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Albendazole encapsulation along with quercetin in chitosan-alginate microspheres enhances bioavailability and sustained release in broilers

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Abstract
The present study investigated the pharmacokinetics of Albendazole (ABZ) administered orally along with quercetin in chitosan-alginate encapsulated microspheres to broilers. Thirty two adult broilers were divided into four groups with eight birds in each group and treated with 10mg/kg b.wt pure albendazole orally to group I and intravenously to group II while groups III received chitosan alginate microspheres impregnated with albendazole (CS-ALG-ABZ) equivalent to deliver 10 mg/kg of ABZ and group IV received chitosan alginate microspheres impregnated with albendazole and quercetin (CS-ALG-ABZ-QUE) equivalent to deliver 10 mg/kg b.wt. of ABZ. Plasma separated from blood samples collected at various intervals for HPLC estimation of ABZ and albendazole sulphoxide (ABZ-SO) to carry out pharmacokinetic analysis by non-compartmental model.

ABZ could not be detected in Group-I at any point of time, while in other three groups ABZ was detectable up to 9-12h after administration. The C_{max} of ABZ in groups II and IV was significantly higher when compared to group III, while t_{max} was significantly higher in group IV when compared to groups II and III. The AUC observed in group IV was significantly (~2.5 fold) higher compared to groups II and III. The volume of distribution and clearance of ABZ in group IV was significantly lower when compared to groups II and III.

Albendazole sulphoxide, the active metabolite of albendazole, could be detected from one hour to 48 h in group IV compared to 0.5 to 36 h in group I, 0.083 to 36 h in group II and 0.5 to 48 h in group III. The pharmacokinetics of ABZ-SO revealed a significantly higher $t_{½}$ and C_{max} in groups III and IV when compared to group I.

The AUC observed in group II, III and IV was significantly higher compared to group I though the AUC observed in group IV was non-significantly lower than that of group III. The MRT observed in group IV was longer than that observed in groups I and II. Similar to its parent compound ABZ-SO also showed similar trend with respect to V_d and clearance.

The results in present study indicate that the quercetin increased the absorption of ABZ and decreased its metabolite formation probably by inhibiting intestinal/hepatic CYPs. The formulation containing quercetin prolonged the absorption and elimination of the active metabolite of ABZ-SO as evidenced by increased C_{max}, AUC, and MRT observed in groups III and IV.

In conclusion ABZ absorption enhanced with relative improvement in bioavailability of its active metabolite albendazole sulphoxide by administering chitosan alginate microspheres impregnated with albendazole along with quercetin.

Keywords: Albendazole, pharmacokinetics, chitosan alginate microspheres, quercetin

Introduction
One of the major causes for the production and economic losses in livestock sector is helminthic infections and to control Benzimidazoles (BZD) are the major class of anthelmintic drugs used in therapeutics [1, 2]. Among the benzimidazoles, albendazole (ABZ) the broad-spectrum and a safe anthelmintic has been used for over 20 years even though it is poorly absorbed from the gastrointestinal tract due to its low aqueous solubility and first pass metabolism [3]. Maximum bioavailability is important parameter for therapeutic use of drugs because the bioavailability directly influences therapeutic efficacy after oral drug administration. Recent approach is formulation of drugs with an attempt to improve bioavailability of active ingredient and there by reduction in cost of therapy and adverse effects [4, 5] In this context Albendazole was selected as a drug for formulating oral drug delivery system to obtain better pharmacological effect with sustained release for prolonged effect against both GI and extra GI helminthic infection.

Chitosan a natural biodegradable polymer forms complex with alginate can be used in the formulation of drug delivery system by encapsulation of active ingredient in chitosan microspheres [6] there by enhancing the bioavailability and efficacy [7]. Chitosan microspheres are used as drug carriers and slowly deliver at the area of interest over desired period of time to maintain an effective local concentration [8]. Chitosan improves the transport of drugs across biological membranes through adhesion and increases paracellular permeation and absorption of drugs *in vitro* and *in vivo* [9, 10, 11]. Chitosan alginate encapsulation is an effective strategy to encapsulate drug and phytochemical combination where phytochemical influences the release of drug from microspheres and also improve bioavailability/effectiveness [12].

