CHAPTER 1
INTRODUCTION

1.1 Statement of problems

Stevia plant (*Stevia rebaudiana Bertoni*) has long been used as a sweetener by the natives in South America. The sweet compounds found in Stevia leaves are diterpene glycosides with differing sugar molecules attached and the main compound is stevioside which is 300 times sweeter than sucrose but it is a non-caloric sweetener. Scientific research has revealed that Stevia may effectively regulating blood sugar and helpful to people with diabetes and hypoglycemia. It also inhibits the growth and reproduction of some bacteria and other infectious organisms, including the bacteria that cause tooth decay and gum disease. Stevia sweetener has now been fully approved and widely used in many counties such as Brazil, Paraguay, Uruguay, central America, The United States, Isreal, Chaina and Japan for use in food, drink and medicine (Crammer and Ikan, 1986). There has never been a complaint that Stevia, in any of its consumable forms, has caused any harmful side effects in the 1,500 years of use in Paraguay and about 20 years in Japan. However, more elaborate safety tests were performed by the researcher during their evaluation of Stevia and their compounds as a possible sweetening agent. No chromosomal effect of stevioside and steviol was observed on cultured blood lymphocytes from healthy donors (Suttajit *et al*, 1993), stevioside at a dose as high as 2.5 g/kg.bw./day affects neither growth nor reproduction in hamsters (Yodyingyuad and Bunyawong, 1991), steviol was negative in reverse mutation assays using *S.typhimurium* TA97,TA98, TA100, TA102, TA104, TA1535, TA1537 and *Escherichia coli* WP2 uvrA/pKM101 and rec-assay using *Bacillus subtilis* (Matsui, *et al.*, 1996), no significant alteration of neoplastic or non-neoplastic was seen in F344 rats (Toyoda,*et al.*, 1997) and Wistar rats (Xili, *et al.*,

1992) receiving stevioside mixed diet. Much of the research data indicated that Stevia and Stevia products were innocuous. However, the aglycone of stevioside, steviol was found to be highly mutagenic toward *Salmonella typhimurium* TM677 (Pezzuto , *et al.*, 1985) and found that steviol at doses of 0.75 and 1.0g/kg.bw./day were highly toxic to both dams and fetuses in hamsters (Wasuntarawat, *et al.*, 1998). These results have created interest concerning the safety and toxicity of Stevia. In Thailand, Stevia extract or stevioside are not allowable for import and commerce by Thai Food and Drug Administration because of its mutagenic and teratogenic activity in some research.

In recent years, some efforts have been made worldwide to encourage a reduction in the consumption of dietary sugar. It is generally known that high sugar consumption is linked to dental caries, obesity and cardiovascular disease. One way of reducing sucrose consumption is to substitute other sweeteners, and Stevia is being investigated as such a sweetener. Stevia has been used for hundreds of years as an alternative sweetener. It is used widely in Japan with no adverse effects. Scientists involved in reviewing Stevia have declared it to be safe for human consumption which has been well known in many parts of the world where it is not banned. This research supports work to convince FDA of the safety of Stevia. It may help in the economic of the country because Stevia is an excellent cash crop and grows well in Thailand. Many authors have demonstrated that Stevia extracts may be used not only for sweetening purposes, but also for its physiologic and therapeutic effects. It is reputed to have therapeutic value in the treatment of patients with diabetes-related obesity, hypertension (Oveido, *et al*, 1970) or cardiac disease (Boech and Humboldt, 1981).

Wingard, *et al* sugested that stevioside is swiftly transformed into steviol, uncovered as a potentially mutagenic chemical, by the action of anaerobic microorganism in the intestine (Wingard, *et al*, 1980). In this study, we have investigated the chronic toxicity and carcinogenic potential of Stevia extract mixed in Matoom (*Aegle marmelos Corr.*) juice fed to male Wistar rats for up to 100 days on aberrant crypt foci (ACF) formation in colon, activity of AST and ALT in serum, GSH content, GST activity and subunit composition in the liver and intestinal mucosa in male

vWistar rats after 100 days administration of Stevia mixed in Matoom juice. The results may provide important information about toxicity in rats treated with Stevia extract mixed in Matoom juice on colon preneoplastic lesion and the xenobiotic-metabolizing enzyme system is generally involved in the metabolic disposition of most xenobiotics leading to modification in the toxicity response.

