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Cornsilk as a Bioavailable Source of Betasitosterol: A Pharmacokinetic Study Using HPTLC

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Abstract: Betasitosterol is one of the major phytoconstituents in cornsilk (style and stigma of *Zea mays* Linn.). This study is an attempt to standardize cornsilk as a bioavailable source of Betasitosterol and it reports the pharmacokinetic evaluation of slurry of cornsilk using HPTLC. The study was conducted on male albino rabbits of New Zealand strain. Blood samples were collected at different time intervals following a single oral administration of cornsilk slurry in water (1 g kg⁻¹ b.wt.). Absorption and elimination of Betasitosterol after ingestion of cornsilk was monitored using changes in the concentration of the marker band in the HPTLC profile. A marker of cornsilk at R_f 0.48, identified as Betasitosterol, is detected in rabbit plasma after 1 h of ingestion of the slurry. The marker reaches maximum concentration at 2.5 h post dose and is not detectable in plasma after 24 h. By applying Wagner-Nelson method, it can be deduced that over a 24 h period 32% of Betasitosterol is absorbed when administered in rabbits in the form of slurry of powdered cornsilk. The pharmacokinetic parameters generated, confirm significant bioavailability of Betasitosterol from cornsilk and the potential use of cornsilk as a natural source of Betasitosterol.

Key words: Betasitosterol, bioavailability, cornsilk, HPTLC, pharmacokinetics

INTRODUCTION

Establishing the pharmacological basis of the efficacy of herbal medicine is a constant challenge. Of particular interest is the question of bioavailability to assess to what degree and how fast compounds are absorbed after administration of a herbal medicine (Ahmad *et al.*, 2006). The therapeutic efficacy and safety of many drugs commonly used in clinical practice can be augmented by individualization of their dosage (Pal and Ganesan, 2004). The same principles that govern the effect of prescription drugs also influence the action of herbal preparations. Knowledge of absorption, distribution, metabolism and excretion for most herbal preparations is presently incomplete or unavailable (Kuruvilla, 2002). It is important to establish markers which can be used to monitor the absorption-elimination process of the herbal preparations. The feasibility of using a phytochemical marker to follow the absorption and elimination of plant slurry in a biological system using HPTLC has already been reported by Sane *et al.* (2004).

Betasitosterol is one of the key phytoconstituents of cornsilk (style and stigma of *Zea mays* Linn.); which has been traditionally used as a diuretic and is reported in ancient medicinal history for its use in treatment of urinary problems like nephritis and prostatitis (Caceres *et al.*,

1987; Doan *et al.*, 1992; Grases *et al.*, 1993; Newall *et al.*, 1996; Al-Ali *et al.*, 2003; Khare, 2004; Mueller and Mechler, 2005; Suzuki *et al.*, 2005; Velazquez *et al.*, 2005). Significant improvements in symptoms and urinary flow parameters in earlier studies have also proven the effectiveness of betasitosterol in the treatment of Benign Prostate Hypertrophy (BPH) (Berges *et al.*, 1995; Buck, 1996; Klippel *et al.*, 1997; Wilt *et al.*, 1999). Betasitosterol has been proven to be a safe, natural and effective nutritional supplement with no known toxicity or adverse drug events (Paniagua *et al.*, 2005). Plant sterols are relatively inexpensive and can safely be used in conjunction with conventional therapies in management of human health (Awad *et al.*, 2007, 2008; Bradford and Award, 2007; Brufaua *et al.*, 2008; Delaney *et al.*, 2004; Gomes *et al.*, 2007; Ju *et al.*, 2004; Li *et al.*, 2007; Li and Sinclair, 2002; Park *et al.*, 2007; Sanders *et al.*, 2000). Therefore, cornsilk as a source of Betasitosterol has great potentials, as a dietary supplement (Awad and Fink, 2000; Muti *et al.*, 2003). Bioavailability and pharmacokinetic data of betasitosterol from cornsilk has not yet been evaluated in either animals or humans. The results of this study will form baseline for research to determine the pharmacological activity and bioavailability of Betasitosterol from cornsilk leading to a possible extrapolation to humans.

MATERIALS AND METHODS

Cornsilk was collected from Mumbai, Maharashtra, India and was authenticated from National Botanical Research Institute (NBRI), Lucknow, India. Betasitosterol standard was procured from Sigma Aldrich Chemie (Steinheim, Germany). The solvents chloroform, toluene, ethyl acetate, methanol and glacial acetic acid were of analytical grade and were purchased from Qualigens Fine Chemicals, India.

