeded into a 96-well tissue culture plate at 2 x 10^4 cells / well and 10 fold serial dilution of avian influenza virus isolate were done with maintenance medium. Growth media was discarded and the cells were washed 3 times with PBS. A volume of 200 µl from each dilution of the virus was inoculated in one column of 96 well TC plate of monolayer MDCK cells and the column No. 12 in the plate was kept as –ve control (normal cells without virus). The inoculated cells were incubated in CO2 incubator at 37ºC and 5% CO2. The inoculated cells were daily observed and CPE was recorded. TCID50 was calculated by Reed and Muench (1938).

6. Cytotoxicity of Quercetin nanoparticles

For determination of the safest dose of the quercetin nanoparticles on the cells, Madin-Darby Canine Kidney (MDCK) cells were seeded into a 96-well tissue culture plate at 2x10^4 cells / well. The quercetin nanoparticles powder were reconstituted in deionized water at concentration of 100 µg/ml then it was serially diluted by 10 fold serial dilution using maintenance medium. The growth media was removed from the cells and the cells were washed 3 times with PBS. Each dilution of the quercetin nanoparticles was inoculated in one column of the plate; the last column in the plate was kept as –ve control (normal cells without treatment). The plate was then incubated in CO2 incubator at 37ºC and 5% CO2 for 48 hours with daily inspection for the alteration of cell morphology. The highest concentration of the quercetin nanoparticles causing no or minimal morphological changes of the cells was recorded compared with normal cells to be used further in the antiviral assay later. The protocol was described by Repetto *et al.* (2008).

7. CPE reduction assay (Antiviral assay) of Quercetin nanoparticles

CPE reduction assay was used for evaluation of the antiviral activity of the quercetin nanoparticles. MDCK cells were seeded at 2 x 10^4 cells / well into a 96 well tissue culture plate the determined safe dose of the quercetin nanoparticles (10µg/ml) was pre-incubated with each dilution of the 10 fold serial dilutions of AI virus at 37ºC for 30 minutes. The growth media was removed from the cells and the cells were washed 3 times with PBS and inoculated with the pre-incubated virus-quercetin nanoparticles mixture then incubated in CO2 incubator at 37ºC and 5% CO2 for 48 hours. After 48 hours post-infection, the inhibition of virus replication was evaluated by the reduction of cytopathic effect (CPE) induced by the virus.

8. Statistical analysis

Differences between groups were analyzed by using Independent Samples tTest. Statistical analysis was performed using the Statistical software package SPSS for Windows (version 20.0; SPSS Inc., Chicago IL, USA). Statistical significance between mean values was set at $P \leq 0.001$.

RESULTS

1.Quercetin nanoparticles characterization using Scanning Electron Microscope (SEM):

The morphology of prepared quercetin nanoparticles was studied using SEM. Quercetin nanoparticles (QUENPS) formed at flow rate 10 ml/ min, S/AS volume ratio 1:12, stirring speed 1300 rpm and drug concentration 5mg/ml showed a particle size range from 182nm to 240nm. QUENPs prepared by solvation/anti-solvation technique using syringe pump showed smaller particles uniform in their size and shape as shown in Figure, 2.