Bioavailability enhancer/ Bio-enhancer administration can improve the bioavailability of active drug molecules by inhibition of CYP3A4 enzymes and/or P-glycoprotein a multi drug resistance transports in the gut wall and liver [13]. One such bioenhancer is quercetin a plant derived flavonoid glycoside which enhances bioavailability of doxorubicin, [14] ranolazine, [15] and valsartan [16] in rats.

Hence, the present study designed to prepare chitosan alginate microspheres impregnated with albendazole along with quercetin and study the pharmacokinetics of albendazole in broilers.

Materials and Methods

Experimental animals

Adult birds were procured from M/s Sneha Hatcheries Pvt. Ltd, Yerraguntla village, Kadapa (Dist), India. They were maintained under standard management conditions with 24 hours free access to feed and water. The experiments were approved by Institutional Animal Ethics Committee (IAEC), College of Veterinary Science, Proddatur, Andhra Pradesh, India.

Drugs and chemicals

Pure technical grade albendazole, albendazole sulfoxide, quercetin were procured from Sigma Aldrich, USA. Acetonitrile and other chemicals of HPLC grade used in the experiment were procured from M/s Merck, Mumbai, India. Water for HPLC was obtained by Millipore water purification system and was filtered through 0.2 µm filter prior to use. All other chemicals used in the study were of analytical grade obtained from Sd fine-CHEM. Ltd, Mumbai and Hi-media, Pvt. Ltd, Mumbai.

Preparation of Albendazole solutions

A 2% ABZ solution for intravenous administration was prepared by dissolving 0.2 g of pure ABZ in a solution containing 0.2 ml of dimethyl sulphoxide (DMSO) and 2.0 ml of propylene glycol and the final volume made up to 10 ml with HPLC water. The formulation was mixed until complete dissolution of the ABZ whereas for oral administration a 2% ABZ suspension was prepared by mixing 0.5 g of pure ABZ along with 0.125 g of carboxy methyl cellulose in 25 ml of HPLC water.

Preparation of Chitosan-alginate encapsulated albendazole microspheres and Chitosan-alginate encapsulated albendazole along with quercetin microspheres

Chitosan-alginate encapsulated albendazole (CS-ALG-ABZ) was prepared as per the procedure described by manuroop *et al.*, 2020 [12]. Albendazole (0.4 g) was mixed with 20 mL of 2.5 per cent sodium alginate. The solution was added drop wise @ 30 mL/h under constant stirring to 100 mL of 0.1 per cent chitosan in 2 per cent acetic acid and 1.5 per cent of calcium chloride solution (pH adjusted to 5.5 using 10 per cent NaOH). Stirring was continued for an hour for the polymerization of chitosan and alginate. Then the microspheres were filtered and washed thrice with distilled water. Finally, acetone was added for drying the microspheres. The drying of microspheres was confirmed by achieving constant weight on consecutive days. The microspheres were stored in air tight container at 4^0C until further use whereas preparation of Chitosan-alginate encapsulated albendazole along with quercetin (CS-ALG-ABZ-QUE) was almost similar except that along with albendazole (0.4 g) and 10 mg of quercetin was also mixed in 20 ml of 2.5% sodium alginate.

Loading efficacy of Albendazole microspheres & albendazole along with quercetin microspheres.

Albendazole concentration of chitosan-alginate encapsulated microspheres was estimated to know the ABZ loading efficiency. A 25 mg of microspheres (CS-ALG-ABZ and CS-ALG-ABZ-QUE separately) were crushed into powder and treated with solution containing 47.5 ml of 0.1 N HCl and 2.5 ml of methanol. The resulting mixture was stirred at 250 rpm for 2 h and the temperature was maintained at 37±0.2ºC. The solution was filtered and filtrate was analysed for ABZ using HPLC at 225 nm.

Experimental Design

Thirty two adult birds weighing about 2-2.5 kg were randomly divided into four groups with eight birds in each group. Group I and II birds were administered with standard albendazole @ 10mg/Kg oral and Intra venous respectively whereas group III and IV birds were administered with Chitosan-alginate encapsulated microspheres with albendazole (10 mg/kg, Oral) and Chitosan-alginate microspheres with encapsulated albendazole and quercetin (10 mg /kg, Oral).

