

Impact of Science on the Economics of Tea Industry

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Session VIII
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Prof. Maqsood Siddiqi is the director of Bose Institute Kolkatta since October, 2001. He is a cancer research scientist and was the Director of Chittaranjan Cancer Institute, Kolkata, before taking up the present assignment. He has been working on various aspects of cancer for the last 25 years and is recognized in his field of environmental carcinogens. His discovery of caffeine derived Nitroso compounds and their high implication in esophageal and stomach cancer in Kashmir has been internationally acclaimed. He has about 50 research paper publications in peer reviewed international research journals on various aspects of cancer. He has earlier been associated with Indian Institute of Medical Sciences in University of Kashmir, in Srinagar and the German Cancer Center in Heidelberg, Germany. His interests include chemo-prevention of cancer using tea polyphenols and other naturally occurring compounds as well as the etiology of environmental and genetically disposed cancer in India. He is well known in establishing a population based cancer registry in Kolkata and for initiating community-based cancer screening programs in rural Bengal. He is a member of large number of professional committees and Academic bodies related to cancer research and cancer control in India and has represented the country in various meetings on cancer research.



Opening Remarks by Session Chairman Dr. Maqsood Siddiqi

Good morning ladies and gentlemen. We have a penultimate session on health and tea. You are already aware that for the past 15 years tea has been variously named as the Elixir of life, the Champagne of the east or the beverage of the Millennium. Although in ancient days, discussing ancient medicine or traditional medicine, experts in both India and China have been talking about medicinal properties of tea and other plant-based compounds, it is only during the past 15 years that we have come to know about the health-beneficiary properties of tea through modern analytical testing systems. In the present session we have four speakers, Prof B.N. Dhawan, Mr. Jhawar, Prof. Hadi and Mr. Juneja, who will deal with Chemopreventive effects of tea against human ailments. The Chairman, then invited Dr. Dhawan to make his comments and then presentation of his paper.

Chapter 35

TEA- HEALTH FOOD OR RASAYANA DRUG?¹

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Prof. B.N.Dhawan, too well known in the field of pharmacology to need any introduction. In the field of medicinal plants he is known as the grand guru of medicinal sciences. Born in 1932 in an illustrious family in Uttar Pradesh, Prof. Dhawan did his M.B.B.S., and M.D. from KG Medical College Lucknow and post-doctoral training in UK. He was a professor of pharmacology at the Motilal Nehru Medical College at Allahabad and later shifted to the Central Drug Research Institute, Lucknow in 1967 as Assistant Director, then Deputy Director, and then he was the director of the C.D.R.I from 1988 to 1992. He was Emeritus Scientist of the CSIR (1992-1997). Prof. Dhawan is the Fellow of all important Academy of Sciences, is the winner of numerous awards, including the prestigious Ranbaxy award in 1988 and the Third World Academy Prize in Medical Sciences in 1998 in India and of Third World Academy of Sciences, Italy. He has studied the biological activity of more than 4000 medicinal plants and published more than 500 papers, edited 14 books and there are 30 patents to his credit.

**Preliminary Remarks by Prof. Dhawan**

Before I present my paper, I would like to make some general comments to place this session on tea and health in proper perspective. Much information has been generated on the health effects of tea during the last 10-15 years and the general public has realized the beneficial properties of tea. However, since the British introduced tea in this country, and the feeling at that time was that anything that the British brought here was meant to harm the locals, an impression persists amongst many people, unfortunately, that tea is harmful and is not a healthy drink.

It is for the tea industry to dispel this erroneous perception with all the data that experts like Dr. Siddiqi and Dr. Juneja have generated, and make people conscious of the fact that tea indeed is a health food. In my paper I purposely chose to refer to tea as food and not to call it as drug. The basic difference is that when talking about food you are talking of health, about something positive. When you talk of drug, you are talking of cure and drugs have a role only when disease has already occurred. Unfortunately, the drug industry is not very much interested in prevention because if there is no disease, they are out of business.

Drug development is an entirely different area. In drug industry you need very strong patents and anything that you discover as drugs, out of tea or any of biological material, will very unlikely yield patents. Therefore after putting up all the cost for developing a drug, my experience is that the original discoverer may land in a situation where no industrialist will be prepared to take up the new technology or market it because tomorrow, somebody will put an ethyl or a methyl group somewhere in the bioactive molecule and break the patent, come up with a new product to throw you out of business. So my suggestion to all scientists and particularly, the pharmacologists would be to try to promote tea as a health food rather than as a drug.

¹Dedicated to Dr. R.O.B. Wijesekera, formerly Special Technical Adviser, Chemical Industries Branch, UNIDO, Vienna on his 80th Birthday

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INTRODUCTION

The health giving properties of tea have been increasingly recognized during the last 2 decades. Many persons in India still consider tea to be an unhealthy drink. This may be because tea was introduced in India during the British rule and there was a general mistrust against culinary practices introduced by the foreigners. In India also the health-giving properties of tea are gradually being accepted.

What is the basic difference between a health food and a drug? A health food has something positive to offer. It can prevent disease(s). Drugs, on the other hand, are taken generally after a disease process has set in. The drug industry, like other industries, is basically profit driven. Its profits depend on the disease burden and hence they would be much less interested in promoting disease-preventing agents. They would take away their main source of business.

Secondly, the highly competitive drug industry survives on strong patents. These will not be easy to obtain on tea or its constituents. I will like to confine the present review to compare the health giving properties of tea with the class of Ayurvedic agents known as *Rasayanas*

WHAT ARE RASAYANAS?

It will be necessary to briefly recapitulate the basic tenets and objectives of *Ayurveda* to place the activities of *Rasayanas* in a proper perspective. The *Yajurveda* (36/24) has stated that the aim of *Ayurveda* is not necessarily to ensure a life span of 100 years but to enable living 100 years fully and actively. Thus, there is a built-in concept of quality of life. *Rasayana* therapy forms one of the 8 main branches of *Ayurveda* and *Charaka* (Su 30/20) separates *Rasayanas* from medicines (*Kaya Chikitsa*). *Agnipurana* (141/6) also classifies *Rasayanas* as *Sarvarogahari Aushadhi*, thus putting them in a separate class of medicaments.

Rasayana drugs have been defined in Ayurvedic texts in various ways. All the definitions, however, convey the concept of attainment of positive health, increased resistance to diseases and assured longevity. They are often confused with the modern Adaptogens. *Rasayanas* have a much broader spectrum of activity and adaptation to environmental stress is only one of them. *Sushruta* (Su 1/7) says that they re-establish youth, strengthen life and brain power and provide capability to counteract diseases. *Sharangdhara* (2/32) offers a more restricted definition, describing them as substances capable of destroying diseases of old age. *Agnipurana* (141/6) improves it by stating that they cure all illnesses and bestow immortality. *Charaka*, however, provides the most comprehensive definition. The *Charaka Samhita* (CS7,8) says that *Rasayana Drugs* lead to fulfillment of life as a whole. They prolong life span, ensure disease-free youthful life with good vigor and control of bodily functions, resounding voice and a glowing complexion. The treatise has classified 34 plants as *Rasayanas*, some of them being *Ayasika* (Dietary) *Rasayanas*. The purpose of the present review is to evaluate available data on tea and its constituents and to see if fulfils the criteria of a dietary or classical *Rasayana*.

MAJOR ACTIVITIES OF RASAYANAS AMENABLE TO LABORATORY EVALUATION

It is evident from the above discussion that *Rasayanas* exhibit multiple activities. Hence it is necessary to employ a battery of tests to uncover their activity profile. Three major activities of *Rasayanas* can be evaluated in animal models. These are:

1. Effect on general bodily and mental functions
2. Altered resistance to diseases caused by stress, toxins, infections, xenobiotics etc.
3. Effect on aging and life span

Several parameters have been used to evaluate

these activities. These can be grouped in following 6 heads:

- Actions on immune system
- Response to stress
- Mental activity
- Selected physiological functions
- Regeneration following injury
- Aging

It will be beyond the purview of the present paper to describe the methods used to screen for these activities in laboratory animals. The necessary details can be obtained from publications on screening models (Dhawan and Srimal 1998, Nodine and Seigler 1964, Seigler and Moyer 1967, Turner 1971)

COMPARISON OF ACTIVITIES OF TEA AND RASAYANAS

Rasayanas re-establish youth, strengthen life and brain and provide capability to counteract diseases (*Sushruta Su1/7*). The Chinese scholar Lu Yu, in a treatise written in 780 AD and entitled *Cha Ching*, states that "tea tempers the spirits, harmonizes the mind, dispels the lassitude, relieves fatigues, awakens thought, prevents drowsiness, refreshes the body and clears the perspective faculties" (Jhawar 2000). The similarities are apparent even from these early descriptions. A comparison of their effects in the experimental paradigms mentioned above and some clinical data should enable a better assessment of the similarities.

A. RESPONSE TO STRESS

Rasayanas modulate responses to all types of stress – Physical (Ramachandran et al 1990), Chemical (Dhawan 1995), Microbiological (Dahanukar et al 1986) or Endogenous like the cancer (Dhuley 1997, Seena et al 1993).

The effect of tea and its constituents has not been so systematically investigated on responses to

various types of stress. There are reports of many clinical and experimental studies, however, from which data can be extrapolated. These are summarized below under 4 heads:

Physical Stress

Reduction of sunburn and DNA damage following ultra-violet exposure of skin by local application of green tea polyphenols has been reported in a study on human volunteers (Elmets et al 2001)

Chemical Stress

Effects of tea constituents have been studied on non-specific as well as specific chemotoxins. In the first category, special mention may be made of the prevention of mutagenic effects of carcinogens and pro-carcinogens (Yamada and Tonita 1994). The effect against specific toxins has been studied in several tissues. Liver is protected against damage by galactosamine, aflatoxins, lipopolysaccharides etc. (He et al 2001). The DNA adduct formation by nitropropane is also inhibited (Shukla and Arora 2002). Renal failure induced by adenine or c-BSA is prevented by green tea polyphenols (Yokozawa et al 1996a) and a beneficial effect has been reported in patients on renal dialysis (Yokozawa et al 1996b). Hot water extract of green tea prevents bone marrow damage by aflatoxin B₁ (Shukla and Arora 2002). Amelioration of b-amyloid neurotoxicity is seen in cultured hippocampal cells (Choi 2001). Kim et al (2001) have observed selective inhibition of prolyl endopeptidase by (-)-epigallocatechin gallate, (-)-epicatechin gallate and (-)-gallocatechin obtained from green tea. These properties may find application in management of patients suffering from Alzheimer's disease. Similarly, the protective effect of tea catechins against 6-hydroxydopamine induced apoptosis in PC-12 cells (Jin et al 2001) suggests possible utility in patients of Parkinson's disease.

Microbiological Stress

Tea extracts have been reported to inhibit the growth of many viruses (Including HIV),

mycoplasma, fungi, protozoa and Gram positive as well as Gram negative bacteria (Miller and Taylor 2001). They can also suppress the emergence of resistance to antibacterial agents (Pillai et al 2001) and potentiate the activity of β -lactam antibiotics. Theasinensin A suppresses antibiotic resistance of methicillin-resistant *Staphylococcus aureus* (Hatano et al 2003). A synergistic effect is observed with some antibacterial agents. This has recently been reported for the effect of leofloxacin against *E. coli* (Isogai et al 2001). The protection against causative organisms of dental caries is well documented (Banerjee 1990).

Response to Endogenous Stress

Maximum information is available against several types of cancer (Katiyar and Mukhtar 1996). Large amount of epidemiological, clinical and experimental data has been accumulated during the last 2-3 decades. For example, there is significantly lower incidence of lung, digestive tract and skin cancer in communities with high consumption of tea, particularly the green tea (Bushman 1998). Green tea has been reported to have anti-cancer activity in certain experimental models as well (Oguni et al 1988). Tea flavonoids can inhibit urethane and NNK (a nicotine derived carcinogen) induced pulmonary neoplasm (Chung et al 2001) as well as induction of apoptosis and anti-clastogenic effect in experimental models of leukemia, gastric carcinoma etc. *In vitro* studies have demonstrated the ability of polyphenols to reverse multi-drug resistance in cancer cell lines (Zhu et al 2001) and synergistic activity with cancer preventing agents like genistein, sulindoc, curcumin etc. and with anti-tumor agents like doxorubicin.

B. EFFECT ON THE IMMUNE SYSTEM

Rasayanas have immuno-stimulant properties (reviewed by Agarwal and Singh 1999). Some of them, in addition, exhibit anti-allergic activity (Baruah et al 1998).

Tea extracts and constituents also share some of these properties. Some pertinent experimental and clinical data have been summarized below:

Melanin-like

Melanin-like pigment from black tea has immuno-stimulant activity (Sava et al 2001)

Black tea

Black tea can reverse EAC induced immuno-suppression (Bhattacharya et al 2002).

Anti-allergic Activity

Tea catechins inhibit PCA in rat and mouse (Kar et al 1981) and autoimmune disease in MRL-fas/fas mouse (Sayama and Oguri 2001). They suppress expression of high affinity IgE receptor FC Epsilon R (Fujimura et al 2001). Green tea polyphenols have beneficial effect in guinea pig allergic rhinitis model (Juneja 2001). Kunishiro et al (2001) have shown facilitation of antigen-specific antibody production through selective augmentation of IL-2 generation *in-vitro* as well as *in-vivo* by tea extracts.

Clinical Data

Oolong tea has beneficial effect in the patients of atopic dermatitis and allergic rhinitis (Uchara et al 2001). There is an improvement in CD4/CD8 ratio with the green tea (Tsuboi et al 2001).