Figure 1. *Stevia rebaudiana*, *Bertoni.* Family: Compositae

Literature review

1.2 *Stevia rebaudiana, Bertoni*

The worldwide demand for high potency sweeteners is expected to increase especially with the new practice of blending different sweeteners, the demand for alternatives is expected to increase. The sweet herb of Paraguay, *Stevia rebaudiana*, Bertoni produces, in its leaves, just such an alternative with the added advantage that Stevia sweeteners are natural plant products. In addition, the sweet steviol glycosides have functional and sensory properties superior to those of many other high potency sweeteners. Stevia is likely to become a major source of high potency sweetener for the growing natural food market in the future. *Stevia rebaudiana*, Bert. is one of 154 members of the genus Stevia and one of only two that produce sweet steviol glycosides (Robinson, 1930; Soejarto, *et al*, 1982). Currently Stevia production is centered in China and the major market is in Japan (Kinghorn and Soejarto, 1985).

1.2.1 The chemistry of the diterpene glycoside sweeteners

The sweet diterpene glycosides of Stevia have been the subject of a number of reviews (Kinghorn and Soejarto, 1985; Crammer and Ikan, 1986; Hanson and De Oliveira, 1993). Although interest in the chemistry of the sweet principles dates from very early in the century, significant progress towards chemical characterization was not made until 1931, with the isolation of stevioside (Bridel and Lavieille, 1931). Subsequent studies have led to the isolation of seven other sweet glycosides of steviol. Typical proportions, on a dry weight basis, for the four major glycosides found in the leaves of wild Stevia plants is 0.3 % dulcoside, 0.6% rebaudioside C, 3.8 % rebaudioside A and 9.1 % stevioside. Two other glycosides that may be present in plant tissue are rebaudioside D, E and rebaudioside B has been detected but is probably an artifact formed during isolation. Structures of these and other diterpenes and diterpene glucosides are presented in Figure 2. Of the four major sweet diterpene glycoside sweeteners present in Stevia leaves only two, stevioside and rebaudioside

A, have had their physical and sensory properties well characterized. Stevioside and rebaudioside A were tested for stability in carbonated beverages and found to be both heat and pH stable (Chang and Cook, 1983) and therefore have a wide range of applications in food products. However, rebaudioside A was subject to degradation upon long term exposure to sunlight. Kinghorn and Soejarto (Kinghorn and Soejarto, 1985) also cite numerous Japanese studies that demonstrate that stevioside is very stable. Phillips has summarized the early sensory research. Stevioside was between 110 and 270 times sweeter than sucrose, rebaudioside A between 150 and 320, and rebaudioside C between 40 and 60. Dulcoside A was 30 times sweeter than sucrose (Phillips, 1989).

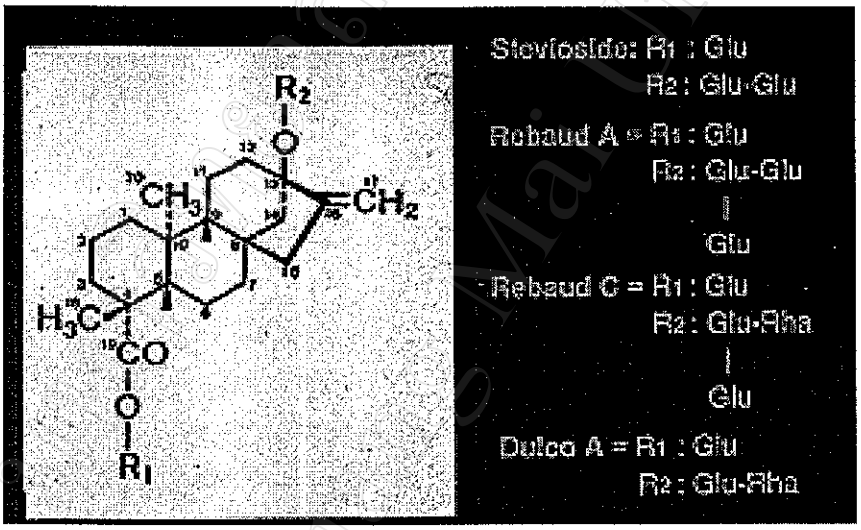


Figure 2. Chemical structure of the steviol-glycosides

(J.E. Brandle, *et al*, 1997)

1.2.2 Biosynthesis of the sweet glycosides

The steviol glycosides are synthesized via melvalonic acid in the same way as all isoprenoid compounds (Figure 3). The steps up to the formation of ent-kaurenoic acid are identical to those used in the synthesis of the plant hormone gibberellic acid. We have cloned and sequenced the copalyl pyrophosphate synthase gene from Stevia that is responsible for the conversion of Geranylgeranyl Pyrophosphate (GGPP) to *ent*-copalyl pyrophosphate (CPP). The hydroxylation of ent-kaurenoic acid at the C13 position to yield steviol is the point of divergence. Following the formation of steviol glycan side chains containing glucose and/or rhamnose are added to the C13 alcohol and C19 carboxylate groups to form the various steviol glycosides (Chappell, 1995; McGarvey and Croteau, 1995).

