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Review Article

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Fruits and Vegetables as Dietary Sources of Antimutagens

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Abstract

Mutation is the process leading to heritable changes in the genetic material of an organism and caused mainly by the external factors, including chemical and physical agents, or can also occur spontaneously due to errors in DNA replication, repair, and recombination. Agents contributing to the mutagenic burden in the environment could be from industrial sources, wide spectrum applications of biocides in the agriculture, and other contaminants. As many of these mutagenic chemicals can induce severe disorders in humans including cancer and a large spectrum of inherited diseases, it is important to detect such mutagenic agents precisely and rapidly, and also look for an approach to combat them. Natural occurring dietary antimutagens primarily from health protective foods such as fruits and vegetables could provide a mechanism to counteract the deleterious effect of these mutagens. The World Health Organization (WHO) indicates that one-third of all cancer deaths are preventable and that diet is closely linked to cancer prevention. These health protective phytochemicals particularly antimutagenic ones could provide an effective solution to these concerns. The current review deals with understanding of the mutagenic events, methods of its analysis and a brief compilation of the existing scientific findings related to the dietary sources having potential to counteract the effects of the mutagenic exposures from different sources. The review would provide an opportunity to look into the science, think about the possible future perspectives and mechanism to translate the outcome of the scientific research for benefits of the mankind.

Keywords

Mutagens, Vegetables, Fruits, Antimutagenicity

Introduction

Mutation is the process leading to heritable changes in the genetic material of an organism. The mutagenesis is primarily caused by the chemical and physical agents called as mutagens. Additionally, mutations can also occur spontaneously due to errors in DNA replication, repair, and recombination. Some mutagenic events can affect only one or a few nucleotides within a gene and hence called as point mutations. These base pair substitutions i.e. the replacement of one base pair with another primarily could be a deletion (the loss of one or more base pairs) or an insertion (the addition of extra base pairs into the DNA sequence). Mutagenic changes that occur in germ line cells can be passed to future generations. Agents contributing to the mutagenic burden in the environment could be from industrial sources, biocides (e.g. insecticides, herbicides, and pesticides) used in the agriculture, and the natural biotic (e.g. toxigenic microbes including

fungi) or abiotic (e.g. radiation) sources. Occasionally dietary agents can also add to the mutagenic burden. For example when protein-rich foods such as meat and fish are cooked, a family of heterocyclic and polycyclic aromatic amines could be formed. The amount of these amines depends on cooking conditions (boiling, barbecuing, frying, and grilling) and or on meat type (beef, chicken, mutton, or pork). These heterocyclic aromatic amines have been reported to induce DNA damage in mammalian cells [1] and are potent multi organ carcinogens in rodents [2]. Besides, high nitrate levels in processed foods may also be a risk factor, possibly through their ability to form N-nitroso compounds *in vivo,* as these chemicals induce tumors in various organs including liver, lung, kidney, bladder, pancreas and tongue [3]. Epigenetic changes in DNA methylation patterns at CpG sites termed as epimutations has emerged as a mechanism involved in tumor progression [4].

As many of these mutagenic chemicals can induce severe disorders in humans including cancer and different inherited diseases, it is important to detect such mutagenic agents precisely and rapidly, and develop strategy for nullifying their action [5-8]. Dietary antimutagenic phytochemicals from health protective foods such as fruits and vegetables could provide means to counteract the deleterious effect of these mutagens. Diet is closely linked to cancer prevention and as per the estimate of the World Health Organization (WHO) around one-third of all cancer deaths are preventable. Numerous epidemiological findings have indicated the potential of dietary phytochemicals as an effective intervention in combating carcinogenesis [9]. Hence, severe adverse events are conceivably less likely to arise in therapeutic settings using natural sources compared to synthetic compounds.

Mutagens and Their Types

Mutagens are either direct or indirect acting. The direct-acting mutagens affect genetic material directly leading to structural change (e.g. sodium azide- NaN_3), whereas indirect acting mutagens works in an indirect manner through the metabolic activation leading to the formation of metabolites or different chemicals which directly acts upon DNA. During this process, the transformation of promutagen into the mutagen takes place primarily by the action of phase I metabolic enzymes, such as the cytochrome P450. The mutagen activation involves N-oxidation by cytochrome P4501A2 followed by the activation by N-acetyltransferase [10]. Some common examples of direct and indirect acting mutagens with their possible mechanism of action have been documented in Tables 1 and 2. Different mutagens work through different mechanism of action as detailed below.