The duration of the entire study from collection of plant material to sample preparation and finally standardizing the method took nearly three months from July 2006 to September 2006. The pharmacokinetic study was conducted as per the following schedule: - Study initiation date: 04-09-06; Experiment commenced on: 11-09-06; Experiment completed on: 13-09-06; Testing facility: Animal Testing Unit (CPCSEA/315); Ramnarain Ruia College; Matunga, Mumbai- 400 019.

Instrument: Camag HPTLC system with Cats 3 Version Software was used for the analysis. Camag Linomat IV was used as spotter and Camag Scanner II with mercury lamp was used for scanning.

Sample and standard preparation: Cornsilk was collected, washed and dried at 45°C to constant weight. Dried cornsilk, free from moisture was powdered, sieved through an 80 mesh (BSS) sieve and stored in an airtight container. Twenty five milligrams of the dried powder was accurately weighed and placed in a stoppered tube and 10 mL of methanol was added, the sample was vortexed for 1-2 min. and left to stand overnight at room temperature (28±2°C). The contents of the tube were filtered through Whatmann filter paper No. 41 (E. Merck, India) and the filtrate was diluted to 25 mL with methanol in a volumetric flask. A stock solution of betasitosterol (1000 µg mL⁻¹) was prepared in methanol.

Chromatography: Chromatography was performed on aluminium backed silica gel 60 F₂₅₄ HPTLC pre-coated plates. Before use, the plates were washed with methanol and activated at 110°C for 2 h. Samples (10 µL) were spotted 10 mm from the edge of the prewashed and activated plates by means of a Camag Linomat IV sample applicator and the plates were developed to a distance of 85 mm in a Camag twin-trough chamber previously equilibrated with the mobile phase; toluene-ethyl acetate-methanol-glacial acetic acid, 8+1+0.5+0.3 (v/v). The chromatographic conditions had previously been optimized to achieve the best resolution and peak shape. Chromatograms were evaluated densitometrically at λ = 366 nm by means of a Camag Scanner II in

fluorescence/reflectance mode using mercury lamp in conjunction with Cats 3 Version Software.

The chromatographic plate of Betasitosterol standard, spiked rabbit plasma and cornsilk extract is shown in Fig. 1. The corresponding HPTLC densitogram is shown in Fig. 2a-c.

Linearity of detector response for betasitosterol standard: Solutions containing Betasitosterol at ten different concentrations (10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 µg mL⁻¹) were prepared in methanol. Each of these solutions (10 µL) was applied to the plate, the plate was developed, and the detector response for the different concentrations was measured.

A graph was plotted of the concentration of Betasitosterol against peak area. The plot was linear in the range from 10 to 100 µg mL⁻¹. This experiment was performed in triplicate and the mean value was used for calculations. The data obtained showed that the response for Betasitosterol standard was linear between 10 µg mL⁻¹ to 100 µg mL⁻¹ with a correlation coefficient of 0.996. The line of regression had a slope of 29.02 and intercept of 89.96. The chromatographic plate of linearity of Betasitosterol standard is shown in Fig. 3.

Linearity of detector response for plasma spiked with betasitosterol standard: Betasitosterol at eight different concentrations (35, 70, 105 (LQC), 120, 140 (MQC), 210,

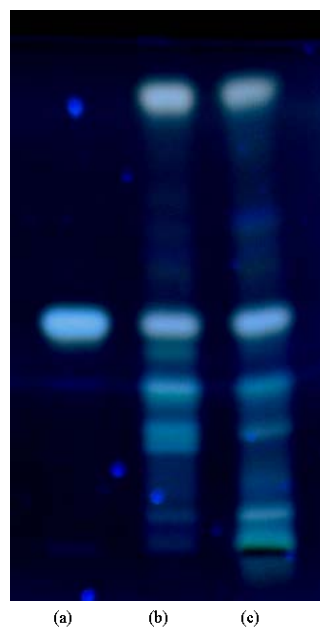


Fig. 1: HPTLC fingerprint showing comparison of Betasitosterol standard, spiked rabbit plasma and cornsilk extract. (a) Betasitosterol, (b) spiked rabbit plasma and (c) cornsilk extract