Blood sample of 0.5ml collected from either left or right tarsal vein at the intervals 0 h, 0.0833 h, 0.25 h, 0.5 h, 1 h, 3 h, 6 h, 9 h, 12 h, 15 h, 24 h, 30 h, 36 h and 48 h for intravenous route treated group whereas 0 h, 0.5 h, 1 h, 3 h, 6 h, 9 h, 12 h, 15 h, 24 h, 30 h, 36 h and 48 h for oral route treated group in to the heparinised eppendorf tube. The collected samples were centrifuged at 2000 rpm for 10 min at 37 ºC and the supernatant (plasma) was harvested and stored at -20 ºC for analysis for albendazole and albendazole sulphoxide.

Assay of Albendazole and albendazole sulphoxide

Albendazole and albendazole sulphoxide estimation in blood samples was carried out as per the methods described by shah *et al.*, 2014 [17] and valois *et al.*, 1994 [18] respectively by using HPLC. Briefly, acetonitrile was added to plasma sample in the ratio of 1:2, after vortex mixing at high speed for 5 min, the tubes were subjected to centrifugation for 15 min at 5000 rpm. The clear supernatant thus obtained was filtered through a 0.2 µm nylon membrane filter and 20 µL of filtrate was injected into the HPLC system.

The HPLC system comprised of C18 reversed-phase column (particle size 5µm, 4.6 mm x 250 mm) as the stationary phase

and photo diode array detector (SPD-M20A). A mixture of 60 parts of acetonitrile and 40 parts of water (pH 3.2 adjusted with 10% ortho-phosphoric acid) as mobile phase at a wave length of 225 nm. The flow rate was adjusted at 1.0 $mLmin^{-1}$ with 10 minutes run time. Chromatographic conditions for albendazole sulphoxide were a mixture of 30 parts of acetonitrile and 70 parts of 0.25 N sodium acetate buffer (NaOAc) (pH adjusted with glacial acetic acid to 5.0) as mobile phase at flow rate of 1.5 ml/min with runtime of 10 minutes at 290 nm respectively. There were no interfering substances in the plasma at the retention time of albendazole and albendazole sulphoxide as evident by the chromatograms obtained for plasma blank and spiked plasma standards. Peak areas were taken for the quantification of albendazole and albendazole sulphoxide in plasma from calibration curves obtained on analysis of blank plasma samples spiked with albendazole (external standards) and analysed as described for the experimental samples. The limit of quantification for albendazole and albendazole sulphoxide was 0.078μgml^{-1}. The method was found to be linear and reproducible in the concentration range of 0.078- 5 $\mu g.ml^{-1}$ albendazole and albendazole sulphoxide.

Pharmacokinetic analysis

Plasma concentration versus time data of albendazole and albendazole sulphoxide obtained in birds were utilized for calculating various pharmacokinetic parameters with an interactive least squares linear regression by non-compartment model using computer software (PK Solver Version 2.0, 2010 by Zhang Yang) [19]. Best-fit model was chosen by using minimal Akaike information criteria estimation [20]. Peak plasma concentration (Cmax) and time to reach peak concentration (t_{max}) were calculated from the actual plasma data of each bird.

Bioavailability of ABZ and ABZ-SO was calculated for orally administered ABZ in different preparation of the birds by using the AUC of standard (group I), chitosan encapsulated albendazole (group III) and chitosan encapsulated with quercetin albendazole (group IV) with AUC of ABZ administered intravenously (group II).

Further the relative bioavailability of albendazole sulphoxide (an active metabolite of ABZ) was calculated to express the bioequivalence of ABZ administered as CS-ALG microspheres and ABZ administered along with quercetin as CS-ALG microspheres with standard ABZ.

Statistical analysis

The plasma concentrations and pharmacokinetic variables of albendazole and albendazole sulphoxide are expressed as mean±S.E. Differences in plasma concentrations and pharmacokinetic data between two groups were analyzed for statistical significance using independent t-test whereas differences in plasma concentrations and pharmacokinetic data between all the groups were analyzed using One-Way ANOVA with Post Hoc test using Tukey's SPSS 17.0. AUC, $t_{½}$ β, C_{max} values were log transformed prior to the analysis. All P values <0.05 were considered statistically significant.

