RESEARCH ARTICLE

Formulation and *In Vitro - In Vivo* Evaluation of Quercetin Loaded Eudragit S100 Microspheres

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Abstract

Background: Sulfur mustard (SM), well known as the mustard gas, when contacts the skin can cause stern blisters and causes systemic toxicity as well, affects eyes, skin, and respiratory tract. Till now, exact therapy to counteract SM-induced systemic toxicity has not been developed yet. SM-induced toxicity can be reduced by cellular glutathione replenishment. Plant flavonoids, for example, quercetin and gossypin possess antioxidant, radical scavenging activity. Employing this attribute, quercetin-loaded Eudragit microspheres were fabricated to enhance the oral bioavailability of the quercetin. **Materials and Methods:** Drug-loaded microspheres were prepared using emulsion-solvent diffusion method. **Results and Discussion:** Particles so obtained were within the micrometer range (48.25 ± 2.01–100.40 ± 3.01 µm for QM1 to QM10 batches). Fourier transform infrared spectra indicated no drug-polymer interaction whereas differential scanning calorimetry thermograms revealed the absence of crystalline drug in the drug-loaded microsphere formulation. Scanning electron micrographs showed that the fabricated microspheres were spherical in shape. Batch QM3 displayed 73% encapsulation efficiency with highest *in vitro* drug release rate (98%). Bioavailability studies showed that quercetin loaded Eudragit S100 microspheres were able to enhance the oral bioavailability of the drug. As per stability studies, the developed formulation was stable when tested under accelerated stability conditions. **Conclusion:** The fabricated quercetin-loaded Eudragit S100 microspheres were able to enhance the bioavailability of the drug in the serum of the animal model.

Key words: Antioxidant, Eudragit S100, oral bioavailability, quercetin, radical scavenging, sulfur mustard

INTRODUCTION

Sulfur mustard (SM) familiarly known as mustard gas is an alkylating agent that causes severe blisters on contact with the skin.[1] SM has also been used as a chemical warfare agent in many occurrences.[2] SM forms ions in the body and alkylates deoxyribonucleic acid (DNA) leading to DNA strand breaks and cell bereavement.[3] SM-induced toxicity mostly affects eyes, skin, and respiratory tract.[4,5] Much attention has been focused to develop pharmacological strategies to counter the toxic effects of SM. These studies included preventing or reversing the SM alkylated critical cell targets, improve calcium regulation, and protect cell-mediated biochemical disruptions.[6-8] Many drug compounds have exhibited promising prophylactic as well as therapeutic protection *in vitro*, their *in vivo* efficiency is yet to be proven.[9,10] SM toxicity can be lessened by replenishing cellular glutathione. Glutathione, cysteine, and other endogenous thiols can reduce toxic effects of SM *in vitro*.[11] Yet, their *in vivo* efficacy has not been documented till now.

Quercetin (3,3',4',5,7-pentahydroxyflavone) (a plant flavonoid) has been reported to have radical scavenging, antioxidant and anticarcinogenic activity and is helpful in the recovery of n-diethyl nitrosamine-induced carcinogenesis,[12,13] human leukemia cell,[14]

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streptozotocin-induced diabetes,[15] chronic renal failure, and reactive oxygen species induced DNA damage.[16] Quercetin and other flavonoids have been shown to modify eicosanoid biosynthesis (anti-prostanoid and anti-inflammatory responses), protect low-density lipoprotein from oxidation (prevent atherosclerotic plaque formation), prevent platelet aggregation (antithrombic effects), and promote relaxation of cardiovascular smooth muscle (antihypertensive and antiarrhythmic effects). Though having above mentioned benefits, quercetin possesses fewer drawbacks also like that of poor aqueous solubility, poor absorption and low bioavailability (1–5%).[17-20]

Recently, microspheres have drawn great attention due to their excellent efficiency in prolonging the half-life of the drug and improving oral bioavailability of the drug *in vivo* by controlling the release rate of the drug from the microspheres.[21-24] Microspheres can also offer advantages such as limiting fluctuation within the therapeutic range, reducing side effects, decreasing dosing frequency, and improving patient compliance.[25,26]

Eudragit polymers are series of acrylate and methacrylate polymers available in different ionic forms. Various grades of Eudragit are insoluble in aqueous media, but they are permeable, and both have pH-independent release profiles, Eudragit S100 is soluble at physiological pH.

