Cruciferous Plants: Phytochemical Toxicity Versus Cancer Chemoprotection

Mohamed E. Assayed*,[1,2] and A. M. Abd El-Aty*,[3]

[1]*Department of Environmental and Radiological Health Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, Colorado 80523, USA;* [2]*Department of Forensic Medicine and Toxicology, College of Veterinary Medicine, Menoufiya University, Sadat City Branch, Egypt;* [3]*Department of Pharmacology, Faculty of Veterinary Medicine, Cairo University, 12211-Giza, Egypt*

Abstract: The Cruciferae (also known as the *Brassicaceae*) are the family of plants that include the various familiar members of the species *Brassica oleracea* (e.g., broccoli, cabbage, cauliflower, kale, Brussels sprouts) as well as many other plants that are widely consumed in various parts of the world. Forage and root brassicas are widely used as winter feeds for cattle and sheep. A striking and characteristic chemical property of cruciferous plants is their high content of glucosinolates (more than 120 types), which often approaches 1% or more of their dry weight. The interest devoted to this group of natural products is caused by the appreciable biological effects of both the intact glucosinolates (GSLs) and especially the complex group of glucosinolate transformation products produced in non-enzymatic and enzymatic reactions. Depending on the concentration and structural types of these compounds, their biological effects can be toxic, antinutritional or beneficial to health. Most serious economic problems in livestock seem to result from rapeseed meal; arising from GSLs or their breakdown products. In contrast, GSLs and their isothiocyanate (ITC) hydrolysis products are reportedly well-known protectors against carcinogenesis. GSLs play further protective and evolutionarily important roles in plants. These include allelopathy (suppression of growth of neighboring plants), specific positive and negative feeding cues for some insects and broad antibiotic properties including nematocidal, antimicrobial, antifungal, antiprotozoal and insecticidal activities. The controversy in the referred actions contributed to crucifers' phytochemicals has been exclusively discussed.

Key Words: *Brassicaceae, Cruciferae,* glucosinolates, GSLs, isothiocyanate, ITC, phytochemicals, toxic, cancer chemoprotection.

INTRODUCTION

The Cruciferae (also known as the *Brassicaceae*) are the family of plants that include the various familiar members of the species *Brassica oleracea* (e.g., broccoli, cabbage, cauliflower, kale, Brussels sprouts) as well as many other plants that are widely consumed in various parts of the world but not very often in the United States, such as oriental cabbage, arugula, watercress, radish, daikon, wasabi and various mustards. Regional patterns of crucifer consumption vary substantially in different parts of the world; a striking example is the huge consumption of daikon (*Raphanus sativus*; 20 kg/y or 55 g/d) in Japan, where it is the most popular vegetable [1].

Forage and root brassicas are widely grown in Britain, and to a lesser extent in Canada, Sweden, and the USA to be used as winter feeds for cattle and sheep. Many common vegetables such as broccoli, Brussels sprouts, cabbage and cauliflower, which are traditionally important constituents of the human diet, belong to this plant family. At the same time the genera *Brassica, Crambe, Sinapis and Raphanus* include important oil- and protein-rich agricultural crops used for the production of plant oils, such as rapeseed oils. The press cake is used as animal feed material. The increased demand for plant oils for technical purposes has recently raised interest in *Camelina sativa* (false flax) and its possible use as an alternative oilseed crop and as a source of materials for animal feed. Glucosinolates and their miscellaneous breakdown products are generally known as mustard oil glucosides or thioglucosides. They confer the distinctive flavor and bitter taste to the food plants. The intensity flavor and taste depends on the glucosinolate concentration of an individual plant and the degree of hydrolysis releasing an array of breakdown products. In oilseed crops [*Brassica napus* (rapeseed of Canola), *B. campestris* (turnip rape) and *Sinapis alba* (white mustard)] the hydrophilic glucosinolates remain in the seed meal after extraction of the oil and hence are present in the seed meal fraction used in animal diets [2].

At present, more than 120 different GSLs have been identified in plants [3]. Although the majorities have been isolated from crucifers, 15 other families of plants are known to contain GSLs. The interest devoted to this group of natural products is caused by the appreciable biological effects of both the intact GSLs and specially the complex group of

*Address correspondence to these authors at the Department of Forensic Medicine and Toxicology, College of Veterinary Medicine, Menoufiya University, Sadat City Branch, Egypt; Tel: +202-25190923;
Fax: +20-482603214; E-mail: memzat@hotmail.com and
Department of Pharmacology, Faculty of Veterinary Medicine, Cairo University, 12211-Giza, Egypt; Tel: +202-27548926; Fax: +202-35725240;
E-mail: abdelaty44@hotmail.com

1389-5575/09 $55.00+.00

glucosinolate transformation products produced in non-enzymatic and enzymatic reactions. Depending on the concentration and structural types of these compounds, their biological effects can be toxic, anti-nutritional or beneficial to health. According to their structure, GSLs have been classified as aliphatic, aromatic, ω-methylthioalkyl and heterocyclic (indole-) glucosinolates [3].

Most serious economic problems in livestock seem to result from rapeseed meal; arising from GSLs or their breakdown products. GSLs are glycosides of B-D thioglucose. The aglycones of these compounds can yield an ITC, thiocyanate, nitrile, or similar compound. Problems of anemia and gastrointestinal upset are noted most often. Reproductive failure and goiter are more insidious. Scouring, rumen stasis, jaundice, decreased milk production, growth retardation, poor conception, poor growth, enlarged thyroids; possibly due to antithyroid factors and anemia are also common clinical signs. Lesions like swollen, pale, or hemorrhagic liver (in poultry fed rapeseed meal), hepatic necrosis, extensive hemosiderosis in spleen and kidney, renal tubular epithelial degeneration are common findings [4].

On the other hand, GSLs and their ITC hydrolysis products are reportedly well-known protectors against carcinogenesis. The relatively large consumption of glucosinolates by many individuals, in comparison with other plants, currently under study as potential sources of chemoprotective activity, adds special significance to these compounds. Since the early 1960s, both natural and synthetic ITCs have attracted considerable and growing attention as important and effective protectors against chemical carcinogenesis in a number of animal models [5-7]. Although only a few GSLs have been examined, largely because adequate quantities of these compounds have been unavailable, some are very effective in inhibiting carcinogenesis [8-10].

1. THE BIZARRE PHYTOCHEMISTRY OF CRUCIFERS

1.1. Glucosinolates and Isothiocyanates

A striking and characteristic chemical property of cruciferous plants is their high content of GSLs, which often approaches 1% or more of their dry weight [11]. GSLs are *β*-thioglucoside N-hydroxysulfates (Fig. **1**) and are the precursors of ITCs (mustard oils). GSLs play protective and evolutionarily important roles in plants. These include allelopathy (suppression of growth of neighboring plants), specific positive and negative feeding cues for some insects and broad antibiotic properties including nematocidal, antimicrobial, antifungal, antiprotozoal and insecticidal activities. GSLs are invariably accompanied in plant cells by the enzyme myrosinase (a *β*-thioglucosidase), which is normally physically segregated from its glucosinolate substrates but is released

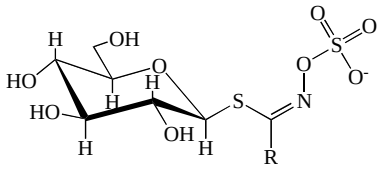

Fig. (1). Glucosinolate structure. Side group *R* varies.

and hydrolyzes GSLs to ITCs and other products when plants are injured by predators or when food is prepared or chewed. This reaction is responsible for the development of the sharp taste of horseradish, mustard and wasabi. In the absence of myrosinase, for example, when plants are cooked and myrosinase is heat inactivated, humans can efficiently convert GSLs to ITCs through the action of the microflora of the gastrointestinal tract [12].

