

Potential of Myristicin, a constituent of Parsley leaf, in Chemoprevention



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Impact of Cancer on the society

Out of a total of about 12 billion dollars NIH budget, National Cancer Institute gets about 3 billions per year

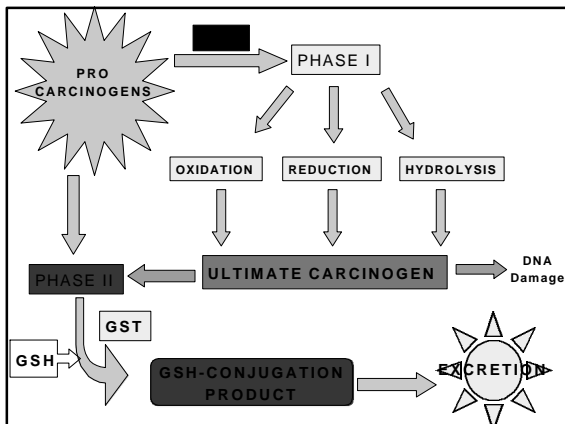
NIH supports 17 different Institutes, besides NCI

Since Dr. Turner has covered to some extent the development of cancer, we will focus mostly on the mechanisms of cancer prevention by a very well studied group of enzymes known as Glutathione S-Transferase (GST)

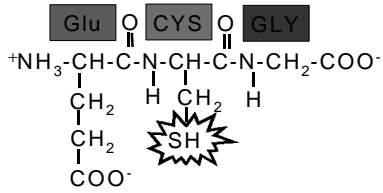
Both biochemical and epidemiological studies indicate that intake of diet rich in fruits and vegetables is directly related to lower incidences of cancer.

What is it in these fruits and vegetables that protect the cells from becoming cancerous?

ROLE OF GSH/GST-DEPENDENT DETOXIFICATION SYSTEM IN CHEMOPREVENTION



Glutathione



GLUTATHIONE and DETOXIFICATION

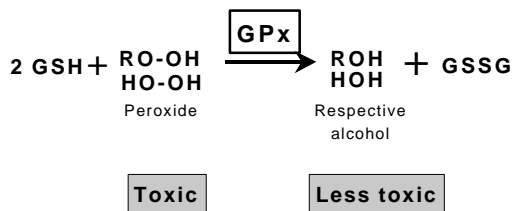
Non enzymatic

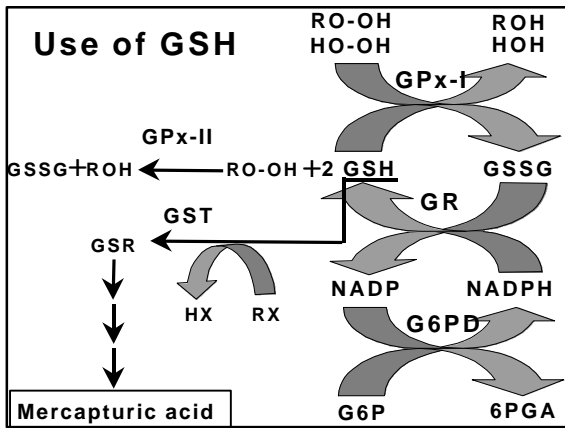
- Major cellular Nucleophile
- Reducing agent
- Free radical Scavenger

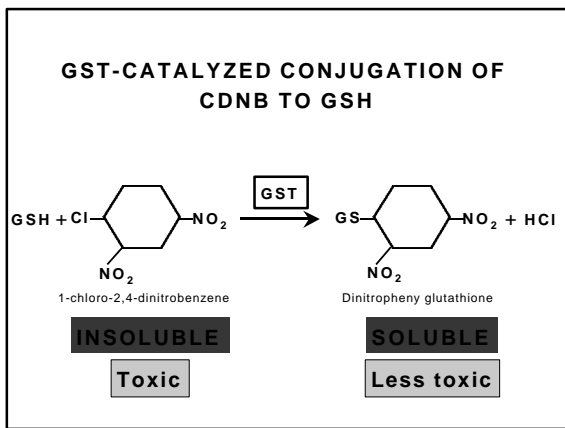
Enzymatic:

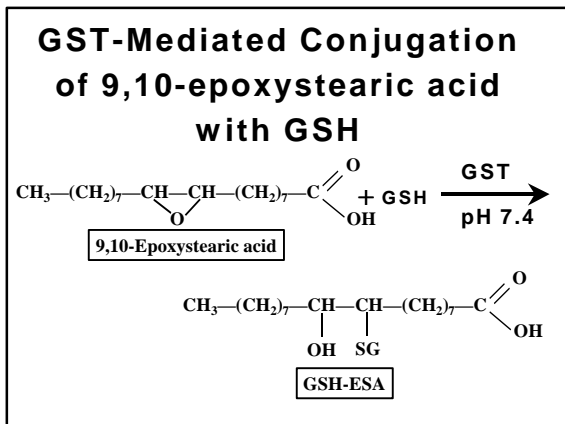
- GSH peroxidase I and II activities
- Glutathione S-Transferase Activity

GLUTATHIONE PEROXIDASE CATALYZED REACTION









FUNCTIONS OF GST

CATALYTIC FUNCTIONS:

- Glutathione S-transferase activity
- Glutathione Peroxidase II activity

NON CATALYTIC FUNCTIONS:

- Reversible biding and transport of several organic compounds
- Irreversible binding and transport of several electrophilic compounds including some carcinogen

CHARACTERISCICS OF GST

1. High Concentrations in Tissues: Rat liver 5%, human liver 3% of soluble proteins
2. Multiple forms: Present in most of the human tissues investigated so far.
3. Multiple Functions: Conjugate wide variety of xenobiotics to glutathione ; Bind covalently and non-covalently to xenobiotics and Express glutathione peroxidase activity towards lipids and nucleic acid hydroperoxide

CHARACTERISCICS OF GST

4. Induction: Inducible by phenolic antioxidants and many other xenobiotics and plants and vegetable chemical compounds.
5. Structure: Most human isoenzymes arise from dimeric combination of several different subunits in the apparent Mr. range of 22K to 29K.
6. Tissue Specific Expression: Some tissues expresses only specific isoenzymes probably to satisfy the specific or unique needs of the tissue.

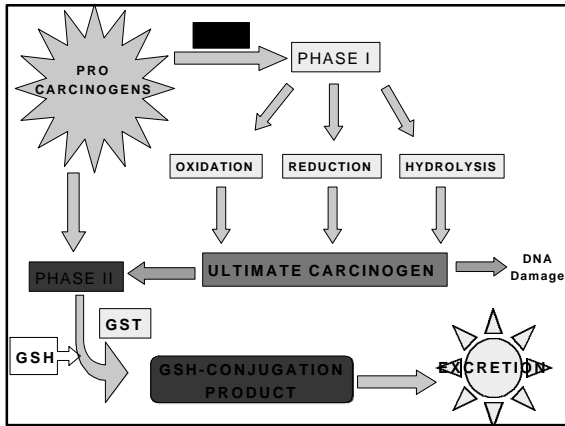
CLASSIFICATION OF GST

PROPERTY	Alpha Class	Mu class	Pi class	Theta Class
Preferred Substrate	Cumene Hydroperoxide	Epoxide substrates	Ethacrynic acid	CDNB
Polymorphism	Yes	Yes	??	??
N-terminal	Blocked	Free	Free	??
Gene	Multiple	Multiple	Two?	?
N-Terminal Sequence				
Human	A E K P K L H Y K N S	P M I L G Y D I R	PPYTVVYFPV	?
Rat	S G K P K L H Y H I P D	P M I L G Y N V R T I	PPYTVVYFPV	?

Some of the Substrates of GST

Class	Example
Halogenitrobenzenes	1,2,4-Trichloro-3-nitro benzene 1,2-dichloro-4-nitro benzene
Aralkyl halides	Benzyl chloride 4-bromobutylbenzene
Aralkyl esters	1-menaphthyl sulfate
Alkyl halides	2-chlorohydrin 1,2-epoxypropane
Alkene halides	Vinyl chloride
Organophosphorus compounds	Methylparathione
Arene oxides	metabolites of naphthalene, benzo[a]pyrene
Steroids	Δ^5 -androstene-3,17-dione

As biochemists, we ask the basic question: How Glutathione S-Transferases are linked to Cancer Prevention?



**ENZYME INDUCTION
AND CHEMOPREVENTION**

The discovery by Wattenberg in 1973 that food additive BHA protected rodents against carcinogens indeed provided the important stimulus to the development of the field of chemoprevention.