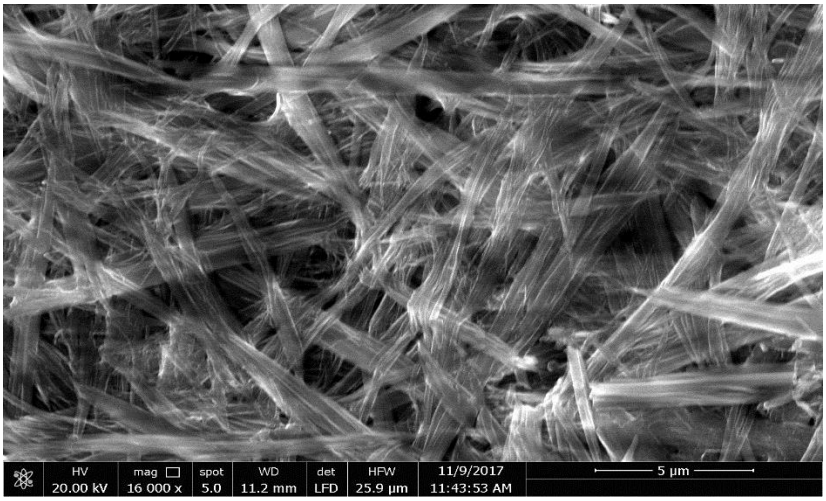

Fig.2: Scanning electron microscope (SEM) photograph showing quercetin nanoparticles

2. AI virus propagation on MDCK

The Microscopic examination of the inoculated MDCK showed the CPE of the highly pathogenic avian influenza virus H5N1; the infected cells showed destruction and complete detachment of the cell monolayer after 48 hours.

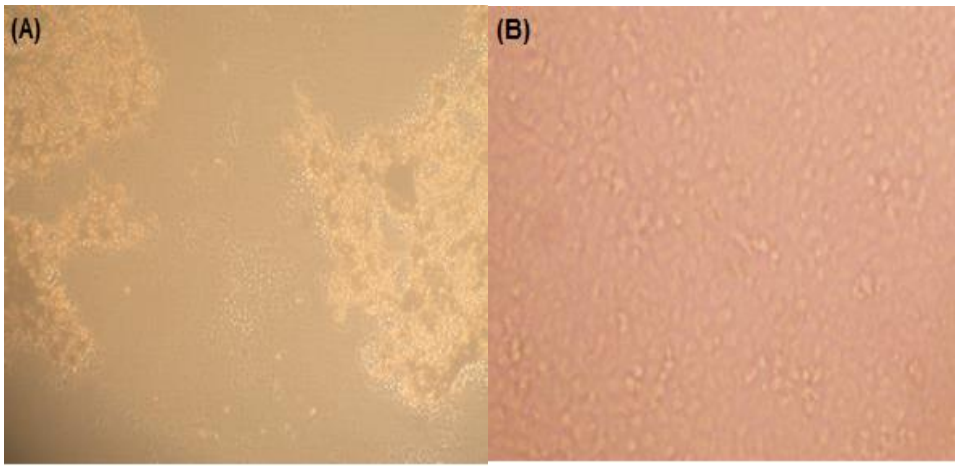

Fig. 3: (A) Showed destructed and detached cell monolayer after 48 hours post inoculation, **(B)** showed normal MDCK (negative cell control) (100 X)

3. AI virus titration on MDCK cells

The infective titer of H5N1 avian influenza virus (A/chicken/Egypt/1575S NLQP/2015) on MDCK was 7 log10 TCID50/ml.

4. Cytotoxicity of Quercetin nanoparticles

The results of cytotoxicity of quercetin nanoparticles revealed that only the concentration of 100 µg/ml showed sloughing of the MDCK cells and vacuoles in the cell monolayer while all the 10 fold serial dilutions from 10^{-1} to 10^{-10} had no significant cytotoxic effect on cells.