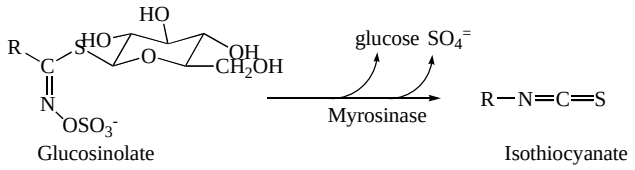

Fig. (2). Conversion of glucosinolates to isothiocyanates by plant myrosinase.

For the producing plants, GSLs are part of an innate defense system. They convey different signals to herbivorous insects in attracting parasitic wasps and favoring or opposing oviposition by insects. The defense system involves also thioglucosidases commonly known as myrosinases. In the intact plant, glucosinolates and myrosinases are sequestered in different compartments. Upon plant damage (for example by insects, or by chewing during ingestion of plant material), an enzymatic reaction takes place catalysed by myrosinases, and resulting in the formation of isothiocyanates, thiocyanates, oxazolidinethiones (including 5-vinyl-2-oxozolidinethione (VOT) and 5-vinyl-1,3-oxyzolodine-2-thione (5-VOT) as well as nitriles and epithionitriles [13].

1.2. Toxicokinetics, Metabolism and Tissue Distribution

Numerous studies describing the metabolism and toxicokinetics of a number of glucosinolates and their metabolites indicate that the metabolism of isothiocyanates produced after myrosinase activity, chiefly the microbial hydrolysis of the glucosinolates in the gastrointestinal tract, shows wide qualitative and quantitative interspecies variations. The *in vivo* metabolism of several natural isothiocyanates has been studied in rodents and humans. For instance, the N-acetylcysteine conjugates of Allyl isothiocyanate (AITC) and phenethyl isothiocyanate (PEITC) are excreted in the urine of mice, although the major metabolite of PEITC in mice is a cyclic mercaptopyruvic conjugate [14]. However, the N-acetylcysteine conjugates are the major urinary metabohites in rats treated with AITC and benzyl isothiocyanate (BITC) [15]. In humans, the N-acetylcysteine conjugates of BITC and PEITC appear to be the only urinary metabolites following ingestion of *brassica* vegetables. Conjugations of ITCs with glutathione appear to be the major metabolic pathway in humans, since most of their urinary metabolites are mercapturic acids or other derivatives from glutathione conjugates [14]. Although the Phase II enzyme glutathione S-transferase-catalyzed conjugation of isothiocyanates is considered to be a natural detoxification process, it has been postulated that this pathway may also be involved in the cytotoxicity of isothiocyanates. The glutathione conjugates of isothiocyanates are usually subject to further degradation to give final metabolites, the N-acetylcysteine conju-

gates of isothiocyanates, by enzymes such as γ-glutamyl transpeptidase, cysteinyl glycinase, and N-acetyl transferase [16]. In contrast, the dog excretes the glycine conjugate, hippuric acid, possibly because of hydroxylation of the benzylic moiety with subsequent oxidation and conjugation of benzoic acid [17,18]. Data from studies in mice demonstrated the excretion of the mercapturic acids for some compounds such as the allyl and phenyl isothiocyanates but these only constituted 6-10 % and 9-15 % of the dose, respectively, and most of the excreted metabolites correspond to cysteine conjugates of thiocyanate (90% for AITC) and the mercaptopyruvic acid conjugate of phenyl isothiocyanates (26 - 32 %) [14,19]. A recent study on the pharmacokinetics of the isothiocyanates indole-3-carbinol as a hydrolysis product of glucobrassicin showed rapid absorption, distribution, and elimination from the plasma and the tissues, falling below the limit of detection within one hour. Six-fold higher concentrations in the liver as compared to the plasma were found. The metabolic route for this compound involves acid condensation to carboxylic acid and a minor oxidative metabolite carboxylaldehyde metabolite [20].

1.3. Phytochemicals Toxicity of Crucifers

Data on the toxicity of individual GSLs for food-producing animal species are very limited, and in most cases only the total glucosinolate content in a given feed material, measured indirectly though the quantification of hydrolysable glucose is available. Only for rapeseed meal or press cakes comprehensive feeding trials in farm animals have been conducted, resulting in the recommendation to restrict the total glucosinolate content to 1 – 1.5 mmol/kg feed for monogastric animals, and to even lower concentrations in feeds for young animals. Forage brassica species are widely used in temperate agricultural systems as a feed for ruminants but unfortunately contain representatives of two groups of plant secondary compounds which have potentially harmful effects to ruminants; as they contain glucosinolates; plant thioglucosides which undergo hydrolysis when the vegetative part of the plants are damaged to yield a range of potentially toxic compounds, including nitriles, isothiocyanates, VOT and thiocyanate ions. They mentioned that glucosinolates, such as sinigrin, and S-methyl cysteine sulphoxide (SMCO), found in forage brassica species, have been implicated in the low intakes observed among lambs consuming such diets [21].

The range of products arising as a result of glucosinolate hydrolysis is dependent on the chemical environment in which hydrolysis occurs as well as the identity of the parent glucosinolates [22]. For example, low pH tends to favor nitrile production, with increasing pH leading to greater production of isothiocyanates. Furthermore, sinigrin hydrolysis yields allyl cyanide (ACN) as the nitrile metabolite while the higher homologue, gluconapin, yields butenyl cyanide. Different nitrile or isothiocyanate compounds differ in their toxicity as a result of their different disposition following ingestion [15,23]. Toxicity, however, is almost certainly associated with the presence of the C-N or N=C=S groups and assumptions can be drawn on the mechanisms of toxicity, if not the magnitude of toxicity of particular compounds, from findings on related compounds [21].

The toxicity of glucosinolate-derived nitriles is generally attributed to their effects on the liver and kidney [24]. A number of histological studies in non-ruminants have demonstrated cellular damage in liver and kidney tissues following nitrile administration [25,26]. Another potential route of nitrile toxicity is suppression of cellular respiration following release of free cyanide in the tissues and consequent inhibition of cytochrome oxidase activity [27-29]. The toxic effects of isothiocyanates include effects on thyroid function [30,31], more direct toxic effects may arise as a result of their extremely electrophilic nature and their capacity to bind the sulphydryl groups of biologically important molecules [32,33].

Additionally, the toxicity of glucosinolates for humans and farm animals has been associated particularly with the formation of thiocyanates, oxazolidinethiones and nitriles. These compounds interfere with iodine uptake (thiocyanates) and the synthesis of the thyroid hormones T3 and T4, (oxazolidinethiones) leading eventually to hypothyroidism and enlargement of the thyroid gland, a disease referred to as "goiter" [34,35]. As a consequence of these changes on thyroid function, the metabolism in almost all tissues, including the reproductive organs is affected. Subsequently, a reduction in the fertility of male and female animals is observed. Moreover, various products of glucosinolate hydrolysis cause irritation of the gastro-intestinal mucosa followed by local necroses, and hepatotoxicity [18,36-39]. Subsequently, the major clinical signs of toxicity described in farm animals include growth retardation, reduction in performance (milk and egg production), impaired reproductive activity, and impairment of liver and kidney functions, the latter being attributed to the formed nitriles [36].

1.4. Toxic Clinical Signs Observed on Livestock

Intoxications following the consumption of glucosinolate-containing plants have been described in all major farm animal species. However, many of the reports refer to old case studies in which the actual intake (dose) and the nature of the glucosinolate or products derived there from are not given. Moreover, the clinical cases described below are reported in relation to the ingestion of rapeseeds and rapeseed meal prior to the implementation of double-low varieties. In general, the common clinical signs following exposure to intoxicating levels of glucosinolates are:

1) Alterations of the thyroid metabolism and enlargement of the thyroid gland following the ingestions of isothiocyanates and oxazolidinethione [40].

