MODULATORY INFLUENCE OF CERTAIN MEDICINAL PLANTS AND SPICES ON XENOBIOTIC ACTIVATING AND DETOXIFYING ENZYMES IN THE LIVER OF MOUSE

Dissertation submitted to Jawaharlal Nehru University
in partial fulfilment of the requirements
for the Degree of

MASTER OF PHILOSOPHY

ANJU GUPTA

CANCER BIOLOGY LABORATORY
SCHOOL OF LIFE SCIENCES
JAWAHARLAL NEHRU UNIVERSITY
NEW DELHI-110067

1990

CERTIFICATE

The research work embodied in this thesis has been carried out in the School of Life Sciences, Jawaharlal Nehru University, New Delhi. The work is original and has not been submitted so far, in part of full, for any other degree or diploma of any University.

Anju Gupta

ANJU GUPTA
Candidate

M Habibulla 23.7.90

PROF. M. HABIBULLA
Dean

SCHOOL OF LIFE SCIENCES

A. Ramesha Rao 23/3/90

PROF. A. RAMESHA RAO
Supervisor

SCHOOL OF LIFE SCIENCES
JAWAHARLAL NEHRU UNIVERSITY
NEW DELHI - 110 067

ACKNOWLEDGEMENT

It gives me immense pleasure to express my deep sense of gratitude to my esteemed supervisor, Dr. A. Ramesha Rao, School of Life Sciences, Jawaharlal Nehru University, New Delhi, for his excellent guidance, constant encouragement, valuable suggestions and keen interest which has helped me in completing this work.

Also, I am very much indebted to Prof. M. Habibulla, Dean, School of Life Sciences, Jawaharlal Nehru University, for providing me with all the necessary experimental facilities.

I owe my special thanks to Mr. Vijay for his time to time laboratory assistance and animal care throughout the tenure of my work. I am very much grateful to Mr. Alexander and Mr. Khan for their technical help in the Central Instrumentation Facility.

Thanks are even due to all my colleagues and friends, Sandhya, Perwez, Saran, Jannu, Sanjeev and Anjali who encouraged and wished me in silence.

Financial assistance in the form of Junior Research Fellowship by U.G.C., New Delhi, is gratefully acknowledged.

Finally, I cannot fail to mention the tremendous support and encouragement given by my parents and husband (Someshwar) on many crucial situations.

Anju Gupta

CONTENTS

LIST OF ABBREVIATIONS USED

cyt.	Cytochrome
DNA	Deoxyribose Nucleic Acid
NADPH	Nicotinamide Adenine Dinucleotide Phosphate (reduced)
MFO	Mixed Function Oxidase
ER	Endoplasmic Reticulum
BHA	Butylated Hydroxy Anisole
BHT	Butylated Hydroxy Toulene
BP	Benzo(a)pyrene
GSH	Glutathione Veduced
SH	Sulphydryl

INTRODUCTION

INTRODUCTION

Since decades mankind is continuously interacting with nature and its products. Daily one inhales, intakes and ingests a variety of substances. In spite of this long exposure still it has managed to survive through disasters like cancer. This has been possible due to the fact that higher organisms including human beings possess the inherited gift of certain internal defense systems. These systems serve to convert various toxic hydrophobic compounds (e.g. chemicals, carcinogens and other useless foreign substances) into hydrophilic products which are easily excreted and eliminated from the body via bile and urine.

The whole system of xenobiotic metabolism comprises of enzymes which carry out two phases:

1. Phase I Reactions (Oxidative) : These reactions insert an exposed reactive functional group (e.g. -OH, -SH, -NH2, -SH, -COOH) into the substrate molecule (The substrate molecular being usually inert).
2. Phase II reactions (conjugative) : They involve an attack on the inserted group to form inactive, excretable compounds which are easily thrown out of the body.

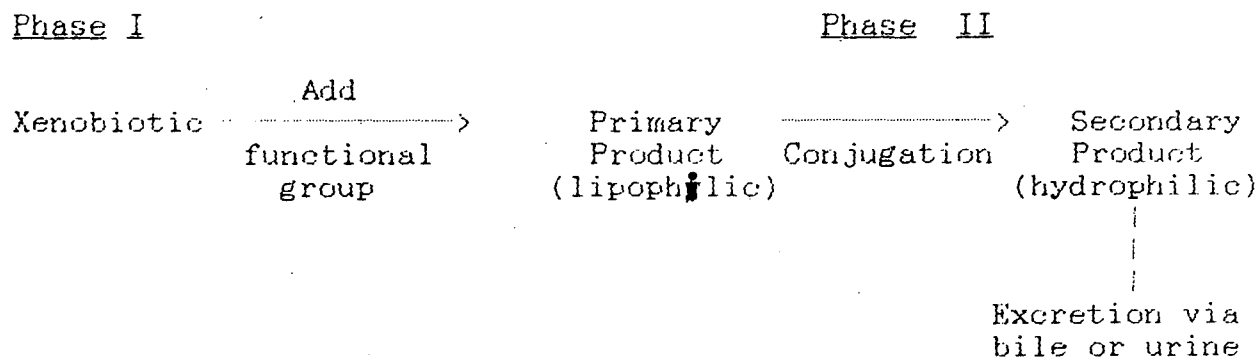


Fig. 1 Integration of Phase I and Phase II Biotransformation Reactions

The ultimate change in the toxicity of the foreign moiety depends on the balance between the two kinds of reactions i.e., activation and detoxification. But it is quite unfortunate that various enzymes involved in these reactions are tissue-specific. In other words, they may enhance the conversion into non-toxic molecules and their subsequent elimination in one tissue and catalyse the production of active electropholic species in the other tissue which in turn attack and mnodify the critical cellular macromolecules (DNA, RNA and membranes). The result is an increase in the toxicity levels.

The wide variety of chemical carcinogens can be of two major kinds :

1. Directly acting carcinogens e.g., mustard gas.
2. Indirectly acting carcinogens: These require metabolic activation before causing any damage, e.g., polycyclic aromatic hydrocarbons. Activation leads to formation of ultimate carcinogens which are much more toxic than the parent compound.

Another way of classifying the carcinogens is based on the mechanism of neoplasia induction :

1. Genotoxic carcinogens which directly interact with the critical target like DNA. The interactions leads to modification in the chemical structure and adduct formation, hence triggering neoplastic transformation of the cell. e.g., Nitrosamines and PAHs.

2. Epigenetic carcinogens which directly produce some other biological effect associated with carcinogenicity, e.g. Purine analogues, estrogen etc.

The entire process of carcinogenesis is a multi step phenomenon. The initial step is the transformation of a normal cell into a cancerous cell, the process being termed as neoplastic conversion. The next step involves the progression of the transformed cell into a detectable tumor. This is called neoplastic development.

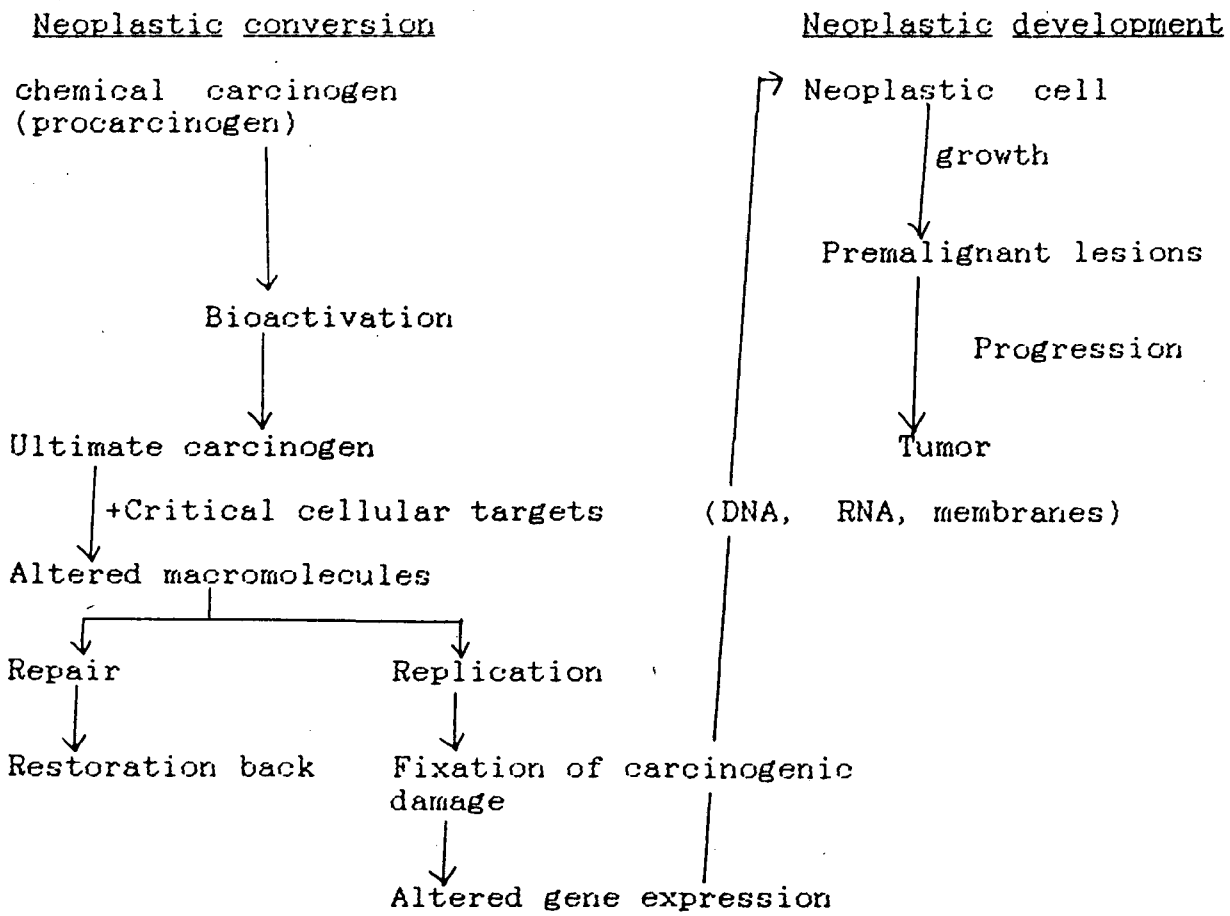


Fig. 2 : Main steps in carcinogenesis.

