Human Serum Albumin Based Nanoparticles of Bridelia Retusa Extract as Vaginal Microbicide

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ABSTRACT SUMMARY:

The present work presents human serum albumin based nanoparticulate system of methanolic extract of stem bark of Bridelia retusa as vaginal microbicide. The nanoformulation showed better cellular uptake as compared to the extract.

INTRODUCTION:

Microbicides represent one of the promising options for protecting women from HIV and STI. Microbicides are expected to provide protection by directly inactivating HIV or preventing HIV from attaching, entering or replicating in susceptible target cells and simultaneously maintaining normal microbial and vaginal defenses. One of the alternate therapies is to explore, identify and isolate new lead molecules from natural plant sources.

Herbal medicines provide rational means for the treatment of many diseases that are obstinate and incurable in other systems of medicine, hence may be a good source of new active agents. The objective of the present research work was to develop Human serum albumin (HSA) based nanoparticulate drug delivery system of Bridelia retusa stem bark methanolic extract (BRSBME) as vaginal microbicide. The Nanocarrier system was further surface modified for enhanced penetration of extract into the vaginal mucosa in the presence of mucus.

EXPERIMENTAL:

Stem bark of Bridelia retusa was extracted with methanol using hot continuous Soxhlet extractor for 18-24h. The ability of BRSBME to inhibit CXCR4 and CCR5 tropic HIV-1 (cell free) and its replication (cell associated) was assessed using high throughput TZM-bl assay. The anti HIV activity was further confirmed using T-lymphoid (PM-1) cell line. The extract was tested for toxicity and monolayer integrity using a transwell epithelial model system. The cytotoxicity and anti HIV activity was then tested on ectocervical tissue of hysterectomized patients using open explant cultures. The extract was also tested for inhibition of sexually transmitted infections (C. albicans, N. gonorrhoeae and H. duc eryi) by agar disc diffusion method.

Nanostructured polymeric carrier system was fabricated for incorporation of BRSBME in Human serum albumin using high pressure homogenization. The method was optimized for product and process variables. The nanosystem was further surface modified using appropriate stealthing agent. The formulations were characterized for particle size and surface properties using PCS, TEM and SEM analysis. Visualization of vaginal uptake was studied in vivo in rat by confocal laser scanning microscopy (CLSM) studies using rhodamine B6 labelled nanoparticles as the fluorescent marker. Safety evaluation was conducted using acute and repeated dose toxicity studies using rats as per the OECD guidelines.
Fig. 1: TEM and SEM images of developed nanoparticles

Fig 2: Confocal scanning microscopy images of rat vagina after administration of extract and developed nanoparticulate formulations
A: Bright field; B: Red channel; C: Overlay of bright field and red channel

Fig 3: Histopathology of rat vaginal after repeated application of NP

Fig 4: Histopathology of rat vaginal after repeated application of SMNP
A: Upper (cervico) vagina; B: Middle vagina; C: Lower (uro) vagina.

RESULTS AND DISCUSSION:
BRSBME exhibited 80% viability at 2 µg/ml and weak inhibition of Cell free (CF) X4 and R5 HIV-1 laboratory strains (IC_{80}: 2.2-2.3 µg/ml) and primary isolates (IC_{80}: 0.4-4 µg/ml) in TZM-bl assay. It showed 55% reduction of HIV-1 in open explant cultures at 2 mg/ml. Activity against N. gonorrhea and H. ducreyi was exhibited by the crude plant extract. With BRSBME showing good HIV activity it was loaded into human serum albumin nanoparticles. Stable, homogenous brown colored BRSBME HSA-based nanoparticles were successfully developed using high pressure homogenization technique and further surface modified.

Colloidal nature of the developed NP was observed from PCS results with mean particle size of bare NP estimated to be 152.4nm with PI of 0.083 while surface modified SMNP showed particle size of 192.6nm with PI of 0.035. SEM and TEM analysis of the formulations confirmed the particle size, spherical shape and coating of the surface modifier. Visualization of vaginal uptake in vivo in rat using CLSM revealed highest fluorescence intensity in epithelium and lamina propria. Better penetration and localization of fluorescent marker compound in epithelium and lamina propria was observed for nanoparticulate formulation as compared to the BRSBME(Fig 2) which reflects an ability for improved localization and ability to deliver the active to the target site. Acute and repeated dose vaginal toxicity studies revealed the nanoformulations to be safe (Fig 3 and 4).

CONCLUSION
Formulation of the herbal active extract as nanocarrier system and its surface modification led to prolonged localization at vaginal site with no significant toxicity. Accumulation of the nanoparticles in the vaginal mucosa has potential for its site-specific targeting opening perspectives for use as vaginal microbicide.

REFERENCES

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