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RESEARCH ARTICLE

EVALUATION AND COMPARISON OF ANTICANCER ACTIVITY OF HERBOMINERAL COMPLEX

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ABSTRACT

Background: Cancer is a bubonic plague to mankind for which presently, there is no cure Chemopreventive plant compounds such as catechin, curcumin affect all phases of the cancer process, i.e., tumor initiation, promotion and progression. But major drawback of these phenolic compound is their poor bioavailability
Objective: Literature resources reveled that formation of complex of these phenolic compound with mineral will increase
Bioavailability and free radical scavenging activity: Which stimulate our interest to form herbomineral complex and evaluate its effect on free radical scavenging and anticancer activity
Material and Method: Herbomineral complex was formed by the process of chelation Phenolic compounds such as Catechin, Curcumin and polyphenols from Trigonella foenum graecum extract chelate the divalent metal ions Zn^{2+}in weakly acidic aqueous solution. Formed complex was screened for anticancer activity on human nesopharyngeal cell line kB. Herbomineral complex of catechin and Trigonella foenum graecum extract showed potent anticancer activity.In the present study an attempt was also made to prove the therapeutic effect of herbomineral complexes of these drug

INTRODUCTION

Cancer is a bubonic plague to mankind for which presently, there is no cure **(**Kothari Manu and Mehta lopa, 1979). Patients confronting a diagnosis of advanced (stage 4) cancer face the statistical reality that conventional chemotherapy can effect a cure only a tiny minority (between 1-3% depending on the malignancy) of all such cases. More often, the reasonable impulse of these patients to investigate alternative treatment options such as herbal medicine (Takimoto *et al*., 2005). Cancer treatment recognizes the importance of botanical medicine. However, despite extensive positive research data from experimental and preclinical studies, and the anecdotal clinical experience of many practitioners, patients and cancer survivors, its potential in this field remains largely untapped and large-scale clinical trials are generally unavailable. The reasons for this are multifactorial, and include historical, political, and cultural factors, and almost invariably a misunderstanding of the core principles of herbal medicine itself. Herbal drugs targ*et all* three phases of the cancer. Herbal drugs are an excellent source of many antioxidants. We need these to fight free radicals Chemopreventive plant compounds affect all phases of the cancer process, i.e., tumor initiation, promotion and progression.

Botanical medicines are complex natural mixtures of pharmacological multitaskers, simultaneously exerting influence on different levels and via different mechanisms green tea polyphenols can influence signal transduction factors, inhibit COX-2, promote cell cycle arrest, increase apoptosis and disable multidrug resistance pumps. Curcumin from turmeric has been found to influence over 60 such molecular targets in the cancer process (Jonathan Treasure 2005). But major drawback of these phenolic compound is their poor bioavailability Literature resources reveled that formation of complex of these phenolic compound with mineral will increase bioavailability and free radical scavenging activity Their ability to chelate transition metal ions, can generate highly reactive oxygen free radicals by Fenton or Haber-Weiss chemistry. In the Fenton reaction the hydroxyl radical ($HO^{\bullet}$) is produced from hydrogen peroxide. In the iron-catalyzed Haber-Weiss reaction the superoxide radical ($O_2^{\bullet-}$) reduces ferric to ferrous ions, which then are again involved in generating of hydroxyl radical. Extremely reactive hydroxyl radicals can interact with many biological macro- and small molecules and therefore lead to lipid peroxidation, DNA damage and polymerization or denaturation of proteins. The binding of transition metal ions by phenolic compounds can stabilize prooxidative activity of those ions. Yet data on complexing of ion metals by tannins isolated from plant extracts are scarce (Magdalena Karamac 2009). Therefore, the aim of this study was to examine their ability to chelate Zn (II). The knowledge of those properties can be applied in future studies aimed at elucidating the mechanisms of antioxidant and anticancer activity.