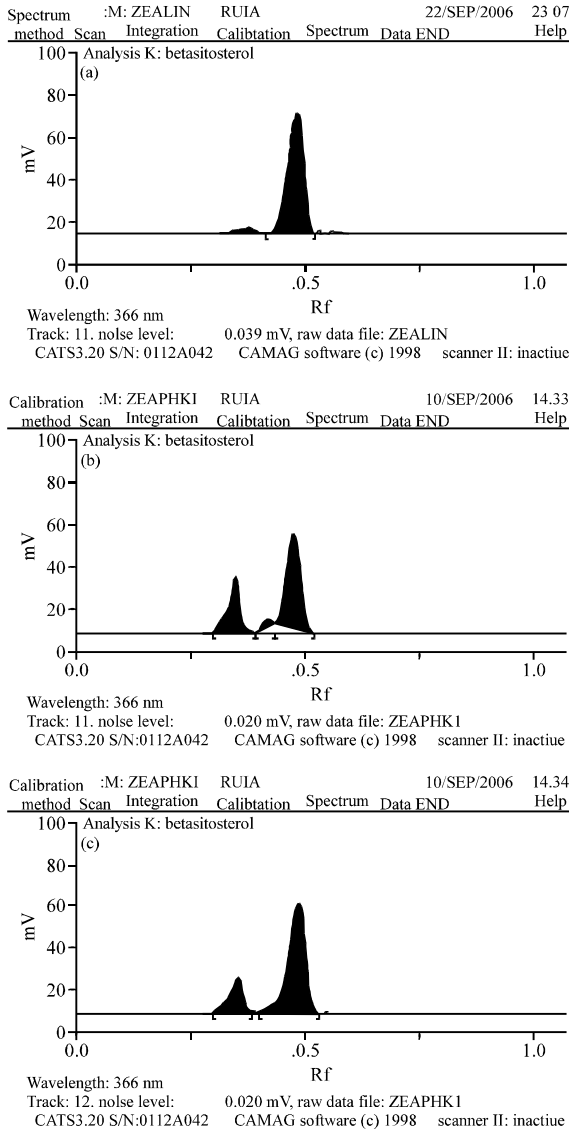


Fig. 2: HPTLC densitograms of Betasitosterol standard, spiked rabbit plasma and cornsilk extract. (The figure has been scanned at 500 dpi resolution power). (a) Betasitosterol, (b) spiked rabbit plasma and (c) cornsilk extract

280 (HQC) and 350 ng) were prepared in methanol. 0.5 mL of blank rabbit plasma was transferred to clean and dry stopper test tubes. Each tube was spiked with the above concentrations of Betasitosterol. Chloroform (5 mL) was added to each test tube and the tubes were shaken well for 10 min and then kept undisturbed for 30 min. The organic layer was isolated carefully and transferred to evaporating tubes. The tubes were placed in a water bath at 50°C and the residue obtained after evaporation was reconstituted in chloroform (200 µL). Each of these solutions (10 µL) was applied to the plate, the plate was

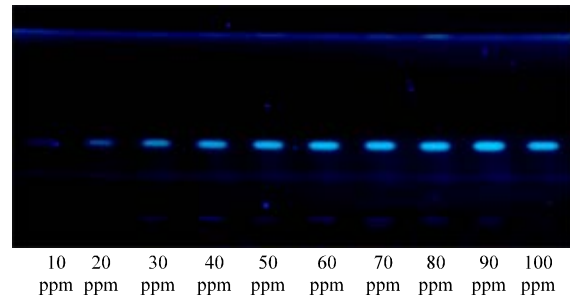


Fig. 3: HPTLC fingerprint showing linearity of Betasitosterol standard after derivatization with Libermann-Burchard reagent under fluorescence (366 nm)

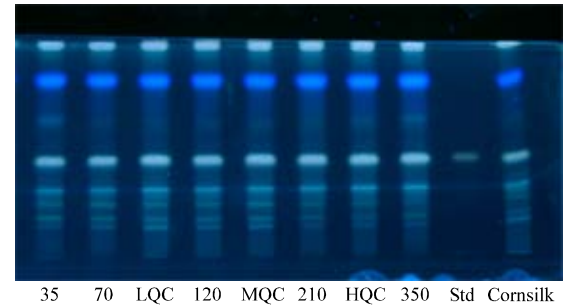


Fig. 4: HPTLC fingerprint showing linearity of plasma spiked with Betasitosterol standard after derivatization with Libermann-Burchard reagent under fluorescence (366 nm)

developed and the detector response for the different concentrations was measured. A graph was plotted of the concentration of Betasitosterol in spiked plasma against peak area. The plot was linear in the range from 35 to 350 ng. This experiment was performed in triplicate and the mean value was used for calculations. The data obtained showed that the response for plasma spiked with Betasitosterol standard was linear between 35 to 350 ng with a correlation coefficient of 0.986. The line of regression had a slope of 5.48 and intercept of 97.96. This linearity was used for calculating the concentration of Betasitosterol in plasma of rabbit administered with cornsilk slurry. The chromatographic plate of linearity of plasma spiked with Betasitosterol standard is shown in Fig. 4.

