AMERICAN JOURNAL OF PHARMTECH RESEARCH
Journal home page: http://www.ajptr.com/

Formulation and Assessment of Mucoadhesive Microspheres of Quercetin Dihydrate Against Mustard Agent Poisoning

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ABSTRACT

In an endeavor to boost the prophylactic action of Quercetin Dihydrate against mustard agent poisoning, mucoadhesive microspheres, were formulated. The microspheres enclosed Quercetin Dihydrate, an adhesive polymer (ethyl cellulose) and a colon specific polymer (Eutragit S100). Microspheres were devised by an oil/water emulsification solvent evaporation method. Two variables polymer concentration and plasticizer concentration for formulation were used. The mean particle sizes of the prepared microspheres were found significantly increasing with polymer concentration and decreased with increasing plasticizer concentration. The drug entrapment effectiveness increased with increasing plasticizer concentration. The Percentage mucoadhesion increases with increasing concentration of ethyl cellulose but not much affected by concentration of eudragit. Drug entrapment efficiency was found in range of 73.35±2.7 (batch R1) to 80.61±1.54 (batch F5). The entire formulations had excellent flow property. The best fit release kinetic model was found to be Higuchi for all formulations, which indicated release from matrix type formulation. The In vivo studies on male wistar rats were conducted and plasma concentration time method was employed to study the influence of quercetin by formulating it as microspheres. By Statistical Analysis through sigma plot, the semi log plot of pure drug (Quercetin) and its microspheres shows linearity which indicates that they follow linear kinetics. Formulation of microspheres produced a sustained effect on its absorption and availability and several parameters are there which concluded that the microspheres of quercetin are better choice for mustard toxicity as compared to pure drug.

Keywords: Quercetindihydrate, mustard agent poisoning, mucoadhesive microspheres, polymers concentration, plasticizer concentration.

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Received 04 December 2015, Accepted 27 January 2016

Please cite this article as: Jat RC *et al*., Formulation and Assessment of Mucoadhesive Microspheres of Quercetin Dihydrate Against Mustard Agent Poisoning. American Journal of PharmTech Research 2016.

INTRODUCTION

The organophosphorus nerve agents and the blistering agents continue to be threats not only as chemical warfare agents, but also from the terrorist organizations. Though the Chemical Weapons Convention is signed and ratified by several countries and the stockpiled chemical warfare agents are being destroyed, still the threat persists from the use of chemical weapons.

The mechanism of action of the nerve agents is clearly understood and effective and accepted treatment protocols are known[1] In spite of research over several decades, no satisfactory prophylactic or treatment regimen has evolved for sulphur mustard (SM), a well known blistering agent. So the search for a better antidote is being pursued the world over.

The SM, commonly known as mustard gas, is chemically bis (2-chloroethyl) sulphide and an alkylating agent that causes serious blisters upon contact with human skin. SM has been used as a chemical warfare agent in many instances.[2-5] SM forms sulphonium ion in the body and alkylates DNA leading to DNA strand breaks and cell death.[6] Due to the high electrophilic property of the sulphonium ion, SM binds to a variety of cellular macromolecules.[7] Eyes, skin and the respiratory tract are the principal target organs of SM toxicity.[6,8]

Antidotes to SM can act by four different mechanisms

(a) Prevention of SM from entering the system (personal decontamination at the site of contact),

(b) Prevention of SM from alkylating critical target molecules mainly DNA,

(c) Retrieval of SM alkylated DNA,

(d) Prevention and reversal of the cascade of secondary biochemical reactions of alkylation.[6-9]

The most effective way of minimizing SM toxicity is by decontamination either by physical adsorption or by chemical decontamination.[17]

One of the important mechanisms of action of SM cytotoxicity is based on the depletion of reduced glutathione (GSH), and subsequent lipid peroxidation and free radical generation.[7,18]

Flavonoids are reported to exhibit a wide variety of biological effects, including antioxidant and free radical scavenging activities. Quercetin and other flavonoids have been shown to modify eicosanoid biosynthesis (antiprostanoid 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, antiarrhythmic effects). In addition, flavonoids have been shown to have antiviral and carcinostatic properties.[19]

The study was aimed to formulate of mucoadhessive quercetin microspheres and evaluate their protective effect against Mustard Agent.

The objective of the research was to develop microspheres containing Quercetin and to study the bioavailability of the microspheres with a view to achieve a controlled drug release with improved bioavailability as compared to pure drug.

MATERIALS AND METHOD

Materials:

Quercetin hydrate was procured from DRDEO,Gwl. (M.P). And all other ingredients were of analytical grade.

Preformulation studies

Preformulation in the broadest sense encompasses all the activities and studies that are required to convert pharmacological substances into a suitable dosage form. It can be defined as an investigation of the physical and chemical properties of a drug substance alone and also when combined with the excipient.

Identification of drug

Quercetin hydrate was identified by several methods like Infrared spectroscopy and Ultraviolet spectroscopy. The drug sample of Quercetin was issued by DRDEO, Gwalior.

Organoleptic Characteristic:

The drug and polymer was visually identified on the basis of organoleptic characteristics.

Melting Point:

The melting point of the drug was determined by capillary fusion method. A capillary was sealed at one end filled with a small amount of drug and the capillary was kept inverted i.e. sealed end downwards into the melting point apparatus. The temperature at which the drug melted was noted down using the thermometer provided.[20]

Solubility Studies

Solubility is the property of solid, liquid or gaseous chemical substances called solute to dissolve in a solid, liquid, or gaseous solvent to form a homogeneous solution of the solute in the solvent. The solubility of a substance fundamentally depends on the used solvent as well as on temperature and pressure. The extent of the solubility of a substance in a specific solvent is measured as the saturation concentration where adding more solute does not increase the concentration of the solution.[21]

Solubility is also one method for purity determination of drug. This study was performed according to IF where solubility study of drug was performed in different solvents e.g. alcohol, methanol, chloroform, water etc. A saturated solution of drug was made in different solvents system by the following method: excess solute was added in a solvent in a volumetric flask and placed in a mechanical shaker for 72 hrs, And on magnetic stirrer for 36 hrs. Samples were withdrawn every 12 hrs until equilibrium was reached. After that, the mixture was filtered, stored at 25 0C for 4 hours in an oven to crystallize out the excess of dissolved solute, leaving saturated solution. The solution was then filtered and then concentration of drug was measured by UV spectrophotometer at 327 nm wavelengths. According to reference of merk index quercetindihydrate soluble in glacial acetic acid, aqueous alkali and ethyl alcohol. 1gm of quercetin hydrochloride dissolves in 290ml of absolute alcohol.

Partition coefficient

The partition coefficient is defined as the ratio of unionized drug distributed between the organic and aqueous phase at equilibrium. For a drug delivery system, Lipophilic/ Hydrophilic balance has been shown to be contributing factors for rate and extent of drug absorption. Partition coefficient provides a means of characterizing, Lipophilic / Hydrophilic nature of drug. The measurement of drug lipophilicity and indication of its ability to cross the lipoidal cell membrane is the oil/water partition coefficient in system such as octanol/water and octanol / buffer.

Partition coefficient of quercetindihydrate was determined by using shake flask method. This relies on the equilibrium distribution of a drug between an oil and aqueous phase. In this study 10 mg of drug was taken in a 60 ml vial and then 20 ml of phosphate buffer pH 7.4 was added to it and shaked it, then 20 ml of n-octanol was added. Octanol layer was less dense than water, so the n-octanol layer was on the top of the water. The system was then shaked for 24 hrs and then it was left to reach equilibrium for 24 hrs in a separating funnel. The two phases were then separated. Then the concentration of drug was measured in each phase of UV spectroscopy at 327 nm. The partition coefficient was calculated by the following equation.

$$Po/w = C\ organic/\ C\ aqueous$$

Spectrophotometric Determination of Quercetin Dihydrate

Ultraviolet Absorption Maxima of Quercetindihydrate 10 mg drug was dissolved in 25 ml absolute alcohol and volume made up to 50 ml with phosphate buffer solution pH 7.4. 0.2 ml of drug solution was taken into a 10 ml volumetric flask and made up the volume to 10 ml with phosphate buffer solution. The absorption maxima were determined to be at 327nm.

Infrared spectrum

The pellets of KBr and drug were prepared and examined under spectrum RX1, Perkin Elmer, Fu R system, UK. The drug sample peaks are similar to reference standard.

Preparation of Calibration Curve Preparation of phosphate buffer pH 7.4 50ml of ethanol was placed in a 100 ml volumetric flask and added 50 ml distilled water to make the final volume. Preparation of Calibration Curve of Quercetindihydrate 10 mg of Quercetin was weighed accurately and dissolved in 25 ml of absolute alcohol in a volumetric flask and volume was made up to 50 ml with the phosphate buffer solution pH 7.4. 200 µg/ml stock solutions were prepared. 2.5 ml of this solution was diluted to 25 ml with phosphate buffer solution pH 7.4 to obtain a sub-stock solution of 20µg/ml. From this sub stock solution, aliquots of 0.5ml, 1ml, 1.5 ml, 2 ml, 2.5ml, 3.0 ml, 3.5 ml, 4 ml were taken into 10 ml volumetric flask and volume was made up to 10 ml with phosphate buffer solution pH 7.4. The absorbance of these solutions was measured at 327 nm against a blank phosphate buffer solution pH 7.4 by spectrophotometrically using shimadzu UV-1700E spectrophotometer. The calibration curve was plotted between concentration and absorbance.

Interaction between Drug and Polymer[22, 23]

There is always possibility of drug excipient interaction in any formulation due to their intimate contact. The drug excipient interaction studies were carried out employing IR Spectroscopic technique. The sample (pure drug and Drug+excipients) was dispersed in KBr and compressed into pellets. IR spectra of drug with and without polymers (Ethyl cellulose and EudragitS 100) were obtained.The pellets were placed in the light path and the spectrum was recorded in the wavelength region of 4000- 400cm-1.

METHOD OF FORMULATION OF MICROSPHERES

Microspheres were prepared by an oil/water emulsification solvent evaporation method as prepared by Kawashima et al. and the composition along with formulation code is as given in Table. 3. We used two formulation variables like polymers concentration (Table 3) and plasticizer concentration (Table 4). In this method the polymer was dissolved in methylene chloride. Then quercetindihydrate was suspended by ultrasonication in the polymer solution. This suspension was poured into 1% w/w PVA solution and an oil/water emulsion was formed by extensive stirring with a three blade propeller at 500 rpm at room temperature. After evaporation of solvent, the system poured into 0.1% w/w PVA solution, while stirring was maintained. After decantation, the microspheres were filtered (whatmann filter paper), washed extensively with distilled water and lyophilized overnight. After that air dry was performed and complete dried in hot air oven for 1-2 hr.

Table- 1 Formulation at 500 rpm and different ratio of polymer

Batch No.	Drug Polymer	Dichloromethane	Ethyl Cellulose	Eudragit S 100
F1	1.00 gm	500 mg	500 mg	10 ml
F2	1.00 gm	1.0 gm	1.0 gm	10 ml
F3	1.00 gm	1.5 gm	500 mg	10 ml
F4	1.00 gm	2.0 gm	1.0 gm	10 ml
F5	1.00 gm	500 mg	1.0 gm	10 ml
F6	1.00 gm	500 mg	1.5 gm	10 ml
F7	1.00 gm	1.0 gm	2.0 gm	10 ml

Table-2 Effect of Different Plasticizer Concentration on F7

Batch No.	Drug: polymer	Plasticizer % v/v	
		DBT	DET
P_1	1:3	-	-
P_2	1:3	-	10
P_3	1:3	-	20
P_4	1:3	10	-
P_5	1:3	20	-

CHARACTERIZATION OF MICROSPHERES

The prepared microspheres were evaluated for their physiochemical characteristics.

Micromeritic studies of microspheres

The microspheres were characterized by their micromeritic properties, such as % yield, bulk density, tapped density, % compressibility, particle size determination, angle of repose, carr‘s index etc.

Particle Size Analysis

Scanning electron microscopy: Scanning electron microscopy (LEO, 430 surface controlled digital SEM) was performed to characterize the surface of formed microspheres. A small amount of microspheres were spread on glass stub. Gold palladium coating on the prepared stub was carried out by using sputter coater. Afterwards, the stub containing the sample was placed in the electron microscope. The scanning electron photomicrograph was taken at the acceleration voltage of 20 kV, chamber pressure of 0.6 mm Hg.

