GENERAL INTRODUCTION
INTRODUCTION

Virtually all aspects of our lives involve daily exposures to xenobiotics that are either present naturally in the environment or have been placed there by humans. Exposure to toxic chemicals has been problem for almost all organisms from the origin of the life. Many toxic chemicals that we encounter from environment are innumerable chemical compounds of natural or synthetic origin. These may include food additives, environmental pollutants, drugs etc. These molecules can serve as agents of inducers of human metabolic systems. To regulate them several methods were adapted by human beings. The primary option selected by human population was use of plants.

Role of plants on health care

It has been estimated that in developed countries such as United States, plant drugs constitute as much as 25% of the total drugs, while in fast developing countries such as China and India, the contribution is as much as 80%. Thus, the economic importance of medicinal plants is much more to countries such as India than to rest of the world. These countries provide two third of the plants used in modern system of medicine and the health care system of rural tribes population depend on indigenous systems of medicine.

Of the 2,50,000 higher plant species on earth, more than 80,000 are medicinal. India is one of the world’s 12 biodiversity centres with the presence of over 45,000 different plant species. India’s diversity is unmatched due to the presence of 16 different agro-climatic zones, 10 vegetation zones, 25 biotic provinces and 426 biomes (habitats of specific species). Of these, about 15,000-20,000 plants have good medicinal value.

The drugs are derived either from the whole plant or from different organs, like leaves, stem, bark, root, flower, seed, etc. Some drugs are prepared from excretory plant product such as gum, resins and latex. Even the Allopathic system of medicine has adopted a number of plant-derived drugs which form an important segment of the modern pharmacopoeia. Some important chemical intermediates needed for manufacturing the modern drugs are also obtained from plants (Eg.
diosgenin, solasodine, β-ionone). Not only that plant-derived drug offers a stable market world wide, but also plants continue to be an important source for new drugs.

Among ancient civilisations, India has been known to be rich repository of medicinal plants. The forest in India is the principal repository of large number of medicinal and aromatic plants, which are largely collected as raw materials for manufacture of drugs and perfumery products. About 8,000 herbal remedies have been codified in Ayurveda. The Rigveda (5000 BC) has recorded 67 medicinal plants, Yajurveda 81 species, Atharvaveda (4500-2500 BC) 290 species, Charak Samhita (700 BC) and Sushrut Samhita (200 BC) had described properties and uses of 1100 and 1270 species respectively, in compounding of drugs and these are still used in the classical formulations, in the Ayurvedic system of medicine. Unfortunately, much of the ancient knowledge and many valuable plants are being lost at an alarming rate. With the rapid depletion of forests, impairing the availability of raw drugs, Ayurveda, like other systems of herbal medicines has reached a very critical phase. About 50% of the tropical forests, the treasure house of plant and animal diversity have already been destroyed. In India, forest cover is disappearing at an annual rate 1.5mha/yr. What is left at present is only 8% as against a mandatory 33% of the geographical area. Many valuable medicinal plants are under the verge of extinction.

**Phytochemicals and it's preventive role of cancer**

The relatively consistent epidemiological finding that the consumption of whole foods of different types such as fruits, vegetables and whole grains is strongly associated with reduced risk of cancer and of other chronic diseases has led to the hypothesis that particular phytochemicals are responsible for the preventive effects observed (Figure-1). In the research conducted, numerous bioactive compounds have been isolated and identified, and their potential health-promoting effects evaluated extensively, both *invitro* and *invivo*.
Quercetin Glycitein Curcumin

Figure-1: Some phytochemicals are responsible for the preventive effects

One of the key problems of research in this field, however, is that purified phytochemicals do not necessarily have the same beneficial health effect as these compounds do when their source is a food or even a complete diet. There is a growing body of evidence that the actions of phytochemicals administered as dietary supplements fail to provide the health benefits that have been observed for diets rich in fruits, vegetables, whole grains, and the like. Although relatively high doses of single bioactive agents may show potent anticarcinogenic effects, the cancer-preventive effects that certain whole foods and diets are shown to have can perhaps better be explained in terms of the chemopreventive properties that interactions between the different dietary ingredients involved to create.
Carcinogenesis is an extremely complex multistep process in which numerous molecular mechanisms play an important role. Cancer-preventive dietary compounds may interfere at a variety of different levels with these processes. The combinations of phytochemicals that natural foods contain can reduce the risk of cancer by affecting different overlapping and complementary mechanisms. Isolated and purified compounds, in contrast, may lose their biological activity or fail to behave in the same way as in the complex matrix that the original item of food represents (Rao et al. 1998; Raveendra et al., 2008). This can be illustrated by the effects of increased intake of carotenoids and vitamin C in diets high in fruits and green and yellow vegetables, which are believed to have cancer-preventive effects.

Flavonoids

Many thousands of different flavonoids are found in plant species, with major dietary sources including fruit, vegetables, tea, chocolate and soy. Total daily intake can range from 50–800 mg. Those flavonoids which have been studied in most detail exhibit many properties which could be protective against heart disease, ageing and cancer. These polyphenolic compounds are classified, according to structure, as flavonols (quercetin, kaempferol), flavones (luteolin, apigenin), flavanones (myricetin, naringin, hesperetin), isoflavones (genistein, daidzein), anthocyanins (cyanidin, pelargonidin, petunidin), catechins (epicatechin, epicatechin-3-gallate) and chalones (xanthohumol). These compounds health-promoting properties are antioxidation, antiviral, anti-allergic, anti-inflammatory, and anticancer activities. Such chemopreventive agents can be effective at different stages of the carcinogenic process, both by blocking initiation and by suppressing the later stages involving promotion, progression, angiogenesis, invasion and metastasis. Several recent reviews have summarised the potential chemopreventive mechanisms for a number of flavonoids (Manson, 2003 & 2005; Sarkar and Li, 2004; Surh, 2003).

Many flavonoids possess antioxidant or free radical scavenging potential, which varies depending on the hydroxylation status of the benzene rings. Examples include quercetin (a flavonol in vegetables, apples and onions), xanthohumol (a chalone in hops and beer) and genistein (an isoflavone in soy). An early study by Duthie et al (1997) reported that quercetin protected human lymphocytes from hydrogen peroxide-induced DNA damage. Similar findings were reported by Wilms
et al (2005), who also found that quercetin protected human lymphocyte DNA from bulky adduct formation following treatment with benzo[a]pyrene. Also in this study, volunteers consumed quercetin-rich blueberry/apple juice for 4 weeks, which led to a significant increase in antioxidant capacity of plasma.

Figure-2: Phytochemicals rich foods
Flavonoids can interact with the aryl hydrocarbon receptor (AhR) as agonists or antagonists, depending on structure and cell context. Such interactions influence the expression of drug metabolising enzymes such as cytochromes P450 (Zhang et al, 2003). They have also been shown to influence the multi-drug resistance phenotype acquired by many tumour cells. Quercetin and silymarin were found to inhibit MRP1/4/5-mediated drug transport from intact erythrocytes with high affinity, in a manner which suggested that they interact at the substrate-binding sites. Such interactions might influence bioavailability of anti-cancer drugs in vivo and could be considered for combination therapies (Wu et al, 2005). In another recent study (Limtrakul et al, 2005), the flavonols, quercetin and kaempferol, reduced P-glycoprotein expression and function in multi-drug resistant human cervical carcinoma KB-IV cells, while the isoflavones, genistein and daidzein, modulated intracellular drug levels by inhibiting function, without affecting expression.

Xanthohumol possesses several useful properties to block carcinogenesis including modulation of enzymes involved in carcinogen metabolism and detoxification (inhibition of Cyp1A, induction of quinone reductase activity), scavenging of ROS, including hydroxyl and peroxyl radicals, along with inhibition of superoxide anion radical formation and nitric oxide production (Gerhauser et al, 2002).

Phytosterols are plant sterols that are structurally similar to cholesterol and that possess anticarcinogenic properties (Smith, 2000). Together with squalene, they represent markers of cholesterol synthesis and absorption and are transported together with cholesterol in serum lipoproteins (Ketomaki et al, 2003). In recent years, functional foods high in phytosterol-ester content for lowering the cholesterol level have been developed. Although phytosterols act as immune modulators and anticancer agents in vitro (Bouic, 2001), the protection (if any) that high concentrations of phytosterol provide against the development of cancer in humans has not been adequately examined, further study of this being needed.

Lignans are plant compounds metabolized in the gut to produce the phytoestrogens, enterolactone and enterodiol. Phytoestrogens have an anticarcinogenic potential through the anti-estrogenic, anti-angiogenic, proapoptotic and anti-oxidant mechanisms established for them (Peeters et al, 2003; Webb et al,
Recent findings suggest that enterolactone is more rapidly metabolized in human colon epithelial cells and/or excreted than enterodiol. Plasma enterolignan concentrations can thus be considered to be good biomarkers of dietary lignan exposure and be used to evaluate the effects of lignans (Kuijsten et al, 2005). A number of in vitro and animal studies support a role for lignan-rich foods and of purified lignans in the modulation of cancer events in the breast, prostate and colon, whereas the findings of epidemiological studies are controversial (Webb et al, 2005). Nevertheless, a tendency for a lower risk of breast cancer to be associated with higher plasma concentrations of enterolactone, restricted almost entirely to estrogen-receptor alpha negative breast cancer has been found, suggesting that dietary lignans may be important in the etiology of breast cancer, particularly in premenopausal women (McCann et al, 2004).

Plant oil contain mostly antioxidants such as α-tocopherol and carotinoids. These antioxidants protect plasma from various oxidative species. The olive oil also contain flavonoid molecules which include single and biphenolic molecules. These molecules can serve as putative nutritional biomarkers and can be used as preventive agents of cancer at initiation, promotion and progression. For example lycopene, a molecule of tomato is more useful for the prevention of cancer after its entry into blood however this molecule must require protection during cooking. All oils can damage lycopene except use of olive oil during cooking. Therefore selection of process of preparation and extraction of molecules is a major phenomenon to not to cause damage to the required ingredient. The molecule isolated through the process at indigenous levels shall help the system from various insults.