As shown in the figure, the neoplastic conversion limitations consists of :

a. Bioactivation of inactive carcinogens into active electropholic moieties (ultimate carcinogens).

b. Modifications in the cellular macromolecules: The resulting electropilic substances react with the critical target sites like DNA, RNA, membranes and proteins to form modified molecules called adducts. This marks the onset of cellular damage. Repair is possible at this stage since cell has certain repair enzyme systems which can restore back normal macromolecules.

c. Fixation of damage : Alternations in theDNA may involve mutations or activation of oncogenes and once this altered and damaged DNA starts replicating, the damage gets more and more fixed.

d. Multiplication resulting in cells with altered genome: Replication of DNA is followed by multiplication. Hence a completely neoplastic cell is formed with altered gene expression.

Neoplastic Development involves:

A. Growth : This is possible with the help of tumor promoters. Hence conditions favourable for the growth of initiated neoplastic cell are developed within the tissue forming premalignant lesions.

B. **Progression** : This process gives rise to a clinically detectable tumor which is capable of manifestation and invasion (Williams and Weisburger, 1986).

Carcinogenesis is a complex process, each of its steps being modified by a variety of factors (both intrinsic and extrinsic). Intrinsic factors include genetic, sex, hormonal status, age etc.) whereas extrinsic factors involve nutrition, mode and route of carcinogen exposure etc.

Of these, xenobiotic chemicals are of our major concern. Depending on the kind of effect they exert on the system and the step at which the effect is brought about, these xenobiotics may act as enhancer or inhibitor of carcinogenesis. Also, there is a possibility that the same chemical serves as inhibitor to one carcinogen and enhancer to other.

We are basically interested in the xenobiotics which bring about inhibition of carcinogenesis and hence can be called as anticarcinogens. This approach to the control of cancer is known as chemoprevention. The field is gaining immense importance day by day. Since last few years many chemicals have been proved to act as potential chemopreventive agents (Wattenberg et al, 1985).

Some of these are obtained from plants and are a major part of our usual diet. The very fact makes us realise the immense need to re-evaluate our nutritional habits.

The present thesis is a minor attempt to screen and assess some plant products that are daily consumed by us in one way or the other for their possible role as chemopreventive agents in cancer control.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

2.1 CHEMICAL CARCINOGENESIS

Induction of cancer by chemicals in experimental animals is a complex process comprised of several distinct steps which may be divided into two mechanistically separate sequences, neoplastic conversion of the cell and neoplastic development. Neoplastic conversion is the process in which a normal cell is transformed into a neoplastic cell and hence corresponds to initiation as defined by Berenblum. This sequence may proceed through stages. The second sequence is neoplastic development which is the process of growth of the neoplastic cell into a tumor and the further acquisition of abnormal properties by that tumor. Promotion was defined by Berenblum (1974) as the facilitation of growth of dormant neoplastic cells into tumors and hence neoplastic development includes promotion but is more comprehensive than that process.

Chemicals operate in a variety of ways in both of these sequences and facilitation of either sequence by a chemical can increase the occurrence of neoplasms. Chemicals that are carcinogenic in animals by the broad definition of increasing the occurrence of neoplasms are an extremely diverse group of agents. In an effort at mechanistic distinction they have been categorised into two basic types, genotoxic carcinogens and epigenetic carcinogens. Genotoxic carcinogens by definition react

with DNA and presumably thereby induce neoplastic conversion of cell. For some genotoxic carcinogens a single exposure is sufficient to ultimately result in cancer, indicating that neoplastic conversion was readily produced. Others require prolonged administration which may be necessary for the production of neoplastic conversion or may be required to facilitate neoplastic development in some way, perhaps through an additional promoting action. Non-genotoxic agents produce neoplasms through a variety of mechanisms; they may enhance the process of neoplastic conversion by indirectly producing genetic alterations or through epigenetic effects in the second sequence of events to increase neoplastic development of preexisting neoplastic cells. As a consequence of enhancement of neoplastic development certain epigenetic carcinogenic agents can augument the effects of genotoxic carcinogens and hence described as carcinogenesis enhancers (Clayson, 1981).

The carcinogenic process may be modified by various intrinsic and extrinsic factors (Table 1) impinging at different steps in the two sequences (Weisburger and Williams, 1982a). Among these factors xenobiotics, both naturally occurring and synthetic, can modify the carcinogenic effects of genotoxic agents through a variety of mechanisms (Table 2). Further, the same agent can exert several effects and consequently, a given chemical may through one action be an inhibitor at one step and through another action be an enhancer at a different step. Moreover, the concentrations available in specific tissues can likewise determine what kinds of effects occur. Additionally, a

chemical can be an inhibitor of the carcinogenecity of the agent, but an enhancer of another. Thus, the influences of a xenobiotic on the genesis of cancer are complex and vary with the specific circumstances of its interaction with a carcinogen.

Table 1: Modifying factors in chemical carcinogenesis.

Intrinsic

- Genetic
- Sex and endocraine status
- Age

Extrinsic

- Mode and regimen of exposure
- Housing
- Nutrition
- Other chemicals

Table 2: Mechanisms of modification of effects of chemical carcinogen

A Modification of effects of carcinogens

1. Alteration of bioavailability
2. Alteration of biotransformation
3. Depletion of active molecular species
4. Alteration of excretion

B. Modification of response of test subject

1. Alteration of susceptibility to biological effect(s) of test agent.
2. Imposition of biological effects that ameliorate or exacerbates those of test agents.

2.1.1 Mechanisms of modification of effects of chemical carcinogens:

1. Alteration of carcinogen availability: An agent that modifies the absorption from the point of entry in animals or uptake into cells of a chemical carcinogen can modulate carcinogenicity. A possible example is the study by Honburger and Tregier (1969) of subcutaneous carcinogenesis in mice by benzo (rst) pentaphene in different vehicles. The latent period for neoplastic development by the same dose was 16 weeks in peanut oil, 37 weeks in lipoprotein and 62 weeks in Ringer's solution.

2. Modification of biotransformation: Many genotoxic chemical carcinogens are procarcinogens that require activation by enzyme systems to reactive species to produce their carcinogenic effects (Miller and Miller, 1981). Modification of these enzyme systems involved in biotransformation can either decrease or increase the carcinogenicity of genotoxic procarcinogens. One of the earlier examples of cancer inhibition was demonstrated by Kensler et al (1941) that riboflavin protected against liver carcinogenicity of aminoazo dyes.

3. Modification of reaction interactions with target site: The reactive type of carcinogen is capable of forming an electrophile (Miller and Miller, 1981). These molecular species bind to various cellular nucleophiles, ultimately resulting in neoplastic conversion of the cell. A critical target for such reactions appears to be DNA.

An agent that competes with relevant macromolecules for the binding of a reactive carcinogen or indirectly generated reactive species could decrease genotoxicity and carcinogenicity. For example, glutathione has been reported to inhibit the liver carcinogenicity of aflatoxin B_1 (Novi, 1981).

4. Modification of expresison of cellular alteration: The adducts formed in DNA by genotoxic carcinogens can be removed by enzymic DNA repair systems and this restoration of DNA reduces mutagenic and carcinogenic effects. Hence an agent that alters repair processes or the rate of cell proliferation can affect the frequency of neoplastic conversion of carcinogen-damaged cells.

2.2 MAJOR METABOLIC PATHWAYS AND ENZYME SYSTEMS

Xenobiotic metabolism can be studied under two phases. The phase I enzymes (oxidative) are almost located in the endoplasmic reticulum, besides a phase II enzyme glucuronyl transferase. Other phase II (conjugative enzymes) are present in the cytoplasm.

Table 3: Reaction classified as phase I and phase II metabolism.

Phase I	Phase II
Oxidation involving cytochrome P 450	glucuronidation/ glucosidation
Oxidation - others	Sulphation
Reduction	Methylation
Hydrolysis	Acetylation
Hydration	amino acid conjugation and
[redacted]	glutathione conjugation
Dethioacetyation	
[redacted]	Fatty acid
Isomerisation	conjugation
[redacted]	
[redacted]	

2.2.1 Phase 1 metabolism

There are two major enzyme systems - cyt. P_{450} system (Mixed Function Oxidase MFO system) and Mixed Function Amine Oxidase System. They add a hydroxyl moiety to the foreign compound.

A. Components of MFO System:

(a) Cytochrome P450: Cy t. P450 is classified as a haeme containing enzyme with iron protoprophyrin IX as the prosthetic group. This is found predominantly in the endoplasmic reticulum and catalyses the following MFO reacton :

$$NADPH + H^+ + O_2 + RH \text{ ----------> } NADP^+ + H_2 + ROH$$

The name cyt. P-450 comes from the fact that the cytochrome has an absorbance maximum at 450 nm when reduced and complexed with carbon monoxide i.e., when it is intact and catalytically functional. When the enzyme gets denatured, it loses its specific peak at 450 nm, it gives an absorbance maximum at 420 nm (like other haemeproteins).

(b) NADPH-cytochrome P-450 reductase: This is a flavin containing enzyme which serves as an intermediate electron transfer between NADPH + H^+ and cytochrome P-450.

(c) Lipids: These are the basic requirements for MFO activity.

(d) Aryl hydrocarbon hydroxylase (AHH): The enzyme is specific for converting many polycyclic aromatic hydrocarbons (chemically inert) to water soluble forms.