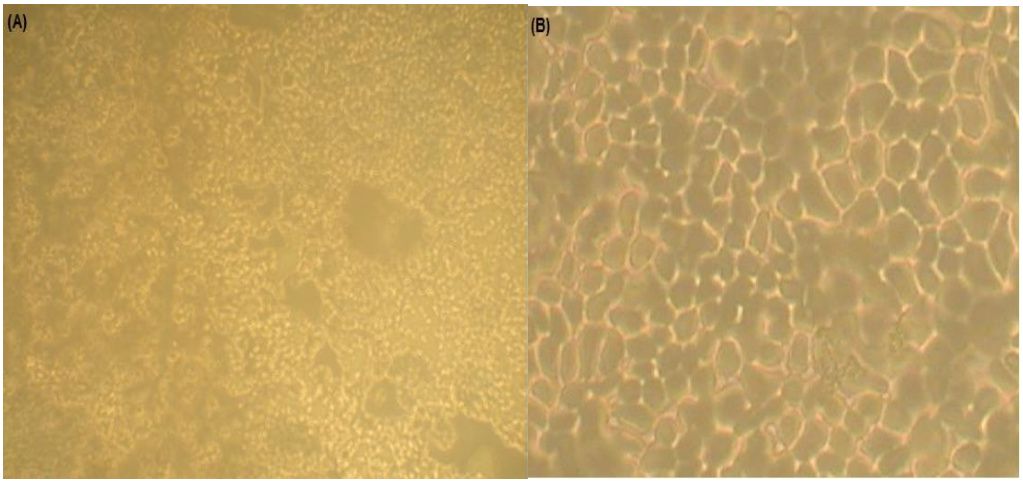

Fig. 4: (A): MDCK cells treated with the quercetin nanoparticles of concentration 100µg/ml had cytotoxic effect on cells which showing sloughing and vacuoles, **(B):** MDCK cells treated with dilution 10µg /ml of quercetin nanoparticles showing no morphological changes in the cells (100 X).

5. CPE reduction assay of Quercetin nanoparticles

The antiviral activity of the quercetin nanoparticles against avian influenza virus was evaluated by CPE inhibition on MDCK cell line and the results of CPE reduction assay revealed that the quercetin nanoparticles had a significant inhibitory effect on the avian influenza virus H5N1 as the virus titer (7 log10 TCID50/ml) decreased significantly (3.67 ± 0.33) after treatment with quercetin nanoparticles at concentration of 10µg/ml in comparison to the MDCK cells infected with the avian influenza virus not treated with quercetin nanoparticles (7.00 ± 0.00), fig (6). The CPE reduction assay was repeated twice with similar results.

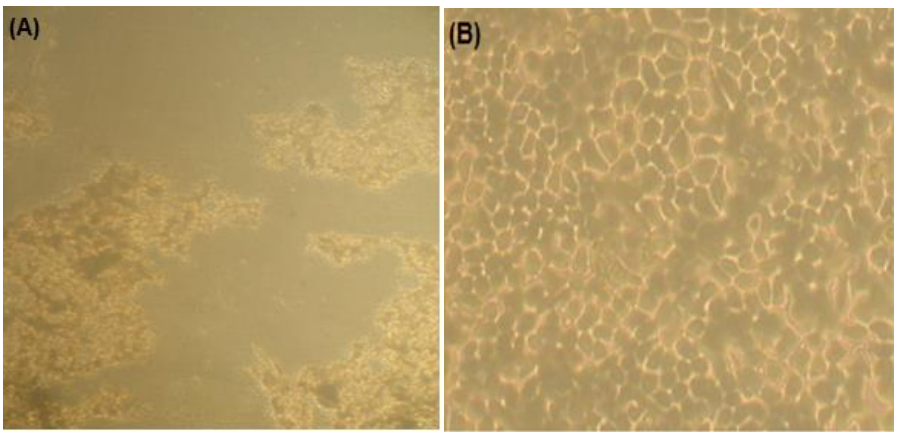

Fig. 5: (A) MDCK cells infected with avian influenza virus not treated with quercetin nanoparticles showing the CPE of AIV; detached and destructed cells, **(B):** MDCK cells infected with avian influenza virus treated with quercetin nanoparticles dilution 10µg/ml showing inhibition of the virus CPE (100 X).