The follow up studies of the same agents by Talaly's group in 1978 led to the startling discovery that administration of these antioxidants produced a marked change in the elevation of GST and other phase II enzymes.

A number of synthetic as well as natural chemical compounds are now known that causes induction of GSTs in many animal models.

**ENZYME INDUCTION
AND CHEMOPREVENTION**

Important Phase II Defense Enzymes

1. **Glutathione S-Transferase:** Inactivation of electrophiles by conjugation to GSH
2. **UDP-Glucosyltransferase:** Inactivation of electrophiles by conjugation to glucuronic acid.
3. **NAD(P)H:Quinone oxidoreductase:** Catalyzes the obligatory two electron reduction of quinones and thus shields the cells against the electrophilicity of quinones and oxidative stress.

The best sources of dietary antioxidants/Phase II enzyme (GST) inducers



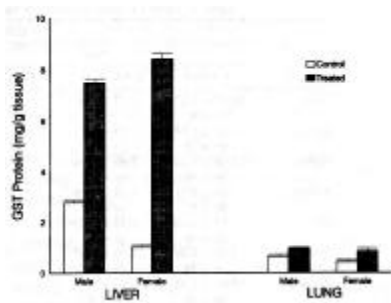
Fruits



Vegetables

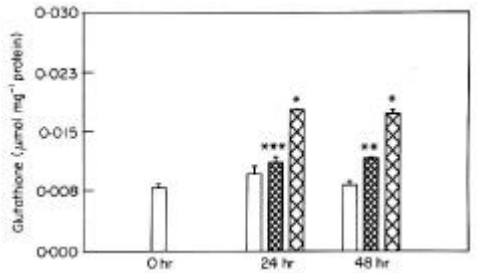
Our past experience with induction of GST in various experimental models

Effect of BHT on the levels of GST in male and female mice



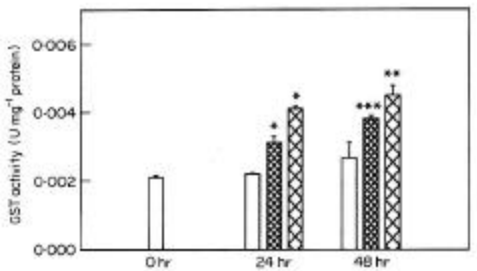
Sharma et al. 1993 *Comp. Biochem. & Physiol* 115C,31

Effect of BHT on GSH levels of rat lens cultured *in vitro*



Ahmad *et al.* 1992 *Exp. Eye Res.* 54, 41

Effect of BHT on Glutathione S-transferase levels of rat lens cultured *in vitro*

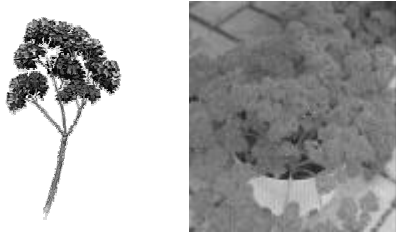


Ahmad *et al.* 1992 *Exp. Eye Res.* 54, 41

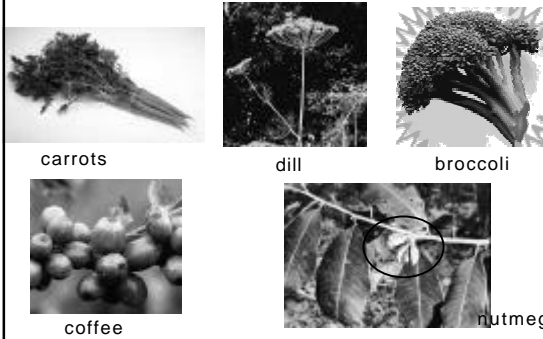
In 1995, we started searching for a natural source that **Exhibits** GST inducible properties similar to or better than BHT

We followed a study published in 1987 by Lam *et al.* that fruits and vegetable oils enhances the GSH/GST levels in mice.