2) Irritation and local necroses of the gastro-intestinal mucosa by alkyl isothiocyanates [41].

3) Growth retardation [42,43].

4) Liver damage with increased enzyme leakage [41,44,45].

5) Impairment of fertility following long-term exposure to glucosinolate containing plants [46,47].

6) Transient impairment of locomotion, behavioural changes and disorientation (rape blindness) probably caused by isothiocyanates [48,49].

The toxic principle of two members of Crucifer weeds; tansy mustard (*Descurainia pinnata brachycarpa)* or flixweed *(Decurainia sophia)* is not known. The plant is most often eaten when it is young and succulent, and it is at this stage that it is most toxic. The toxicity of the plant varies from year to year. The neurological signs such as blindness, head-pressing seen with tansy mustard poisoning are suggestive of sulfate poisoning. Other toxic signs include difficult chewing and swallowing because the animal's tongue appears to be paralyzed, weight loss, severe photosensitization occurs in cattle grazing Sophia (flixweed) and tansy mustard in early spring [50].

1.4.1. Toxic Effects in Horses

In horses, there is only one case report dealing with rapeseed oil intake in association with respiratory disease [51], but this report does not provide information on the clinical signs induced. Nonetheless, ingestion of certain mustards and possibly other members of the Brassica family by mares during late pregnancy could cause a condition called "Congenital Hypothyroid Dysmaturity Syndrome" in foals. The syndrome most often results when the consumption of mustards occurs in the last 2 to 3 months of pregnancy. Signs of the condition are: 1) Abnormally long pregnancy; 2) Foals commonly born with facial and lower jaw deformities; 3) Foals born with the fine skin and soft, silky hair coat of premature foals even though they are carried longer than normal; and 4) Deformities of the limbs (incomplete ossification of the cuboidal bones of the limbs, flexural deformities of the forelimbs, and commonly ruptured digital extensors. 5) Some had hydrocephalus and some patent urachus. The abortion and fatality rate of the syndrome is quite high and many of the foals born alive have to be destroyed because of severe deformities. This syndrome most often occurs in mares that are bred late in the breeding season and fed hay year-around that is contaminated with mustard plants. However, it can also occur when mares are kept stalled during the winter and then let out in the early spring to exercise yards or weedy pastures that contain winter annuals in the mustard family, such as Blue mustard, Jim Hill mustard, Shepherd spurse (*Capsella bursa-pastoris*), Blue mustard (*Chorispora tenella*) and Flixweed (*Descurainia sophia*). Other Brassica species commonly incriminated are Clasping pepperweed, (*Lepidium perfoliatum*) Field pennycress (*Thlaspi arvense*) and Tumble mustard (Jim Hill mustard, *Sisymbrium altissimum*). There is also a risk of this syndrome if a significant amount of mustards are present in the alfalfa or grass hay that is fed in late pregnancy. Mustards are often found in newly seeded alfalfa fields and first cutting hay [52,53].

1.4.2. Toxic Effects in Ruminants

Glucosinolate poisoning in cattle has been reported by several authors [46,49,54-58]. Clinical symptoms include poor productivity, reduced fertility (prolonged interval from calving to conception) and poor body condition [46].

Growing cattle tolerated dietary concentrations of glucosinolates up to a level of 10 - 15 mmol/kg and did not show any detrimental effects on growth and feed conversion [59]. Calves tolerated a level of 7.71 mmol/kg [37]. Cows showed signs of toxicity, thyroid dysfunction and depressed fertility following a daily intake of 44 mmol/day (equivalent to 31 mmol/kg dry matter) [47].

Although ruminants are generally considered to be less sensitive to glucosinolates in their feed, as compared to monogastric species; including pre-ruminant calves, as the rumen flora can degrade various glucosinolate breakdown products, however, a case of an acute intoxication of cattle by *Brassica oleracea ssp. Acephala* was described, resulting in acute death within 24 hours of two cows and acute illness in one cow out of thirty exposed animals (the glucosinolate concentrations are not given). The primary pathological lesions were vascular damage and oedema of the rumen mucosa [54].

Photosensitization; as an important clinical sign; was later reported in a number of cases of *Brassica* intoxications in cattle [56].

A reduction in fertility and a mild suppressive effect on thyroid function were found after feeding cows a ration containing more than 3 kg of double-low rapeseed per day during three consecutive lactations [47]. The authors suggested Glucosinolates as undesirable substances in animal feed that an amount of 3 kg/day could be considered as maximal tolerable inclusion rate of double low rapeseed in the diet of dairy cows.

1.4.3. Toxic Effects in Small Ruminants

A report of glucosinolate poisoning in lambs mentions swayback, anaemia and visibly enlarged thyroid glands as major clinical signs [46]. Lambs feeding on Brassica developed hypothyroidism [60]. Body weight losses were reported in sheep following a glucosinolate intake of 2.5 – 7.6 mmol/day (equivalent to 1.2 - 2.2 mmol/kg feed dry matter) [61]. In lambs, dietary levels of up to 10 mmol/kg affected digestibility only insignificantly without any clinical signs of intoxication, whereas levels above 10 mmol/kg reduce growth [62]. Glucosinolate intakes of 0.24 - 0.69 μmol/day affected thyroid function in lambs prior to weaning, whereas after weaning only much higher levels of 1.6 - 3.9 mmol/day affected thyroid function. This relatively high tolerance of sheep suggests that at least sheep for fattening might tolerate diets with low glucosinolate rapeseed as the sole protein source [63].

Ewes showed an impaired fertility with significantly reduced oestradiol levels following dietary exposure to 1.2 - 1.6 mmol/kg dry matter [61]. In male animals fed high glucosinolate *Brassica juncea* meal (glucosinolate amount not known), reduced testosterone levels and impaired semen quality were reported. These effects were reversible when the diet was supplemented with iodine [64].

1.4.4. Adverse Effects in Pigs

Pigs are among the most sensitive animal species regarding acute adverse effects of glucosinolates. Typical effects attributable to rapeseed meal produced from rape with high glucosinolate levels, were a delayed sexual maturity, impaired conception rates and a decrease in the number of piglets born alive, and weaned as well as reduced feed intake and growth retardation were consistently reported [43]. Feeding diets containing either 100 g (equivalent to 2 mmol

total glucosinolates/kg) or 250 g (equivalent to 10 mmol total glucosinolates/kg diet) of rapeseed meal per kg feed to sows in late gestation and during lactation resulted in a slight decrease in litter weight and hypothyroidism in the piglets, probably due to a reduced iodine excretion with the sows' milk [65,66]. Similar symptoms including hypothyroidism, characterized by low levels of thyroid hormones and increase in thyroid gland weight were observed by previous scientists [40]. Dietary levels between 9 - 10.1 mmol/kg induce iodine deficiency and an increase in the serum level of T3 and T4 followed by thyroid hypertrophy [36,37]. From these experimental data it was concluded that dietary total glucosinolate levels for pigs should remain below 2.1 mmol/kg diet and that when diets containing glucosinolates are used, sufficient iodine (1000 μg/kg diet) should be supplemented [66,67].

1.4.5. Adverse Effects in Poultry

Feeding rapeseed meal containing high glucosinolate levels (the concentration is not reported) in amounts of 200 g/kg diet to laying hens resulted in liver haemorrhage, liver enlargement, reticulolysis (lysis of the endoplasmatic reticulum of hepatocytes) and lymphoproliferation. The high glucosinolate level di*et al*so significantly reduced egg production and plasma urate levels [41]. At lower concentrations, glucosinolates decreased only feed intake and weight gain [42]. Little effect on growth rates of broiler chicks was reported at total glucosinolate levels of 2 – 4 mmol/kg of diet (equivalent to about 20 % Canola meal in the diet), although a significant reduction in daily weight gain was evident when the inclusion rates in the diet exceeded 10 mmol/kg [36,37].