C. EFFECT ON PHYSIOLOGICAL FUNCTIONS

Rasayanas improve physical performance and stamina (Grover et al 1995), optimize food utilization (Pushpangadan et al 1995), are potent anti-oxidants (Rastogi et al 1995) and can restore disturbed carbohydrate and lipid metabolism (Khanna et al 1994).

Tea extracts and constituents share many of these properties. Massive data is some on some of these activities of products from tea but only a brief summary can be included in the present review under 6 heads:

Work Performance

Tea extracts facilitate skeletomotor function by action on L calcium channels and are known to alleviate post-game fatigue in athletes (Pushpangadan and Latha 2002). They have been given to horses in Tibet to increase their capacity to work (Emboden 1971).

Food Intake and Body Weight

Green tea ethanolic extract inhibits gastric and pancreatic lipases and stimulates thermogenesis. It exerted a weight reducing effect in clinical studies in moderately obese patients. (Chantre and Lairon (2002). Intra-peritoneal administration of (-)-epigallocatechin-3 gallate produced a reversible 20-30% reduction in body weight in 2-7 days, due to a reduction in food intake (Kao et al 2000). Anti-obesity effect has been reported in female mice also. More data is required to evaluate this effect.

Carbohydrate Metabolism

Hypoglycemic activity has in a been reported variety of experimental models including normal, streptozotocin, fructose or alloxan diabetic rats and KKA mice (Sugahara et al 2001). Aldose reductase activity is inhibited in streptozotocin diabetic rats thereby slowing the progress of nephropathy and cataract (Sakai et al 2001). The secretion of insulin is not affected in diabetic animals (Sugahara et al 2001). It, however, caused more than 15-fold increase in sensitivity to insulin in an *in-vitro* epididymal fat cell assay. The main active ingredient was (-)-epigallocatechin gallate (Anderson and Polansky 2002). A lowering of blood glucose has also been reported with a polysaccharide from tea, which is coordinated with rare earth metals (Wang et al 2001). Clinically, lowering of blood sugar and HbA_{1c} (Fukino et al 2001) and slowing the progress of nephropathy in patients of diabetes mellitus has been reported (Takaro et al 2001)

Lipid Metabolism

Yang et al (2001) have reported a reduction in

raised levels of serum cholesterol and triglycerides, fat storage in the liver and heart and in the weight gain in rats on a high sucrose diet. Catechins prevent atherosclerosis in hamsters on a high fat diet (Vinson et al 2001) in apoprotein E deficient mice by inhibiting activity of p-glycoprotein (Miura et al 2001). Cholesterol biosynthesis is prevented by selective inhibition of squalene peroxides (Abe et al 2001). Clinical data supporting hypolipidemic activity of tea constituents include lower levels of serum cholesterol LDL and VLDL along with a raised level of HDL, reduction in atherogenic index (Imai and Nakachi 1995) and lowered level of the adhesion molecule P-selectin (Hodgson et al 2001). Green tea extract enhances neutral endopeptidase activity in SK-N-SH cells, thereby preventing the formation of amyloid plaques (Melzig and Jaika (2003). Oolong tea suppresses oxidation of LDL in a dose-dependant manner (Kurihara et al 2003).

Detoxification Mechanisms

Tea polyphenols induce xenobiotic detoxifying enzymes like Cytochrome P450 1A₁, 1A₂, 2B₁ and UDP-glucuronyl transferase (Weisburger 2000). They can also prevent heavy metal toxicity by chelation (Liao et al 2001).

Anti-oxidant Activity

Tea polyphenols effectively scavenge the reactive oxygen species (ROS) which are a major contributory factor in cellular injury by (Yang and Wang 1993)

D. EFFECT ON CNS ACTIVITY

Rasayanas facilitate learning and consolidation of memory, antagonize CNS effects of stress, are anxiolytic and are capable of interacting with some neurotransmitter mechanisms (Dhawan and Singh 2002).

Tea constituents improve learning and memory in senescence accelerated mice, specially the older animals (Unno et al 2001). They can antagonize 6-

hydroxydopamine induced apoptosis in PC-12 neurons (Jin et al 2001), which is an *in-vitro* model of Parkinsonism. The toxicity of nitric oxide (NO) on hippocampal neurons is also antagonized (Nagai et al 2001). The thearubigin fraction can block the paralytic effect of botulinum (Sato et al 2001a) and tetanus (Sato et al 2001b) neurotoxins. Theanine protects against ischaemic delayed neuronal death (Kakuda 2001). The activity of several enzymes linked to monoamine neurotransmitters, like tyrosinase, COMT and MAO, can also be affected (Siddiqui et al 2001). (-) Epicatechin gallate inhibits neurosphere adhesion, cell migration and neurite outgrowth in rat neurospheres (Chen et al 2003). It might affect neural stem cell survival or differentiation.

E. FACILITATION OF TISSUE REGENERATION

Rasayanams reduce time for regeneration of damaged tissues and also lead to a better functional recovery after injury due to ischaemia re-perfusion (Singh et al 2000), toxin (Tandon et al 1995) or surgical resection (Saxena et al 1997).

The data with tea constituents is rather limited and further in-depth studies are strongly indicated. Green tea has been reported to facilitate recovery from ischaemia re-perfusion injury of forebrain in gerbils (Kakuda 2001) and of brain and gastric mucosa in rats (Yagi et al 2001). Similarly, repair of DNA damage by mutagenic agents is facilitated. No data is available on the effect on recovery of organs like liver following partial resection.

F. ANTI-AGING EFFECT

Rasayanams improve life span, help retain efficient task performance and retard development of biochemical markers of aging (Pattan 2003).

Large amount of epidemiological data is available from well-planned surveys in tea drinkers but there

have been very few experimental studies. Sadakota (1995) has reported lower mortality rate in Japanese women practitioners of traditional tea ceremony. The incidence of debilitating and killer diseases is also less. Kanis et al (1999) reported protection against hip fracture in a population based study and Hegarty et al (2000) found significantly higher bone density in spine and hip region of 65-75 year old tea drinkers. The Dutch cohort study (Geleijnse et al 1999) suggests lower risk of death from coronary artery disease or stroke in tea drinkers. In a Boston study also, the risk of heart attack was assessed to be lower in persons drinking one or more cup of tea daily (Sesso et al 1999). Bushman (1990) has observed, in a review of cohort studies, a lower incidence of cancer of esophagus, stomach, colon and pancreas in green tea drinkers. It is not easy to undertake such studies in tea drinking countries like India because control cohorts of non-tea drinking persons are difficult to obtain.

TEA VERSUS OTHER HEALTH DRINKS

Karakaya et al (2001) undertook an interesting study in Turkey. They compared the anti-oxidant activity of various health drinks with the phenolic content and observed a good co-relation ($r_2 = 0.95$). They also found that phenolic contents per serving were higher in liquid than in solid foods. They gave the highest ranking to black tea among the commonly consumed liquid food in that country. It was followed in descending order by instant coffee, cola drinks, red wine, carrot juice, apricot nectar, Turkish coffee, grape molasses and white wine. In a more recent study, Parmar et al (2003) have also shown that anti-oxidant activity of tea is comparable with fruits and vegetables. They have concluded that tea seems to fit the description of an ideal component of a healthy dietary habit. Further such studies are indicated in other countries to compare tea with locally consumed liquid foods.

DOES THE COLOR OF TEA MATTER?

Majority of tea drinkers (~ 78%) take black tea as

such or with milk (~ 50%). The polyphenol content of both is similar and largely consists of theaflavins and thearubins. The next commonly used tea is the green tea (~ 20%). It is rich in the original polyphenols like (-)-epigallocatechin and other catechins. The red (Oolong) tea is mainly used in certain parts of China and would account for only ~2% of total consumption. The polyphenol oxidase partially converts catechins to other polyphenols. The use of the least processed form of tea, the white tea, would be hardly 1%. It contains 9 major constituents of green tea, even though their relative percentage may vary. Nishizawa and Van (2001) have made the important observation that all varieties of tea have similar physiological effects even though the composition of polyphenols etc. may differ.

THE EPILOGUE: HOW MANY CUPS?

Nothing describes this better than the following English translation of an old Chinese poem by Jhawar (2000):

*“One cup does all disorders cure
With two, your troubles will be fewer
Thrice, to the bone more vigour give
With four, forever you will live
As young as on your day of birth”*

CONCLUDING REMARKS

This brief review clearly indicates that tea constituents exhibit, to a significant degree, all the beneficial properties ascribed to *Rasayanas* in the *Ayurveda*. It can be safely predicted that if tea were available in India when the major Ayurvedic treatises were being compiled, it would have found place as a *Rasayana*. Its introduction in India during the 18th century was too late for inclusion in Ayurvedic texts. It is suggested that the Indian tea industry should promote tea as a *Rasayana* rather than just as a refreshing drink or even as health food. It is also necessary that adequate funding should be made available for experimental, clinical and epidemiological studies in few areas where more information is required.

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Introduction of Mr. Jhavar by Session Chairman Dr. Siddiqi

Mr. Jhavar is one man, in my view, who has promoted the positive aspects of tea for health. He has motivated a greater number of scientist including me to take up tea and study the chemo preventive properties against cancer, he has been the moving spirit behind a lot of new projects which have come up in Calcutta. In several laboratories we have groups working, including Bose Institute, Cancer Institute, IICB or Indian Institute of Chemical Biology and other institutions in town and also in various other places in the country, his book on tea and human health has been very well received, both by scientist, general readers as well as the industry which was released at the India International Millennium Tea Conference in the year 2000. I request Mr. Jhavar to deliver his presentation.

Chapter 36**TEA & HEALTH: GENERATING CONSUMER AWARENESS IN INDIA****R. S. Jhavar***

Mr. R.S. Jhavar is the whole time Director of Eveready Industries of the Williamson Magor Group. He is the former Chairman of Indian Tea Association for two terms, former Regional President of Indo-American Chamber of Commerce and the Vice President of Tea Board of India. Currently he is associated with the Bengal Chamber of Commerce in India. He is also a distinguished member of Institute of Chartered Accountants of India and an Associate Member of Chartered institute of Management Accounts, London.

Mr. Jhavar is the recipient from the President for Proficiency in Chartered Accountancy. He was also the President of Calcutta Branch of Chartered Institute of Management Accounts, London for three terms and was awarded a special Citation in appreciation of his services to the institute. He is now the President of Calcutta Society of Management. He is the recipient of numerous awards including one from the India International Society for Excellence in Management and also from the Institute of Economics Studies.

The last decade has been quite eventful for tea as a beverage. It is, however, essential to continue our efforts to highlight the health benefits of this beverage and make tea popular among the common man in India.



The first International Seminar on Tea was held in Washington in September 1993, which mainly discussed the qualities of 'green tea' and brought out the health benefits of tea for the first time at an international forum. It took another five years to hold the Second Seminar in 1998 at the same place where the benefits of both green and black tea were discussed. The year 1998 became a historic day for the beverage when scientists from different parts of the world categorically said that 'black tea' also

has all the properties or equally good health-giving properties, as that of green tea.

Simultaneously, the FAO took the lead and, along with four tea-producing countries and three tea-consuming countries of the world, took up research projects worth \$ 4-5 million to go into all aspects of tea. These projects came out with similar conclusions that tea has all the health-giving properties for preventing chronic and non-chronic diseases. At the same time independent studies were carried out in various countries, Including India, China and Japan, where experts came to the

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conclusion that tea has the properties to help prevent certain chronic and non-chronic diseases. Leading scientists and research organizations took up research in India, including the Bose Institute and the NTRF and their findings led to the conclusions that regular consumption of tea helps in preventing diseases, like cancer, cardiovascular ailments, blood pressure, cataract, diabetes, renal and dental problems, and helps in absorption of body fat. It checks environmental pollution by weaning away heavy smokers. More recent reports also indicate that tea helps fighting HIV/AIDS. These reports, however, need to be authenticated.

It is beyond doubt that most of these maladies are caused by changes in life-style — our food habits — our craze for junk food, drinking, smoking, work pressure and lack of exercise etc. If tea has all these beneficial qualities, it should be of great assistance to the mankind. It will also help us cut down the spiraling health-care cost, which is one of our major problems in the developing and the developed countries today.

The impact of the chronic diseases has also been clearly brought out by the WHO in one of its recent reports. It says that on one hand the life expectancy of mankind has gone up significantly, but chronic diseases have taken an increase in toll of human lives — 50% of it account for Cancer and cardiovascular diseases. HIV-AIDS has compounded the problems. Half a century ago, the life expectancy had an average of 50 years. Today, it is 60 years and is projected to reach 71 by the year 2020. I happened to read somewhere that 75 years hence, the life expectancy could even go up to 150 years! I understand replacing the genes by healthier ones can play an important part in it.

If tea has this unique role, then we should take advantage of it and allow the mankind follow their own lifestyle, while they remain immune to chronic

diseases. It is also most essential that we let the common man know about it.

Discoveries of modern science and technology have made life so much more comfortable, but at the same time, there is a psychological fear about the possibilities of dreaded diseases. Quality of life can be influenced by intake of food and beverages. Except for alcohol, no serious research has taken place in food and beverages in the past. Tea here is the biggest winner. Awareness is still lacking and there are lots of misconceptions about tea in India that must be removed. The Chinese conception of olden days about tea is today getting a scientific confirmation.

