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Pharmacokinetics of quercetin loaded biodegradable chitosan nanoparticles following oral administration in rabbits

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Abstract

Infectious diseases contribute major sickness of the residential area and resistance towards synthetic anti-bacterials is a major concern. Quercetin is a bioactive flavonoid nutraceutical. Its biopharmaceutical applications are limited due to poor solubility and low bioavailability. Quercetin loaded poly lactic Co glycolic acid (PLGA) nanoparticles were prepared by the solvent displacement method. Nanoparticles were characterized and their antibacterial potential was found *in vitro* against gram positive and negative bacteria using the broth dilution method. Comparative pharmacokinetics of plain quercetin and quercetin loaded nanoparticles were conducted in rabbits. Plasma samples were analyzed for the quercetin using spectrophotometer. The values of pharmacokinetic parameters such as AUC, clearance, Vd, Tmax and Cmax were 183.4±3.94 h.mg/L, 0.13±0.01 L/h, 1.53±0.78 L, 11.19±0.28h, and 5.98±1.24 mg/L respectively for pure quercetin and were 1616 ± 5.71 h.mg/L, 0.01 ± 0.00 L/h, 0.50 ± 0.03 L, 18.50 ± 0.34h, and 27.65 ± 1.21 mg/L respectively for quercetin nanoparticles. This study indicated that the pharmacokinetics of quercetin nanoparticles has been significantly improved.

Keywords

Nutraceuticals
Pharmacokinetics
Poly lactic Co glycolic acid
Quercetin
Rabbits

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Introduction

Bacterial infections are the most usual reason of diseases worldwide. Resistance has been emerged against most of synthetic drugs used for these infections. In addition, synthetic drugs also cause various adverse effects on human health. Efforts are focused on the discovery of other therapeutic options for treating infections (Woźnicka et al., 2013). The conventional drug delivery systems are deprived of target specificity that's why drugs are distributed in all body tissues resulting in other side effects. That's why there should be a dosage form which ensure targeted delivery of drugs (Wang et al., 2014).

Quercetin, a nutraceutical is mainly present in onions and green tea. Quercetin has various beneficial pharmacological activities such as anti-cancer, anti-oxidant, and anti-platelet (Bagad & Khan, 2015). Encapsulation of quercetin in poly lactide-Co-glycolide (PLGA) nanoparticles enhances its antibacterial efficiency hence providing an alternative approach for resolution of solubility, stability and bioavailability issues associated with simple administration of quercetin (Kumar et al., 2016). PLGA nanoparticles are innovative and suitable drug delivery system for quercetin delivery. PLGA is a biodegradable and biocompatible polymer which degrades to lactic acid and glycolic acid in the body (Makadia & Siegel, 2011; Danhier et al., 2012). We

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have reported the use PLGA nanoparticles in detoxification (Nguyen et al., 2016; Muhammad et al., 2017) as well as in drug delivery (Akhtar et al., 2019; Anwar et al., 2020). Quercetin loaded PLGA nanoparticles are easy to prepare and can be administered by a number of routes, but the oral one is the most convenient, common and has greater patient compliance (Roger et al., 2010).

Despite of having a broad range of therapeutic effects, quercetin use in clinical practice is limited owing to little oral bioavailability, hydrophobicity, and insolubility. Certain drugs, when administered orally, lead to deterioration of active ingredients due to low stomach pH. These problems can be overcome by encapsulating the drug in polymeric nanoparticles. Polymeric nanoparticles can be easily internalized and transported through colon (Mukhopadhyay & Prajapati, 2015). Keeping in mind the above scenario, the study was aimed to enhance the solubility and oral bioavailability of quercetin. For this purpose, quercetin was encapsulated in PLGA nanoparticles. In vitro antibacterial potential was determined. Comparative pharmacokinetics study was done in rabbits to determine the significant difference between quercetin and quercetin loaded PLGA nanoparticles.

Materials and Methods

Materials: Poly lactic co-glycolic acid, Resomer ® RG 502 H, (MW= 7000-17,000 and acid-terminated) was purchased from Sigma Aldrich (St Loius, USA). Chitosan was purchased from Sigma-Aldrich (St Loius, USA) with MW=140,000-220,000 and white mushrooms as biological source. Polyvinyl alcohol (PVA) 1500 was purchased from Duksan pure chemicals, Korea. Nutrient broth was purchased from lab M. Limited, Lancashire, UK. Dimethyl sulfoxide (DMSO), acetone, methanol, acetic acid and ascorbic acid were purchased from Sigma-Aldrich. All the chemicals used in study were of analytical grade.