Optical microscopy:

The size of microspheres was determined using an optical microscope magnification 10X (Magnus MLX-DX) fitted with an ocular micrometer and stage micrometer. The mean particle size was determined by measuring 200-300 particles.

% Yield of microspheres

The prepared microspheres were collected and weighed. The measured weight was divided by the total amount of all non-volatile components which were used for the preparation of microspheres multiply by 100 gives the % yield of microspheres.

The yield of microspheres was calculated by the following formula

%Yield = Actual weight of product obtained/Total weight of recipient and drug X 100

Tapped density

The prepared microspheres were weighed, collected, and poured into a 5 ml of graduated cylinder. This system was tapped 100 times from 2.5 cm height and then measured the volume of filled microspheres.

Tapped density was calculated by using the following formula:

Tapped Density = Mass of microspheres/Volume of microspheres after tapping

Bulk Density = Mass of microspheres/ Bulk Volume of microspheres

% **Compressibility index**

The prepared microspheres were weighed, collected, and poured in to a 5 ml of graduated cylinder. This system was tapped 100 times and then measured the volume of filled microspheres. It is the ratio of the volume before tapping which was filled in the graduated cylinder and after tapped volume.

% Compressibility index = 1-V/V0 X 100

Where V and Vo are the volume of the samples after tapping and before tapping.

Angle of repose

The angle of repose of different formulation was measured according to fixed funnel standing method (n=3)

Q = tan-1 h/r

Where Q is angle of repose, r is radius and h is height.

Carr's index and Hausner's ratio

Compressibility index (CI) or Carr's index value of micro particles was computed according to the fallowing equation.

Carr's index = Tapped density – Bulk density / Tapped density X 100

Hausner's ratio= Tapped density/ Bulk density

Drug Entrapment Efficiency

The 10 mg of prepared microspheres were crushed in a glass mortar and the powdered microspheres were suspended in a 10 ml of methanol after 24 hours, the solution was filtered and

the filtrate was analyzed after suitable dilutions using UV spectrophotometer (ShimadzuUV-1700E series) at λmax 268 nm.

The amount of drug entrapped in the microspheres was calculated by the following formula:

Drug entrapment efficiency = Practical drug content/ Theoretical drug content X 100

Swelling Index

A known weight (30mg) of various formulations were placed in 5ml measuring cylinder with phosphate buffer (pH 6.8) and allowed to swell for the 16 hrs at 37±0.8 C. After a selected time intervals, the microspheres were withdrawn, blotted to remove extra water and weighed. If wet weight of microspheres is Wg and dry weight of microspheres is Wo than the swelling index (SI) was calculated by using the following formula.

$$SI = Wg – Wo/ Wg \times 100$$

Mucoadhesion Study

The in vitro mucoadhesive test was carried out using small intestine from chicken. The small intestinal tissue was excised and flushed with saline. Five centimeter segment of jejunum were everted using a glass rod. Ligature was placed at both ends of the segment. 100 microspheres were scattered uniformly on the everted sac from the position of 2 cm above. Then the sac was suspended in a 10ml tube containing 8 ml of saline by the wire, to immerse in the saline completely. The sac were incubated at 370C and agitated horizontally. The sac were taken out of the medium after immersion for 0.5, 1, 1.5, 2, 2.5 and 3 hrs, immediately repositioned as before in a similar tube containing 8ml of fresh saline and unbound microspheres were counted. The adhering percent was presented by the following equation.

Mucoadhesion = No. of microspheres adhered / No. of microspheres applied × 100

In-vitro Release

The drug release study was performed using USP XXIV dissolution apparatus at 37°C±0.5°C at 100 rpm using 900 ml of simulated gastric fluid, phosphate buffer pH 6.8, phosphate buffer pH7. Microspheres 100mg were tied in non- reacting muslin cloth and suspended with nylon thread in the dissolution media, 5 ml of sample solution was withdrawn at predetermined time intervals, diluted suitably and analyzed by UV spectrophotometer (Shimadzu UV- 1700E). An equal amount of fresh dissolution medium was replaced immediately after each withdrawal of test sample to maintain the sink condition.

Kinetics of Drug Release

The zero-order rate (Equation 1) describes systems where drug release is independent of its concentration and this is applicable to the dosage forms like transdermal system, coated forms,

osmotic system, as well as matrix tablets with low soluble drugs. The first-order equation (Equation 2) describes systems in which the release is dependent on its concentration (generally seen for water-soluble drugs in porous matrix). _Higuchi developed an Equation 3 for the release of a drug from a homogeneous polymer matrix type deliver system that indicates the amount of drug release is proportional to the square root of time. If the release of drug from the matrix, when plotted against square root of time, shows a straight line, it indicates that the release pattern is obeying Higuchi‘s kinetics.

Zero order release equation

Qt= k0 t -(1)

First order release equation

In Qt = In Qo - k1 t -(2)

Higuchi‘s square root of time equation

Qt = kH t1/2 -(3)

Korsmeyer and Peppas equation

F = (Mt/M) = Km tn-(4)

Hixon—Crowell equation

Wo1/3–Wt1/3=Kst -(5)

Where Qt is the amount of drug released at time t; Qo is the initial amount of the drug in the formulation; k0, k1,kH, and km are release rate constants for zero-order, first-order, Higuchi and korsmeyer model rate constant of equations respectively.

Where, Wo is the initial of drug in the microsphere, Wt is the remaining amount of drug in microspheres at time t and Ks is a constant incorporating the surface-volume relation.

Table 3: Interpretation of diffusion drug release from microspheres

Release Exponent (n)	Drug transport mechanism	Rate at function of time
≤0.5	Fickian diffusion	$t^{0.5}$
0.5<n<1.0	Non Fickian diffusion/ Anomolous transport	t^{n-1}
1.0	Case –II transport	Zero order release
Higher than 1.0	Sure case-II transport	t^{n-1}

IN-VIVO STUDY

Animals

Randomly wistar rats male / female (250-300g. B.W) from central animal facility of Shri Ram College of Pharmacy, Banmore, M.P., India [891/AC/05/CPCSEA] and was maintained in polypropylene cages on dust free rice husk as the bedding material and in condition of controlled temperature (22±2oC) and acclimatized to 12/12 h light/dark cycle. Free access to food and water

was allowed until 2h before the experiment. The care and maintenance of animals were as per the approved guidelines of the ―Committee for the purpose of control and supervision of experiments on animals (CPCSEA). All animal experiments were carried out with the approval of institutional animal‘s ethical committee.

EXPERIMENTAL DESIGN

A total of 8 animals were equally divided into two groups (n=4).

GROUP –I: Quercetin (100mg/kg B.W oral)

GROUP –II: Microspheres of Quercetin (100mg/kg B.W oral)

PROCEDURE

Dosing

Animals were divided into two groups of 4 animals in each. The first group received quercetin solution orally of dose 100 mg/kg B.W of animal. The II group received quercetin microspheres solution orally of dose 100 mg/kg B.W of animal.

Figure 1: Dosing of animals.

Collection of Blood Samples

For the collection of blood samples, animals were anaesthetized individually with the help of ether in dessicator. Then, using heparinised capillary, blood was collected from retro orbital plexus at different time intervals of 4 hrs. up to 32 hrs. of each animal of both groups about 0.5 ml. Individual blood samples were kept in eppendorff which were rinsed with EDTA and left at room temperature.

Separation of Plasma

All the samples were centrifuged for 10 min at 10,000g. Then, plasma was separated above and collected with the help of micropipette leaving RBC pellets at bottom. Then, accurate measured ml

(5-1) of acetonitrile was added to the samples for the precipitation of proteins. Again all the samples were centrifuged for 10 mins. at 10,000g. and again plasma was separated and collected in individual eppendorff. Then, all the samples were analysed spectrophotometrically by UV-VIS Spectrophometer for concentration of drug.

Calculation of Area under the Curve (AUC) using Trapezoidal Rule

We can directly calculate the AUC from conc. versus time data. We need to use different approach. The most simple, common approach is a numerical approximation method called the Trapezoidal rule.

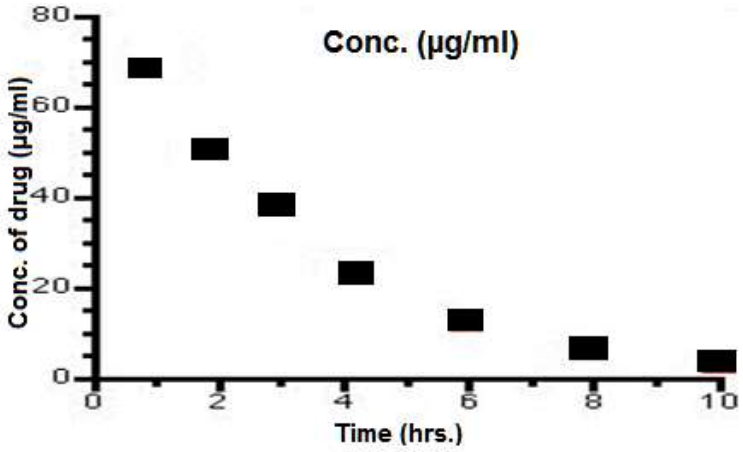


Figure 2: Linear Plot of Cp versus Time showing Typical Data Points

We can calculate the AUC of each segment if we consider the segments to be trapezoids. The area of each segment can be calculated by multiplying the average concentration by the segment width. For the segment from Cp2 to Cp3

$$\text{For example: } AUC_{2\text{-}3} = \frac{Cp_2 + Cp_3}{2} \bullet (t_3 - t_2)$$

This segment is illustrated in Figure 3: below

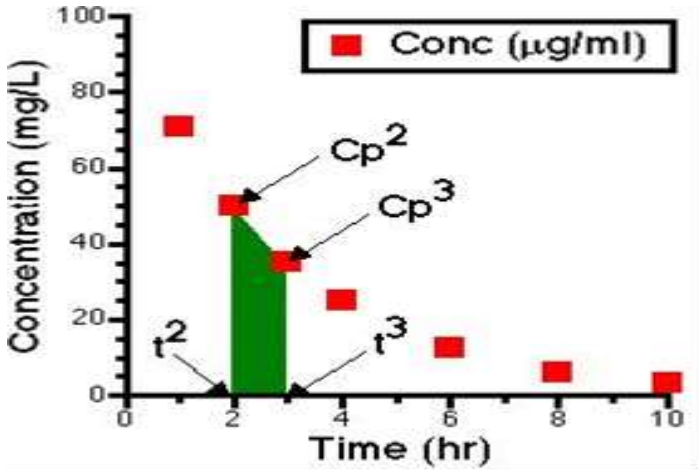


Figure 3: Linear Plot of Cp versus Time showing One Trapezoid

The area from the first to last data point can then be calculated by adding the areas together.

$$AUC_{1-n} = \sum \left\{ \frac{Cp_1 + Cp_2}{2} \bullet (t_2 - t_1) \right\} + \left\{ \frac{Cp_2 + Cp_3}{2} \bullet (t_3 - t_2) \right\} + \ldots$$

Summation of data point information (non-calculus).

To finish this calculation we have two more areas to consider. The first and the last segments.

After a rapid IV bolus, the first segment can be calculated after determining the zero plasma concentration Cp0 by extrapolation.

Thus,

$$AUC_{0-1} = \left\{ \frac{Cp_0 + Cp_1}{2} \bullet (t_1) \right\}$$

If we assume that the last data points follow a single exponential decline (a straight line on semi-log graph paper) the final segment can be calculated from the equation above from at last to infinity.

$$AUC_{t_{last} - \infty} = \int_{t=t_{last}}^{t=\infty} Cp \bullet dt = \frac{Cp_{last}}{kel}$$

Thus the total AUC can be calculated as

$$AUC_{0-\infty} = AUC_{0-1} + AUC_{1-last} + AUC_{last-\infty}$$

$$= \frac{Cp_0 + Cp_1}{2} \bullet t_1 + \frac{Cp_1 + Cp_2}{2} \bullet (t_2 - t_1)$$

$$+ \frac{Cp_2 + Cp_3}{2} \bullet (t_3 - t_2) + \ldots + \frac{Cp_{last}}{kel}$$

Antidote Activity

Animals: Female Swiss albino mice (25-30 g) were obtained from the central animal facility of DRDE, Gwalior and were maintained in polypropylene cages on rodent pellet condition of controlled temperature (22±2 oC)and acclimatized to 12/12 h light/dark cycle. Free access to food and water were allowed until 2h before the experiment. The care and maintenance of the animals were as per the approved guidelines of the "Committee for the purpose of control and supervision of experiments on animals (CPCSEA)". A day before dermal exposure, hairs on the back of the animal was closely clipped using a pair of scissors. Food and water were provided 2h after the

experiment. All experiments on animals were conducted according to the guidelines of establishment's ethical committee on animal experimentation.