Apart from water, tea is the only drink that has no intoxication, no extra caffeine like that in coffee, and no harmful preservatives and no risk of pollution. If what has been mentioned about tea is correct, then we have to find out how to go about creating a sense of awareness and ensure that it reaches the common man. We have to minimize the gap between laboratory findings and their availability for the common man. This is very important from the industry viewpoint. There have been many discussions, but somehow the desired level of decision making and its implementation, has been missed.

The issue, to my mind, can be tackled at national level by:

- a) Holding industry-level Seminars at District, Panchayat and Zila Parisad levels,
- b) Making use of the distribution outlets — tea shops, dhabas, restaurants (numbering an estimated 5 million countrywide) to carry the message of health benefits of tea
- c) Involving schools, colleges, doctors, women's organizations, hotels and catering management institutions,
- d) Getting chefs to prepare menus using tea in leading hotels,
- e) Collecting all the facts about tea, consolidate

findings of research on tea and presenting these to the Ministry of commerce so that programmes can be initiated through the Tea Board of India, as they do in Japan,

- f) Strengthening the *"Piyo More Chai"* campaign
- g) Involving the Health Ministry and the Ministry of Rural development for generic campaigns to create public awareness,
- h) Putting up advertisements in print & electronic media.

And at international level by:

- a) Involving the WHO, UNICEF, FAO
- b) Involving other international agencies like NGOs, the Lions and the Rotary Clubs,
- c) Making tea perfumes, lotions, hair creams, slimming, beauty-care & skin-care products

(There was a craze for tea at the recent Tea Convention in Paris— the center of world fashions and beauty.)

We have to utilize today's craze for "natural products" and reach for the Youth — let them know that tea is a trendy drink.

It should be kept in mind that tea is not a "food" but a supplement to food, which can be taken with

food — as with bread, chappatis and biscuits. The U.S. Food and Drug Administration are being persuaded by the tea organizations in the U.S. to declare tea as a part of food and give it its proper recognition.

The future of tea depends on the tea industry itself. The industry has an important role to play to disseminate information about the health - giving properties of tea. They should also maintain the quality of tea and not aim at too much profitability. Establishment of common tea-testing laboratories and a mention of flavonoid/anti-oxidant percentages on tea packets are also important aspects that can be considered.

Per capita consumption of tea in India is still very low at about 650 - 700 gms. There is considerable scope to increase this. An increase of about 10 - 20 per cent will be a quantum leap.

I have no doubt that the International Conference on Global Advances on Tea science will bridge the gap between the research findings in laboratories and their utilization for the benefit of the consumer. It is a challenge for the industry as well as for the mankind.

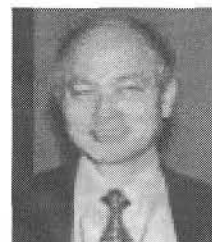


Chapter 37

HEALTH BENEFITS AND INDUSTRIAL APPLICATIONS OF TEA CATECHINS

Yukihiko Hara*

Dr. Yukihiko Hara was born in Japan in 1943, graduated in Agricultural Biological Chemistry and received his Ph.D. degree (1990) from Tokyo University. He joined Mitsui Norin Company in 1967 and became the Director of its Food Research Institute, Fujeida City in 1983. He is Vice President of Tokyo Food Techno and Senior Adviser of Mitsui Norin Co. Ltd. A recipient of the 1996 Merit Award from the Japanese Ministry of Science & Technology for his pioneering work on tea catechins, Dr. Hara is author and co-author of many papers, journal articles, book chapters and books. He is a member of the Japanese Cancer Association, the Japanese Society of Nutrition & Food Science, The Japanese Society of food Science & Technology and The Japan Society of Bioscience, Biotechnology & Agro-chemistry.

**INTRODUCTION**

Various health beneficial functions of tea catechins have been investigated for last 25 years. In order to make use of tea catechins on a large industrial scale, the following factors are essential:

- 1) investigation of the physiological actions in vitro, in vivo, and/or in humans;
- 2) extraction of tea catechins on an industrial scale; and
- 3) impregnation of commercial products with tea catechins and proof of the utility thereof.

In all cases, the following three difficulties have to be overcome:

- 1) the taste of tea catechins is extremely pungent,
- 2) tea catechins tend to stain other components with dark color as time passes,
- 3) the cost of tea catechins tends to be expensive because of the sophisticated purification process.

Above points will be discussed with various examples of successful commercial realization.

R&D FOR THE INDUSTRIALIZATION OF TEA CATECHINS

Following are notable physiological functions of tea catechins.

Anti-Oxidative Action on Edible Fats and Oils

The major tea catechin, epigallocatechin gallate (EGCG), was confirmed to have 20 times more potent anti-oxidative activity than α -tocopherol in the AOM system in which heated lard mixed with these antioxidants was oxidized with bubbling air.

Radical Scavenging Actions Against Harmful Radicals

Potent radical scavenging action of tea catechins was confirmed with DPPH radicals. Among catechins, galloyl catechins show twice as much potency as catechins without gallate moiety. In this system, EGCG was nearly 20 times more effective than α -tocopherol. Similar scavenging potency was observed against superoxide anion or hydroxy radical as in the case of DPPH.

Anti-Bacterial Action Against Foodborne Bacteria

Minimum inhibitory concentrations (MIC) of tea catechins against such food-borne bacteria as

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Staphylococcus aureus, *Vibrio parahaemolyticus* etc. were determined. The growth of these bacteria was suppressed by tea catechins at concentrations less than that of a normal drinking brew.

Anti-Carious Action

Dental caries occurs by the infection and growth of the bacterium, *Streptococcus mutans*, on the dental surface. Tea catechins inhibit the plaque-forming enzyme of *S. mutans*. At less than a normal drinking concentration, tea catechins suppress the growth of *S. mutans* and hence prevent dental plaque formation and caries.

Inactivation of Influenza Virus

By incubating the flu virus with tea catechins at a few ppm concentration, the virus loses infectivity to cells. A very trace amount of tea catechins interacts instantly with the glycoprotein of the viral surface and renders it non-infective. After the virus was adsorbed inside the cells (infection), tea catechins could not inhibit the proliferation of the virus.

Suppression of Blood Glucose Increase

The intake of starch or sucrose increases the blood glucose level due to the activity of α -amylase or sucrase. Following oral intake, tea catechins interact with α -amylase or sucrase in the intestine and thereby suppress an increase of the blood glucose level. Thus, the intake of tea catechins could be an effective diet regimen.

Suppression of Hypertension and Brain Stroke

Tea catechins inhibit the activity of hypertensive enzyme (angiotensin 1 converting enzyme) in the blood stream and suppress the excessive increase of tension. The hypotensive effect was proved in spontaneously hypertensive rats (SHR) and stroke prone SHR by the feeding of catechins in their diet.

Suppression of Cholesterol and Fat Increase

Tea catechins inhibit the micellation of lipids in the intestine and hence suppress cholesterol absorption in the body. By administering catechins

to animals or humans consuming a high fat diet, the increase of the blood cholesterol level, in particular that of LDL-cholesterol, was suppressed.

Anti-Tumor Action

Various in vitro and animal experiments show that tea catechins suppress the process of carcinogenesis in various ways. The effects range from the inhibition of initiation, promotion and progression in the process of tumor formation and even metastasis or angiogenesis of tumors.

Improvement of Intestinal Flora

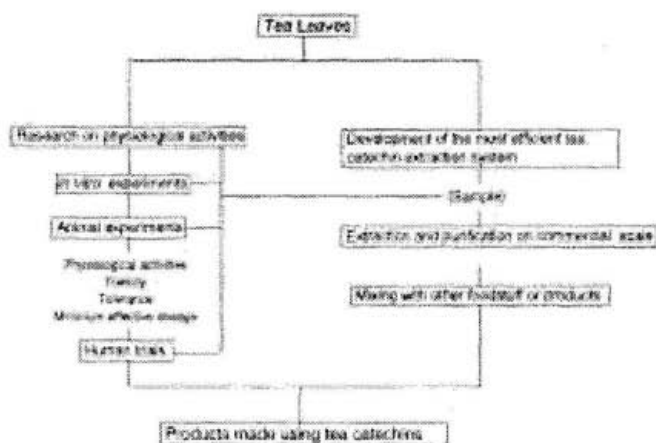
By administering catechins over a period of a few weeks in the diet of people in long-term care, remarkably favorable changes of fecal parameters were observed. Significant increase of lactic acid bacteria and decrease of putrefactive, odorous compounds in the feces were confirmed in human trials.

Deodorant Action

Catechins were confirmed to trap formaldehyde and other obnoxious gases, which cause so-called sick house syndrome and lower the concentration of these harmful gases in the atmosphere of the house. As opposed to other formaldehyde catchers, tea catechins do not release the volatile gas after scavenging. There are plans to make use of these effects in various housing materials and items.

SCHEMATIC EXAMPLE OF R&D PROCESS

Fig. 1. Tea catechins - Research and Development



EXAMPLES OF CATECHIN IMPREGNATED COMMERCIAL PRODUCTS

Catechin Capsules

A range of health supplements that offer all-round benefits of health maintenance and prevention of illness.

'Catechin ACE' is a popular, high-grade health supplement consisting of a blend of catechins and vitamins, giving increased radical scavenging activity.

'Catechin 100 Plus Oligo' is a tablet combining oligo sugar with tea catechin, which regulates the condition of the intestinal flora much more effectively than lactic acid beverages such as oligo and others.

'Catechin 100' containing catechin only; also works to maintain the health, prevent colds etc., and has many avid users.

'Catechin 50' is another product that is now being introduced to long-term care facilities since it is known to reduce unpleasant fecal odors.

Catechin Eggs

Tea catechins lower cholesterol in the blood, inhibit oxidation and protect against heart disease, as has been confirmed in rats and clinical trials. We harnessed these effects by feeding catechin to chickens to produce an out-standing, low-fat egg. 'Catechin eggs' are sold throughout Japan. Now wider applications, such as in the area of livestock, 'Catechin chicken', are also being researched.

Catechin Candy

Tea catechins have been proven to play a useful role in various aspects of oral hygiene, such as preventing tooth decay and the influenza virus. 'Catechin candy' fortified with tea catechins is now being sold throughout Japan.

Catechin Anti-Flu-Air-Filter

This product is an air filter impregnated with tea catechins. Research has shown that in a room where an air cleaner fitted with this filter is used, almost all viruses are trapped and rendered inactive. A virus-free environment can be achieved easily with this novel product. Other applications could be, for example, to combine this filter with a hepa-filter, or put into a drying machine that is used for taking the moisture out of futons (bedding mats).

Catechin Car-Air-Filter

World-recognized automobile company, Suzuki Motor Corp. and we have developed an inventive Catechin Air Filter that reduces tobacco and other odorous volatiles inside the car. This filter is used widely in various vehicles of Suzuki, for example, Suzuki MR Wagon.

Catechin Mask

A catechin filter has been fitted to this mask. It can be used to guard against influenza, and also has a preventative effect against hay-fever.

Catechin in Cosmetics

Tea catechins can protect the skin from the harmful effects of UV rays and guard against pigmentation. Tea catechins have been used in some cosmetics and soap products and further wider applications are expected in the future.

Preservative for Seafood Products

Tea catechins are used to prevent the dissolution by oxidization of the color component in red fish (carotenoid), the deterioration of fish oil, an increase in bacteria, fishy odors etc. The freshness of dried salted fish can be preserved notably by the addition of just a small amount of tea catechins to the salty water during the production of this product.

Kitchen Disinfectant and Deodorizer

A spray for kitchen use containing catechins dissolved in ethanol, disinfects and deodorizes.

Breath Freshener

In tablet form and packaged in a small-sized container for use as a mouth-freshener after meals, this product puts to work the odor-reducing action of tea catechins on fish and garlic odors.

Tonic with Radical Scavenging Effect

A health tonic product that combines the healthy effects of tea catechins with anti-oxidative vitamins. Packed in glass bottles, one dose require 50ml.

Catechin Beverage

A healthy, thirst-quenching beverage fortified with catechins and other natural ingredients (500ml bottle).

Tea-dyed Antibacterial Clothing

Towels, socks, T-shirts etc., manufactured using tea-dyed cotton. The antibacterial action of tea catechins lasts for a considerably long time, even

after many washes.

Mollusk Spray

A unique and safe liquid catechin spray for gardening use, that kills slugs when sprayed on directly.

Formaldehyde-Scavenging Materials and Products

These products make use of a remarkable formaldehyde scavenging ability by tea catechins. Tea catechins are extremely effective at scavenging even the smallest amount of formaldehyde present in the environment or in a room. Moreover they do not release the formaldehyde after scavenging. Expectations are that the safety of the environment of new buildings will improve greatly. Applications for all housing materials (plywood, adhesives, paints, tatami floor mats etc.) are under study.



Chapter 38

PRO-OXIDANT ACTIVITY OF TEA POLYPHENOLS : IMPLICATIONS FOR ANTI-CANCER PROPERTIES

Sonish Azam^a, Naghma Hadi^a, Asfar S. Azmi^a, N.U. Khan^b and S.M. Hadi^a

Prof Hadi has been the professor of biochemistry at Aligarh University. He has a very distinguished career in the field of biochemistry in general, particularly in molecular biology and & gene cloning. He switched to polyphenols and worked on a number of polyphenols for a long time. I am very happy that finally he has come to polyphenols from tea. He has recently proposed a mechanism about how tea acts. So it would be very interesting to hear him.

Prof. Hadi has worked for a number of years abroad. He was with Case Western School of Medicine and Brookhaven National Laboratory in New York, USA. He was also associated with University of Leeds in United Kingdom and University of Innsbrook in Australia. Besides, he has more than 5 years association with Department of Micro Biology at Biozentrum University, at Basel, Switzerland. He spends summer at Basel even now whenever he gets time from his laboratory in India.