Preparation of Quercetin loaded nanoparticles: PLGA nanoparticles were prepared following solvent displacement method with some modifications (Tefas et al., 2015). Five percent aqueous polyvinyl alcohol solution was prepared. Poly lactic co glycolic acid (100mg) and quercetin (10mg) were added in 10mL of acetone. This solution was mixed homogeneously with vortex mixer. Polyvinyl alcohol (20mL) solution was taken in a beaker on magnetic stirrer under 1200 rpm at 25°C. PLGA and quercetin solution was added in PVA solution drop wise for homogenous distribution of drug and polymer in the formulation.

Purification of nanoparticles: After freshly preparing the dispersion of nanoparticles, it was kept on magnetic stirrer for 4 hours at 40°C, so that the organic phase (acetone) was completely removed. The remaining dispersion was then centrifuged at 25000 rpm for half an hour at 25°C. The pellet of nanoparticles was settled down. Then pellet was dispersed in distilled water on vortex mixer. The dispersion was sonicated for 10 minutes for further complete homogenization.

Characterization of quercetin loaded PLGA nanoparticles

Size and potential of nanoparticles: The prepared nanoparticles were characterized for size, potential and poly dispersity index by using Zetasizer Nano-ZS90.

Standardization of UV-visible spectrophotometric method for quercetin detection: The different dilutions of concentrations 5µg/mL, 10µg/mL, 15µg/mL, 20µg/mL, and 25µg/mL were prepared in methanol. The maximum wavelength (λmax) of quercetin was determined by taking wavelength scan at UV-visible spectrophotometer. A standard curve was created using concentration and relative absorbance data.

Determination of encapsulating efficiency: Quercetin loaded nanoparticles were centrifuged at speed of 25000 rpm for 30 minutes at 25°C. The resulting supernatant was analyzed at 300 nm by using ultraviolet visible spectrophotometer. For the determination of encapsulating efficiency, the standard curve (described above) was used. It was calculated by following the given formula; (Bagad & Khan, 2015).

$$\%\ \text{Encapsulating Efficiency} = \frac{Quercetin\ weight\ in\ nanoparticles}{Initial\ weight\ of\ quercetin\ used} \times 100$$

***In vitro* determination of minimum inhibitory concentration:** Using broth dilution method with minor modifications, MIC of quercetin loaded PLGA nanoparticles was determined. Stock solution of QueNPs was prepared in 10% dimethyl sulfoxide (DMSO). Then different dilutions of QueNPs (1-100 ug/ml) were prepared in DMSO. In each dilution, 0.1 mL of tested strains inoculum (*Staphylococcus aureus*, *Escherichia coli*) was poured and incubated for 24 h at 37 °C. The presence of turbidity showed growth of bacteria. The concentration at which there was no growth of bacteria (no turbidity) was regarded as MIC of quercetin nanoparticles. Dilutions of 10% dimethyl sulfoxide (DMSO) were also incubated with tested strains to serve as a negative control (Tuo et al., 1923; Jaisinghani, 2017; Altaf et al., 2019; Lin et al., 2020).

Pharmacokinetic study of quercetin nanoparticles: The *in vivo* pharmacokinetics study was performed using rabbits as experimental animals. All the protocols and procedures followed for animals handling, drug administration and sampling were reviewed and approved by Institutional Biosafety and Bioethics Committee (IBC), University of Agriculture, Faisalabad. Rabbits (n=6) were purchased from local market of Faisalabad and kept in animal house of Institute of Physiology and Pharmacology, University of Agriculture, Faisalabad, Pakistan. Animals were acclimatized for one week and 25mg/kg pure quercetin

in distilled water was given orally to each rabbit. The blood samples were collected at 0 h, 0.5 h, 1 h, 2 h, 4 h, and 8 h, 12 h, and 24 h intervals in the heparinized vials. Blood was collected and analyzed for quercetin determination by UV-visible spectrophotometer as described above. After one week of wash out period, 25mg/kg QueNPs were administered orally to all the rabbits. The blood samples were collected and processed as described above (Biasutto et al., 2010).