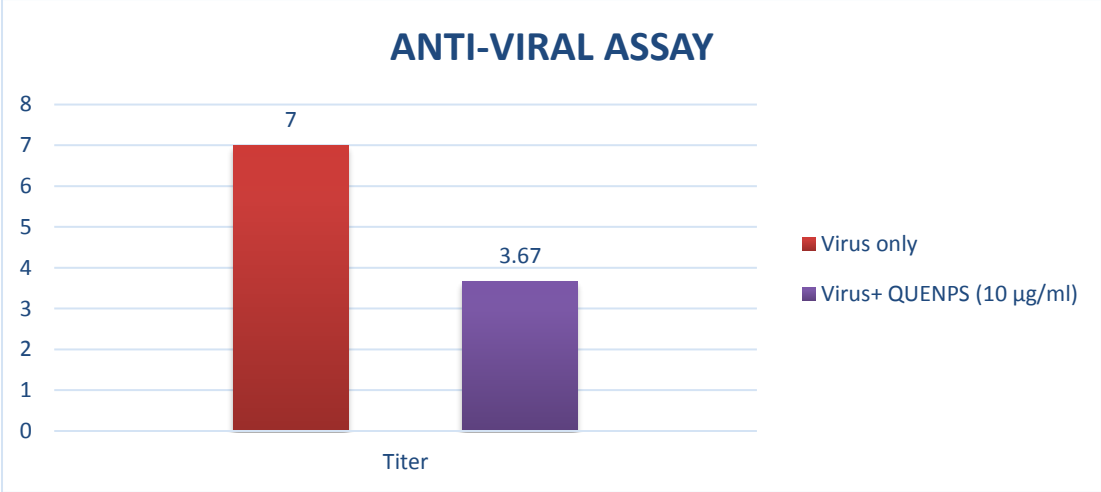

Fig. 6: Anti-viral (anti-cytopathic) effect represented as Log 10 TCID 50/ml. QUENPS: quercetin nanoparticles

DISCUSSION

Recently, a number of research reports highlighted the anti-influenza activity of quercetin and its derivatives and proved that quercetin has potent inhibitory effect on influenza virus infection either through acting as anti-influenza targets, specifically targeting viral proteins such as HA (Choi *et al.*, 2009), NA (Gong *et al.*, 2009) and M2 (Moorthy *et al.*, 2014) and viral messenger RNA (mRNA) replication (Nakazawa *et al.*, 2008 and Clark *et al.*, 2014) or through playing an important role in stimulating the cellular immune response (Gao *et al.*, 2001; Jassim and Naji 2003; Chu *et al.*, 2007 and Gansukh *et al.*, 2016). The mentioned unique mechanism of quercetin in combating the influenza virus beside its great potential to overcome drug resistance through its robust stable binding ability with emergent H7N9 neuraminidase mutant nominate this compound to become on the top of the most promising antivirals against influenza virus in the near future (liu *et al.*, 2015), one of the key challenges related to its use as an anti-influenza remedy is its low solubility and poor bioavailability. Nanotechnology offers an optimal solution for overcoming the limitation of quercetin in pharmaceuticals via its formulation in the form of nanoparticles, nanocapsules or micelles which appears to enhance the solubility and bioavailability of quercetin to maximize its benefits and provide optimize its effectiveness (Wina and Feng 2006 and Gansukh *et al.*, 2017).

Multidisciplinary research offers great opportunities for solving the scientific issues, from this prespective the current study is designed to fabricate quercetin nanoparticles and determine its efficacy as a new antiviral against H5N1 influenza virus and as a new approach within the continuous efforts for facing the potential influenza pandemic threating the globe. Here, quercetin nanoparticles prepared by solvation/anti-solvation technique using syringe

pump showed smaller particles with uniform in their size and shape. The mentioned behavior can be explained by main factors including, the concentration influence on the viscosity and the nuclei number formed in the interface of solvent/anti-solvent (Al-Jameel and Abd El-Rahman 2017). Moreover, fabrication of quercetin nanoparticles is influenced by important parameters including solvent to anti-solvent ratio, stirring speed and flow rate. Enhancing of the former parameters reduces the particle size of quercetin (Kakran *et al.,* 2012). Regarding with the solvent / anti-solvent ratio parameter, the given particles size in the current study was182-240 nm at S/AS ratio of 1:12 and this result was better compared to that obtained by Kakran *et al.* (2011) (220nm at S/AS ratio of 1:25).

