Formulations and Evaluation of Green Tea Extract Based Lozenges for In-Vitro Anti-Cancer Activity

1. **Chirag Goda*, 2. Ashish Kandalkar, 3. Manish R. Bhise**
1. Ibn SinaNational College for Medical Studies, Jaddah, Saudi Arabia
2. Late Shri Ramraoji Gawande Institute of Pharmacy, Akola, (MS) India
Correspondence author: chirag.goda@gmail.com, ashishkandalkar1@gmail.com
3. SGSPS, Institute of Pharmacy, Akola, (MS) India.

Abstract:

Objectives: The goal of this study is to develop and test green tea extract lozenges for anticancer activity and medication delivery into the buccal cavity. It helps with oxidative and inflammatory disorders, metabolic syndrome, arthritis, anxiety, and hyperlipidemia treatment. Anticancer activity of green tea extract lozenges was investigated. The goal of this study is to develop and test green tea extract lozenges for anticancer activity and medication delivery into the buccal cavity. It helps with oxidative and inflammatory disorders, metabolic syndrome, arthritis, anxiety, and hyperlipidemia treatment. Anticancer activity of green tea extract lozenges was investigated.

Method: Solid dispersion of green tea extract was prepared using β-cyclodextrin to enhance the solubility of green tea extract. The prepared solid dispersion of green tea extract was analysed for solubility enhancement then formulated with the same, liquid glucose, sucrose and corn syrup by heat, and congealing technique. The prepared lozenges were evaluated for drug-excipient incompatibility study, colour, odour, taste and hardness.

Results: The results of the compatibility study showed that there was no interaction between the selected drug and excipients. In-vivo studies of the lozenges were optimized based on in in-vitro drug release, drug Stability studies revealed that the formulation was stable, green tea extract having greatest anticancer activity 46% towards prostate cancer cell, 4% towards liver cancer cell, lungs cancer cell 28% and average of 62% towards colon cancer cell compared to standard anticancer drugs like 5-Fu, Mito-C and Paclitaxel.

Conclusion: From the present work, it was concluded that the green tea extract's lozenges can be considered as a suitable delivery system for the treatment variety of Cancer.

Keywords: Lozenges, Anticancer, Tumour, Normal cells, green tea extract.

Tob Regul Sci.™ 2022; 8(1): 1097-1113
DOI: doi.org/10.18001/TRS.8.1.89

Introduction

Amongst the numerous routes of transport, oral path is possibly the maximum desired to the patient and the clinician alike. But, peroral management of drugs have risks together with hepatic first bypass metabolism and enzymatic degradation inside the gastrointestinal tract, that restrict oral administration of sure instructions of medicine.[1] The oral mucosa consists of an outermost layer of stratified squamous epithelium, that is blanketed with mucous and consists of stratum distendum, stratum filamentosum, stratum suprabasale and stratum basale. The area under the basal lamina is blanketed with lamina propria and submucosa. The epithelium serves because the mechanical barrier that protects underlying tissues, while the lamina propria provides a mechanical guide and additionally incorporates the blood vessels and nerves. A few areas of the oral mucosa are keratinized. [2] The oral mucosa in standard is really leaky epithelia intermediate among that of the epidermis and the intestinal mucosa. It's far predicted that the permeability of the buccal mucosa is 4-4000 times more than that of skin. As indicative by way of the huge range on this stated price, there are significant variations in permeability among one of a kind regions of the oral cavity due to the various systems and functions of different oral mucosae3 the oral mucosa in popular is fairly leaky epithelia intermediate between that of the epidermis and the intestinal mucosa. It's far expected that the permeability of the buccal mucosa is 4-4000 times extra than that of pores and skin. As indicative through the wide variety in this said value, there are giant differences in permeability among unique areas of the oral cavity because of the numerous structures and features of various oral mucosae4 in preferred, the permeability of the oral mucosae decreases in the order of sublingual more than buccal and buccal more than palatal.[5] this rank order is primarily based at the relative thickness and diploma of keratinization of these tissues, with the sublingual mucosa is enormously thin and non-keratinized, the buccal thicker and non-keratinized, and palatal is intermediate in thickness however keratinized. [6] there are permeation pathways for passive drug transport throughout the oral mucosa: paracellular and transcellular routes. Permeants can use these routes simultaneously, but one path is normally favored over the alternative relying at the physiochemical residences of the diffusant. Because the intercellular areas and cytoplasm are hydrophilic in individual, lipophilic compounds could have low solubilities on this environment. [7] the barriers consisting of saliva, mucus, membrane coating granules, basement membrane and so on retard the price and extent of drug absorption through the buccal mucosa. The primary penetration barrier exists in the outermost region to one third of the epithelium. [8] in non-keratinized epithelia, the build up of lipids and cytokeratins in the keratinocytes are less evident and the change in morphology is far much less marked than in keratinized epithelia. The mature cells within the outer part of non-keratinized epithelia turn out to be big and flat maintain nuclei and other organelles and the cytokeratins do no longer aggregate to form bundles of filaments as seen in keratinizing epithelia. [9] although the superficial