EXPERIMENTAL

MATERIAL AND METHOD

- Method of testing: sulforhodamine B assay
- Parameter reported: GI50, TGI, LC50
- Vehicle used : Dimethyl sulfoxide (DMSO)
- Source of cell line: NCCS, pune
- Positive control drug: doxorubicin (Adriamycin, ADR) Mfg: pharmacia

Cell line details

- Cell line – KB
- Human tissue of origin – Neso- pharynge
- Cells/Well – $5*10^3$

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Table 1. Defination of parameters for invitro testing

Definations	
GI50	Growth inhibition of 50%(GI50) calculated from[(Ti-Tz)/(C-Tz)]x100=50, drug concentration resulting in a 50% reduction in the net protein increase
TGI	Drug concentration resulting in total growth inhibition (TGI), calculated from Ti=Tz
LC50	Concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment (concentration of drug causing lethality to 50 % of the cells) ascompared to that at the beginning) indicating a net loss of cells following treatment is calculated from [(Ti-Tz)/ Tz]x 100 = -50
Gi50≤ **20**µg/ml is considered as active for extracts and GI50≤ 10µg/ml is considered as active for pure compounds	

METHODOLOGY

The cell lines were grown in RPMI 1640 medium containing 10% fetal bovine serum and 2mM L-glutamine. For present screening experiment, cells were inoculated into 96 well microtiter plates in 100µL at plating densities as shown in the study details above, depending on the doubling time of individual cell lines. After cell innoculation, the microtiter plates were incubated at 37ºc, 5%CO_2, 95% air and 100% relative humidity for 24h prior to addition of experimental drugs**.** After 24 h, one 96 well plate containing $5*10^3$ cells/well was fixed in situ with TCA, to represent a measurement of the cell population at the time of drug addition (Tz). Experimental drugs were initially solubilized in dimethyl sulfoxide at 100mg/ml and diluted to 1mg/ml using water and stored frozen prior to use. At the time of drug addition, an aliquote of frozen concentrate (1mg/ml) was thawed and diluted to 100µg/ml, 200µg/ml, 400µg/ml and 800µg/ml with complete medium containing test article. Aliquots of 10µl of these different drug dilutions were added to the appropriate microtiter wells already containing 90µl of medium, resulting in the required final drug concentrations i.e. 10µg/ml, 20µg/ml, 40µg/ml, 80µg/ml. (Vanicha vichai 2006)

End point measurement

After compound addition, plates were incubated at standard conditions for 48 hours and assay was terminated by the addition of cold TCA. Cells were fixed in situ by the gentle addition of 50 µl of cold 30 % (w/v) TCA (final concentration, 10% TCA) and incubated for 60 minutes at 4ºC. The supernatant was discarded; the plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (50µl) at 0.4% (w/v) in 1% acetic acid was added to each of the wells, and plates were incubated for 20 minutes at room temperature. After staining, unbound dye was recovered and the residual dye was removed by washing five times with 1% acetic acid. The plates were air dried. Bound stain was subsequently eluted with 10mM trizma base, and the absorbance was read on an plate reader at a wavelength of 540nm with 690 nm reference wavelength.

Percent growth was calculated on a plate-by-plate basis for test wells relative to control wells. Percent growth was expressed as the ratio of average absorbance of the test well to the average absorbance of the control wells* 100.

Using the six absorbance measurements (time zero (Tz), control growth (C), and test growth in the presence of drug at the four concentration levels (Ti), the percentage growth was calculated at each of the drug concentration levels. Percentage growth inhibition was calculated as:

[(Ti-Tz)/(C-Tz)] x 100 for concentrations for which Ti>/=Tz (Ti-Tz) positive or zero
[(Ti-Tz)/Tz] x100 for concentrations for which Ti<Tz (Ti-Tz) negative

The dose response parameters were calculated for each test article. Growth inhibition of 50% (GI50) was calculated from [(Ti-Tz)/(C-Tz)] x100= 50, which is the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. The drug concentration resulting in total growth inhibition (TGI) was calculated from Ti=Tz. The LC50 (concentration of drug resulting in a 50 % reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of cells following treatment is calculated from [(Ti- Tz)/Tz] x 100= -50

Values were calculated for each of these three parameters, the level of activity was reached; however, if the effect was not reached or was exceeded, the values for that parameter were expressed as greater or less than the maximum or minimum concentration tested (Skehn1990)