In turkeys fed rapeseed products for 16 weeks (glucosinolate concentration not given), liver cirrhosis accompanied by hydropericardium was observed. Histological examination revealed an extensive fibrosis, characterized mainly by reticular fibers, and centrolobular degeneration of parenchymal liver cells. At twelve weeks, multiple focal necrotic sites appeared, preceding the ultimate fibrotic degeneration [44]. In a recent 37-day study, starting with 1-day broiler chickens, the effect of *Camelina sativa* expeller cake (5 and 10 %) on their growth performance and their meat quality was investigated. Generally, at both concentrations *Camelina sativa* cake significantly impaired the growth of the broiler chickens in a linear fashion between day 15 and day 37 as well as their feed intake. However, the relative organ weights of the thyroid glands were not increased, and there were no gross signs of liver toxicity in these animals [68].

1.5. Tissue Residues of Glucosinolates and their Metabolites

Following exposure of farm animals to forages and concentrates containing glucosinolates, a carry over of glucosinolates and their associated breakdown products into edible tissues, milk and eggs has been described, but the rate of carry-over is very low. The measurable residues in dairy milk corresponding to approximately 0.1 % of the given glucosinolate dose, the residues in muscle tissues and organs were even lower. In certain breeds of laying hens, excretion of glucosinolate-derived compounds may convey an undesirable fishy taint to the eggs. However, all measured concentrations in animal-derived products are much lower than those found in vegetables for human consumption, and are unlikely to induce adverse health effects in consumers [47].

1.6. Mutagenicity, Genotoxicity and Carcinogenicity

Glucosinolates and some of their metabolites have been shown to be mutagenic and weakly genotoxic [18,36-39]. For example, crude juice extracts of a number of Brassica vegetables all caused genotoxic effects in the absence of metabolic activation measured as point mutations in Salmonella strains, repairable DNA damage in *E. coli* K-12 cells, and clastogenic effects in cultured mammalian cells. In mammalian cells, chromosomal aberrations and sister chromatid exchanges were also described [69]. A high oral dose of AITC (25 mg/kg in corn oil given 5 times/week by gavage for 103 weeks in F344 rats resulted in increased incidences of epithelial hyperplasia and transitional-cell papillomas of the urinary bladder in males, whereas in females subcutaneous fibrosarcomas were observed at the same dose. The reviewers concluded that under the conditions of this bioassay, AITC was carcinogenic for male F344/N rats, causing transitional-cell papillomas in the urinary bladder. Evidence for associating allyl isothiocyanate with subcutaneous fibrosarcomas in female F344/N rats was vague. Allyl isothiocyanate was not carcinogenic for B6C3F1 mice of either sex [70]. In contrast, phenylethyl isothiocyanate was shown to be an inhibitor of tumor formation at several sites in rats and in lungs of A/J mice in various assays [71-74]. However, investigations with indole-3-carbinol gave conflicting results: when administered before or at the same time as a chemical carcinogen, it was found to inhibit the developments of cancers of the breast, stomach, colon, lung and liver [75]. In contrast, in other studies in which indole-3-carbinol was administered after the carcinogen (post initiation) an enhancement of cancer of the liver, thyroid, colon and uterus was observed in rats [72,76,77]. The exact mechanisms underlying these controversial results are not entirely elucidated, but the therapeutic use of these com-pounds has been questioned, despite the increasing epidemi-ological evidence that higher intake of cruciferous vegetables is associated with a decreased cancer risk in humans [78].

2. CRUCIFEROUS PLANTS: CHEMOPROTECTION AGAINST CARCINOGENESIS

2.1. Preface

Two types of DNA-damaging agents can evoke neoplastic transformations, i.e., electrophiles, largely of exogenous origin, and reactive oxygen species, originating in part from exogenous sources but arising also in considerable quantities from normal cellular oxidations. Most electrophiles require metabolic activation, usually by phase I enzymes (cytochrome P450); they convert generally harmless procarcinogens to highly reactive electrophilic ultimate carcinogens that can damage susceptible centers of DNA bases and initiate carcinogenesis. DNA and other macromolecules are principally protected against damage caused by electrophiles and reactive oxygen species by a family of phase II enzymes. By a variety of mechanisms including conjugation with endogenous compounds (e.g., glutathione, glucuronic acid), phase II enzymes inactivate these agents and promote their excretion.

In addition, glutathione, the principal and most abundant small-molecule cellular antioxidant, which is similarly regulated by phase II enzymes, plays a major role in protection against electrophiles and reactive oxygen species. Thus, whether malignancy will ensue when a cell is exposed to a potential carcinogen is determined largely by the balance of activities of phase I enzymes that activate carcinogens and phase II enzymes that nearly always detoxify reactive carcinogens [79]. It is therefore of considerable importance that both families of enzymes are highly inducible in many tissues and that their activities can be regulated by a wide variety of chemical agents belonging to nine chemical classes, among which dietary phytochemicals are especially important [80]. Furthermore, although some inducers elevate both phase I and phase II enzymes (bifunctional inducers), others selectively induce only phase II enzymes (monofunctional inducers) [81]. Actually, a voluminous literature now supports the attitude that induction of phase II enzymes is an important and sufficient mechanism for achieving protection against the toxic and neoplastic effects of many carcinogens [79,82]. However, the mechanisms responsible for protective effects against carcinogenesis are multiple and probably involve complex interactions as well as these mechanisms are incompletely understood. In recent years, however, much evidence has accumulated suggesting that the induction of phase II detoxication enzymes (e.g., glutathione transferases, glucuronosyltransferases, NAD(P)H-quinine reductase, epoxide hydrolase) is a major strategy for achieving protection against the toxic and neoplastic effects of mutagens and carcinogens [83].

2.2. Epidemiologic Evidence for the Correlation Between Crucifer C*onsumption and Cancer Risk*

Epidemiologic evidence relating cancer risk reduction to the consumption of specific types of fruits and vegetables and to crucifers in particular has been available in the last two decades. A conclusion was established that "a dose-response relationship was also encountered in analyses of each of the following for cancer of the colon: sauerkraut, coleslaw, Brussels sprouts, and broccoli" [84]. Recent comprehensive reviews and other numerous studies were reported; implying to show a specific protective effect of crucifers, especially brassicas, have cautioned: "It is not yet possible to decide whether the protective effect is attributable to brassica vegetables per se or to vegetables in general" [1,85]. Since these reviews were published, further studies continue to report an inverse association between crucifer consumption and cancer. Most authors observed highly significant cancer risk reduction with increasing crucifer intake in cohorts that developed prostate cancer [86,87], breast cancer [88], as well as crucifer-associated reduction in non-Hodgkin's lymphoma in women [89].

2.3. Distribution of Phase II Enzyme Inducers Among Plants: Role of Isothiocyanates and Sulforaphane

When organic extracts of various edible plants belonging to several plant families were examined for phase II inducer activities, striking differences were observed [90].

A simple cell culture system designed to detect and quantify phase II enzyme inducer activity led to the identification of sulforaphane as the principal and exceedingly potent phase II enzyme inducer in broccoli [5]. Studies in animals have demonstrated that sulforaphane induces phase II enzymes *in vivo* and blocks chemically induced mammary tumor formation in rats [1,85], confirming the hypothesis that induction is associated with chemoprotection.

Such observations have led Talalay and coworkers [9,79,91] to conduct a series of studies on the effects of cruciferous vegetable extracts on phase II enzyme inductions and animal tumorigenesis. They have developed an *in vitro* assay to distinguish bifunctional phytochemicals that induce both phase I and phase II enzyme systems from monofunctional phytochemicals that induce only phase II enzymes. They then used this assay to demonstrate that *Brassica* vegetables are particularly rich sources of monofunctional phase II inducers and to identify the isothiocyanate sulforaphane as the principal phase II inducer in broccoli extracts. They also demonstrated that sulforaphane is a dose-related inhibitor of carcinogen-induced mammary tumorigenesis in rats.