**Antioxidant molecules of plants**

Alpha-tocopherol is a major lipid soluble antioxidant vitamin and free radical scavenger, present as an integral component of cellular membranes and having important biological functions (Burton, 1994). This vitamin is known to protect cellular membranes from oxidative damage and lipid peroxidation (Machlin and Bendich, 1987; Carpenter, 1991). Supplementation of this antioxidant is also reported to decrease oxidative DNA damage (Duthe et al, 1996). In view of these properties, alpha-tocopherol is considered to be a potential chemopreventive agent for cancer. Experimental studies have revealed the protective effect of this vitamin against the
development of sarcomas and carcinomas at different sites, although reports of the contrary also exist (Das, 1994). The protective effect of alpha-tocopherol on carcinogenesis of the uterine cervix has not yet been well documented, even though some experimental studies and human trials, including our own, are ongoing. In a pilot study noted regression of precancer lesions of the uterine cervix (CIN I and CIN II). Supplemental vitamin E also improved the serum level of vitamin E and induced mitogenic response of peripheral blood lymphocytes (PBL) (Ganguly et al. 2001).

Quercetin, a major representative of the flavonol subclass of flavonoids, is a common non-nutrient component of plant food (Singleton, 1981) which is an integral part of human diet. It was reported to be one of the most potent mutagens in Ames test (Mac Gregor, 1980) but later studies revealed its antimutagenic action (Huang et al., 1983; Malavielli et al., 1996). Although a number of flavonols found in edible plants (including quercetin) are mutagenic and genotoxic in invitro assays, the results of their carcinogenicity in experimental animal models were inconsistent (Mac Gregor, 1986). There are reports which suggest that quercetin inhibits the carcinogenic process indicating that this flavonol may be considered as a chemopreventive agent (Elangovan 1994). The action of quercetin is however dose dependent and site specific. Quercetin is known to be a dietary antioxidant that can prevent oxidation of low density lipoproteins by scavenging free oxygen radicals (Hollman et al., 1996; Candlish and Das, 1996) and is shown to protect from oxidative stress (Skaper et al., 1997).

Biochemical and Molecular Action of Nutrients in Various Diseases:

Evidence shows that the consumption of soybean preparations (soybean chips, soybean paste, soy sauce) inhibits tumorigenesis in experimental animals (Baggott et al. 1990, Nagahara et al. 1992). Various soybean preparations fed to rodents resulted in a reduced incidence and delayed appearance of rat mammary carcinogenesis induced by dimethylbenz[a]anthracene (DMBA) or methylnitrosourea (Baggott et al. 1990, Barnes et al. 1990) and inhibition of mouse forestomach neoplasia induced by benzo[a]pyrene (Nagahara et al. 1992). Recent work involving chemoprevention by soy focused on its major phytoestrogens, genistein and daidzein, and mechanisms explaining protective effects. These studies have suggested that alteration of the ontogeny of the mammary gland (Lamartiniere et al. 1998), alteration in hormonal
status and regulation of the menstrual cycle (Cassidy et al. 1994), protease inhibition (Maki et al. 1994), or antioxidant activity (Record et al. 1995).

The protective effects of soy may also be exerted in other steps involved mechanistically in cancer development, such as phase I and II metabolism of carcinogens. In a recent study, soy flour and soy protein isolate increased phase II enzyme activity. Cai and Wei (1996) showed small but significant increases in hepatic Glutathione S-transferase (GST) activity of mice fed genistein as well as the antioxidant enzymes glutathione peroxidase (GSH Px, E.C. 1.11.1.9) and glutathione reductase (GSH Rd, E.C. 1.6.4.2) in skin and small intestine.

**Enzyme-based molecular anticancer diet**

Approaches to cancer prevention necessarily focus on eliminating cigarette smoking or improving diet and exercise patterns, both of which are believed to contribute to about one-third of annual cancer deaths (Bailar III, and Gornik, 1997; McGinnis, and Foege, 1993). Dietary factors, for example, have been estimated to account for up to 80% of cancers of the large bowel, and breast, prostate, and even lung cancer may have a dietary component; to a variable extent, eating and drinking habits can be said to have some role to play in many if not all cancers. Table-I assigns the modes of action of a range of phytochemicals in protecting humans against.

The evident protective effect of consuming plants as foods raises the theoretical possibility that their specific micronutrient or phytochemical constituents might have beneficial effects as chemopreventive agents, either as naturally occurring dietary constituents or pharmaceuticals that could be used to control cancer incidence (Peto et al, 1981; Gerhauser et al, 1997). In the absence of knowledge of the specific mechanisms of action of many phytochemicals, components of their use as chemopreventive agents speculate that they could manipulate the activity of enzymes that break down mutagens and carcinogens to reduce lifetime cancer risk.
Table 1. Modes of action of a range of phytochemicals in protecting humans against cancer

<table>
<thead>
<tr>
<th>Mode of action</th>
<th>End result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reducing or blocking activation of carcinogen</td>
<td>Many carcinogens need to be activated through a system of enzymes in the body. Some phytochemicals reduce the activation of these phase 1 enzymes, resulting in reduced or inactivation of carcinogen</td>
</tr>
<tr>
<td>Increases activation of phase 2 enzymes</td>
<td>These enzymes can detoxify and eliminate carcinogens</td>
</tr>
<tr>
<td>Enhancing DNA repair</td>
<td>Reversing and preventing DNA damage</td>
</tr>
<tr>
<td>Controlling oncogene expression</td>
<td>Resulting in reduced expression of mutated genes</td>
</tr>
<tr>
<td>Modulating cell signalling</td>
<td>Increasing communication between damaged cells and cancerous cells</td>
</tr>
<tr>
<td>Promoting cell differentiation</td>
<td>Reversing undifferentiated cancerous cells back to normally growing cells</td>
</tr>
<tr>
<td>Angiogenesis</td>
<td>Reducing the growth of cancer</td>
</tr>
<tr>
<td>Enhancing immune surveillance</td>
<td>Increasing the capacity to recognize and eliminate cancerous cells</td>
</tr>
</tbody>
</table>

Modified from Prospects for preventing cancer and heart disease-Dreosti

They suggest that phase-II post-oxidative enzymes, such as GST, UDPGT and acetyl transferase, promote health by detoxifying xenobiotics, while phase-I oxidative enzymes, mainly cytochrome P450 (CYP) and FAD-containing monooxygenases, raise cancer risk by activating carcinogens. This rather simplistic dichotomy has in turn suggested that plant foods rich in key nutrients or phytochemicals might be used to reduce the risk of cancer through two enzyme-based strategies: boosting the “good” detoxifying enzymes, or inhibiting the “bad” activating enzymes (Morse and Stoner, 1993). Fig-3: illustrates these strategies. In this scheme, phytochemical-containing fruits such as grapes and vegetables, like cauliflower, kale, and broccoli stimulate phase-II induction, whereas the tea, garlic, and onion cause phase-I inhibition. These strategies were extrapolated from epidemiological observations on populations consuming diets varying in quantity and type of plant foods containing large numbers of chemical components capable of modulating the activity of metabolizing enzymes in phase-I & II. It is known that, dietary magic bullets can produce health benefits or harmful outcomes, depending on circumstances that cannot yet be predicted (Fig-3). Given this situation, the effects of single nutrient or phytochemical components isolated from whole plant foods on xenobiotic metabolism and cancer risk are also uncertain.
**Phytochemicals and Defense enzymes**

The boosting strategy involves large-scale induction of phase-II metabolizing enzymes that detoxify xenobiotics, thereby accelerating the elimination of toxicants and protecting cells against mutagenesis and neoplasia (Morse and Stoner, 1993). Much attention has focused on resveratrol, a phytoalexin found in grapes and other food products that boosts phase-II linked activities (Jang et al, 1997), and cruciferous (mustard family) vegetables of the genus Brassica, such as broccoli, kale, cabbage, brussels sprouts and cauliflower. US health authorities have recommended consumption of these vegetables for cancer prevention since the early 1980s (National Research Council Diet, 1992). An alternative anticancer hypothesis is to inhibit the typical phase-I bioactivating enzymes (Morse and Stoner, 1993; Garnet-Payrastre et al, 2000). The difficulty with these strategies is that they ignore the complexity of metabolizing enzyme systems.

![Fig-3: Effect of nutrients in the modulation of health conditions.](image)

The use of isolated naturally occurring dietary constituents such as isothiocyanates or individual drugs such as disulfiram, for example, also elicit contrary effects that can be highly undesirable. The reasons for both proposed strategies also must be considered in the context of genetic polymorphisms, which may differentially modulate the effects of any one dietary factor on individual. **Table-2** assign three major classes of phytochemicals and the phytochemicals in each class.
Table-2. Three major classes of phytochemicals and the phytochemicals in each class

<table>
<thead>
<tr>
<th>Phytochemical class</th>
<th>Phytochemicals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terpenoids</td>
<td>Monoterpenoids, iridoids, sesquiterpenoids, sesquiterpene lactones, diterpenoids, triterpenoid saponins, steroid saponins, cardenolides, bufadienolides, phytosterols, cucurbitacins, nortriterpenoids, triterpenoids, carotenoids, limonoids</td>
</tr>
<tr>
<td>Phenolic metabolites</td>
<td>Anthocyanins, anthochlors, benzofurans, chromones, coumarins, flavonoids, flavonones, flavonols, isoflavonoids, lignans, phenols, phenolic acid, phenolic ketones, phenyl-propanoids, quinonoids, stilbenoids, tannins, xanthones</td>
</tr>
<tr>
<td>Alkaloids and other nitrogen-containing constituents</td>
<td>Amaryllidacea, betalain, diterpenoid, indole, isoquinoline, lycopodium, monoterpenes, sesquiterpene, peptide, pyrrolidine, piperidine, pyrrolizidine, quinoline, quinolizidine, steroidal, tropane compounds, -non-protein amino acids, amines, cyanogenic glycosides, glucosinilates, purines, pyrimines</td>
</tr>
</tbody>
</table>

Adapted from Phytochemicals: Nutraceuticals and human health-Dillard and German

Enzyme upregulators are already consumed by humans as food additives such as BHA [2(3)- tert-butyl-4-hydroxyanisole], medicines such as oltipraz, or natural constituents of vegetables such as glucoraphanin, bioprecursor of sulforaphane. These compounds might confer protection against cancer by raising the activity of post-oxidative enzymes has been widely accepted during the last two decades (Morse and Stoner, 1993). Hence researchers have created hybrid plants specifically to produce higher amounts of single phytochemical-inducers (Faulkner et al, 1998). Such efforts ignore evidence suggesting that each phase-II enzyme is involved in electrophilic species generation and, therefore, should be considered as an “activating system” for specific chemical classes: halogenated hydrocarbons by glutathione S-transferases, for example, or polycyclic aromatic hydrocarbons (PAHs) by epoxide hydrolases or sulphotransferases (Guengerich, 1985). In other words, the bioactivation or bioinactivation of a specific compound depends on the nature of the compound itself. In general, manipulation of the activity of one or more post-oxidative enzymes can either increase or reduce the bioactivation of specific compounds. Whereas induction increases the detoxification of certain protoxics and promutagens/procarcinogens, thereby favoring chemoprevention, it also increases the bioactivation of countless other foreign chemicals. Since humans are exposed to a myriad of potentially harmful
molecules, any modification of the activity of these enzymes could actually lead to an increase in toxicological risk (Cantelli-Forti et al. 1998).