Pretreatment of blood samples for analysis: The quercetin concentration in blood was determined following the method of (Biasutto et al., 2010). 1 mL blood from each dilution and samples were taken in mini test tubes. Then 0.1mL of ascorbic acid solution (0.01 M) and 0.1mL of acetic acid solution (0.6M) were added in each mini test tube and was mixed well. Acetone (4 mL) was added to them and sonicated for 2 min. All samples were centrifuged at 3000 rpm for 5 min. Supernatant was removed and absorbance was determined by UV-visible spectrophotometer.

Determination of pharmacokinetic parameters: The Pharmacokinetic parameters were determined by linear regression method using APO software version 3.2 (A Mediware Product). The classical clinical pharmacokinetics parameters were recorded e.g. area under the curve, volume of distribution, absorption and elimination half-life, clearance etc.

Statistical analysis: All values were expressed in terms of average ± SEM. Pharmacokinetic data was subjected to analysis of variance (ANOVA) followed by student's t test to check the significance ($p \leq 0.05$).

Results

Characterization of Nanoparticles: The behavior of nanoparticles based drug delivery systems depends on the physical and chemical properties of the nanoparticles, like entrapment efficiency, zeta potential, polydispersity index and particle size. The prepared formulation exhibited diameter of 243.3 nm and polydispersity index of 0.131 as represented in Figure 1(A) and 1(C). The zeta potential value of prepared nanoparticles was -1.83mV as shown in Figure 1(B). The solubility of quercetin has also been increased in the form of nanoparticles versus in conventional form as shown in Figure 1(D).

Entrapment efficiency: Entrapment efficiency was calculated from standard curve of known dilutions of quercetin as represented in Figure 2(A) and 2(B). The encapsulation efficiency of the prepared nanoparticles was 84% as determined by using above mentioned formula.

Determination of minimum inhibitory concentration: Figure 3 showed the minimum inhibitory concentration of pure quercetin, quercetin loaded nanoparticles, 10% DMSO (as negative control) and commercially available ampicillin against tested strains of bacteria. The results revealed that the MIC of pure quercetin and QueNPs were less against the tested strains in comparison to commercially available ampicillin.

***In vivo* pharmacokinetic study of quercetin in rabbit blood:** *In vivo* pharmacokinetic study of quercetin in rabbits was done by orally administering pure quercetin and quercetin loaded PLGA nanoparticles at a dose rate of 25mg/kg body weight. The mean concentration-time curve of the pure quercetin and quercetin PLGA nanoparticles have been shown in Figure 4, demonstrating that the concentration-time curve was best described by two-compartment model for quercetin. The pharmacokinetic parameters of pure quercetin showed that Cmax of quercetin in rabbit blood was 5.98 ± 1.24 mg/L while Tmax was 11.19 ± 0.28 h. While the pharmacokinetic parameters of QueNPs showed that quercetin was absorbed at a higher concentration in rabbit, with Cmax of 27.65 ± 1.21 mg/L and Tmax of 18.50 ± 0.34h. The values of pharmacokinetic parameters such as area under curve, clearance, volume of distribution, time to peak concentration (Tmax.), peak concentration (Cmax.) were 183.4±3.94 h.mg/L, 0.13±0.01 L/h, 1.53±0.78 L, 11.19±0.28h, and 5.98±1.24 mg/L respectively for pure quercetin and were 1616±5.71 h.mg/L, 0.01±0.00 L/h, 0.50±0.03 L, 18.50±0.34h, and 27.65±1.21 mg/L respectively for QueNP (as shown in Table 1). The other pharmacokinetic parameters of QueNPs and pure quercetin has been described in Table 1.

Discussion

Nanoparticles are very important drug carrier systems (Aziz et al., 2020). Preparation of nanoparticles depends upon the interaction of PLGA with aqueous phase. Nanoparticle size depends upon ratio of PLGA and quercetin and stirring speed. Biological performance of nanoparticles will vary based upon the size characteristics. The behavior of nanoparticle based drug delivery systems depends on the physical and chemical properties of the nanoparticles, like entrapment efficiency, zeta potential, polydispersity index and particle size. The prepared nanoparticles exhibited diameter of 243.3 nm which is ideal size of nanoparticles for biological applications. The size distribution curve for QueNPs was represented in Figure 1(A). In the curve it was estimated that the prepared nanoparticles showed unimodal and narrow curve of size distribution system. Passive targeting for nanoparticles was supposed because the particle size of the prepared formulation was within 100 to 300nm range.