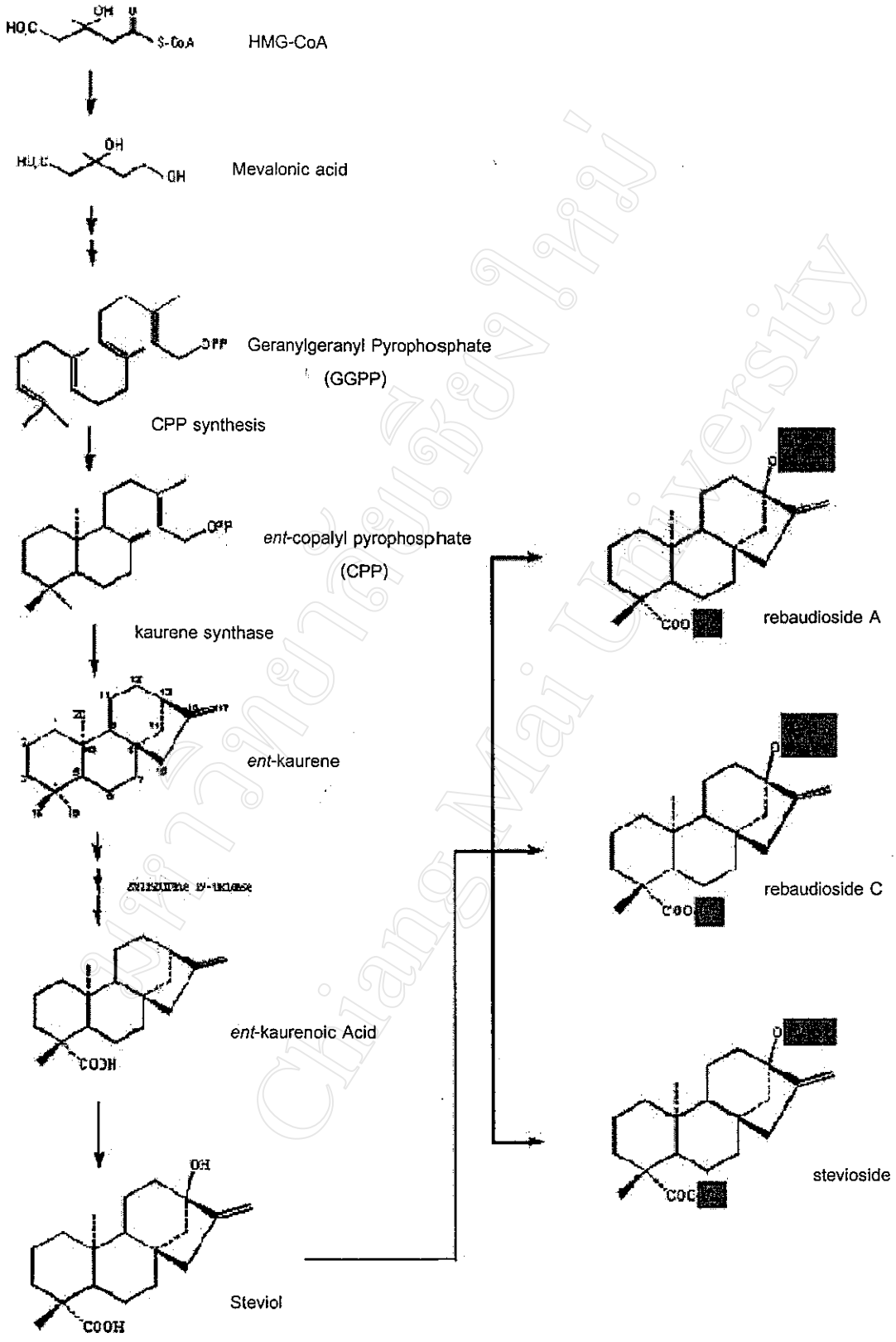


Figure 3. Biosynthetic Pathway for Synthesis of steviol glycosides

(J.E. Brandle, *et al*, 1997)