To answer an interesting question about the safety of quercetin nanoparticles on the cells here, cytotoxicity assay were performed on MDCK cells to determine the safest dose of quercetin nanoparticles used in the cells. Although quercetin nanoparticles at concentration of 100µg/ml showed sloughing of the MDCK cells, the all 10 fold serial dilutions from 10^{-1} to 10^{-10} had no significant cytotoxic effect on cells and this is an excellent opportunity to introduce the most safe and effective nanoparticle concentration for drug delivery.

Our study is considered the preliminary report to investigate the antiviral activity of quercetin nanopaticles (particle size 182-240nm) on H5N1 AIV by CPE reduction assay on MDCK cell line, the results revealed that, the determined safe dilution 10µg/ml had a significant inhibitory effect on the avian influenza virus H5N1 (EPI573317) affecting its replication through reducing the virus titer from 7.00 ± 0.00 (log 10 TCID 50/ml) to 3.67 ± 0.33 (log 10 TCID 50/ml). The former findings were supported by a previous study that confirmed that, quercetin could inhibit the entry of different divergent strains of H5N1 pseudviruses through interferening with the function of HA envelope protein (specifically targeting HA2 subunit) (Wu *et al.,* 2016). Furthermore, the former author and co-collaborates call for serious efforts regarding the modification of quercetin to enhance its pharmacokinetics and pharmacodynamics which inspired us to conduct our study and our preliminary results encouraged us to take initiative steps in the in-vivo studies later.

CONCLUSION

In our study, we found that, quercetin nanoparticles possessed anti-influenza activity and subsequently it may be considered as an excellent formulation for the enhancement of antiviral potency, reducing drug dosage and overcoming the emergence of drug resistance. It is expected that quercetin nanoparticles may be the drug of choice in treatment and prevention of influenza infection in the near future.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

ACKNOWLEDGEMENT

The authors would like to thank members of RLQP, Animal Health Research Institute for their help. The authors also thank the head of Naqaa foundation for scientific research, technology and development for her help and support.

REFERENCES

Al-Jameel, S.S. and Abd El-Rahman, S.N. (2017): Effect of quercetin nanoparticles on the kidney of the streptozotocin-induced diabetes in male rats: A histological study and serum biochemical alterations. African Journal of Biotechnology. 16(39): 1944–1952.

Cai, X.; Fang, Z.; Dou, J.; Yu, A. and Zhai, G. (2013): Bioavailability of quercetin problems and promise. Curr Med Chem. 20 (20): 2572–2582.

Choi, H.J.; Song, J.H.; Park, K.S. and Kwon, D.H. (2009): Inhibitory effects of quercetin 3-rhamnoside on influenza A virus replication. Eur J Pharm. Sci. 37: 329–333.

Chu, Z.Y.; Chu, M. and Teng, Y. (2007): Effect of baicalin on in vivo anti-virus. Zhongguo Zhong Yao Za Zhi. 32: 2413–2415.

Clark, M.P.; Ledeboer, M.W. and Davies I. (2014): Discovery of a Novel, First- in-Class, Orally Bioavailable Azaindole Inhibitor (VX-787) of Influenza PB2. J Med Chem. 57: 6668–6678.

Collins, P.J.; Haire, L.F.; Lin, Y.P.; Liu, J.; Russell, R.J.; Walker P.A.; Skehel, J.J.; Martin, S.R.; Hay, A.J. and Gamblin, S.J. (2008): Crystal structures of oseltamivir-resistant influenza virus neuraminidase mutants. Nature 453: 1258-1262.

Dajas, F. (2012): Life or death: Neuroprotective and anticancer effects of quercetin. J. Ethnopharmacol. 143, 383–396.