The difficulty of determining how isolated dietary factors might affect metabolizing enzymes is illustrated by inconsistencies in studies on cruciferous vegetables. Although consumption of such vegetables is, on balance, associated with reduced cancer risk, epidemiological data show that a high intake of these plant foods in the form of vegetable mixtures (Tannenbaum, 1998) or single plants (e.g. broccoli, cabbage or Brussels sprouts) (Lampe et al. 2000), can exert cancer-enhancing effects due to their content of enzyme inducers that activate procarcinogens such as polycyclic aromatic hydrocarbons and aromatic amines in tobacco. On this basis, the authoritative refusal of a former President of the US, George Bush, to eat broccoli may be understood to have little effect on his cancer risk.

Even with the recent success of such novel compounds as Prostatin, the interest shown by pharmaceutical companies in the development of drugs from plants has fluctuated over the years. After an initial flourish in the 1940s and 50s leading to the launch of resperine from Rauwolfia spp., and diosgenin from Dioscorea spp., very little interest was shown after 1960 (Fellows, 1991). In 1974, only one US company was investigating plant-derived drugs, and by 1980 there were none (Farnsworth and Soejarto, 1985). New analytical techniques and the commercial success of Taxol once again witnessed a renewed interest in plants as sources of new commercial drugs in the 1990s. In 1993, 223 companies worldwide were investigating plants as sources of bioactivity and novel compounds (Fellows, 1991). Today, major government initiatives including the International Cooperative Biodiversity Group Program (ICBG) as well as countless independent investigations are underway in which ethnobotanists are collaborating with healers, and recording traditional and folk knowledge of medicinal plant use in various parts of the world (Rosenthal, 2001). Yet, to date fewer than 5% of the approximately 250,000 species of higher plants have been exhaustively studied for their potential pharmacological activity.

Many of our most valuable plant-derived drugs, such as digitoxin and tubocurarine, used to treat specific conditions remain unsurpassed in their respective fields and cannot be synthesized. The isolation of opium alkaloids such as morphine in the early part of the 19th century also heralded the beginning of the reductionist
approach whereby plant extracts were replaced by pure active compounds isolated from a plant. Important drugs introduced this way include digitoxin, and later digoxin, from foxglove (Digitalis purpurea L.); quinine from Chinchona (Chinchona spp.); cocaine from coca bushes (Erythroxylum coca Lam.); and atropine and hyoscine from the deadly Solanaceous nightshade family.

In contrast to the ethnobotanical approach, recent advances in molecular pharmacology, combinatorial chemistry and genomic have led to the development of increasingly competitive modalities. Again the sudden cyclic wane in the interest of natural products in some drug discovery programs is occurring. Mapping the chemical sequences for human DNA, the chemical "code" that makes up the foundation of human life, is the most recent example of a pioneering breakthrough that scientists expect to lead to new cures for cancers, heart disease, drug addiction and mental illness (Peltonen and McKusiek, 2001). At the same time, however, periodic discoveries of novel molecules from marine organisms such as bryostatin and potent new chemotherapeutic agents from plants is sure to maintain interest in natural products research (Plotkin, 2000).

Hence to study the phytochemicals of above of any present shall be studied in the selected medicinal plant *Henneaspermus*. This plant is also used in various preperations of Ayurveda for several remedies.

*Hybanthus enneaspermus* (Ratanpurus)

<table>
<thead>
<tr>
<th>Family</th>
<th>: VIOLACEAE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genus</td>
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</tr>
<tr>
<td>Specific epithet</td>
<td>: Enneaspermus</td>
</tr>
<tr>
<td>Kingdom</td>
<td>: Plantae</td>
</tr>
<tr>
<td>Phylum</td>
<td>: Magnoliophyta</td>
</tr>
<tr>
<td>Class</td>
<td>: Magnoliopsida</td>
</tr>
<tr>
<td>Order</td>
<td>: Violales</td>
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<tr>
<td>Family</td>
<td>: Violaceae</td>
</tr>
<tr>
<td>Genus</td>
<td>: Hybanthus</td>
</tr>
</tbody>
</table>

*Hybanthus enneaspermus* (L.) F. Muell., 1876
Common name: Spade Flower, Pink ladies slipper

- Hindi      : Ratan purush  
- Bengali    : Munbora    
- Kannada    : Purusharathna  
- Malayalam  : Orilathamatai  
- Telugu     : Ratnapurusha  
- Marathi    : Rathanparas  
- Sanskrit   : Rathnapurusha  

Botanical name: *Hybanthus enneaspermus*

Family: *Violaceae* (Violet family)

Synonyms: *lonidium suffruticosum*

*Hybanthus enneaspermus* is an erect shrub of Violaceae family medicinal plant. Throughout the tropics and subtropics of India from Uttar Pradesh to Bengal southwards to Tamil Nadu and Kerala.

It is a small perennial herb with woody base and numerous diffuse or ascending branches. Leaves are subsessile, linear to oblanceolate, margins are entire or serrate, stipules are gland-tipped. Flowers are solitary, axillary, red, or purple; petals are unequal, the lowest much larger than the others with an orbicular or obovate limb with a long claw. Fruit is a small subglobose capsule. Seeds are ovoid, longitudinally striate, yellowish-white.

![Hybanthus enneespermus](image1)

**Figure-4:** *Hybanthus enneespermus* (A) Plant and (B) Spade flower
Because of its free radical scavenging nature it has been subjected to intensive phytochemical screening to determine its medicinal usage. Research on these plants has suggests that this herb has medicinal properties such as anti-tumor, uterotonic, and HIV inhibition. Their natural function may be host defense, a speculation supported by antibacterial and antimicrobial activity.

**Medicinal Uses:**

The whole plant is considered to possess tonic, diuretic and demulcent properties. The leaves and tender branches are demulcent and are used as a decoction and are used to prepare a cooling liniment for the head. The dried powdered leaves are used to treat asthma. The root is diuretic and administered as an infusion to treat gonorrhoea and urinary infections: it is also used to treat bowel complaints in children. The fruit is reportedly used to treat scorpion stings.

**Concept of Oxidative Stress and Biomarkers**

As shown in **Figure-5** Oxidative stress has been defined as a disturbance in the balance between the production of ROS, or free radicals and antioxidant defenses, which may lead to tissue injury (Halliwell B., 1994). Also, free radical can be defined as a chemical species that contains unpaired electrons in their outer orbit and thus can react virtually with all cell components (Collier A. 1992; Przekwas M. 2003; Slater TF, 1984). Although, ROS are crucial to normal biological processes, they are potentially dangerous (Toyokuni S., 1999; Zimmerman JJ., 1998) and are commonly referred to as prooxidants (Mates JM., et al 1999). The RO intermediates, including superoxide and hydroxyl radicals as well as hydrogen peroxide can cause direct cellular injury by inducing lipid and protein peroxidation and damage to nucleic acid (Richard C., et al 1990; Takeda K., et al 1984).

The cell organelle, mitochondria which reduces O₂ can leak ROS into the cytoplasm and cause cellular damage by oxidizing a variety of biologically important molecules, including DNA, proteins, lipids, and carbohydrates (Przekwas M, et al 2003). Lipid and protein peroxidation reactions play an important role in the pathogenesis of a variety diseases (Przekwas M, et al 2003). Also, Oxidative stress (OS) caused by an imbalance of oxidants and antioxidants is capable of inflicting injury on membrane lipids, proteins and nucleic acids (Toyokuni S, 1999;...
Zimmerman, JJ, 1998). For the prevention of diseases and control of aging, evaluation and control of oxidative stress invivo may become essential. A wide variety of functional assays are used in the field of research related with oxidative stress. To reflect minor changes in the pro-oxidant/antioxidant status under normal, nonpathological conditions in humans might be of special interest for some biomarkers.

The biomarkers were originally produced due to any biochemical, histological, or physiological alterations or manifestations of environmental stress (NRC, 1987). They have been classified as biomarkers of exposure to a toxicant, biomarkers of effects of exposure, or biomarkers of susceptibility to the effects of exposure (Peakall and Shugart, 1993).

![Diagram showing Reactive oxygen species (ROS) (red) and Free radicals (black) copies are more in number than copies of biomarkers (blue). This imbalance leads to cellular damage.](image)

**Figure-5: Elevation of ROS, Free radicals and depletion of defense enzymes due to cell damage**

To relate effects on individuals to higher levels of biological organization, the biomarker response should be related to a degree of impairment of growth, reproductive output, or metabolic function which directly affects the survival of the organism and which can be attributed to exposure to a known amount of the specific contaminant (Depledge and Fossi, 1994). The effects of neuroactive compounds, which have been used as insecticides for the last 50 years, and the exposure to neurotoxicants can be assessed through the measurement of biomarkers related to their target activity or detoxification processes (Casida and Quistad, 1998).
The chemical nature of free radicals and its role in cancer

The terms free radicals, oxidative stress and antioxidants are now a days relatively common in our everyday life, as there is an increasing tendency in the media to advertise new foods with claimed medicinal effects on human health (Mandel et al., 2005). However, this was not always the case, and in the 1950’s free radicals and antioxidants were almost unheard of in the clinical and biological sciences, not to say among the general public, despite chemists had known about them for years in the contexts of radiation, polymer and combustion technologies (Gutteridge and Halliwell, 2000). In biology, free radicals are mostly classified depending on whether the unpaired electrons lay on oxygen or nitrogen atoms. Hence, oxygen-derived free radicals are called reactive oxygen species (ROS) and are the main cause of oxidative stress. The most notorious ROS representatives are the hydroxyl and superoxide radicals. Of note, although hydrogen peroxide (H$_2$O$_2$) is not a free radical, it is also considered a ROS member as it is normally converted into hydroxyl radicals in vivo in the presence of copper and iron ions (Gutteridge and Halliwell, 2000).