layers of the oral epithelium represent the number one barrier to the entry of the materials from the exterior, it's miles evident that the basement membrane additionally performs a position in limiting the connective tissue. A similar mechanism seems to perform in the contrary direction. The fee at the ingredients of the basal lamina may restrict the charge of penetration of lipophilic compounds which could traverse the superficial epithelial barrier relatively without problems. [10] the epithelial cells of buccal mucosa are surrounded via the intercellular ground substance called mucus with the thickness varies from 40 μm to 300 μm. Although the sublingual glands and minor salivary glands contribute simplest approximately 10% of all saliva collectively they produce most of the people of mucus and are vital in keeping the mucin layer over the oral mucosa[11]. The mucosal ground has a salivary coating predicted to be 70μm thick which act as unstirred layer. In the saliva there is excessive molecular weight mucin named that may bind to the surface of the oral mucosa with a purpose to maintain hydration, provide lubrication, pay attention defensive molecules including secretory immunoglobulins, and restriction the attachments of micro-organisms. [12] management of a drug thru buccal mucosa to the systemic movement is defined as buccal delivery. Regardless of, the buccal mucosa is considerably less permeable than the sublingual mucosa and commonly not capable of provide rapid drug absorption or exact bioavailability, it's far relatively more permeable than skin and also gives different advantages over alternative delivery routes.[13] Alternatively drug released from medical chewing gum and lozenges, if does not get absorbed through oral hollow space membrane than may be swallowed and entered within the stomach in a dissolved or a dispersed form in saliva. Thus the drug would be available to gastro intestinal tract for absorption.[14] alternatively drug released from medical chewing gum and lozenges, if does no longer get absorbed through oral hollow space membrane than may be swallowed and entered in the stomach in a dissolved or a dispersed form in saliva. Consequently, the drug might be available to gastro intestinal tract for absorption. [15] the frequency of a particular most cancers can also depend upon the gender factor. Skin most cancers is the maximum common form of malignancy for each ladies and men, the second one most common type in guys is prostate most cancers and in ladies, the breast cancer. Its frequency does no longer equate to most cancers mortality. Pores and skin cancers are frequently curable. Lung most cancers is the main reason of dying in both ladies and men.[16]

MATERIALS AND METHOD

Materials

Green tea leaves were collected Kangara (Himachal Pradesh)and extracted at regional research lab Jammu

Drug Profile

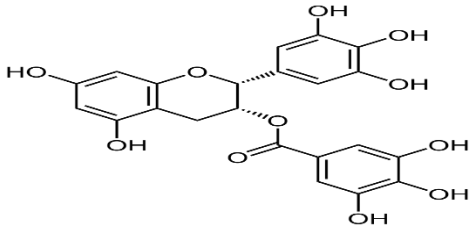

Figure-1 Epigallo catechin chemical structure

Synonym: 3-O-galloyl-(–)-epigallocatechin

Molecular Weight: 458.372 g/mol

Description:

Epigallocatechin gallate, also known as epigallocatechin-3-gallate, is the ester of epigallocatechin and gallic acid, and is a type of catechin. [17]

Method

Extraction of green tea leaves: [18]

The purpose of extraction procedures for crude drugs is to attain therapeutically desired portion and eliminate the inert material by treatment with selective solvent known as menstruum. The extract thus obtained may be ready for use as a medicinal agent as with certain tinctures or fluid extracts, or it may be further processed to be incorporated in any dosage forms such as tablets, capsules or it may be fractionated to isolate individual chemical entities. Thus standardization of extraction procedures contributes significantly to the final quality of the drug. Cell lines and cell culture preparation.

Complexation with cyclodextrins[19]:

Inclusion complexes occur when aqueous solution of cyclodextrin is shaken with drug molecules or its solutions. In aqueous solution the hydrophobic cavities of cyclodextrin are occupied by water molecules, which appropriate drug molecules that are less polar than water can replace.

Solubility of sparingly soluble drugs can be improved by molecular encapsulation with cyclodextrin. When drug molecules are accommodated into cyclodextrin cavities whose outer surfaces are hydrophilic, the drug molecules are hydrophilized, i.e. the hydrophobic drug acquires a hydrophilic molecular wrapping with cyclodextrin.

The solubility of manadione in cyclodextrin solution shows a maximum of 4-fold increase, whereas it the manadione cyclodextrin complex is dispersed in water, an approximately 10-fold increase is observed.

Cell lines and cell culture preparation[20]

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 green tea extract[21]

The lozenges were placed in the dissolution apparatus's,throughout the experiment, paddles were utilized as a spinning device at a speed of 50 rpm, and the temperature was kept at 37.010 0C. The dissolution procedure was carried out with a 6.4 pH phosphate buffer. To maintain sink conditions, 5mL of samples were pipette out and replaced with fresh dissolving fluid at predefined time intervals. The absorbance of the removed samples was measured using a spectrophotometer.

Formulation of Lozenges [22]

Accurate amount of green tea extract was transferred to a beaker, and was mixed with accurately weighed lactose. Liquid glucose, sucrose and corn syrup were weighed and mixed thoroughly, then passed through sieve no. 60. This produces a mass of the required consistency. The mass was rolled on lozenge board and cut to required size. The lozenges were dried in a hot air oven. Details are mentioned in table no. 1

Table-1 Formulation of Lozenges

Sr. No	Ingredients	Quantity
1	Green tea extract	100 mg
2	Liquid glucose	800 mg
3	Sucrose	50 mg
4	Corn syrup	50 mg

Evaluation of Lozenges[23]

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 25^0C at 80% relative humidity for 6-12 months, 37^0C at 80% humidity for 3 months, and 25^0C 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:

Colour:

Lozenges are checked for the colour 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 lozenges 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 green tea extract Lozenges

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 Lozenges 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 lozenges 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

Lozenges thickness:

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

Friability:

It is a proportion of mechanical strength of tablets. Roche friabilator was utilized to decide the friability by following method. Pre weighed capsules (20 tablets) were put in the friabilator. The tables were turned at 25 rpm for 4 minutes (100 revolutions). Toward the finish of test, the tablets 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 tablets

W2 = Weight of the 20 tablets after testing

Table- 3 Evaluation of Lozenges

Parameters	Green tea extract
Weight variation (mg)	3004±2.12
Thickness (mm)	3.22±0.04
Hardness (kg/cm2)	8.34±0.3
Friability (%)	0.16
Content uniformity	93.33

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

Preparation of standard solution:

Estimation of green tea extract in 6.4 pH phosphate buffer.

100 mg of green tea extractwas 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 green tea extract in 6.4 pH phosphate buffer.