(c) Cytochrome b5: The exact role of cytochrome b_5 in MFO reaction is not yet clear but there is a concept that it may act as a transducer of reducing equivalents between NADPH-cyt. P450 reductase and cyt. P450.

B. Microsomal FAD-containing monoxygenase (MFMO): This is also present in the endoplasmic reticulum and oxidises nucleophilic nitrogen and sulphur compounds.

(d) Epoxide hydratase: The enzyme serves to catalyse hydration of arene oxides and aliphalic epoxides to their corresponding transdihydro diols. The close association of epoxide hydratase with cytochrome P450 may play an important role in detoxification of those reactive epoxides.

2.2.2 Phase II metabolism: Phase II reactions include the conjugation of xenobiotic compounds (produced by Phase I reactions) with endogenous compounds to give excretable hydrophilic conjugates.

(a) Uridine diphosphate glucuronosyl transferase (UDPGT): The enzyme is located in the microsomal membrane and catalyses the production of glucuronide conjugates. The resulting conjugates are excreted from the body via bile or urine.

(b) Glutathione S-transferases: GSTS are a group of proteins with overlapping substrate specificities and ligand--binding capacities. They catalyse nucleophilic attack by the thiol group of glutathione on xenobiotics as epoxides, halogennitro benzeme and sulphobromophthalein. The enzyme is

located in the cytosol and also promotes the ester formation from hydrophilic compounds as a first step in mercapturic acid formation. The GSH conjugates (thio esters) are mostly excreted in the bile unlike other Phase II products (Fig.3).

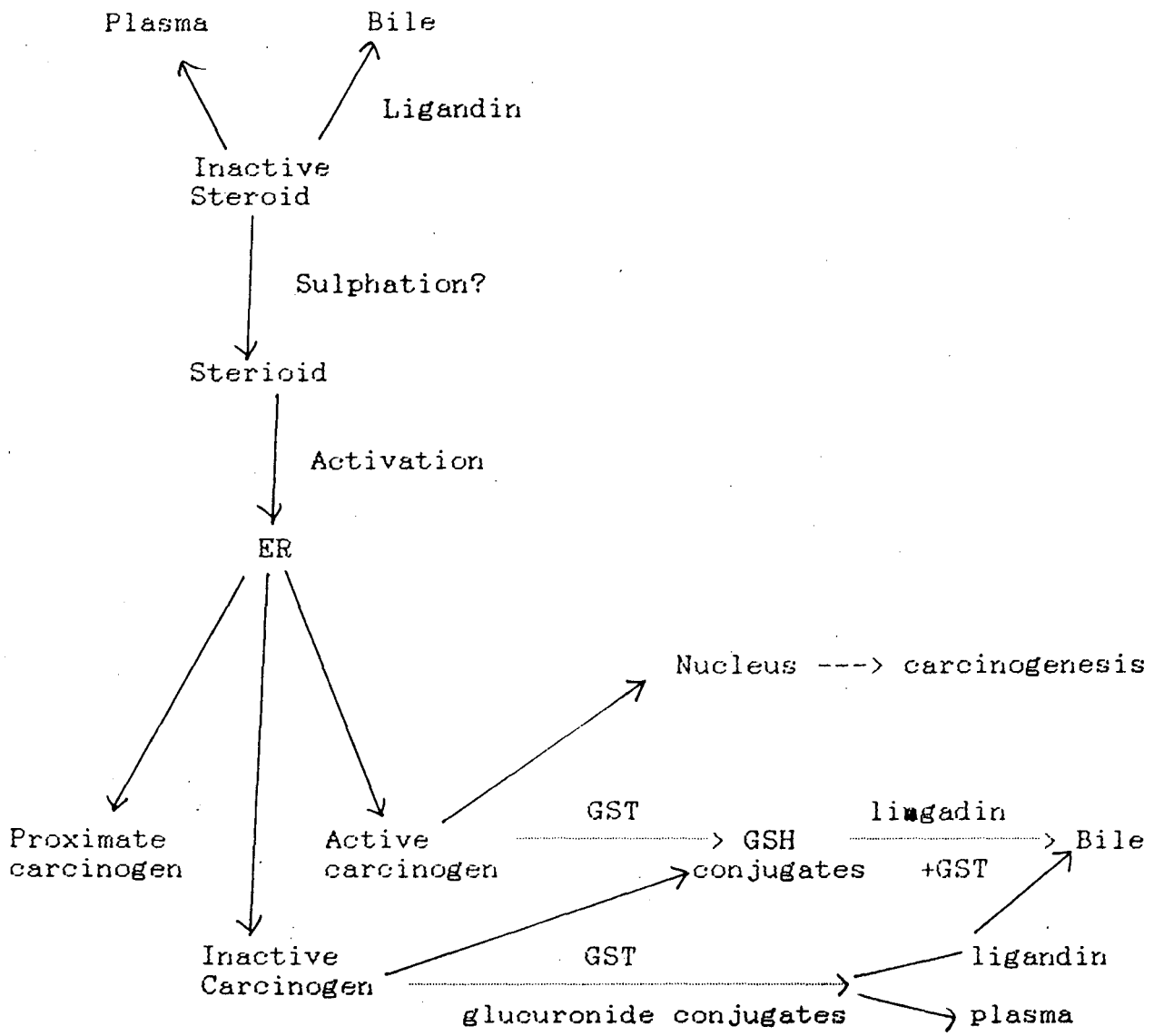


Fig. 3: Role of GST in carcinogenesis.

GST is present in multiple forms which are differentiated on the basis of 2 sub units that each iso enzyme contains. So far seven subunits have been defined which can be distinguished by subunit molecular weight and isolectric point. All the isoenzymes show 1-chloro-2,4-dinitrobenzene (CDNB) as the universal substrate.

(c) NAD(P)H oxidoreductase/DT diaphorase (DTD): This is mainly present in the cytosolic fraction of liver. This is a flavoenzyme and catalyses a 2 electron reduction leading to conversion of quinone into non-toxic hydroquinones. These are further converted into sulphate/glucuronyl conjugates in the presence of other phase II enzymes. Other important conjugation enzymes of phase II metabolism are :

1) Sulphotransferase - that catalyse sulphation of functional groups.

2) Methyltransferases - which methylate amines, phenols and sulphydryl containing compounds.

3) Acetyl transferases - that are responsible for acetylation and

4) Amino acids that conjugate to carboxylic acid group to form amides.

All the conjugates are eliminated in urine.

TH-7114

2.3 **CHEMOPREVENTION**: a potential means of cancer control :

Chemoprevention is the most desirable means of cancer control in which the administration of one or more chemically defined agents inhibits the preneoplastic events which usually lead to the development of clinically detected cancer (Wattenburg et al, 1985). When one evaluates a chemoprevention comnpound, considerations of its projected use are highly relevant. If the compound is very potent but has significant toxicity, it may nevertheless be useful for high risk individuals but not for the general population. If a compound has modest protective effects and little toxicity, considerations of its primary use for individuals at lesser risk to neoplasia would be appropriate.

2.3.1 Chemopreventive compounds: One of the most impressive findings in the field of chemoprevention is the very large number of compounds that have been demonstrated to prevent the occurrence of cancer.

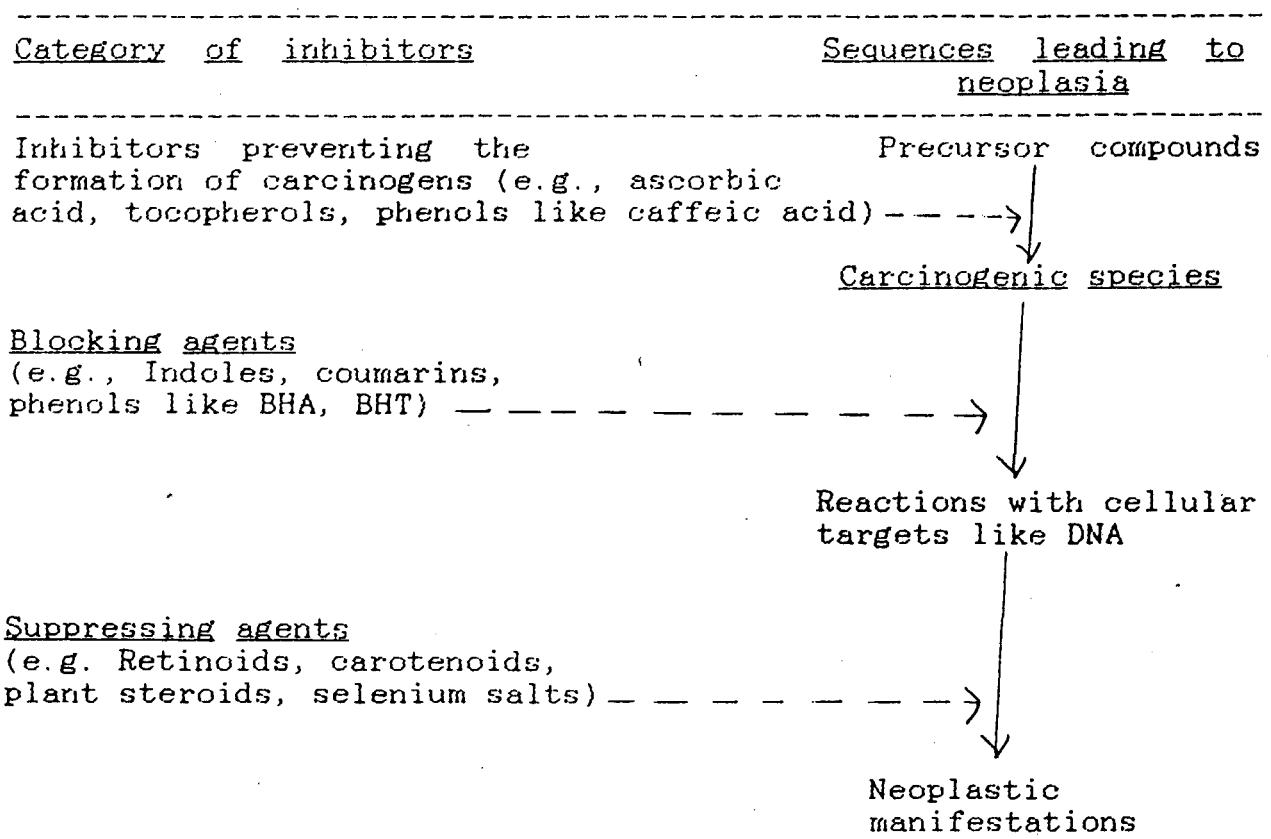
Depending on the time of action, the chemopreventive agents can be placed into 3 broad categories (in Table 4).