Cancer as a disease of free radicals overload

Free radicals are normally produced at low levels within the cells and play important physiological roles. Furthermore, their levels and subcellular localization are normally regulated within the cell by a plethora of antioxidant systems that continuously sustain the intracellular reducing environment (Gutteridge and Halliwell, 2000). Nevertheless, it is thought that one in two males or three females that develop cancer by the age of 85 owe this burden to a lifetime exposure to low levels of free radicals (DePinho, 2000). In agreement with this, the fact that cancer is not any more a disease of the elder seems to be accounted for, at least in part, by the numerous insults of modern life that result in increased ROS levels within our body (Halliwell, 2002) (Figure-6).
These include repeated exposure to ultraviolet (UV) light (Thomas-Ahner et al., 2007) as well as to PAHs contained in cigarette- and car exhaust smoke, eating of benzpyrene present in overcooked food, breathing of traces of asbestos and nickel spread in the environment, and also the development of chronic inflammatory conditions (Halliwell, 2002).

The chronic inflammatory conditions derived from continuous exposure to chemicals produce high levels of free radicals which are not only contribute to about 25% of cancer cases worldwide, but also increase the risk of cancer in healthy individuals in more than two hundred-fold in certain cases (Hussain et al., 2003). Accordingly, it is estimated that about 80% of cancers would be avoided if people changes their lifestyle (McKinnell et al., 2006). Thus, apart from the clear-cut example of inflammation, whose carcinogenic effects are mostly driven by the production of free radicals and certain inflammatory cytokines (Baniyash, 2006; Hussain et al., 2003; Karin, 2006; Philip et al., 2004), free radicals are currently emerging as potent carcinogens at all cancer stages, namely initiation, promotion and progression. Furthermore, exposure of several cancer cell lines to inflammation or chemically-induced ROS boosts their migratory and invasive behaviors (Okada et al., 2006; Payne et al., 2005; Polytarchou et al., 2005), indicating a likely role of free radicals in promoting cancer cells to the autonomous and invasive phenotype.

**Detoxification enzymes and cancer: a matter of equilibrium**

The cooperative functions of class I and II detoxification enzymes are critical for the cellular disposal of compounds that frequently have carcinogenic effects (i.e. dioxins, PAHs). Accordingly, reduced activity of some of these enzymes has been
found to predispose to some cancer types, which is believed to be accounted for by a general lack of xenobiotic detoxification (Dalhoff et al., 2005). Accordingly, GSTs are currently emerging as new promising targets in cancer therapy (Guengerich, 2005; Townsend et al., 2005; Turella et al., 2005; McIlwain et al., 2006). The expression of class I and GST enzymes in normal tissues would be expected to have an anti-cancerous role due to efficient xenobiotic disposal, the sole expression of GST proteins in some tissues might conversely predispose to cell transformation through inhibition of two pathways with known tumor-suppressive roles (Bulavin and Fornace, 2004; Kennedy et al., 2003). For instance, the homozygous deletion of the GSTM1 gene has been shown to predispose to lung, bladder and colorectal carcinomas. Similarly, reduced GSTP activity has been correlated with higher prostate cancer risk. Of note, class II enzyme alterations as those just mentioned here would not be only expected to affect xenobiotic clearance (Dalhoff et al., 2005), but also to lead to enhanced accumulation of alkylating xenobiotic electrophiles due to insufficient conjugation, which may likely account in the first place for the carcinogenic effect of such loss-of-function alterations. However, in contrast with the assumed anti-cancerous activity of these detoxification enzymes, it has been recently found as well that the overexpression of wild-type (WT) or gain-of-function alleles of some of these enzymes can both predispose to some cancer types and also interfere with cancer treatment. For instance, high CYP expression has been correlated with increased azoximethane-induced bladder cancer risk, which is probably due to the accumulation of higher levels of azoximethane electrophiles (McKinnell et al., 2006), and has also been proposed to predispose to breast cancer (Tsuchiya et al., 2005). Similarly, gain-of-function N-acetyl transferase (NAT) alleles have been shown to predispose to bladder cancer (Dalhoff et al., 2005). However, the most notorious examples are the GST isoforms GSTM and GSTP. Hence, whereas GSTM expression reduces the risk of cancer in tissues normally exposed to a high xenobiotic burden (i.e. lung, colon; see above), in which high expression of CYPs is also expected, GSTM has been conversely shown to predispose to breast cancer (Parl, 2005), as well as to be associated with ovary cancer progression and worse survival (Beeghly et al., 2006). Furthermore, GSTM expression in several cancer types precludes efficient therapy (McIlwain et al., 2006), and GSTM overexpressing human cancer cells are resistant to apoptosis. Similarly, GSTP has been shown to be a prevalent protein in
many solid tumors and is normally found overexpressed in drug-resistant cancers (Townsend et al., 2005).

**Primary evidence for the role of GST against cancers**

Primary evidence for the role of GST and GSH in drug resistance comes from three sources: 1) analysis of tumor cells from patients before and after the onset of clinical drug resistance, where an increase in GSH and GST occurs after development of resistance (Schisselbauer et al., 1990); 2) an increase in GSH/GST after the selection of acquired resistance to anticancer agents in tumor cell lines (Tew, 1994; Hayes and Pulford, 1995); and 3) analysis of resistance expressed after transfection of particular GST genes into cell lines (Hayes and Pulford, 1995).

Primary colon and rectal cancers (CRC) are tumors that occur at high frequency in the United States and in all European countries, including Bulgaria (American Cancer Society, 2004; Finish Cancer Registry, 1997; National Oncological Centre, 1998). Each year more than 3000 new cases of colorectal cancers are diagnosed in Bulgaria. Despite the progress in early diagnosis and the improvement of treatment modalities, more than 2200 cancer-related deaths continue to occur each year (National Oncological Centre - Bulgaria, 1998). Heterogeneous levels of expression of GST-pi in the cytoplasm of tumour cells of colorectal carcinoma, which could be due to different genetic or epigenetic factors, this must be due to the formation of ROS. These ROS are found to induce the expression of the genes of GST-pi and other phase II xenobiotic-biotransforming enzymes (Hoensch H et al., 2002; Tew KD and Ronai Z., 1999; O'Brien ML and Tew KD, 1996). Extensive deletions in GSTM1 and GSTT1 result in complete loss of enzyme function, which possibly influence colorectal cancer susceptibility. Therefore, a high number of studies have been performed to assess whether GSTM1-deficiency or other GST polymorphisms are associated with colorectal cancer susceptibility (d'Errico, et al., 1996; Strange and Fryer, 1999; Cotton S.C et al., 2000; de Jong et al., 2002).

Prostate cancer (CaP) is the most commonly diagnosed cancer in U.S. men, and the second leading cause of cancer-related mortality (Landig SH et al., 1999). The underlying mechanisms of carcinogenesis and progression of the disease remain largely obscure (Harries et al., 1997). Androgens are known to be central to the
development of the prostate and to influence the etiology of this cancer (Yager and Liehr, 1996; Preston-Martin et al., 1990; Henderson et al., 1991). Nutritional and other external factors appear to be important. GSTP1 is overexpressed in many preneoplastic and neoplastic lesions (Satoh K et al., 1998) and in most tumor cells resistant to chemotherapeutic drugs, (Black and Wolf, 1991) and its transcription is inactivated by DNA hypermethylation in early stages of prostate carcinogenesis (Lee et al., 1994). Limited data suggest a possible role of PAH, a known substrate of GST, in the development of prostate cancer (Tolbert, 1997). A number of carcinogenic PAHs are present in cigarette smoke (Sundberg et al., 1998). The role of smoking in prostate cancer is still unclear but is biologically plausible, especially for fatal disease (Giovannucci et al., 1999; Eichholzer et al., 1999).

Breast cancer is the most prevalent cancer among women in Western countries, and its prevalence is also increasing in Asia (Parkin et al., 1997; Yoo et al., 2002). The identification of susceptibility factors that predispose individuals to breast cancer (for example, if they are exposed to particular environmental agents) could possibly give further insight into the etiology of this malignancy. Inherited differences in the capacity to metabolize environmental carcinogens have recently been suggested to modify individual susceptibility to breast cancer. Therefore, the identification of new breast cancer susceptibility genes would yield new insight into breast tumorigenesis, and could provide targets for the future development of therapeutics. The inherited metabolic capacity of GSTs have been related to the individual breast cancer risk (Helzlsouer et al., 1998). GSTs are a superfamily of enzymes that are involved in conjugation with reactive intermediates to soluble glutathione, and therefore, play an important role in the detoxification of endogenous and exogenous toxicants. GSTM1 can detoxify carcinogenic PAHs, such as benzo[a]pyrene (BaP) and mycotoxin aflatoxin, while GSTTI can detoxify smaller reactive hydrocarbons, such as ethylene oxide and diepoxynbutane. Glutathione S-transferase P1 is a major GST, which is ubiquitously expressed in both normal and tumor breast tissue (Forrester et al., 1990; Shea et al., 1990; Albin et al., 1993).

GST-α is found at high concentrations in the human liver and is released quickly and in large quantities into the bloodstream during hepatocellular damage (Beckett and Hayes, 1993). Because the half-life of GST-α in plasma is 1h (Beckett et
al., 1985), its concentration will follow changes in hepatocellular damage more rapidly.

Most cases of lung cancer appear in smokers, reflecting the carcinogenicity of long-standing exposure to tobacco smoke. But only a fraction of smokers will finally die from lung cancer, depending on the extent of carcinogen exposure, environmental burdens, and endogenous host factors, like bioactivation or detoxification of foreign compounds. Since carcinogens like epoxides of polycyclic aromatic hydrocarbons are substrates of GSTM1, the presence of this enzyme should offer some protection against lung cancer, while individuals lacking GSTM1 activity may be at higher risk for the toxic effects of xenobiotics incorporated by smoking. The first epidemiological studies on GST class \( \pi \) and lung cancer risk demonstrated a significantly lower proportion of individuals active in the \( \text{ex vivo} \) TSO conjugation assay among lung cancer patients as compared to a control group (Seidegard, et al., 1986 and 1990). In patients suffering from cancer, enzyme activities may be altered due to the disease or its treatment; e.g., expression of GST class \( \mu \) is hormonally controlled (Mankowitz et al., 1990) and induced, e.g., by Phenobarbital or by propylthiouracil.