These studies leave no doubt that sulforaphane does indeed induce phase II enzymes and inhibit carcinogenesis under these conditions. Under debate, however, is the clinical significance of induction of such enzyme systems by single phytochemicals. Both phase I and phase II systems are highly multifunctional and inducible by a wide variety of dietary compounds. Forty-nine of such compounds have been identified in cabbage, among them several that have been tested and found mutagenic or carcinogenic in animal test systems. Thus, cruciferous and other vegetables contain some phytochemicals that are carcinogenic and others that are anticarcinogenic in test systems [92]. This confusing situation is further complicated by the ability of both phase I and phase II enzyme systems to inactivate some carcinogens, but activate others, depending on circumstances. Chemicals that induce activating enzymes also will induce activation of any other compounds present that are metabolized by the same system; the same is true of substances that induce inactivation [11].

Allyl isothiocyanates (AITC) is widely present in cruciferous vegetables such as cabbage, broccoli, kale, cauliflower, and horseradish. It is also commonly used in the human diet as a flavor agent [93]. Like other isothiocyanates, AITC inhibits microsomal enzyme activities [94]. Previous studies have shown that liver microsomes, obtained from rats that were fed a diet containing AITC, metabolize nitrosamines to a lesser extent than those of the untreated rats. AITC and its glucosinolate precursor, sinigrin, given in the diet, also inhibit hepatic DNA methylation induced by the tobacco-specific nitrosamine 4-(methylnitrosamino)-i-(3-pyridyl)-i-butanone in rats [95-97]. These results suggest the potential of AITC in modulating the carcinogenic activities of nitrosamines, since many aryl alkyl isothiocyanates structurally related to AITC are known to be inhibitors of lung tumorigenesis induced by 4-methylnitrosamino)-i-(3-pyridyl)-i–butanone [16]. It was shown recently that AITC inhibits the growth of human cancer cells *in vitro* [98]. Furthermore, several authors have reported that AITC induces the Phase II detoxification enzyme glutathione S-transferases [99-100]. Further support for the anticarcinogenic activity of isothiocyanates was afforded by the synthesis of a large number of isothiocyanate analogs on the basis of their poten-

cies as phase II enzyme inducers and the finding that these compounds also inhibited mammary tumor formation in rats evoked by DMBA [101]. On the other hand, chronic treatment with high doses of AITC induces urinary bladder tumors in rats [102]. The diverse biochemical and biological activities of AITC and its wide consumption imply its potential effects on human health.

Consequently, Cruciferae, and particularly the brassicas, were proven to be especially rich in enzyme inducer activities, whereas many other plant families were generally much poorer sources. The importance of developing glucosinolates and isothiocyanates as chemoprotectors received considerable impetus from the totally independent and unexpected bioassay-guided discovery that the principal inducer of phase II detoxication enzymes in broccoli, and especially in 3-days-old broccoli sprouts, was an unusual isothiocyanate, i.e. sulforaphane [1-isothiocyanato-(4R)-methylsulfinyl)-butane; $CH_3S(CH_2)_4NCS$] that blocked mammary tumor formation in rats treated with dimethyl benz[a]anthracene (DMBA) [10]. Sulforaphane is an extremely potent inducer of phase II enzymes, perhaps the most potent naturally occurring inducer described to date [89].

REFERENCES

[1] Verhoeven, D.T.H.; Verhagen, H.; Goldbohm, R.A.; van den Brandt, P.A.; van Poppel, G. A review of mechanisms underlying anticarcinogenicity by brassica vegetables. *Chem. Biol. Interact.*, **1997**, *103*, 79–129.

[2] Matthäus, B.; Zubr, J. Variability of specific components in *Camelina sativa* oilseed cakes. *Ind. Crops Prod.*, **2000,** *12*, 9-18.

[3] Fahey, J.W.; Zalcmann, A.T.; Talalay, P. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry*, **2001**, *56*, 5-51.

[4] Beasley, V. In: *Veterinary Toxicology*, (Ed.) Publisher: International Veterinary Information Service (www.ivis.org), Ithaca, New York, USA, **1999**.

[5] Zhang, Y.; Talalay, P. Anticarcinogenic activities of organic isothiocyanates: chemistry and mechanisms. *Cancer Res. (suppl.)* **1994,** *54*, 1976s-1981s.

[6] Hecht, S.S. Chemoprevention by isothiocyanates. *J. Cell. Biochem. Suppl*., **1995**, *22*, 195–209.

[7] Hecht, S.S. Chemoprevention by modifiers of carcinogen metabolism. In: "*Phytochemicals as Bioactive Agents*", Bidlack, W.R.; Omaye, S.T.; Meskin M.S.; Topham, D.K.W., Eds, Technomic Publishing Co.: Lancaster, PA, **2000**, pp. 43–74.

[8] Wattenberg, L.W.; Hanley, A.B.; Barany, G.; Sparnins, V.L.; Lam, L.K.T. and Fenwick, G.R. (1986): Inhibition of carcinogenesis by some minor dietary constituents. In: *Diet, Nutrition and Cancer* (Hayashi, Y.; Nagao, H.; Sugimura, T.; Tokayama, S.; Tomatis, L.; Wattenberg, L. W.; Wogan, G. N.; Eds, Japan Science Society Press: Tokyo, Japan, **1986**, pp. 193–203

[9] Talalay, P.; Zhang, Y. Chemoprotection against cancer by isothiocyanates and glucosinolates. *Biochem. Soc. Trans.*, **1996**, *24*, 806–810.

[10] Fahey, J.W.; Zhang, Y.; Talalay, P. Broccoli sprouts: an exceptionally rich source of inducers of enzymes that protect against chemical carcinogens. *Proc. Natl. Acad. Sci. USA*, **1997**, *94*, 10367–10372.

[11] Rosa, E.A.S.; Heaney, R.K.; Fenwick, G.R.; Portas, C.A.M. Glucosinolates in crop plants. *Hortic. Rev.* **1997,** *19*, 99–215.

[12] Shapiro, T.A.; Fahey, J.W.; Wade, K.L.; Stephenson, K.K.; Talalay, P. Chemoprotective glucosinolates and isothiocyanates of broccoli sprouts: metabolism and excretion in humans. *Cancer Epidemiol. Biomarkers Prev.*, **2001,** *10*, 501–508.

[13] Kliebenstein, D.J.; Kroymann, J.; Mitchell-Olds, T. The glucosinolate-myrosinase system in an ecological and evolutionary context. *Curr. Opin. Plant Biol.*, **2005,** *8*, 264-271.

[14] Eklind, K.I.; Morse, M.A.; Chung, F-L. Distribution and metabolism of the natural anticarcinogen phenethyl isothiocyanate in NJ mice. *Carcinogenesis*, **1990**, *11*, 2033-2036.

[15] Mennicke, W.H.; Gorler, K.; Krumbiegel, G. Metabolism of some naturally occurring isothiocyanates in the rat. *Xenobiotica* **1983,** *13*, 203-207.

[16] Chung, F-L.; Morse, M.A.; Eklind, K.I. New potential chemopreventive agents for lung carcinogenesis of tobacco-specific nitrosamine**.** *Cancer Res*. (Suppl.), **1992**, *52*, 2719s-2722s.

[17] Gorler, K.; Krumbiegel, G.; Mennicke, W.H.; Siehl, H.U. The metabolism of benzyl isothiocyanate and its cysteine conjugate in ginea-pegs and rabbits. *Xenobiotica*, **1982**, *12*, 535-542.

