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STUDY OF ANTIBACTERIAL EFFECT AND ANALGESIC EFFECT FROM EXTRACTION OF CAFFEINE FROM COFEA ARABICA AND TASTEMASKING BY INCLUSION COMPLEXATION OF BETACYCLODEXTRINE

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ABSTRACT
Caffeine is an xantheine derivative mild CNS activity largly found in Cofee *Cofea Arabica*, tea *Thea sinsensis*, which is the largest consumed drink after water in the world. Here the research work is an attempt to mask the taste of bitter tasted caffeine which is an anti psychotic drug and analgesic with antihypertensive and diuretic drug. In this innovative work betacyclodextrine was taken in different molecular weight ratio with caffeine in respective parameter. Pure drug was dissolute in distilled water and compared with physical mixture, kneading mixture. The solvent taken for the drug and betacyclodextrin complexation with comparison to phase solubility study with different solvent. After solvent optimization, temperature was optimized. The dissolution study was carried out and compared. The taste masking property was analyzed by scientific committee / ethical committee. Human volunteer was tasted the pure drug which was bitter in taste. After taste masking by beta cyclodextrine complexation, it was reported by volunteer to be masked bitter taste. The caffeine was extracted by the following method. First the leaves of coffee and fruit pulp, seeds were collected from medicinal/ herbal garden of Jeypore College of Pharmacy, which was confirmed by Swaminathan Research centre, Jeypore, as Cofea Arabica plant. It was percolated in hot water overnights and filtered. The collected sample was placed in separated funnel and added chloroform. After swirling it was stood for time till two phases separated and the chloroform was collected and evaporated till caffeine was found in crystal form. The complexed drug was found to have different improved physical characteristics like bulk density, tapped density, carrs' index, angle of repose. XRD report shown the complexed drug to have more stabilized than pure drug. As the complex shows fuse peaks at low intensities indicating more stable and soluble, compare to the pure drug having intensed peaks showng crystalline nature which indicates its non soluble nature. Micromeritics study of pure drug measured by tapped density, bulk density, angle of repose, carr's index, hausner's ratio which found to be 0.1742,0.2632,33.82%,1.52,33.52 respectively. After complexation it optimized to 0.293,0.374,23.43%,30.064°,1.306 of above parameters respectively. The antimicrobial test was conducted and the analgesic effect was reported, which found to be satisfactory.

KEYWORDS: Caffeine, comlexation, XRD, taste masking, kneading, partition coefficient, analgesic, antibacterial effect.

MANUSCRIPT
Caffeine is a central nervous system (CNS) stimulant of the methylxanthine class. It is a bitter, white crystalline purine, a methylxanthine alkaloid bases of deoxyribonucleic acid. The coffee tree, scientifically known as Coffea Arabica.

Extraction of Caffeine from Tea/ coffee leaves
Principle
In the method of extraction the solution of these dissolved compounds is referred to as the extract. In the case of Caffeine extraction from tea powder, the solubility of caffeine in water is 22mg/ml at 25°C, 180mg/ml at 80°C, and 670mg/ml at 100°C. Here the organic solvent Chloroform is used to extract caffeine from aqueous extract of tea powder because caffeine is more soluble in chloroform (140mg/ml) than it is in water (22mg/ml). The chloroform - caffeine mixture can then be separated on the basis of the different densities of chloroform and water because chloroform is much denser than water and insoluble in it. Residual water is separated from chloroform by draining out the chloroform through separating funnel, thus chloroform passed through the funnel while polar solvents such as water is still remains in the funnel. Water and chloroform is slightly soluble in each other. So, after separating the

solvents, residual water will remain the organic layer. The anhydrous sodium sulfite is used for the removal of water from organic layer.

OBJECTIVE

CAFFEINE has poor flow properties. Cyclodextrine plays an important role in formulation development due to its effect on solubility, dissolution rate and absorption of drug. So, the soubity of CAFFEINE is significanty enhanced by forming compex with β-cyclodextrin (BCD).