SUMMARY

It is believed that anticancer and apoptosis inducing properties of green tea are mediated by its polyphenolic constituents particularly catechins. A number of reports have shown that green tea polyphenol (-)- epigallocatechin-3-gallate (EGCG) is among the most effective chemopreventive and apoptosis-inducing agents present in the beverage. Plant polyphenols are naturally occurring antioxidants but they also exhibit prooxidant properties. Over the last several years we have shown that various classes of plant polyphenols including flavonoids, curcuminoids and tannins are capable of catalyzing oxidative DNA cleavage particularly in the presence of transition metal ions such as copper and iron. With a view to understand the chemical basis of various pharmacological properties of green tea, in this paper we have compared the prooxidant properties of green tea polyphenols- EGCG and EC ((-)- epicatechin). The rate of oxidative DNA degradation as well as hydroxyl radical and superoxide anion formation

was found to be greater in the case of EGCG as compared with EC. It was also shown that copper mediated oxidation of EC and EGCG possibly leads to the formation of polymerized polyphenols. Further, it was indicated that copper oxidized catechins were more efficient prooxidants as compared with their unoxidized forms. These results correlate with the observation by others that EGCG is the most effective apoptosis inducing polyphenol present in green tea. They are also in support of our hypothesis that prooxidant action of plant polyphenols may be an important mechanism of their anticancer properties. A model for binding of Cu(II) to EC has been presented where the formation of quinone and a quinone methide has been proposed.

Keywords: India; green tea catechins; DNA cleavage; antioxidant; prooxidant; Cu(II) binding.

INTRODUCTION

There has been increasing realization in recent years that several plant derived polyphenolic compounds may possess anticancer and apoptosis-inducing properties (Mukhtar et al., 1998; Clement et al., 1998). Therefore, the role of plant-

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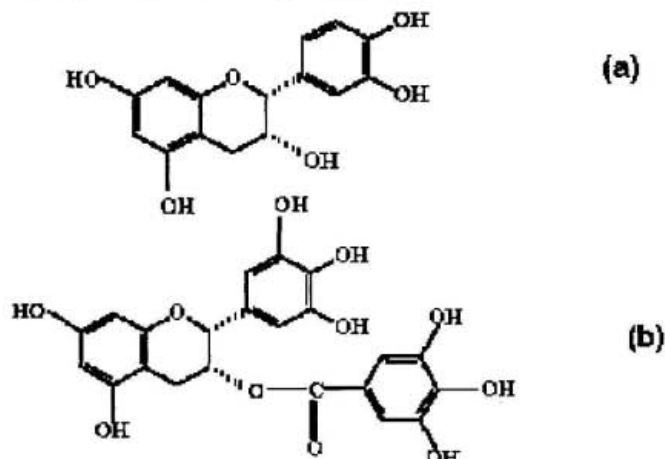
derived polyphenols in chemoprevention of cancer has emerged as an interesting area of research. The data in literature points to the possible role of green tea as a chemopreventive agent against different types of cancers (Picard, 1996; Sato, 1999; Sadzuka et al., 1998; Otsuka et al., 1998). Tea (*Camillia sinensis*) is the second most common beverage in the world next to water (Wei et al., 1999). Although both green and black teas are derived from *Camillia sinensis*, it is the production process that differentiates the two types of teas. Green tea contains polyphenols that include flavanols, flavandiols, flavonoids and phenolic acids. Most of the green tea polyphenols are flavanols, commonly known as catechins. The primary catechins in green tea are - epicatechin (EC), (-)-epicatechin-3-gallate (ECG), (-) epigallocatechin (EGC), (-)-epigallocatechin-3-gallate (EGCG), (+)-gallocatechin and (+)-catechin. It is believed that much of the anticancer effect of green tea is mediated by its polyphenolic constituents (Ahmad et al., 1998; Katiyar & Mukhtar, 1996). During the manufacture of black tea these polyphenols undergo polyphenol oxidase catalyzed oxidative polymerization giving rise to the formation of theaflavins and thearubigins in the process referred to as 'tea fermentation' (Wei et al., 1999). However, it is considered that black tea is not as effective in its chemopreventive properties. Other studies have shown that black tea polyphenols-theaflavins exhibit stronger anticarcinogenic activity than EGCG. Thus, the molecular mechanisms of cancer chemopreventive effects of tea polyphenols are not completely understood (Lin & Liang, 2000).

Plant polyphenols are natural antioxidants and most of their pharmacological properties are considered to be due to their antioxidant action (Ames et al., 1995). This is generally considered to reflect their ability to scavenge endogenously generated oxygen radicals or those radicals formed by various xenobiotics, radiation etc. However, some data in the literature suggest that the antioxidant properties of the polyphenolic compounds may not fully account for their chemopreventive effects (Gali et

al., 1992) Most plant polyphenols possess both antioxidant as well as prooxidant properties (Inoue et al., 1994) and we have earlier proposed that the prooxidant action of polyphenolics may be an important mechanism of their anticancer and apoptosis inducing-properties (Hadi et al., 2000).

In our laboratory we have confirmed that a number of plant polyphenols like flavonoids, tannins and trans-stilbenes possess oxidative DNA cleavage properties either alone or in the presence of transition metal ions such as copper (known to be a normal component of chromatin) (Rahman et al., 1989; Khan & Hadi, 1998; Ahsan & Hadi, 1998; Ahmad et al., 2000). It is to be noted that a number of reports have shown the green tea polyphenol EGCG to be among the most effective apoptosis inducing agents present in green tea (Chen et al., 1998). With a view to understand the chemical basis of various pharmacological properties of green tea, in this paper we have compared the prooxidant properties of green tea polyphenols - EGCG and EC (Fig. 1). Our results indicate that of the two EGCG is more effective as a prooxidant. It is also the more efficient reducer of Cu(II) to Cu(I), a reaction which leads to the formation of reactive oxygen species such as the hydroxyl radical (Rahman et al., 1990). A model of Cu(II) binding to epicatechin has also been proposed.

Fig. 1. Structures of (a) (-)-epicatechin; (b) (-)-epigallocatechin-3-gallate



MATERIALS AND METHODS

Calf thymus DNA (sodium salt, average molecular weight 1×10^6), bathocuproine, S_1 nuclease, (-)-epicatechin (EC) and (-)-epigallocatechin-3-gallate (EGCG) were purchased from Sigma Chemical Company (St. Louis, MO). Supercoiled plasmid pBR322 DNA was prepared according to the standard methods (Maniatis et al., 1982). All other chemicals were of analytical grade.

Copper Oxidation of Green Tea Catechins

Catechins (EC & EGCG) and Cu(II) (4 mM each) were incubated overnight in a final volume of 200 μ l. 100 mg of chelex in 1 ml of 10 mM NaCl was centrifuged at 2500 rpm for 10 min. The supernatant was removed and 1 ml of distilled water was added to the pellet. 200 μ l of this suspension was added to the overnight incubated sample of catechin and Cu(II). After 10 min shaking the sample was centrifuged at 2500 rpm for 10 min. The supernatant containing oxidized catechins was collected.

Thin Layer Chromatography

Copper oxidized catechins were applied on to silica gel (25 μ m layer thickness) plates for thin layer chromatography (TLC) along with the standard compounds. A mixture of toluene – ethyl acetate (1:8) was used as the solvent (Wei et al., 1999). Polyphenols were detected by exposure to iodine as well as under UV (254 nm).

Reaction of Catechins and Copper Oxidized Catechins with Calf Thymus DNA and Digestion with S_1 - Nuclease

Reaction mixtures (0.5 ml) contained 10 mM Tris-HCl (pH 7.5), 500 μ g DNA, cupric chloride and polyphenols as indicated. Incubation was performed at room temperature for 1 hour. All solutions were sterilized before use. Single strand specific nuclease digestion was performed as described previously (Naseem & Hadi, 1987). Acid soluble deoxyribonucleotides were determined colorimetrically (Schneider, 1957).

Reaction with Plasmid PBR322 DNA

Reaction mixtures (30 μ l) contained 10 mM Tris-HCl, pH 7.5, 0.5 μ g plasmid DNA and other components as described in the figure legend. Incubation at room temperature was carried out for 1 hour. After the incubation 10 μ l of a solution containing 40 mM EDTA, 0.05% bromophenol blue tracking dye and 50% (v/v) glycerol was added and the solution was subjected to electrophoresis on 1% agarose gel. The gel was stained with ethidium bromide (0.5 mg/l), viewed and photographed on a UV transilluminator.

Stoichiometric Titration of EC and EGCG

The concentration of Cu(I) produced in the EC/EGCG-Cu(II) reaction mixture was determined by titration with bathocuproine. Bathocuproine complexes with Cu(I) to form a Cu (Bathocuproine)₂⁺ which has an absorption peak at 480 nm (Nebesar, 1964). EC and EGCG (10 μ M each) in 10 mM Tris-HCl, (pH 7.5) was mixed with varying concentrations of CuCl₂ (2.5 μ M – 50 μ M) and 0.3 mM bathocuproine solution in a total volume of 3 ml. The bathocuproine – Cu(I) complex was determined by measuring at 480 nm.

Assay of Active Oxygen Species

Superoxide anion was detected by the reduction of nitroblue tetrazolium (NBT) essentially as described by Nakayama *et al.* (Nakayama et al., 1983). A typical assay mixture contained 50 mM potassium phosphate buffer (pH 7.8), 33 μ M NBT, 0.1 mM EDTA and 0.06 % Triton X-100 in a total volume of 3.0 ml. After mixing, absorbance was recorded at 560 nm against a blank, which did not contain the compound, at different time intervals.

In order to compare the hydroxyl radical production by increasing concentrations of EC and EGCG in the presence of 100 μ M Cu(II), the method of Quinlan and Guttridge (Quinlan & Guttridge, 1987) was followed. Calf thymus

DNA (200 μg) was used as substrate and the malondialdehyde generated from deoxyribose radicals was assayed as described earlier (Singh et al., 1998).

RESULTS

Interaction of Cu (II) with EC and EGCG.

We have previously shown that plant polyphenols are able to reduce Cu(II) to Cu(I) (Bhatt & Hadi, 1994). To assess the relative efficacy of reduction of Cu(II) by EC and EGCG, the experiment shown in Fig. 3 was performed. Increasing concentrations of Cu(II) were added to a fixed concentration of catechins in the presence of Cu(I) specific sequestering agent bathocuproine. Job plots of equivalents of Cu(II)/ [EC] or [EGCG] vs. absorption of bathocuproine-Cu(I) complex at 480 nm reveal that there is no clear stoichiometry of reduction of Cu(II) to Cu(I) in both cases as a clear plateau is not observed (Fig. 3). It would appear that both EC and EGCG can reduce more than 1 mole of Cu(II) per mole of EC/ EGCG. It can, however, be inferred from the figure that EGCG is a more effective reducer of Cu(II) as compared to EC.

Fig. 2. Absorption spectra of EC and EGCG in the presence and absence of Cu(II). The concentration of (A) EC and (B) EGCG in a total volume of 2 ml reaction mixture containing 10 mM Tris-HCl (pH 7.5) was 200 μM . EC/ EGCG alone (—); EC/ EGCG in the presence of 200 μM Cu(II) (---)

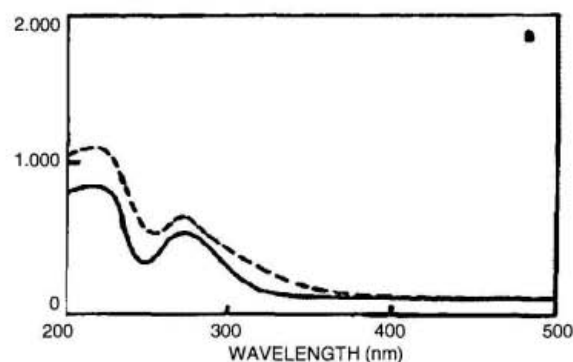
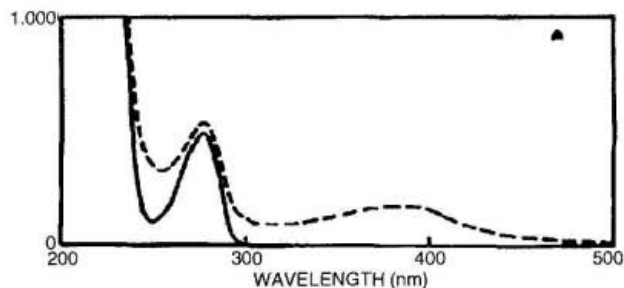
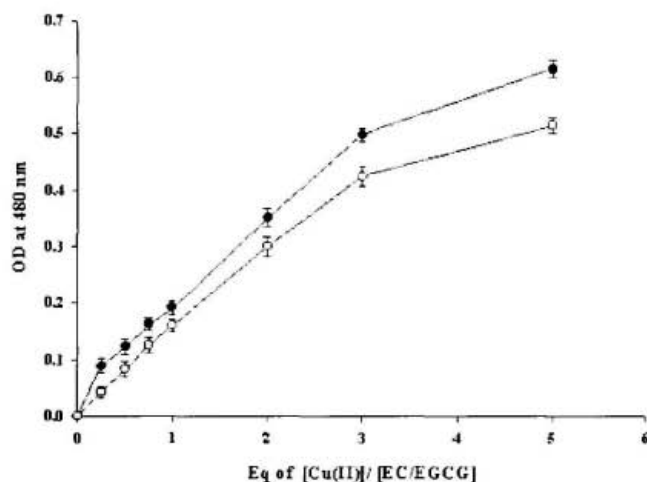


Fig. 3. Detection of stoichiometry of EC and EGCG. The concentration of EGCG (●) and EC (○) was 10 μM in the presence of 0.3 mM bathocuproine. The absorbance at 480 nm vs. equivalent of Cu(II) per molar equivalents of EC/ EGCG is plotted

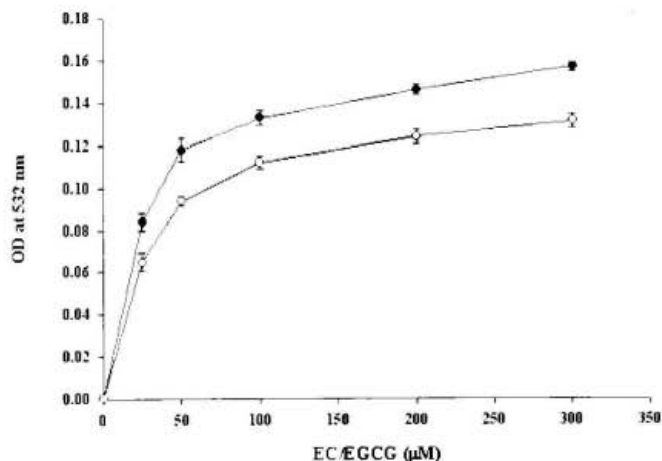


As Cu(II) is reduced to Cu(I), it was, therefore, of interest to determine whether a complex with Cu(II) is formed. Fig. 2 (A & B), shows the absorption spectra of catechins (EC & EGCG) alone and on the addition of Cu(II). The absorption maxima of EC and EGCG lie in the range of 200–280 nm. On addition of copper an enhancement in the absorption spectra of EC and EGCG is observed. As seen in Fig 2 (A) in the case of EC, a peak at 380 nm also appears which is indicative of the formation of a quinone (Harbone, 1973).