Recently, dosage forms that can precisely control the release rates and target drugs to a specific body site have made an enormous impact in the formulation and development of novel drug delivery systems. Microspheres form an important part of such novel drug delivery systems.[27-29] They have varied applications and are prepared using assorted polymers.[30] Thus, it is proposed to fabricate quercetin loaded Eudragit microsphere to enhance the oral bioavailability of the selected model drug against SM.

MATERIALS AND METHODS

Materials

Quercetin dihydrate was acquired from DRDE, Gwalior, India, Eudragit S100 was purchased from Evonik Industries, Mumbai, India. All other solvents used were of analytical grade and purchased from Merck Limited, Mumbai, India.

Methods

Quercetin loaded Eudragit microspheres were prepared by the method reported by Kawashima *et al*. with slight modification.[31] Different rations of Eudragit S100 and quercetin dihydrate were dissolved in dimethyl sulfoxide [Table 1]. The organic phase (10 ml) was poured into 500 ml of water containing various concentrations of polyvinyl alcohol (hot) under mechanical stirring with four-blade propellers for 2 h at 1600 rpm to evaporate the solvent. After evaporation of the solvent, microspheres were collected by filtration and washed 3 times with double distilled water. The collected microspheres were dried at room temperature for 12 h and were stored in desiccator. 10 formulations were developed accordingly to the conditions listed in Table 1.

Particle size, shape, and surface morphology

The fabricated microspheres particle size and size distribution were measured using zetasizer (Nano series, Malvern Instruments, England). The prepared drug-loaded microspheres were dispersed in distilled water by sonication and vortexed for 30 s, and the resulting homogenized suspension was analyzed for average particle size, polydispersity index, and zeta potential using the zetasizer.

Table 1: Formulation code, particle size, pdi and % encapsulation efficiency of quercetin loaded Eudragit microspheres

Batch code	Drug: polymer ratio (mg) (quercetin: ES100)	Stirring rate (rpm)	Average particle size (mm)±SD	Polydispersity index	% Encapsulation efficiency
QM1	1:1	900	48.25±2.01	0.078±0.014	68.03±1.15
QM2	1:2	900	53.17±1.67	0.083±0.020	70.17±1.37
QM3	1:3	1000	60.89±1.91	0.085±0.023	73.48±1.77
QM4	1:4	1000	66.34±2.31	0.089±0.017	71.51±2.04
QM5	1:5	1000	72.16±1.93	0.090±0.027	68.29±2.71
QM6	1:3	800	81.02±2.41	0.092±0.029	65.06±1.76
QM7	1:3	1100	90.12±2.74	0.095±0.013	64.73±2.13
QM8	1:3	1200	98.51±2.97	0.097±0.019	63.38±2.43
QM9	1:3	1400	99.59±2.99	0.098±0.010	62.29±1.46
QM10	1:3	1500	100.40±3.01	0.099±0.023	61.02±1.02

*Mean±SD, *n*=3. SD: Standard deviation

Qualitative assessment of shape and surface morphology of the developed formulations was observed through scanning electron microscopy (SEM) by sprinkling particles on one side the adhesive stub which was then coated with conductive gold through an autocoater and was examined under SEM, LEO-430, UK.

Drug-excipient interaction and thermogravimetric analysis

Possible chemical interactions between the drug and polymer in the microsphere formulation can be investigated by analyzing the infrared (IR) spectra. For this, samples of the formulations were crushed with potassium bromide, and pellets were formed. The spectra of quercetin, Eudragit, placebo microspheres, and drug-loaded microspheres were recorded in the range of 4000–400 cm^{-1} using Perkin-Elmer, BX-II series, UK. Thermal behavior of the drug and polymer and the behavior of the drug in the fabricated microspheres was determined employing differential scanning calorimetry (DSC). Thermal analysis was performed using DSC-60, Shimadzu, Japan. Samples were placed into aluminum containers and heated in a temperature range of 20–400°C under nitrogen gas flow by applying minimum pressure, an empty aluminum pan was used as a reference.