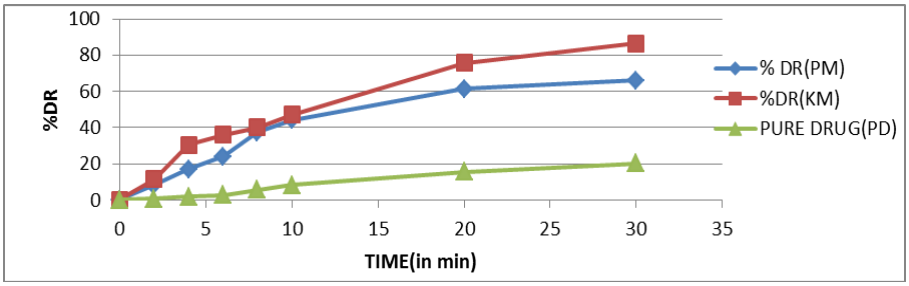
EXPERIMENTAL METHOD

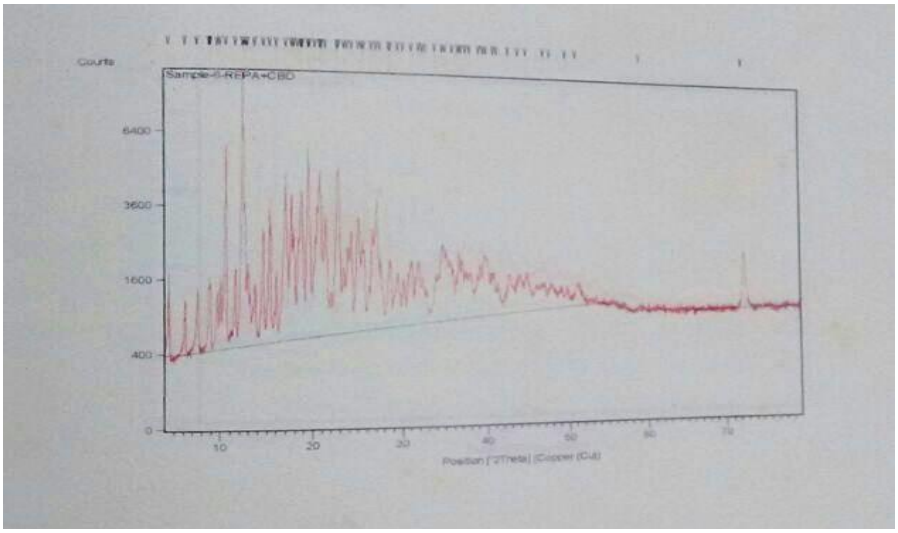
Micromeritics study of pure drug (CAFFEINE) measured by tapped density, bulk density, ang e of repose, carr's index, hausner's ratio which found to be 0.1742,0.2632,33.82%,1.52,33.52 respectively. After complexation it optimized to 0.294,0.384,23.43%,30.064°,1.306 of above parameters respective y. he ca ibration curve of CAFFEINE with 0.1N HCL and distilled waters with enhanced ratio calibrated a straight line with regression value of 0.999 at 273 nm. Solubility study with solvents distilled water and 0.1N HCl found as 15.78, 84.29mg/100mL respectively. Dissolution of pure drug was found to 20.18%DR after 30min. Complexation made by physical mixture (PM) and kneading method (KM). Phase solubility study shown 5.76,6.24,6.88,7.74, 7.06, 5.54 mg/100ml with molar concentration of BCD 0.5,1,1.5,2.0,2.5,3 respectively. Which was optimized at 1:2. In PM and KM % drug content found 83.82 and 85.62 respective y. he neading method was optimized by a tering so vents at various temperature which shown 15m ethano at 45 was the maximum. The complexation was confirmed by XRD and dissolution carried out at IMMT, BBSR. The fuse peak confirmed the complexation. The optimized dissolution rate found to be 86.43% compared to pure CAFFEINE of 20.18 at 30min.