Drug Treatment:

LD50 of Sulphur mustard (8.1mg/kg) was administered percutaneously (p.c) to groups of mice. SM was diluted in PEG-300 and the dilution were made in such a manner that the quantity applied was between 25 to 50 µ liter. The diluted solution was smeared uniformly on the back of the animals on a circular area of 1.0 cm diameter, using a gas tight syringe (Hayward Apparatus, USA). After SM administration animals were kept in a well–ventilated room for 24 h and then shifted to the experimental room for further monitoring. Quercetin(100mg/kg) orally and its microsphere formulation ((100mg/kg) was given orally 30 min prior to SM exposure (8.1mg/kg).

Grouping of Animals

Group- I : - Naive control

Group- II : - SM

Group- III : - Quercetin+ SM

Group- IV : - Q microspheres + SM

The body weights of the animals were recorded daily up to 14 days. After 14 days animals were anaesthetized with ether for collection of blood from orbital sinus, and then sacrificed by cervical dislocation for the removal of vital organ. Various haematological and biochemical analysis were carried out.

Hematological and Biochemical Evaluation in Blood

Hematology: The hematological variables, viz., RBC, WBC, and Hb were measured by using Sysmex hematology auto analyzer (Model K 4500, Japan).

Blood glutathione assay: Blood GSH was estimated by the method of Beutleret al. (1963). In which 0.2 mL fresh blood was collected from each animal and1.8 mL distilled water was added to it and 3 mL of precipitating solution was added to mixture. The mixture was then allowed to stand for approximately 5 min and then filtered. 2.0 mL of filtrate was added to 8.0 mL of phosphate solution in cuvette and 1.0 mL DTNB reagent was added to cuvette and the optical density (OD) was measured at 412nm.

Hematological and Biochemical Evaluation in Serum

Enzymes assay: Plasma SGPT, SGOT and ALP level was estimated by using commercial kits (E-coline, Merck, India) as per the manufacturer instructions.

Thiol assay: The reaction mixture containing 900 µL 2mM Na 2 EDTA in 0.2M Na2HPO4, 20 µL 10mM DTNB in 0.2M Na2HPO4 and 100 µL serum was incubated at room temperature for 5

min; the absorbance was read at 412 nm. Appropriate sample and reagent blanks were prepared simultaneously and the respective absorbance was noted. Corrected absorbance values were used to calculate serum protein thiol content using a molar extinction coefficient of 1600/M/cm and values were expressed as mM. The calibration curve was produced using GSH dissolved in phosphate-buffered saline (PBS).

GST assay: One mL of reaction mixture containing 850 µL of 0.1M phosphate buffer, pH 6.5, 50 µL 20mM CDNB (1-chloro 2,4-dinitrobenzene) and 50 µL 20mM GSH was pre-incubated at 37oC for 10min. Reaction was started by adding 50 µL serum and GST activity was assayed kinetically. Reaction was followed at one minute intervals for five minutes by measuring the absorbance at 340nm. GST was determined by using a molar extinction coefficient of 9.6/mM/cm and GST activity was expressed as IU/L.

MDA assay: The reaction mixture containing 1mL 0.67% thiobarbituric acid (TBA), 1 mL 20% tricarboxylic acid (TCA), and 100 µL serum was incubated at 100oC for 20 min and centrifuged at 12,000 rpm for 5 min. The absorbance of the supernatant was read at 532 nm and MDA concentration was determined by using a molar extinction coefficient of 1.56×10 5 /M/cm and the values were expressed as mM.

Hematological and Biochemical Evaluation in Tissue

Lipid Peroxidation Assay: A portion of the liver was used for biochemical estimation. Liver lipid peroxidation was determined by measuring the level of MDA according to the method of Ohkawaet al., 1979. 2mL of suspension medium was taken from the supernatants of the 10% tissue homogenate in 1.15% KCl and centrifuged at 10,000 rpm. 1mL of 30% TCA followed by 1mL of 0.8% TBA were added to it. The tubes were covered with aluminum foil and kept in shaking water bath for 30 minutes at 800 C, after 30 min, tubes were taken out and kept in ice cold water for 10 min. They were then centrifuged at 3000 rpm for 15 min. The absorbance of supernatant was read at 540 nm at room temperature against blank. Blank consisted of 2mL distilled water, 1mL TBA, and 1mL TCA.

GSH Assay: Tissue GSH was determined by the method of Sedlak and Lindsay (1968). A portion of the reperfused liver tissue (300-600 mg) homogenized in 5-8 mL of 0.02M EDTA and then 4mL of cold distill water was added to it. After mixing 1mL of 50% TCA was added to it and shaken for 10min and centrifuged at 6000 rpm for 15 min. 4 mL of 0.4 M tris buffer was mixed with 2 mL of supernatant and 0.1mL of 0.01M DTNB. The absorbance of this resulting mixture was read at 410 nm at room temperature against reagent blank.

Statistical Analysis

Statistical evaluations were made using one-way ANOVA followed by Dunnet's test. A probability of 0.05 and less was taken as statistically significant. The analyses were carried out using sigma stat for windows version 2.03 (SPSS Inc.USA).

RESULT AND DISCUSSION

Preformulation studies

Identification of drug

Organoleptic Characteristic:

Organoleptic characteristics of the drug were found within standard limits as shown in Table 6.

Table 4: Physical Properties of the Drug

S.No.	Properties	Organoleptic Properties Observed
1.	Color	Yellowish crystalline powder
2.	Odor	Odourless
3.	Taste	Tasteless
4.	State	Crystalline powder

Melting Point:

Melting point of drug was determined by capillary fusion method and it is enlisted in Table 7.

Table 5: Melting Point of Drug

S. No.	Drug	Melting Point ^{0}C (Literature)	Melting Point^{0}C (Practical)
1	Quercetin	316	332

Solubility Studies: Solubility profile of Quercetin is depicted in Table 1.8.

Table 6: Solubility Studies of Quercetin

S. No.	Solvent	Querectin
1.	Ethanol	+++
2.	Methanol	+++
3.	Water	++
4.	n-hexane	++
5.	DMSO	+++
6.	PBS (pH 7.2)	+++

Partition coefficient: Partition coefficient was determined in Octanol /phosphate buffer pH 7.4 system and showed that Quercetin is more hydrophilic Table 9.

Medium	Partition coefficient
Octanol /phosphate buffer pH 7.4	0.52

Spectrophotometric Determination of Quercetin Dihydrate

Ultraviolet Absorption Maxima of Quercetin dihydrate

The sample was scanned in the range of 200-400 nm using Systronic, Double beam spectrophotometer 2203 to determine the λmax. The spectra showed the maximum absorbance at 327 nm λmax. The UV spectrum is shown in Figure 4.

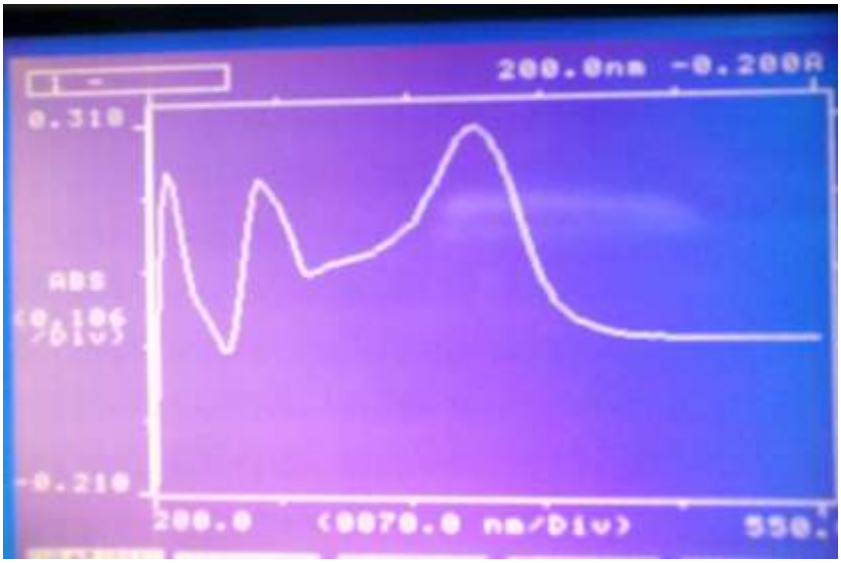


Figure 4- Absorbance curve of Quercetin hydrate

Infrared spectrum

IR graph of Drug sample was found concordant with that of the reference sample Figure 5.

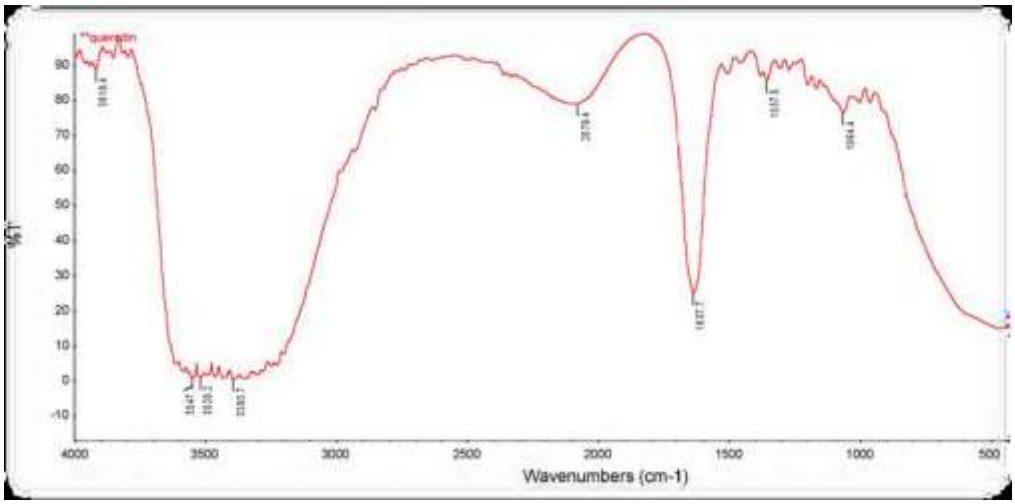


Figure 5: FT-IR Spectrum of Drug. (Quercetin)

Preparation of Calibration Curve of Quercetin dihydrate:

Standard curve of Quercetin dihydrate was prepared in phosphate buffer (pH 7.4). 1µg/ml to 8µg/ml concentration range solution were prepared. The absorbance of each solution was noted at 327 nm. This standard curve was linearly regressed. (Figure 6)

Table-10: Standard curve of Quercetin Dihydrate

S. NO.	Concentration	Absorbance
1	0	0
2	1	0.018
3	2	0.036

4	3	0.057
5	4	0.079
6	5	0.091
7	6	0.109
8	7	0.128

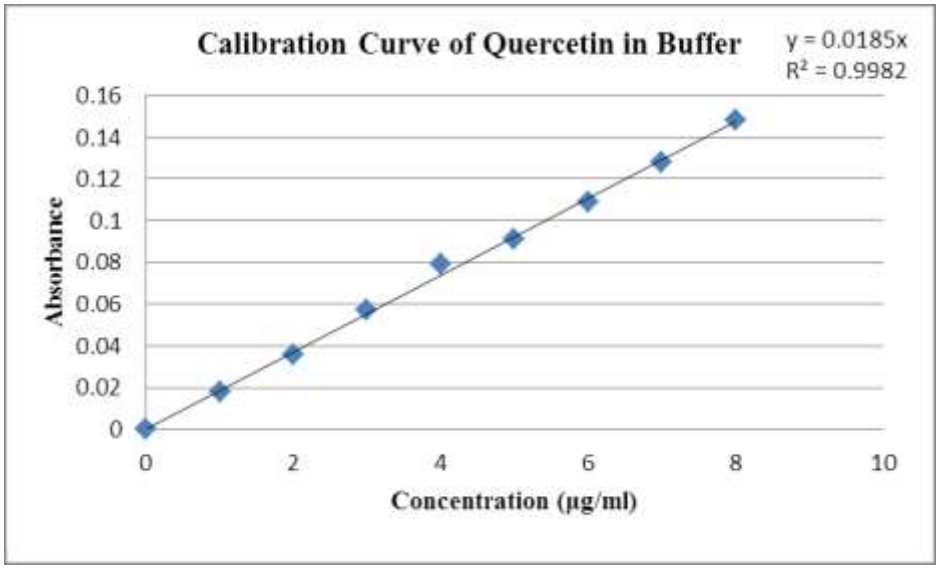


Figure 6: Standard/calibration curve of Quercetin Dihydrate in phosphate buffer (pH 7.4)

Table-8 Statistical parameters of Standard curve

S.No.	Parameters	Statistical parameter for Quercetin dihydrate in phosphate buffer pH 7.4
1.	Regression coefficient (R^2)	0.9982
2.	Slope (m)	0.0185
3.	Equation of line	Y=0.0185 X

Interaction between Drug and Polymer

The drug excipient interaction studies were carried out employing FT-IR Spectroscopic technique and the principle peaks of Quercetin were found identical to the standard depicting no harmful interaction (Figures 7,8 and 9). Quercetin pure drug and the formulation F1 subjected for FT-IR spectroscopic analysis for compatibility studies and to ascertain whether there is any interaction between the drug and the polymers used. The IR spectra of Quercetin and drug-loaded microspheres were found to be identical. The major characteristic IR absorption peaks of Quercetin as (1100-1600 cm−1) i.e. C=O (1664 cm−1) and O-H phenolic bands (1200-1400) were present in drug loaded microspheres. The FT-IR spectra of the pure drug and formulation F1 indicated that characteristics peaks of Quercetin were not altered without any change in their position after successful entrapment in microspheres, indicating no chemical interactions between the drug and carriers used. FT-IR spectra of the microspheres showed all the Quercetin

characteristics absorption bands suggesting the absence of interactions between the drug and the other components of the formulations. These results indicate the method used to prepare microspheres does not affect the physicochemical properties of the systems.