Alkylating agents

These agents react with DNA bases directly and transfer an alkyl group to form its mono-adducts. N-methyl-N′-nitro-N-nitrosoguanidine (MNNG) and ethyl methanesulfonate (EMS) are the well-known alkylating agents. Alkylation of bases results in their mispairing eventually affecting the primary structure of the translated protein when these changes take place in exon (i.e. transcribing) regions of the DNA [11]. The most frequent location of alkylation in DNA by EMS is at guanine, leading to the formation of O^6-alkylguanine [12]. During DNA replication thymine is placed instead of cytosine opposite O^6-alkylguanine by the DNA polymerase. Thus during subsequent row of replication, the original G:C pair can become A:T pair. The process is called transition mutation. Similarly, MNNG acts by adding alkyl group to O^6 of guanine or O^4 of thymine leading to G:C to A:T transition [13, 14].

Table 1: Some common direct acting chemical mutagens and their mechanisms of action.

Mutagen	Mechanism of action	Ref. no.
Acridine	Binds DNA tightly but reversibly through intercalation (at low concentrations)	[154]
9-aminoacridine	Induces frameshift mutations where a single base, especially guanine, is repeated	[154]
Doxorubicin	Induces G:C–T:A transversions, and also undergoes electron reduction leading to the generation of free radical species	[155, 156]
Ethyl methanesulfonate	An alkylating agent. At low concentrations alters a base in DNA, and may induce DNA strand breaks and lesions as a consequence of depurination	[157, 158]
Methyl methanesulfonate	An alkylating agent. Modifies guanine and adenine to cause base mispairing and replication blocks, respectively	[159]
N-methyl-N′-nitro-N-nitrosoguanidine	Leads to the alkylation of purines and pyrimidines. Leads to formation of O^6-methylguanine	[14, 155, 160]
4-nitro-o-phenylenediamine	Induces frameshift mutations	[155]
1-nitropyrene	Forms DNA adduct N-(deoxyguanosine-8-yl)-1-aminopyrene	[161]
4-nitroquinoline-N-oxide	A base substitution agent, principally acting at G residues. Induce mainly GC to AT transitions	[162]
Sodium azide (NaN_3)	Mutagenicity is mediated through the production of L-azidoadenine that interacts with DNA and causes point mutations in the genome, Induces G:C→A:T transitions	[14, 155]

Note: Table modified and drawn from ref. 25.

Base analogs

Such chemicals can substitute for a normal base in nucleic acid. It can be either purine or pyrimidine analogue. These molecules have structure similar to normal DNA bases and hence, can substitute a base in genetic material, leading to transitions and tautomerization. The common examples are 5-bromouracil (5-BrU), and 2-amino-purine (2-AP). Primarily, 5-bromouracil (5-BrU) is an analogue of thymine. 5-BrU exists in tautomeric forms. The keto form pairs with adenine whereas enol form pairs with guanine.

These tautomeric forms frequently interchange so base pairing properties can become altered at any time. The base pair will change from an A:T to a G:C or from a G:C to an A:T pair after a number of replication cycles. 2-AP is an analogue of guanine or adenine and most commonly pairs with thymine but can also pair with cytosine [7].

Table 2: Some common indirect acting chemical mutagens and their mechanisms of action.

Mutagen	Mechanism of action	Ref. no.
N-acetyl-2-aminofluorene	Reacts with guanines at the C8 position in DNA to form a structure that interferes with DNA replication	[163]
2-aminoanthracene	Its electrophilic reactive metabolites form DNA adducts	[164, 165]
2-aminofluorene	Gets converted to reactive carcinogenic ester 2-acetylaminofluorene-N-sulfate, which can attack guanine residues in nucleic acids	[166]
Aflatoxin B1 (AFB1)	Stimulates the release of free radicals, which cause chromosomal aberrations	[167]
Benzo(α)pyrene	An active mutagen is benzo[a] pyrene-7, 8-diol-9, 10-epoxide (BPDE). Major adducts of BP-DNA are BPDE-deoxyguanosine (dG) and 9-OH-BP-dG-derived adducts	[168]
Cyclophosphamide	Alkylate DNA and also leads to free radical production	[48]

Note: Table modified and drawn from ref 25.

