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Potential Herbal Anti-Cancer Drug Formulations Using Modern Drug Delivery Methods

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Objectives: The goal of the current effort is to create and assess chewing gum containing a potassium salt of curcumin extract for its ability to carry medication into the buccal cavity and fight cancer. It helps with the treatment of metabolic syndrome, arthritis, anxiety, and hyperlipidemia as well as oxidative and inflammatory diseases. Chewing gums containing curcumin were created to examine its anticancer properties.
Method: To increase the solubility of potassium Curcuminate, a solid dispersion of the compound was created utilising -cyclodextrin. For the purpose of improving solubility, the obtained solid dispersion of potassium curcuminate was examined. Curcuminate Sucrose, castor oil, chewing gum base, potassium curcuminate, and other ingredients were then combined to create chewing gums. The prepared chewing gum was assessed for colour, flavour, hardness, and drug-excipient compatibility.
Results: The compatibility study's findings demonstrated that there was no interaction between the chosen medicine and excipients. When compared to curcumin and other common anticancer medications like 5-Fu, Mito-C, and Paclitaxel, potassium curcuminate has greater anticancer activity: 85% against prostate cancer cells, 80% against liver cancer cells, and 92% on average against colon cancer cells. In-vivo studies of the chewing gums were optimised based on in-vitro drug release.
Conclusion: According to the results of this study, chewing gum containing the potassium salt of the cancer-fighting compound curcumin can be deemed a good delivery mechanism.

Keywords*: Chewing gums, Cancer, Anticancer, Tumor, Normal cells, Curcumin.*

Introduction

The oral route is arguably the one that both patients and doctors favour among the other administration methods. Drugs administered orally, however, have drawbacks such hepatic first pass metabolism and gastrointestinal enzymatic degradation that make oral administration of some medication types inappropriate. [1] The outermost layer of the oral mucosa is made up of stratum distendum, stratum filamentosum, stratum suprabasale, and stratum basale, all of which are stratified squamous epithelium and are mucous-covered. Lamina propria and submucosa cover the area beneath the basal lamina. The lamina propria serves as a mechanical support and also carries the blood vessels and nerves, whilst the epithelium acts as a mechanical barrier to protect underlying tissues. The oral mucosa contains keratinized areas. [2] In general, the oral mucosa is an intermediate layer of leaky epithelia between the epidermis and the intestinal mucosa. The buccal mucosa's permeability is thought to be 4–4000 times greater than that of skin. Because of the various forms and functions of various oral mucosae, there are significant variances in permeability between different parts of the oral cavity, as seen by the wide range in this reported value. [3] In general, the oral mucosa is an intermediate layer of leaky epithelia between the epidermis and the intestinal mucosa. The buccal mucosa's permeability is thought to be 4–4000 times greater than that of skin. Because of the various forms and functions of various oral mucosae, there are significant variances in permeability between different parts of the oral cavity, as seen by the wide range in this reported value. [4] In general, buccal