(i) Which prevent the formation of carcinogens from precursor substances.

(ii) Compounds that inhibit by preventing the carcinogenic species from reaching/reacting with the critical target sites in tissues. They are called blocking agents.

(iii) These inhibitors suppress the expression of so called cancer cells. They are termed as suppressing agents.

Table 4: Classification of chemopreventive agents on the basis of the time at which they exert their protective effects (Wattenburg et al).



Compounds belonging to over 20 different classes of chemicals have been shown to have chemopreventive capacities (Table 5). The great chemical diversity is a positive feature in that it indicates the likelihood that a variety of approaches can be made to prevention and the options of selecting optimal compounds will be large. Some of these inhibitors are naturally occurring constitutents of food.

The various categories of chemopreventive agents (Table 5) are discussed as follows :

a) Inhibitors effective against complete carcinogens:

(i) Compounds inhibiting the formation of carcinogens: A major focus of this group of inhibitors has been on prevention of the formation of nitroso carcinogens from the reactions of precursor amines or amides with nitrites. Ascrobic acid is effective in inhibiting formation of these carcinogens both in vitro and in vivo.

(ii) Blocking agents: A large and diverse grop of compounds both naturally occurring and synthetic fall into this category of inhibitors. An understanding of their mechanisms of action is based on the contributions of the Millers and others to the field of chemical carcinogenesis. Blocking agents can be placed into 3 groups according to their mechanism of action. One groups acts simply by inhibiting the activation of carcinogen to its ultimate carcinogenic form. For example, inhibition of dimethylhydrazine induced neoplasia by disulfiram (Wattenburg and Weisburger, 1977). The second group is effective by virtue of inducing

increases in activity of enzyme systems having the capacity to enhance carcinogen detoxification. The inhibitors of this group are of particular interest because they have the capacity to inhibit a wide range of carcinogens. The third group of blocking agents has the capacity to act by scavenging the reactive forms of carcinogens. Physiological nucleophiles, such as GSH fall into this group. Recently, xenobiotic compoounds present in plant constitutents of the diet have been shown to scavenge the ultimate carcinogenic form of BP. Ellagic acid has been shown to be highly potent in this regard (Yagi and Conney, 1982). Two general categories of blocking agents that enhance carcinogen detoxification systems have been identified in studies of inhibition of carcinogenesis. They are designated as type A and type B inhibitors. Both type A and B inhibitors induce an increase in activity of multiple enzymes, presumably by reacting with receptors. The type A inhibitors induce an increase in Phase II enxzymes i.e., conjugating enzymes and some related systems. These inhibitors have been shown to inhibit carcinogenesis resulting from administration of BP, DMBA, diethylnitrosamine, uracil mustard, urethan etc.

Type B inhibitors characterically induced pronounced increases in microsomal monooxygenase activities. A prototype of this class of inhibitors is β-naphthoflavone. Type B inhibitors also enhance the activity of major conjugating systems. They have been shown to inhibit the occurrence of neoplasia resulting from administration of BP, DMBA, aflatoxin etc. These inhibitors are complicated in that the the microsomal monoxygenase system can both activate and

detoxify chemical carcinogens. The classic example of this is with the aromatic amines. With these compounds, ring hydroxylation of the nitrogen is not an activation reaction (detoxification) whereas hydroxylation of nitrogen is an activation reaction.

(iii) Suppressing agents: Suppressing agents are compounds that inhibit carcinogenesis when administered subsequent to a course of carcinogen administrations that would result in the occurrence of cancer. The number of classes of comnpounds that act as suppressing agents is smaller than that of blocking agents. The most extensively studied suppressing agents are the retinoids.

Table 5: Inhibitors of carcinogen-induced neoplasia

Catetgory of inhibitor	Chemical class	Inhibitor compounds
1. Compounds preventing formation of carcinogen from precursor compounds	Reductive acids	ascrobic acid
	Tocopherols	∝-tocopherol
	Phenols	Caffeic acid, ferulic acid
2. Blocking agents	Phenols	BHA, BHT, ellagic acid
	Indoles	Indole-3-carbinol
	Aromatic isothiocyanqtes	Phenyl isothiocyanates
	Coumarins	Coumarin
	Flavones	β-naphtho flavone
	Dithiothiones	5-(2-pyrazinyl) -4-methyl-1,4 dithiol-3-thione
	Diterpenes	Kahweol palmitate
	Dithiocarbamates	Disulfram
	Phenothiazines	Phenothiazine
	Barbiturates	Phenobarbital
	Trimethylquinolines	Ethoxygquin
3. Suppressing agents	Retinoids and carotenoids	β-carotene, retinyl acetate
	Selenium salts	Sodium selenite
	Protease inhibitors	antipain
	Inhibitor of arachidonic acid metabolism	Indomethacin aspirin
	Cyanates and isothiocyanates	Sodium cyanate
	Phenol's	BHA
	Plant sterols	β-sitosterol
	Methylated xanthines	Caffeine
	others	fumaric acid

Compounds inhibiting tumor promotion (Table 6)

The vast majority of studies on inhibition of tumor promotion have focussed on inhibition of promotion of epidermal neoplasia in mouse as a result of topical administrtation of TPA. A major hypothesis concerning tumor promotion has been that attack by oxygen radicals may play a role in its causation. Hence inhibitors that prevent attack by oxygen radicals inhibit tumor promotion. For example, protease inhibitors prevent formation of oxygen radicals by TPA and inhibit tumor promotion.

Table 6: Inhibitors of tumor promotion of the mouse skin

Class of inhibitor	Compound
Retinoids	All-trans retinoic acid, 13-cis-retinoic acid
Protease inhibitors	Leupeptin
Inhibitors of arachidonic acid metabolism	Dexamethasone, Indomethacin
Phenols	2-tert-butylhydroxyanisole 3-tert-butylhydroxyanisole
Synthetic compound with superoxide dismutase activity	Copper (II)3,5-diisopropylsalicylic acid
Cyclic nucleotides or inhibitors of phosphodiesterase activity	Cyclic AMP
Polyamines	Putrescine
Modulation of calcium metabolism	1 α-25-dihydroxy vitamin D_3
Benzodiazepines	Diazepam
Others	Quercetin, -difluoromethylornithrine (DFMO)

2.4 MODULATION OF CARCINOGENESIS VIA CHANGES IN THE LEVELS OF METABOLIC ENZYMES

The review is mainly restricted to those xenobiotics that are called blocking agents.

These agents are classified into 2 classes. The former class enhances phase II enzyme system wheras the latter one promotes both phase I and phase II systems, the net effects deciding inhibition or promotion of carcinogenesis (Wattenberg, 1983).

2.5 ANTIOXIDANT DEFENSE MECHANISMS

Exposure to radiation or xenobiotics leads to the production of free radicals. These free radicals subsequently attack on the biological membranes and other critical macromolecules hence damaging them extensively. To tackle this situation, cells have certain inherent features which are termed as antioxidant-defense systems. They are classified as follows :

2.5.1 Superoxide dismutase (SOD): This catalyses the reduction of superoxide free radical ($O_2^{\cdot -}$) to H_2O_2 and O_2.

2.5.2 Catalase: This enzyme is present in peroxisomes. It catalyses the decomposition of hydrogen peroxide hence preventing the formation of free radicals.

$$2H_2O_2 \xrightarrow{\text{catalase}} H_2O + O_2$$

2.5.3 Biological antioxidants: These include various endogenous nucleophilic compounds which render free radicals inaztive for e.g., carotenes, ascorbate, tocopherols and thiols

(SH containing). The radicals abstract a hydrogen atom from these compounds hence protecting the usual critical targets like DNA, RNA and membranes.

Glutathione-dependent system: This is the most important defense system. Glutathione is a widely occurring non-protein thiol (90% of total SH content). It is a strong antioxidant which maintains the SH group of SH-depednent enzymes in reduced state. Besides this, GSH scavenges free radicals nonenzymatically leading to a reduction in the caused damage. GSH also serves as a substrate for glutathione S-transferases.

Considering the importance of reduced glutathione in preventing carcinogenesis, the cells continuously restore back this reduced form. Usually GSH is enzymatically oxidised to its disulphide (GSSG) which can be reduced back to GSH by GSH reductase in the presence of $NADPH_2$.

2.6 DIET AND CANCER

Besides chemoprevention, at present several factors in the diet are seen as tumor promotors or antipromotors. The item most clearly established as a promotor is dietary fat. The possible antipromotors include dietary fiberi Vitamin C, A, E, the trace element selenium and certain compounds in vegetables like cabbage and cauliflower (Wattenburg et al). Evidence from clinical studies and laboratory experiments suggest that some of the vegetable compounds may act at more than one point in the carcinogenic sequence, affecting enzymes that detoxify initiating carcinogens and also serving as antipromotors.

There are two major sources of evidence suggesting a role of dietary factors in cancer: epidemiological studies and laboratory experiments with rodents. The studies indicate a diversity and widespread occurrence of these compounds in food which makes it impossible to consume a diet that does not contain inhibitors of carcinogenesis (Cohen et al, 1987).