Intercalating agents

They mimic base pairs and are able to insert between DNA bases at the core of the DNA double helix. This results in single-nucleotide pair insertions and deletions leading to frame-shift mutations. Its common example include acridines such as proflavine and quinacrine; exo 8, 9 epoxide of aflatoxin B1; and ethidium bromide. 9-Aminoacridine (9-AA) binds to DNA non-covalently by intercalation and causes frame shift mutations [15].

Antimutagens and Mechanism of Action

Looking for the compounds with antimutagenic properties is currently an intriguing area of research. Such compounds are under consideration to be used in combination with chemotherapeutic drugs having mutagenic potential to reduce its negative effect. The concept is quite relevant for the drugs being applied in cancer therapy because most of these drugs are DNA damaging agents and hence potential mutagens too. Many of these drugs may induce mutation in bystander cells and thus can cause secondary oncogenesis later in the patients that underwent chemical and/or radiation therapies even after the cure of the primary cancer. Such events are quite noticeable in case of cancers where therapeutic success rate is comparatively high. Therefore, searching for compounds with antimutagenic potency and understanding its mode of action bears immense significance [16]. Some antimutagens function at extracellular level by inactivating mutagenic agents and thus prevent their reach to the target (i.e. DNA), whereas others act within the cell and participate in mutation suppression after DNA damage by influencing genome repair and replication [17-19]. The first type of antimutagens are termed as 'desmutagens', whereas later as a 'bioantimutagens'. In recent studies some of the natural dietary constituents have been found to work through suppression of error prone pathway and also through up-regulation of genes that have been found to prevent mistranslation error [20, 21]. Occasionally certain compounds may exhibit dual nature and display both antimutagenic and mutagenic effects as reported in case of β-carotene, which possesses the ability to both scavenge and produce free radicals [22]. Such compounds have been termed as "Janus mutagens", after the Roman god who had one head with two faces looking in opposite directions [23-25]. Several antitumor compounds also displayed antimutagenicity [26-28].

Mechanisms of antimutagenic action

Direct physical interaction with mutagens

This is actually based upon direct chemical interaction between an antimutagenic compound and a mutagen before it induces DNA damage. Sulfhydryl compounds, such as cysteine was found to interact with 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX) and thus reduce its mutagenicity [29]. Phenolics too were reported to work through direct interaction [19, 30]. They work through two mechanisms. An extracellular mechanism involves interference with the cytochrome P450-mediated metabolism of these mutagens and the interaction with active mutagenic metabolites [31]. Their intracellular action is related to the protection of DNA from electrophilic mutagens [31]. Antimutagenic properties of gallic acid when assayed by the Ames test, were found to be possibly due to its nucleophilic property leading to the scavenging of the electrophilic mutagens [32]. Besides the gallic acid can also bind or insert into the outer membrane transporters and lead to the blockage of a mutagen that was transferred into the cytosol.

Blockage of mutagen binding to the target

Certain antimutagens work by blocking the mutagen binding to DNA [33]. Two synthesized β-amino ketones have demonstrated their antimutagenic action against MNNG and 9-aminoacridine (9-AA) induced mutagenicity through this mechanism [33].

Inhibition of promutagen bioactivation

Some compounds have been reported to inhibit promutagen bioactivation as reported in case of synthesized nitrogen- and oxygen-containing heterocyclic compounds against NaN_3 and MNNG induced mutagenicity in the Ames and *Escherichia coli* WP2 bacterial reverse mutation assays. They were reported to work through possible inhibition of L-azidoalanine and O^6-methylguanine formation [34]. Similarly, *Terminalia arjuna* constituents were reported to suppress the mutagenic effect of the aromatic amine, i.e., 2-aminofluorene (2-AF) by inhibiting its metabolic activation [35].