1.2.4 Safety of Stevia sweeteners

Stevia sweeteners have a long history of use in South America and now in Japan and there are no reports of adverse effects. Nonetheless, the safety of Stevia sweeteners has been the subject of controversy for a number of years (e.g. Bonvie, *et al*, 1997; Pendergast, 1991). Planas and Kuc (Planas and Kuc, 1968) reported that 5 % solution of Stevia leaf extract had a strong anti-fertility effect in both male and female rats. Subsequent studies conducted to confirm this result have all been negative (Sincholle and Marcorelles, 1989; Yodyingyuad and Bunyawong, 1991). In studies of acute toxicity, a LD_{50} of 8.2 g kg^{-1} for a refined stevioside extract was cited by Katayama, *et al*. (Katayama, *et al*, 1979). An acceptable daily stevioside intake of 7.9 mg kg^{-1} was suggested by Xili, *et al*. (Xili, *et al*, 1992). Yodyingyuad and Bunyawong reported that neither growth nor reproduction were affected in hamsters fed pure stevioside at levels up to 2.5 g kg^{-1} day^{-1} for 4 months (Yodyingyuad and Bunyawong, 1991). Stevioside and rebaudioside A are both non-carcinogenic (Das, *et al*, 1992). Pezzuto and co-workers (Pezzuto, *et al*, 1985) reported that metabolically activated steviol is mutagenic, a result that has been confirmed in another more recent study (Matsui, *et al*, 1996). Kinghorn and Soejarto (Kinghorn and Soejarto, 1985) and Kinghorn (Kinghorn, 1992) conducted two reviews of the literature related to safety of Stevia sweeteners and concluded that Stevia leaves and stevioside are safe for human consumption. However, the activated steviol metabolite that is mutagenic has not yet been identified and it is not known if the activation of steviol actually occurs in humans (Procinska, *et al*, 1991; Matsui, *et al*, 1996). Matsui *et al*. (Matsui, *et al*, 1996) concluded that further work is required to determine what risk steviol glycosides pose to humans. The production of remarkably high levels of one class of secondary metabolite is of significant interest for chemists, biochemists and geneticists and may prove to be a foundation for the production of new metabolites in the future. Because the safety of Stevia for human consumption remains controversial, there is a clear need for further experimentation with respect to the metabolic fate of steviol glycosides.

1.3 Aberrant Crypt Foci Formation

Colon cancer incidence in Thailand has been increasing in recent year. Epidemiological studies have shown that dietary habits such as the consumption of foods high in fat, low in fiber and low in calcium are associated with the development of colon cancer in humans (Weisburger *et al*, 1977; Greenward and Lanza, 1985; Doll and Peto, 1981). It has been recognized that environmental carcinogens can contribute to the development of colon cancer. Laboratory investigations into the role of diet in carcinogenesis and cancer prevention are essential for developing.

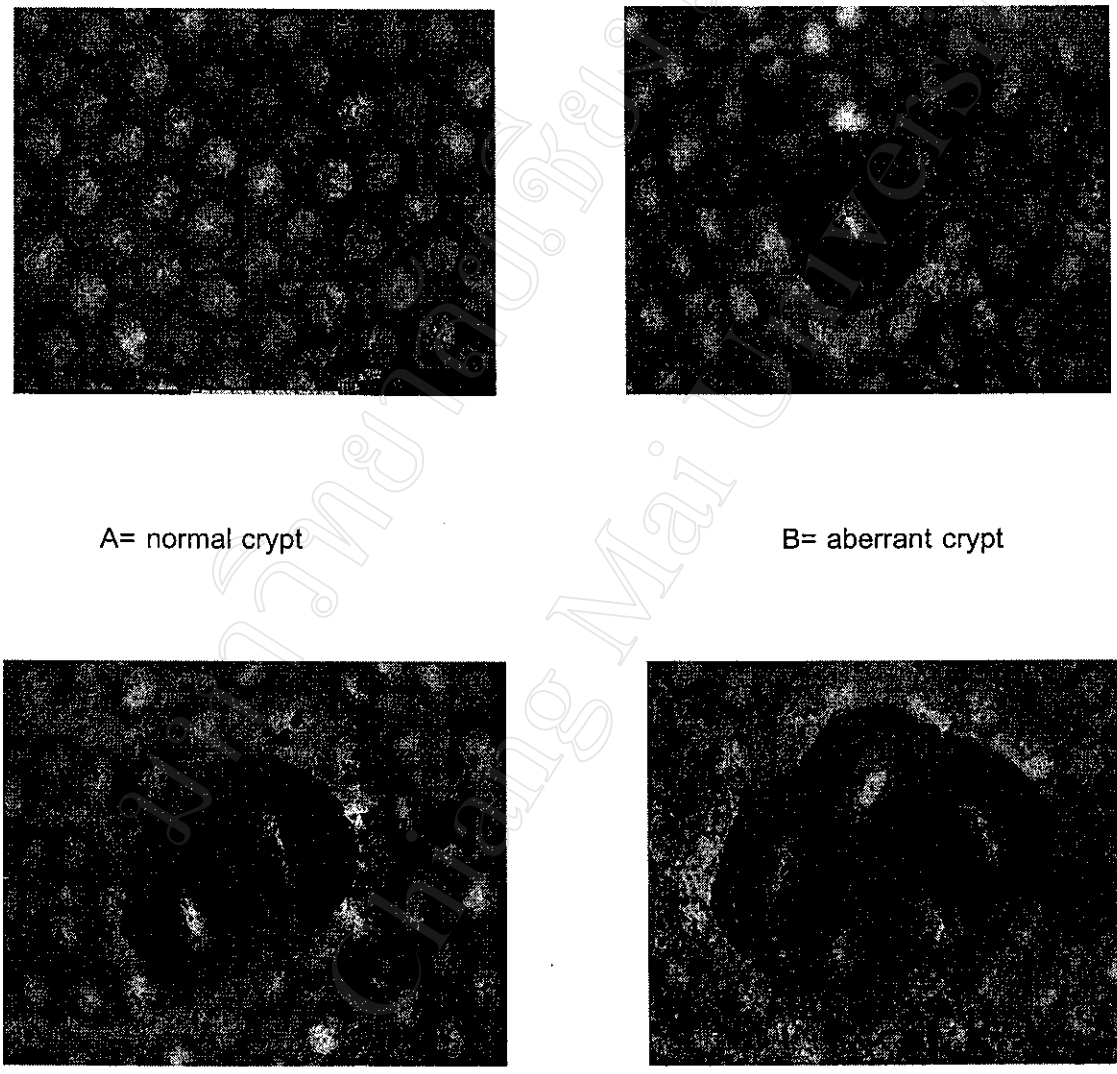
Aberrant crypt foci (ACF) were first identified in the colon of carcinogen treated rodents by a simple methodological approach (Bird, 1987). These crypts termed aberrant crypts were present only in carcinogen treated rodent colon. ACF in the colon of rodents and humans are regarded as a putative precursor lesion for colon cancer (Pretlow, *et al*, 1991) and as useful biomarkers for detecting the modulatory effects of xenobiotics on colon carcinogenesis (Wargovich, *et al*, 2000). Their number and growth features could be used to identify modulators of colon carcinogenesis, to identify the underlying cellular and molecular events leading to tumor development, and to quantify the stepwise development of colon cancer.