mucosae are more permeable than sublingual, and buccal mucosae are more permeable than palatal. [5] The sublingual mucosa is relatively thin and non-keratinized, the buccal mucosa is thicker and non-keratinized, and the palatal mucosa is intermediate in thickness but keratinized. These tissues are ranked according to their relative thickness and degree of keratinization. [6] The paracellular and transcellular routes are the two penetration routes for passive drug transport over the oral mucosa. These two routes can be used simultaneously by permeants, although depending on the physiochemical characteristics of the diffusant, one is typically chosen over the other. Since the cytoplasm and intercellular gaps have a hydrophilic nature, lipophilic substances would have poor solubilities in this setting. [7] The pace and amount of drug absorption through the buccal mucosa are slowed down by barriers such saliva, mucus, membrane coating granules, basement membrane, etc. Within the outermost quarter to a third of the epithelium, there lies the major penetration barrier. [8] The buildup of lipids and cytokeratins in the keratinocytes is less obvious and the change in morphology is much less pronounced in non-keratinized epithelia than in keratinized epithelia. The cytokeratins do not consolidate to form bundles of filaments as seen in keratinizing epithelia, and the mature cells in the outer region of non-keratinized epithelia grow big and flat and retain their nuclei and other organelles. [9] Although the basement membrane is also a factor in controlling connective tissue, the oral epithelium's superficial layers still serve as the main barrier to the entry of substances from the outside. The opposing direction appears to be controlled by a comparable process. Lipophilic substances that can reasonably easily cross the superficial epithelial barrier may have their rate of penetration slowed down by the charge on the basal lamina's constituents. [10] The buccal mucosa's epithelial cells are encircled by mucus, an intercellular ground substance that can range in thickness from 40 m to 300 m. Even though the small salivary glands and sublingual glands together only provide around 10% of all saliva, they create the majority of mucus and are essential for preserving the mucin layer over the oral mucosa. [11] A salivary layer that is unstirred and is thought to be 70 m thick covers the mucosal surface. High molecular weight mucin, found in saliva, can bind to the surface of the oral mucosa to prevent drying out, lubricate, concentrate protective chemicals like secretory immunoglobulins, and prevent microbial adhesion.[12] Buccal delivery is the process of administering a medicine to the systemic circulation through the buccal mucosa. The buccal mucosa is substantially more permeable than skin and has additional benefits over other delivery methods, although being significantly less permeable than the sublingual mucosa and typically not able to enable quick drug absorption or adequate bioavailability. [13] A different option is for the medicine released from chewing gum to be eaten and delivered into the stomach in a dissolved or dispersed form in saliva if it is not absorbed through the oral cavity membrane. As a result, the medicine would be available for absorption by the gastro intestinal tract. [14] As an alternative, medication released from medical chewing gum and lozenges can be ingested and received into the stomach in a dissolved or dispersed form by saliva if it is not absorbed through the oral cavity barrier. As a result, the medicine would be available for absorption by the gastro intestinal tract. [15] The gender factor may affect how frequently a certain cancer occurs. For both sexes, skin cancer is the most prevalent kind of malignancy, followed by breast cancer in women and prostate cancer in men. Its frequency is not a direct indicator of cancer mortality. Skin malignancies can frequently be cured. The main cause of death for both men and women is lung cancer. [16]

Materials And Method

Materials

Potassium di Hydrogen Othophosphate was obtained from Qualigens Fine Chemicals, Sodium Hydroxide was gotten from Qualigens Fine Chemicals, Liquid Glucose from Laxmi Confectionaries Ltd, Akola, and Curcumin from CHR-Hansen Pvt Ltd, Mumbai

Drug Profile

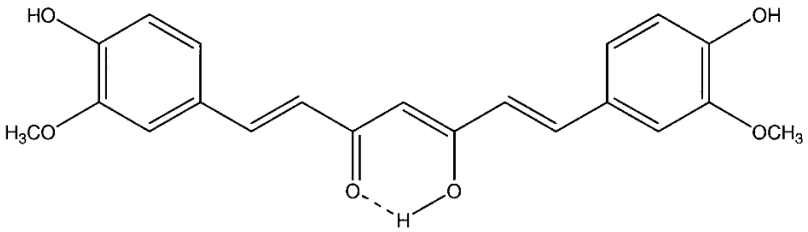

Figure-1 Curcumin chemical structure

Synonym: Diferuloyl Methane
Molecular Weight: 368.37
Description:
Curcumin occurs as orange yellowish powder It is practically insoluble in
Water[17]

Method

Preparation of potassium curcuminate salt: [18]

Curcumin diferuloyl methane is insoluble in water. It is considered worthwhile to prepare water soluble alkali phenates of curcumin which might possess enhanced activity compared to curcumin.

The procedure followed to prepare alkali salt of the curcumin is as follows:

Curcumin was added to alcoholic potassium hydroxide (1:2 moles respectively) and stirred to homogeneous thin paste which soon liquefied to a deep red solution. After adding an excess of about 5% curcumin and thorough stirring, the liquid was dried in a current of air at 400 C. The dried mass was than dissolved in minimum quantity of cold water and filtered under reduced pressure. The filtrate containing the potassium phenates were evaporated to dryness as above and finally in vacuum desiccators to constant weights.

Solid dispersion of potassium curcuminate was prepared using β-cyclodextrin to enhance the solubility of potassium Curcuminate.

Cell lines and cell culture preparation[19]

PC-3, HEP-2, A-549, 502713, HCT-15 and SW-620 cell lines were obtained from Indian Institute of Integrated Medicine, Jammu. cells were cultured in 2-3 ml of 0.05% trypsin-EDTA. And addition of 10 ml of 5% serum containing RPMI 1640 medium.