Antimutagenicity through antioxidant mechanism

Not always but in some cases mutagens act through

the generation of reactive oxygen species (ROS). In these cases scavenging of the ROS seems to be the principal mechanism of antimutagenicity [36-38]. Lipoic acid (LA) is one such example which has shown potent antioxidant activity and also antimutagenicity against mitomycin-C induced mutations in human peripheral lymphocytes [39-42]. Similarly, antimutagenic activity of the lichen extracts was found to be closely related to its antioxidant capacity [43-45]. *Acacia salicina* extracts too provided protection against DNA strand break induced by the hydroxyl radical, and also significantly decreased mutagenicity induced by 4-nitro-O-phenylenediamine [46]. In another study, synthetic antimutagens like organoselenium compounds protected against genotoxicity and oxidative stress induced by an indirect-acting mutagen cyclophosphamide (CP) possibly through multiple antioxidant mechanisms such as activation of superoxide dismutase (SOD) and catalase, restoration of the level of glutathione (GSH), and the removal of ROS [47]. CP is known to work through DNA- alkylating mechanism and also free radicals production [48]. Bichalcophenes too significantly decreased the mutagenicity induced by sodium azide (NaN_3) and Benzo(α)pyrene (BP) [49].

However, not in all the cases, the antimutagenicity and antioxidant capacity are correlated. In the case of many foods such as different cultivars of apple, honey, and various vegetables, the extracted or purified bio-actives did not show good correlation between the antioxidant and antimutagenic activities [20, 21, 50-55]. However, some antimutagenic compounds which are not potent antioxidants on their own but can be converted into molecules that display good antioxidant activity as reported in case of several amino acid conjugates of curcumin demonstrated very high antimutagenic activity against NaN_3 and methyl methanesulfonate (MMS) in Ames test [56].

Methodology to Assess Mutagenicity and Antimutagenicity

The screening strategy for mutagenic events and antimutagenicity potential of test compound relies upon the standard *in vitro* and *in vivo* assays. Depending upon the mode of screen a wide variety of genetic damage such as gene mutation, chromosomal damage, and aneuploidy can be detected by some of these assays. Both *in vitro* and *in vivo* testing methods are basically used to identify the same endpoints [57]. In antimutagenicity assay, cells are treated with the potential antimutagenic test compound along with a known standard mutagen prior to analysis.

These assessments protocol has been divided into three phases. Phase 1 is based upon *in vitro* tests involving cultured bacterial and mammalian cells. Phase 2 involves *in vivo* assessment of activity in somatic cells. Phase 3 assays screen for germ cell mutagens [58, 59]. Phase 1 assays are primarily used for the identification of gene mutations and chromosome alterations. In the early mutagenicity assessment, two or three different tests in bacteria and mammalian cells should be used. The bacterial mutation assays such as *Salmonella typhimurium* and *E. coli* WP2 reverse mutation tests are useful tool for point mutations identification. Mammalian mutation assays are useful especially in case of bactericidal compounds and agents acting preferentially on the replication system in mammals. Common Phase 1 *in vitro* mammalian tests include: the mouse lymphoma thymidine kinase (TK) gene mutation assay, which detect compounds that induce forward gene mutations in the *tk* gene of the L5178Y mouse lymphoma cell line, and the hypoxanthine guanine phosphorybosyl transferase (*hprt*) gene mutation assay, which identifies agents that cause gene mutations in the *hprt* gene of a suitable cell line, such as Chinese hamster cells [57-60]. These phase 1 assays have many advantages, including their simplicity, relatively low cost, sensitivity, and flexibility to different experimental settings [61]. In addition, such tests may also provide some clues about the possible mechanisms of mutagenicity. Phase 2 *in vivo* assays can be used in the verification of the positive results obtained Phase 1 testing. The common procedure is screening for cytogenetic damage by metaphase analysis assay or the micronucleus test. Other *in vivo* assays include transgenic animal assays for point mutations, which can be used for the simultaneous detection of mutagenic effects in various tissues; DNA strand breakage assays, such as comet assay (also called as the single-cell gel electrophoresis assay), which detect single- and double-strand breaks, repair induced breaks and alkali-labile lesions; and the liver unscheduled DNA synthesis (UDS) test, which is useful for the measurement of the repair of DNA lesions [57, 58]. Compounds that give positive results for mutagenic potential in somatic cells *in vivo* should be further tested with germ cells. Phase 3 comprises of two classes of assays. Class 1 includes assays in germ cells per se, such as gene mutation tests in transgenic animals; paternal germinal mutation in the expanded simple tandem repeat (ESTR) test; and chromosomal aberration tests, whereas class 2 assays deal with the identification of alterations in offspring of mutagen exposed animals by testing for gene mutations in the ESTR assay; mouse visible specific locus test for detecting and quantifying the induction of heritable point mutations in mammals; the biochemical specific locus test for detection of mutations originating in the germ line of a mammalian species; and the dominant lethal test for chromosome or gene mutations [58, 62].