100 mg of green tea extractwas 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 readings are shown in table no. 4

Table-4 Standard curve data for green tea extract by UV-Visible spectrophotometer

Serial No	Concentration in µg/ml	Average Absorbance (n=3)
1	50	0.060±0.002
2	100	0.120±0.004
3	150	0.176±0.007
4	200	0.235±0.006
5	250	0.300±0.014
6	300	0.355±0.008
7	350	0.418±0.008

8	400	0.060±0.007

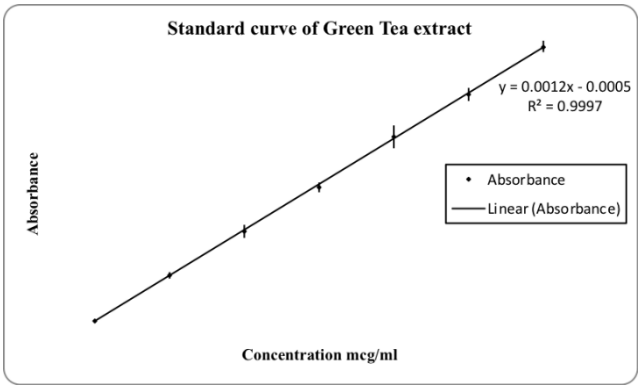

Figure-2 Standard Curve of Green tea extract

Dissolution studies: [14]

In vitro dissolution studies for green tea extract

The vessel was filled with 200 ml phosphate buffer (6.4) and the extract 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 Lozenges.

This process was continued for 30min. 5 ml sample of the buffer solution was withdrawn at a regular interval of 2 min and every time it was replaced with equal amount of phosphate buffer, thus the samples were collected at 2, 4, 6 upto 30 minutes. Results are shown in table-5

Table-5: *In vitro* dissolution profile of green tea extract

Time in Minutes	Average Percent Drug Release
2	5.233±0.252
4	6.167±0.289
6	15.500±0.500
8	34.333±1.155
10	41.333±1.155
12	57.733±0.643
14	64.667±1.528
16	71.833±0.764
18	78.267±0.751
20	82.567±0.603
22	88.133±0.231
24	92.500±0.500

26	94.233±0.252
30	94.333±0.289

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

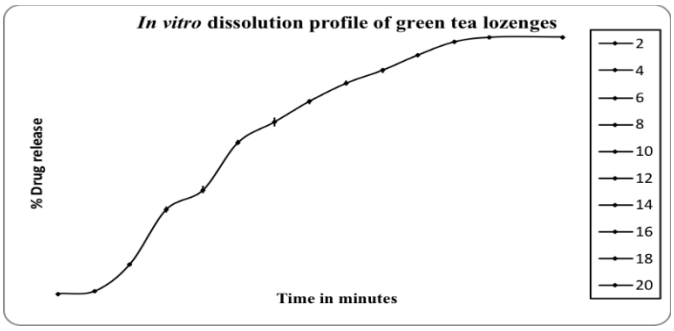

Figure-3 Drug release from green tea extract

Drug Release Kinetics:

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

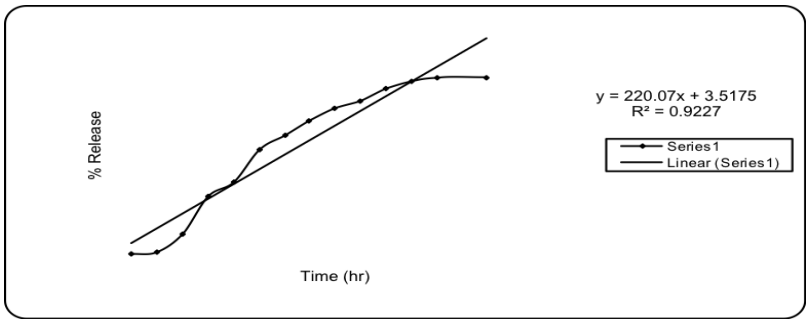

Figure 4: Zero order model for drug release from green tea lozenges

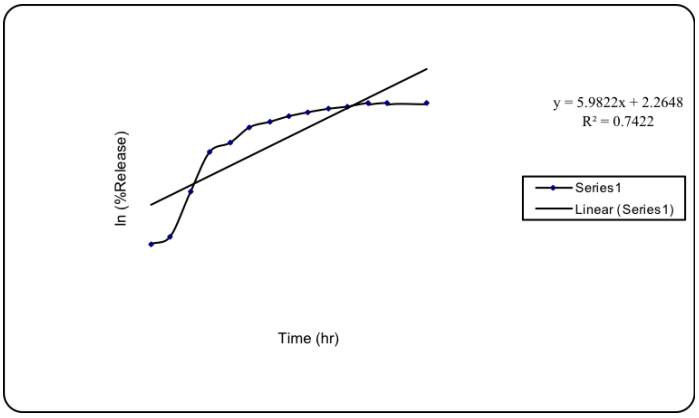

Figure 5: First order model for drug release from green tea lozenges

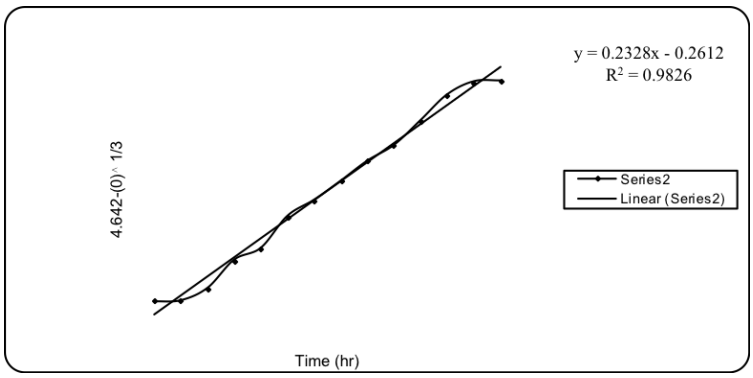

Figure 6: Hixson Cromwell model for drug release from green tea lozenges

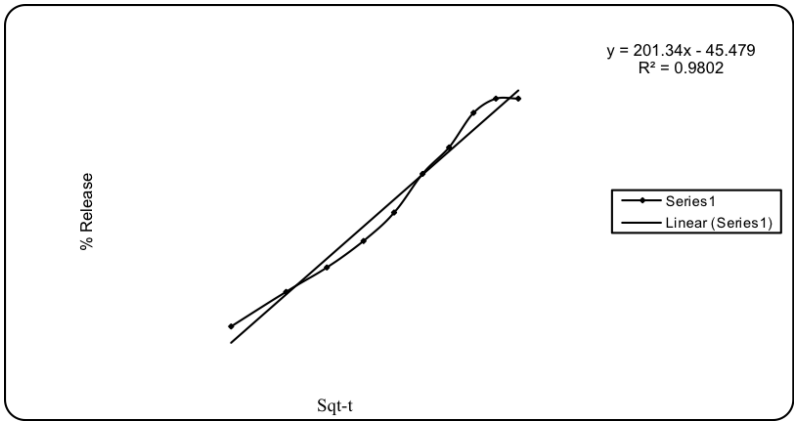

Figure 7: Higuchi square root model for drug release from green tea lozenges

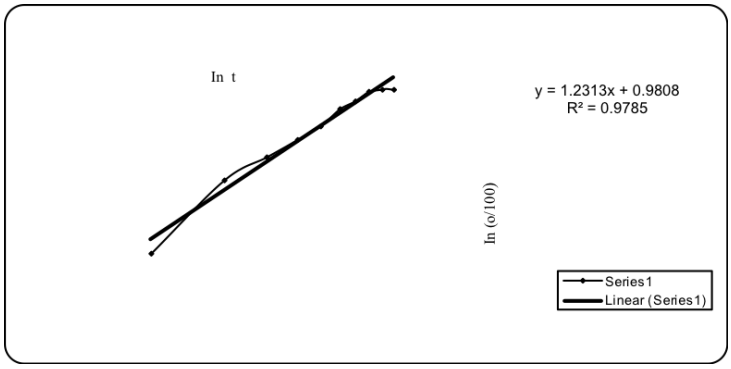

Figure 8: Korsemeyer model for drug release from green tea lozenges

Table-6 Comparative study of drug release from different models for green tea extract lozenges