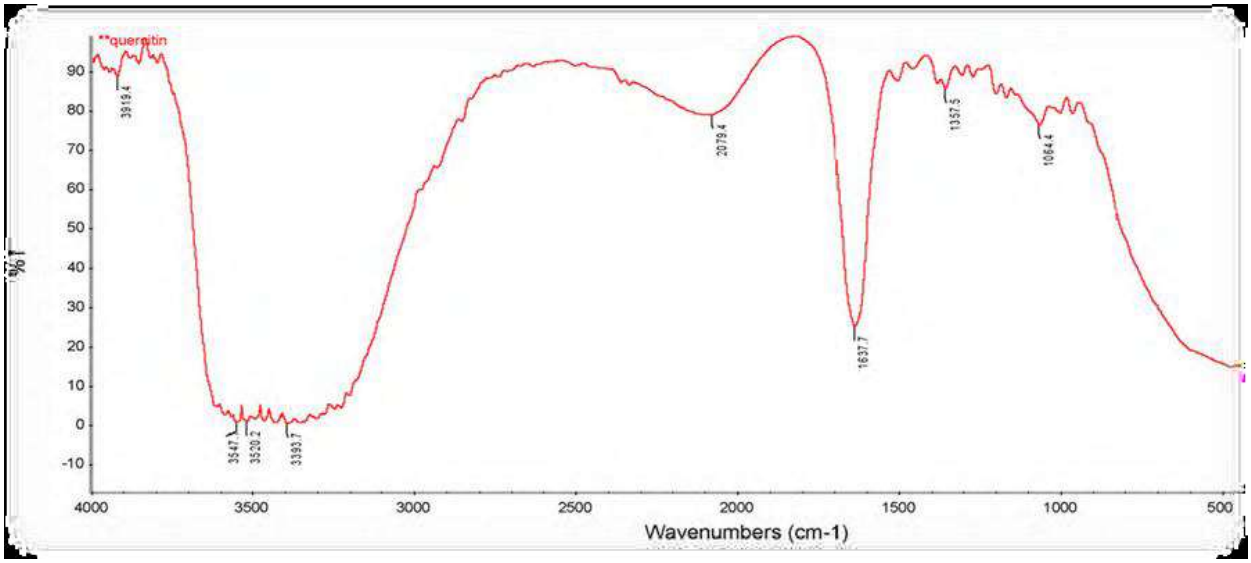


Figure 7: FT-IR Spectrum of Drug. (Quercetin)

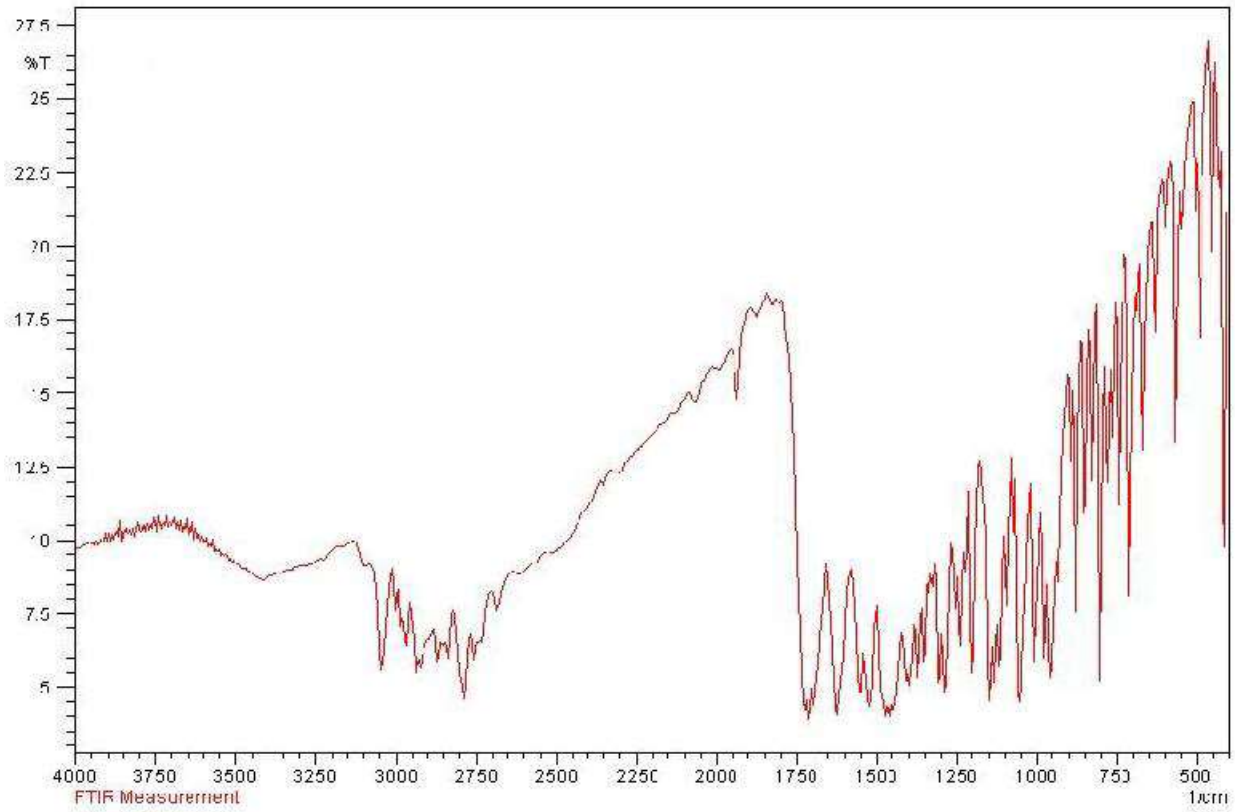


Figure 8: FT-IR Spectrum of Eudragit S 100

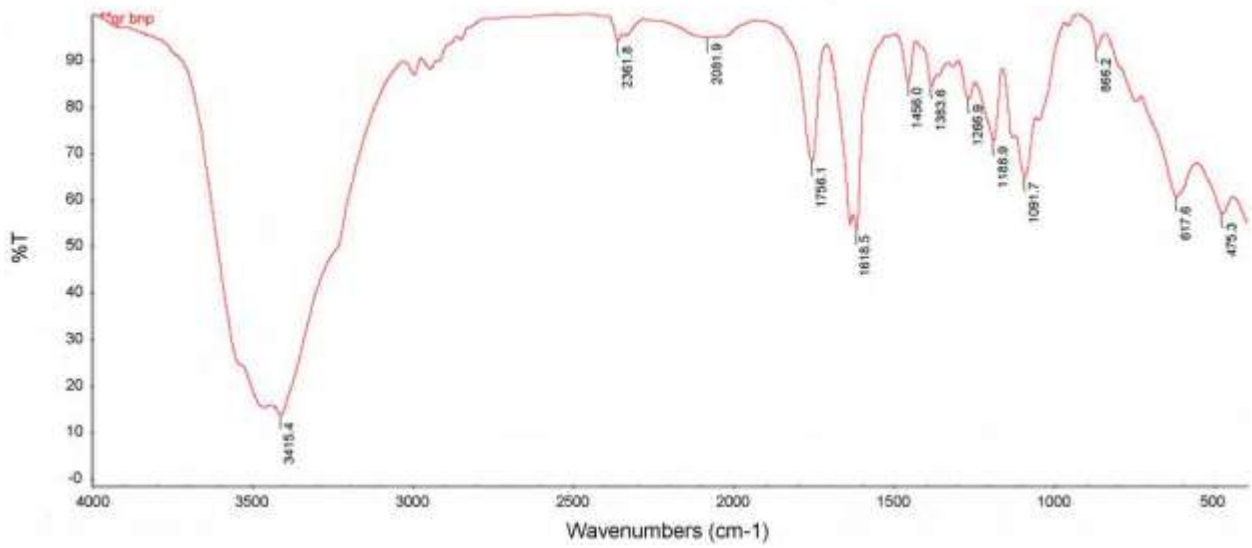


Figure 9: FTIR Spectrum of Quercetin loaded eudragit microspheres. (F1)

CHARACTERIZATION OF MICROSPHERS

Micromeritic study

Particle size

The mean particle sizes of the prepared microspheres are found significantly increasing with polymer concentration and decreasing with increasing plasticizer concentration (table- 13). The range of particle size was 30.49±1.33 (batch F5) to 44.02±1.90 (batch F7) (table- 12)

Drug entrapment efficiency

The drug entrapment efficiency was found in range of 73.35±2.7 (batch P1) (table- 13) to 80.61±1.54 (batch F5) (table- 12).

Angle of repose

The angle of repose shows the flow ability of the materials. The range of angle of repose was found 19.72±1.33 (batch-F5) (table- 12) to 23.01±1.6 (batch-P1) (table- 13). Formulation F5 has free flowing and others have good flowing property.

Carr's index

The Carr's index was found in the range of 6.94±1.01 (batch-F1) to 14.45±1.17 (batch-F7) (table-12). All formulations have excellent flow property.

Percentage compressibility

Compressibility index ranging from 8.35±2.4 (batch-P1) to 5.21±1.99(batch-P5) (table- 13). The C.I. decreases with increasing amount of plasticizer and amount of polymer.

Drug entrapment efficiency

The drug entrapment efficiency was found in the range of 73.35 ± 2.7 (batch-F7) to 80.61 ± 1.54 (batch-F5) (table- 12). The drug entrapment efficiency increases with plasticizer concentration (table- 13).

Percentage Mucoadhesion

Percentage mucoadhesion was found in the range of 62.5 ± 1.81 (batch-F1) to 74.9 ± 1.13 (batch-F4) (table- 12). The Percentage mucoadhesion increase with concentration of ethyl cellulose but not more affect concentration of eudragit.