Davis, J.M.; Murphy, E.A.; McClellan, J.L.; Carmichael, M.D. and Gangemi, J.D. (2008): Quercetin reduces susceptibility to influenza infection following stressful exercise. Am J Physiol Regul Integr Comp Physiol. 295: R505–9.

Delgado, L.; Fernandes, I.; Gonzalez-Manzano, S.; de Freitas, V.; Mateus, N. and Santos-Buelga, C. (2014): Anti-proliferative effects of quercetin and catechin metabolites. Food Funct., 5, 797–803.

Gansukh, E.; Kazibwe Z.; Pandurangan, M.; Judy, G. and Kim, D.H. (2016): Probing the impact of quercetin-7-O-glucoside on influenza virus replication influence. Phytomedicine. 23: 958–967.

Gansukh, E.; Muthu M.; Paul, D.; Ethiraj, G.; Chun, S. and Gopal, J. (2017): Nature nominee quercetin's anti-influenza combat strategy-demonstrations and remonstrations. Rev Med Virol. 27:e1930. https://doi.org/10.1002/ rmv.1930.

Gao, Z.H.; Huang, K.X. and Xu, H.B. (2001): Protective effects of flavonoids in the roots of Scutellaria baicalensis Georgi against hydrogen peroxide induced oxidative stress in HS-SY5Y cells. Pharmacol Res. 43: 173–178.

Ge, H.; Wang, Y.F.; Xu, J.; Gu, Q.; Liu, H.B.; Xiao, P.G.; Zhou, J.; Liu, Y.; Yang, Z. and Su, H. (2010): Anti-influenza agents from traditional Chinese medicine. Nat. Prod. Rep. 27, 1758–1780.

Gong, J.Z.; Fang, H. and Li, M.Y. (2009): Potential Targets and Their Relevant Inhibitors in Anti-influenza Fields. Curr Med Chem. 16: 3716–3739.

Ha, S.Y.; Youn, H.; Song, C.S.; Kang, S.C.; Bae, J.J.; Kim, H.T.; Lee, K.M.; Eom, T.H.; Kim, I.S. and Kwak, J.H. (2014): Antiviral effect of flavonol glycosides isolated from the leaf of Zanthoxylum piperitum on influenza virus. J. Microbiol. 52, 340–344.

Ho, J.Y.; Chang, H.W.; Lin, C.F.; Liu, C.J.; Hsieh, C.F. and Horng, J.T. (2014): Characterization of the anti-influenza activity of the Chinese herbal plant Paeonia lactiflora. Viruses. 6, 1861–1875.

Jassim, S.A.A. and Naji, M.A. (2003): Novel antiviral agents: a medicinal plant perspective. J. Appl Microbiol. 95: 412–427.

Kakran, M.; Sahoo, N.G. and Li, L. (2011): Dissolution enhancement of quercetin through nanofibrication, complexation, and solid dispersion. Colloids and Surfaces B:Biointerfaces. 88(1): 121–130.

Kakran, M.; Sahoo, N.G.; Li, L. and Judeh, Z. (2012): Fabrication of quercetin nanoparticles by antisolvent precipitation method for enhanced dissolution. Powder Technology. 223: 59–64.

Kumar, P.; Khanna, M.; Srivastava, V.; Tyagi, Y.K.; Raj, H.G. and Ravi, K. (2005): Effect of quercetin supplementation on lung antioxidants after experimental influenza virus infection. Exp Lung Res. 31: 449–459.

Kunyanga, C.N.; Imungi, J.K.; Okoth, M.W.; Biesalski, H.K. and Vadivel, V. (2011): Flavonoid content in ethanolic extracts of selected raw and traditionally processed indigenous foods consumed by vulnerable groups of Kenya: antioxidant and type II diabetes-related functional properties. Int J. Food Sci Nutr. 62: 465–473.