The ratio in incidence between high- and low-risk occurrence could be as great as 500:1. The high incidence in special areas indicates the importance of environmental factors in esophageal carcinogenesis. However, only a small part of individuals in the high-risk area for esophageal cancer develop into esophageal cancer, although all the residents in that area share very similar environment-related risk factors and lifestyle. GSTs polymorphism may be responsible for the higher-risk for esophageal cancer in this population. GSTM1 and GSTT1 null genotypes have been reported to enhance the risk of developing gastric, colorectal, and lung cancers (Chenevix-Trench et al., 1995; Deakin et al., 1996), although other studies did not show such a genetic predisposition (Katoh et al., 1996). Again, in normal esophageal epithelium, GSTP1 was the mean isoform for GST (Peters et al., 1993).

Therefore, variations in the expression of GSTs due to heritable genetic polymorphisms probably modulate the process of carcinogenesis by altering the exposure levels of tobacco-derived carcinogenesis. Head and neck squamous cell carcinoma (HNSCC) is one of the most prevalent diseases among the population
throughout the world. Head and neck cancer (HNC) is commonly associated with tobacco use alone and also in combination with alcohol consumption. Genetic predisposition may play an important role for the development of cancer of the head and neck that is not fully understood at the present time (Notani, 2000). Environmental factors may interact with the host’s genetic material resulting in an accumulation of a series of detrimental genetic alteration that lead to invasive cancer. It is hypothesized that individuals with the GST M1 null genotype have an impaired ability to detoxify carcinogens resulting in a high risk for the development of head and neck cancer. A link between GST M1-null and laryngeal cancer was first suggested by Lafuente et al., (1993).

Salivary gland carcinoma (SGC) is a rare malignancy with an incidence rate of approximately 1 per 100,000 population per year in the United States (Carvalho et al., 2005). SGC may arise in major or minor salivary glands and may have a variety of histologic and biologic characteristics. The parotid gland is the most common anatomic site of origin, and mucoepidermoid carcinomas and adenoid cystic carcinomas are the most frequently occurring histologic types (Spiro RH, 1986; Bell RB et al., 2005). The GSTT1 null genotype was associated with a significantly increased risk of SGC (Sayaka Kondo et al., 2009).

Role of GSTM1, T1, and P1 polymorphisms for cancer risk, especially in acute leukemia, has been reported not only in adults but also in children (Hayes and Pulford, 1995; Krajinovic et al., 1999; Balta et al., 2003; Barnette et al., 2004; Davies et al., 2001). Davies et al. (2001) reported that GSTM1 null genotype was a significant risk factor for childhood acute myeloblastic leukemia. GSTM1 null genotype, mutant genotype of GSTP1, and CYP2C92 allele may have a critical role in the development of childhood cancer (Öznur Duzovali et al., 2008).

Finally oxidative stress plays a role in the pathogenesis of asthma, and the GST superfamily is important. We hypothesize that polymorphisms of GST genes functioning in antioxidant pathways are determinants of asthma development. Several members of the GST superfamily, notably the GSTP1 gene and GSTM1 gene, are expressed in the respiratory tract and have common functional variant alleles (Hayes and Strange, 1995, 2000; Strange et al., 2001). GSTM1 deficiency has been found to be consistently associated with moderate increased risk for lung, bladder cancer
(dErrico et al., 1996; Rebbeck, 1997), and various other malignancies (Baxter et al., 2002; Srivastava et al., 2005; Jain et al., 2006).

**Glutathione-S-Transferases (GSTs--EC.2.5.1.18)**

The Glutathione-S-transferases (GSTs), have been assigned the EC number, EC 2.5.1.18, by the Nomenclature Committee of the International Union of Biochemistry, since they are part of the transferases, class number 2, their number starts with a 2. These enzymes were first identified more than 50 years ago as GSH binding proteins (Booth et al., 1961).

However, Gallagher et al., (1996) reported that GST detoxifies a number of environmental carcinogens and epoxide intermediates. Thus, the GST assay was suggested as a useful tool for biomonitoring oxidative stress (Di Giulio et al., 1993). GSTs are enzymes that participate in cellular detoxification of endogenous as well as foreign electrophilic compounds, function in the cellular detoxification systems and are evolved to protect cells against ROS by conjugating the reactive molecules to the nucleophile scavenging tripeptide glutathione (GSH, γ-glu-cys-gly).

As on today two hundred and four GSTs have been identified in many different species, ranging from primitive bacteria to humans (http://www.au.expasy.org; Buetler et al., 1992). They are expressed at high levels in mammalian liver constituting up to 4% of the total soluble proteins (Eaton et al., 1999). These enzymes have been identified in different organisms by chromatography, enzymatic activity, and immunological methods, such as amino acid sequencing and molecular cloning (Buetler et al., 1992). The vast number of different GST subunits implies their importance in the protection of cells against a wide variety of potentially harmful substances that they can encounter although GSTs have now been purified from a number of species (Escherichia coli to humans).

In those species so far investigated, soluble forms of GSTs are homodimers that consist of two identical subunits or heterodimers that consist of two nonidentical subunits with distinct substrate specificities (Mannervik and Jenssen 1982) having molecular weight from 20,000 to 25,000 Da. Each subunit consists of 200 – 240 amino acids and one catalytic site. GSTs are composed of subunits of the same class.
and are heterodimeric or homodimeric combinations of GSTs (Baldwin et al., 1996; Sheehan et al., 2001).

Each subunit has a characteristic enzyme activity, which is expressed independently of the other subunit (Ostlund Farrants et al., 1987). The GSTs in addition to their enzymatic activities, bind with high affinity to a variety of hydrophobic compounds such as heme, bilirubin, hormones and drugs, which suggests that they may serve as intracellular carrier proteins for the transport of various ligands. A marked increase in GST activity has been observed in tumor cells resistant to anticancer drugs (Daniel 1993). GST protect organisms from toxic chemicals once they are ingested or absorbed from the environment (Clark, 1989). GSTs are involved in the first enzymatic step in formation of mercapturic acids (N-acetyl L-cysteine S-conjugates), which are excreted by mammals. These enzymes are a family of essential multifunctional proteins with important physiological functions (Baldwin et al., 1996; Aceto et al., 1991; Vidal et al., 2002). GSTs structural multiplicity was determined (Thyagaraju et al., 1994). They can intracellularly detoxifying xenobiotics and metabolites (Stenersen et al., 1987; Petrivalsky et al., 1997; Mannervik et al., 1988) by converting toxic compounds into a water soluble, nonreactive conjugates (Figure-6) which may easily be excreted (Clark, 1989). The enzymes do this by catalyzing the conjugation of GSH to the xenobiotics and then in turn makes the xenobiotics more hydrophilic and easily excrete by the organism (Vidal et al., 2002). GSTs also act as intracellular carrier proteins of certain organic molecules (Mannervik et al., 1988).
### Table-3: Glutathione S-transferases and their genes (Rat)

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Type of gene</th>
<th>Type of GST</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>gsta1</td>
<td>Glutathione S-transferase alpha 1</td>
</tr>
<tr>
<td>2</td>
<td>gsta2</td>
<td>Glutathione S-transferase alpha 2</td>
</tr>
<tr>
<td>3</td>
<td>gsta3</td>
<td>Glutathione S-transferase alpha 3</td>
</tr>
<tr>
<td>4</td>
<td>gsta4</td>
<td>Glutathione S-transferase alpha 4</td>
</tr>
<tr>
<td>5</td>
<td>gstk1</td>
<td>Glutathione S-transferase kappa 1</td>
</tr>
<tr>
<td>6</td>
<td>gstm1</td>
<td>Glutathione S-transferase mu 1</td>
</tr>
<tr>
<td>7</td>
<td>gstm2</td>
<td>Glutathione S-transferase mu 2</td>
</tr>
<tr>
<td>8</td>
<td>gstm3</td>
<td>Glutathione S-transferase mu 3</td>
</tr>
<tr>
<td>9</td>
<td>gstm4</td>
<td>Glutathione S-transferase mu 4</td>
</tr>
<tr>
<td>10</td>
<td>gstm5</td>
<td>Glutathione S-transferase mu 5</td>
</tr>
<tr>
<td>11</td>
<td>gsto1</td>
<td>Glutathione S-transferase omega 1</td>
</tr>
<tr>
<td>12</td>
<td>gstp1</td>
<td>Glutathione S-transferase pi 1</td>
</tr>
<tr>
<td>13</td>
<td>gstt1</td>
<td>Glutathione S-transferase theta 1</td>
</tr>
<tr>
<td>14</td>
<td>gstt2</td>
<td>Glutathione S-transferase theta 2</td>
</tr>
<tr>
<td>15</td>
<td>gstt3</td>
<td>Glutathione S-transferase theta 3</td>
</tr>
<tr>
<td>16</td>
<td>mgst1</td>
<td>Microsomal glutathione S-transferase 1</td>
</tr>
<tr>
<td>17</td>
<td>mgst2</td>
<td>Microsomal glutathione S-transferase 2</td>
</tr>
<tr>
<td>18</td>
<td>mgst3</td>
<td>Microsomal glutathione S-transferase 3</td>
</tr>
</tbody>
</table>

Glutathione-S-transferases detoxify xenobiotic compounds in three ways. First, they may bind glutathione to the substrate and form the urinary metabolite mercapturic acid. Second, GST alone may bind to the xenobiotic to render it inactive and are thus termed ligandins. Third, it may form covalent bonds with xenobiotics and are themselves rendered inactive in a suicide reaction. Compounds conjugated by GST may be excreted in the urine, bile, or feces. The GSTs are encoded by eight different gene families: alpha, chi (or omega), mu, pi, theta, zeta, and kappa (Hayes, 1995).

The cells are also protected from reactive epoxides and oxygen species by GSTs (Yuen et al., 2001). Chemical stress on the body may actually cause production of ROS which results in oxidative stress on cells, which in turn activates GST activity.
The role of GSTs as an antioxidant enzyme is to reduce the lipid peroxides to form their respective alcohols by using GSH and thereby prevent oxidative damage in the cells (Barata et al., 2005). In rats, an antioxidant responsive element (ARE) has been identified in the 5' flanking region of the GST Ya subunit gene (Rushmore et al., 1991). The ARE is a cis-acting regulatory element that is responsive to oxidative stress due to protect the cells against oxidative stress (Rushmore et al., 1991; Feng et al., 2001).