Table 9- Micromeritic studies of microspheres (Formulation F)

Parameter	F1	F2	F3	F4	F5	F6	F7
Particle size diameter in (µm)	35.41±1.21	32.11±1.21	34.59±1.21	39.01±1.33	30.49±1.21	39.07±1.05	44.02±1.90
Drug entrapment efficiency (%)	77.34±1.54	79.92±1.54	78.05±1.54	76.68±0.69	80.61±1.54	76.38±0.69	73.35±2.7
Angle of repose	20.86±1.86	20.01±1.29	20.34±1.46	20.51±1.07	19.72±1.33	20.72±1.07	23.01±1.61
Bulk density (gm/cm^3)	1.167±0.011	1.217±0.013	1.193±0.031	1.098±0.037	1.233±0.011	1.067±0.019	0.823±0.016
Tapped density (gm/cm^3)	1.254±0.023	1.331±0.057	1.283±0.096	1.201±0.020	1.374±0.055	1.211±0.072	0.962±0.019
Carr's index (%)	6.94±1.01	8.56±0.98	7.02±0.79	8.58±1.23	10.26±1.11	11.89±1.43	14.45±1.17
% compressibility	5.34±1.99	5.29±1.99	5.37±1.99	6.80±1.77	5.21±1.99	6.92±1.77	8.35±2.4
% yield	79.63±1.76	82.97±1.76	80.32±1.76	78.24±1.01	84.18±1.76	78.15±1.01	71.96±2.0
% Mucoadhesion	62.5 ±1.81	65.7 ± 1.22	68.2 ± 1.40	74.9 ± 1.13	62.9 ± 1.62	63.3 ± 1.07	66.2 ± 1.29

Table 10- Micromeritic studies of microspheres (Formulation P)

Parameter	P1	P2	P3	P4	P5
Particle size diameter in (µm)	44.02±1.90	37.27±1.05	32.89±1.21	38.77±1.05	33.99±1.21
Drug entrapment efficiency (%)	73.35±2.7	77.29±0.69	79.62±1.54	76.38±0.69	78.15±1.54
Angle of repose	23.01±1.61	21.52±1.07	20.09±1.33	20.92±1.07	20.26±1.33
Bulk density (gm/cm^3)	0.823±0.016	1.098±0.019	1.203±0.011	1.024±0.021	1.153±0.072
Tapped density (gm/cm^3)	0.962±0.019	1.211±0.089	1.294±0.076	1.125±0.072	1.263±0.055
Carr's index (%)	14.45±1.17	9.33±1.43	7.03±1.29	8.98±1.43	8.71±1.11
% compressibility	8.35±2.4	6.52±1.77	5.29±1.99	6.92±1.77	5.21±1.99
% yield	71.96±2.0	79.22±1.01	82.69±1.76	78.35±1.01	81.02±1.76

In-vitro Drug release study

All release kinetic models were applied on all formulation. The best fit model was found to be Higuchi for all formulation. The best model was based on residual sum of squares. If the release of drug from the matrix, when plotted against square root of time, shows a straight line, having highest R value and lowest Residual sum of square (RSS), it indicates that the release pattern is obeying Higuchi kinetics.

On increase the concentration of polymer (number of biopolymer molecules per unit), in the vicinity of core capsule, as a result more densely cross-linked gel structure would probably be formed result in better incorporation efficiency as shown in table-. Surfactant concentration and stirring speed increases than the size of microspheres should be decreases. Drug release from batch P slightly increases due to decrease particle size and increase surface area (table- 14) & (table- 15). At gastric pH drug release negligible and at pH 6.8-7.2 drug releases controlled.

Table 11-Cumulative percentage drug release of F formulation

Time (Hrs.)	Cumulative Percentage Drug Release						
	F1	F2	F3	F4	F5	F6	F7
1	6.032	6.62	6.03	5.44	7.98	6.42	5.06
2	10.51	13.43	12.26	10.70	14.98	12.06	11.09
3	17.51	18.87	18.29	17.99	21.01	15.96	15.56
4	26.66	28.02	25.69	24.99	29.96	23.94	22.96
6	34.83	36.78	35.61	34.92	38.91	34.05	31.91
8	42.62	44.36	45.14	45.01	48.06	42.62	41.25
12	52.73	53.71	55.46	55.91	57.99	51.96	50.98
16	67.13	69.47	68.87	67.97	71.03	66.80	65.77
20	73.95	75.50	73.55	72.06	79.01	71.86	71.02
24	87.76	90.88	88.54	82.96	92.04	82.95	77.06

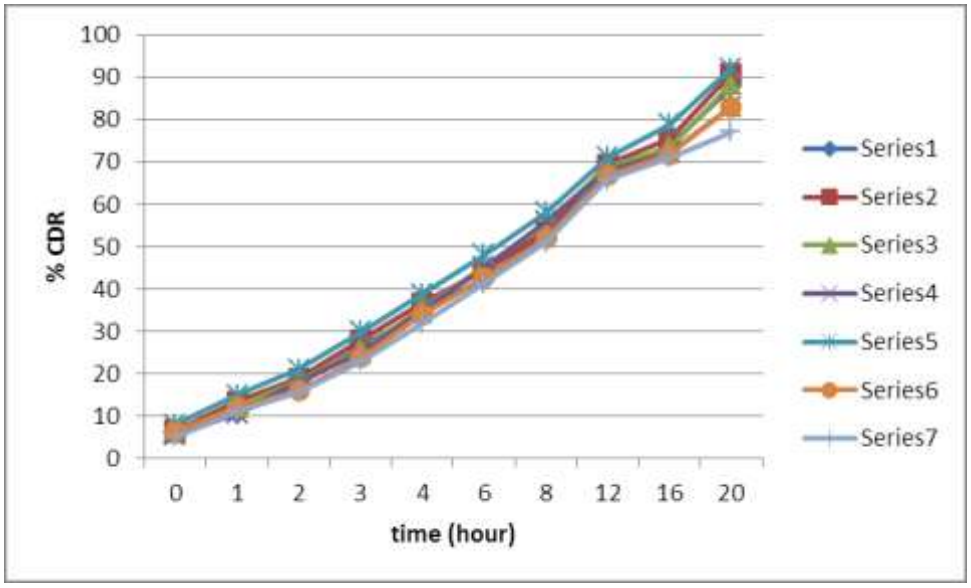
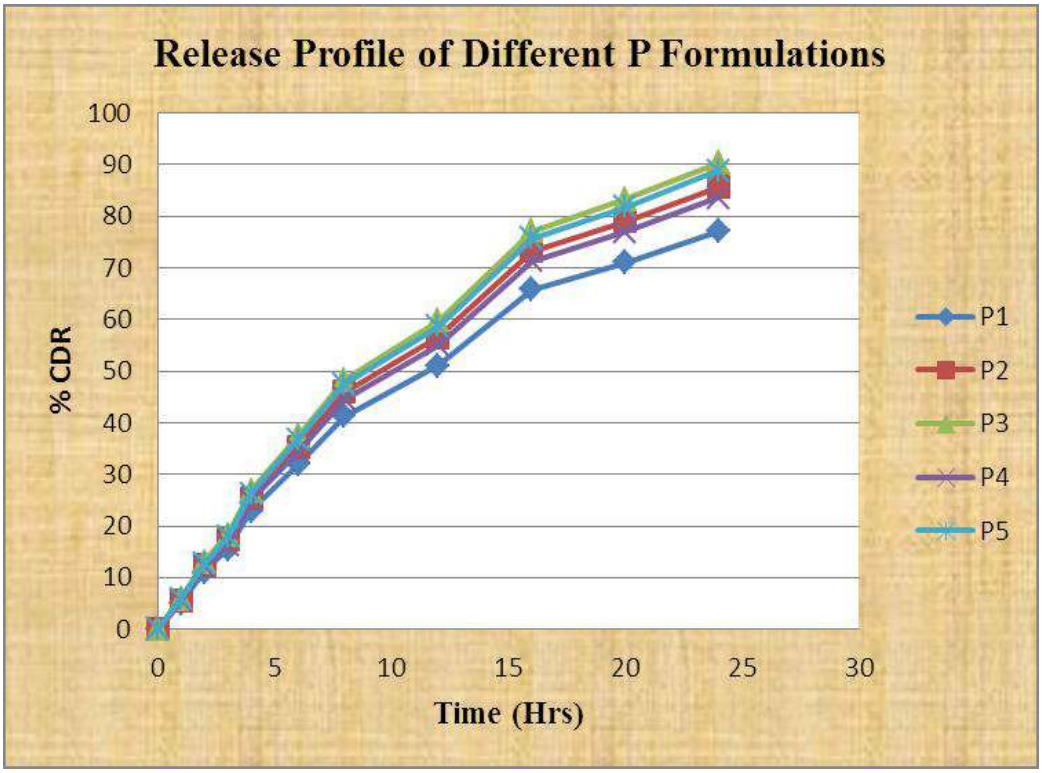


Table 12-Cumulative percentage drug release of P formulation

Time (Hrs.)	Cumulative Percentage Drug Release				
	P1	P2	P3	P4	P5
1	5.06	5.64	5.83	5.45	5.83
2	11.09	12.24	13.03	12.06	12.64
3	15.57	17.30	18.29	16.34	17.90
4	22.96	25.47	26.85	24.91	26.27
6	31.91	35.39	37.36	34.44	36.78
8	41.25	45.91	48.26	44.56	47.48
12	50.98	56.60	59.74	55.07	58.57
16	66.77	73.14	77.05	71.22	75.69
20	71.02	78.98	83.28	76.86	81.72
24	77.06	85.60	90.29	83.48	88.73



Kinetic Modeling of Drug Release Profiles

The result obtaining in vitro release studies were plotted in different model of data treatment as follows.

1. Cumulative percent drug released Vs. time (zero order rate kinetics)
2. Log Cumulative percent drug retained Vs. time (first order rate kinetics)
3. Log Cumulative percent drug released Vs. square root of time (Higuichi‘s classical diffusion equation)
4. Log Cumulative percent drug released Vs. time (Peppas exponential equation)
5. (Percentage retained)1/3 Vs. time (Hixon—Crowell Erosion Equation)

Table 13: Kinetic modeling of drug release for formulations of P group

Batch Code	R^2 value						Best Fit Model
	Zero Order	First Order	Higuchi	Hixon Crowell	Korsmeyer Peppas	n value	
P1	0.924	0.9964	0.9947	0.8489	0.9749	0.8485	I order
P2	0.9253	0.9962	0.9946	0.8492	0.9822	0.8497	I order
P3	0.9255	0.9874	0.9949	0.8482	0.9815	0.8522	Higuchi
P4	0.9265	0.9958	0.9944	0.8497	0.982	0.8525	I order
P5	0.9257	0.7682	0.9947	0.8493	0.9823	0.8502	Higuchi

Table 14: Kinetic modeling of drug release for formulations of F group

Batch Code	R^2 value						Best Fit Model
	Zero Order	First Order	Higuchi	Hixon Crowell	Korsmeyer Peppas	n value	
F1	0.9396	0.9646	0.995	0.862	0.9812	0.8335	Higuchi
F2	0.93	0.9422	0.994	0.8663	0.984	0.7919	Higuchi
F3	0.928	0.9583	0.9953	0.8562	0.9833	0.8212	Higuchi
F4	0.9129	0.9917	0.9944	0.8342	0.9763	0.8471	Higuchi
F5	0.9676	0.9521	0.997	0.8627	0.9885	0.7505	Higuchi
F6	0.9311	0.9893	0.9953	0.8644	0.9884	0.8075	Higuchi
F7	0.9251	0.9964	0.9947	0.8489	0.9749	0.8485	I order

IN-VIVO STUDY

The In vivo studies on male wistar rats were conducted and plasma concentration time method was employed to study the influence of quercetin by formulating it as microspheres. The aim was to determine whether microspheres as NDDS, sustains the bioavailability of quercetin. By carrying out the Spectrophotometric assay of periodic plasma samples of plots, which were previously administered with the microspheres, the plasma concentration time profile was obtained. The same data was collected for the pure drug administered to the rats.

By Statistical Analysis through sigma plot, the semi log plot of pure drug (Quercetin) (Figure 15) and its microspheres (Figure 19) shows linearity which indicates that they follow linear kinetics.

In-vitro antioxidant and photo protective properties and interaction with model membranes of three new quercetin esters. Quercetin is well known to possess the strongest protective effect against UV light-induced lipoperoxidation. However, the absolute water insolubility of quercetin is a key step that may limit its bioavailability and, thus, its _in vivo‘ employment as a photo protective agent.

Quercetin has been proved to have antidotal effect on toxicity of mustard agents and can produce more beneficial effects if it is being incorporated as microspheres instead of pure drug being formulated as any conventional dosages form.

Thus, it can be concluded that the absorption kinetics and availability of quercetin is modified and altered by formulating it as microspheres. It is strongly indicated that formulation of microspheres of quercetin produced a sustained effect on its absorption and availability and several parameters (Table 20) are there which concluded that the microspheres of quercetin are better choice for mustard toxicity as compared to pure drug.

This study indicates the better performance of microspheres as compared to pure drug. Here the availability of microspheres is more than the pure drug by 66.9% and its Cmax is also higher. The MRT of microspheres is nearly about 17.77-18 hrs. as compared to pure drug which is only 14.899 hrs.