In vitro dissolution studies for Potassium curcuminate[20]

The vessel was filled with 200 ml phosphate buffer (6.4) and the gum was placed in the inner perforated vessel. The metal bob was attached to the rod, the height of rod and bob was previously adjusted so that the bob completely touches the bottom of the perforated vessel.

The apparatus was switched on and the bob was allowed to impact on the chewing gum.

This process was continued for 2 hours. 5 ml sample of the buffer solution was withdrawn at a regular interval of 10 min and every time it was replaced with equal amount of phosphate buffer, thus the samples were collected at 10, 20, 30 up-to 120 minutes

Formulation of Chewing gum [21]

The chewing gums with chewing gum base and drug and glucose were prepared by the method described below. Numbers of trials were extensively carried out to arrive at the correct concentration of each ingredient in order to have a desirable consistency as well as non-adhering properties.

In case of the chewing gums various plasticizers such as soyabean oil cottonseed oil, castor oil, diethyl phthalate, dibutyl phthalate were tried. The optimum concentration was decided for each plasticizer and two of them which showed good result, were selected. The two selected plasticizer castor oil and dibutyl phthalate were used subsequently bees wax was often incorporated in the gum bases in order to improve the chewability that is to give desired consistency to product prepared from the gum bases.

The chewing gum formulation is always sweetened. This is obvious as the product is used for the chewing and hence the taste of the product must be considered. In this study the model drug selected has bitter taste and hence large quantities of sucrose is added to mask the taste.

Talc was employed in the gum base as an anti-sticking agent. The purpose is to avoid adhering of the gum to dental surfaces. The concentration of talc was fixed to 5 to 10 % of total gum base composition.

table no. 1

Table-1 Formulation of Chewing Gum

Potassium curcuminate	100 mg
Chewing Gum Base	800 mg
Sucrose	150 mg
Castor oil	0.5 ml

Evaluation of Chewing Gums:[22]

Stability testing:

The purpose of this testing is to determine the physical and chemical stabilities of medicament, flavour, candy base and colour both under accelerated temperature and humidity conditions and at ambient storage conditions. This testing will enable the formulator to predict the acceptable shelf life of the product in a relatively short period of time and make changes as required to eliminate any incompatibilities that may influence product stability.

Elevated temperature and elevated humidity testing:

Elevated temperature and elevated humidity testing is initiated as soon as product is manufactured. Product should be tested at elevated temperatures and elevated humidity conditions. Testing conditions generally utilized by the product development laboratory include 250C at 80% relative humidity for 6-12 months, 370C at 80% humidity for 3 months, and 250C at 70% relative humidity for 6-12 months. The elevated humidity studies are carried out both at constant humidity and in humidity cabinets with day and night cycling. Elevated humidity tests are vital for ascertaining medicament stability and candy stickiness including surface graining characteristics

Physical stability:

Concurrent with the chemical stability evaluation, a physical stability study is carried out on the product in order to determine what factors will detract from organoleptic appeal of the product and how long these changes will take place to occur. A routine physical stability evaluation includes:

Color:

Chewing gums are checked for the color stability by keeping them in direct sunlight and at elevated temperatures to determine if the colours are light fast, also changes occurring due to presence of medicaments are to be evaluated. Table no. 2

Odour:

Changes in the odour of flavours at elevated temperature are evaluated by sealing the Chewing gums in glass bottles and determining if any odour is there.

Taste:

The product is tasted and compared to production controls in order to determine if any flavour change have occurred. Many small flavour changes that cannot be detected via gas –liquid chromatography can be ascertained when lozenge is tasted. Any change in the surface texture is also evaluated during the taste evaluation. Result mentioned in table no.2.