In 1995, We selected to study the effect of parsley leaf oil metabolite, MYRISTICIN, on the induction of GSH-GST in mice

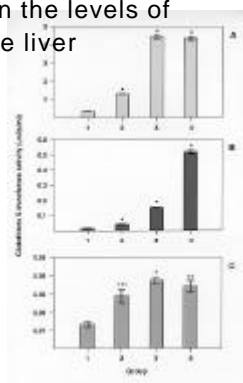


Other source of Myristicin



Effect of Myristicin on the levels of GST in mouse liver

1, 2, 3 & 4 = dose
A, B, C = substrates

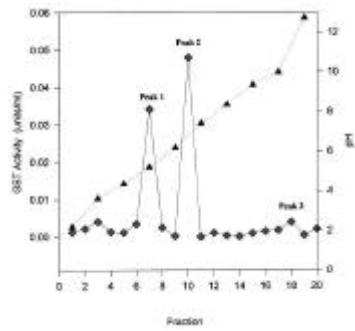


Ahmad et. al. 1997 *Biochem. Biophys. Res. Comm.* 236: 825

In 1999 we investigated the effect of myristicin in rat tissues to see if the GST induction could be achieved in rats tissues

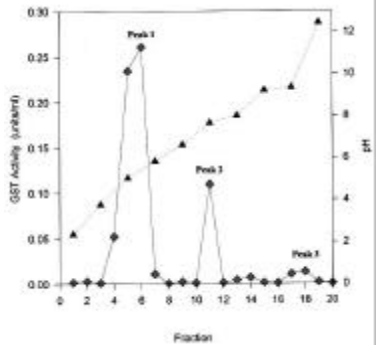
Effect of Myristicin on Rat liver GST

Control Group

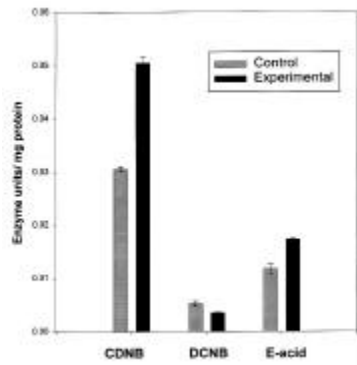


Effect of Myristicin on Rat liver GST

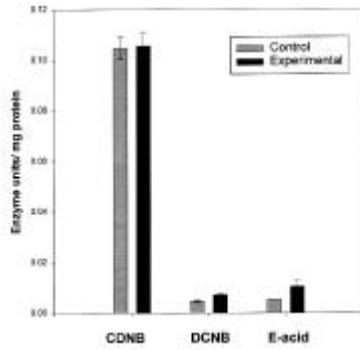
Exp. Group



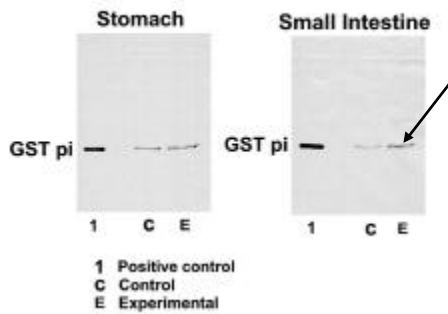
Effect of Myristicin on Rat lung GST



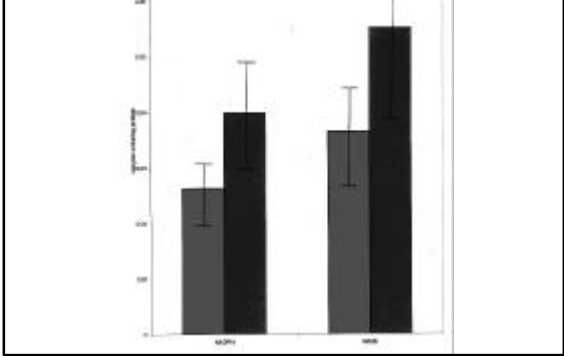
Effect of Myristicin on Rat small Intestine GST