Nanoparticles showed polydispersity index of 0.131 (Figure 1C). Polydispersity index is a measure of proper distribution of nanoparticles within the formulation. In the nanoparticles formulation, polydispersity index was an indicative of similar sized mono-dispersion system of

Table 1: Comparative Pharmacokinetic parameters of pure Quercetin and Quercetin loaded PLGA nanoparticles.

Parameter	Unit	Quercetin Mean ± SEM	Quercetin loaded nanoparticles Mean ± SEM
t1/2 (elimination)	H	7.85 ± 3.50[a]	23 ± 0.22[b]
Vd Steady state	L	1.53 ± 0.78 [a]	0.50 ± 0.03 [b]
CL	L/h	0.13 ± 0.01 [a]	0.01 ± 0.00 [b]
Tmax.	H	11.19 ± 0.28 [a]	18.50 ± 0.34 [b]
Cmax.	mg/L	5.98 ± 1.24 [a]	27.65 ± 1.21 [b]
AUC	h.mg/L	183.4 ± 3.94 [a]	1616 ± 5.71 [b]
MRT	H	22.49 ± 1.26 [a]	44.57 ± 0.64 [b]

Values bearing different alphabet superscripts in rows are significantly different ($p \leq 0.05$)

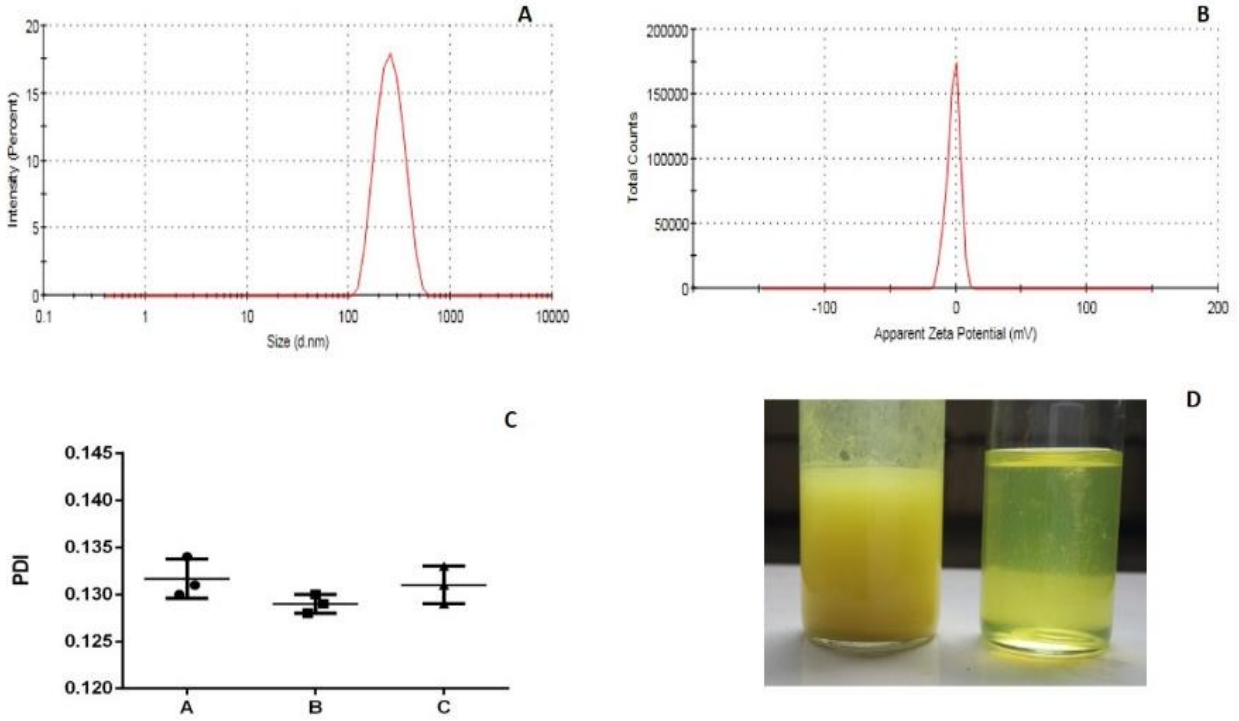

Figure 1: Characteristics of prepared nanoparticles: (A): size of nanoparticles, (B): Zeta potential of nanoparticles, (C) Polydispersity index, (D) Solubility of quercetin versus nanoparticles of quercetin.