Percentage encapsulation efficiency

Percentage encapsulation efficiency of the quercetin loaded microspheres was determined by Vortexing 10 mg of microspheres dissolved in 25 ml of dichloromethane for 10 min. Ethyl alcohol was added, and Vortexing was carried for another 5 min followed by centrifugation at a speed of 3000 rpm for 10 min to allow settling of the polymer as a precipitate. The supernatant containing drug was diluted by ethanol and absorbance was measured at 370 nm by ultraviolet (UV) spectrophotometer (Shimadzu 1800, Japan). Drug loading and encapsulation of the microspheres were calculated by the following formula:[32]

$$\% \text{ Entrapment efficiency} = \frac{\text{Practical drug content}}{\text{Theoretical drug content}} \times 100$$

In vitro drug release studies

Quercetin release from the microspheres was determined in 900 ml phosphate buffer saline (pH 7.4) using USP XXIII dissolution apparatus (paddle type). A weighed amount of quercetin-loaded microspheres equivalent to 50 mg of drug was kept in a non-reacting cloth having smaller mesh size than the microspheres and tied with a nylon thread to evade any seepage of the microspheres. A glass bead was placed in the mesh along with the formulation to induce sinking of the microspheres in the dissolution medium which was maintained at 37°C ± 0.5°C. At specific time intervals, 5 ml aliquots were withdrawn, diluted with the same medium and the concentration of quercetin was determined using UV double beam spectrophotometer (Shimadzu, UV-1800 series, Japan) at 370 nm.[33]

Oral bioavailability studies

To assess the oral bioavailability of quercetin, plain drug, and drug-loaded microspheres were subjected to animal studies. 9 female Swiss albino mice weighing 25–30 g were selected, divided into three groups with 3 animals in each group and were housed in propylene cages under standard storage conditions, supplied with unlimited food and water. The animal experiment has approval from the IAEC and CPCSEA with approval number 891/AC/05/CPCSEA. Food and water supply was withheld before 2 h of the commencement of the experiments. Quercetin (plain drug) was dissolved in polyethylene glycol-400 to minimize the lethal action of the SM. Quercetin loaded microspheres were administered through per oral route. The experimental design and dosing of the formulations are divided as given below:

Group I: Received a single dose of SM (19.1 mg/kg) through percutaneous route.

Group II: Received quercetin (plain drug: 200 mg/kg) through peroral route.

Group III: Received quercetin loaded microspheres (at a drug dose of 200 mg/kg) through peroral route.

The blood samples (0.4–0.5 ml) were collected on making an incision at the tip of the tail at the intervals up to 480 min into Eppendorf tubes containing heparin to prevent coagulation. The collected blood samples were then coagulated, plasma was separated by centrifugation (RM12C DX Microcentrifuge, Remi Electronics Ltd., India) at 10000 rpm for 15 min. Serum samples were analyzed spectrophotometrically (Shimadzu-1800, Japan) to assess the drug concentration in serum and plasma concentration-time response curves were plotted.[34]

Stability studies

To assess the stability of drug as well as formulation, stability studies were conducted following the WHO guidelines.[35] The fabricated batches were stored at 25 ± 2°C/60 ± 5% relative humidity (RH) and under accelerated storage conditions (40 ± 2°C/75 ± 5% RH) for a period of 6 months and all the sample batches were analyzed for any deviation in particle size, % entrapment efficiency and *in vitro* drug release.

RESULTS AND DISCUSSION

Formulation development, particle size, and surface morphology analysis

An increase in the polymer concentration leads to the increase in the particle size which is evident in Table 1. The particle size

increases due to the increase in viscosity leading to increase in the emulsion droplet size ultimately leading to increase in the particle size of the microsphere.[36] This increase in size can also be attributed to the diminished shearing efficiency at the high concentration of the polymer. From the size distribution pattern of the fabricated microspheres, it can be concluded that Eudragit S100 concentration in higher amounts promoted the development of a homogeneous formulation.