In mammal, the crypt constitutes a structural unit of the colonic epithelial. In the crypts, epithelial cells are produced constantly from a lower layer and migrated out of the crypt to the surface where they are extruded. The size, shape and cellularity depends heavily on the rate of proliferation, migration and loss of cells. Almost if not all, of the target cells of the colon specific carcinogens are the proliferative epithelial cells. Both direct and indirect acting carcinogens following metabolic activation may cause methylation to nuclear DNA of the colonic epithelial layer (Chang, 1984).

The methodological approach that led to the identification of ACF was based on the premise that preneoplastic changes occur in single crypts. These changes must accompany aberrant growth and instability within the crypts, leading to altered crypt morphology. This includes changes in crypt width, height and presumably the thickness of the cell wall lining the crypt. It was reasoned that early changes affecting

the luminal opening and epithelial lining of the crypts could be identified topographically.

The characteristics of ACF were distinguished from the surrounding normal crypts when stained with 0.2% methylene blue by their increase size, thicker epithelial cell lining and increase pericryptal space thicker (Bird, 1987) as shown in Figure 4.



A= normal crypt

B= aberrant crypt

C= 2 crypts/focus

D= multiple crypts

Figure 4. Morphological of ACF

1.4 Serum Aminotransferase Enzyme

Discovery of elevated values for transaminases in serum (serum aspartate aminotransferase and serum alanine aminotransferase) in subjectively unaffected individuals is one clinical problem that has increased due to more frequent collection of blood samples. The most common cause for slightly elevated values for only transaminases is liver steatosis, due to alcoholism, drugs, overweight and diabetes (Sherwood, *et al*, 2001).

1.4.1 Aspartate aminotransferase (AST)

Aspartate aminotransferase (AST) is also known as serum glutamic oxaloacetic transaminase (SGOT). AST is found in the heart, liver, skeletal muscle, kidney, pancreas, spleen, lung, red blood cells, and brain tissue. When disease or injury affects these tissues, the cells are destroyed and AST is released into the bloodstream. The determination of AST levels aids primarily in the diagnosis of liver disease. The amount of AST is directly related to the number of cells affected by the disease or injury (Jacobs and David, 1996). Although not specific for liver disease, it can be used in combination with other enzymes to monitor the course of various liver disorders. Chronic, silent hepatitis (hepatitis C) is sometimes the cause of elevated AST. In alcoholic hepatitis, caused by excessive alcohol ingestion, AST values are usually moderately elevated (Nalpas, *et al*, 1986); in acute viral hepatitis, AST levels can rise to over 20 times normal. Increased activity of AST in serum is observed in myocardial infarction after 20-36 hours of onset and hence used as a supporting evidence in the diagnosis of myocardial infarction. Values are usually less than 10 times the upper limit of normal (ULN). In cases of cirrhosis , the AST level is related to the amount of active inflammation of the liver. Determination of AST also assists in early recognition of toxic hepatitis that results from exposure to drugs toxic to the liver, like acetaminophen and cholesterol lowering medications (Pagana, *et al*, 1998).