The GSTs exist in multiple isoforms in almost all organisms to protect the cells from various insults of nature. In contrast the presence of a wide range of GST isozymes with a differential and overlapping substrate specificity has been detected in a wide variety of species, including man (Kamisaka et al., 1975), rat (Pabst et al., 1973; Askelof, 1975; Igarashi et al., 1986), rat brain (Thyagaraju et al., 1996) mouse (Clark et al., 1973; Lee et al., 1981; Igarashi et al., 1986), rabbit (Igarashi et al., 1986) hamster (Smith et al., 1980; Igarashi et al., 1986), guinea pig (Irwin et al., 1980; Di Ilio et al., 1982; Igarashi et al., 1986; Oshino et al., 1990), chicken (Yeung and 1980), (Chang et al., 1990), cow (Saneto et al., 1980), monkey (Asaoka et al., 1977), trout (Nimmo and 1985), shark (Sugiyama et al., 1981), little skate (Fourmman and Bend, 1984), grass grub (Clark et al., 1973), house fly (Clark et al., 1973), American cockroach (Clark et al., 1973), corn (Mozer et al., 1983) and sheep (Clark et al., 1973; Reddy et al., 1983; Ünsal and 1991; Abu-Hijleh, 1993).

**GST FAMILIES**

Glutathione S-Transferases are of interest to pharmacologists and toxicologists because they provide targets for antiasthmatic and antitumor drug therapies (Evans et al., 1991; Matsushita et al., 1998; Jakobsson et al., 1999; Ruscoe et al., 2001), and they metabolize cancer chemotherapeutic agents, insecticides, herbicides, carcinogens, and by-products of oxidative stress. Overexpression of GST in mammalian tumor cells has been implicated with resistance to various anticancer agents and chemical carcinogens (Hayes and Pulford, 1995). Furthermore, elevated levels of GST have been associated with tolerance of insecticides and with herbicide selectivity (Ranson et al., 2001; Edwards and Dixon, 2004). Three major families of proteins that are widely distributed in nature exhibit glutathione transferase activity.
One major reason of individual variation of GST activity is due to existence of polymorphism in these genes as stated in Table-3. There are three GST families, Microsomal GSTs, Mitochondrial GSTs, Cytosolic GSTs (Figure-7). Two of these, the cytosolic and mitochondrial GST, comprise soluble enzymes that are only distantly related (Ladner et al., 2004; Robinson et al., 2004).

Figure-7: Families of glutathione s-transferases

The third family comprises microsomal GST and is now referred to as membrane-associated proteins in eicosanoid and glutathione (MAPEG) metabolism (Jakobsson et al., 1999). A further distinct family of transferases exists, represented by the bacterial fosfomycin (FOS) resistance proteins, FosA and FosB (Armstrong, 2000). Two of these the cytosolic and mitochondrial GSTs are soluble enzymes that are only distantly related (Ladner, et al., 2004; Robinson et al., 2004).
Table-4: Classification of Glutathione S-transferases related to various organisms

<table>
<thead>
<tr>
<th>Class of Glutathione S-transferase</th>
<th>Organisms Found in</th>
</tr>
</thead>
<tbody>
<tr>
<td>α</td>
<td>Human, Rabbit, Mouse, Rat and chicken (Buetler, 1992)</td>
</tr>
<tr>
<td>β</td>
<td>Insects</td>
</tr>
<tr>
<td>δ</td>
<td>Referred to as class-I GSTs in insects, D.Melanogaster, Musca domestica, Lucilla Cuprina, and Anaphels gambiae (Laugarre, 1999)</td>
</tr>
<tr>
<td>κ</td>
<td>Mammals</td>
</tr>
<tr>
<td>µ</td>
<td>Humans, Rat, Hamster, Mouse, Guinea pig, S.Japonicum, S.Mansoni, F.Hepatica (Buetler, 1992)</td>
</tr>
<tr>
<td>ω</td>
<td>Referred to as class-II GSTs in insects</td>
</tr>
<tr>
<td>σ</td>
<td>Referred to as class II GSTs in insects, mammals, mollusk lens, octopus, and squid lens (Buetler, 1992)</td>
</tr>
<tr>
<td>θ</td>
<td>Referred to as class II GSTs in insects, mammals, yeast, ecoli, bacteria, and plants (Buetler, 1992)</td>
</tr>
<tr>
<td>ξ</td>
<td>Referred to as class-II GSTs in insects</td>
</tr>
</tbody>
</table>

Cytosolic and mitochondrial GSTs share some similarities in their three-dimensional fold (Ladner et al., 2004) but bear no structural resemblance to the MAPEG enzymes (Holm et al., 2002). However, all three families contain the conjugation of GSH with 1-chloro-2, 4-dinitrobenzene (CDNB) and exhibit glutathione peroxidase activity toward cumene hydroperoxide (CuOOH). The cytosolic GST and MAPEG enzymes catalyze isomerization of various unsaturated compounds (Jakobsson et al., 1999; Khojasteh-Bakht et al., 1999; Dixon et al., 2000).
and are intimately involved in the synthesis of prostaglandins and leukotrienes (Thyagaraju, 1998; Hayes and McLellan, 1999; Jakobsson et al., 1999).

ACTIVE SITES OF GSTS AND GSH BINDING MODES

![Diagram of GST active sites](image)

Figure-8: Two typical views of two active sites of a dimeric Glutathione S-Transferase with their substrates

The GSTs possess two binding sites. They are G and H sites. The H-site is a small in size and which determine to bind to substrate in specific manner Figure-8 (Hayes et al., 1995). Alpha, mu, pi, sigma, and theta have similar activity sites in each subunit. The A site is localized in C-terminous end (Hayes, 1995). The G site is localized in N-terminus end and that is used for the binding of glutathione, a primary substrate (Sheehan et al., 2001).

In the alpha, mu, pi, and sigma classes, the G-site is facilitated by a conserved tyrosine, while in the theta class; a serine residue facilitates the G-site (Hayes et al., 1995). In addition, it has also been shown that the glutathione binding domain (G-site) is highly conserved (not identical) in all classes. The domain I of a subunit is interlinked to domain II of another subunit with a small linker sequence.
Figure-9: Domain structure of GST subunits

Three-dimensional structures of individual GST subunits are shown. The N-terminal domain 1 is coloured blue, while the C-terminal domain 2 is red.

Figure-10: Representation of the highly conserved core \( \beta \beta \alpha \) motif which is responsible for the recognition of the \( \gamma \)-glutamyl residue of GSH. Glutathione and the side chains located.

The relationship among nutrients, cancers and GSTs lacking at present and it requires attention of ayurvedic doctors in the analysis of various phytochemicals.

DETOXIFICATION THROUGH THE MERCAPTURIC ACID PATHWAY

The primary role of GSTs is to augment the detoxification of exogenous (xenobiotic and their metabolites) and endogenous (primary products of oxidative stress) toxic compounds in the phase II reaction of drug detoxification pathway. They do so by conjugating the electrophilic center of toxic, hydrophobic compounds to the sulfur atom of GSH and the nature of the reaction is nucleophilic and the
resultant water soluble S-conjugate is processed and eliminated, via the classical mercapturic acid pathway (Fig- 11)

The GSTs achieve detoxication by catalyzing the conjugation of reduced glutathione to various electrophilic substrates. GSH conjugation is the first step in mercapturic acid synthesis, which aids in the protection of the cell by enhancing the excretion of toxic metabolites from both animals and humans. Therefore, the levels of GSTs have been suggested as important determinant of the susceptibility of organisms or tissues to pharmacological or physiological changes.

Exogenous substrates for soluble GST include drugs, industrial intermediates, pesticides, herbicides, environmental pollutants and carcinogens. The cancer chemotherapeutic agents adriamycin, 1,3-bis (2-chloroethyl)-1-nitrosourea (BCNU), busulfan, carmustine, chlorambucil, cis-platin, crotonyloxymethyl-2-cyclohexenone (COMC-6), cyclophosphamide, ethacrynic acid, melphalan, mitozantrone, and thiotepa are detoxified by GST (Hayes JD and Pulford DJ, 1995, Hamilton D.S et al., 2003).

Figure-11: Metabolic and transport steps in mercapturic acid biosynthesis
The GSH S-conjugate is made intracellularly and then transported out of the cell for subsequent degradation by the ectoproteins like gGT and the dipeptidases. The cysteine S-conjugate that is formed is transported back into the cell and N-acetylated to form mercapturic acid. Ac CoA- coenzyme A; Cys- cysteine; Glu- glutamate; Gly- glycine; R- Xenobiotic, S-R- thiol-conjugated Xenobiotic; SH- sulphydryl group.

Environmental chemicals and their metabolites detoxified by GST include acrolein, atrazine, DDT, inorganic arsenic, lindane, malathion, methyl parathion, mucosaldehyde, and tridiphane (Hayes and Pulford, 1995, Abel and Bammler; Abel and Opp, 2004).

A large number of epoxides, such as the antibiotic fosfomycin and those derived from environmental carcinogens, are detoxified by GST. The latter group includes epoxides formed from aflatoxin B1, 1-nitropyrene, 4-nitroquinoline, PAHs and styrene by the actions of cytochromes P450 in the liver, lung, gastrointestinal tract, and other organs.

Reactions of Glutathione S-Transferases

The reactions catalyzed by GSTs can be classified broadly as the catalytic and non-catalytic reactions. In each of these reactions GSH is the nucleophilic reactant towards an electrophilic substrate (Mannervik, 1986). Catalytic reactions are subdivided into as follows:

**Conjugation:** GSTs can conjugate GSH with compounds that possess an electrophilic center. The electrophilic functional group to a compound can be provided by a carbon, nitrogen or a sulphur atom, aliphatic and aromatic halides, unsaturated carbonyls, organic nitrate enters and organic thiocyanates. The formation of a thioether bond between electrophiles and GSH almost always yields a conjugate that is more stable thermodynamically than the parental compound. Thus from the functional point of view, GSH conjugation is thought to be of value not only to remove harmful electrophilic moieties from the cell but also to increase the solubility of hydrophobic xenobiotics and there by preventing their partitioning into membrane lipid. The exact mechanism of GST-catalyzed conjugation reaction is not yet clarified unequivocally and is still at the level of debate. Of late, in proposed that the most likely method involved is lowering of pKa value of GSH from 9 in aqueous solution to 6.5 in the
bound state, which results in the formation of the potent nucleophilic species, the thiolate anion (GST). The stability to this anion is provided by the hydrogen bonding with tyrosine in the α, μ, π and σ classes at the N-terminus of the polypeptide. A notable structural feature of rGST M4 is the Cys-115 residue in place of the Tyr-115 of other μGSTs. This sub class of rodent μGSTs with redox-active Cys-115 residues could have specialized physiological functions in response to oxidative stress.