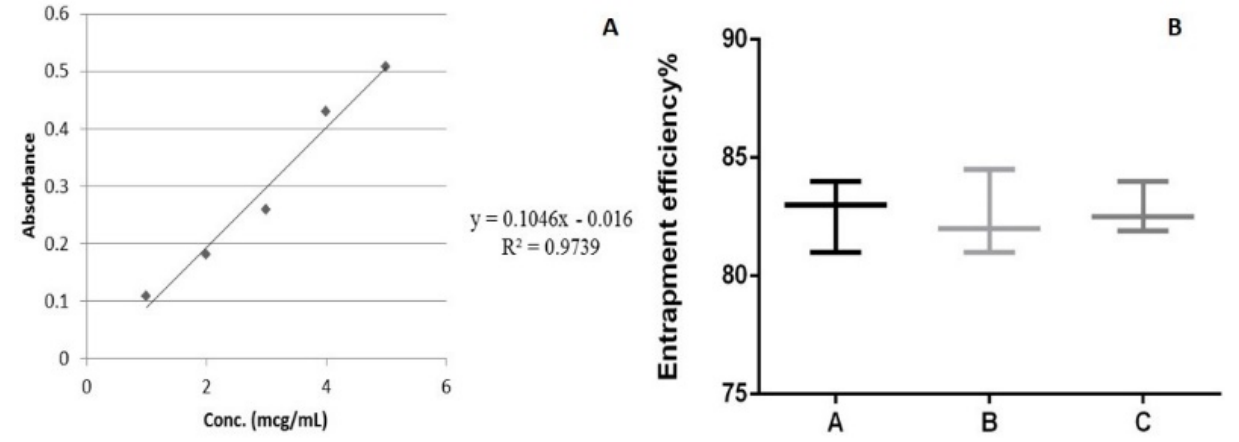

Figure 2: UV-Visible absorbance of quercetin: (A): Standard curve of quercetin by UV-visible spectroscopy, (B): Entrapment efficiency of nanoparticles.

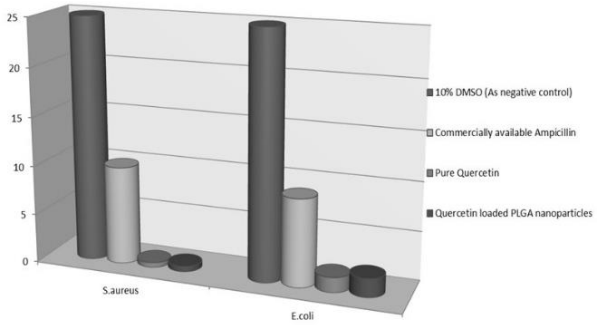

Figure 3: Minimum inhibitory concentration of Quercetin and Ampicillin against *S. aureus* and *E.coli.*

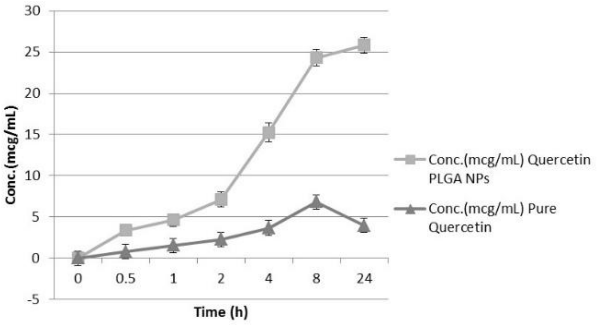

Figure 4: Plasma concentration versus time profile of pure quercetin and quercetin loaded nanoparticles.

nanoparticles (Figure 1C). The results are comparable with another study which involved QueNPs preparations using a solvent displacement method and the prepared formulation exhibited mean diameter 399.6 nm with narrow distribution ploydispersity index 0.262 (Pool et al., 2012). The study utilized higher MW (60kDa) PLGA. The higher MW of the PLGA used in this study increased the viscosity of the internal phase, leading to decrease net shear stress hence producing large nanoparticles.