These aspects appeal for the need of reviewing our food habits that are rich in spices and different flavouring plant products. The mechanism by which these plant products affect cancer causation/control still lies to be revealed.

2.6.1 Bitter gourd: bittergourd (Momerdica chirantia) is a member of the family cucurbitaceae. The fruit is bitter, cooling, digestible, laxative, antipyletic, antihelminthic, appetiser, cures blood diseases, anaemia, ulcers etc. The juice of the fruit is recommended for snake bite and in cholera. It is also used as a laxative and as an ointment for sores. The juice of the plant is applied externally to the scalp in pustular eruptions whereas the root is used in opthalmia.

2.6.2 Brassica: Brassica belongs to the family cruciferae. The genus consists of many species out of which two are described as follows :

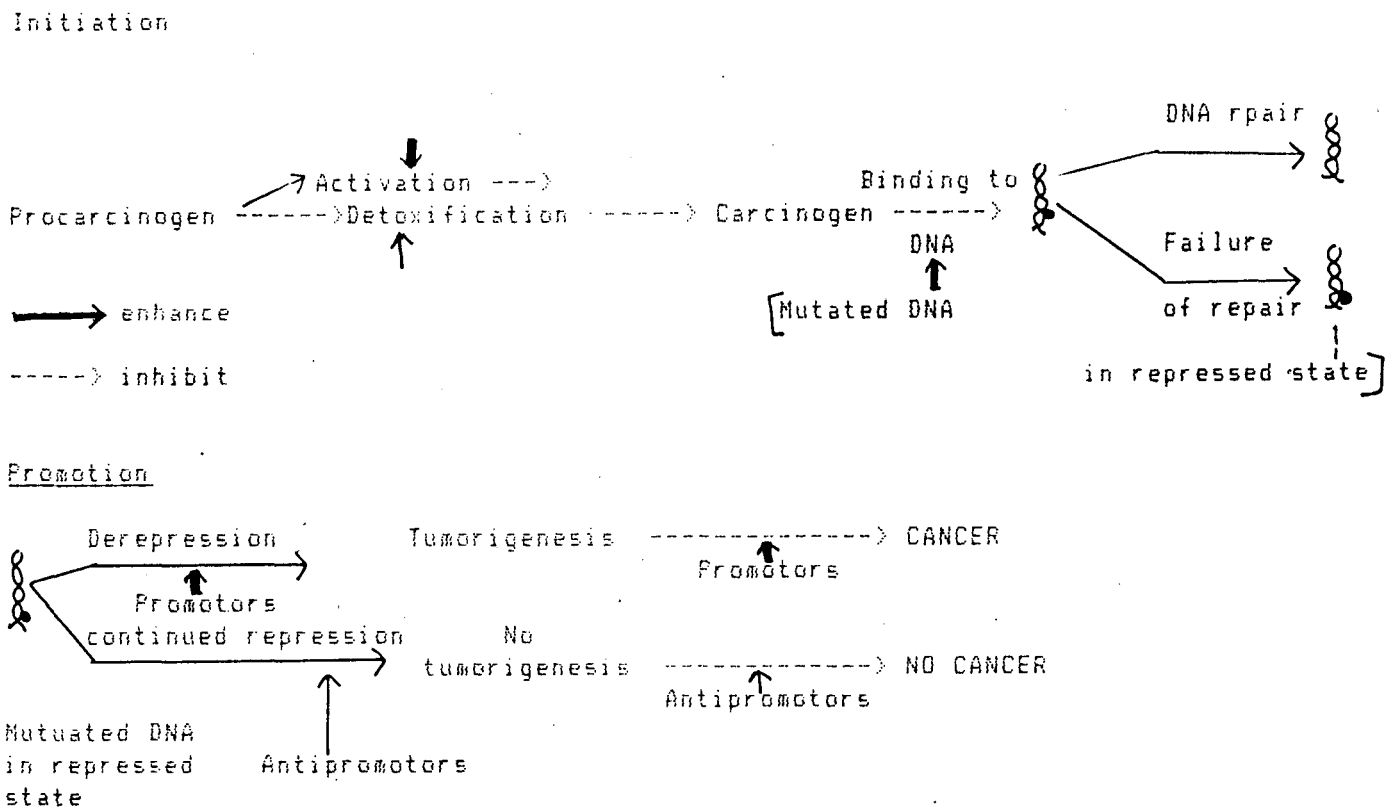
a) Brassica napus: The root of the plant is embollient and diuretic. Its juice is useful in chronic coughs and bronchial catarrh.

b) B. Campestris: It is oily, bitter, hot and sharp taste, stomachacic, cures skin eruptions, itching, good in piles, inflammation, ulcers, epilepsy, toothache stops vomiting. The oil from its seeds mixed with camphor seems as an efficient embrocation in muscular rheumatism, stiff neck. It is also used in dengue fever and rubbed on chest in bronchitis. The oil contains glycerides of erucic acid.

2.6.3 Methi (Trigonella foenum graecum) Umbelliferae: The seeds are hot, with a sharp bitter taste, antipyretic, anti-helmithic, increase appetite, astringent cures leprosy, vomiting, bronchitis, piles, removes bad taste from mouth, helpful in heart disease. It contains alkaloid trigonelline. Powdered seeds are used in veterinary practice. The seeds are also used in chronic cough and enlargements of the liver and spleen. Fenugreek seeds are carminative.

2.6.4 Cardamom (Elletaria cardamum): It belongs to the family scitamineae. The seeds are bitter, cooling, pungent, fragrant, useful in asthma, bronchitis, piles, disease of kidney etc. They are also given in snake bite and snake venom. The fruit is applied to the tumors of uterus.

2.6.5 Sandalwood (Santalum album): This belongs to the family Santalaceae. The wood is bitter, cooling, exhilarating, antipyretic, useful in diseases of heart, thirst, burning sensation, bronchitis, vaginal discharges and small pox. The oil is used in skin disease. The wood with water is used for local inflammations.



Promotors : high fat diet

Antipromotors : vitamin A,C,E and selenium etc.

Fig. 4 Influence of dietery factors in carcinogenesis

Table 7: Nutritional factors involved in certain human cancers

Type of cancer	Postulated source and genotoxic carcinogens	Epigenetic factors	Protective or inhibiting elements
Oesophageal, oral cavity	Tobacco (nitrosamines, polycyclic aromatic hydrocarbons)	Alcohol	Green and yellow vetetables, fruits high in vitamin A,C,E
Stomach	Dried, salted fish, pickled vegetables, smoked fish (nitrite + promutagen)	Salt (NaCl), low intake fresh fruits and vegetables	Increased intake of fresh vegetables and fruits, vitamins C & E
		Low intake of Vitamin C	Vitamin C & E
Pancreas	Tobacco	Alcohol ? coffee ?	Fresh fruits, vegetables, low-fat diet
Large bowel: colon	Carcinogens formed in fruit and broiled meat and fish (heterocyclic amines)	High levels of dietary fat, bile acids.	Cereal bran fiber, certain cruiferous vegetables, selenium, calcium, olive oil? fish oil? Exercise? Fibre?
Rectum	??	Alcohol?	
Breast	Fried meat/fish	High fat diet Hormone imbalance?	Low fat diet, olive oil, fish oils
Endometrium	??	Postmenopausal estrogen intake, obesity	-do-
Ovary	??	High fat diet	Low fat diet
Prostate	??	High fat diet	Low fat diet, adequate zinc and selenium.

MATERIALS AND METHODS

MATERIALS AND METHODS

3.1 ANIMALS

Randombred male Swiss albino mice (young adults) body weight (25-30 gm) maintained in the air conditioned Animal facility at Jawaharlal Nehru University were employed in the experiments. Animals were kept in plastic cages lined with rice-husk padding and were provided with standard mouse feed (Hindustan Lever Ltd., India) and tap water ad libitum unless otherwise stated.

3.2 CHEMICALS

Bovine serum albumin (BSA), 1-chloro-2,4-dinitrobenzene (CDNB), 3,3'-Dithiobis, 2-nitrobenzoic acid (DNTB), 2(3)-tert-butyl-4-hydroxyanisole (BHA), 5-sulfosalcylic acid, reduced Nicotinamide adenine dinucleotide (NADH), reduced glutathione (GSH), sodium dithionite have been obtained from Sigma Chemical Co., USA. While Commassie Brilliant Blue (G-250) were obtained from Merck, Germany. Brassica species from Pusa Institute, Delhi - E. alba from CSIR, Delhi - and the rest of the plant materials used were purchased afresh from local markets.

3.3 TREATMENT SCHEDULE FOR ANIMALS

The general protocol followed for the hepatic studies employing various test materials in depicted in the following table. The duration of treatment in case of short term genotoxicity studies is ten days for all the test materials.

The animals maintained on their respective diets were weighed before and after the treatment to note any change in weight due to the individual treatment (A loss in weight indicates toxicity of the materials). They were starved overnight prior to the termination of the experiments i.e., sacrificing.

Table 8: Experimental design for hepatic studies

Experiment	Test substance	Mode of of Exposure	Doses employed		Duration of treatment (Days)
1	Brassica napus	Oral		1%	10D
	Brassica campestris	Oral		1%	10D
2	Bitter gourd (Momerdica chirantia)	Oral	1%	2%	10D
3	Methi (Trigonella foenum-graecum)	Oral		2%	10D
4	Cardamom (Elettaria cardamum)	Oral	1%	2%	10D
5	Sandalwood (Santalum album)	Oral	1%	2%	10D

3.4 PREPARATION OF TEST DIETS AND EXTRACTS

1. Brasicca extracts: The seeds of the two species tested were provided (individually). These powders were diluted with distilled water so as to get the required doses of Brassica napus (1%) and Brassica campestris (1%).