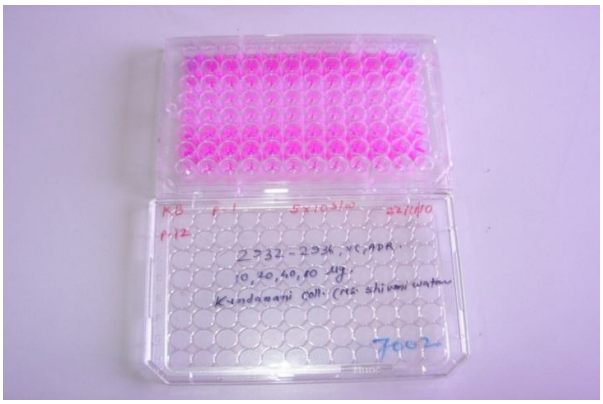

Figure 1. Image of microtiter plate

Observation

Invitro testing of anticancer activity was carried out in collaboration with Tata memorial centre (Advanced centre for Treatment, Research and Education in cancer). After performing experiment, the observations obtained are given in table as follows

Table 2. Reports of invitro testing for Anticancer activity

Reports of *in vitro* testing for Anticancer Activity

Human Neso-Pharyngeal Cancer Cell Line KB

% Growth as compared to control

Drug concentration (µg/ml)

	Experiment 1				Experiment 2				Experiment 3				Average Values			
	10	20	40	80	10	20	40	80	10	20	40	80	10	20	40	8
A	100.0	100.0	100.0	97.8	100.0	100.0	100.0	97.6	100.0	100.0	100.0	100.0	100.0	100.0	100.0	98
AC	17.1	16.9	14.1	11.6	15.0	14.6	11.9	8.7	12.4	16.9	14.9	12.4	14.9	16.1	13.6	10
B	17.9	15.7	14.7	13.4	15.2	12.7	10.3	8.5	11.4	15.9	15.5	15.3	14.8	14.8	13.5	12
C	15.7	14.1	12.3	11.1	13.7	13.8	11.1	7.7	13.1	14.5	15.1	10.8	14.2	14.2	12.8	9.
CC	100.0	89.8	82.9	71.2	93.8	80.1	76.0	62.6	86.8	91.9	90.0	76.4	93.6	87.3	82.9	70
ADR	3.6	-0.8	-14.9	-31.6	1.1	-6.9	-18.7	-28.3	-3.7	-7.2	-2.4	-23.2	0.4	-5.0	-12.0	-27

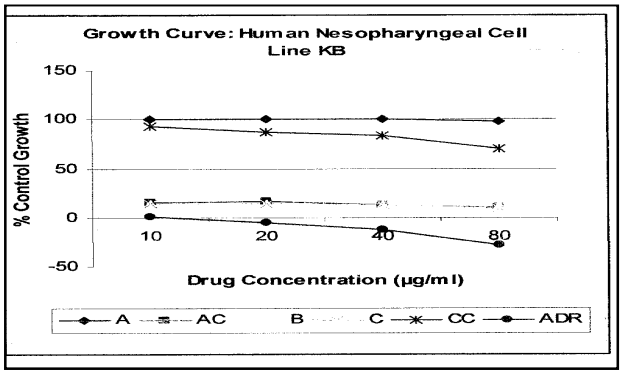

Figure 2. Growth curve of human nesopharyngeal cell line

Table 3. Parameters calculated from graph of Human Neso pharyngeal cell line

Human Neso-pharyngeal Cancer Cell Line K
Parameters calculated from graph:

	µg/ml values for		
	LC50	TGI	GI50
A	>80	>80	>80
AC	>80	75.8	<10
B	>80	77.3	<10
C	>80	74.1	<10
CC	>80	>80	>80
ADR	>80	41.0	<10

Where,
A= Catechin
Ac= Herbomineral complex of catechin or chelaed catechin
B= Herbomineral complex of *Trigonella foenum graecum* extract
C= Curcumin
Cc=Herbomineral complex of curcumin or chelaed curcumin

RESULT AND DISCUSSION

Catechin, curcumin and *Trigonella foenum graecum* extract and their herbomineral complex with zinc were evaluated and compared for anticancer activity. Herbomineral complex of Catechin and *Trigonella foenum graecum* extract showed potent anticancer activity. Formation of herbomineral complex of catechin and *Trigonella foenum graecum* extract with zinc showed significant increased in activity of catechin and *Trigonella foenum graecum* extract.