Comparison of Prooxidant Properties of EC and EGCG

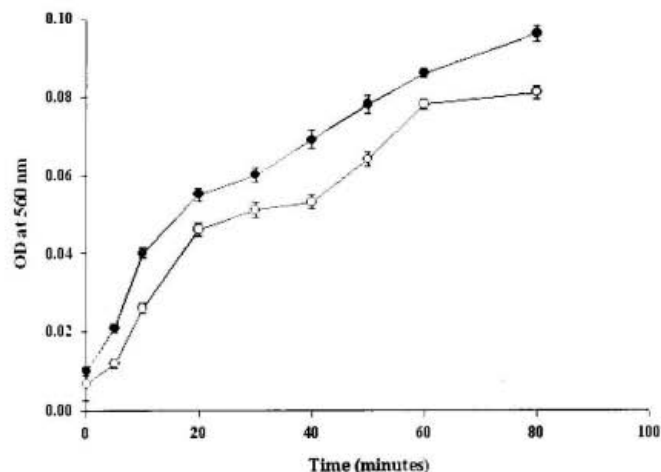
It has been previously elucidated that during the reduction of Cu(II) to Cu(I), reactive oxygen species such as hydroxyl radicals are formed which serve as proximal DNA cleaving agent (Rahman et al., 1989). Therefore, the capacity of EC and EGCG to generate hydroxyl radicals in the presence of Cu(II) was compared (Fig 4). This assay is based on the fact that degradation of DNA by hydroxyl radicals results in the release of TBA (thiobarbituric acid) reactive material, which forms a colored adduct with TBA readable at 532 nm (Quinlan & Gutteridge, 1987). Increasing concentrations of both the compounds lead to a progressively increased formation of hydroxyl radicals. However, at all the concentrations tested, the formation of TBA reactive material was greater in the case of EGCG, indicating that it is relatively more efficient than EC in generating hydroxyl radical.

Fig. 4. Formation of hydroxyl radicals as a function of EC and EGCG concentration in the presence of Cu(II). The reaction mixtures were as described in 'Methods' containing 100 μ M Cu(II) and increasing concentrations of EGCG (●) and EC (○). The incubation was at 37°C for 1 hour



It is known that generation of the O_2^- anion may lead to the formation of H_2O_2 . The addition of a second electron to the O_2^- anion gives the peroxide ion ($2O_2^-$), which has no unpaired electron and is not a radical. However, at neutral pH the peroxide ion immediately protonates to give hydrogen peroxide (H_2O_2). Alternatively, in aqueous solution the superoxide anion undergoes dismutation to form H_2O_2 and O_2 (Halliwell & Gutteridge, 1984). Therefore, the rate of generation of superoxide anion by EC and EGCG was compared. The increase in absorption at 560 nm is observed on reduction of nitroblue tetrazolium (NBT) by superoxide anion. From Fig. 5 it is evident that the production of superoxide radical increases with the increasing time of incubation. It is seen that EGCG is the more efficient producer of superoxide radical as compared to EC.

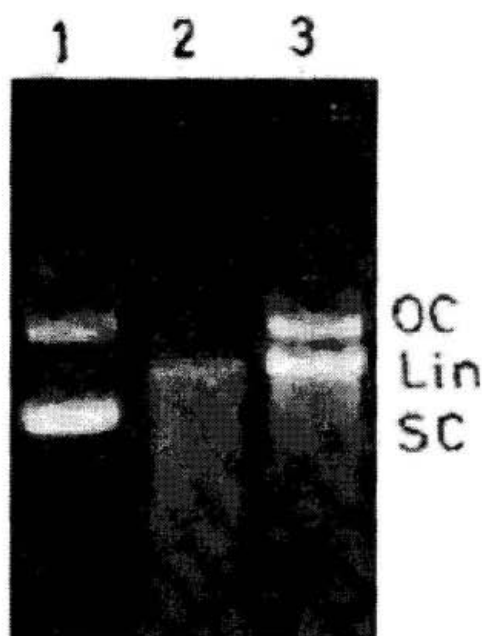
Fig. 5. Photogeneration of superoxide anion by EC and EGCG on illumination under fluorescent light. The concentration of EGCG (●) and EC (○) was 60 μ M. The two samples were placed 10 cm from the light source. Details are given in 'Methods'



It is known from our previous results that polyphenols-Cu(II) system mediates DNA cleavage through the generation of reactive oxygen species such as the hydroxyl radicals

(Rahman et al., 1989; Ahmad et al., 2000) Thus, the cleavage of supercoiled pBR322 plasmid DNA by EC/ EGCG-Cu(II) systems was compared. Fig. 6 shows the ethidium bromide stained banding pattern of pBR322 DNA tested with EGCG or EC in the presence of Cu(II). As can be seen, the greater degrading effect is caused by EGCG where the complete conversion of supercoiled DNA to linear forms and progressively smaller heterogenous sized fragments is seen (lane 2). In the case of EC the supercoiled DNA is converted to open circular and linear forms with some of the DNA also converted to smaller sized fragments (lane 3). These results correlate with the finding (Figs. 4 & 5), that EGCG is a more efficient producer of the reactive oxygen species.

Fig. 6. Agarose electrophoretic pattern of ethidium bromide stained pBR322 DNA after the treatment with EC and EGCG in the presence of Cu(II). Reaction mixture containing EC/EGCG and Cu(II) (100 μ M each) was incubated for 1 hour. Lanes (1) DNA alone; (2) DNA + EGCG + Cu(II); (3) DNA + EC + Cu(II). The positions of open circle (OC), linear (Lin) and supercoiled (SC) are indicated



TLC of Catechins (EC And EGCG) and Their Copper Mediated Oxidized Forms

EC and EGCG were oxidized by copper as given in 'Methods'. The unoxidized and copper oxidized catechins were applied on a silica gel plate for thin layer chromatography. Fig. 7 shows the relative movement of the catechins on the plate in toluene–ethyl acetate (1:8) solvent. Results indicate that the unoxidized forms are readily mobile (lanes a & c) where as a major portion of the copper oxidized catechins remains stationary (lanes b & d). These results are similar to those obtained when water extracts of green tea and black tea are subjected to TLC. The extract of green tea shows the presence of EC and EGCG whereas the polyphenols of black tea do not show any movement (results not shown). Thus copper mediated oxidation of EC and EGCG possibly leads to the formation of polymerized forms of catechins similar to those present in black tea.

Fig. 7. TLC profile of catechins (EC and EGCG) and their copper mediated oxidized forms. Lane a: EC; Lane b: copper oxidized EC; Lane c: EGCG; Lane d: copper oxidized EGCG.



Degradation of Calf Thymus DNA by EC/ EGCG and Their Copper Oxidized Forms

To compare the extent of DNA degradation by catechins with their copper oxidized forms in the presence of Cu(II), double stranded calf thymus DNA was used as the substrate. The reaction was assessed by recording the proportion of double stranded DNA converted to acid soluble nucleotides by S_1 nuclease. Control experiments (data not shown) established that heat-denatured DNA underwent 100% hydrolysis following the treatment with S_1 -nuclease, whereas only 9% of native form was hydrolyzed. Table I shows the extent of DNA degradation by the catechins under study in the absence and presence of Cu(II). It is seen from the results that the relative rates of DNA hydrolysis between EGCG, oxidized EGCG, EC and oxidized EC in the presence of Cu(II) are quite similar. However, in the absence of copper the oxidized EGCG and oxidized EC show considerably enhanced rate as compared to their unoxidized forms (11.2 vs. 7.68 & 10.93 vs. 2.26 respectively).

Table 1. S_1 nuclease hydrolysis following damage to calf thymus DNA by EGCG/ oxidized EGCG/ EC and oxidized EC in the presence of copper ions.

Compounds	% DNA Hydrolysis	
	With copper	Without copper
EGCG	17.97 ± 0.77	7.68 ± 0.091
Ox EGCG	19.87 ± 0.93	11.20 ± 0.085
EC	16.35 ± 0.82	2.26 ± 0.071
Ox EC	16.35 ± 0.75	10.93 ± 0.52

Reaction conditions were as described in Materials and methods. The concentrations of polyphenols and Cu(II) were 200 μ g/ ml and 100 μ M respectively.

Ox EGCG – Copper oxidized EGCG

Ox EC – Copper oxidized EC

* All values are expressed as Mean \pm SE for three different experiments.

DISCUSSION

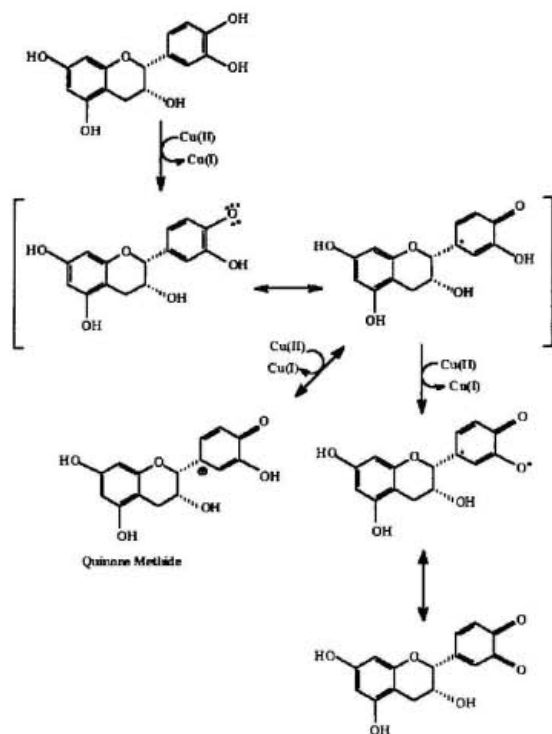
The above studies lead to the following major conclusions. (i) Similar to several other classes

of polyphenols, both EC and EGCG exhibit prooxidant properties such as the generation of superoxide anion and the hydroxyl radical. Both the polyphenols lead to oxidative DNA cleavage in the presence of copper ions. (ii) Copper mediated oxidation of EC and EGCG possibly leads to the formation of polymerized polyphenols. It is indicated that copper oxidized catechins are better prooxidants as compared to their unoxidized forms.

In a previous study from this laboratory, reactivities of plant flavonoids with different hydroxyl substituents for the cleavage of DNA in the presence of copper ions was studied (Jain et al., 1999). It was concluded that the rate of DNA cleavage depended on the number of hydroxyl groups on the flavonoid molecule. Further, the presence of orthodihydroxy groups was particularly important in enhancing the activity of a compound. This was considered to be related to the relative efficacy of Cu(II) chelation at these positions. In the studies presented above EGCG has been shown to be a more efficient producer of hydroxyl radicals in the presence of Cu(II) leading to a greater rate of DNA cleavage as compared with EC. EGCG offers a number of possibilities of orthochelation of Cu(II) and is thus more efficient than EC as an oxidative DNA cleaving agent.

Green tea contains polyphenolic compounds such as EC, EGC and EGCG. During manufacture of black tea these gallic catechins undergo oxidation. The catechin quinones react in several complex manners. The quinones derived from a simple catechin or its gallate may react with a quinone derived from gallic catechin or its gallate to form seven membered ring compounds known as theaflavins (Wei et al., 1999). As shown above copper mediated formation of quinones is also indicated in the case of EC (Fig. 8). It is possible that such oxidation may also lead to the formation of theaflavins

Fig. 8. Proposed model of binding of Cu(II) to (-)-epicatechin (EC)



or similar polymers. The hallmark of apoptosis is internucleosomal DNA fragmentation, which distinguishes it from necrosis. Most clinically used anticancer drugs can activate late events of apoptosis (DNA degradation and morphological changes) and essential signaling pathways differ between pharmacological cell death and physiological induction of programmed cell death (Smets, 1994). On the basis of our own observations and those of others a mechanism of DNA fragmentation involving mobilization of intracellular and extracellular copper has been proposed (Hadi et al., 2000). This is based on a number of observations including the facts that copper is an essential constituent of chromatin and that copper ion levels are elevated in a number of malignancies (Ebadi and Swanson, 1998; Yoshida et al., 1993). It would appear from the results of Table I that copper mediated oxidation of EC and EGCG transforms these compounds into even more potent prooxidant DNA cleaving agents that are active even in the absence of copper ions.