Some of these assays are discussed below.

The Ames test (*S. typhimurium*/microsome assay)

The Ames test uses several genetically engineered strains of the bacterium *S. typhimurium* that carry mutations in genes involved in histidine biosynthesis. These strains are auxotrophic mutants, i.e. they require histidine for growth, but cannot produce it. The method tests the capability of the test compound in creating mutations that result in a reversion to a "prototrophic" state, so that the cells can grow on a histidine-free medium. It is one of the most widely used short-term mutagenicity/antimutagenicity test [63, 64]. The test detect mutagenic agents acting with different mutation mechanisms, such as base-pair substitution and frame shift mutations using specific strains. Similarly, antimutagenic activity of compounds against induced mutations can also be evaluated [64]. Rat liver extract is added to activate the indirect acting mutagens such as benzo[α]pyrene. The Ames test was initially developed using

agar plates (the plate incorporation technique), as described above. Later a popular alternative method called 'fluctuation method' was also developed. In this method by including a pH indicator, the frequency of mutation is counted in microplates as the number of wells which have changed color due to drop in pH due to metabolic processes of reproducing bacteria. The fluctuation method is comparable to the traditional pour plate method in terms of sensitivity and accuracy [65]. The test serves as a quick and convenient assay to estimate the carcinogenic potential of a compound because standard carcinogen assays on mice and rats are time-consuming and expensive. However, false-positives and false-negatives are known. Early studies showed that 50-70% of known carcinogens may be identified via this test [66-68]. Also the dose response curve using varying concentrations of chemical is almost always linear, indicating that there is no threshold concentration for mutagenesis [66, 67]. However, some proposed that organisms can tolerate low level of mutagens due to protective mechanisms such as DNA repair, and threshold may exist for certain chemical mutagens [63, 64].

E. coli WP2 tryptophan reverse mutation assay

It detects *trp*– to *trp*C reversion at a site blocking a step in the biosynthesis of tryptophan prior to the formation of anthranilic acid. *E. coli* strain WP2 is a radiation resistant derivative of *E. coli* B/r which was the strain used by Luria and Delbrück [69]. This assay is primarily useful in the detection of A/T base pair damage [70]. The target site for a site specific back mutation is an ochre (UAA) nonsense mutation [71, 72]. The assay is unable to detect frame shift mutations [73]. Like Ames test this assay has also comparatively higher rate of spontaneous tryptophan revertant colonies per plate [70].

SOS chromotest

In this assay *E. coli* PQ37 mutant strain allows the assessment of DNA changes induced by various mutagens through a colorimetric assay by means of fusion of a SOS regulon gene (responsive to the genotoxic compounds) with a reporter gene β-galactosidase (*lacZ*) [74]. Two genes play a key role in the SOS response: *lexA* encodes a repressor, and *recA* encodes a protein able to cleave the LexA repressor (by autocatalytic mechanism) upon activation by an SOS inducing signal. By including a lactose analog which yields a colored compound upon degradation, an easily observable or quantifiable change in colour is obtained. Since the chemical tested may inhibit protein synthesis at higher concentrations, which would lead to an underestimation of β-galactosidase induction, alkaline phosphatase is assayed simultaneously in order to scale the data to survivability of the cells. The test is performed over a few hours in columns of a 96-well microplate. The test is comparable in accuracy and sensitivity to other established methods such as the Ames test.