Results

Characterization of microspheres

Shape and size

The microsphere synthesized using chitosan-alginate-albendazole and quercetin complex was spherical (fig:1) with a diameter ranging from 0.475 mm to 0.552 mm. The mean diameters of various microspheres synthesized are presented in table 1. There was no significant difference between the mean diameter of CS-ALG, CS-ALG-ABZ and CS-ALG-ABZ-QUE.

A B

Fig 1: A) Chitosan-alginate- albendazole microsphere; B) Chitosan-alginate- albendazole- quercetin microsphere

Table 1: Mean diameter (mm) of chitosan-alginate encapsulated compounds

Encapsulated compounds	Mean diameter (mm)
CS-ALG	0.475±0.036[a]
CS-ALG-ABZ	0.512±0.033[a]
CS-ALG-ABZ- QUE	0.552±0.035[a]

Values are mean ± SE. Means with same superscripts do not differ significantly (P<0.05) different. One way ANOVA followed by Duncans *post-hoc* test using IBM SPSS software 19.0 version. CS = Chitosan; ALG = Alginate; ABZ = Albendazole, QUE= Quercetin

Drug loading

The concentration of albendazole in chitosan encapsulated microspheres was 5.25 mg and chitosan encapsulated albendazole with quercetin microspheres was 6.75 mg, per 25 mg of formulations.

Assay of albendazole and albendazole sulphoxide and pharmacokinetic analysis

Albendazole and ABZ-SO estimated in plasma samples collected at different time intervals (0, 0.5, 1, 3, 6, 9, 12, 15, 24, 30, 36 and 48 hours of post dosing) after oral

administration of standard ABZ (10 mg/kg b.wt) to the group I broilers. Albendazole was not detected at any time point, whereas ABZ-SO was detected from the collected plasma samples and mean plasma level of ABZ-SO in group I is graphically presented in Fig.2. Various pharmacokinetic parameters estimated by noncompartmental analysis of plasma concentrations of ABZ-SO in group I of birds are summarized in Table 2. The various pharmacokinetic parameters of ABZ-SO observed in group I were elimination rate constant (β) (0.09±0.01), elimination half-life (7.26±0.6 h), Cmax (1.08±0.16 µg/ml), T_{max} (6.00±0.56 h), area under plasma concentration time curve (15.10±1.94 µg/h.ml). Other pharmacokinetic parameters like AUMC, MRT, Vd, Cl_B obtained in the present study was 183.75±23.94 µg/h^2.ml, 12.28±0.78 h, 7.67±0.95 L/Kg and 0.72±0.07 L/Kg/h respectively.

The plasma samples collected at different time intervals 0 (blank), 5, 15, 30 minutes, 1, 3, 6, 9, 12, 15, 24, 30, 36 and 48 hours of post dosing after intravenous administration of standard ABZ (10 mg/kg b.wt) to group II birds and estimated for ABZ and ABZ-SO to carry out pharmacokinetic analysis. The mean plasma ABZ concentrations of group II birds was 0.32±0.02 µg/ml, which was achieved at 0.083 h and gradually declined to 0.01±0.00 µg/ml at 9 h (Fig. 2) whereas, the ABZ-SO mean peak plasma concentration of 1.61±0.08 µg/ml was achieved at 6 h and gradually declined to 0.17±0.04 µg/ml at 36 h (Fig. 2). When data was analysed by noncompartmental method the mean values for ABZ & ABZ-SO for elimination half-life ($t_{½}$), maximum plasma concentration (Cmax), area under plasma drug concentration curve ($AUC_{0-\infty}$) were found to be 1.81 ± 0.17 h and 8.78 ± 1.02 h; 0.32 ± 0.02 and 1.63 ± 0.07 µg/mL and 0.53 ± 0.04 and 30.0 ± 2.08 µg/h.mL respectively. The other pharmacokinetic parameters ABZ and ABZ-SO are presented in Table 2.

After oral administration of ABZ at 10 mg/kg b.wt as chitosan-alginate encapsulated microspheres to birds of group III, the ABZ mean peak plasma concentration of 0.18±0.04 µg/mL was achieved at 1h and it gradually declined to 0.01±0.00 µg/mL at 9 h (Fig 2), whereas the ABZ-SO mean peak plasma concentration of 1.87±0.22 µg/ml was achieved at 6 h and it gradually declined to 0.16±0.01 µg/ml at 48 h (Fig. 2). When data was analyzed by non-compartmental method the mean values of ABZ for elimination half-life ($t_{½}$), the mean peak plasma concentration (Cmax) the mean area under plasma drug concentration curve was 1.84±0.37 h, 0.19±0.04 µg/ml, 0.44±0.06 µg/h.ml respectively. The same for ABZ-SO were 10.78±0.72 h, 1.95±0.21 µg/ml, and 42.12±4.59 µg/h.ml respectively. The other pharmacokinetic parameters were presented in table 2.