Western Blots of Affinity Purified GST

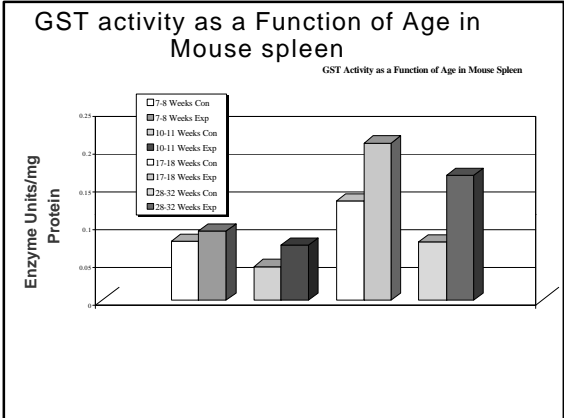


Effect of Myristicin on Rat liver NAD(P)H:Quinone reductase

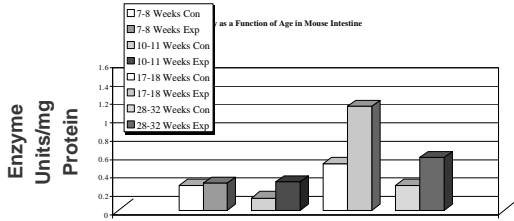


We have recently started studies to investigate the effect of induction of GST by myristicin on various strains of mice, age of mice and also on sex.

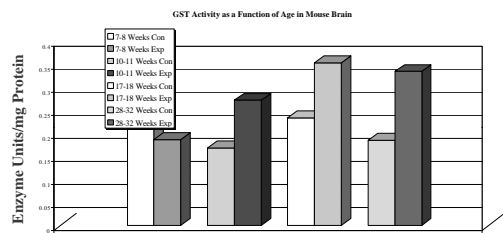
GST activity as a Function of Age in Mouse spleen



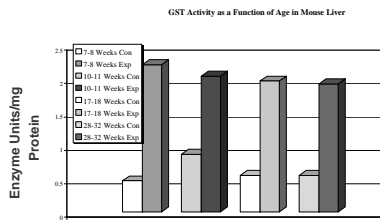
GST activity as a Function of Age in Mouse Intestine



GST activity as a Function of Age in Mouse Brain



GST activity as a Function of Age in Mouse Liver

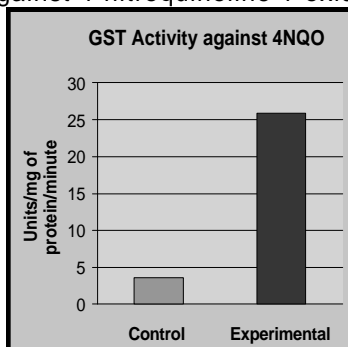


Effect of mice strain and gender on the Induction of GST activity

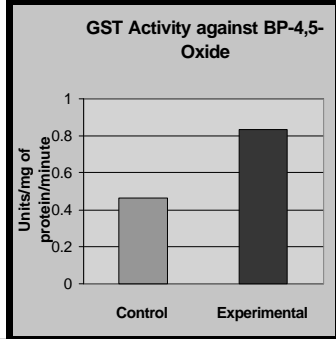
- In liver, there is appreciable increase in GST activity for CBA/J, C57BL and A/J mice.
- For BALB/C, Increase in GST activity seems to be insignificant in liver.
- In fact, GST induction seems to be insignificant in most of the organs of BALB/C tested.
- Gender doesn't seem to affect GST induction in liver. However, it seems to have some effect on GST induction on other tissues?

In 2000 We took the mice study one level further to demonstrate that the Myristicin-induced GST is capable of metabolizing at least two known carcinogens.

Effect of Myristicin-induced GST against 4-nitroquinoline 1-oxide.



Effect of Myristicin-induced GST against Benzo(a)pyrene-4,5-oxide



CONCLUSIONS

- Myristicin tends to induce the GST levels in many mouse and rat tissues.
- Carcinogens are efficiently metabolized by the induced GST liver.
- Myristicin appears to have a terrific potential for being an excellent chemopreventive agent.

FUTURE PLAN?

- To establish with confidence the induction of specific isoenzymes in various mouse tissues.
- To determine the capacity of the induced GST to metabolize carcinogenic compounds.
- To investigate the effect of induced GST in protection against chemical carcinogenesis in animal models.

FUTURE PLAN?

- To investigate the structure-function relationship of the induced GST.
- To investigate the effect of other phytochemicals such as citrus limonoids and flavonoids on the induction of phase II enzymes.

The History of Cancer Treatment

2000 BC	Here, eat these seeds
1000 BC	Seeds are heathen. Here, say this prayer.
1850 AD	Prayer is superstition! Here, have the surgeon cut it out.
1940 AD	There's more than surgery, add radiation.
1985 AD	There's more than surgery and radiation, add chemotherapy.
2000 AD	No better? Here, eat these seeds!