2. Bitter gourd extract: Kknown amount of bitter gourd (fresh) was crushed into a pulp which was further squeezed to get the stock extract. This extract was diluted with distilled water to get the required doses (1% and 2%).

3. Methi extract: Methi seeds were ground into a fine powder first in a pestle mortar and then mixie. After sieving, known amount of this powder was dissolved in known volume of distilled water so as to get the required doses (1% and 2%) in a volume of Ø.1 ml.

4. Cardamom extract: Cardamom seeds were ground into a fine powder. Known amount of the powder was dissolved in known volumes of distilled water so as to get the required doses (Ø.Ø1 mg/ml and Ø.Ø2 mg/ml) in a volume of Ø.1 ml.

5. Sandalwood oil extract: Pure sandalwood oil was obntained from Karnataka Emporium, New Delhi. KNown volumes of the oil was suspended in known volume of distilled water to form an emulsion of required concentrations (1% and 2%) in a volume of Ø.1 ml.

3.5 PREPARATION OF VARIOUS SUBFRACTIONS OF LIVER

The animals were sacrificed by cervical dislocation and their livers were rapidly excised, perfused and rinsed in Ø.9% NaCl. These were then weighed quickly and homogenised in chilled homogenising media containing Ø.154 M KCl and 5Ø mM Tris-HCl (pH 7.4) to yield a 1Ø% (w/v) homeogenate. This was subjected to centrifugation at 1Ø,ØØØ x g (11,5ØØ rpm) for 2Ø minutes at $4^{o}C$ (using RC-5 Sorvall centrifuge). The resultant supernatant (viz., the post-mitochondrial supernatant) is further centrifuged at 1ØØ,ØØØ x g (4Ø K) for 1 hour at $4^{o}C$ (using Beckman L87ØM ultracentrifuge). The supernatant which forms the cytosolic (soluble) fraction of liver was used for the assay of total GST enzyme.

The pellet formed in the above differential centrifugation represents the microsomal fraction. It was resuspended in the homogenizing buffer (2 ml) and was used for the assays of cytochrome b5 and cytochrome P45Ø enzymes.

3.6 PARAMETERS OF HEPATIC STUDIES

3.6.1 Determination of cytochrome b_5 and cytochrome P45Ø

The microsomal suspension was used as such for the assay of cytochrome b5 and cytochrome P45Ø contents by Omura and Sato method (1964).

A Cytochrome b_5

Principle : The haemeprotein (prosthetic group of cytochrome b^5) is readily estimated from its redox spectrum of NaDH-reduced versus oxidised cytochrome. The reduction of cyt. b5 is catalysed by the presence of a microsomal flavoprotein enzymes, NADH cyt. b_5 reductase.

Procedure : 1 ml of the microsomal suspension was diluted with 3.8 ml of homogenizing-buffer. This suspension was divided into both the cuvettes which were then scanned between 400 and 500 nm for baseline adjustment. 100 λ of NADH (2 mg in 500 λ of homogenising buffer) and 100 λ of the buffer were then added to the test and reference curvettes respectively and the contents were properly mixed. After 2 minutes, the redox spectrum between 400 and 500 nm was recorded. The absorption difference (ΔA) between the peak at around 424 nm and the trough at 409 nm in the redox spectrum was directly obtained from Schimadzu UV 260 spectrohotometer. The cytochrome b_5 content expressed as nanomoles/mg microsomal protein was obtained from the formula :

$$= \frac{\Delta A(424-409) \times 1000}{185 \times \text{protein in mg}}$$

where 185 nm^{-1} cm^{-1} represent the extinction coefficient of cytochrome b_5.

B Cytochrome P450

Principle : The cyt P450 reduced by sodium dithionite forms an adduct with carbon monoxide which has an absorbance peak at 450 nm.

Procedue : This was assayed from the same samples as above. After the estimation of b5 the contents of both the cuvettes were mixed and reduced with few mg of sodium dithionite. The contents were again divided into the 2 cuvettes which were then adjusted for baseline correction betweeen 450 nm and 490 nm. The contents of the sample cuvette were gased gently for about few seconds (30-40 bubbles) with CO (CO was generated by the reaction of conc. Sulphuric acid and formic acid in a ratio of 2:1). This leads to the formation of an adduct of cyt. P450 which has an absorbance maximum at 450 nm.

The cytochrome P450 content expressd as nanomoles/mg microsomal protein was obtained from the formula :

$$= \frac{\Delta A\ (490-450) \times 1000}{91 \times \text{protein in mg}}$$

where 91 $nm^{-1}\ cm^{-1}$ is the molar extinction coefficient of cyt P450. The A was directly obtained from the spectrophotometer (Shimadzu UV-260).

3.6.2 Estimation of GST activity: The enzyme has the ability to conjugate GSH with CDNB which causes a change (increase) in absorbance at 340 nm.

Procedure: The cytosolic GST activity was determined spectrophotometrically (in a Schimadzu sepctrophotometer) by the procedure described by Habig et al (1974). The reaction mixture contained 0.1 ml of both the substrates, (1 mM GSH and 1 mM CDNB in ethanol), 1 ml of 0.1 M phosphate buyffer (pH = 6.5) and water

was added to make a final volume of 2.9 ml. The reaction mixture was then incubated at 25°C for 5 min. and the reaction was started by adding Ø.1 ml of diluted cytosol (diluted with Ø.1 M $Na\text{-}PO_4$ buffer pH = 6.5 in the ratio of 1:9). The enzyme activity was followed for 3 min against a blank containing complete assay mixture minus the enzyme.

The specific activity of GST is evaluated using the formula:

$$= \frac{\text{Change in absorbance/min x 3 x 1ØØØ}}{\text{9.6 x protein in mg}}$$

where 3 is the sample volume. 9.6 is the molar extinction coefficient value of GST. The activity was expressed as μmoles of CDNB-GSH conjugate formed/min/mig protien.

3.6.3 Estimation of Acid soluble sulphydryl content

Principle : The acid soluble SH gps (non-protien thiols of which GSH forms 9Ø%) react with DTNB to yield a yellow coloured complex which has a specific absorbance at 412 nm.

Procedure : The assay was done by Ellman's method (1959) as modified by Sparnin et al (1982). Hmogenates were precipitated with Ø.1 ml of 25% trichloroacetic acid (TCA) and the precipitate was removed after cenrifugation. Free SH gps were assayed by the addition of 2.7 ml of Ø.1 mM DTNB in Ø.1 M phosphate buffer (pH = 8.Ø) to Ø.3 ml of the supernatant. The absorbance was read at 412 nm which was then used in the formula to give the sulphydryl

content:

$$\frac{\text{Conc std}}{\text{OD std}} \times \text{OD sample} = \text{Conc sample}$$

where conc std and std were obtained from the standard curve of GSH (glutathione).

3.6.4 Estimation of protein levels

Protein content in various subfractions was determined by Bradford's method (1976) using BSA as standard.

Principle : The dye Commassie Brilliant Blue G-250 binds to protein to form a blue protein-dye complex which has an absorbance at 595 nm.

Procedure :

Preparation of Bradford's reagent : 100 mg of commassie Blue G-200 dye was dissolved in 50 ml of 95% ethanol and then 100 ml of 85% (w/v) phosphoric acid. This was diluted with distilled water in the ratio of 1:4.

5 ml of the reagent was added to 0.1 ml of various tissue subfractions and the absorbance was read at 595 nm against a blank containing 0.1 ml of phosphate buffer and 5 ml of the reagent. The concentrations of protein was evaluated from the standard curve made with BSA and was expressed as mg/gm of tissue.

3.7 STATISTICAL ANALYSIS

To see whether the values of various parameters (of hepatic studies) assessed in different treated groups significantly differ from their respective control mean values they were subjected to student's t-test (Makroudian, 1969 and Ipsen and Feigl, 1970).

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

4.1 EXPERIMENT I : EFFECT OF KARELLA

Results

Administration of Karella extract orally at doses of 1% and 2% to mice did not alter their body weights or liver weights. The acid soluble SH fractions were significantly enhanced as compared to their respective control values. The alterations in various hepatic enzyme levels by Karella extract are depicted in Table 1Ø. Significant increase in the level of GST along with significant decrease in cyt. b_5 and P-45Ø were observed as compared to control values.

Discussion

Karella is strongly recommended for the treatment of diseases like anaemia and ulcers. These medicinal aspects of Karella directed us to test the material for its anticarcinogenic properties too. The present study has shown positive results with Karella which makes it a potential future chemopreventive agent. It is a potent inducer of Phase I and Phase II enzymes. In addition, it has also enhanced the components of antioxidant defense system as evidenced by the enhanced SH levels. However, the mechanism of enzyme induction by Karella still has to be revealed.