1.4.2 Alanine aminotransferase (ALT)

Alanine aminotransferase (ALT) is an enzyme found mainly in the liver, but also in smaller amounts in the kidneys, heart, muscles, and pancreas. ALT formerly was called serum glutamic pyruvic transaminase (SGPT). ALT is measured to determine if the liver is damaged or diseased. Low levels of ALT are normally found in the blood. However, when the liver is damaged or diseased, it releases ALT into the bloodstream, causing levels of the enzyme to rise. Although ALT is found in organs other than the liver, most increases in ALT levels are due to liver damage. Increased ALT activity is observed in hepatitis and cirrhosis. Values may be increased to >10 times – 100 times ULN in hepatitis. The ALT test often is done along with other tests that can determine if the liver is damaged, including aspartate aminotransferase (AST), alkaline phosphatase, lactic dehydrogenase (LDH), and bilirubin. ALT is the test usually used to detect liver injury (Jacobs and David, 1996).

1.5 Glutathione

Glutathione (g-glutamylcysteinylglycine, GSH) (Figure. 5) is a sulfhydryl (-SH) antioxidant, antitoxin, and enzyme cofactor. GSH plays a role in such diverse biological processes as protein synthesis, enzyme catalysis, transmembrane transport, receptor action, intermediary metabolism, and cell maturation. GSH is ubiquitous in animals, plants, and microorganisms, and being water soluble is found mainly in the cell cytosol and other aqueous phases of the living system (Kosower and Kosower, 1978; Lomaestro and Malone, 1995). Glutathione status is homeostatically controlled, being continually self-adjusting with respect to the balance between GSH synthesis (by GSH synthetase enzymes), its recycling from GSSG (by GSH reductase), and its utilization (by peroxidases, transferases, transhydrogenases, and transpeptidases).

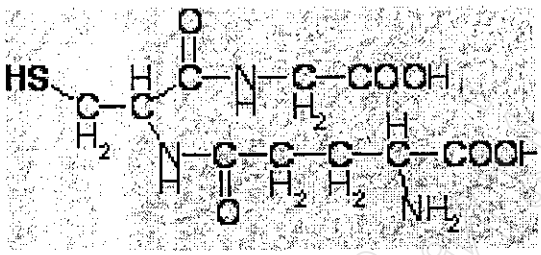


Figure 5. Structure of glutathione (GSH, γ-L-glutamyl-l-cysteinylglycine)

Many evidence indicated that the cumulative damaging effects of oxygen radicals and other oxidants are principal contributors to degenerative diseases, and to the progressive loss of organ functions that they recognize as aging (Cross, 1987). GSH also helps protect against exogenous oxidative insults as shown in Figure 7. The functional diversity of the molecule is rendered possible by the chemical properties of the thiol group which can participate in redox transitions, thiol exchange reactions, thioether formation, and may act as radical scavenger.

The oxidized form is a sulfur-sulfur linked compound, known as glutathione disulfide or GSSG. The GSSG/GSH ratio may be a sensitive indicator of oxidative stress. Glutathione is present inside cells mainly in its reduced (electron-rich, antioxidant) GSH form. Intracellular GSH status appears to be a sensitive indicator of the cell's overall health, and of its ability to resist toxic challenge. GSH abnormalities linked to tissue and organ system breakdown, and explores the possibilities for GSH replacement therapy to benefit degenerative conditions. Experimental GSH depletion can trigger suicide of the cell by a process known as apoptosis (Duke, *et al*, 1996; Slater, *et al*, 1995). GSH depletion has been suggested to represent an important contributory factor to liver injury, and to enhanced morbidity related to liver hypofunction (Lomaestro and Malone, 1995). Oxidative stressors such as cigarette smoke, atmospheric pollutants, and other inhaled environmental toxins results in depletion of GSH and other antioxidants from the lungs. (Pacht, *et al*, 1991; Bunnell

and Pacht, 1993). Many pharmaceutical products are oxidants capable of depleting GSH from the liver, kidneys, heart, and other tissues (Hoyumpa, 1996). The popular over-the-counter drug acetaminophen is a potent oxidant. It depletes GSH from the cells of the liver, and by so doing renders the liver more vulnerable to toxic damage.