Table-2 Study for stability of potassium curcuminate Chewing gums

Parameters	Appearance		Colour		Dissolution	
First day 4°C	--	--	--	--	--	--
Room Temp	--	--	--	--	--	--
37° C	--	--	--	--	--	--
50° C	--	--	--	--	--	--
1st Month 4° C	--	--	--	--	--	--
Room Temp	--	--	--	--	--	--
37° C	--	--	--	--	--	--

50° C	--	--	--	--	+	--
2nd Month 4° C	--	--	--	--	--	--
Room Temp	--	--	--	--	--	--
37° C	--	--	--	--	--	--
50° C	--	--	--	--	+	--
3rd Month 4° C	--	--	--	--	--	--
Room Temp	--	--	--	--	--	--
37° C	--	--	--	--	--	--
50° C	--	--	--	--	+	--

-- No Change + Slight change

Weight variation test:

Twenty Chewing gum were taken and their weight was decided exclusively and all things considered on a computerized weighing balance. The typical load of one not set in stone from the system weight. The weight variety test would be a agreeable strategy for deciding the medication content consistency. The percent deviation was determined utilizing the accompanying equation. The outcomes are introduced in

% Deviation =

(Individual weight – Average weight /

Average weight) × 100

Hardness:

Compressed tablet Chewing gum are tested for proper hardness using Pfizer hardness tester. The force required to penetrate the lozenge is used as measure of chewiness, surface harness and stability. Results are mentioned in table no. 3

Chewing gum thickness:

Chewing gum thickness is a significant trademark in repeating appearance. Twenty chewing gum were taken and their thickness was recorded utilizing Digital Micrometer. The typical thickness for Chewing gum was determined and given standard deviation. The outcomes are introduced in Table no-3

Friability:

It is a proportion of mechanical strength of Chewing gum. Roche friabilator was utilized to decide the friability by following method. Pre weighed Chewing gum (20) were put in the friabilator. The Chewing gums were turned at 25 rpm for 4 minutes (100 revolutions). Toward the finish of test, the Chewing gums were re-gauged, misfortune in the heaviness of capsules is the proportion of friability and is communicated in Table no.3

% Friability = [(W1 – W2) / W1] × 100

Where,

W1 = Initial weight of 20 chewing gums

W2 = Weight of the 20 chewing gum after testing

Table- 3 Evaluation of Chewing gum

Parameters	**Potassium Curcuminate**
Weight variation (mg)	2804±2.16
Thickness (mm)	3.23±0.08
Hardness (kg/cm2)	1.40±0.45
Friability (%)	0.11
Content uniformity	95.21

*All information is offered in Mean ± SD, n=3, SD = standard deviation

Preparation of standard solution:

Estimation of Potassium Curcuminate in 6.4 pH phosphate buffer.

100 mg of potassium curcuminate was dissolved in 100 ml of 6.4 pH phosphate buffer; the resulting solution was subsequently diluted with 6.4 pH phosphate buffer to obtain series of diluted concentrations i.e. 50, to 500 mcg/ml the absorbance of above diluted concentrations was measured at 464 nm using 6.4 pH buffer as blank.

Estimation of potassium curcuminate in 6.4 pH phosphate buffer.

100 mg of potassium curcuminate was dissolved in 100 ml of 6.4 pH phosphate buffer; the resulting solution was subsequently diluted with 6.4 pH phosphate buffer to obtain series of diluted concentrations i.e. 50, to 350 mcg/ml the absorbance of above diluted concentrations was measured at 272 nm using 6.4 pH buffer as blank. Standard curve data reading are shown in table no.4

Table-4 Standard curve data for potassium curcuminate by UV-Visible spectrophotometer

Serial No	Concentration in µg/ml	Average Absorbance (n=3)
1	50	0.079±0.004
2	100	0.156±0.007
3	150	0.235±0.008
4	200	0.316±0.011
5	250	0.393±0.007
6	300	0.465±0.008
7	350	0.549±0.007
8	400	0.625±0.008

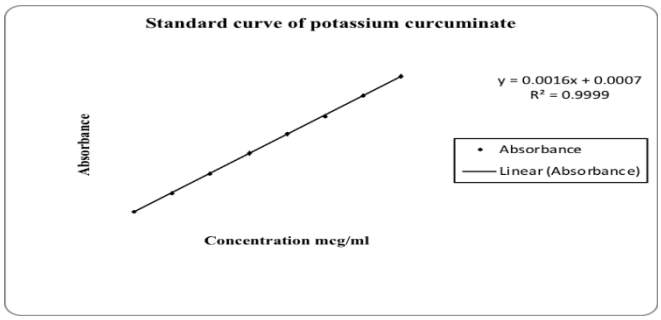

Figure-2 Standard Curve of Potassium Curcuminate

Dissolution studies:[14]

In vitro dissolution studies for Potassium curcuminate

The vessel was filled with 200 ml phosphate buffer (6.4) and the Chewing gums was placed in the inner perforated vessel. The metal bob was attached to the rod, the height of rod and bob was previously adjusted so that the bob completely touches the bottom of the perforated vessel.