The pdi was in the range of 0.078 ± 0.014–0.099 ± 0.023 and suggested a monodisperse size distribution. Stirring speed at 1000 rpm resulted in optimum particle sized microspheres (QM3, 60.89 ± 1.91 µm) with high entrapment efficiency of 73.48 ± 1.77% but at further higher speeds, snags such as drug leaching (low entrapment of 61.02 ± 1.02% for QM8) and particle agglomeration occurred resulted in increased particle size.

From scanning electron photomicrographs, it can be assessed that the drug-loaded microspheres (QM3) possesses a discrete, rough, spherical surface due to the crystal of the drug attached on the surface of the microspheres leading to the rapid release of the drug [Figure 1].

Drug-excipient interaction studies

Fourier transform IR (FTIR) analysis

From the FTIR spectra [Figure 2], one can confirm the chemical stability of the drug in a formulation, here, the IR spectra of quercetin exhibited a broadened phenolic −OH band at 3407/cm, −CO stretching at 1670/cm, an aromatic bending and stretching about at 1100 and 1600/cm, and a −OH phenolic bending about at 1200 and 1400/cm.

IR spectra of Eudragit S-100 showed a characteristic peak at 1731/cm. A small peak of quercetin −CO stretching at 1670/cm can be observed around the characteristic peak of Eudragit around 1730/cm thus concluding with the FTIR analysis, it can be inferred that the main characteristic peaks of quercetin were extant in the entire microspheres which established its presence without any interaction with the excipients.

DSC analysis

The physical state of the drug in polymer matrix ascertains it release characteristics; this feature can be adjudged with DSC analysis about the nature and interaction of the encapsulated drug in the matrix of the polymer. Quercetin as a pure drug showed a sharp endothermic peak at 318°C and a broad endothermic peak for dehydration from 101 to 108°C. Eudragit S-100 exhibited two endothermic peaks at 81.79°C and 230°C whereas the physical mixture of the drug and polymer showed a reduced peak intensity of quercetin in the mix from 318°C to 310°C. Quercetin loaded Eudragit S-100 microspheres showed a characteristic melting peak for Eudragit at 93°C, possibly due to the dilution effect of the amorphous polymer, the peak for the drug was not protuberant due to the dispersion of the drug in the microspheres indicative of the absence of any crystalline form of the drug [Figure 3].

Percentage encapsulation efficiency

Percentage encapsulation efficiency of the quercetin loaded microspheres is summarized in Table 1 according to which it can be summed that the formulation QM3 (1:3 ratio) showed 73% entrapment of the drug which was the best among all the prepared batches. The entrapment efficiency recorded a decline after QM3 batch, reason for which can be the saturation effect of the polymer and different stirring rate due to which drug might have leached out consequently lowering the encapsulation efficiency of the fabricated microspheres.[37]

In vitro release

The drug release from the quercetin loaded microspheres is presented in Figure 4, according to which the drug release from the microspheres recorded a retarding rate when the polymer concentration is increased, which might be due to the formation of rigid polymer matrix at higher polymer

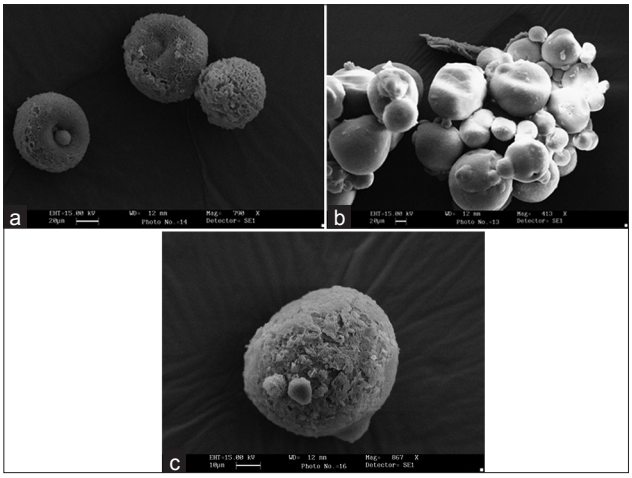

Figure 1: (a-c) Scanning electron microscopy images of quercetin-loaded Eudragit microspheres (QM3)