**Oxidation-reduction:** The oxidation-reduction reaction spans around the selenium - independent glutathione peroxidase activity of GPx II. This type of reaction is thought to occur in two steps and proceeds via the formation of sulfenic acid and represents nucleophilic attack by GSH on electrophilic oxygen.

\[
\text{ROOH} + \text{GSH} \rightarrow \text{ROH} + (\text{GSOH}) \\
(\text{GSOH}) + \text{GSH} \rightarrow \text{GSSG} + \text{H}_2\text{O}
\]

The reduction of organic hydroperoxides and endoperoxides by GPx II forms an important antioxidant defense mechanism of cellular systems as these compounds directly or indirectly, through their decomposition to reactive free radicals, damage vital biomacromolecules. In man the α and the θ class excel in this activity. In rat specific isoform studies have shown that GSTs1-2, 2-2, 5-5 and 7-7 show activity toward lipid peroxides. GST 5-5 also shows activity towards DNA hydro peroxides and GST 8-8, shows high conjugative activity toward 4-hydroxyxynonenal, a potent aldehyde product of lipid peroxidation (Hoff et al., 1992 & 1993). The reduction of endoperoxides prostaglandin H2 to PGI2 a is catalysed by GST 1-1. GST 6-6 is involved in the biosynthesis of leukotrienes.

**Isomerization:** GSTs also catalyze the reaction of positional isomerization. In this reaction GSTs act catalytically and hence do not undergo metabolism. For example 5-ketosteroids to 4-ketosteroids and PGH2 to biologically active PGE2 and PGD2 (Keen et al., 1978). The other possible enzymatic functions are in the metabolism of ethanol and the catalysis of disulfide exchange with non polar disulfides.

**Thiolysis:** GSTs catalyse the thiolysis of p-nitrophenyl acetate and Methyl para thione. Incomplete detoxification by GST occurs with certain esters, ethers and organic phosphorous when conjugation leads to cleavage of the substrate with only one of the two products being conjugated. This process has been called thiolysis and
in the case of p-Nitrophenol acetate, it results in the release of p-nitrophenol. Thiolysis represents incomplete detoxification because the unconjugated cleavage product still provides a chemical threat to the cell (Keen et al., 1978)

XENOBIOTIC GST SUBSTRATES (INDUCERS)

GSTs were discovered as enzymes catalyzing aromatic nucleophilic displacement reactions in which the sulfhydryl group of glutathione is conjugated with an aryl group of the electrophilic substrate. Halogenated nitrobenzenes are representative examples. Since the discovery of the first GST-catalyzed reactions, a very large number of electrophilic chemical compounds have been found to serve as substrate for one or several GSTs (Mannervik, B., 1985; Mannervik, B. and Danielson, U.H., 1988).

![Figure-12: Types of inducers of defense enzymes](image-url)
The electrophilic center of a substrate is often an activated carbon atom, but may also be sulfur, oxygen or nitrogen (Keen, J.H et al., 1976). To this present investigation Acrylamide (AC)(Figure-13) (monofunctional inducer Figure-12) was selected as xenobiotic substrate or inducer.

ACRYLAMIDE

Acrylamide (AC) is an odorless, white, crystalline solid. Synonyms include acrylic amide, acrylic acid amide, ethylenecarboxamide, propenamide and propenoic acid amide.

![Figure-13: The chemical structure of Acrylamide (AC)](image)

The cancer potency of acrylamide was estimated from dose-response data of multiple acrylamide-responding tumor sites observed in four long-term drinking water studies, two in male rats and two in female rats (Johnson et al., 1986; Friedman et al., 1995). These tumor sites were the mammary gland, thyroid, central nervous system, oral cavity, uterus and clitoral gland in female rats, and thyroid, testis (mesothelioma), and central nervous system in male rats. Epidemiological data are inadequate for characterizing the dose-response relationship for acrylamide, although there is one study (Marsh et al., 1999) that provides a basis for checking the quantitative consistency of cancer potency derived from the animal data.

Acrylamide is a multisite carcinogen. In mice, in studies examining only the lung and skin, acrylamide induced lung and/or skin tumors (Bull et al., 1984a,b; Robinson et al., 1986). In female rats acrylamide induced tumors of the mammary gland, thyroid, central nervous system, oral cavity, uterus and clitoral gland and, in male rats, tumors of the thyroid, testis (mesothelioma), and central nervous system (Johnson et al., 1986; Friedman et al., 1995). In addition to inducing cancers of the rat testes, acrylamide caused male reproductive toxicity, affecting male fertility in mice and rats, and causing heritable genetic mutations in male mice (CERHR, 2004a,b).
Additionally, the central nervous system is a target not only for acrylamide-induced tumorigenicity, but also neurotoxicity. Human occupational studies and animal experiments have demonstrated acrylamide to be a potent neurotoxicant (CERHR, 2004; U.S. EPA, 1991).

In addition to the animal bioassays, occupational and dietary epidemiological studies have been published that provide some quantitative information on acrylamide exposure and cancer response (Mucci et al., 2003; 2004; Bosetti et al., 2002; Pelucchi et al., 2003). Because of methodological deficiencies, the dietary studies are unsuitable for risk assessment (Hagmar and Tornqvist, 2003; Dybing and Sanner, 2003; Erdreich and Friedman, 2004; Koehler, 2004). While the occupational studies are not of sufficient power or reliability (Erdreich and Friedman, 2004; Koehler, 2004), they potentially can be used to derive an upper bound estimate on cancer potency (OEHHA, 2003). The most reliable epidemiological study for this purpose is the study of acrylamide-exposed workers by Marsh et al. (1999).

**ACRYLAMIDE IN CIGARETTE SMOKE**

Acrylamide (AC) is a component of cigarette smoke and AC content in mainstream cigarette smoke has been estimated at 1.1–2.34 μg per cigarette (Smith et al., 2000). Smoking is a source of human inhalation exposure and secondhand smoke could contribute to AC in indoor air, although no data were found on indoor air levels of acrylamide from environmental tobacco smoke. Boettcher et al. (2005) measured the AC and AC metabolites in human urine and reported median levels in smokers (n=13) about four times higher than in non-smokers (n=16) indicating that cigarette smoke is clearly an important source of acrylamide exposure.

**ACRYLAMIDE FORMATION IN FOODS DURING PROCESSING**

In early 2002, high concentrations of AC were reported in certain fried, baked and deep-fried foods (Swedish National Food Agency, 2002). This discovery dramatically increased the interest in nonindustrial sources of acrylamide exposure to the general public. Subsequent research in many European countries and the United States determined that AC is formed primarily in carbohydrate-rich foods prepared or cooked at high temperatures (i.e., >120° C) (Tareke et al., 2002; 2000). The predominant chemistry involves a Maillard reaction, a nonenzymatic browning
reaction that occurs by a condensation of the amino group of the amino acid, asparagine, and the carbonyl group of reducing sugars (fructose and glucose) during high-temperature heating (Mottram et al., 2002; Stadler et al., 2002). Thus, browned crispy crusts in foods like French fries, potato chips, crackers, pretzel-like snacks, cereals, and browned breads tend to have the highest levels of AC. Acrylamide has been detected in some food products that are processed at temperatures in the 98° - 116° C range and in high moisture conditions (e.g., canned black olives [not oil cured] and prune juice) (Roach et al., 2003), so there are other pathways of formation that do not involve temperatures over 120° C and crispiness and these are being further evaluated (JIFSAN, 2004). Dybing et al. (2005) list AC concentrations in various foods in the United States as determined by the U.S. Food and Drug Administration (U.S. FDA, 2006a) in Table-5 and, in Table-6, in foods in Europe from data compiled by the Institute for Reference Materials and Measurements (IRMM, 2004).

Table-5: Summary of acrylamide levels in food (ppb) derived from the FDA data collected from 2002 through October 1, 2003

<table>
<thead>
<tr>
<th>Food commodity</th>
<th>n</th>
<th>Minimum</th>
<th>25%</th>
<th>Median</th>
<th>75%</th>
<th>Maximum</th>
<th>St. Dev</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baby food and infant formula</td>
<td>36</td>
<td>0.0</td>
<td>0.0</td>
<td>10.0</td>
<td>31.8</td>
<td>13.0</td>
<td>36.6</td>
</tr>
<tr>
<td>French fries and chips</td>
<td>97.0</td>
<td>0.0</td>
<td>20.0</td>
<td>220.0</td>
<td>318.0</td>
<td>462.0</td>
<td>2762.0</td>
</tr>
<tr>
<td>Protein foods</td>
<td>21.0</td>
<td>0.0</td>
<td>0.0</td>
<td>10.0</td>
<td>25.0</td>
<td>116.0</td>
<td>27.7</td>
</tr>
<tr>
<td>Bread and bakery products (a)</td>
<td>49.0</td>
<td>0.0</td>
<td>15.0</td>
<td>34.0</td>
<td>96.0</td>
<td>432.0</td>
<td>107.9</td>
</tr>
<tr>
<td>Cereals and muesli</td>
<td>23.0</td>
<td>11.0</td>
<td>49.0</td>
<td>77.0</td>
<td>166.0</td>
<td>1057.0</td>
<td>249.1</td>
</tr>
<tr>
<td>Gravies and seasonings</td>
<td>13.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>151.0</td>
<td>43.4</td>
</tr>
<tr>
<td>Crackers and snack foods</td>
<td>32.0</td>
<td>12.0</td>
<td>92.5</td>
<td>169.0</td>
<td>302.3</td>
<td>1243.0</td>
<td>331.1</td>
</tr>
<tr>
<td>Nuts and butters</td>
<td>13.0</td>
<td>0.0</td>
<td>28.0</td>
<td>89.0</td>
<td>236.0</td>
<td>457.0</td>
<td>143.0</td>
</tr>
<tr>
<td>Chocolate products</td>
<td>14.0</td>
<td>0.0</td>
<td>2.5</td>
<td>20.5</td>
<td>84.3</td>
<td>909.0</td>
<td>243.6</td>
</tr>
<tr>
<td>Canned foods</td>
<td>33.0</td>
<td>0.0</td>
<td>0.0</td>
<td>10.0</td>
<td>70.0</td>
<td>1925.0</td>
<td>411.7</td>
</tr>
<tr>
<td>Coffee, ground</td>
<td>59.0</td>
<td>37.0</td>
<td>158.0</td>
<td>205.0</td>
<td>299.0</td>
<td>539.0</td>
<td>106.3</td>
</tr>
<tr>
<td>Coffee, brewed</td>
<td>20.0</td>
<td>3.0</td>
<td>6.0</td>
<td>6.5</td>
<td>8.0</td>
<td>13.0</td>
<td>2.4</td>
</tr>
<tr>
<td>Miscellaneous(b)</td>
<td>41.0</td>
<td>0.0</td>
<td>0.0</td>
<td>10.0</td>
<td>43.0</td>
<td>5399.0</td>
<td>1018.8</td>
</tr>
</tbody>
</table>

a. Includes cookies, pies and pastry, bagels.
b. Hot beverages other than coffee (Postum, caffeine-free coffee substitute), frozen vegetables, dried foods, dairy, juice and other miscellaneous.
Data were calculated from the data published by the FDA on the Internet ("Exploratory Data on Acrylamide in Food," March 2004 [http://www.cfsan.fda.gov/~dms/acyrdata.html]). The database contains data collected from 2002 through October 1, 2003.