The apparatus was switched on and the bob was allowed to impact on the Chewing gums.

This process was continued for 2 hours. 5 ml sample of the buffer solution was withdrawn at a regular interval of 10 min and every time it was replaced with equal amount of phosphate buffer, thus the samples were collected at 10, 20, 30 upto 60 minutes. Results are shown in table-5

Table-5: *In vitro* dissolution profile of potassium curcuminate

Time in Minutes	Average Percent Drug Release
5	4.733±0.681
10	12.400±0.529
15	14.833±0.289
20	24.500±0.500
25	36.000±1.000
30	39.667±0.577
35	49.667±1.528
40	64.000±1.732
45	71.000±2.646
50	82.000±3.000
55	92.833±1.041
60	92.833±0.764

*All information is offered in Mean ± SD, n=3, SD = standard deviation

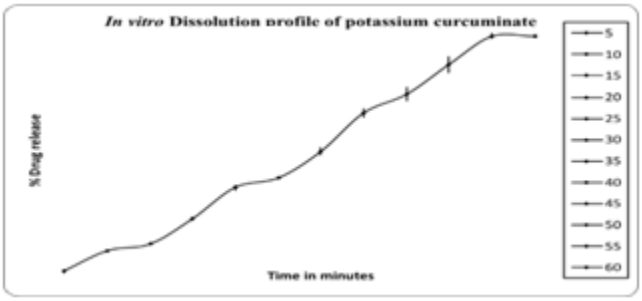

Figure-3 Drug release from potassium curcuminate

Drug Release Kinetics:

The mechanism of drug release from Chewing gums was determined by fitting the in vitro release profiles of optimized batches with zero order, first order, Hixson, Higuichi and Korsmeyer models. The obtained correlation coefficient values are given in the Table-6 & figure4-8

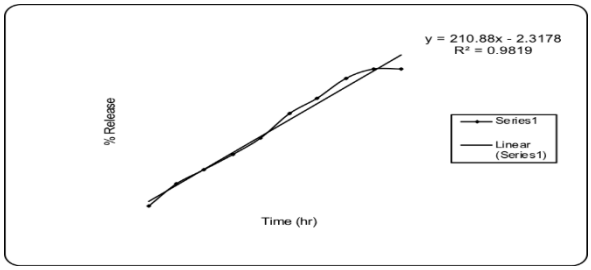

Figure -4 Zero order model for drug release from potassium curcuminate Chewing gums

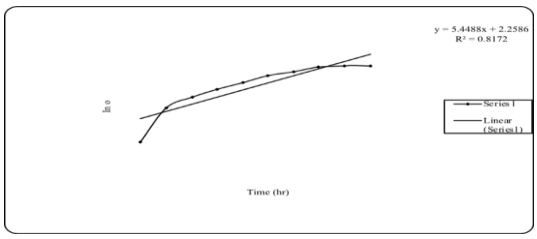

Figure-5 First order model for drug release from potassium curcuminate Chewing gums

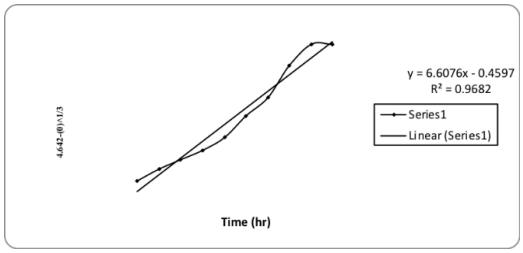

Figure-6 Hixson Cromwell model for drug release from potassium curcuminate Chewing gum