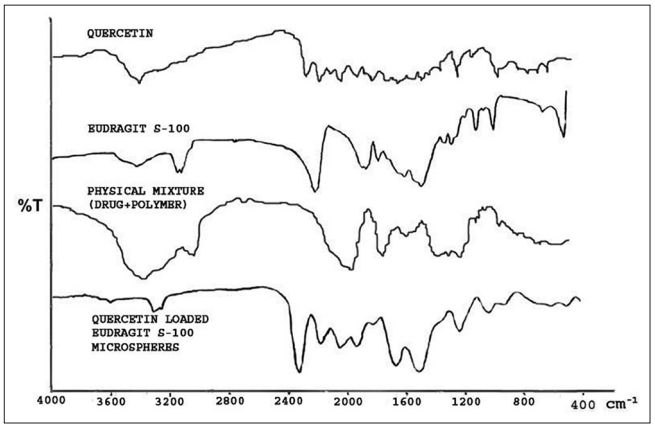

Figure 2: Fourier transform infrared spectrum of quercetin, Eudragit S100, physical mixture of drug with polymer and drug-loaded microspheres

concentration which leads to low polymer dissolution thus causing a slower drug release from the microspheres. QM3 exhibited a better drug release profile (98.76 ± 1.04%) at a drug-polymer ratio of 1:3 and a stirring rate of 1000 rpm.

Oral bioavailability studies

The oral bioavailability of quercetin in QM3 was assessed and was compared with the bioavailability of the pure drug. C_{max} of quercetin in the microspheres was found to be 0.21 µg/ml, and for control formulation, it was 0.055 µg/ml. T_{max} for test and control formulation was 4 h. It is evident from Figure 5 that the oral absorption of quercetin increases when loaded with the microspheres. Area under the first moment curve and area under the zero moment curve was 3.9 µg.h/L and 0.050 µg.h/L, respectively, with a mean residence time of 4.5 h [Table 2].

Stability studies

A slight increase in particle size (from 60.89 ± 1.91 µm to 69.71 ± 1.43 µm) was observed in the selected QM3 batch when tested for stability under accelerated storage conditions mainly due to the aggregation of particles. A decline in the % entrapment efficiency was evident in the selected batch (73– 61%) due to the drug leaching out the microspheres. *In vitro* drug release studies [Figure 6] showed that there were no significant changes in the drug release from the drug-loaded microspheres (QM3 initial batch showed a drug release of 98.76 ± 1.04%, QM3 after 3 months of storage showed a drug release of 98.45 ± 1.08%, and QM3 after 6 months of storage showed a drug release of 98.23 ± 2.01%).

Thus, from the stability studies, the fabricated microspheres can be rendered stable under the testing conditions.

CONCLUSION

From the study discussed above, it can be concluded that the fabricated quercetin microspheres can be used as a useful source to counteract SM-induced toxicity. Eudragit based microspheres showed an excellent drug release profile releasing drug up to 99% with an optimum particle size of 60.89 ± 1.91 µm for the selected batch QM3. There was no drug-polymer interaction as revealed from the FTIR studies. DSC analysis claims that the drug was completely assimilated in the polymer in the formulation and no crystalline form was present. Drug entrapment

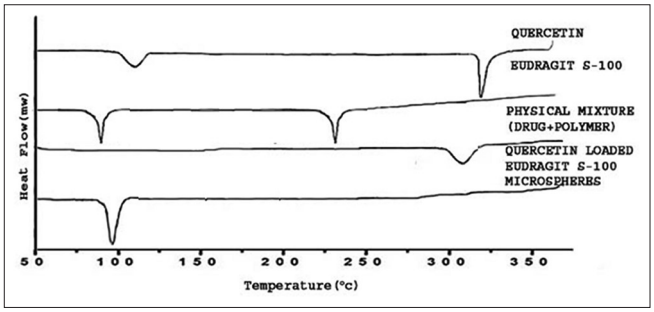

Figure 3: Differential scanning calorimetry thermogram of quercetin, Eudragit S100, physical mixture of drug with polymer and drug-loaded microspheres

Table 2: Pharmacokinetics parameters of QM3 and control

Parameter	Value
C_{max} (test)	0.21 µg/ml
C_{max} (control)	0.055 µg/ml
T_{max} (test)	4 h
T_{max} (control)	4 h
AUMC	3.9 µg.h/L
AUC	0.050 µg.h/L
MRT	4.5 h