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Chapter 39

PREVENTIVE EFFECTS OF GREEN TEA POLYPHENOLS AGAINST OXIDATIVE STRESS OF RENAL DISEASE

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ABSTRACT

Renal disease largely associated with other various diseases is affected by free radical induced oxidative stress. Green tea polyphenols, known for their powerful antioxidant and active free radical scavenger activities, were examined against the oxidative stress of renal disease and related complications in rat models and humans. The polyphenols strongly inhibited the oxidative stress of free radicals and decreased the cell injury in renal epithelial cell line. The polyphenols have relieved the high oxidation stress condition and renal hypertension by inhibiting the production of oxidative uremic toxins and improving the renal blood circulating state, respectively. The application of polyphenols improved the renal function by inhibiting the mesangial cells proliferation. The polyphenols were found effective in easing the pains concerned with renal disease. The antioxidant properties of polyphenols against oxidative stress and related complications of renal disease have established the activity of green tea in regulating renal function.

Keywords: antioxidant; glomerular function; glomerular sclerosis; renal; hypertension; uremia

INTRODUCTION

Renal disease is a growing menace of the world population. In United States the patients with end stage renal diseases are rising at the rate of 8% annually for the last 10 years. Nearly 20 million people have been noticed with the impaired kidney function based on serum creatinine and urinary albumin values. Renal disease is associated with various diseases such as diabetes (36%) followed by hypertension (24%) and glomerulonephritis (14%). Almost 3% of the world population is suffering with diabetes mellitus. The U.S. (6.2%), Japan (5.3%) and India (3.1%) have large percentage of diabetic patients in their population. The world statistics invariably suggests that renal disease is yet another life style related disease of modern society.

Functional disorders of the kidneys may lead to the accumulation of waste metabolites such as urea and guanidine compounds, which can cause a state of oxidative stress in renal disease. The oxidative stress generally recognized as "uremia" is mediated by free radical species. Also a decrease in antioxidative enzyme activities, such as super oxide dismutase (SOD), catalase and gultathione peroxidase (GSH-Px), and an increase in the amount of hydroxyl radical (OH) in animal model of renal disease suggest the weakening of

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free radical scavenging system in renal disease (Yokozawa et al. 1999). Therefore, it is believed that the substances, which have strong free radical scavenging activities, may ameliorate the oxidative stress conditions in renal disease (Diamond et al. 1986; Rehan et al. 1984; Shah and Walker 1988; Yokozawa et al. 1993b).

Green tea polyphenols have been recognized as a powerful antioxidant and active scavenger of free radicals (Chen and Ho 1995; Chu and Juneja 1998; Koketsu 1997; Hara 2001; Nakagawa et al. 2002; Serafini et al. 1996; Xie et al. 1993), and are found to be effective in the prevention of various free radical induced and mediated diseases like cancer, cardiovascular diseases and arthritis. Our laboratory in collaboration with Dr. Yokozawa group (Toyama Medical and Pharmaceutical University, Toyama, Japan) has exclusively examined the antioxidative and physiological functions of green tea polyphenols against renal disease using animal models and humans. A refined form of green tea polyphenols 'Sunphenon®' (Taiyo Kagaku Co., Ltd, Japan) was used in all our studies.

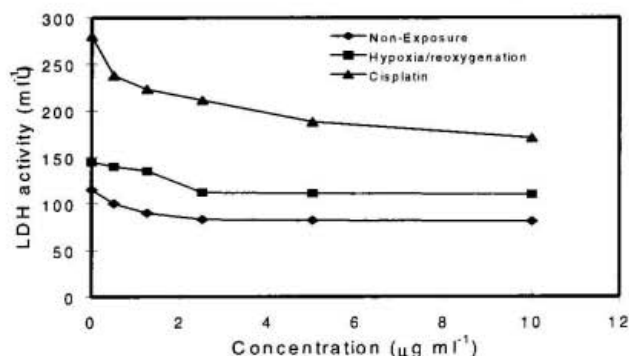
PREVENTION OF OXIDATIVE STRESS

Oxidative stress is linked to tissue damage and the development of several chronic diseases including the renal disease. Renal disease is known to occur as a result of reperfusion after certain period of blood flow blockage (Yokozawa et al. 1997a). In the case of ischemia-reperfusion the generation of super oxide (O_2^-) in renal proximal tubule cells increases leading to lipid peroxidation and cell and tissue injury. In addition, recent studies have indicated that nitric oxide (NO) is also produced in proximal tubules as a result of ischemia-reperfusion. Excess NO produced during ischemia-reperfusion is considered to act as a toxic radical and to cause renal dysfunction like O_2^- . NO and O_2^- cause ischemic renal injury individually and they work together to bring about

further damage. The degree of damage increases several folds when the two radicals react forming peroxynitrite (ONOO), which can lead to a series of toxic reactions with biomolecules such as proteins, lipids and nucleic acids. Further, the succession of renal disease is then associated with the accumulation of highly oxidative uremic toxins. Hence, the symptoms of uremia, high oxidative stress conditions are a common phenomenon in the renal disease patients (Fillit et al. 1981; Flament et al. 1986; Giardini et al. 1984; Kuroda et al. 1985). The uremic toxins are mainly produced by the involvement of hydroxyl radicals (Ienaga et al. 1991; Nakamura et al. 1991; Yokozawa et al. 1991a,b, 1992, 1997b, c). The free radical species mediated oxidative stresses are, therefore, the main factors in the occurrence and progression of renal disease (Yokozawa et al. 1996, 1997a, 1998, 2000).

The effect of green tea extract on oxidative stress damage in renal disease was assessed using a porcine kidney-derived cultured epithelial cell line, LLC-PK₁₀ (Yokozawa et al. 1997a and 1999). This line had the nature of a proximal uriniferous tubule and known to be severely injured in ischemic acute renal disease. When LLC-PK₁ cells were cultured under hypoxic conditions (<2% oxygen) before reoxygenation was applied (95% air, 5%CO₂), the leakage of lactate dehydrogenase (LDH) into the medium increased. This phenomenon was inhibited by dimethyl sulfoxide, a free radical scavenger, suggesting the involvement of free radicals. Similarly, cisplatin, an anti-tumor drug, was known to induce acute renal failure by decreasing the radical scavenger SOD. The induction of oxidative stress by hypoxic /reoxygenation and cisplatin in LLC-PK₁ cells was inhibited dose- dependently by Sunphenon (Fig. 1).

Fig. 1. Effect of green tea polyphenols on lactate dehydrogenase (LDH) leakage from LLC-PK₁ cells exposed to hypoxia/reoxygenation or cisplatin (Yokozawa et al. 1997, 1999)



In another study, a hydrophilic azo compound, namely 2,2'-azobis-(2-amidinopropane) dihydrochloride (AAPH), was used to generate peroxy radicals in LLC-PK₁ line (Yokozawa et al. 2000). AAPH is known to generate peroxy radicals via interaction with carbon-centered radicals and molecular oxygen, eventually causing the oxidation of lipid and protein in biomolecules. Green tea polyphenols have significantly decreased the AAPH induced lipid peroxidation in the cell line and dramatically increased the cell viability (Fig. 2).

Fig. 2. Effect of green tea polyphenols on thiobarbituric acid-reactive substances (TBARS) and cell viability of LLC-PK₁ cells treated with AAPH (Yokozawa et al. 2000)

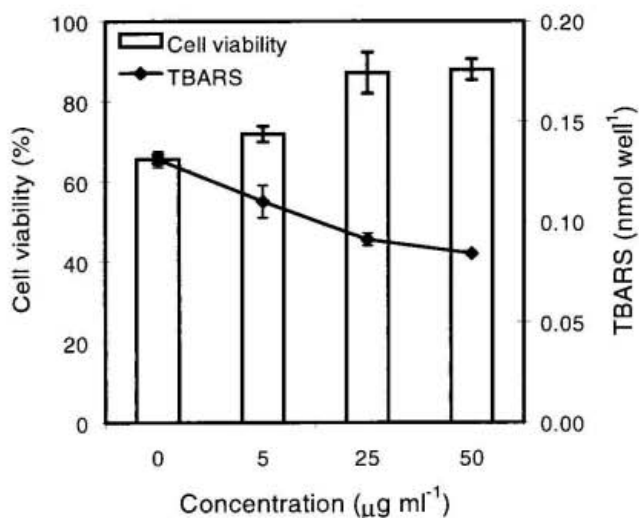
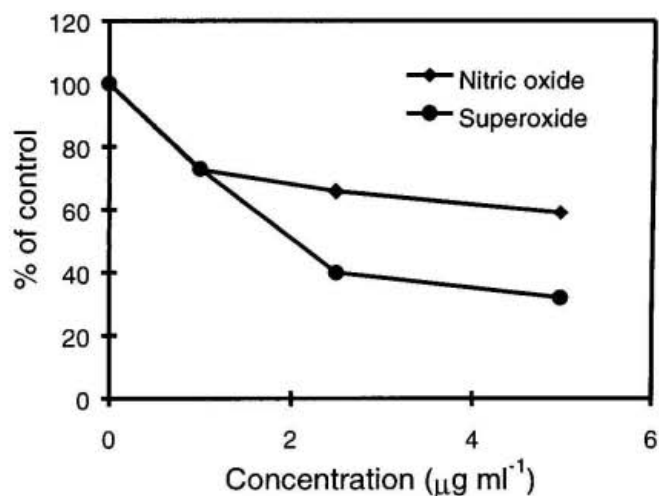


Fig. 3. Effect of green tea extract on the inhibition of NO and O₂⁻ (Nakagawa and Yokozawa 2002)



Green tea polyphenols also showed direct scavenging of NO and O₂⁻ radicals (Fig. 3) (Nakagawa and Yokozawa 2002). These studies have confirmed the beneficial effects of green tea polyphenols in relieving the oxidative stress in renal disease.

The suppression of oxidative stress and free radical scavenging activity of green tea are mainly due to composition of low molecular polyphenols namely epigallocatechin gallate (EGCg), epigallocatechin (EGC), gallic acid (GA), gallic acid gallate (GAG), epicatechin gallate (ECg), epicatechin (EC), gallic acid (GA) and catechin (C). These polyphenols are largely known as polyphenols and structurally they belong to flavan-3-ol group. Chemically they are highly reactive, with properties of metal chelation, oxidative radical scavenging, nitrosation inhibition etc. The polyphenols liberate a hydrogen radical from the hydroxyl groups of 3'- and 4'-positions in B ring, and the hydrogen radical joins with other free radicals (eg. lipid peroxide) to become stabilized. On the other hand, the catechin itself changes to a phenoxyl radical, then the transfer of the radical electron occurs by contribution of the resonance structure of the benzene ring and the carbons at the 3' and 4'-positions form a double bond with a remaining

oxygen atom to form a ketone structure. The bond between C-3' and C-4' of the B ring is oxidatively cleaved leaving one radical electron each on both of the carbons. The radical electron of C-3' forms a lactone ring with the alcoholic hydroxyl group of the 3-position of the C ring in the same way that hydroxyl carboxylic acid forms an intramolecular ester. Another radical electron of C-4' captures the hydroxyl radical existing in the reaction system and becomes stable by the formation of carboxylic acid. By this reaction process, a polyphenol can scavenge four radicals per mole, and thus the above reaction may explain the mechanism of the antioxidative action of polyphenols.

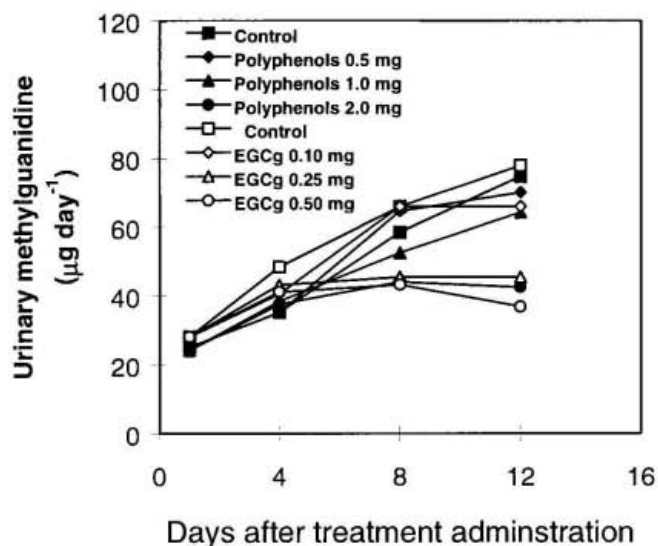
INHIBITION OF UREMIA

At the inception of chronic renal failure the concentration of uremic toxins increases leading to the state of uremia and a high oxidation stress condition (Sakanaka and Kim 1997; Giovannetti et al. 1973). The toxins currently known include methylguanidine, guanidinosuccinic acid, dimethylamine, myoinositol and β_2 -microglobulin. Among these toxins, methylguanidine has been pertinent toxin to induce the uremic condition (Giovannetti et al. 1968). Examination of urine specimens from the chronic renal disease patients revealed that methylguanidine is produced from creatinine via creatol (5-hydroxycreatinine) by the OH (Ienaga et al. 1991; Nakamura et al. 1991; Yokozawa et al. 1991a,b). Green tea polyphenols known for their active free radical scavenging activity have inhibited the production of methylguanidine and thus alleviated chronic renal failure both in animals (Sakanaka and Kim 1997; Yokozawa et al. 1992, 1994, 1996a, 1997b, c) and in humans (Sakanaka and Kim 1997; Yokozawa et al. 1996b).