rpoB-rifampicin resistance assay

The $Rif^S \rightarrow Rif^R$ (rifampicin sensitive to resistant) test is based on acquisition of rifampicin resistance by *E. coli* MG1655 cells upon mutagen exposure (Figure 1). The *rpoB* gene encodes the β-subunit of RNA polymerase (subunits: α2, β, β', and ω) that has many hot spots for mutations [20, 21, 55]. Mutation in this gene reduces its binding to rifampicin and thus results in acquisition of rifampicin resistance by *E. coli* mutants [20, 21, 55]. This assay is advantageous due to its simplicity in deployment, the ability to provide a wide spectrum of forward mutations and low level of spontaneous mutations (approximately $1/10^8$ cells) (Figure 1). It has been extensively used recently to screen the antimutagenic potential of different foods particularly fruits, vegetables and other allied food products at Food Technology Division, Bhabha Atomic Research Centre, Mumbai, India (Figure 2) [20, 50-55, 75-79]. The assay is better for scoring antimutagenicity over mutagenicity. This is because *rpoB* has many hot spots of mutation and hence detecting precise site of mutation due to any mutagenic assault becomes cumbersome. However, for analyzing antimutagenicity this does not create a limiting condition, albeit it provides a comprehensive window to screen the antimutagenic potential.

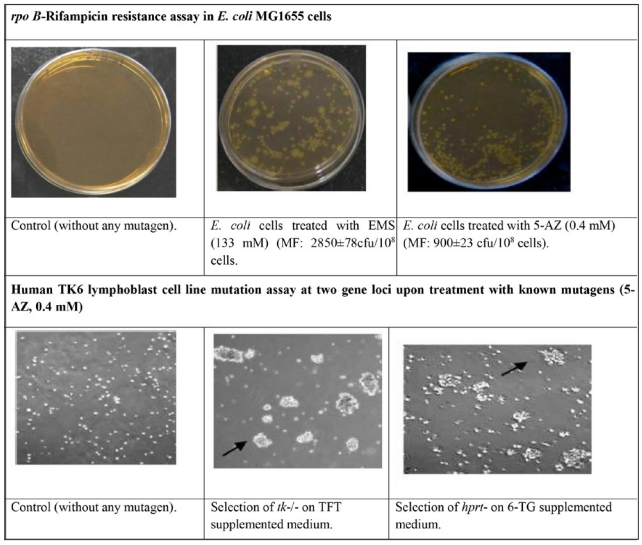

Figure 1: Recently standardized mutagenicity assay systems (MF: Mutation frequency; TFT: Trifluorothymidine; 6-TG: 6-thioguanine).

Vibrio harveyi assay

The test employs a series of genetically modified *Vibrio harveyi* strains. The bacterium is naturally sensitive to neomycin; however, antibiotic-resistant mutants may arise. The frequency of appearance of mutants increases in the presence of mutagens in a dose–response manner, which forms the basis of this assay [80-88]. It is also not pathogenic to humans, and hence is safe to work with. This was also found to be significantly more sensitive to mutagenic treatments than *E. coli* [80]. *V. harveyi* cell envelope is significantly more permeable for large molecules, including most mutagens, and hence penetration of mutagens into *V. harveyi* cells is more efficient relative to many other bacteria used in mutagenic assays [80]. Besides being a tool for mutagenic detection, *V. harveyi* possesses several features making this bacterium a useful bio indicator of mutagenic pollution in natural water samples particularly marine water due to higher level of salt tolerance [81].

Yeast mutagenicity assay

Yeasts being eukaryotes have chromosome structure and DNA repair processes quite similar to those in mammals. *Saccharomyces cerevisiae* strains have endogenous cytochrome