		ZERO ORDER		FIRST ORDER		HIGUCHI		HIXSON CROMWELL		KORSMEYER	
time (t) h	Observed % rel.	Calculated % rel. (Q)	RS	Calculated % rel. (Q)	RS	Calculated % rel. (Q)	RS	Calculated % rel. (Q)	RS	Calculated % rel. (Q)	RS
0.033	5.233	10.853	31.583	11.754	42.521	-1.880	50.598	-3.678	79.419	4.672	0.315
0.067	6.167	18.189	144.532	14.348	66.935	13.669	56.289	10.941	22.793	11.016	23.520
0.100	15.500	25.525	100.491	17.514	4.058	25.601	102.022	24.117	74.252	18.196	7.267
0.133	34.333	32.860	2.170	21.379	167.805	35.659	1.758	35.925	2.533	25.977	69.822
0.167	41.333	40.196	1.294	26.097	232.136	44.521	10.161	46.439	26.072	34.240	50.318
0.200	57.733	47.532	104.077	31.856	669.613	52.533	27.048	55.736	3.990	42.907	219.827
0.233	64.667	54.867	96.030	38.886	664.618	59.900	22.720	63.889	0.605	51.925	162.342
0.267	71.833	62.203	92.747	47.468	593.675	66.758	25.764	70.974	0.738	61.256	111.876
0.300	78.267	69.539	76.181	57.943	413.051	73.198	25.689	77.066	1.442	70.869	54.725
0.333	82.567	76.874	32.405	70.730	140.112	79.290	10.737	82.239	0.107	80.739	3.339
0.367	88.133	84.210	15.394	86.338	3.222	85.084	9.298	86.570	2.444	90.848	7.367
0.400	92.500	91.546	0.911	105.391	166.185	90.620	3.534	90.132	5.607	101.177	75.286
0.433	94.233	98.881	21.602	128.649	1184.428	95.930	2.879	93.001	1.518	111.713	305.525
0.500	94.333	113.553	369.376	191.694	9479.086	105.969	135.394	96.960	6.899	133.357	1522.862
SSR			**1088.793**		**13827.445**		**483.890**		**228.418**		**2614.391**

SSR/	1180.	18630.	501.1	232.6	2791.
R^2	008	349	29	52	065

ZERO	Q =220.07t + 3.5175	R^2 =	0.923	
FIRST	lnQ = 5.9822t+2.2648	R^2 =	0.742	
HIXSON	$4.642-(Q)^{1/3}=0.2328t-0.2612$		R^2 =	0.982
HIGUCHI	$Q =205.61\ t^{1/2}-39.419$	R^2 =	0.966	
KORSMEYER	ln (Q/100) = 1.2376 lnt+1.1457	R^2 =	0.937	

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:[24]

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 37^0C 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 -70^0C 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 being 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 50 µl aliquote 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 150 µl of Dimethyl 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 culture was 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%
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

Green tea extractexhibits targeted anticancer effect against Prostate, Liver, Lungs and colon cancer cell line Anticancer effect of Green tea extractwas estimated by MTT assay and morphological studies. The results of the MTT assay, Sulforhodamine bio assay revealed wide anticancer activity of the Green tea extracttowards Prostate, Liver, Lungs and Colon cancer cells. Green tea extract having greatest anticancer activity 46% towards prostate cancer cell, 4% towards liver cancer cell, lungs cancer cell 28% and average of 62% towards colon cancer cell compared standard anticancer drugs like 5-Fu, Mito-C and Paclitaxel.

CONCLUSION

Lozenges 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 formGreen tea extractas lozenges 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 theGreen tea extractlozenges can be considered as a suitable delivery system for the treatment variety of Cancer.

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