For group I (quercetin)

Table 15: Plasma Conc. Data after Oral Administration of Drug (100 mg / kg. B.W)

S. No	Time (hrs.)	Conc. of drug (µg/ml)	Cumulative conc. of drug (µg/ml)	$\frac{Xu}{Xu^{32}}$	1- (Xu / Xu^{32})	Log (1- (Xu / Xu^{32}))
1	0	00 ± 00	00	00	00	00
2	0 - 4	89.667 ± .012	89.667	0.1875	0.8125	0.09017
3	4 – 8	119.85 ± .065	209.517	0.4382	0.5618	0.25041
4	8 - 12	85.671 ± .016	295.188	0.6174	0.3826	0.41725
5	12 - 16	55.781 ± .092	350.969	0.7341	0.2659	0.57528
6	16 - 20	42.921 ± .15	393.89	0.8239	0.1761	0.76548
7	20 - 24	33.306 ± .042	427.196	0.8935	0.1065	0.97265
8	24 - 28	27.491 ± .046	454.687	0.9514	0.0486	1.31336
9	28 - 32	23.38 ± .18	478.067	1	00	00

Mean ± S.E (n = 4)

$P \leq 0.05$ is taken as significant.

AUC Curve of Pure Drug (Quercetin)

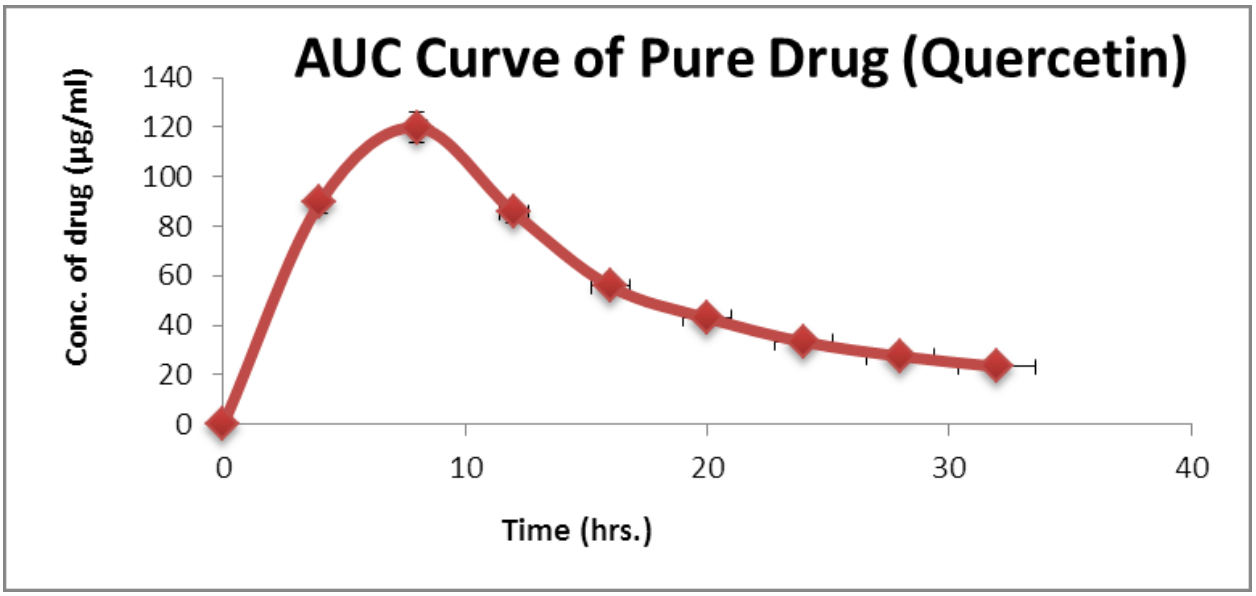


Figure 12: AUC Curve of Pure Drug (Quercetin)

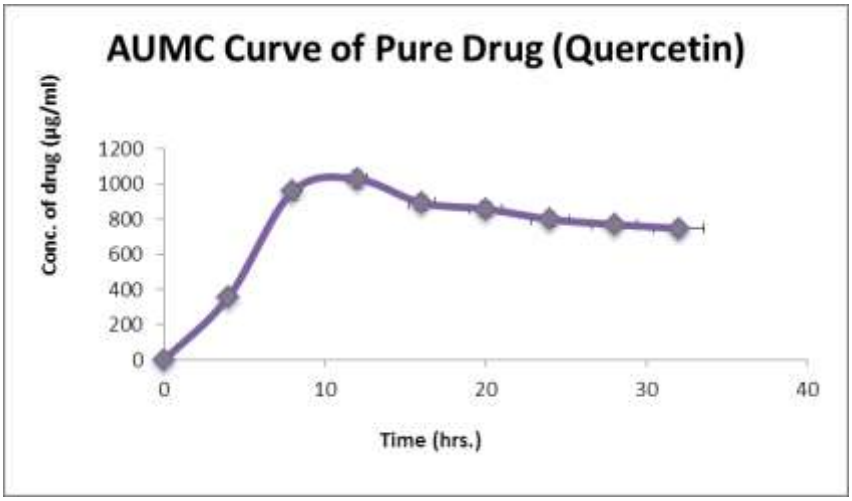


Figure 13: AUMC Curve of Pure Drug (Quercetin)

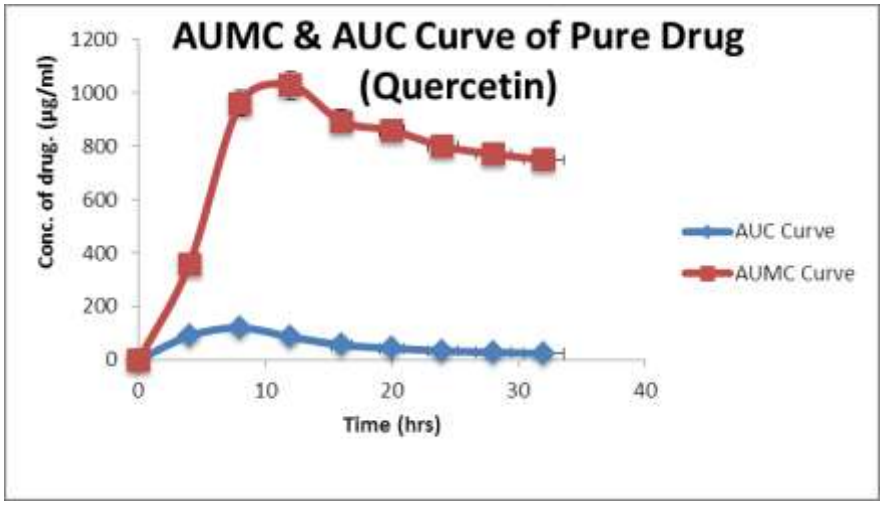


Figure 14: AUC & AUMC Curve of Pure Drug (Quercetin)

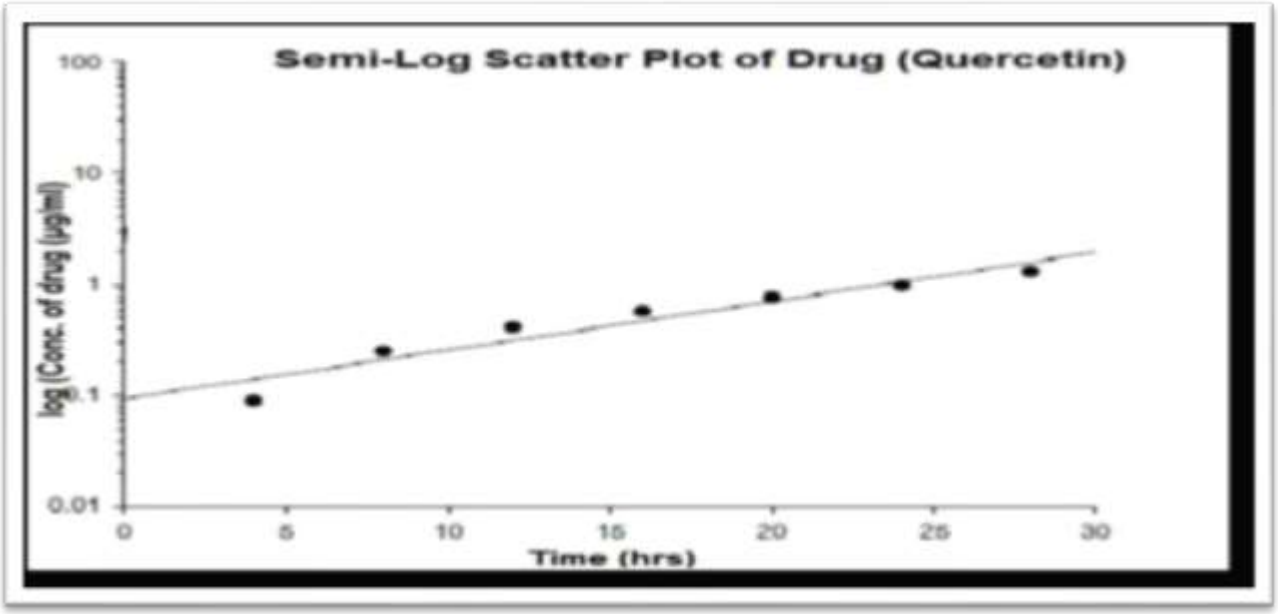


Figure 15: Semi-Log Plot of Drug (Quercetin)

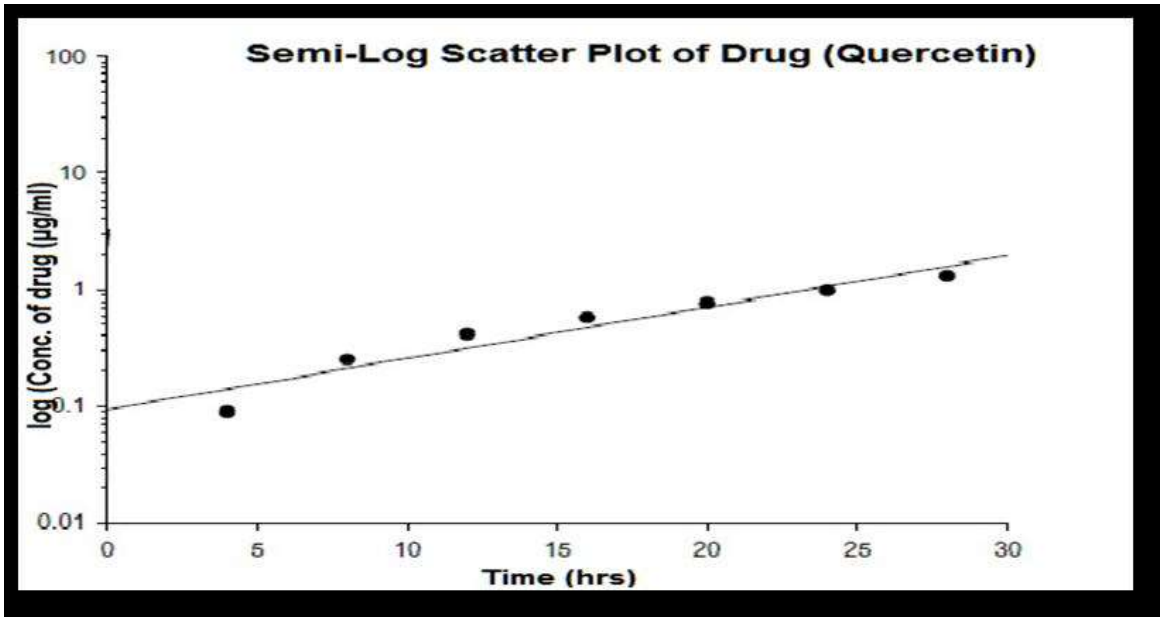


Figure 16: AUC Curve of Microspheres (Quercetin)