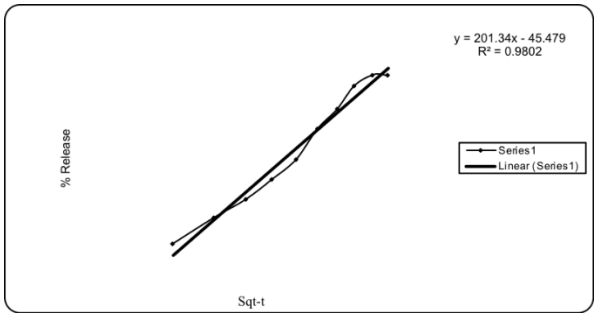

Figure-7 Higuchi square root model for drug release from potassium curcuminate Chewing gum

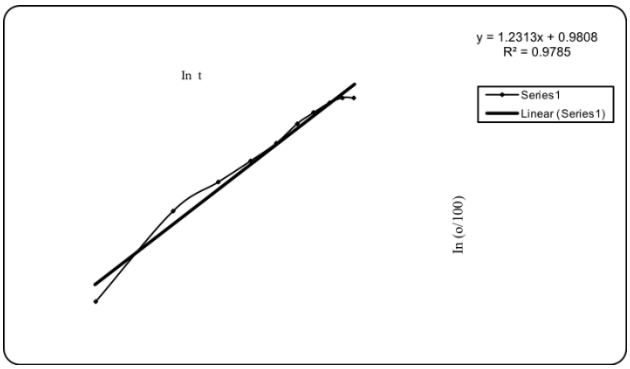

Figure-8 Korsemeyer model for drug release from potassium curcuminate Chewing gum

Table-6 Comparative study of drug release from different models for potassium curcuminate Chewing gum

		ZERO		FIRST		HIGUCHI		HIXSON		KORSMEYER	
time	Observed % rel.	Calculated % rel. (Q)	RS	Calculated % rel. (Q)	RS	Calculated % rel. (Q)	RS	Calculated % rel. (Q)	RS	Calculated % rel. (Q)	RS
0.05					53.77		32.39		191.9		
0	5.233	8.226	8.957	12.567	6	-0.458	1	-8.621	55	6.668	2.058
0.10	19.16								45.57		12.33
0	7	18.770	0.157	16.502	7.101	18.190	0.953	12.416	1	15.655	1
0.15	28.66				48.95		14.69				
0	7	29.314	0.419	21.670	6	32.500	2	30.545	3.528	25.792	8.266
0.20	38.66				104.2		34.76		53.51		
0	7	39.858	1.420	28.456	57	44.563	7	45.982	6	36.755	3.655
0.25	49.33				143.1		34.31		92.35		
0	3	50.402	1.142	37.367	82	55.191	2	58.944	6	48.377	0.914

0.30	65.00		16.43		253.7				21.58		19.77
0	0	60.946	3	49.070	77	64.799	0.040	69.646	2	60.553	6
0.35	75.33		14.77		118.7						
0	3	71.490	0	64.436	41	73.635	2.883	78.305	8.829	73.209	4.511
0.40	88.00		35.59		11.45		37.70				
0	0	82.034	1	84.616	3	81.860	5	85.137	8.195	86.292	2.916
0.45	93.66			111.11	304.4		16.66		10.93		37.12
0	7	92.578	1.185	4	22	89.584	8	90.360	5	99.760	9
0.50	93.66	103.12	89.40	145.91	2729.		10.38			113.57	396.4
0	7	2	7	1	518	96.890	9	94.188	0.272	9	98
SSR			**169.4 82**		**3775. 185**		**184.8 01**		**436.7 40**		**488.0 56**
SSR/ R^2			**172.6 06**		**4619. 659**		**188.5 34**		**451.0 85**		**498.7 80**

ZERO	Q = 210.88t-2.3178	R^2 = 0.9819
FIRST	lnQ = 5.4488T+2.2586	R^2 = 0.8172
HIXSON	4.642-(Q)1/3 = 0.3106t-0.3225	R^2 = 0.9493
HIGUCHI	Q = 201.34 $t^{1/2}$-45.479	R^2 = 0.9802
KORSMEYER	ln (Q/100) = 1.2313lnt+0.9808	R^2 = 0.9785

Procedure for in vitro analysis of anti-cancer activity[23]

The criteria for selection of a cell line for use in the interim panel were as follows.

a) Adaptability to growth on a single medium (RPMI-1640 plus 5% fetal bovine serum and 2mM glutamine):

b) A negative test for mycoplasma and mouse antibody production.

c) Isoenzyme and karyotype profiles verifying the human origin of cells.

d) Mass doubling that allows for harnessing of approximately 3x 107 cells twice a week: and

e) Suitability for use with microculture assays.