AUMC: Area under the moment curve, AUC: Area under the curve, MRT: Mean residence time

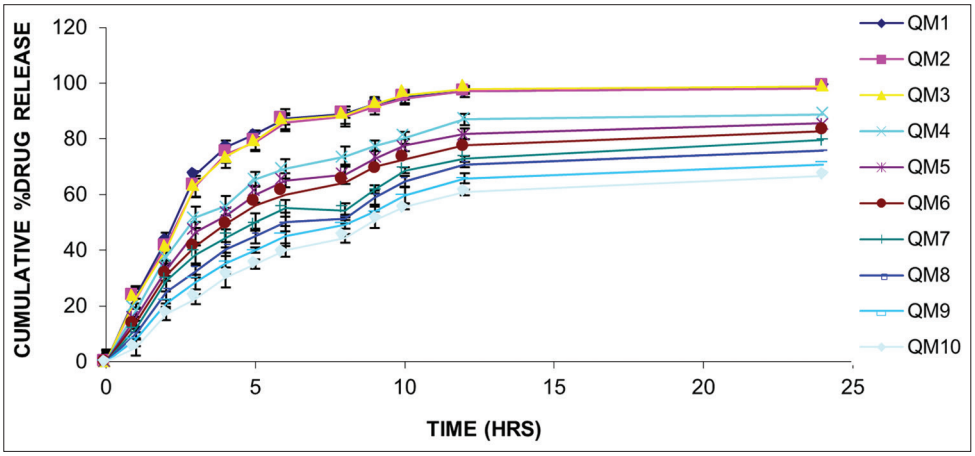

Figure 4: Cumulative % drug release profile of quercetin-loaded Eudragit microspheres (all batches)

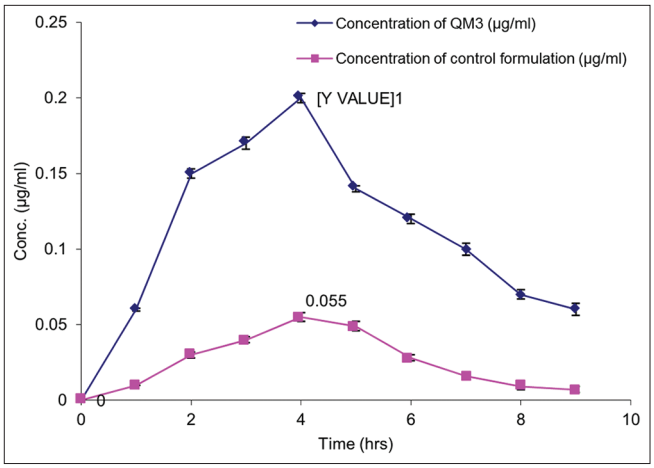

Figure 5: *In vivo* bioavailability studies of formulation QM3 and control

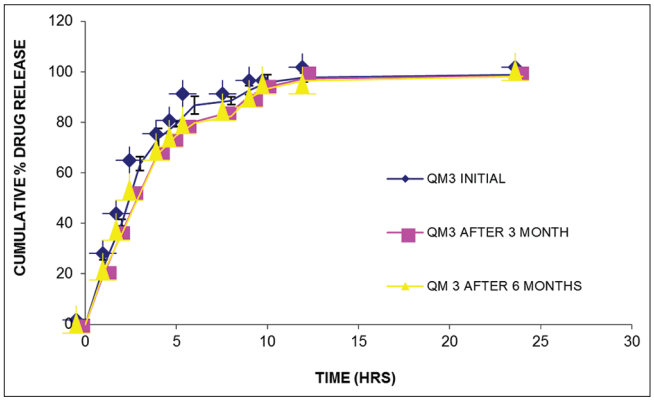

Figure 6: Comparative *in vitro* release profile of QM3 before and after stability testing

was 73% for a formulation which has 1:3 drug-polymer ratio and was further selected for oral bioavailability studies. Eudragit S100 based microspheres enhanced the oral bioavailability of the drug which is confirmed by the results obtained. Stability studies rendered quercetin loaded Eudragit microspheres stable when evaluated for particle size, drug entrapment and *in vitro* drug release. Future perspective of the study indicates to assess the antioxidant, and protective value of quercetin-loaded Eudragit microspheres against SM-induced systemic toxicity.