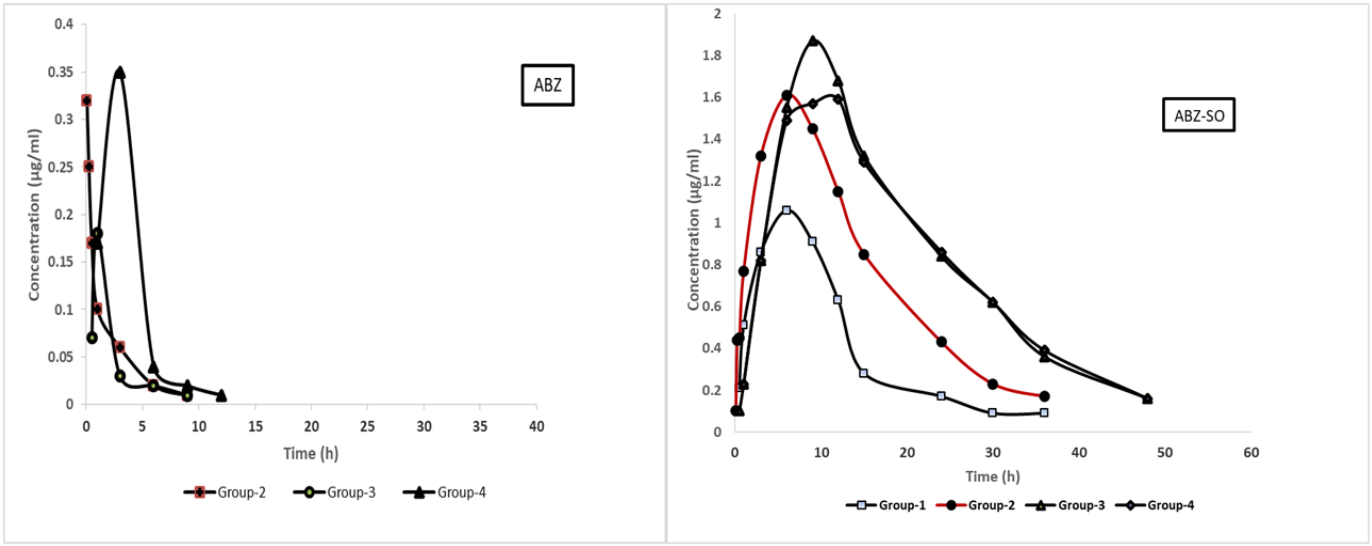
After oral administration of ABZ (10 mg/kg) along with quercetin as chitosan encapsulated microspheres to group IV birds, the mean peak plasma concentration of ABZ 0.35±0.02 µg/mL was achieved at 3 h and it gradually declined to 0.01±0.00 µg/mL at 12 h (Fig. 2) in the same group ABZ-SO mean peak plasma concentration of 1.59±0.08 µg/mL was achieved at 12 h and it gradually declined to 0.16±0.04 µg/mL at 48 h. (Fig. 2). When data was analyzed by non-compartmental method the mean values of ABZ for elimination half-life ($t_{½}$), maximum plasma concentration (C_{max}), and area under plasma drug concentration ($AUC_{0-\infty}$) were recorded as 1.62±0.13 h, 0.35±0.03 µg/mL, and 1.31±0.09 µg/h.mL respectively. The same for ABZ-SO were 11.93±1.41 h, 1.72±0.06 µg/mL, and 43.06±3.88 µg/h.mL, respectively. The other pharmacokinetic parameters were showed in table 2.