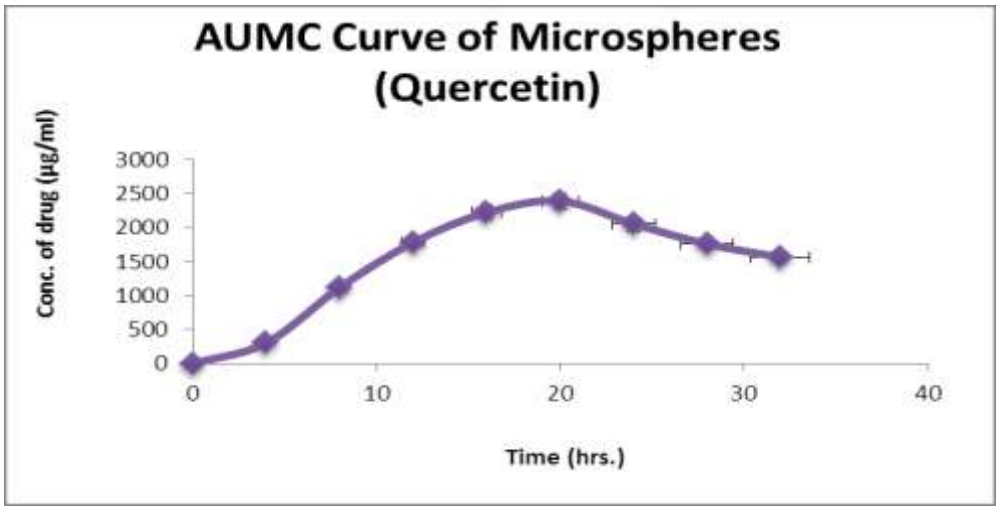


Figure 17: AUMC Curve of Microspheres (Quercetin)

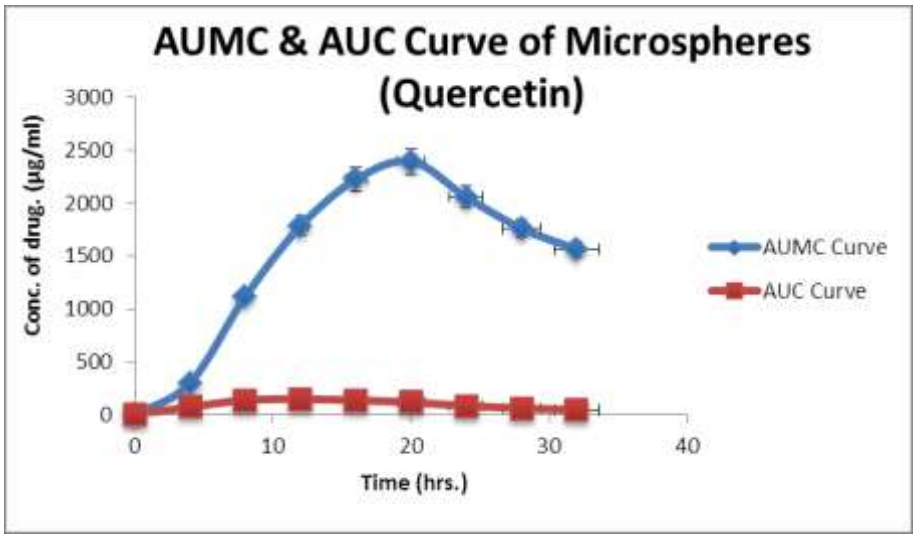


Figure 18: AUC AUMC Curve of Microspheres (Quercetin)

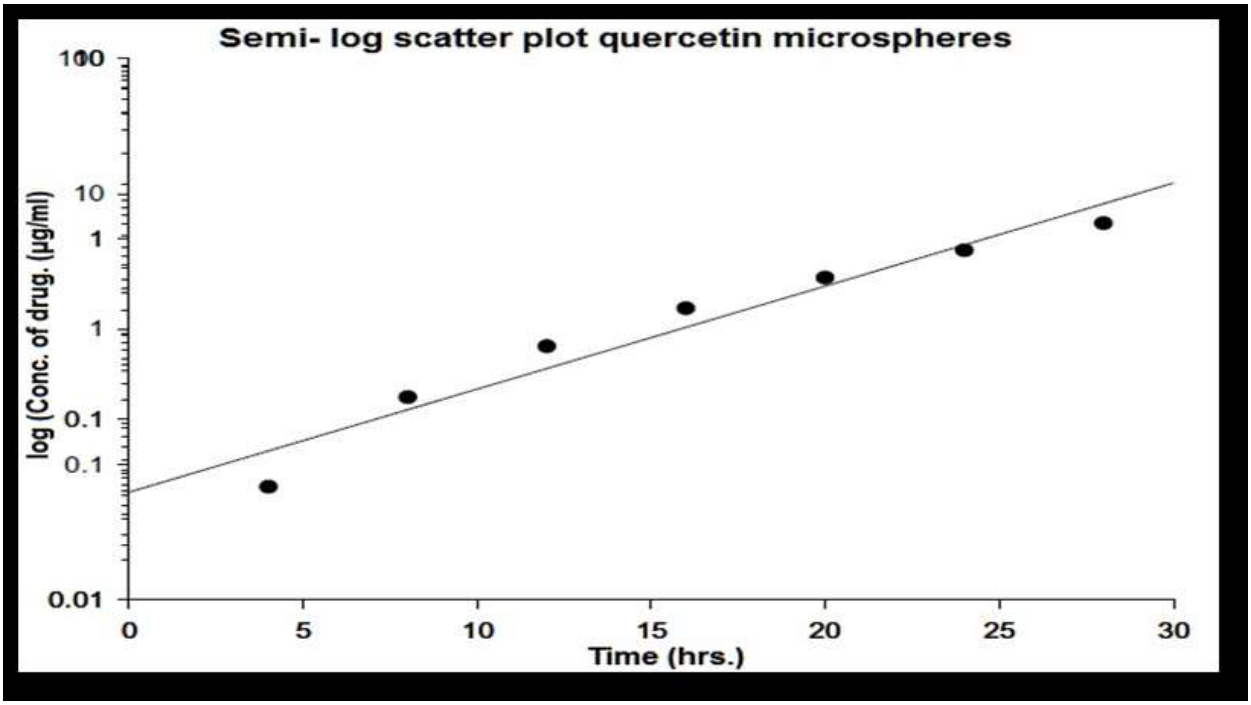


Figure19: Semi-Log Plot of Microspheres (Quercetin)

For group II (microspheres F5of quercetin)

Note: Kinetic Modeling of Drug Release profiles

The results obtaining in vitro release studies were plotted in different model of data treatment as follows

1. Cumulative percent drug released vs. time (zero order rate kinetics)

2. Log cumulative percent drug retained vs. time (First order rate kinetics)

3. Log cumulative percent drug released vs. square root of time (Higuchi‘s classical diffusion equation)

4. Log of cumulative % release vs. log time (Peppas exponential equation)

Table 19: Parameters of Bioavailability

S. No.	Dosage form	Cmax (µg/ml)	Tmax (hrs.)	AUC (µg/ml/hr)
1	Pure Drug	119.85	6-8	1865.504
2	Microspheres	148.725	9-10	3109.565

Antidote Activity

As per the literature the estimated LD50 of SM is 8.1mg/kg. In SM treated group body weight significant ($p<0.05$) decrease when compare to control group. The percentage increase in body weight was significant ($p<0.05$) in pure Quercetin and quercetin microsphere treated groups compared to SM group. In microsphere treated group significant change was found in liver weight compared to quercetin alone treated group.

Figure 22 shows, WBC, RBC count and Hemoglobin level after 8.1mg/kg of SM. There was significant ($p<0.05$) fall in the WBC, RBC and Hb count in SM treated group compared to healthy control mice. A moderate improvement in WBC, RBC and Hbbcount was observed in quercetin treated mice and in both Quercetin microsphere treated mice changes were significant ($p<0.05$) as compared to SM treated mice treated with microspheres formulation.

Figure 24 summaries, the changes in the reduced glutathione levels in blood. There was significant decrease ($p<0.05$) in blood glutathione level in SM treated group compared to healthy control mice. In quercetin and microspheres formulation of Quercetin pretreated groups, blood GSH level was also significantly increased ($p<0.05$) with respect to SM treated group.

Figure 23 shows, the activities of serum SGPT, SGOT and ALP enzymes in all treatment groups. There was a significant increase ($p<0.05$) was observed in SM treated group compared to naive group. Quercetin and microspheres formulation of quercetin pretreated groups show significant decrease ($p<0.05$) in SGPT, SGOT and ALP activities compared to SM treated mice.

Figure 21 Summaries, the changes in the serum total thiol, lipid peroxidation and GST levels of animals of all groups. There was significant decrease in GST and total thiol level in SM treated mice ($P<0.05$) compared to Quercetin treated mice did not showed significant increase ($p<0.05$) in serum GST and total thiol level compared to SM treated but in Quercetin microsphere showed ($p<0.05$) significant increase ($p<0.05$) compared to SM. MDA level was significantly increased in SM treated group with respect to healthy control group ($p<0.05$) and significant change has been observed by quercetin and microspheres treated group compare to SM.

Figure 25 summaries, the effect of various treatments on liver reduced glutathione levels in mice. There was significant decrease in reduced glutathione level in SM treated group ($p<0.05$) compared to naive group. Quercetin and its formulation significant increase ($p<0.05$) in hepatic GSH level compared to SM treated group.

In order to evaluate the effect of treatments on lipoperoxidation, MDA levels were assayed in tissue (Figure 26). Lipid peroxidation increased ($p<0.05$)significantly in SM in treated mice compared to naïve group ($p<0.05$). MDA level was decreased significantly($p<0.05$)in quercetin and its formulation pretreated groups compared to SM treated mice.

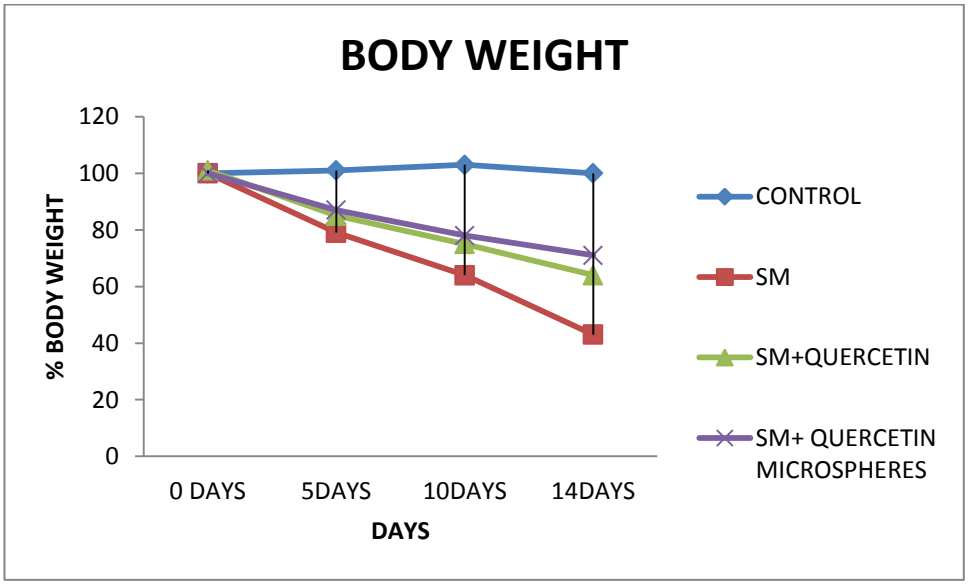
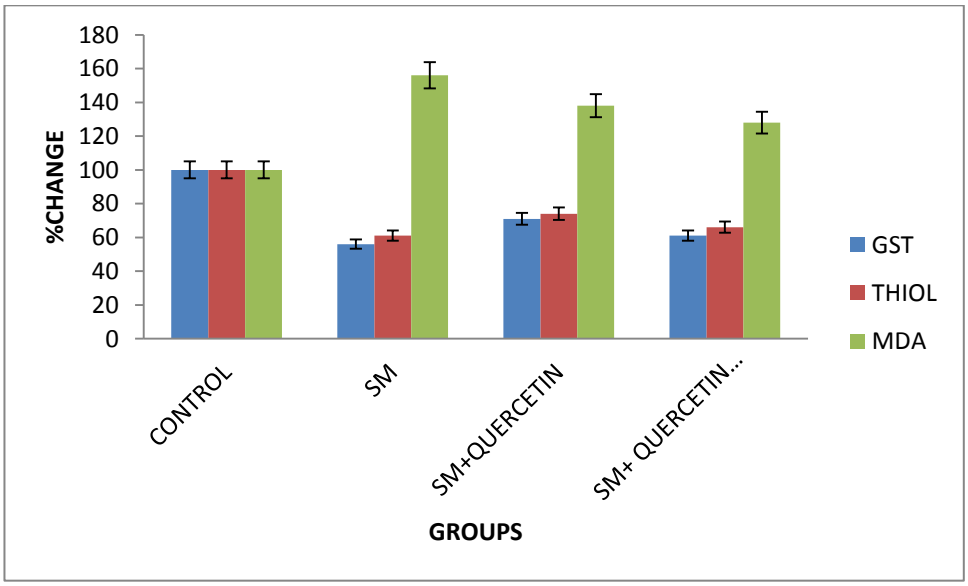