Leonov, H.; Astrahan, P.; Krugliak, M. and Arkin, I.T. (2011): How do aminoadamantanes block the influenza M2 channel, and how does resistance develop? J. Am. Chem. Soc. 133, 9903–9911.

Li, M. and Xu, Z. (2008): Quercetin in a lotus leaves extract may be responsible for antibacterial activity. Arch. Pharm. Res. 31, 640–644.

Liu, Z.; Zhao, J.; Li, W.; Wang, X.;Xu, J.; Xie,J.; Tao, K.; Shen, L. and Zhang, R. (2015): Molecular Docking of Potential Inhibitors for Influenza H7N9 Computational and Mathematical Methods in Medicine Volume 2015, Article ID 480764, 8 pages doi:10.1155/2015/480764.

Liu, Z.K.; Zhao, J.; Li, W.; Shen, L.; Huang, S.; Tang, J.; Duan, J.; Fang, F.; Huang, Y.; Chang, H.; Chen, Z. and Zhang, R. (2016): Computational screen and experimental validation of anti-influenza effects of quercetin and chlorogenic acid from traditional Chinese medicine. Scientific Reports 6, Article number: 19095 (2016) doi:10.1038/srep19095.

Manach, C.; Williamson, G.; Morand, C.; Scalbert, A. and Remesy, C. (2005): Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. Am J Clin Nutr. 81: 230S-242S.

Moorthy, N.S.H.N.; Poongavanam, V. and Pratheepa, V. (2014): Viral M2 Ion Channel Protein: A Promising Target for Anti-influenza Drug Discovery. Mini Reviews in Medicinal Chemistry. 14: 819–830.

Muthuri, S.G.; Myles, P.R.; Venkatesan, S.; Leonardi-Bee, J. and Nguyen-Van-Tam, J.S. (2013): Impact of neuraminidase inhibitor treatment on outcomes of public health importance during the 2009–2010 influenza A (H1N1) pandemic: a systematic review and metaanalysis in hospitalized patients. J Infect Dis. 207(4): 553-563.

Nakazawa, M.; Kadowaki, S.E.; Watanabe, I.; Kadowaki, Y.; Takei, M. and Fukuda, H.PA. (2008): Subunit of RNA polymerase as a promising target for anti-influenza virus agents. Antiviral Res. 78: 194–201.

Nguyen, H.T.; Nguyen, T.; Mishin, V.P.; Sleeman, K.; Balish, A.; Jones, J.; Creanga, A.; Marjuki, H.; Uyeki, T.M.; Nguyen, D.H.; Nguyen, D.T.; Do, H.T.; Klimov, A.I.; Davis, C.T. and Gubareva, L.V. (2013): Antiviral Susceptibility of Highly Pathogenic Avian Influenza A (H5N1) Viruses Isolated from Poultry, Vietnam, 2009–2011. Emerging Infectious Diseases www.cdc.gov/eid .19(12): 1963–1971.

Nishimuro, H.; Ohnishi, H.; Sato, M.; Ohnishi-Kameyama, M.; Matsunaga, I.; Naito, S.; Ippoushi, K.; Oike, H.; Nagata,T. and Akasaka, H. (2015): Estimate daily intake and seasonal food sources of quercetin in Japan . Nutrients 7: 2345–2358.

Ratnam, D.V.; Ankola, D.D.; Bhardwaj, V.; Sahana, D.K. and Kumar, M.N.V.R. (2006): Role of antioxidants in prophylaxis and therapy: A

pharmaceutical perspective. J Control Release. 113: 189–207.

Reed, L.J. and Muench, H. (1938): A simple method of estimating fifty percent endpoints. Am. J. Hyg. 27: 493–497.

Repetto, G.; Ana del Peso and Zurita, J.L. (2008): Neutral red uptake assay for the estimation of cell viability/ cytotoxicity. Nature Protocols, 3 (7), 1125– 1131.

Robaszkiewicz, A.; Balcerczyk, A. and Bartosz, G. (2007): Antioxidative and prooxidative effects of quercetin on A549 cells. Cell Biol. Int. 31, 1245–1250.