[18] Conaway, C.C.; Yang, Y.M.; Chung, F.L. Isothiocyanates as cancer chemopreventive agents: their biological activities and metabolism in rodents and humans. *Curr.Drug Metab*., **2002**, *3*, 233-255.

[19] Bollard, M.; Stribbling, S.; Mitchell, S.; Caldwell, J. The disposition of allyl isothiocyanate in the rat and mouse. *Food Chem. Toxicol*., **1997**, *35*, 933-943.

[20] Anderton, M.J.; Manson, M.M.; Verschoyle, R.D.; Gescher, A.; Lamb, J.H.; Farmer, P.B.; Steward, W.P.; Williams, M.L. Pharmacokinetics and tissue disposition of indole-3-carbinol and its acid condensation products after oral administration to mice. *Clin. Cancer Res*., **2004**, *10*, 5233-5241.

[21] Duncan, A.J.; Milne, J.A. Effects of oral administration of brassica secondary metabolites, allyl cyanide, allyl isothiocyanate and dimethyl disulphide, on the voluntary food intake and metabolism of sheep. *Br. J. Nutr*., **1993**, *70*, 631-645.

[22] Fenwick, G.R.; Heaney, R.K. Glucosinolates and their break-down products in cruciferous crops, foods and feeding stuffs. *Food Chem.* **1983,** *11*, 249-271.

[23] Silver, E.H.; Kuttab, S.H.; Hasan, T. Structural considerations in the metabolism of nitriles to cyanide *in vivo*. *Drug Metab. Dispos.* **1982,** *10*, 495-498.

[24] Srivastava, V.K.; Philbrick, D.J.; Hill, D.C. Responses of rats and chicks to rapeseed meal subjected to different enzymatic treatments. *Can. J. Anim. Sci.*, **1975,** *55*, 331-335.

[25] Nishie, K.; Daxenbichler, M.E. Toxicology of glucosinolates, related compounds (nitriles, R-goitrin, isothiocyanates) and vitamin U found in Cruciferae. *Food Cosmet. Toxicol*., **1980,** *18*, 159-172.

[26] Gould, D.H.; Fettman, M.J.; Daxenbichler, M.E.; Bartuska, B.M. Functional and structural alterations of the rat kidney induced by the naturally occurring organonitrile, 2s-I-cyano-2-hydroxy-3, 4-epithiobutane. *Toxicol. Appl. Pharmacol.*, **1985**, *78*, 190-201.

[27] Willhite, C.C.; Smith, R.P. The role of cyanide liberation in the acute toxicity of aliphatic nitriles. *Toxicol. Appl. Pharmacol.*, **1981,** *59*, 589-602.

[28] Ahmed, P.; Farooqui, M.Y. Comparative toxicities of aliphatic nitriles. *Toxicol. Lett.*, **1982**, *12*, 157-163.

[29] Tanii, H.; Hashimoto, K. Studies on the mechanism of acute toxicity of nitriles in mice. *Arch. Toxicol.* **1984,** 55, 47-54.

[30] Langer, P. Study of the chemical representatives of the goitrogenic activity of raw cabbage. *Physiologia Bohemoslovenica* **1964,** *3*, 542-549.

[31] Langer, P.; Stolc, V. Goitrogenic activity of allylisothiocyanate - a widespread natural mustard oil. *Endocrinology*, **1965,** *76*, 151-155.

[32] Kawakishi, S.; Namiki, M. Oxidative cleavage of the disulfide bond of cystine by the action of ally1 isothiocyanate. *J. Agric. Food Chem*., **1982**, *30*, 618-620.

[33] Kawakishi, S.; Goto, T.; Namiki, M. Oxidative scission of the disulphide bond of cystine and polypeptides by the action of allyl isothiocyanates. *Agric. Biol. Chem*., **1983**, *47*, 2071-2076.

[34] Griffiths, D.W.; Birch, A.N.A.; Hillmann, J.R. Antinutritional compounds in the Brassicaceae -Analysis, biosynthesis, chemistry and dietary effects. *J. Hortic. Sci. Biotechnol*., **1998**, *73*, 1-18.

[35] Halkier, B.A.; Gershenzon, J. Biology and biochemistry of glucosinolate. *Annu. Rev. Plant Biol*., **2006**, *57*, 303-333.

[36] Mawson, R.; Heaney, R.K.; Zdunczyk, Z.; Kozlowska, H. Rapeseed meal glucosinolates and their antinutritional effects. Part 3. Animal growth and performance. *Nahrung* **1994a,** *38*, 167-177.

[37] Mawson, R.; Heaney, R.K.; Zdunczyk, Z.; Kozlowska, H. Rapeseed meal glucosinolates and their antinutritional effects. Part 4. Goitrogenicity and internal organs abnormalities in animals. *Nahrung* **1994b,** *38*, 178-191.

[38] Mithen, R.F.; Dekker, M.; Verkerk, R.; Rabot, S.; Johnson, I.T. The nutritional significance, biosynthesis and bioavailability of

glucosinolates in human food. *J. Sci. Food Agric*. **2000,** *80*, 967-984.

[39] Burel, C.; Boujard, T.; Kaushik, S.J.; Boeuf,G.; Mol, K.A.; Van der, G.S.; Darras, V.M.; Kuhn, E.R.; Pradet-Balade, B.; Querat, B.; Quinsac, A.; Krouti, M.; Ribaillier, D. Effects of rapeseed meal-glucosinolates on thyroid metabolism and feed utilization in rainbow trout. *Gen. Comp. Endocrinol*., **2001**, *124*, 343-358.

[40] Spiegel, C.; Bestetti, G.E.; Rossi, G.L.; Blum, J.W. Normal circulating triiodothyronine concentrations are maintained despite severe hypothyroidism in growing pigs fed rapeseed presscake meal. *J. Nutr*. **1993,** *123*, 1554-1561.

[41] Martland, M.F.; Butler, E.J.; Fenwick, G.R. Rapeseed induced liver haemorrhage reticulolysis and biochemical changes in laying hens: the effects of feeding high and low glucosinolate meals. *Res. Vet. Sci*. **1984,** *63*, 298-309.

[42] Kloss, P.; Jeffrey, E.; Wallig, M.; Tumbleson, M.; Parsons, C.; Johnson, L.; Reuber, M. Efficacy of feeding glucosinolate-extracted crambe meal to broiler chicks. *Poult. Sci.* **1994,** *73*, 1542-1551.

[43] Schöne, F.; Rudolph, B.; Kirchheim, U.; Knapp, G. Counteracting the negative effects of rapeseed and rapeseed press cake in pig diets. *Br. J. Nutr*. **1997a,** *78*, 947-939.

[44] Umemura, T.; Yamashiro, S.; Bhatnagar, M.K.; Moody, D.L.; Slinger, S.J. Liver fibrosis of the turkey on rapeseed products. *Res. Vet. Sci*. **1977,** *23*, 139-145.

[45] Campbell, L.D. Incidence of liver haemorrhage among white leghorn strains fed on diets containing different types of rapeseed meals. *Br. Poult. Sci.*, **1979**, *20*, 239-246.

[46] Taljaard, T.L. Cabbage poisoning in ruminants. *J. South African Vet. Assoc*. **1993,** *64*, 96-100.

[47] Ahlin, K.A.; Emanuelson, M.; Wiktorson, H. Rapeseed products from double- low cultivars as feed for dairy cows: effects of long term feeding on thyroid function, fertility and animal health. *Acta Vet. Scand.*, **1994**, *35*, 37-53.

[48] Schmid, A.; Schmid, H. Rapeseed poisoning of wild herbivores. *Tierarzl. Prax*. **1992,** *20*, 321-325.

[49] Rodriguez, R.A.; Maciel, M.G.; de Ochoteco, M.B. Blindness in Holando Argentine calves due to ingestion of turnip (*Brassica campestris*). *Vet. Arg.,* **1997,** *14*, 601-605.