Figure 20: Effect of pretreatment of quercetin and quercetin microspheres on body weight against sulphur mustard

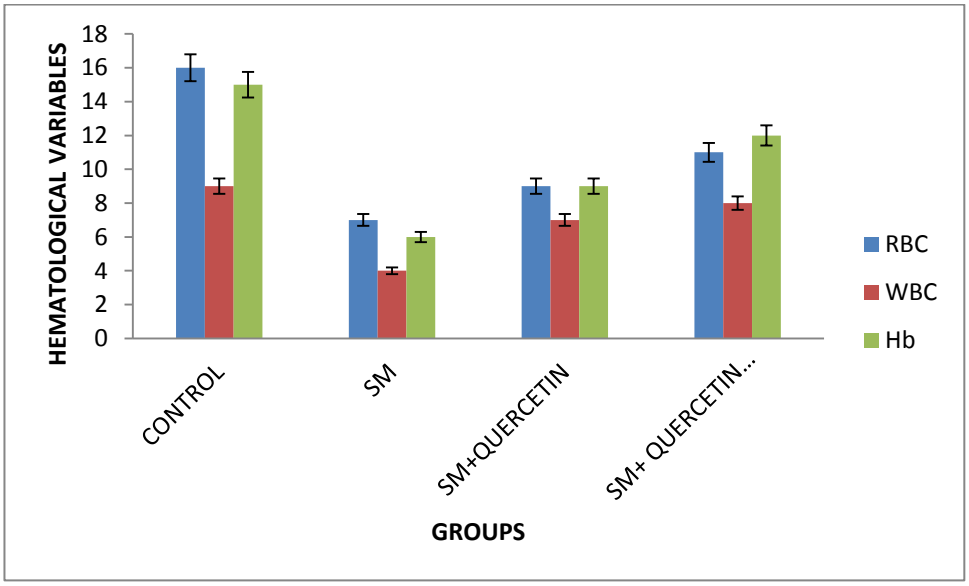


*P<0.05, compared to control group (One way ANOVA followed by Dunnett's t test).

#P<0.05, compared to Positive control group (One way ANOVA followed by Dunnett's t test).

Control value for: GST=0.248±0.051U/L, Total thiol=0.193±0.7mM, MDA=0.22±0.08X10^{-3}mM

Figure 21 - Effect of pretreatment of quercetin and quercetin microspheres on GST, THIOL,MDA against sulphur mustard

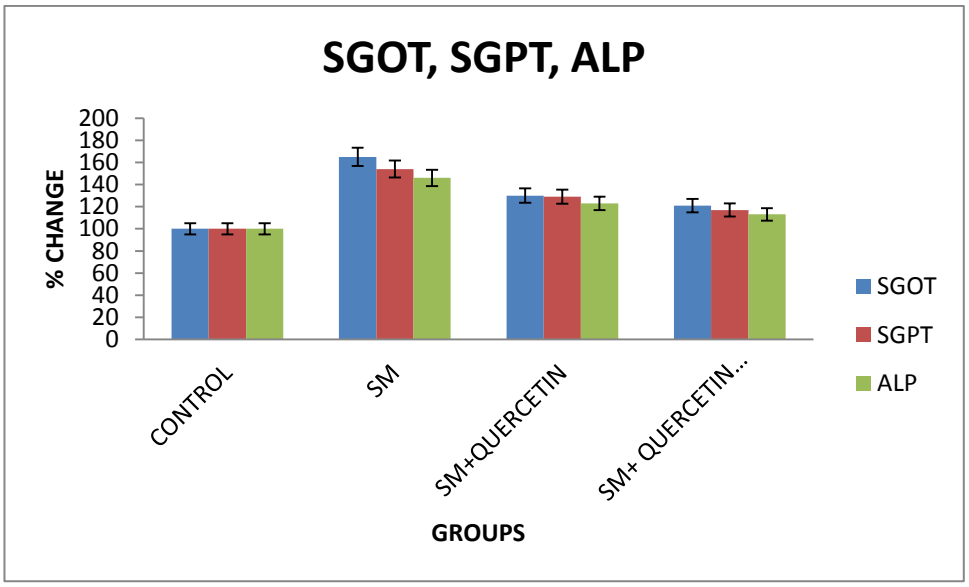


*P<0.05, compared to control group (One way ANOVA followed by Dunnett's t test).

#P<0.05, compared to Positive control group (One way ANOVA followed by Dunnett's t test).

Control value for:WBC=13.4±0.7X10^3 cell/µl, RBC=8.3X10^6 cells/µl, Hb=13.2±0.4g/dl

Figure 22 - Effect of pretreatment of quercetin and quercetin microspheres on RBC, WBC, Hb against sulphur mustard

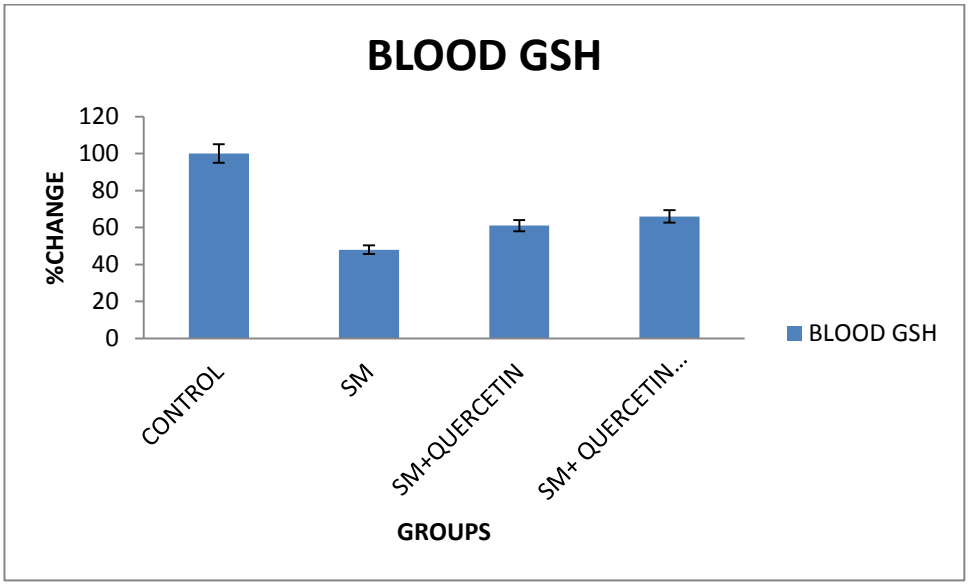


*P<0.05, compared to control group (One way ANOVA followed by Dunnett's t test).

#P<0.05, compared to Positive control group (One way ANOVA followed by Dunnett's t test).

Control value for: SGPT = 25.0±1.011 U/L, SGOT = 26.1±1.021 U/L, ALP = 29.2±2.1 U/L.

Figure 23 - Effect of pretreatment of quercetin and quercetin microspheres on SGOT, SGPT, ALP against sulphur mustard

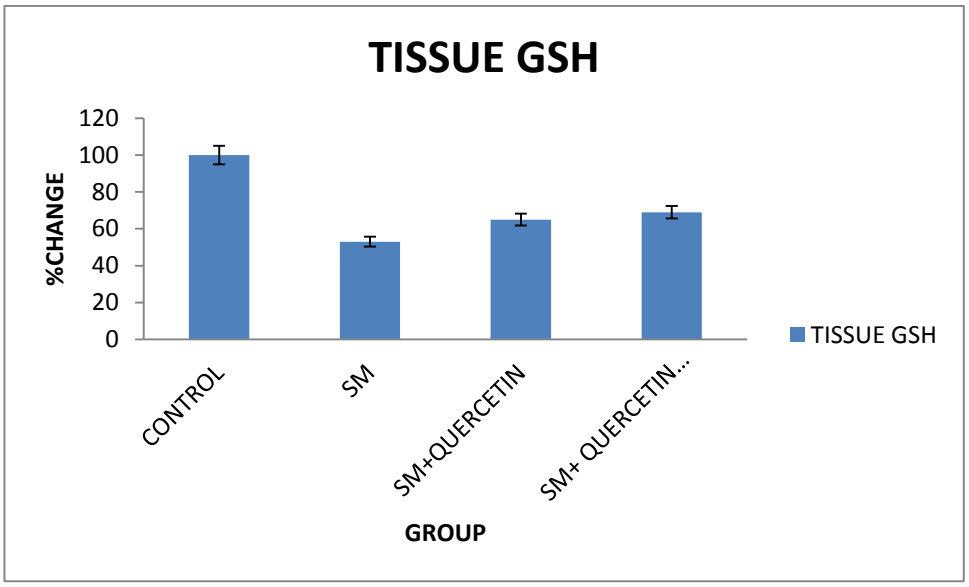


*P<0.05, compared to control group (One way ANOVA followed by Dunnett's t test).

#P<0.05, compared to Positive control group (One way ANOVA followed by Dunnett's t test).

Control value for: Blood GSH=0.075±0.05µg/dl

Figure 24 - Effect of pretreatment of quercetin and quercetin microspheres on Blood GSH against sulphur mustard

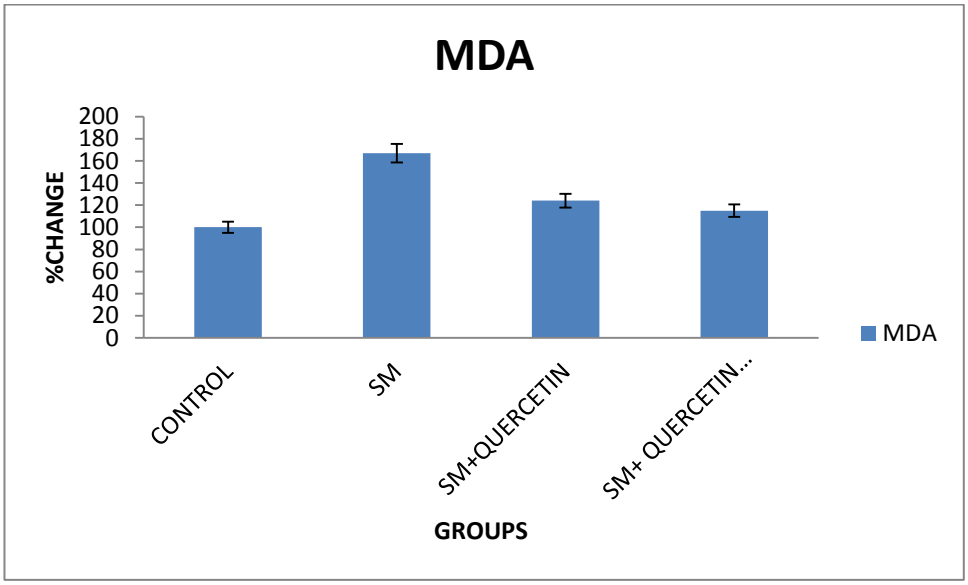


*P<0.05, compared to control group (One way ANOVA followed by Dunnett's t test).

#P<0.05, compared to Positive control group (One way ANOVA followed by Dunnett's t test).

Control value for: Hepatic GSH=7.2±0.4 µmoles/gm tissue

Figure 25 - Effect of pretreatment of quercetin and quercetin microspheres on Tissue GSH against sulphur mustard



*P<0.05, compared to control group (One way ANOVA followed by Dunnett's t test).

#P<0.05, compared to Positive control group (One way ANOVA followed by Dunnett's t test).

Control value for: hepatic MDA=4.43±0.5nmol/gm tissue

Figure 26 - Effect of pretreatment of quercetin and quercetin microspheres on tissue MDA against sulphur mustard

CONCLUSION

The present work was aimed at exploitation of pH-sensitive polymer Eudragit S100 for colon-specific delivery of Quercetin Dihydrate and, further, at achieving mucoadhesion of the core microspheres by use of mucoadhesive polymer ethyl cellulose. The best fit release kinetic model was found to be Higuchi for all formulations. In conclusion, the In vivo studies on male wistar rats were conducted and plasma concentration time method was employed to study the influence of quercetin by formulating it as microspheres. The semi log plot of pure drug and its microspheres showed linearity which indicates that they follow linear kinetics. Thus, it can be concluded that the absorption kinetics and availability of quercetin is modified and altered by formulating it as microspheres. Quercetin-microspheres more effectively showed the antidote activity against sulphur mustard.

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