REFERENCES

1. Momeni AZ, Enshaeih S, Meghdadi M, Amindjavaheri M. Skin manifestations of mustard gas. A clinical study of 535 patients exposed to mustard gas. Arch Dermatol 1992;128:775-80.
2. Smith WJ, Dunn MA. Medical defence against blistering chemical warfare agents. Arch Dermatol 1991;127:1207-13.
3. Fox M, Scott D. The genetic toxicology of nitrogen and sulphur mustard. Mutat Res 1980;75:131-68.
4. Somani SM, Babu SR. Toxicodynamics of sulfur mustard. Int J Clin Pharmacol Ther Toxicol 1989;27:419-35.
5. Wormser U. Toxicology of mustard gas. Trends Pharmacol Sci 1991;12:164-7.
6. Papirmeister B, Feister AJ, Robinson SL, Ford RO. Medical Defense against Mustard Gas: Toxic Mechanisms and Pharmacological Implications. Boca, Raton: CRC Press; 1991. p. 359.
7. Casillas RP, Kiser RC, Truxall JA, Singer AW, Shumaker SS, Niamuth NA, *et al.* Therapeutic approaches to dermatotoxicity by sulfur mustard: I, modulation of sulfur mustard induced cutaneous injury in mouse ear vesicant model. J Appl Toxicol 2000;20:S145-51.
8. Sawyer TW, Lundy PM, Weiss MT. Protective effect of an inhibitor of nitric oxide synthase on sulfur mustard toxicity *in vitro*. Toxicol Appl Pharmacol 1996;141:138-44.
9. Sawyer TW. Characterization of protective effect of the protective effect of L-nitroarginine methyl ester (L-NAME) against the toxicity of sulphur mustard *in vitro*. Toxicology 1998;131:21-32.
10. Sawyer TW, Risk D. Effects of selected arginine analogues on sulphur mustard toxicity in human and hairless guinea pig skin keratinocytes. Toxicol Appl Pharmacol 2000;163:75-85.
11. Wilde PE, Upshall DG. Cysteine esters protect cultured rodent lung slices from sulphur mustard. Hum Exp Toxicol 1994;13:743-8.
12. Middleton E, Kandaswami C. The Impact of Plant Flavonoids on Mammalian Biology: Implications for Immunity, Inflammation and Cancer. London: Chapman & Hall; 1993.
13. Sánchez-Pérez Y, Carrasco-Legleu C, García-Cuellar C, Pérez-Carreón J, Hernández-García S, Salcido-Neyoy M, *et al.* Oxidative stress in carcinogenesis. Correlation between lipid peroxidation and induction of preneoplastic lesions in rat hepatocarcinogenesis. Cancer Lett 2005;217:25-32.
14. Chen J, Kang J, Da W, Ou Y. Combination with water-soluble antioxidants increases the anticancer activity of Quercetin in human leukemia cells. Pharmazie 2004;59:859-63.
15. Anjaneyulu M, Chopra K. Quercetin attenuates thermal hyperalgesia and cold allodynia in STZ induced diabetic rates. Indian J Exp Biol 2004;42:766-9.
16. Cemeli E, Schmid TE, Anderson D. Modulations of flavonoids of DNA damages induced by estrogen like compounds. Environ Mol Mutagen 2004;44:420-6.
17. Reinboth M, Wolffram S, Abraham G, Ungemach FR, Cermak R. Oral bioavailability of Quercetin from different Quercetin glycosides in dogs. Br J Nutr 2010;104:198-203.
18. Graefe EU, Wittig J, Drewelow B, Riethling AK, Mueller S, Wukasch I, *et al*. Relative systemic availability of the flavonoids Quercetin and rutin in humans. Arch Pharm Pharm Med Chem 1999;332:20.