Determination of measuring zeta potential of nanoparticles is a method for figuring out the nanoparticles surface charge within the formulation. During the preparation steps of nanoparticles, a surface charge is imparted onto nanoparticles that attract ionic layer of contrary charge nanoparticle. This dual layer of ions moves along with nanoparticles. The superficial electrical potential on the circumference of the double layer is known to be the zeta potential of that particles. The values of zeta potential greatly vary between +100 mV to -100 mV. Zeta potential is the determinative factor for stability of nanoparticles dispersion.

The nanoparticles dispersion is said to be highly stable if its zeta potential values are within ±25mV range. The nanoparticles dispersions, having low values of zeta potential lead to accumulation of nanoparticles due to inter particles van der waal's interactions. Meanwhile, the nanoparticles dispersion having higher values of zeta potential lead to the most stable formulation, because strong inter-particle repulsion decreases the potential of nanoparticles aggregation. The zeta potential value of the prepared formulation was -1.83 mV as shown in Figure 1(B). The negative zeta potential is important in prolongation of nanoparticles circulation within the body fluids and also helpful in drug delivery systems as we previously described in another study (Akhtar et al., 2020b). This negative charge value is attributed to the carboxyl group of PLGA (Platel et al., 2016). Another study the QueNPs formulation prepared by utilizing nano-precipitation (solvent displacement) technique showed nanoparticles diameter of 148.6 nm, poly-dispersity index 0.088 and zeta potential value of -27 mV (Pimple et al., 2012).

The encapsulation efficiency of nanoparticles was 84% (Figure 2B), which is comparatively higher in comparison to other reported studies (Pool et al., 2012) where 41.36% of encapsulation efficiency of quercetin in PLGA nanoparticles was obtained. The results are comparable with another study (Akhtar et al., 2020a). Our higher entrapment efficiency might be attributed to relatively higher PLGA nanoparticle size to accommodate more quercetin.

In this study, the MIC of free quercetin and QueNPs were evaluated by broth micro dilution technique. In the broth micro dilution technique, MIC was determined with spectrophotometric analysis due to formation of turbidity. That's why quercetin gave absorbance value at 570 nm. The solution of pure quercetin in DMSO produced insoluble turbidity formation and resulted in false positive outcomes. Nanoparticles are the efficient carrier system for lipophilic drugs, so the above condition was not investigated for quercetin PLGA nanoparticles.

The results of minimum inhibitory concentration revealed that values of MIC for pure quercetin and quercetin PLGA nanoparticles were too small in comparison to commercially available ampicillin (Figure 4). We may conclude that quercetin in PLGA nanoparticles can be used clinically against different infectious diseases caused by the tested strains. The results of 10% DMSO showed that DMSO had antibacterial activity at very high concentrations.

The results showed that there was no considerable difference in MIC of pure quercetin and quercetin PLGA nanoparticles. We may conclude that PLGA nanoparticles did not significantly decrease the MIC values. This could be answered by the sustained, programmed and passive erosion of quercetin from PLGA nanoparticles. (ARASOĞLU et al., 2017). Observed that the antibacterial activity depends on the amount of dose and not on the sustained and controlled release of quercetin. However, the sustained and

controlled release behavior of QueNPs helps in maintaining specific concentration of quercetin for longer time period which is quite important in microbial inhibition.

Another study utilized the colony count disc diffusion method for determining the susceptibility of bacterial strains. This study showed that *E. coli* was more susceptible to QueNPs than *Micrococcus tetragenus*. The MIC value of *E.coli* was 5µg/mL whereas in this study the MIC value of *E.coli* was 1.89µg/mL. (Sun et al., 2016).