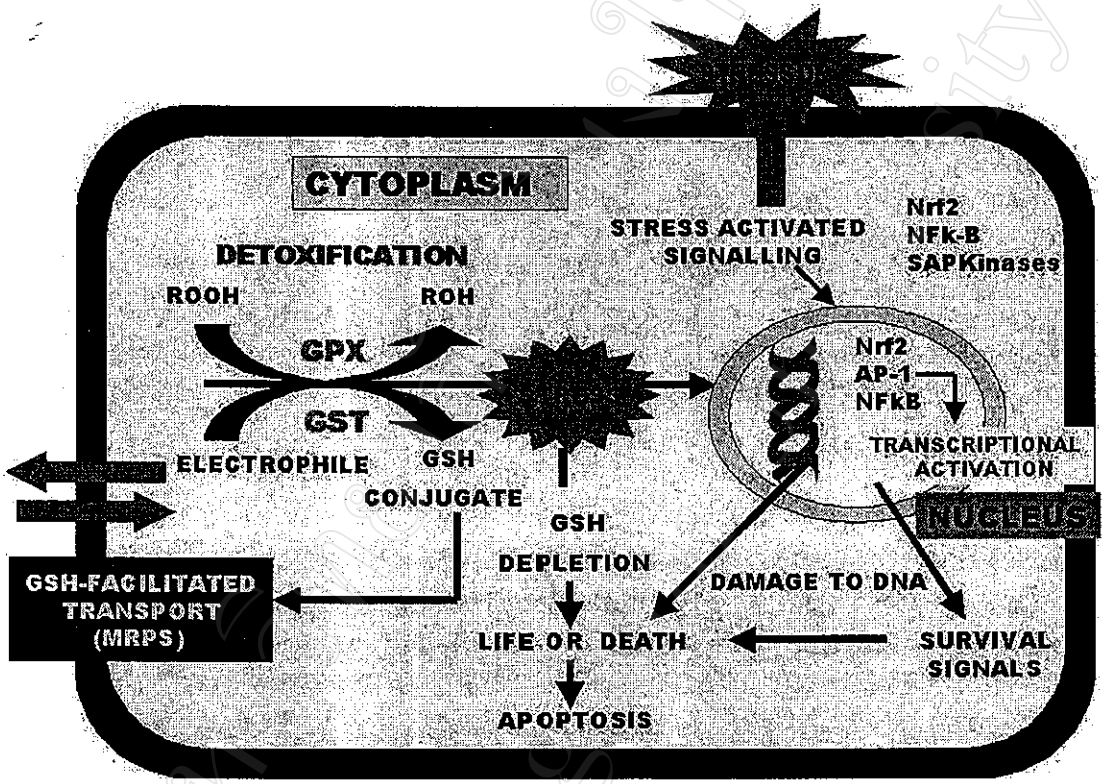


Figure 6. The role of glutathione-dependent pathways in cell survival
(C. Roland Wolf, *et al*, 1999)

1.6 Glutathione-S-transferase (EC 2.5.1.18)

Glutathione S-transferases (GSTs) are ubiquitous multifunctional proteins involved in the detoxification of endogenous and xenobiotic compounds using either glutathione conjugation, glutathione peroxidase activity or passive/sacrificial binding. This process involves conjugation of glutathione (GSH) with electrophilic metabolites and extrusion of the conjugate out of the cell for further metabolism. Some GSTs have

also specific roles in the binding and transport of ligands such as bile acids, haematin and fatty acids (Abramowitz *et al*, 1988). This enzyme promotes the displacement by the sulfhydryl group of GSH on electrophilic groups of generally hydrophobic molecules. The reaction products are commonly thiolesters and their formations represent the first step in mercapturic acid synthesis, which are readily excreted. An ATP-dependent efflux pump that mediates the export of GSH conjugates from cell has recently been described. The reaction can be shown as;

$$R + GSH \xrightarrow{GST} R\text{-}S\text{-}G$$

R ; elctrophilic xenobiotic

1.6.1 Glutathione-S-transferase Isoenzymes

GST are a super-gene family of isoenzymes that are responsible for catalyzing the conjugation of wide range of structurally diverse electrophilic xenobiotics and endogenous substrates with GSH, facilitating their excretion from the body. At least five separate gene families of GST exist; these include both cytosolic (named Alpha, Mu, Pi and Theta) and a microsomal GST (Mannervik *et al*, 1985). Cytosolic GST are abundant soluble homo-or hetero–dimers consisting of polypeptides of about 25 kDa. Another form, microsomal GST, have 17 kDa subunits attached in the endoplasmic reticulum membrane.

Based on sequence similarity, mobility on SDS/PAGE and isolectric points, members of the Alpha family are designated Ya (or 1) and Yc (or 2) subunit; members of the Mu family are Yb1 (or 3), Yb2 (or 4), Yb3 (or 6) and Yn subunit; the Pi family appears to be a single pi gene whose product is designated Yp (or 7) subunit and the more recently recovered Theta, including subunit 5, 12 and Yr. They are expressed in a tissue- and development-specific fashion. It is expected that individuality in the levels of expression of specific isoenzymes may lead to individuality in the response to

external toxins and carcinogens. The three-dimensional structures of GST each subunit binds two molecules of a competitive inhibitor S-hexylglutathione is shown in figure 7.