Once a line had been established as suitable, the number of cells was massively expanded in minimal number of passages and the cells were cryopreserved in a large repository of ampoules each containing 1x106 cells to provide a consistent frozen stock for future use. Once the growth in the in the new stock is established at the second or third passage, the older passage line is replaced with the new stock established at the second or third passage, the older passage line is replaced with the new stock for use in the screening laboratory.

Cell line maintenance:

Cells are maintained in multiple of T150 tissue culture flasks. Cells for each inoculation day are maintained separately (no common reagents) and passaged on separate days to prevent catastrophic loss of growing cell line stocks to microbial contamination. Additional backup flasks of cells are also maintained. For each cell line, the seeding density per flask is determined for production of healthy culture of 70% to 90% after 7 days for continued routine maintenance. These seeding densities than utilized twice a week to maintain sufficient cells for anti-cancer drug screening.

Preparation and inoculation of cells:

All of the adherent cell lines are detached from the culture flasks by addition of 2-3 ml of 0.05% trypsin-EDTA. Thereafter trypsin is inactivated by addition of 10 ml of 5% serum containing RPMI 1640 medium. Cells are separated into single cell suspension by gentle pipetting action then counted using trypan blue exclusion on a hemacytometer or by Coulter counter which is used when viability as determined by trypan- blue exclusion routinely greater than 97%. After counting dilutions were made to give the appropriate cell densities for inoculation onto the micrometer plate. Cells were inoculated in a volume of 100µl per well at densities between 5000 and 40000 cells per well. Cells were counted diluted and inoculated onto microculture plates within 4 hours period on 2 days each week. The micrometer plates containing the cells are preincubated for approximately 24 hours at 370C to allow stabilization prior to addition of drug.

Solubilization and dilution of samples:

For the initial screening of pure compounds each agent is routinely tested at five 10 fold dilutions starting from a maximum concentration of 104 M. Alternatively a maximum of 10 M can be selected if solubility permits. All samples are initially solubilized in dimethyl sulfoxide (DMSO) or water at 400 times the desired final maximum test concentration. Drug stocks are not filtered or sterilized, but microbial contamination is controlled by addition of gentamicin to the drug diluent. Multiple aliquots are stored at frozen at -700C to provide uniform samples for initial for tests as well as retests, if required just prior to preparation of the drug dilutions in cell-culture medium. These frozen concentrates are thawed at room temperature for 5 minutes. The concentrates are then diluted with complete medium containing 50 µg/ml gentamicin to twice the desire final concentrations.

Drug incubation:

Immediately after preparation of these intermediate dilutions 100µL aliquots of each dilution were added to the appropriate microtiter plate wells according to the format. As the microtiter wells already contain the cells in 100µL of medium, the final drug concentration tested is 50% of that in the intermediate dilutions. Agents are than added immediately to the cultures in the microtiter plates. During development of these procedures, drug incubation time was 1, 2, 3, 4 or 6 days at 37 ^{0}C in an atmosphere of 5% CO2 and 100% relative humidity. The plates were been assayed for the cellular growth and viability by microculture assay by tetrazolium assay or by SRB assay. In the current screening procedure, the cultures were incubated with test agents for 2 days and the end point is measured by the SRB assay.

Microculture tetrazolium assay.

The MTT assay is based on metabolic reduction of 3-(4, 5- dimethyl thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). A 50µl aliquot of MTT solution (1mg/ml) in RPMI-1640 medium, with no serum or glutamine, is added directly to all the appropriate microtiter plate wells containing cells complete growth medium and test agents. The culture is then incubated for 4 hrs to allow for MTT metabolism to formazan. After this time the supernatant is aspired and 150µl of Dimithyl sulfoxide is added to dissolve the formazan. Plates are agitated on plate shaker to ensure a homogenous solution, and the optical densities are read on an automated spectrophotometric plate reader.