The categories were used as given by the FDA. For coffee, only data for roasted coffee were used (total sample number \( n = 439 \)). Source: Dybing et al. (2005).

Table 6: Acrylamide levels in food (ppb) as collected by the European Union Joint Research Center (updated June 2004)

<table>
<thead>
<tr>
<th>Food commodity</th>
<th>N</th>
<th>Min</th>
<th>25%</th>
<th>Median</th>
<th>75%</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>French fries</td>
<td>741.0</td>
<td>5.0</td>
<td>90.0</td>
<td>178.0</td>
<td>326.0</td>
<td>2228.0</td>
</tr>
<tr>
<td>Chips</td>
<td>569.0</td>
<td>5.0</td>
<td>378.0</td>
<td>600.0</td>
<td>980.0</td>
<td>3770.0</td>
</tr>
<tr>
<td>Potato fritter</td>
<td>75.0</td>
<td>15.0</td>
<td>215.0</td>
<td>492.0</td>
<td>797.6</td>
<td>2779.0</td>
</tr>
<tr>
<td>Fine bakery ware</td>
<td>485</td>
<td>5.0</td>
<td>67.0</td>
<td>160.0</td>
<td>366.0</td>
<td>3324.0</td>
</tr>
<tr>
<td>Gingerbread</td>
<td>414.0</td>
<td>5.0</td>
<td>152.0</td>
<td>298.5</td>
<td>650.7</td>
<td>7834.0</td>
</tr>
<tr>
<td>Crispbread</td>
<td>261.0</td>
<td>5.0</td>
<td>81.0</td>
<td>251.0</td>
<td>602.0</td>
<td>2838.0</td>
</tr>
<tr>
<td>Infant biscuits</td>
<td>63.0</td>
<td>5.0</td>
<td>64.3</td>
<td>90.0</td>
<td>275.1</td>
<td>910.0</td>
</tr>
<tr>
<td>Diabetics’ cakes and biscuits</td>
<td>212.0</td>
<td>5.0</td>
<td>92.5</td>
<td>291.5</td>
<td>772.3</td>
<td>3044.0</td>
</tr>
<tr>
<td>Breakfast cereals</td>
<td>162.0</td>
<td>5.0</td>
<td>30.0</td>
<td>60.0</td>
<td>152.5</td>
<td>846.0</td>
</tr>
<tr>
<td>Coffee, roasted</td>
<td>102.0</td>
<td>79.0</td>
<td>192.0</td>
<td>264.0</td>
<td>337.0</td>
<td>975.0</td>
</tr>
<tr>
<td>Coffee, substitutes</td>
<td>50.0</td>
<td>115.6</td>
<td>439.4</td>
<td>739.0</td>
<td>1321.8</td>
<td>2955.0</td>
</tr>
</tbody>
</table>

Note: Data were calculated from the monitoring database on acrylamide levels in food (http://www.irmm.jrc.be/) maintained by the IRMM, together with the Directorate General for Health and Consumer Affairs.

This database comprises 3442 samples of acrylamide levels in food products throughout the EU, including the data collection from the Confédération des Industries Agro-Alimentaires de l’Union Européenne.

The categories were used as given in the data collection. Source: Dybing et al. (2005).
Two physiologically based toxicokinetic (PBTK) models for acrylamide were available from the literature (Kirman et al., 2003).

A diagram of the Kirman et al. (2003) model is presented in Figure-14. This model simulates the distribution of AC and GA within five compartments—arterial blood, venous blood, liver, lung and all other tissues lumped together.

Figure-14: Schematic representation of physiologically based toxicokinetic model of acrylamide and its metabolite, glycidamide activity in tissues.

Among these two GA can serve as better carcinogen. And AC' can damage DNA in testicular tissues germ cells (Laxminarasiah, 2009). Therefore a study is required on the use of AC in the generation of damage to kidney and testis of mice. The study on mice using the AC as source inducer of chemical damage was not well documented.
AIM AND SCOPE
AIM AND SCOPE

Living organisms encounter a wide range of chemical structures among the xenobiotics to which they are exposed. Understanding the functional role of physiologically active molecules in the male reproductive tract is essential to decipher mechanisms involved in the regulation of spermatogenesis, where by modeling alterations in their functions, one can predict predispositions to testicular disease states, causes of infertility and possibilities to inhibit germ cell production.

Male germ cells are extremely vulnerable to stress as their membranes are rich in unsaturated fatty acids and their GSTs a part of the adaptive response of germ cells to oxidative stress. Two pools of germ cell GSTs were detected and purified. One was a plasma membrane pool and another was a cytosolic pool. Cytosolic GSTs could salvage 70% of the lipid peroxidation products formed and were comparatively more effective in salvaging these products as compared to plasma membrane GSTs.

Consequent to earlier studies demonstrating the binding of sperm GSTs to the zona pellucida, the mechanism of non-covalent peripheral attachment of the GSTs on sperm surface was worked out. Metabolic labeling studies in germ cells showed that 10% of the total cellular GSTs were transported to the membranes while 90% remained as a cytosolic pool.

High susceptibility of sperm to lipid peroxidation and the association between ROS production and male infertility, support the importance of the antioxidant system in the testis. Male infertility and abnormal progeny outcome are some of the consequences resulting from the exposure of germ cells to stressors such as environmental chemicals and drugs during the male germ cell development, cells have different abilities to cope with diverse types of stress such as oxidative stress, and protein and DNA damage.

Testicular cancer is a disease in which cells become malignant in one or both testicles. Testicles produce and store sperm and are the body’s main source of male hormones. These hormones control the development of reproductive organs and male characteristics. An estimated 7,400 men in United States will be diagnosed with testicular cancer in 1999. Cancer of the testicles is not a common cancer overall, and
is responsible for less than one per cent of all cancer deaths. However, it is the most
common cancer in men aged 20 to 34. It is important for men to be aware of the
disease so that they can recognise the symptoms. If caught early, testicular cancer can
usually be treated and cured.

GSTs function catalytically to conjugate GSH with a variety of electrophilic
substrates (Ketterer et al., 1994). The proteins of Mu sub family have similar
catalytic specificities and mechanisms, are all cysteine rich are found mainly in testis
and share characteristics that distinguish them from other GSTs. From an
evolutionary stand point these Mu-class genes are the most divergent as explained in
introduction.

Toxicity is of major concern for cancer / anticancer drugs / chemicals. The
toxicants may have the properties of severity in toxicity, dose-limiting toxicity, acute
versus chronic toxicity, cumulative toxicity and scheduled dependent toxicity. The
recommended doses of drugs / chemicals are determined according to the toxicity end
point. Haematological toxicities represent the main toxicity of cytotoxic (Chatelut et
al., 2003).

The human beings are used to expose to various chemicals either directly in
factories or indirectly in streets and fields. The chemicals entering into the biological
systems are either degraded, or modified and gets involved in modification of the
existing metabolism. During this metabolism the modified molecules become
activated and further cause damage to proteins or nucleic acids and tissues. Due to
this, normal function of the individual varies, and creates abnormality in the
biological systems. Some of the chemicals that involved are Phenobarbital,
3-Methylcholanthrene, and acrylamide (Devi et al, 1998).

The excess concentration of these chemicals can cause damage to defense
system and modifies tissue and it leads to cancer. To encounter the above damage the
organisms have defense enzymes like mixed function oxygenase, superoxide
dismutase, catalase, peroxidases, glutathione-transferases, cysteine transferases etc.
These enzymes can participate either to catabolise the molecules or excrete them from
the body. At the beginning the above chemicals interacts with internal GSH to
conjugate in the presence of GST. These enzymes are induced for secondary defense
by using glutathione as primary substrate and the other chemical as secondary substrate.

3-Methylcholanthrene and acrylamide are chemical toxicants (Thygaraju et al., 2003; Devi et al., 2002a & b; Dixit et al., 1981; Vasundhara et al., 2002). Acrylamide's neurotoxic properties have been well documented. Now this study will focus on after exposure reproductive effects. The enzymes induced in the presence of these chemicals are varied and are used as marker proteins to detect the chemical toxicity and carcinogeneity. The studies conducted on these lines are minimum and are yet to reach to the common man.

In this context, the present study is aimed to reveal "The effect of selected chemical toxicant acrylamide on the induction of glutathione S-transferases" and to investigate the sustained induction of GSTs in kidney and testis of mice with selected dose & time intervals.

The plant extract are used to cure various diseases. This fact is known to all people since the ancient period. However the plant extract of H E which contain more of phytochemicals require further elucidation. Therefore this plant extract use and its active principle characterization was selected for analysis in control mice and acrylamide treated animals.

Therefore to find the use of GST subunits as prognostic markers in kidney and testis the following objectives were studied.

1. To purify and characterize the glutathione S-transferases of mice kidney and testis.
2. To study the effect of acrylamide on the induction of GSTs of mice kidney and testis.
3. To study the effect of Hybanthus enneaspermus active principle extract on acrylamide treated GSTs of mice kidney and testis
4. To study tissue modifications of mice kidney and testis by the effect acrylamide and acrylamide and Hybanthus enneaspermus.

To study the above aims, experiments were conducted on Kidney and testis GSTs purification and their induction, and modifications of tissues by acrylamide and Hybanthus enneaspermus active principle treatment.