Figure 7. The three-dimensional structure of two subunits in the dimer of GST and GSH-binding site (Ellie A., 2001)

1.6.2 GST as a tumor marker and susceptibility to toxic chemical

GST function catalytically to conjugate glutathione (GSH) with a wide variety of electrophilic substrates but also may serve as intracellular stoichiometric binding proteins for structurally diverse lipophilic ligands. The proteins are considered to serve in a detoxification capacity to protect cells from those types of noxious substances. Dietary components or substances from the environment that induce elevated levels of GST and other phase II drug metabolizing enzymes in rodents, are therefore thought to represent chemoprotective or anticarcinogenic agents for human beings. By virtue of GST increases, these inducer compounds may indeed ameliorate the activity of cytotoxic agents but could also adversely affect responses to administered drugs and cancer chemotherapeutic agents. It has been demonstrated that GST activity can be increased in extrahepatic organs after feeding mice with the anticarcinogen butylated hydroxyanisole (BHA) (Nijhof and Peter, 1992). Enhancing activity of GST detoxification

potential could increase the capacity to withstand the burden of daily exposure to toxicants and carcinogen (Carr, 1985). Furthermore, changes in GST isoenzyme profile without alteration of the total enzyme activity may result in a different risk of DNA damage due to the different substrate specificities of each of the various GST isoenzyme (Pickelt and Lu, 1989).

There is a general pattern of GST isoenzymes specificity with regard to resistance to individual classes of drugs. The alpha class is most likely to be associated with alkylating agents, and in particular, nitrogen mustards. On the contrary, the mu form of GST has been shown to be elevated in a nitrosourea-resistant subline, and there is direct evidence that it catalyzes a denitrosation reaction that detoxifies this class of drugs (Smith *et al*, 1989). GST pi is elevated in a number of cell lines selected for resistance to doxorubicin (Bastist *et al*, 1986), and transfection of the GST pi full-length cDNA conferred low-level resistance in a particular dose range to doxorubicin selectively (Nakagawa *et al*, 1990).

The concentration of the various GST isoenzymes in normal and tumoral tissues is important because these enzymes play a central role in the detoxification of many electrophilic toxic compounds, including carcinogens and cytotoxic drugs. Given the great diversity of GST functions, several authors (Satoh *et al*, 1985; Soma *et al*, 1986) have postulated that the variability in the expression of these enzymes could be a factor in the susceptibility of various tissues to toxins and carcinogens, and they suggested that these enzymes may be used as potential prognostic markers in carcinogenesis. It has been postulated that the GST enzymes and the genes encoding these may be involved in susceptibility to cancer (Rebbeck, 1997). GST-μ and τ have been shown to be genetically polymorphic and are not expressed in 40-50% of the human population. Several studies have suggested that cigarette smokers deficient in the expression of GST-μ are at increased risk for lung (Seidegard *et al*, 1990), bladder (Bell *et al*, 1993), and larynx (Lafuente *et al*, 1993) cancer. Zhong *et al*. reported study of colorectal cancer and *GSTM1*, *GSTT1* found a significantly raised relative risk associated with the *GSTM1* and *GSTT1* null genotype. In mouse, GST-μ was detected

in all tissues investigated but was markedly decreased in mouse colon adenocarcinoma Co38, compared to normal colon. It is possible that GST-μ could play a key role in the malignant phenotype of this mouse colon (Massaad *et al*, 1992). Increased expression of the human π class GST has recently been associated with malignant transformation in rat (Kitahara *et al*, 1984) and human (Moscow *et al*, 1989) tumors. Elevated levels of GST- π protein and RNA have been found in human lung (Ilio *et al*, 1985), breast (Moscow *et al*, 1988), colon and gastric carcinomas (Kodate *et al*, 1986) in comparison with normal surrounding tissue. Silencer activity may provide an explanation for the circumstance that various agents that are effective in primary cultures of adult human hepatocytes are unable to induce GST-α expression in HepG2 cells. Instead, in HepG2 cells the phorbol ester TPA and calcium ionophores elicit a dramatic downregulation of GST- α mRNA by a posttranscriptional mechanism which can be inhibited by inhibitors of protein kinase C. The loss of GST- α expression observed in several human cancers could therefore be explained by loss of HNF-1 activity, activation of the silencers, or posttranscriptional downregulation due to activation of a protein kinase C dependent pathway (Clairmont *et al*, 1994; Eickelmann *et al*, 1995).

1.7 Objectives of the study

To investigate the toxicity of 35 and 100 days administration of Matoom juice containing Stevia extract as sweetener to male Wistar rats in the following effects in initiation and promotion stage;

1. Aberrant crypt foci formation in colon at initiation and promotion stage
2. AST and ALT activities in serum
3. GSH content in liver and intestinal mucosa
4. GST activity and subunit composition in liver and intestinal mucosa