In rats, Yokozawa et al (1992, 1993b) examined the effect of green tea polyphenols on adenine-induced renal disease. They examined the urinary methylguanidine excretion as an index of scavenging reaction. The rats were administered

with different doses of green tea polyphenols (as Sunphenon®) or only the EGCg orally for 14 days after the adenine administration for 20 days. A dose depended decrease in methylguanidine excretion was observed (Fig. 4), whereas a dose of 0.5 mg day⁻¹ of green tea polyphenol or the EGCg did not show any appreciable inhibition in the production of methylguanidine. However, an increase in the dose to 1.0 or 2.0 mg day⁻¹ strongly inhibited the production of methylguanidine. The methylguanidine production was about 40% lower compared to control group with the administration of 2 mg green tea polyphenols at 12 days (32nd day) after adenine-induced chronic renal failure. While a similar decrease (40%) or much further inhibition (about 50%) was noticed with the administration of exclusively EGCg at the rate of 0.25 and 0.5 mg day⁻¹, respectively. These results suggested that EGCg, the powerful antioxidant component of green tea polyphenols, has strong inhibitory effect on methylguanidine production and thereby may induce the recovery from oxidative stress.

Fig. 4. Effect of different doses of green tea polyphenols (Sunphenon®) (closed symbols) or EGCg (open symbols) on the urinary methylguanidine excretion in adenine-induced renal disease rats (Yokozawa et al. 1992)



The clinical efficacy of green tea polyphenols in suppression of creatinine and methylguanidine production was observed in 50 dialysis patients (Yokozawa et al. 1996b). The patients were administered twice a day 200 mg green tea polyphenols in the form of either jelly or capsules consecutively for six months. The blood samples were collected just before dialysis of each month and analyzed for the creatinine and methylguanidine levels. The creatinine level was significantly lowered after three months of the administration, showing almost 8% decrease in 5-6 months (Table 1). A decrease in methylguanidine preceded the decrease of creatinine, reaching a significantly low level within a month. In 5 months the level of methylguanidine was 20% lower than the initial level. These results suggested that green tea polyphenols influenced the radicals involved in the production of methylguanidine from creatinine, which was clearly evident in the ratio of methylguanidine / creatinine. Concurrently a significant decrease in β_2 -microglobulin was also observed within one month of green tea administration in the patients with high oxidative stress (Table 1).

Table 1. Changes in creatinine (Cr), methylguanidine (MG), MG/Cr ratio and β_2 -microglobulin in serum during the application of green tea polyphenols (Sunphenon®) in dialysis patients (Yokozawa et al. 1996b)

Duration of treatment month	Creatinine (Cr)mg dl ⁻¹	Methyl-guanidine (MG) β g dl ⁻¹	MG/Cr globulin (x 10 ⁻³)	β_2 -Micro mg dl ⁻¹
0	13.51	56.43	4.12	39.0
1	13.33	53.65*	3.99	35.0***
2	13.28	51.92**	3.86*	37.5
3	2.81***	48.66***	3.78**	36.4**
4	12.65***	49.12***	3.87*	36.1***
5	12.37***	45.06***	3.62**	35.7**
6	12.43***	48.41***	3.85*	35.4**

Significantly different from the pre-treatment value: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Aggressive removal of β_2 -microglobulin is desirable to prevent the complications of prolonged dialysis including the amyloidosis. It was noteworthy that the administration of green tea polyphenols has relieved 35 to 100% of the pain in shoulder, knee, hips, cubitus, coxa and fingers of dialysis patients (Table 2).

Table 2. Effect of green tea polyphenols (Sunphenon®) on arthralgia in dialysis patients. Values in parenthesis indicate the percentage to the total patients (Yokozawa et al. 1996b)

	Number of patients with arthralgia			
	Disappearance	Relief	No change	Total
Shoulder	5 (31)	1(6)	10 (63)	16
Knee	4 (31)	1(8)	8 (62)	13
Finger	3 (23)	4 (31)	6 (46)	13
Coxa	5 (50)	1(10)	4 (40)	10
Hip	5 (83)	1(17)	0 (0)	6
Cubitus	1(100)	0 (0)	0 (0)	1

These results suggest that the green tea polyphenols are very effective in the suppression of the oxidative stress and the post dialysis arthralgia in dialysis patients.

INHIBITORY EFFECT ON PROLIFERATION OF MESANGIAL CELLS

Glomerulorenal disease and diabetic nephropathy are recognized as the main functional disorders underlying in the development of renal disease. Histological studies have characterized these disorders with deteriorated glomerular filtration by excessive proliferation of mesangial cells.

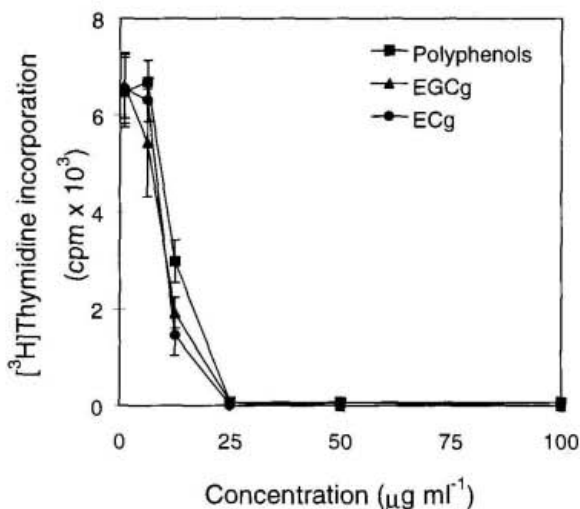
Nearly 70-80% of chronic renal disease patients have the background of either glomerulorenal disease or diabetic nephropathy disorders. These disorders, which are associated with deteriorated glomerular filtration, are characterized histologically by excessive proliferation of mesangial cells. Grond et al. (1985) suggested that mesangial cells affect the hemodynamics of glomerular capillaries via vascular contraction and relaxation thereby regulates the glomerular filtration. Apparently it suggests that the proliferation of mesangial cells

may interfere with the function of glomerular filtration (Kashgarian and Sterzel 1992).

Yokozawa et al (1993a) have examined the effect of green tea polyphenols using Sunphenon® on the proliferation of mesangial cells. They determined the proliferation in terms of [³H]thymidine uptake in cultured mouse mesangial cells. Mesangial cells were isolated from mouse renal glomeruli and cultured in a medium with D-valine MEM containing 20% fetal calf serum (FCS) for 10 days. For the measurement of [³H]thymidine uptake, the cultured mesangial cells were seeded in a 96-well microtiter plate at a density of 10⁴ cells/well in 0.2 ml D-valine MEM containing 20% FCS and incubated for 48 hours with or without Sunphenon® (6.25 to 200 µg ml⁻¹). Twelve hours prior to the end of incubation period, the cultures were pulsed with 1 µCi of [³H]thymidine. At the end of incubation period, the cells were harvested and the radioactivity was measured. Simultaneously the effect of individual polyphenols (EGCg and Ecg) on mesangial cell proliferation was also examined in similar method.

A concentration depended decrease in the uptake of [³H]thymidine was observed with polyphenols in mesangial cells (Fig. 5).

Fig. 5. Effect of green tea polyphenols (Sunphenon®) and its components on the proliferation of mesangial cells (Yokozawa et al. 1993a)



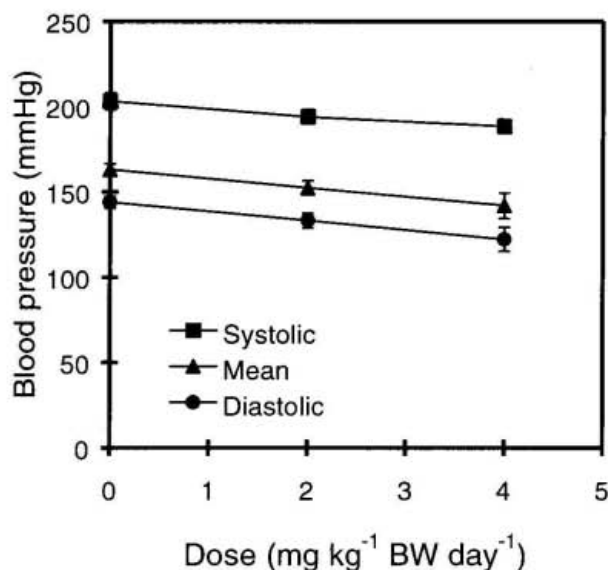
A dose of 25 mg ml⁻¹ of polyphenols EGCg or ECg has completely inhibited the uptake. The EGCg exerted inhibitory effects even at relatively low concentrations. Since EGCg is the strongest antioxidant of all the green tea polyphenols, the inhibitory effects could be attributed to this major component present in Sunphenon®. These results suggest that green tea polyphenols inhibit the proliferation of mesangial cells and could improve the function of glomerular filtration. This was noticed when an oral administration of ECg induced an increase in the glomerular filtration in rats (Oura and Yokozawa 1990).

INHIBITION OF RENAL HYPERTENSION

Besides relieving the oxidative stress and improving the glomerular filtration during the renal disease, the green tea polyphenols were also found to be effective in controlling the renal hypertension by manipulating the blood pressure in kidneys. The kinin-kallikrein system seems to be involved in the mechanism of blood pressure regulation by its mutual action with other vasoactive systems such as the renin-angiotensin-aldosterone, sympathetic nerve vasopression and prostaglandin systems, and by its direct action on cardiovascular system and the mechanism for water and sodium (Abe 1981; Abe et al. 1978; Levinsky 1979). Decreased production by the kinin-kallikrein system in patients with essential hypertension has been reported, suggesting its involvement in the etiology of this condition (Margolius et al. 1974; Zineer et al. 1976). On the other hand, prostaglandin E₂ (PGE₂) is thought to be involved not only with maintaining the blood flow but also with sodium metabolism, and it has been suggested to act as a modulator of the hemodynamic changes associated with hypertension (Coleman et al. 1975).

Yokozawa et al (1994) examined the changes in blood pressure, kallikrein and PGE₂ with the administration of green tea polyphenols in adenine induced renal failure rats. After the induction of renal failure, the rats were administered green tea polyphenols (as Sunphenon®) at the dose of 2 or 4 mg kg⁻¹ BW for 24 consecutive days. The polyphenols were dissolved in water and given to the rats as drinking water. A 24-hour urine sample was collected and analysed for kallikrein and PGE₂. Simultaneously, systolic, mean and diastolic blood pressures were determined by a tail-pulse pick-up method. The administration of green tea polyphenols has significantly reduced the systolic, mean and diastolic blood pressure (Fig. 6).

Fig. 6. Effect of green tea polyphenols (Sunphenon®) on systolic, mean and diastolic blood pressure in adenine-induced renal disease rats. Significantly different from the control value: *p<0.05 (Yokozawa et al. 1994)



On the other hand, the green tea polyphenol administration significantly increased the urinary kallikrein level, the excretion of PGE₂ and sodium (Table 3).

Table 3. Effect of green tea polyphenols (Sunphenon®) on the urinary excretion of PGE₂, kallikrein and sodium (Yokozawa et al. 1994)

	Dose of green tea polyphenols (mg kg ⁻¹ BW day ⁻¹)		
	0	2	4
PGE ₂ (ng day ⁻¹)	8.00	14.87 ^{***}	20.07 ^{***}
Kallikrein (mU day ⁻¹)	14.87	21.44 ^{**}	31.81 ^{***}
Na (mM day ⁻¹)	1.50	1.72	1.96 [*]

Significantly different from the control value: *p<0.05, **p<0.01, ***p<0.001

These data suggest that green tea polyphenols may ameliorate the development of hypertension by improving the renal circulating state. Stokes and Kokko (1977) have found in a study on isolated perfused tubules that PGE₂ caused direct tubular inhibition of sodium reabsorption. Ruilope et al. (1982) have demonstrated the protective role of renal PGE₂ in the maintenance of hypertension. Thus, it appears that the antihypertensive effect of green tea polyphenols resulted from its direct action in the kidney.

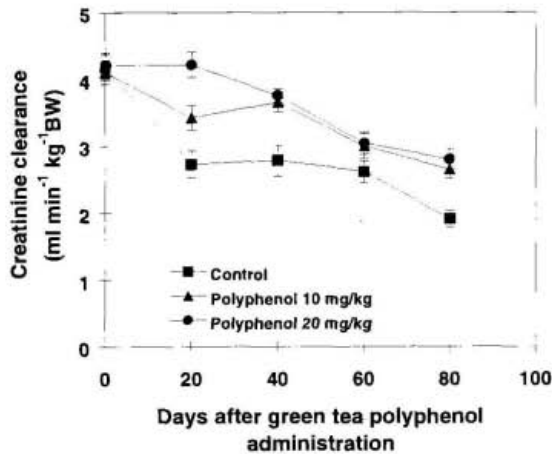
INHIBITION OF RENAL TISSUE LESIONS

To analyse the effect of green tea polyphenol on renal tissue lesions, Yokozawa et al. (1996a) examined the mesangial proliferation and glomerular sclerosis index in nephrectomized rats. An experimental model to induce non-inflammatory renal disease was established by the excision of a part of kidney. It was earlier pointed out that, following the subtotal nephrectomy, growth factors may simultaneously induce glomerular hypertrophy and mesangial proliferation, the former leading to a disorder in the glomerular basement membrane or epithelial cells resulting in protein leakage, and the latter leading to glomerular sclerosis. In the study, the urinary creatinine clearance, protein excretion and oxidative activities were measured with the administration of 10 or 20 mg kg⁻¹ body weight green tea polyphenol (as Sunphenon®) for 80 consecutive days.