Sulforhodamine bio assay:

Adherent cell cultures were fixed in situ by adding 50 µl of cold 50 % (wt/vol) trichloroacetic acid (TCA) and incubating for 60 minutes at 40C. The supernatant is then discarded and the plates were washed five times with deionized water and dried. One hundred microliters of SRB solution (0.4% wt/vol in acetic acid) is added to each microtiter well and the culture is incubated for 10 minutes at room temperature. Unbound SRB is removed by washing five times with 1 % acetic acid. Then the plates are air dried. Bound stain is solubilized with Tris buffer and the optical densities are read.

Data Calculations.

Unprocessed optical density data from each microtiter plate are automatically transferred from the plate reader to a microcomputer, where the background optical density (OD) measurements (i.e. complete medium plus stain minus cells) are subtracted from the appropriate control well values and where the appropriate drug- blank measurements (i.e. complete medium plus test compound dilution plus stain, minus cells) are subtracted from appropriate test well values. The values for mean + SD of data from replicate wells were calculated. Data are expressed in terms of % T/C [(OD of treated cells/ OD of control cells) x 100] as measure of cells viability and survival in the presence of test materials. Calculations are also made for the concentration of test agents giving a T/C value of 50% or 50% growth inhibition (IC50) and a T/C value of 10% or 90% growth inhibition (IC90).

With the SRB assay, a measure is also made of the cells population density at time (the time at which drugs are added) from two extra reference plates of inoculated cells fixed with TCA just prior to drug addition to the test plates. Thus we have three measurements controls optical density (C), test optical density (T) and optical density at tie zero (T0).

Using these measurements, cellular responses can be calculated for growth stimulations for no drug effect and for growth inhibition. If T is greater than or equal to T0, the calculation is 100x [(T-T0)/(C-T0). If T is less than T0, cell killing has occurred and can be calculated from 100x [(T-T0)/T0]. Growth inhibition of 50% (GI50) was calculated from 100 x [(T-T0)/C-T0)] = 50, which is the drug concentration causing a 50% reduction in the net protein increase in control cells during the drug incubation. The drug concentration resulting in total growth

inhibition (TGI) was calculated from T = T0. Where the amount of protein at the end of drug incubation is equal to the amount of protein at the end of drug incubation is equal to the amount at the beginning. The final calculation, LC50, is the concentration of drug causing a 50% reduction in the measured protein at the end of the drug incubation, compared with that at the beginning. Indicating a net loss of cells following drug treatment. LC50 is calculated from 100 x [(T-T0)/T0] = -50. Results are shown in table no. 7

Table-7 Comparative *in vitro* cytotoxicity evaluation against human cancer cell lines with fixed concentration

Name of Drug	Conc. (µg/ml)	PC-3	HEP-2	A-549	502713	HCT-15	SW-620
		Prostate	**Liver**	**Lung**	**Colon**		
Green tea extract	100	46%	4%	28%	**94%**	**83%**	9%
Potassium curcuminate	100	**85%**	**80%**	52%	**92%**	**89%**	**95%**
Curcumin	100	58%	32%	36%	**74%**	**82%**	51%
5-Fu	1x10-4M	51%	24%	32%	65%	44%	40%
Mito-C	1x10-5M	60%	69%	24%	85%	66%	62%
Paclitaxel	1x10-6M	26%	-	37%	71%	73%	-

Results

potassium curcuminate exhibits targeted anticancer effect against Prostate, Liver, Lungs and colon cancer cell line Anticancer effect of potassium curcuminate was estimated by MTT assay and morphological studies. The results of the MTT assay, Sulforhodamine bio assay revealed wide anticancer activity of the potassium curcuminate towards Prostate, Liver, Lungs and Colon cancer cells. Potassium curcuminate greatest anticancer activity 85% towards prostate cancer cell, 80% towards liver cancer cell, and average of 92% towards colon cancer cell compared to curcumin and standard anticancer drugs like 5-Fu, Mito-C and Paclitaxel.

Conclusion

Chewing gum show many benefits over the other dose type of these are; direct simple to geriatric and pediatric populace, has great taste, it draws out the time of medication in the oral cavity to deliver a particular impact, arranged effectively and no need water consumption for organization, this study planned to form potassium curcuminate as Chewing gum to further develop conveyance to treat oral thrush. The pre-arranged definitions were exposed to different physical and Chemical tests like assay, weight variation in-vitro drug release. Finally, it was concluded that the potassium salt of Curcumin Chewing gum can be considered as a suitable delivery system for the treatment variety of Cancer.

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