RESULT AND DISCUSION

Comparative Dissolution Data of Kneading Mixture, Physical Mixture, Pure Drug With Distilled water

Time(min)	%DR(PM)	%DR(KM)	Pure Drug(PD)
0	0	0	0
2	8.3	11.58	0.76
4	17	30.29	1.9
6	24.1	35.89	2.75
8	37.5	40.09	5.63
10	44.21	47.2	8.25
20	61.48	75.74	15.6
30	65.94	86.43	20.18





(XRD of β-CD and CAFFEINE mixture indicating peaks below 2500 conforming the complexation)

TASTE MASKING

The taste masking property was analyzed by scientific committee / ethical committee. Human volunteer was tasted the pure drug which was bitter in taste. After taste masking by beta cyclodextrine complexation, it was reported by volunteer to be masked bitter taste.

Experimental Methods

Antibacterial Activity

Modified agar diffusion method Disc diffusion assay A was used for determination of the antibacterial activity. Nutrient agar was inoculated with microbial cell suspension (200µl in 20ml medium) and poured into sterile Petri dishes. Both compounds extracted from coffee were dissolved in chloroformto reach a final concentration of 2mg/ml, to be tested. Sterile filter paper discs 5mm in diameter were impregnated with 20µl (10µl + 10µl in case of combination) of caffeine and placed on the inoculated agar surface. A standard 6mm disc consisting of gentamycin 10µg/disc was used as positive control. The plates were incubated overnight at 37°C for 24 hours after pre-incubation for 2 hours in a refrigerator. Antibacterial activity was evaluated by measuring the zones of inhibition at the end of incubation period. Individual experiment was tested in triplicate.

Second method

Meal preparation

For this purpose, food containing potato, boneless chicken cooking, masala, tomato and salt was prepared. The prepared meal samples were divided into 8 lots (control, 1 % complexed BCD caffeine, 2 % complexed BCD caffeine, 0.1 % sodium benzoate, 0.1 % sodium benzoate +1 % complexed BCD caffeine, 1% complexed BCD caffeine + autoclaved at 121°C for 5 min, 2% complexed BCD caffeine + autoclaved at 121°C for 5 min and autoclaved only at 121°C for 15min). The treated samples were packed in Tetra pack pouches using HEKELMAN Vacuum System Boxer 42 and stored at room temperature for a period of 90 days. They were analyzed for total bacterial, fungal, coliform, salmonella and sensory quality initially and after 15 days interval for a total period of 3 months.

Microbiological assessment

The prepared meals were tested for microbiological parameters i.e. total bacterial counts (TBC), coliform group of bacteria, fecal coliform, Salmonella, and total fungal counts.

Total bacterial counts (TBC)

TBC were determined by dilution plate method using nutrient agar media (Feng et al. 2002). The colonies were counted by colony counter and TBC were calculated by multiplying average number of colonies by dilution factor and reported as number of colonies g^{-1} of sample.

Total coliform count

The samples were transferred to Lauryl tryptose broth (LSB) (1:3 ratio) and incubated at 35°C for 24 h. The tubes were examined and a reaction for gas production was recorded. The gas negative tubes were re-incubated for next 24 h and checked for any positive gas production. Coliform counts were calculated with the help of most probable number (MPN) (Feng et al. 2002).

Total fungal count

Total fungal count in the samples was determined by the dilution plate method (US FDA 1998). Fifty gram samples were mixed with appropriate amount of 0.1% peptone water to the weighed sample to achieve 10^{-1} dilution, homogenized in a stomacher for 2 min. Dilutions upto 10^{-6} was prepared in 0.1 % peptone water. Hundred micro-liters of each dilution were pipetted aseptically on pre-poured, solidified Dichloran rose bengal chloramphenicol (DRBC) agar plates and spreaded with a sterile, bent glass rod. The petri plates were incubated in inverted position at 25 ±1°C for 5 days. Data on total fungal counts were calculated as TFC g^{-1} of meal sample.

Micro-organisms used for antibacterial activities

Different micro-organisms tested for antimicrobial activities were *Staphylococcus aureus* (Gram-positive, clinical isolates and, *Salmonella typhi* (Gram-negative, clinical isolates), *Escherichia coli* and *Candida albicans* (Fungus).