Catechin and phenolic compounds from extract have been described to show a wide range of chemopreventive effects. The signal transduction pathways may be related to the chemopreventive activity of phenolic compounds. These effects are believed to be mediated by the regulation of signaling pathways, including NF-kB, AP-1, and MAPK. By modulating cell signaling pathways, polyphenols activate cell death signals and induce apoptosis in preneoplastic or neoplastic cells, thus inhibiting cancer development and/or progression (Wattenberg1985 and Surh 2003).

Where as pharmacokinetic zinc showed that, after absorption Zn potentially bound to protein 10 % metallothionine. Zn is primarily stored in RBCs, WBCs, muscle, bone skin, kidneys, liver, pancreas, retina; prostate etc. the extent of binding is 10% plasma albumin, 30-40 % to alpha 2 macroglobulins or transferring and 1% to amino acids like histidine and cysteine. Zinc serve as structural ions in transcription factors and is stored and transferred in metallothioneins. Has a role in the metabolism of RNA and DNA, signal transduction and gene expression. It also regulates apoptosis. Cells in salivary gland, immune system and intestine use zinc signaling as one way to communicate with other cells (Ramanujam ?).

Hence when chemopreventive activity of phenolic compound and pharmacokinetic of zinc was compared chelation of zinc with phenolic provide and regulate signaling pathway for their chemopreventive activity. Thus increase anticancer activity. The matrix metalloproteinases (MMPs) are a family of zinc-dependent proteinases involved in the degradation of the extracellular matrix. The extracellular matrix is an important feature in a variety of biological processes such as embryonic development, tissue remodeling and tissue repair. The MMPs have been implicated in the processes of tumor growth, invasion, and metastasis; are frequently over expressed in malignant tumors; and have been associated with an aggressive malignant phenotype and adverse prognosis in patients with cancer. But also can process a number of bioactive molecules. They are known to be involved in the cleavage of cell surface receptors, the release of apoptotic ligands (such as the FAS ligand), and chemokine/cytokine in/activation. MMPs are also thought to play a major role on cell behaviors such as cell proliferation, migration (adhesion/dispersion), differentiation, angiogenesis, apoptosis and host defense. Zinc controlled degradation of extracellular matrix (Sheng-Teng Huang *et al*., 2011). A number of MMP inhibitors are being developed for the treatment of cancer. The most extensively studied class of MMP inhibitors which bind covalently to the zinc atom at the MMP-active site and inhibit tumor growth and metastases these enzymes were initially associated with the invasive properties of tumour cells, owing to their ability to degrade all major protein components of the extracellular matrix (ECM) and basement membranes. Matrix metalloproteinases (MMPs), which are secreted by both tumor and stromal cells, are involved in degradation of extracellular matrix. This degradation is key for metastatic and angiogenic processes.

MMP-2, like other MMPs, is a zinc-dependent endopeptidase involved in the degradation of the ECM. As zinc is essential for endopeptidase proteolytic capacity to degrade the ECM, compounds with zinc-chelating groups (Talbot 1996) are often used to inhibit the MMP activity chelation of the zinc ion in the active site of the MMPs and act as competitive MMP-inhibitors and exert anti-angiogenesis effect (Raffetto and Khalil 2008). Since the direct co-incubation of purified MMP-2 protein with phenolic compound inhibited the MMP-2 activity, by chelating the zinc and directly inhibiting MMP-2 activity.

In addition to the direct inhibitory effect on MMP-2 activity mediated by zinc chelation, the treatment of endothelial cells with phenolic compound for 24 hours could inhibit the secretion of MMP-2 and therefore further reduced extracellular MMP-2 activity (Huang *et al*., 2009). The cellular secretory process allows rapid mobilization and utilization of MMP-2 enzymes in the early phase of angiogenesis. As a potential anti-cancer drug, the zinc-chelating effect of phenolic compound may also plays a role on preventing the early tumor promotion. Phenolic compounds have been found to exhibit antioxidative activity. Besides scavenging free radicals, antioxidants may inhibit signaling enzymes such as protein kinase C (PKC) that play a crucial role in tumor promotion (Gopalakrishna and Gundimeda 2002). Phenolic compounds can inactivate PKC by chelating the zinc and and block the signal transduction induced by tumor promoters. The redox-mediated inactivation of PKC may, at least in part, be responsible for the antioxidant-induced inhibition of tumor promotion and cell growth. Oxidative stress also represents an important stimulus that widely contributes to tumor angiogenesis mediating the angiogenic switch that can be produced by cancer cells and thus contribute to neoplastic transformation and angiogenesis (Cavallin *et al*., 2009). In summary, we found that phenolic compounds can inhibit the activity and the secretion of MMP-2 in human vascular endothelial cells. They also inhibit the tube formation and migration of human vascular endothelial cells in a dose-dependent manner. The zinc-chelating activity of Catechin and phenolic compound and also elucidating the important role of zinc in the process of angiogenesis. Compared with either MMPI that bind mainly to active zinc-containing domain of specific protein targets, the zinc-chelating effect of Catechin and phenolic compound is much more wide-ranging and therefore may be considered as a more effective anti-angiogenic or anti-cancer drug with greater potential. It is known that phenolic compounds exhibits multiple effects in a variety of tissues and cells, which may simultaneously trigger various molecular mechanisms that help to increase the effectiveness when it is used to inhibit cancer development.