Table 2: Pharmacokinetic parameters of ABZ and ABZ-SO after single oral administration of ABZ (10 mg/kg) in different groups of birds (n=8)

Parameter	Group-I (ABZ oral)		Group-II (ABZ I.V)		Group-III (ABZ-CS-ALG oral)		Group-IV (ABZ-CS-ALG-QUE oral)	
	ABZ	ABZ-SO	ABZ	ABZ-SO	ABZ	ABZ-SO	ABZ	ABZ-SO
β	ND	0.09±0.01^{b}	0.42±0.05^{A}	0.08±0.01^{ab}	0.44±0.04^{A}	0.07±0.01^{a}	0.45±0.03^{A}	0.06±0.01^{a}
$t_{½}$	ND	7.26±0.61^{a}	1.81±0.17^{A}	8.78±1.02^{ab}	1.84±0.37^{A}	10.78±0.72^{ab}	1.62±0.13^{A}	11.93±1.41^{b}
Tmax	ND	6.00±0.57^{a}	0.08±0.00^{A}	6.37±0.37^{a}	1.00±0.00^{B}	8.63±0.89^{ab}	3.00±0.00^{C}	10.50±0.80^{b}
Cmax	ND	1.08±0.15^{a}	0.32±0.02^{B}	1.63±0.07^{b}	0.19±0.04^{A}	1.95±0.21^{b}	0.35±0.03^{B}	1.72±0.06^{b}
AUC_{0-t}	ND	14.17±1.96^{a}	0.50±0.04^{A}	27.42±1.34^{b}	0.411±0.06^{A}	39.60±4.36^{c}	1.28±0.09^{B}	36.50±2.25^{bc}
$AUC_{0-\infty}$	ND	15.10±1.94^{a}	0.53±0.04^{A}	30.04±2.08^{b}	0.44±0.06^{A}	42.12±4.59^{bc}	1.31±0.09^{B}	43.06±3.88^{c}
AUMC	ND	183.75±23.94^{a}	1.24±0.16^{A}	471.7±76.1^{ab}	1.19±0.25^{A}	839.76±113.24^{bc}	4.37±0.34^{B}	982.62±172.30^{c}
MRT	ND	12.28±0.78^{a}	2.28±0.17^{A}	15.09±1.45^{ab}	2.87±0.59^{A}	19.54±1.07^{bc}	3.35±0.14^{A}	21.66±1.91^{c}
Vd	ND	7.67±0.95^{b}	50.03±5.44^{AB}	4.16±0.27^{a}	71.89±17.97^{B}	3.98±0.44^{a}	18.63±2.02^{A}	3.95±0.24^{a}
Cl_B	ND	0.72±0.07	19.52±1.47^{B}	0.35±0.03	27.18±4.39^{B}	0.26±0.03	7.97±0.63^{A}	0.24±0.02
RB		-		-		299.8±37.2^{a}		283.5±31.3^{a}

Values are represented as mean±S.E on mean of 8 observations. Means with different alphabets as superscripts differs significantly $p<0.05$, capital alphabets for albendazole and small alphabets for albendazole sulphoxide.

ABZ: Albendazole
ABZ-SO: Albendazole sulphoxide
CS: Chitosan
ALG: Alginate
β: Elimination rate constant
$t_{½}$: Elimination half life
Vd: Volume of distribution
ND: Not Detected
Cl_B: Total body clearance
MRT: Mean Residence Time
RB: Relative bioavailability
Tmax: Time of maximum concentration in plasma
Cmax: Maximum (peak) plasma concentration
AUC: Area under the plasma concentration time curve
AUC_{0-t}: Area under the plasma concentration time curve from 0 h to 24h
$AUC_{0-\infty}$: Area under the plasma concentration time curve from 0 h to infinity



Albendazole (ABZ) Albendazole sulphoxide (ABZ-SO)

Fig 2: Semi logarithmic plot of ABZ and ABZ-SO plasma concentration vs time curve following oral and intravenous administration of ABZ (10mg/kg) to various group of birds. Each point represents mean ± S.E of eight broilers.

Discussion

Helminthiasis is one of the major reason for production lose and also causing mortality and morbidity in livestock. Benzimidazoles are most popular and widely used broad spectrum anthelmintic agents with high efficiency and wide margin of safety.

Albendazole is one of a versatile member of benzimidazole family that has gained popularity due to its efficacy and safety however, because of chemical and bacterial degradation associated with digesta and uncontrolled absorption and elimination, its therapeutic concentration may not be achieved systemically. Controlled drug delivery system like chitosan-alginate microsphere impregnated along with bio enhancer like quercetin would be a novel way to develop more efficacious preparation over the conventional one.