Quercetin levels in whole blood were determined after oral administration of pure quercetin and QueNPs by UV-visible spectrophotometric analysis (shown in figure 2A). The classical pharmacokinetic parameters were determined by APO software. The mean plasma concentration-time profile of the investigated pure quercetin and QueNPs were shown in Figure 4, demonstrating that the concentration-time profile was best described by two-compartment model for quercetin. Pharmacokinetic parameters of pure quercetin and quercetin PLGA nanoparticles were compared by drawing their average concentration curves versus respective time intervals. The values of pharmacokinetic parameters such as area under curve, clearance, volume of distribution, time to peak concentration, peak concentration were 183.4 ± 3.94 h.mg/L, 0.13 ± 0.01 L/h, 1.53 ± 0.78 L, 11.19 ± 0.28h, and 5.98 ± 1.24 mg/L respectively for pure quercetin while values for QueNP were 1616 ± 5.71 h.mg/L, 0.01 ± 0.00 L/h, 0.50 ± 0.03 L, 18.50 ± 0.34h, and 27.65 ± 1.21 mg/L respectively. The elimination half-life of Quercetin was increased (23±0.22 h) significantly after loading in PLGA nanoparticles in comparison to plane quercetin (7.85 ± 3.50 h).

The increase in Cmax, Tmax and half-life values after administering QueNPs can be explained by controlled and sustained release of quercetin from PLGA nanoparticles. PLGA prevents the rapid degradation of quercetin in gastrointestinal tract as well which aids in greater concentration of quercetin in blood. PLGA nanoparticles also contribute to enhanced and easy penetration of QueNPs across the gastrointestinal membrane. That's why the values of clearance and volume of distribution of QueNPs were decreased in comparison to pure quercetin.

Conclusion: Present study suggests that quercetin loaded nanoparticles might prove a better therapeutic alternative for treatment of bacterial infections. Further better pharmacokinetic profile was achieved which suggest that this formulation might be used as controlled release preparation with enhanced bioavailability.

References

Akhtar B, Muhammad F, Aslam B and Saleemi MK 2019. Toxicity Studies of Oral and Transdermal Formulations of Gentamicin Loaded PLGA Nanoparticles in Animal Model. *Pakistan Veterinary Journal*, DOI: 10.29261/pakvetj/2019.121.

Akhtar B, Muhammad F, Aslam B, Saleemi MK and Sharif A 2020a. Biodegradable nanoparticle based transdermal patches for gentamicin delivery: Formulation, characterization and pharmacokinetics in rabbits. *Journal of Drug Delivery Science and Technology*, 101680.

Akhtar B, Muhammad F, Aslam B, Saleemi MK and Sharif A 2020b. Pharmacokinetic profile of chitosan modified poly lactic co-glycolic acid biodegradable nanoparticles following oral delivery of gentamicin in rabbits. *International journal of biological macromolecules*, **164**;1493-1500.

Altaf M, Ijaz M, Iqbal MK, Rehman A, Avais M, Ghaffar A and Ayyub RM 2019. Molecular Characterization of Methicillin Resistant Staphylococcus aureus (MRSA) and Associated Risk Factors with the Occurrence of Goat Mastitis. *Pakistan Veterinary Journal*, **40** (1); 1-6.

Anwar M, Muhammad F, Akhtar B, ur Rehman S and Saleemi MK 2020. Nephroprotective effects of curcumin loaded chitosan nanoparticles in cypermethrin induced renal toxicity in rabbits. *Environmental Science and Pollution Research*, **27**, 14771-14779.

Arasoğlu T, Derman S, MANSUROĞLU B, UZUNOĞLU D, KOÇYİĞİT BS, GÜMÜŞ B, Acar T and Tuncer B 2017. Preparation, characterization, and enhanced antimicrobial activity: quercetin-loaded PLGA nanoparticles against foodborne pathogens. *Turkish Journal of Biology*, **41**, 127-140.

Aziz S, Abdullah S, Abbas K and Zia MA 2020. Effects of Engineered Zinc Oxide Nanoparticles on Freshwater Fish, Labeo rohita: Characterization of ZnO Nanoparticles, Acute Toxicity and Oxidative Stress. *Pakistan Veterianry Journal*, DOI: 10.29261/pakvetj/2020.030.

Bagad M and Khan ZA 2015. Poly (n-butylcyanoacrylate) nanoparticles for oral delivery of quercetin: preparation, characterization, and pharmacokinetics and biodistribution studies in Wistar rats. *International journal of nanomedicine*, **10**, 3921.

Biasutto L, Marotta E, Garbisa S, Zoratti M and Paradisi C 2010. Determination of quercetin and resveratrol in whole blood—implications for bioavailability studies. *Molecules*, **15**, 6570-6579.