Somerset, S.M. and Johannot, L. (2008): Dietary flavonoid sources in Asutralian adults. Nutrition and Cancer 60:442–449.

Spanakis, N.; Pitiriga, V.; Gennimata, V. and Tsakris, A. (2014): A review of neuraminidase inhibitor susceptibility in influenza strains. Expert Rev. AntiInfect. Ther. 12, 1325–1336.

Wiczkowski, W.; Romaszko, J.; Bucinski, A.; Szawara-Nowak, D.; Honke, J.; Zielinski, H. and Piskula, M.K. (2008): Quercetin from shallots (Alliumcepa L. var. aggregatum) is more bioavailable than its glucosides. Journal of Nutrition 138: 885–888.

Wina, K.Y. and Feng, S.S. (2006): In vitro and in vivo studies on vitamin E TPGS-emulsified poly (D, L-lactic-co-glycolic acid) nanoparticles for paclitaxel formulation. Biomaterials. 27(10), 2285–2291.

Wu, W.; Li, R.; Li, X.; He, J.; Jiang, S. ; Liu, S. and Yang, J. (2016): Quercetin as an Antiviral Agent Inhibits Influenza A Virus (IAV) Entry. Viruses 2016;8 (1) ,6. doi:10.3390/v8010006.

Yang, Z.F.; Bai, L.P.; Huang,W.B.; Li, X.Z.; Zhao, S.S.; Zhong, N.S. and Jiang, Z.H. (2014): Comparison of in vitro antiviral activity of tea polyphenols against influenza A and B viruses and structure-activity relationship analysis. Fitoterapia. 93: 47–53.

Zakaryan, H.; Arabyan, E.; Oo2, A. and Zandi, K. (2017): Flavonoids: promising natural compounds against viral infections. Arch Virol 2017; DOI 10.1007/s00705-017-3417-y.

فعالية جزيئات النانوكوريستين كمضاد جديد للفيروسات ضد تكاثر فيروس الانفلوانزا H5N1

همت شفيق السيد ، وسام حسن ماضى ، محمد عفيفى سيف ، وسام أحمد توفيق

E-mail: hemat.elsayed@yahoo.com Assiut University web-site: www.aun.edu.eg

الظهور المتزايد فى الآونة الأخيرة للسلالات المتحورة من فيروس الانفلونزا H5N1 يتطلب الحاجة الملحة لأدوية جديدة ومؤثرة ضد عدوى الانفلونزا. الكوريستين مرشح طبيعى واعد للعلاج والوقاية من عدوى الأنفلونزا ولكن لسوء الحظ قلة القابيلية للذوبان وضعف الحيوية البيولوجية للكوريستين يعيق استخداماتة فى المجال الطبى. تقنية النانوتكنولوجى تتيح حل ابتكارى لتعزيز القابلية للذوبان والحيوية البيولوجية الخاصة بالكوريستين وبالتالى يعزز من تأثيره العلاجى. فى الدراسة الجارية جزيئات الكوريستين النانوية تزواحت فى حجمها من ١٨٢- ٢٤٠nm بمعدل تدفق ١٠ مل / دقيقة ، نسبة حجم 1:12 solvent /antisolvent ، سرعة التحريك ١٣٠٠ دورة في الدقيقة وتركيز الدواء ٥ ملجم / مل .الجرعة الآمنة من جزيئات النانوكوريستين ١٠ ميكروجرام/مل كان لها تأثير ملحوظ على عترة H5N1 (EPI573317) عن طريق الحد من تتر الفيروس والتغيرات المرضية على خلايا MDCK . يمكن استخدام جزيئات النانوكوريستين كمثبط واعد جديد لـ H5N1 AIV ومن المتوقع أن يكون على رأس المرشحين كمضاد للفيروسات ضد فيروس الأنفلونزا في المستقبل القريب.