It was reported that flavonoid quercetin has strong inhibitory effect on cytochrome P450 (CYP3A4 and CYP2C9) activity [21] and/or also P-glycoprotein a multidrug resistant transporter in gut wall and liver [13] In view of the above facts, present study was undertaken to prepare CS-ALG microspheres impregnated with ABZ alone and along with quercetin and evaluate the pharmacokinetics of ABZ administered orally along with quercetin as chitosan alginate encapsulated microspheres in broilers at therapeutic single oral dose of 10mg/kg of body weight.

Chitosan alginate microspheres prepared with albendazole alone and albendazole along with quercetin were spherical with diameter of 0.475±0.036 mm (CS-ALG), 0.512±0.033 (CS-ALG-ABZ) and 0.552±0.035 (CS-ALG-ABZ-QUE). The synthesised CS-ALG microspheres size is almost similar to the size of CS-ALG microspheres prepared by Dash and Mishra, 2013 [22], and manuroop *et al.*, 2020 [12]. Concentration of ABZ in CS-ALG-ABZ microspheres was 5.25 mg and ABZ and Quercetin in CS-ALG-ABZ-QUE microsphere were 5.19 mg and 6.75 mg respectively for 25 mg of microspheres. Pharmacokinetic study conducted by administering 10 mg of Albendazole alone and microspheres equivalent to contain 10 mg of ABZ to various groups of birds.

In group- I pure ABZ treated orally to birds ABZ could not be detected at any time interval since ABZ undergoes first pass metabolism by liver microsomal enzymes [23, 24] and converted to ABZ-SO, which is major pharmacologically active metabolite and subsequently further transformed by CYP450 enzyme to an inactive metabolite. Due to this extensive metabolism the parent compound is undetectable in blood plasma, in various animal species and human beings [25, 26]. For this reason, many pharmacokinetic studies are developed with plasma concentrations of ABZ-SO [27, 28]. Thus the anthelmintic effect observed after oral administration of ABZ can be attributed to the presence and activity of ABZ-SO. Further there are reports of erratic/irregular absorption of ABZ administered orally [29]. Hence, in group I, pharmacokinetic parameters for ABZ could not be calculated. The other three Groups where ABZ was given as intravenous (group II), chitosan- alginate microsphere (group III) orally, and chitosan-alginate along with quercetin microspheres (group IV) orally, the pharmacokinetic parameters were analyzed by non-compartmental analysis. ABZ was detectable up to 9 h in group II (i.v route) and group III (chitosan-alginate microspheres), while it was detectable up to 12 h in group IV (chitosan-alginate with quercetin microspheres). The increased detection time for ABZ in group IV could be attributed to the increased intestinal absorption and inhibition of gut P-gp and reduced first pass metabolism by quercetin [14].

The elimination half-life and elimination rate constant of ABZ in all the groups were statistically similar. The peak plasma concentration (C_{max}) was significantly ($p<0.05$) higher in intravenous group (Group II) and quercetin added chitosan-alginate microspheres group (group IV) when compared to the chitosan-alginate microspheres group (group III). This can be attributed to the ability of quercetin to enhancing the absorption of co-administered drugs. Shin *et al.*, 2006 [30] reported that co-administration of quercetin with tamoxifen increased the C_{max} of tamoxifen. Which can also be attributed to the muco-adhesive property of the microspheres. The time taken to attain peak plasma concentration (t_{max}) of ABZ in group IV was significantly ($p<0.05$) higher when compared to the other groups indicating the prolonged absorption of ABZ, due to the presence of quercetin in the formulation.

Area under concentration time curve (AUC) is an important parameter to calculate the bioavailability of a drug and to determine the clearance of a drug from the body. The AUC_{0-t} and $AUC_{0-\infty}$ were significantly ($p<0.05$) higher (~2.5 fold) in group IV when compared to the groups II and III. Previous report by Dupuy *et al.*, 2003 [31] reveal that AUC of moxidectin was greater when used concomitantly with quercetin in lambs. Hence, it could be reasoned that quercetin influenced the absorption, metabolism and elimination of ABZ. In the present study the AUC recorded in group IV was highest and this indicates that more amount of drug was present for more time in the body.