[50] Knight, A.P.; Stegelmeier, B.L.; Woods L.W.; Tiwary, A.K. *7th International Symposium on Poisonous Plants*, Logan, UT (abstract) p. 95. http://ddr.nal.usda.gov/bitstream/10113/9257/1/-IND43954300.pdf **2005**.

[51] Dixon, P.M.; McGorum, B. Oilseed rape and equine respiratory disease. *Vet Rec*., **1990**, *126*, 585.

[52] Gay, C.C. www.vetmed.wsu.edu/depts-fdiu/MustardFoalsReport.-pdf **2004**.

[53] Hines M.T. www.vetmed.wsu.edu/depts-fdiu/MustardFoals-Report. pdf **2004**.

[54] Mason, R.W.; Lucas, P. Acute poisoning in cattle after eating old non-viable seed of chou moellier (*Brassica oleracea convar. Acephala*). *Aust. Vet. J.* **1983,** *60*, 272-273.

[55] Lardy, G.P.; Kerley, M.S. Effect of increasing the dietary level of rapeseed meal on intake by growing beef steers. *J. Anim. Sci*. **1994,** *72*, 1936-1942.

[56] Morton, J.M.; Campbell, P.H. Disease signs reported in south-eastern Australian dairy cattle while grazing Brassica species. *Aust. Vet. J*. **1997,** *75*, 109-113.

[57] Hill, F.I.; Ebbett, P.C. Polioencephalomalacia in cattle in New Zealand fed chou moellier (*Brassica oleracea*). *N. Z. Vet. J.*, **1997,** *45*, 37-39.

[58] Katamoto, H.; Nishiguchi, S.; Harada, K.; Ueyama, I.; Fujita, T.; Watanabe, O. Suspected oriental mustard (Brassica juncea) intoxication in cattle. *Vet. Rec*., **2001**, *149*, 215-216.

[59] Bush, R.S.; Ncholson, J.W.G.; MacIntyre, T.M.; McQueen, R.E. A comparison of Candle and Tower rapeseed meal in lamb, sheep and beef steer rations. *Can. J. Anim. Sci*., **1978**, *58*, 369–376.

[60] Cox-Ganser, J.M.; Jung, G.A.; Pushkin, R.T.; Reid, R.L. Evaluation of Brassicas in grazing systems for sheeep: II. Blood composition and nutrient status. *J Anim Sci*., **1994**, *72*, 1832-1841.

[61] Mandiki, S.N.M.; Derycke, G.; Bister, J.L.; Mabon, N.; Wathelet, J.P.; Marlier, M.; Paquay, R. Chemical changes and influence of rapeseed antinutritional factors on gestating and lactating ewes. Part1: Animal performance and plasma hormones and glucose. *Anim. Feed Sci. Technol.* **2002,** *98*, 25-35.

[62] Stanford, K.; Wallins, G.L.; Smart,W.G.; McAllister, A.A. Effect of feeding canola screenings on apparent digestibility, growth performance and carcass characteristics of feedlot lambs. *Can. J. Anim. Sci.* **2000,** *80*, 355–365.

[63] Mabon, N.; Mandiki, S.N.M.; Derycke, G.; Bister, J.-L.; Wathelet, J.P.; Paquay, R.; Marlier, M. Chemical changes and influence of rapeseed antinutritional factor on lamb physiology and performance. Part 3. Antinutritional factor in plasma and organ. *Anim. Feed Sci. Technol.* **2000,** *85*, 111–120.

[64] Pattanaik, A.K.; Khan, S.A.; Mohanty D.N.; Vashaney, V.P. Nutritional performance, clinical chemistry and semen characteristics of goats fed mustard (*Brassica juncea*) cake based supplements with or without iodine. *Small Rumin. Res.* **2004,** *54*, 173-182.

[65] Schöne, F.; Leiterer, M.; Jahreis, G.; Rudolph, B. Effect of rapeseed feedstuff with different glucosinolate conaten and iodine administration on gestating and lactating sow. *Zentralbl. Veterinarmed. A*, **1997,** *44*, 325-339.

[66] Schöne, F.; Leiterer, M.; Hartung, H.; Jahreis, G.; Tischendorf, F. Rapeseed glucosinolates and iodine in sows affect the milk iodine concentration and the iodine status of piglets. *Br. J. Nutr*. **2001,** *85*, 659-670.

[67] Opalka, M.; Dusza, L.; Koziorowski, M.; Staszkiewicz, J.; Lipinski, K.; Tywonczuk, J. Effect of long-term feeding with graded levels of low glucosinolate rapeseed meal on endocrine status of gilts and their piglets. *Livestock Prod. Sci.*, **2001,** *69*, 233-243.

[68] Ryhänen, E.-L.; Perttilä, S.; Tupasela, T.; Valaja, J.; Eriksson, C.; Larkka, K. Effect of *Camelina sativa* expeller cake on performance and meat quality of broilers. *J. Sci. Food Agric*., **2007,** *87*, 1489-1494.

[69] Kassie, F.; Pool-Zobel, B.; Parzefall, W.; Knasmuller, S. Genotoxic effects of benzyl isothiocyanate, a natural chemopreventive agent. *Mutagenesis*, **1999**, *14*, 595-604.

[70] NTP (National Toxicology Program). NTP carcinogenesis bioassay of allyl isothiocyanate (CAS No. 57-06-7) in F344N rats and B6C3F1 mice (gavage study). *Technical Report Series No. 234*. http://ntp.niehs.nih.gov/ntp/htdocs/LT_rpts/tr234.pdf **1982**.

[71] Chung, F-L.; Kelloff, G.; Steele, V.; Pittman, B.; Zang, E.; Jiao, D.; Rigotty, J.; Choi, C.I.; Rivenson, A. Chemopreventive efficacy of arylalkyl isothiocyanates and Nacetylcysteine for lung tumorigenesis in Fischer rats. *Cancer Res*., **1996**, *56*, 772-778.

[72] Stoner, G.; Casto, B.; Ralston, S.; Roebuck, B.; Pereira, C.; Bailey, G. Development of a multi-organ rat model for evaluating chemopreventive agents: efficacy of indole-3-carbinol. *Carcinogenesis*, **2002,** *23*, 265-272.

[73] Adam-Rodwell, G.; Morse, M.A.; Stoner, G.D. The effects of phenethyl isothiocyanate on benzo[a]pyrene-induced tumors and DNA adducts in A/J mouse lung**.** *Cancer Lett.*, **1993**, *71*, 35-42.

[74] Rao, C.V.; Rivenson, A.; Simi, B.; Hamid, R.; Kelloff, G.J.; Steele, V.; Reddy, B.S. Enhancement of experimental colon carcinogenesis by dietary 6-phenylhexyl isothiocyanates. *Cancer Res.,* **1995,** *55*, 4311-4318.

[75] Guo, D.; Schut, H.A.; Davis, C.D.; Snyderwine, E.G.; Bailey, G.S.; Dashwood, R.H. Protection by chlorophyllin and indole-3-carbinol against 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP)-induced DNA adducts and colonic aberrant crypts in the F344 rat. *Carcinognesis*, **1995**, *16*, 2931-2937.

[76] Kim D.J.; Lee, K.K.; Han, B.S.; Ahn, B.; Bae, J.H.; Jang, J.J. Biphasic modifying effect of indole-3-carbinol on diethylnitrosamine-induced preneoplastic glutathione S-transferase placenta form-positive liver cell foci in Sprague Dawley rat. *Jpn. J. Cancer Res*., **1994,** *85*, 578-583.