Danhier F, Ansorena E, Silva JM, Coco R, Le Breton A and Préat V 2012. PLGA-based nanoparticles: an overview of biomedical applications. *Journal of controlled release*, **161**, 505-522.

Jaisinghani RN 2017. Antibacterial properties of quercetin. *Microbiology Research*, **8**, doi.org/10.4081/mr.2017.6877.

Kumar VD, Verma PRP and Singh SK 2016. Morphological and in vitro antibacterial efficacy of quercetin loaded nanoparticles against food-borne microorganisms. *LWT-Food Science and Technology*, **66**, 638-650.

Lin X, Rafique A, Fayyaz T, Bashir W, Luqman M, Zahid FM and Zhou K 2020. Appraisal of Cymbopogon citratus (Lemon grass) for Antibacterial Activity of Against Uropathogens. *Pakistan Veterinary Journal* http://dx.doi.org/10.29261/pakvetj/2020.040

Makadia HK and Siegel SJ 2011. Poly lactic-co-glycolic acid (PLGA) as biodegradable controlled drug delivery carrier. *Polymers*, **3**, 1377-1397.

Muhammad F, Nguyen TDT, Raza A, Akhtar B and Aryal S 2017. A review on nanoparticle-based technologies for biodetoxification. *Drug and Chemical Toxicology*, **40**, 489-497.

Mukhopadhyay P and Prajapati A 2015. Quercetin in anti-diabetic research and strategies for improved quercetin bioavailability using polymer-based carriers–a review. *RSC Advances*, **5**, 97547-97562.

Nguyen TDT, Pitchaimani A, Koirala MB, Muhammad F and Aryal S 2016. Engineered biomimetic nanoabsorbent for cellular detoxification of chemotherapeutics. *RSC Advances*, **6**, 33003-33008.

Pimple S, Manjappa AS, Ukawala M and Murthy R 2012. PLGA nanoparticles loaded with etoposide and quercetin dihydrate individually: in vitro cell line study to ensure advantage of combination therapy. *Cancer nanotechnology*, **3**, 25-36.

Platel A, Carpentier R, Becart E, Mordacq G, Betbeder D and Nesslany F 2016. Influence of the surface charge of PLGA nanoparticles on their in vitro genotoxicity, cytotoxicity, ROS production and endocytosis. *Journal of Applied Toxicology*, **36**, 434-444.

Pool H, Quintanar D, Figueroa JdD, Marinho Mano C, Bechara JEH, Godínez LA and Mendoza S 2012. Antioxidant effects of quercetin and catechin encapsulated into PLGA nanoparticles. *Journal of Nanomaterials*, **145380**, 12 pages doi:10.1155/2012/145380.

Roger E, Lagarce F, Garcion E and Benoit J-P 2010. Biopharmaceutical parameters to consider in order to alter the fate of nanocarriers after oral delivery. *Nanomedicine*, **5**, 287-306.

Sun D, Li N, Zhang W, Yang E, Mou Z, Zhao Z, Liu H and Wang W 2016. Quercetin-loaded PLGA nanoparticles: a highly effective antibacterial agent in vitro and anti-infection application in vivo. *Journal of Nanoparticle Research*, **18**, 3, doi.org/10.1007/s11051-015-3310-0.

Tefas LR, TOMUŢĂ I, Achim M and Vlase L 2015. Development and optimization of quercetin-loaded PLGA nanoparticles by experimental design. *Clujul Medical*, **88** (2), 214-23.

Tuo X, Wang S, Cui D, Wang F, Wang H, Liu Y and Wang H 2020. Antibiotic Resistance Profiles and Virulence Markers of Escherichia coli Strains Isolated from Diarrheal Lambs in Gansu and Qinghai, China. *Pakistan Veterinary Journal*, **40** (1), 123-126.

Wang S, Su R, Nie S, Sun M, Zhang J, Wu D and Moustaid-Moussa N 2014. Application of nanotechnology in improving bioavailability and bioactivity of diet-derived phytochemicals. The *Journal of Nutritional Biochemistry*, **25**, 363-376.

Woźnicka E, Kuźniar A, Nowak D, Nykiel E, Kopacz M, Gruszecka J and Golec K 2013. Comparative study on the antibacterial activity of some flavonoids and their sulfonic derivatives. *Acta Poloniae Pharmaceutica*, **70**, 567-571.