1. MEAN $(\bar{x})$ $= \dfrac{x_1 + x_2 + x_3 + \ldots\ldots + x_n}{n \text{ (no. of samples)}}$

2. Standard deviation (SD) $= \sqrt{\dfrac{\sum_{i}^{n=1} (x_i - \bar{x})^2}{(n-1)}}$

x_i = individual sample

3. Standard error of mean (SEM) $= \dfrac{\text{Standard deviation (SD)}}{\sqrt{n}}$

Fig. 5: Steps involved in calculations

Table 9: Effects of administration of various modulators on hepatic enzyme levels

Group	Treatment Details	Glutathione S-Transferase (µmoles cDNB conjugate/min/ mg protein)	Cytochrome b_5 (nmoles/mg protein)	Cytochrome P 450 (nmoles/mg protein)
I	Control	26.Ø4±Ø.62	421.89±3Ø.51	2Ø5.92±2Ø.41
II	BHA (Ø.5%)	15Ø.49±9.36	2.85±Ø.Ø1	6.62±Ø.Ø4
III	Cardamom (Elletaria cardamum -Ø.Ø1 mg/ml)	85.94±3.2	11.78±Ø.6	23.9Ø±1.1
IV	Cardamom (Elletaria cardamum -Ø.Ø2 mg/ml)	86.29±3.6	13.6Ø±Ø.Ø9	1Ø.99±Ø.Ø8
V	Brassica napus (1%)	94.Ø2±1.23	26.Ø1±Ø.Ø72	63.21±1.96
VI	Brassica campestris(1%)	11Ø.Ø4±3.89	6.3±Ø.Ø29	12.24±Ø.Ø56
VII	Karella (Momerdica charantia-1%)	44.82±Ø.82	31.63±Ø.63	1ØØ.95±3.54
VIII	Karella (Momerdica charantia-2%)	53.42±2.Ø1	11.82±Ø.Ø55	23.2Ø±Ø.Ø64
IX	Sandalwood oil (Santalum album-1%)	1Ø7.99±2.37	3.Ø8±Ø.ØØ5	7.67±Ø.ØØ7
X	Sandalwood oil (Santalum album -2%)	4126.86±31.Ø4	11.86±Ø.Ø49	23.1Ø±Ø.Ø1Ø
XI	Methi (Trigonella foenum-graecum- 2%)	136.62±8.65	26.Ø4±Ø.95	76.5±2. 53

Values expressed as Mean ± SEM of 8 animals.

Table 10: Effect of modulators on hepatic system

Group	Treatment details	Protein content (mg/gm tissue)		Total -SH content (μmoles/gm tissue)
		cytosolic	microsomal	
I	Control (Normal diet)	104.6±3.65	50.8±1.82	6.02±0.046
II	BHA (0.5%)	29.9±1.56	350.1±28.73	12.08±0.051
III	Cardamom (Elletaria cardamum -0.01 mg/ml)	45.1±0.80	149.6±8.75	6.18±0.038
IV	Cardamom (Elletaria cardamum -0.02 mg/ml)	5.2±0.027	106.6±2.26	12.05±0.049
V	Brassica napus (1%)	55.5±2.06	96.4±3.43	9.04±2.89
VI	Brassica campestris (1%)	42.0±1.75	136.5±8.54	27.11±0.93
VII	Bitter gourd (Momerdica chirantia-2%)	125.6±3.76	29.3±0.85	9.06±0.094
VIII	Bitter gourd (Momerdica chirantia-1%)	110.0±3.21	57.7±1.48	21.1±0.76
IX	Sandalwood oil (Santalum album-1%)	94.6±2.89	339.6±29.08	6.08±0.054
X	Sandalwood oil (Santalum album-2%)	102.5±3.05	245.5±21.26	15.06±0.62
XI	Methi (Trigonella foenum-graecum-2%)	46.6±1.26	26.8±0.94	18.1±0.66

4.2 EXPERIMENT 2 : EFFECT OF MUSTARD SEED

Results

The changes imparted by the oral administration of mustard seeds (two varieties - Brassica napus and Brassica campestris at the dose of 1% each) are depicted in the tables. While body weights and liver weights remained unaffected by the treatment, the increase exhibited in SH content was significant. Similarly, GST levels were enhanced with a significant decreasse in the levels of cyt. b_5 and cyt. P-450.

Discussion

Many species of the genus Brassica were thoroughly worked out for their anticarcinogenic properties in different model systems. As it has been suggested that the anticarcinogenic properties of Brassica vegetables could be attributed to the presence of indole glucosinolates (glucobrassicins) and isothiocyanates (Wattenburg, 1983 and McDanell et al; 1988), the possible chemopreventative aspects of other species of this genus and other cruciferous plants need to be tested.

In our present study, two species of mustard seed, namely, Brassica napus and Brassica campestris were tested which gave positive results recommending them as good chemopreventive agents in future. In fact, Brassica campestris proved to be a better candidate in this case.

4.3 EXPERIMENT 3 : EFFECT OF METHI

Results

Extracts of methi when administered orally to mice at a dose of 2% did not signifiantly alter the body weights or the liver weights as compared to untreated animals. Highly enhanced SH levels as compared to control values were observed. The tables represent the changes brought by methi administration in the hepatic enzyme levels. The GST levels were significantly increased whereas the cyt.b_5 and cyt. P-450 contents were decreased to a large extent. Also, an enhancement in the -SH levels was observed.

Discussion

Methi seeds have been proved to be helpful in heart disease. Also, they cure leprosy, bronchitis etc. In the present study, it was tested whether the seeds had any anticarcinogen effect. From the current data obtained, it can be inferred that methi has distinct effects on the hepatic enzyme systems. The results show potential induction of both Phase I (GST) and Phase II (cyt. P-450 and cyt. b_5) enzymes. Also, there is an increase in the SH levels which makes it an excellent test material for further modulatory studies. Methi seeds contain alkaloid trigonelline. However, further studies are needed to find out the exact mechanism by which methi exerts its chemopreventive effect.

4.4 EXPERIMENT 4 : EFFECT OF CARDAMOM

Results

Effects due to oral administration of crdamom extracts (Ø.Ø1 mg/ml and Ø.Ø2 mg/ml) to mice are depicted in the tables. The parameters of body weight and liver weight did not get affected by the cardamom treatment. But the treatment increased the levels of GST significantly, on the other hand, it had negative effect on the levels of cyt. b_5 and P-45Ø. Also, it elevated the SH levels over the control values.

Discussion

Cardamom seeds are useful in asthma, bronchitis, diseases of kidney etc. Further, the fruit is applied to the tumors of uterus. This prompted us to incorporate cardamom seeds in the biochemical studies associated with chemoprevention of cancer. In fact, the present experiment demonstrated positive effects of cardamom administration on Phase I, Phase II enzymes as well as on the antioxidant defense mechanism. The results indicated that cardamom enhanced the GST levels with a significant decrease in the levels of cyt. b_5 and cyt. P-45Ø.

The exact mechanism of action still has to be elucidated further.

4.5 EXPERIMENT 5 : EFFECT OF SANDALWOOD OIL

Results

Changes induced by the oral administration of Sandalwood oil (1% and 2%) are represented in the tables. No changes were brought about in the body weight or liver weight. Significant enhancement in the SH levels in the animals of treated groups over the control values was observed. The levels of GST were also significantly increase along with simultaneous decrease in cyt. b_5 and cyt. P-45Ø levels.

Discussion

Sandalwood is highly useful in disease of heart, bronchitis and small pox. Besides, the oil is used in skin disease. In the present study sandalwood was assessed for the chemopreventive properties and the studies indicated that sandalwood is a potent inducer of Phase I and Phase II enzymes along with the antioxidant defense system. The protective effects exhibited by sandalwood oil may be attributed to its influence on GSH dependent antioxidant system. However, further experimentation is needed to elucidate the mechanistic concepts involved via which sandalwood oil could be rendering protection against hepatotoxicity.

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

In the present study an attempt has been made to evaluate and assess the potentiality of plant products (viz., bitter gourd mustard, methi, sandalwood and cardamom) that are consumed frequently by Indians on a large scale as vegetables, spices and condiments for their possible chemopreventive properties. Many of the currently used antioxidants like BHA, BHA have shown to be carcinogenic. The need for possible alternatives to these compounds is therefore realized.

The assessment of the test materials (administered at different doses to mice) has primarily been based on their modulatory influences on major xenobiotic metabolic pathways as well as components of antioxidants defense system. These constitute hepatic levels of GST (phase II enzyme); cyt. b_5, cyt P-450 (phase I enzymes); acid soluble SH fractions. All the studies have been performed on randombred, young adults male Swiss albino mice. The following table depicts the summation of overall effects brought by various substances tested in the present study.

A. Cruciferous plant products: Modulatory influences on carcinogen metabolism due to the exposure of mice to mustard seed (two varieties: Brassica campestris and Brassica napus) orally at a concentration of 1% were evaluated and compared with the aforementioned studies as parameters.

Both the varieties have shown to possess significant enhancing effects on phase II (GST) enzyme and antioxidant defense system whereas a decrease in the levels of phase I enzymes (cytochrome b_5 and P-450) was observed in both the cases. In fact Brassica campestris was found to be a better inducer of the parameters tested. This study therefore suggests a profound use of mustard seeds in future modulatory studies of chemical carcinogenesis.

B. Vegetables: Modulatory influences on hepatic enzyme levels and antioxidant defense mechanism due to the administration of Karella extract (1% and 2%) to mice were evaluated in this study. It had shown to significantly enhance the phase II (GST) enzyme and simultaneously lower the levels of phase I (cyt. b_5 and P-450) enzymes. It had also shown to strengthen the antioxidant defense mechanism by significantly enhancing the SH levels. The present study clearly favours Karella as strong candidate for future modulatory studies.

C. Spices and condiments: Changes imparted in the hepatic system by the administration of methi (2%), sandalwood (1% and 2%) and cardamom (1% and 2%) were evaluated in this study. All the materials enhanced the GST levels significantly whereas the levels of cytochrome b_5 and P-450 were lowered. The study also demonstrated the positive influence of the materials in strengthening the hepatic antioxidant defense system. The consistent effects imparted by methi, sandalwood and cardamom on almost all the major hepatic enzymes studied strongly favour employment of the compounds in future modulatory studies.

The present research strongly recommend at least five new compounds, viz., methi, sandalwood, cardamom, karella and mustard seed as potential substances for future research, employing different model systems of chemical carcinogenesis before extrapolating our findings to the human situation.

In view of the fact that many of the substances tested in the present study are routinely consumed by different human populations as vegetables, spices and condiments, many of the findings and modulations observed in this study might already be operating in these people. The cancer incidence scene in such populations could be essentially an outcome of the interplay of various environmental carcinogens and these (and other) modulators. Thus, the present study opened new vistas in elucidating a positive relation between diet and nutrition and cancer control.