Animal treatment: Male albino rabbits of New Zealand strain were starved for 18 h before administration of cornsilk. A blank sample (0 h) of blood (2 mL) was collected from the marginal ear vein before dosing and then the rabbit was fed orally with 1 g kg⁻¹ body weight of cornsilk powder suspended in 10 mL distilled water, using a number 10 gavage needle. After administration of the

suspension, blood samples at 1, 2, 2.5, 3, 4, 8, 24 and 48 h were collected in heparinized eppendorf tubes.

Extraction from plasma: The blood samples were centrifuged at 1500 g for 15 min and 0.5 mL of plasma was transferred to clean and dry stoppered test tubes. Chloroform (5 mL) was added to each test tube, shaken well for 10 min and then kept undisturbed for 30 min. The organic layer was isolated carefully and transferred to evaporating tubes. The tubes were placed in a water bath at 50°C and the residue obtained after evaporation was reconstituted in chloroform (200 µL), vortexed for 2 minutes and was used for HPTLC analysis (Sethi, 1996).

RESULTS

Use of precoated silica gel HPTLC plates with toluene-ethyl acetate-methanol-glacial acetic acid, 8+1+0.5+0.3 (v/v) as mobile phase resulted in good separation of Betasitosterol band from the matrix components. The chromatographic plate and overlay of scanned densitograms showing response during pharmacokinetic evaluation is depicted in Fig. 5 and 6, respectively.

A marker of corn silk at $R_f = 0.48$ is detected in rabbit plasma after 1 h of ingestion of the plant slurry. The marker reaches maximum concentration at 2.5 h post dose and is not detectable in plasma after 24 h post dose. Absorption and elimination of the marker after ingestion of cornsilk is followed using changes in the concentration of the marker in the HPTLC profile. The marker could be identified as Betasitosterol using the corresponding bands obtained for standard betasitosterol spiked in plasma and those obtained in the plant extract. A graph showing the absorption-elimination pattern of Betasitosterol from cornsilk ($R_f = 0.48$) in rabbit plasma is given in Fig. 7 as a plot of peak area against time.

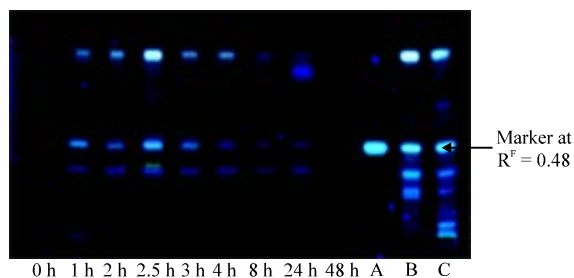


Fig. 5: HPTLC plate showing response during pharmacokinetic evaluation after derivatization with Liebermann-Burchard reagent under fluorescence (366 nm)

Using the linearity of response obtained for plasma spiked with betasitosterol the concentration of Betasitosterol in plasma of rabbit administered with cornsilk slurry was calculated. The results obtained are listed in Table 1. The pharmacokinetic parameters generated for cornsilk after oral administration using the absorption-elimination curve are listed in the Table 2.

Table 1: Concentration of betasitosterol in plasma of rabbits administered with slurry of cornsilk

Time (h)	Peak area	Conc. (ng mL ⁻¹)
0	0	0.00
1	570.4	86.12
2	733.5	115.86
2.5	955.8	156.38
3	627.5	96.53
4	357.1	47.24
8	217.7	21.83
24	43	0.00
48	0	0.00

Table 2: Pharmacokinetic parameters

Parameters	Values
C_{max}	156.38 ng mL ⁻¹
T_{max}	2.5 h
AUC_{0-4}	485.36 ng mL ⁻¹ * h
$AUC_{0-\infty}$	553.06 ng mL ⁻¹ * h
$t_{1/2}$	2.15 h
K_{el}	0.3224

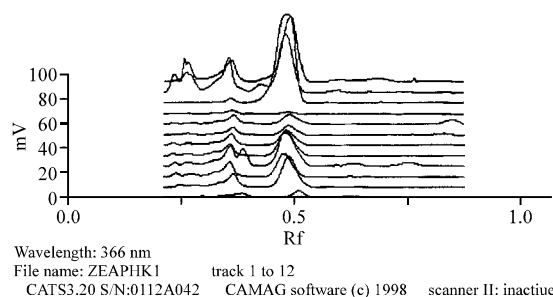


Fig. 6: Overlay of scanned densitograms showing response of marker obtained in rabbit plasma after administration of cornsilk. (a) Betasitosterol, (b) spiked rabbit plasma and (c) cornsilk extract