One recent study investigated the kinetics of alkaline degradation of the curcuminoids. The authors of this study observed that bisdemethoxycurcumin [BDMC] was less susceptible to degradation at p^H 10.2 than curcumin [C] or demethoxycurcumin [DMC] (Shoba *et al*., 1998).

- In serum free buffer solutions, Curcumin was observed to decompose in a pH-dependent manner, with faster reactions at neutral-basic conditions.

- Curcumin showed greater stability in serum but has low plasma and tissue levels and poor absorption

Trans-6-(4'-hydroxy-3'-methoxyphenyl)-2,4-dioxo-5-hexanalwas predicted as the major degradation product (Price and Buescher 1997) Curcumin zinc complex was prepared in presence of buffer at neutral or physiological p H, and also lower plasma, blood and tissue levels due to poor absorption These factors may be responsible for degradation of Curcumin. Hence Curcumin complex does not show significant anticancer activity. One more benefit of chelation is increase bioavailability of parent drug compound. Hence chelation not only showed anticancer activity invitro but also gives promise to show activity invivo.

Zinc is a divalent cation exhibiting important role in health and disease as evidenced by the role of Zn in functional capacity more than 200 metallic enzymes including carbonic anhydrase, carboxypeptidases, alcohol dehydrogenases, alkaline phosphatases and RNA polymerases etc. It also required to maintain the structure of nucleic acid protein, cell membrane and also exerts vital role in various physiological function such as cell growth, division, maturation and reproduction, wound healing and host immunity. Pharmacokinetics study of Zn showed that, Zn excreted mainly in faces and only traces found in the urine, since kidney has little role in regulating body Zn content. Endogenous Zn reabsorbed in ileum and colon creating enterohepatic circulation of Zn. The peak plasma concentration occurs in approximately 2 hours.

From the above kinetics of Zn, it was shown that, herbomineral complex formed by using zinc, also get reabsorbed in kidney, thus increase its bioavailablity, and gives anticancer activity for longer period of time. Catechin excretion occurs through kidney where as 90% Zn is excreted through faeces, hence Catechin complex is reabsorbed and gives potent anticancer activity where as Curcumin and Zn both excreted through faeces, hence there is no significant effect of zinc reabsorption on Curcumin complex and activity is not accelerated by forming Curcumin zinc complex. Flavonoids are metal ion chelator, hence flavonoids present in *Trigonella foenum graecum* extract, formed complex with Zn and get reabsorbed by kidney, and thus gives potent anticancer activity.

Conclusion

The unique properties of herbomineral complexes tend to offer advantages in the discovery and development of new drugs. Additionally the effects of mineral such as zinc can be highly specific and can be modulated by recruiting cellular processes. These can be useful probes of cellular function. Understanding these interactions can lead the way toward rational design of herbomineral formulation and implementation of new co-therapies. These agents can modify both DNA and RNA with a high degree of region chemical, sequential, and conformational specificity. Cell selectivity in mRNA expression makes it an attractive target. These complexes can be potent and highly selective ligands of cell surface receptors. Studies of toxicity mechanisms may provide insights into potential therapeutic approaches. These complexes have shown potent anti-cancer activities in a variety of screens. Thus, have a significant health benefit and growth potential. New agents are likely to find unique market niches due to unique mechanisms of action or pharmacokinetic properties that complement other therapeutics

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