Nephrectomized rats receiving oral green tea polyphenol exhibited milder lesions. The decrease in creatinine clearance was also significantly

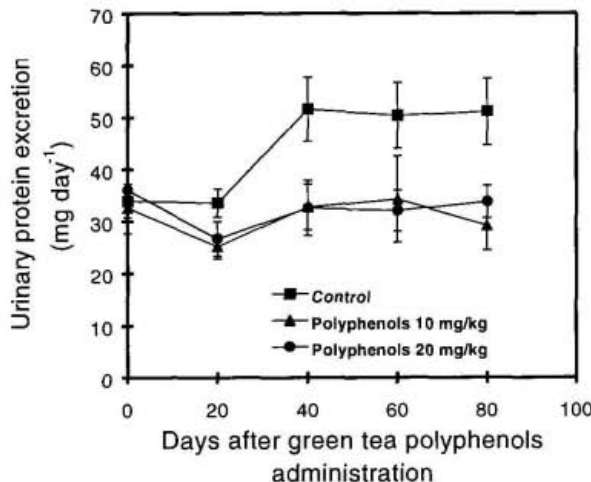
reversed after administration of green tea polyphenols (Fig. 7).

Fig. 7. Effect of green tea polyphenols (Sunphenon®) on creatinine clearance in nephrectomized rats (Yokozawa et al. 1996a)



These results suggested that green tea polyphenols have inhibited the mesangial cell proliferation to retain the function of the glomeruli and thereby inhibiting the progression of glomerular sclerosis. The study also showed that green tea polyphenols suppressed the leakage of urinary protein (Fig. 8), suggesting that the green tea polyphenols also delayed the progression of glomerular hypertrophy.

Fig. 8. Effect of green tea polyphenols (Sunphenon®) on urinary protein excretion in nephrectomized rats (Yokozawa et al. 1996a)



Schrier et al (1988) and Harris et al (1988) have suggested that free radicals are involved in various ways in the occurrence and progression of renal disease. The renal disease model produced by partial resection of the renal parenchyma, as used in this study, results in swelling of the remaining kidney tissue, which might cause increased oxygen consumption and enhanced ATP synthesis, where active free radicals could involve. In such scenario, the measurements of the antioxidative enzymes such as SOD, catalase and GSH-Px activities, may suggest the level of free radical-scavenging activity in the system. In the current study the activities of SOD and catalase were significantly higher in the rats given green tea polyphenol after nephrectomy (Table 4). Since these rats showed low activity of GSH-Px (an enzyme which presents in mitochondrial matrix and eliminates H₂O₂, like the enzyme catalase), it was speculated that the site of action of green tea polyphenol is the peroxisome.

Table 4. Effect of green tea polyphenols (Sunphenon®) on the activities of reactive oxygen species-scavenging enzymes in rats after excision of 3/4 of their kidney volume (Yokozawa et al. 1996a)

	Dose of Sunphenon (mg kg ⁻¹ BW day ⁻¹)		
	0	10	20
SOD (U mg ⁻¹ protein)	8.75	10.68 ^{**}	11.66 ^{***}
Catalase (U mg ⁻¹ protein)	142.7	213.2 ^{***}	224.4 ^{***}
GSH-Px (U mg ⁻¹ protein)	69.63	71.91	76.97 [*]

Significantly different from the control value: *p<0.05, **p<0.01, ***p<0.001

These results suggest that green tea polyphenols maintain the enzyme activities, which are related to free radical scavenger action, thereby inhibit the renal tissue lesions.

CONCLUSIONS

Green tea, a simple refreshing beverage, was believed to have therapeutic uses for many centuries. Recently enormous research findings

have confirmed the therapeutic functions of green tea extract in preventing wide range of diseases, in particular the diseases concerned with modern life (Weisburger and Chung 2002) like cancer (Katiyar and Mukthar 1996; Yang and Wang 1993), cardiovascular diseases (Ross 1993) and allergy (Matsuo et al. 2000). Nevertheless, the green tea extract has been noted for a number of physiological functions such as antibacterial (Juneja et al. 2000), antiviral (Ebina 1991), anticariogenic (Ahn et al. 1991), antimutagenic (Nakagawa et al. 2002), antiatherogenic (Luo et al. 1997), anticarcinogenic (Katiyar and Mukthar 1996; Yang and Wang 1993; Weisburger 1997) and so on. Therefore, if listed, the functions of green tea in prevention of various diseases will be endless. Scientists recognized that these wide ranges of physiological functions in green tea are due to the low molecular weight polyphenols, which are abundant especially in green tea. They also recognized that the efficacy of green tea polyphenols over others was related to its powerful antioxidant and free radical scavenging activities.

Our studies particularly examined the effect of green tea polyphenols on renal disease. We examined the effect of refined green tea polyphenols (Sunphenon®, Taiyo Kagaku Co. Ltd, Japan) on the oxidative stress of renal disease and related complications both in animal models and human. The studies showed that green tea polyphenols can strongly inhibit free radical induced oxidative stress and can alleviate many complications related to renal disease. Green tea polyphenols were found effective in 1) the inhibition of mesangial cells proliferation improving the glomerular function, 2) the inhibition of methylguanidine production, a prominent uremic toxin causes uremia, relieving the oxidative stress in dialysis patients, 3) the regulation of blood pressure suppressing the hypertension, and 4) scavenging the free radicals preventing the renal tissue lesions and relieving the pain from renal

disease complications. These studies suggested not only the therapeutic use of green tea polyphenols in renal disease but also extended its physiological functions as 'antinephropathic' activity.

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Concluding Remarks of Dr. Siddiqi, Chairman Technical Session 8

Ladies and gentlemen we come to the end of this session which started with an inspiring and thought provoking lecture by Dr. Dhawan who advises us that we should not call tea compounds as drugs. Rightly, he related the properties of tea with the concept of the rasayana in Charak Samhita and other ancient scriptures. In a way, his talk combined ayurveda, Indian philosophy, modern science and of course predictive science — which way we are going, what we should be doing, thank you Dr. Dhawan.

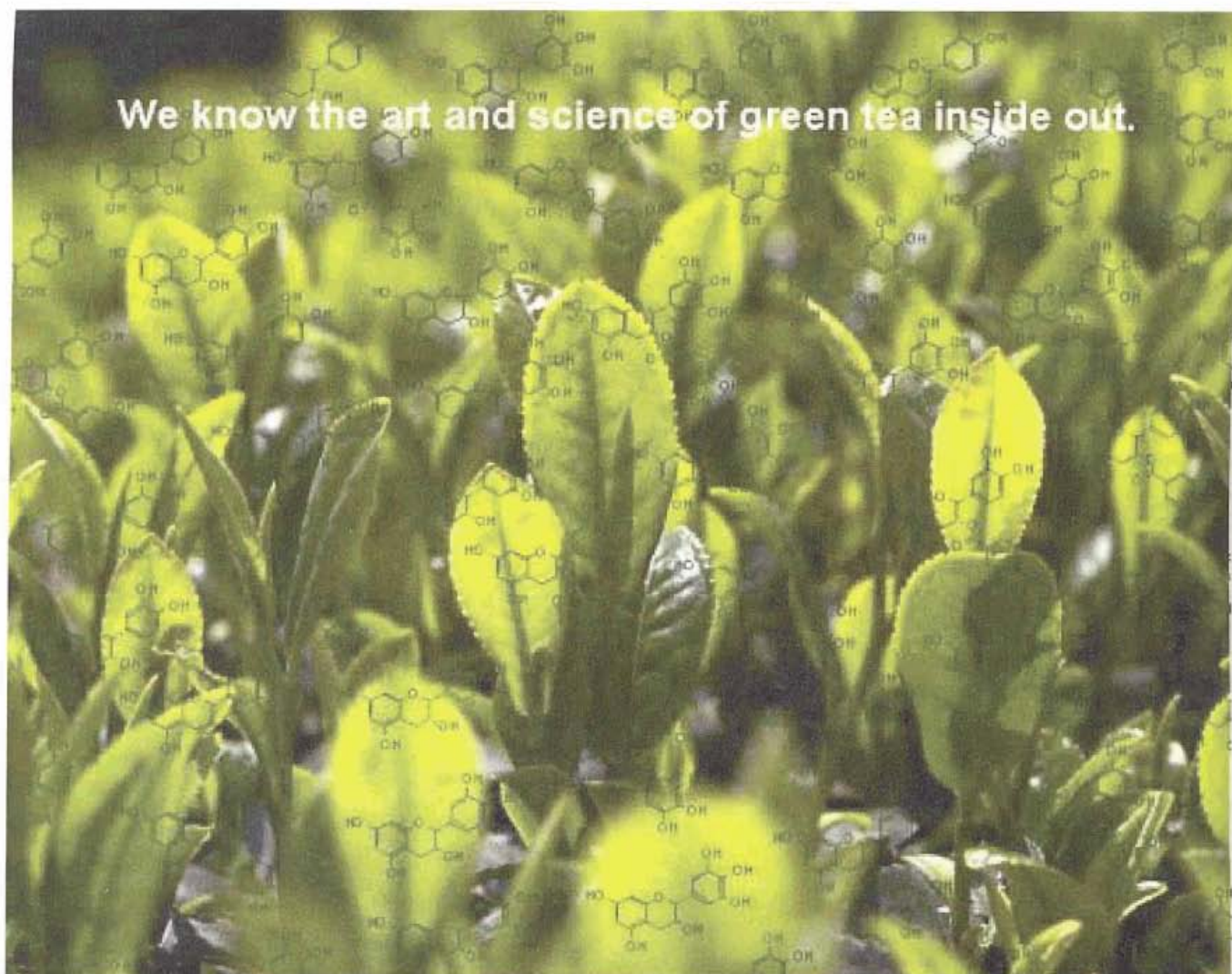
Mr. Jhawar's lecture was also very interesting and thought provoking, he dealt with the problems of consumer awareness and suggested that we must take consumer education to rural areas. According to his estimate there are 5 million tea shops or chai ghars or cafes which are the right place for propagating the positive aspects of tea. That is one way the consumer will be more aware and will be attracted more and more towards tea. The point of view that Mr. Jhawar brought up is that it the right time to make the consumer aware of the level of catechins as the Unique Selling Point (USP) of tea. The consumer should also be told the right method of the preparation of tea. Thank you Mr. Jhawar for focusing on the health effects of tea for better marketing. I think that all of us have taken your message in the right path.

Dr. Hara's presentation is an excellent integration of pharmaceutical properties of tea with its industrial utilisation. It shows the way to exploit knowledge of basic biochemistry, separating individual components of tea and utilising them for specific products. This information is as relevant to Health session as to the session on value addition, where it was initially presented.

Prof. Hadi introduced another dimension by proposing a hypothesis on the pro-oxidant activity. He suggested that perhaps it is the pro-oxidant activity which has a pre eminent role in anti cancer property of tea. He also showed excellent data proving that catechins are able to mobilize copper from the cells. Thank you Prof Hadi .

Dr. Juneja made an excellent presentation on clinical studies on tea constituents done by his group. He provided a very good perspective on clinical study on catechins which he calls the magic bullets — how they act in human diseases, the importance of methyl guanidine in the action of catechins was well focussed. Dr. Juneja also dealt with the superoxide dismutase (SOD) and showed very convincingly how the longevity of the members of the animal kingdom depends upon the superoxide dismutase activity. I am highly impressed by that sir. Reduction in phospholipid oxidation is also very important: the data he showed on anti-obesity is marvellous. Thank you Dr. Juneja on enlightening us (At this stage Dr. Juneja informed that their patented product "Sunpheron", is a mixture of tea catechins. It is 80 percent polyphenols However, there are different grades e.g without caffeine).

The discussion on the importance of tea constituents points to the need of an accredited laboratory for analysis of tea catechins. I have seen some tea packets which state the catechin levels but without the inter laboratory quality control , there is no guarantee. So I would suggest to Dr. Jain that this is the time to suggest to the Tea Board and other regulatory bodies while we talk of health and tea, we must have an accredited laboratory to provide good quality control and excellent analytical abilities for tea contents. The content of positive chemicals in tea would become more relevant to the people in time to come. Thank you very much for your patience.



Sunphenon: High quality green tea polyphenols rich in catechins. Recipient of the 2000 IFT Industrial Achievement Award, Decaffeinated green tea polyphenols (Sunphenon DCF-I). **Suntheanine:** Unique amino acid found in green tea (L-theanine) which promotes relaxation without drowsiness. Honored with the 1998 FIE Fcord Ingredient Research Award. **Matcha Powder:** Natural green tea leaf powder rich in polyphenols, vitamins, minerals, fiber and chlorophyll. Revolutionary health ingredients used in dietary supplements, food, beverages and confections. Enough to suggest it's time you turned over a new leaf. For more information, please visit Taiyo's website <http://taiyokagaku.co.jp>

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