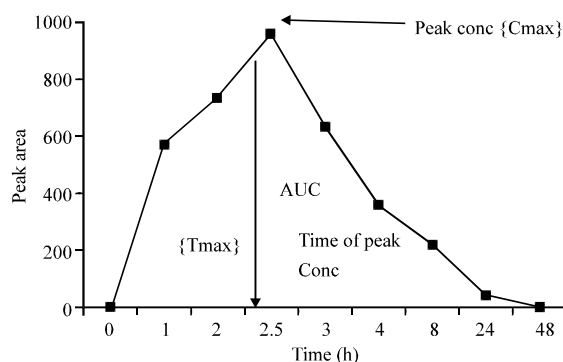


Fig. 7: Pattern of absorption and elimination of the phytochemical marker of cornsilk

Table 3: Blood concentrations and associated data for betasitosterol

Time (h)	C _p	[AUC] _{0-t} ^a	[AUC] _{0-∞} ^b	k[AUC] _{0-t} ^c	C _p +k[AUC] _{0-t} ^d	Ab/Ab [∞]	1-Ab/Ab [∞]	Drug absorbed (%)
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	86.12	43.06	43.06	13.88	99.99	0.15	1.00	15.15
2	115.86	100.99	144.05	46.43	162.29	0.25	0.75	24.59
2.5	156.38	68.06	212.11	68.37	224.75	0.34	0.66	34.05
3	96.53	63.23	275.34	88.75	185.29	0.28	0.72	28.07
4	47.24	71.89	347.22	111.92	159.16	0.24	0.76	24.11
8	21.83	138.14	485.36	156.44	178.28	0.27	0.73	27.01
24	0	174.64	660	212.74	212.74	0.32	0.68	32.23
48	0	0	660	212.74	212.74	0.32	0.68	32.23

where, C_{max} = Maximum plasma concentration, t_{max} = Time for maximum plasma concentration, AUC_{0-t} = Area under plasma concentration time curve 0 to t h, AUC_{0-∞} = Area under plasma concentration time curve 0 to ∞, t_{1/2} = Elimination half life, K_e = Elimination rate constant.

Assuming that the phytoconstituent (Betasitosterol) follows a one compartment model, the rate of absorption is determined by Wagner-Nelson method (Shargel and Yu, 1999). The percentage absorbed into the blood at various time intervals is listed in Table 3 where, C_p = plasma drug concentration, Ab = amount of drug absorbed, Ab[∞] = amount of drug absorbed at time t = ∞, Ab/Ab[∞] = fraction of drug absorbed, 1-Ab/Ab[∞] = fraction of drug unabsorbed, AUC = area under curve, t_n and t_{n-1} are time points.

In the present bioavailability study, 48 h is considered as infinity, because the blood concentration at that point already is below detection levels. By applying Wagner-Nelson method (Shargel and Yu, 1999) it can be deduced that over a 24 h period 32% of Betasitosterol is absorbed when administered in rabbits in the form of slurry of powdered cornsilk.

DISCUSSION

The bioavailability of Betasitosterol in humans is generally reported to be low (Gould, 1955; Borgstrom, 1968; Gould *et al.*, 1969). Bio availability studies of betasitosterol from cornsilk in rabbits has not been reported earlier. Also preclinical pharmacokinetic data and related findings for such a kind of study has not been documented. Recent data suggest that extrapolation of *in vivo* preclinical pharmacokinetic data tends to be the most accurate method for predicting human pharmacokinetic parameters (Jolivet and Ward, 2005).

Also since, most of the cornsilk is thrown as a waste, cornsilk as a source of Betasitosterol has great potentials, as a dietary supplement (Awad and Fink, 2000; Muti *et al.*, 2003). Therefore, it will be of great therapeutic interest to evaluate the relative bioavailability of Betasitosterol from cornsilk powder with that from a formulation of Betasitosterol.

CONCLUSION

The study proves the bioavailability of betasitosterol and establishes the pharmacokinetic parameters for Betasitosterol from powdered cornsilk in rabbit. The study also demonstrates the feasibility of using HPTLC as a tool for evaluation of the pharmacokinetics of herbal formulations. The results of the study are of value in establishing the pharmacological basis for the bioavailability of Betasitosterol from cornsilk in humans. The current study demonstrates the bioavailability of Betasitosterol from powdered cornsilk and suggests the use of cornsilk as a food supplement in human.

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