19. Shaji J, Iyer S. Novel double loaded Quercetin liposomes: Evidence of superior therapeutic potency against CCl4 induced hepatotoxicity – A comparative study. Asian J Pharm Clin Res 2012;5:104-8.
20. Kumari A, Yadav SK, Pakade YB, Singh B, Yadav SC. Development of biodegradable nanoparticles for delivery of Quercetin. Colloids Surf B Biointerfaces 2010;80:184-92.
21. Wang LY, Gu YH, Zhou QZ, Ma GH, Wan YH, Su ZG, *et al.* Preparation and characterization of uniform-sized chitosan microspheres containing insulin by membrane emulsification and a two-step solidification process. Colloids Surf B Biointerfaces 2006;50:126-35.
22. Uddin MN, Patel NJ, Bhowmik T, D'Souza B, Akalkotkar A, Etzlar F, *et al.* Enhanced bioavailability of orally administered antisense oligonucleotide to nuclear factor kappa B mRNA after microencapsulation with albumin. J Drug Target 2013;21:450-7.
23. Hu LD, Xing QB, Shang C, Liu W, Liu C, Luo ZL, *et al.* Preparation of rosiglitazone maleate sustained-release floating microspheres for improved bioavailability. Pharmazie 2010;65:477-80.
24. Blanco MD, Bernardo MV, Sastre RL, Olmo R, Muñiz E, Teijón JM. Preparation of bupivacaine-loaded poly(ε-caprolactone) microspheres by spray drying: Drug release studies and biocompatibility. Eur J Pharm Biopharm 2003;55:229-36.
25. Davis SS, Illum L. Polymeric microspheres as drug carriers. BioMat 1988;9:111-5.
26. Ritschel WA. Biopharmaceutic and pharmacokinetic aspects in the design of controlled release peroral drug delivery systems. Drug Dev Ind Pharm 1989;15:1073-103.
27. Gohel MC, Amin AF. Formulation optimization of controlled release diclofenac sodium microspheres using factorial design. J Control Release 1998;51:115-22.
28. Woo BH, Jiang G, Jo YW, DeLuca PP. Preparation and characterization of a composite PLGA and poly (acryloyl hydroxymethyl starch) microsphere system for protein delivery. Pharm Res 2001;18:1600-6.
29. Capan Y, Jiang G, Giovagnoli S, Na KH, DeLuca PP. Preparation and characterization of poly(D,L-lactide-co-glycolide) microspheres for controlled release of human growth hormone. AAPS PharmSciTech 2003;4:E28.
30. Vasir JK, Tambwekar K, Garg S. Bioadhesive microspheres as a controlled drug delivery system. Int J Pharm 2003;255:13-32.
31. Kawashima T, Niwa T, Takeuchi H, Itoh Y. Hollow microspheres for use as a floating controlled drug delivery system in the stomach. J Pharm Sci 1992;81:135-40.
32. Paulo C, Jose MS. Modeling and comparison of dissolution profiles. Eur J Pharm Sci 2001;13:123-33.
33. Mundargi RC, Srirangarajan S, Agnihotri SA, Patil SA, Ravindra S, Setty SB, *et al*. Development and evaluation of novel biodegradable microspheres based on poly(d,l-lactide-co-glycolide) and poly(epsilon-caprolactone) for controlled delivery of doxycycline in the treatment of human periodontal pocket: *In vitro* and *in vivo* studies. J Control Rel 2007;119:59-68.
34. Vijayaraghavan R, Gautam A, Sharma M, Satish HT, Pant SC, Ganesan K, *et al.* Comparative evaluation of some flavonoids and tocopherol acetate against the systemic toxicity induced by sulphur mustard. Indian J Pharmacol 2008;40:114-20.
35. Fell J, Digenis CG. Imaging and behavior of solid oral dosage forms *in-vivo*. Int J Pharm 1984;22:1-15.
36. Chawla A, Sharma P, Pawar P. Eudragit S-100 coated sodium alginate microspheres of naproxen sodium: Formulation, optimization and *in vitro* evaluation. Acta Pharm 2012;62:529-45.
37. Vaidya A, Jain A, Khare P, Agrawal RK, Jain SK. Metronidazole loaded pectin microspheres for colon targeting. J Pharm Sci 2009;98:4229-36.

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