[77] Yoshida, M.; Katashima, S.; Ando, J.; Tanaka, T.; Uematsu, F.; Nakae, D.; Maekawa, A. Dietary indole-3-carbinol promotes endometrial adenocarcinoma development in rats initiated with N-ethyl-N'-nitro-N-nitrosoguanidine, with induction of cytochrome P450s in the liver and consequent modulation of estrogen metabolism. *Carcinogenesis*, **2004,** *25*, 2257-2264.

[78] Higdon, J.V.; Delage, B.; Williams, D.E.; Dashwood, R.H. Cruciferous vegetables and human cancer risk: epidemiologic evidence and mechanistic basis. *Pharmacol. Res*., **2007**, *55*, 224-236.

[79] Talalay, P. Chemoprotection against cancer by induction of phase 2 enzymes**.** *Biofactors*, **2000,** *12*, 5–11.

[80] Khachik, F.; Bertram J.S.; Huang, M.T.; Fahey, J.W.; Talalay, P. Dietary carotenoids and their metabolites as potentially useful chemoprotective agents against cancer. In: *"Antioxidant Food Sup-*

plements in Human Health"; Packer, L.; Hiramatsu, M.; Yoshikawa, T., Eds.; Academic Press: San Diego, CA, **1999,** pp. 203-229.

[81] Prochaska, H.J.; Talalay, P. Regulatory mechanisms of monofunctional and bifunctional anticarcinogenic enzyme inducers in murine liver. *Cancer Res.*, **1988,** *48*, 4776-4782

[82] Hayes, J.D.; McLellan, L.I. Glutathione and glutathione-dependent enzymes represent a coordinated regulated defense against oxidative stress. *Free Radic. Res*., **1999**, *31*, 273-300.

[83] Steinmetz, K.A.; Potter, J.A. Vegetables, fruit, and cancer prevention: a review. *J. Am. Diet Assoc.* **1996,** *96*, 1027–1039.

[84] Graham, S.; Dayal, H; Swanson, M.; Mittelman, A.; Wilkinson, G. Diet in the epidemiology of cancer of the colon and rectum. *J. Natl. Cancer Inst.*, **1978**, *61*, 709–714

[85] Verhoeven, D.T.H.; Goldbohm, R.A.; van Poppel, G.; Verhagen, H.; van den Brandt, P.A. Epidemiological studies on brassica vegetables and cancer risk**.** *Cancer Epidemiol. Biomarkers Prev*., **1996,** *5*, 733–748.

[86] Jain, M.G.; Hislop, G.T.; Howe, G.R.; Ghadirian, P. Plant foods, antioxidants, and prostate cancer risk: findings from case-control studies in Canada. *Nutr. Cancer*, **1999**, *34*, 173–184.

[87] Kolonel, L.N.; Hankin, J.H.; Whittemore, A.S.; Wu, A.H.; Gallagher, R.P.; Wilkens, L.R.; John, E.M.; Howe, G.R.; Dreon, D.M.; West, D.W.; Paffenberger, R.S., Jr. Vegetables, fruits, legumes and prostate cancer: a multiethnic case-control study. *Cancer Epidemiol. Biomarkers Prev.* **2000,** *9*, 795 - 804.

[88] Terry, P.; Wolk, A.; Persson, I.; Magnusson, C. Brassica vegetables and breast cancer risk. *JAMA*, **2001,** *285*, 2975–2977.

[89] Zhang, S.M.; Hunter, D.J.; Rosner, B.A.; Giovannucci, E.L.; Colditz, G.A.; Speizer, F.E.; Willett, W.C. Intakes of fruits, vegetables, and related nutrients and the risk of non-Hodgkin's lymphoma among women**.** *Cancer Epidemiol. Biomarkers Prev*., **2000,** *9*, 477–485.

[90] Zhang, Y.; Kensler, T.W.; Cho, C.G.; Posner, G.H.; Talalay, P. Anticarcinogenic activities of sulforaphane and structurally related synthetic norbornyl isothiocyanates. *Proc. Natl. Acad. Sci. USA*, **1994,** *91*, 3147–3150.

[91] Talalay, P.; Fahey, J.W. Phytochemicals from Cruciferous Plants Protect against Cancer by Modulating Carcinogen Metabolism. In: *American Institute for Cancer Research 11th Annual Research Conference on Diet, Nutrition and Cancer* held in Washington, DC, July, 16-17. **2001**.

[92] Nestle, M. Broccoli sprouts as inducers of carcinogen-detoxifying enzyme systems: clinical, dietary, and policy implications. *Proc. Natl. Acad. Sci. USA*, **1997,** *94*, 11149–11151.

[93] Daxenbichler, M.E.; Spencer, G.F.; Carlson, D.G.; Rose, G.B.; Brinker, A.B.; Powell, R.G. Glucosinolate composition of seeds from 297 species of wild plants. *Phytochemistry*, **1991**, *30*, 2623-2638.

[94] Guo, Z.; Smith, T.J.; Wang, E.; Eklind, K.I.; Chung, F-L.; Yang, C.S. Structure-activity relationships of arylalkyl isothiocyanates for the inhibition of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone metabolism and the modulation of xenobiotic-metabolizing enzymes in rats and mice. *Carcinogenesis*, **1993**, *14*, 1167-1173.

[95] Chung, F-L.; Juchatz, A.; Vitarius, J.; Hecht, S.S. Effects of dietary compounds on alpha-hydroxylation of N-nitrosopyrrolidine and N'-nitrosonornicotine in rat target tissues**.** *Cancer Res*., **1984**, *44*, 2924-2928.

[96] Chung, F-L.; Wang, M. Y.; Hecht, S.S. Effects of dietary indoles and isothiocyanates on N-nitrosodimethylamine and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone alpha-hydroxylation and DNA methylation in rat liver. *Carcinogenesis*, **1985**, *6*, 539-543.

[97] Morse, M.A.; Wang, C-X.; Amin, S.G.; Hecht, S.S.; Chung, F-L. Effects of dietary sinigrin or indole-3-carbinol on O6-methylguanine-DNA-transmethylase activity and 4-(methyllnitrosamino)-1-(3-pyridyl)-1-butanone-induced DNA methylation and tumorigenicity in F344 rats. *Carcinogenesis*, **1988,** *9*, 1891-1895.

[98] Hasegawa, T.; Nishino, H.; Iwashima, A. Isothiocyanates inhibit cell cycle progression of HeLa cells at G2/M phase. *Anticancer Drugs*, **1993**, *4*, 273-279.

[99] Bogaards, J.J.; van Ommen, B.; Falke, H.E.; Willems, M.I.; van Bladeren, P. Glutathione S-transferase subunit induction patterns of Brussels sprouts, allyl isothiocyanate and goitrin in rat liver and small intestinal mucosa: a new approach for the identification of inducing xenobiotics. *Food Chem. Toxicol.*, **1990**, *28*, 81-88.

[100] Hara, A.; Sakai, N.; Yamada, H.; Tanaka, T.; Kato, K.; Mori, H.; Sato, K. Induction of glutathione S-transferase, placental type in T9 glioma cells by dibutyryladenosine 3',5'-cyclic monophosphate and modification of its expression by naturally occurring isothiocyanates. *Acta Neuropathol*., **1989**, *79*, 144-148.

[101] Posner, G.H.; Cho, C.G.; Green, J.V.; Zhang, Y.; Talalay, P. Design and synthesis of bifunctional isothiocyanate analogs of sulforaphane: correlation between structure and potency as inducers of anticarcinogenic detoxication enzymes. *J. Med. Chem*., **1994,** *37*, 170 - 176.

[102] Dunnick, J.K.; Prejean, J.D.; Haseman, J.; Thomson, R.B.; Giles, H.D.; McConnell, E.E. Carcinogenesis bioassay of allyl isothiocyanates. *Fundam. AppI. Toxicol*., **1982**, *2*, 114-120.

Received: 19 August, 2009 Revised: 25 September, 2009 Accepted: 25 September, 2009