Table 11: Summary of overall effects imparted by substances tested

Test material	Phase I parameter GST	Phase II parameters cyt. b_5	cyt. P-450	Antioxidant defense parameter SH level
Bitter gourd	↑	↓	↓	↑
Methi	↑	↓	↓	↑
Sandalwood oil	↑	↓	↓	↑
Brassica napus	↑	↓	↓	↑
Brassica campestris	↑	↓	↓	↑
Cardamom	↑	↓	↓	↑

REFERENCES

REFERENCES

Ander, M.W., Lash, L., Dekant, W., Elfarra, A.A. and Dohn, D.R. Biosynthesis and Biotransformation of glutathione S-transferse S-conjugates to toxic metabolites. Crit. Res. Toxicology. 18: 311-341, 1988.

Arthur, J.R., morrice, P.C., Nicol, F., Beedows, S.E., Boyel, R., Hayes, J.D. and Beckett, G.J. The effects of selenium and copper deficiences on glutathione S-transferase and glutathione peroxidase in rat liver. Biochem. J. 248: 539-544, 1987.

Benson, A.M., Cha, Y.N., Bueding, E., Heine, H.S. and Talay, P. Elevation of extrahepatic glutathione S-transferase activities and epoxide hydratase activities by 2(3)-tert-butyl-4-hydroxyanisole. Cancer Res. 39: 2971-2173, 1979.

Benson, A.M., Batzinger, R. P., Ou, S.Y.L., Bueding, E., Cha, Y.N. and Talalay, P. Elevation of Hepatic glutathione S-transferases activities and protection against mutagenic metabolites of Benzo(a)pyrene by dietary antioxidants. Cancer Research, 38: 4486-4495, 1978.

Boyland, E. and Chasseaud, L.F. The role of glutathione and glutathione-S-transferases in mercapturic acid biosynthesis. Adv. Enzymol. 32 173-219, 1969.

Bradford, M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248-254, 1976.

Cairns, J. The origin of human cancer. Nature 289: 353-357, 1981.

Cerutti, P.A., Nygaard, D.F. and Simic, M.G. (eds.) Anticarcinogenesis and radiation protection. Plenum Press, N.Y., 1987.

Cha, Y.N. and Bueding, E. Effects of 2(3)-tert-butyl-4-hydroxyanisole administration on the activities of several hepatic microsomal and cytoplasmic enzymes in mice. Biochem. Pharmacol. 28: 1917-1921, 1979.

Ellmann, G.L., Tissue sulphydryl groups. Arch. Biochem. Biophys. 82: 70-77, 1959.

Greenwald, P., Cuellen, J.W. and McKenno, J.W. Cancer prevention and control: from research through applications. J. Natl. Cancer Inst. 79 389-400, 1987.

Habig, W.H., Pabst, M.J. and Jakoby, W.H. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. J. Biol. Chem. **249**: 7130-7139, 1974.

Hernandez, O. and Bend, J.R. Metabolism of epoxides. In: Metabolic basis of detoxification (eds., William B. Jakoby, John R.,. Bend and John, Caldwell), pp.207-228, Academic Press, N.Y., 1982.

Howong-ka and Agarwal, K.C. Role of non-protein thiols in enzymatic reduction of 2-nitroimidazoles. Biochem. Biophys. Res. Communication. **46**: 1187-1193, 1972.

Jakoby, W.B. (ed.) Enzymatic basis of detoxification I & II. Academic Press, N.Y., 1980.

Jakoby, W.B. and Habig, W.H. Glutathione S-transferases: An overview. Methods in Enzymol. **113**: 495-547, 1985.

Jakoby, W.B. and Habig, W.H. Glutathione S-transferses in: Enzymatic basis of detoxification (ed. William B. Jakoby), 11: pp.63-93. Academic Press, N.., 1980.

Jocelyn, P.C. (ed.) Biochemistry of -SH group. Academic Press, London, 1972.

Ketley, J.N., Habig, W.H. and Jakoby, W.B. Binding of non-substrate ligandins to the glutathione S-transferases. J. Biol. Chem. **250**: 8670-8673, 1975.

Ketterer, B. Detoxification reactions of glutathione and glutathione transferases. Xenobiotica, **16**: 957-973, 1986.

L.H. Longley, Cook. Statistical problems. College outline series, Barnes & Noble, Inc., New York.

Lu, A.Y.H. and West, S.B. Multiplicity of mammalian microsomal cytochrome P-450. Pharmacol. Rev. **31**: 277-295, 1980.

Medicinal Plants of India, **Vol.I**, Indian Council of Medical Research Publication.

Meister, A. and Anderson, M.E. Glutathione. Ann. Rev. Biochem. **52**: 711-760, 1983.

Meister, A. Modulation of glutathione levels and metabolism. In: Anticarcinogenesis and Radiation Protection (ed., Peter-A . Cerutti, Oddvar, F. Nyggard and Michael G. Simic), pp.361-371, Plenum Press, N.Y., 1987.

Miller, E.C. and Miller, Miller, J.A. Mechanisms of chemical carcinogenesis. Cancer **47**: 1055-1064, 1981.

Miller, J.A. and Miller, E.C. The metabolic activation of carcinogenic aromatic amines and amides. Prog. Exp. Tumor Res. 11:273-3Ø1, 1969.

Mitchell, J.B. and Russo, A. The role of glutathione in radiation and drug induced cytotoxicity. Br. J. Cancer. 5: Suppl. 96-1Ø4, 1987.

Nersert, D.W. The genetic regulation of drugmetabolsing enzymes. Drug Metabl. Disp., 16: 1-8, 1986.

Newberne, P.M. and Suphakarn, V. Influence of the antioxidants vitamins C and E and of selenium on cancer. In: Vitamins, Nutrition and Cancer (ed. K.N. Prasad), pp.46-67, Korgel, Basel, 1984.

Newmark, H.L. Plant phenolics as inhibitors of mutational and precarcinogenic events. Can. J. Physiol. Pharmacol. 65: 461-466, 1987.

Novi, A.M. Regression of aflatoxin B_1-induced hepato-cellular carcinomas by reduced glutathione. Science, 212: 541-542, 1981.

Omura, J. and Sato, 2. The carbon monoxide binding pigment of liver microsomes. J. Biol. Chem. 239: 237Ø-2378, 1964.

Orrenius, S. and Moldeus, P. The multiple roles of glutathione in drug metabolism. Trends Pharm. Sci. 5: 432-435, 1984.

Prasad, K.N. (ed.) Vitamins, Nutrition and Cancer. Karger, Basel, 1984.

Prochaska, H.J., De-Long, M.J. and Talalay, P. On the mechanism of iduction of cancer protective enzymes: a unifying proposal. Proc. Natl. Acad. Sci. , U.S.A. 82: 8232-8236, 1985.

Prochaska, H.J. and Talalay, P. Regulatory mechanisms of neorofunctional and bifunctional anticarcinogenic enzyme induces in murine liver. Cancer Res., 48: 4776-4782, 1988.

Prochaska, J.R. The glutathione peroxidase activity of glutathione S-transferases. Biochem. Biophys. Acta 611: 87-98, 198Ø.

Ramanna Kumari, M.V. Ph.D. Thesis. Submitted to the Jawaharlal Nehru University, New Delhi, India, 1989.

Schenkman, J.B., Jansson, I. and Robie-Suh, K.M. The many roles of cytochrome b_5 in hepatic microsomes. Life Sci. 19: 611-624, 1976.

Shalini, V.K. and Srinivas, L. Lipid peroxide induced DNA damage: Protection by turmeric (Curcuma longa). Mol. Cell. Biochem. **77**: 3-10, 1987.

Sims, P. and Grover, P.L. Epoxides in PAH metabolism and carcinogenesis. Adv. Cancer Res. **20**: 166-274, 1974.

Smith, G.J., Ohl, V.J. and Litwack, G. Ligandin, the glutathione S-transferases and chemically induced hepatocarcinogenesis: A review. Cancer Res. **37**: 8-14, 1977.

Sparnius, V.L., Venegus, P.L. and Wattenberg, L.W. Glutathione S-transferases activity by compounds inhibiting chemical carcinogenesis and by dietary constitutents. J. Natl. Cancer Inst. **68**: 493-496, 1982.

Troll, W. and Wiesner, R. The role of oxygen radicals as a possible mechanism of tumor promotion. Ann. Rev. Pharmacol. Toxicol. **25**: 509-528, 1985.

Wattenberg, L.W. Inhibition of carcinogenic and toxic effects of polycyclic hydrocarbons by phenolic antioxidants and ethoxyquin. J. Natl. Cancer Inst. **48**: 1425-1430, 1972.

Wattenberg, L.W. Inhibition of carcinogenic and toxic effects of polycyclic aromatic hydrocarbons by several sulphur containing compounds. J. Natl. Cancer Inst. **52**: 1583-1587, 1974.

Wattenberg, L.W. Inhibition of neoplasia. Cancer Res. (Suppl.) **43**: 2448 S-2453 S, 1983.

Wattenberg, L.W. Chemoprevention of cancer. Cancer Res. **45**: 1-8, 1985.

Wattenberg, L.W. Sparnins, V.L. and Barany, G. Inhibition of N-nitrosodiethyl amine carcinogenesis in mice by naturally occurring organosulphur compounds and monterpenes. Cancer Res., **49**: 2689-2692,. 1989.

Weisburger, J.H. and William, G.M. Metabolism of chemical carcinogens. In: Cancer: A comprehensive treatise (ed. F.F. Becker). 2nd ed. pp.241-233.

Wickramasinghe, R.H. (ed.) The Cytochrome P-450 Proteins: Environmental and Genetic aspects. Munster, West Germany, 1979.

Williams, G.M. Modulation of chemical carcinogenesis by xenobiotics. Fondam. Appl. Toxicol. **4**: 325-344, 1984.

Zedeck, M.S. and Lipkin, M. (Eds.) Inhibition of tumor induction and development. Plenum Press, N.Y., 1981.