Antimicrobial activity of complexed BCD caffeine/extract by well diffusion susceptibility method

The antibacterial activity of different solvent extracted samples of complexed BCD caffeine was carried by disc diffusion assay. Briefly, for disc diffusion assay, filter paper discs (Whatman no. 1) of 8 mm diameter were prepared and sterilized. Using sterile forceps, these discs were aseptically placed over nutrient agar plates seeded with the respective test microorganisms. Two different concentrations of complexed BCD caffeine extracts/(6 and 12 µg in DMSO) were aseptically transferred to these discs. The plates were incubated in an upright position at 37°C for 24 h. The diameters of inhibition zones (in mm) were measured. For antifungal activity, the selected fungi were grown on Czapeck dox agar (CDA) medium and plates were incubated at 37°C. The mycelial discs of 5 mm diameter were cut along with adhering agar from the 7 days old cultures and were used as inoculums throughout the present study. Radial growths of fungi in terms of average diameter (mm) were recorded on the 5th day. The data was used for calculating percent inhibition of mycelial growth according to the following formula

$$\%\text{Mycelialzoneofinhibition} = dc - dtdc \times 100$$

Where dc and dt are average diameters of mycelia colony of control and treated sets, respectively.

For antibiotic sensitivity testing, the cultures were prepared in sterile nutrient broth for 16–18 h at 37°C. The cultures were aseptically swabbed on the surface of sterile Nutrient Agar plates. Different antibiotics (Arithromycine, Ciprofloxacin at 50 µg concentration for Gram-positive and Gram-negative bacteria; 50 µg Clotrimazol for fungus in DMSO) were aseptically placed over the seeded agar plates. The plates were incubated at 37°C for 24h and the diameter of the inhibition zones (in mm) were measured.

RESULTS

The total bacterial count of control was more compared with samples with 1% or 2% complexed BCD caffeine extracts. The sealed pouches of control sample were found swollen within 24 h and considered sp ed. After 15 days storage period, gas was produced in some of the samples treated with 1 or 2% omplexed BCD caffeine extract. Both treatments were discarded from the trial and not tested for further storage period. The initial bacterial count in the sample treated with 0.1% sodium benzoate was 2.5×10^1 and slightly increased to 6.1×10^1 TBC g^{-1} of meal sample after 15 days storage period. After 30 days, this lot was also discarded due to the swelling of the pouches. Samples treated with both 1% complexed BCD caffeine extract and 0.1% sodium benzoate were found safe for consumption and kept for further 45 days storage period. Negligible bacterial counts were noted till 45 days storage period, however, later on, some pouches were filled with gas and discarded. No bacterial growth was noted in samples treated with combination of 1% complexed BCD caffeine extract + autoclaved and 2% complexed BCD caffeine extract + autoclaved during the 90 days storage period and was found safe for human consumption. Similar results were also recorded for the meal samples autoclaved for 15 min at 121°C without the addition of caffeine extract.

Analgesic effect of complexed BCD caffeine

Experimental design

Animals were randomly assigned to a con-trol (saline solution) or a treated group (caffeine).

Tail flick

An analgesimeter was used to perform the tail-flick test. The light from a project bulb situated beneath the platform where the animal was placed, was focused through a small hole on the ventral part of the tail at a point about 4 cm from the tip. Withdrawal of the tail exposed a photocell to the light, which turned off the thermal stimulus and automatically stopped the clock. The intensity was regulated so that the reaction time var-ied between 2 and 4 s. The analgesia was tested before and 15, 30 and 45 min after treatment. Each value was derived from the mean of three consecutive readings in which the light was focused on three adjacent points of the tail.

The result concluded in satisfactory analgesic effect of complexed BCD caffeine.

CONCLUSION

After making complexation with BCD at the dissolution rate was found to be 86.43. the XRD report of complex showing it fused peak below 2500 shows its amorphous characteristics. After taste masking by beta cyclodextrine complexation, it was reported by volunteer to be masked bitter taste It was concluded with high dissolution rate and better micromeritics property. The anti microbial effects and analgesic effect found to be satisfactory.

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