Volume of distribution (V_d) is important parameter to know the extent of penetration of drug in the body tissues and to compute optimal dosage regimen of a drug that must be given to produce and maintain its therapeutic concentration in body. In groups II and III a significantly ($p<0.05$) higher volume of distribution was observed indicating the high lipid solubility, low ionization and/or low plasma binding capacity of ABZ when compared to the group IV which showed least volume of distribution in which quercetin was added to ABZ. These results are consistent with previous reports of Li and Choi 2009 [32] in which increase in the oral bioavailability of doxorubicin was attributed to the decreased the V_d.

Clearance describes how quickly the drugs are eliminated, metabolized and/or distributed throughout the body. groups II and III exhibited significantly ($p<0.05$) high clearance than that of group IV. The low clearance of ABZ in group IV can be attributed to the ability of quercetin to inhibit the metabolizing enzymes CYPs.

ABZ-SO was detectable from 0.5 h post administration in groups I, II and III and remained up to 36 h in group I and II and 48 h in group III. In group IV ABZ-SO could be detected beginning from one hour until 48 h post administration. The elimination rate constant was significantly ($p<0.05$) lowered in groups III and IV compared to that of group I. The elimination half-life was significantly ($p<0.05$) higher in groups III and IV when compared to that of group I and this indicated the prolonged absorption and elimination of ABZ which was also reflected by the presence of metabolite for prolonged time in modified formulations containing chitosan-alginate with quercetin.

The C_{max} of active metabolite ABZ-SO was significantly ($p<0.05$) higher in groups III and IV when compared to group I which can be attributed to complete/enhanced absorption of the parent drug as evidenced by the significant increase the C_{max} of ABZ in same groups. Kim *et al.*, 2009 [33] reported that quercetin increased the C_{max} of fexofenadine via the inhibition of P-gp mediated efflux during the absorption phase in the intestine. The C_{max} of ABZ-SO in modified formulation was non significantly higher when compared to group II that received ABZ by i.v route. The t_{max} of group IV was significantly ($p<0.05$) higher when compared to group II, indicating the prolonged and increased absorption of ABZ which was subsequently metabolized to ABZ-SO.

The AUC observed in groups II, III and IV was significantly ($p<0.05$) higher when compared to group I which indicates that more amount of metabolite was present for prolonged time. Among these three groups, group III showed significantly ($p<0.05$) higher AUC value when compared to Group II, indicating the extensive and prolonged absorption, with subsequent metabolism of drug. The AUC of ABZ-SO observed in group IV was none significantly lower than that of group III due to the inhibitory activity of quercetin on CYPs [14] which resulted in reduced metabolite formation.

The MRT observed in group IV was higher than that observed in groups I & II indicating the ability of quercetin to retain the metabolite for prolonged time. The prolonged MRT might be the result of the inhibition of CYP450 by quercetin as reported by Kumar *et al.*, 1981 [34] and Rahman *et al.*, 1994 [35]. This finding is in line with the plasma-concentration time profile in group IV, in which the initial concentration was detected only after one hour of administration. Further ABZ-SO could be detected up to 48 h in group IV. This phenomenon of slow and prolonged metabolite formulation by quercetin is due to its inhibitory activity on CYP3A4 and slow and study absorption from microspheres. Choi *et al.*, 2011 [14] reported increased oral bioavailability of doxorubicin due to enhanced absorption in the intestinal tract via the inhibition of P-gp and reduced first-pass metabolism via the inhibition of CYP3A subfamily in the small intestine and/or in liver rather than reduced renal and/ or hepatic elimination of doxorubicin by quercetin.

ABZ-SO showed similar trend as parent compound ABZ with respect to V_d and Cl whereas groups II, III and IV showed decrease in V_d and Cl as compared with group I. The decreased in V_d and Cl of group IV may be due to the presence of quercetin which can be correlated with findings of Li and Choi 2009 [32] that is the increase oral bioavailability of etoposide was mainly due to the decreased V_d and Cl.

Conclusion

The present study found that there is increase in absorption of ABZ and relative bioavailability of ABZ-SO when ABZ is entrapped in chitosan along with quercetin. Keeping in view of the enhanced absorption of albendazole and improved relative bioavailability of albendazole sulfoxide from microsphere formulations containing chitosan and quercetin, further appropriate studies can be performed to identify their efficacy in combatting systemic helminthic infections

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