P450, and therefore, can be very useful testing promutagens [89]. Two assay systems have been described for yeast. One of these detects insertion/deletion mutations and other base substitutions. The assay for insertion/deletion mutations uses a variety of different simple repeats placed in frame with URA3 such that insertions or deletions lead to a selectable Ura (-) phenotype; essentially all such mutations are in the simple repeat sequence. The assay for base substitution mutations uses a series of six strains with different mutations in one essential codon of the CYC1 gene. Because only true reversions lead to a selectable phenotype, the bases mutated in any reversion event are known [90]. Also an "indirect" reporter assay system based on recombinant yeast containing both a sensor and a reporter plasmid has been developed. The sensor plasmid contains a gene encoding the artificial transcription factor of the *E. coli* LexA DNA binding domain fused to the transcriptional activation domain of yeast Gal4p, which is regulated by the DNA damage-inducible RNR2 promoter. The reporter plasmid contains the *E. coli lacZ* gene with the LexA binding site in the 5'-upstream region, allowing transcriptional activation by the induced LexA-GAL4 protein. To increase the sensitivity of this reporter system amongst several deletion yeast strains enhanced induction of reporter activity was observed in DNA repair-deficient mag1Delta cells [91].

Human lymphoblast mutation (HLM) assay

The TK6 human lymphoblast cell line contains two widely used selectable markers: the heterozygous *tk* locus on chromosome 17q and the X-linked, hemizygous *hprt* locus. The *tk* gene is involved in the synthesis of thymidine kinase 1, a cytosolic phosphotransferase enzyme required in pyrimidine salvage pathway for phosphorylation of deoxythymidine to deoxythymidine 5'-monophosphate (dTMP). It can also phosphorylate pyrimidine analogue such as trifluorothymidine (TFT), which possibly blocks DNA replication upon incorporation, rendering $tk^{-/-}$ mutants to survive the cytotoxicity. The *hprt* gene is involved in the synthesis of the enzyme hypoxanthine-guanine phosphoribosyltransferase (HGPRT) having role in purine salvage pathway. HGPRT catalyzes the conversion of hypoxanthine to inosine monophosphate (IMP) and guanine to guanosine monophosphate (GMP) via transfer of the 5-phosphoribosyl group from 5-phosphoribosyl 1-pyrophosphate (PRPP). This enzyme provides an alternative to energy-expensive *de novo* synthesis of nucleotides by maintaining intracellular purine nucleotide pool in stressed cells. In addition to its normal substrates, HGPRT can also catalyze the transformation of purine analogues such as 6-thioguanine (6-TG) to 6-thioguanine monophosphate (TGMP). Intracellular accumulation of TGMP hampers the *de novo* synthesis of guanine which is carried out by inosine monophosphate dehydrogenase. TGMP gets phosphorylated to thioguanine diphosphate (TGDP) and thioguanine triphosphate (TGTP). The TGMP, TGDP and TGTP are collectively called 6thioguanine nucleotides (6-TGN). 6-TGN is cytotoxic to cells as it incorporates into DNA biosynthesis leading to strand breakage [92]. However the $hprt^{-}$ mutants lacking the functional HGPRT enzyme cannot phosphoribosylate 6-TG and hence these mutant cells are resistant to the cytotoxic effects of 6-TG. For performing the assay the wild type cells ($tk^{+/-}$ or $hprt^{+}$) are selected in CHAT (cytidine, hypoxanthine, aminopterin and thymidine) medium where aminopterin blocks the *de novo* synthesis of nucleotides by inhibiting the dihydrofolate reductase enzyme. Cells were further grown in CHT medium (without aminopterin) for preferential enrichment of $tk^{+/-}$ *or* $hprt^{+}$ population. This selected cell population is further grown in the presence of test (mutagenic/ antimutagenic) compound and later selected with TFT and 6-thioguanine (6-TG) for $tk^{-/-}$ and $hprt^{-}$ cells, respectively. Known mutagens are used as positive control. The mutant cells divide and form cellular aggregates (in suspension) through *de novo* synthesis of nucleotides which can be visualized under an inverted microscope (Figure 1). The result is expressed as the relative number of mutant cells to the total number of seeded cells [54, 77].

Drosophila mutagenicity assay

Mutagen-sensitive (mus) mutations in *Drosophila melanogaster* render developing flies hypersensitive to the lethal effects of DNA-damaging agents and hence, serve as sensitive *in vivo* indicators of a wide range of mutagens and genotoxic carcinogens. That assay measures the survival of DNA repair-deficient mus homozygotes relative to their repair-proficient heterozygous siblings. Those two classes of fly are easily distinguished from one another by their phenotypic markers. In addition, the heterozygotes serve as a relatively mutagen-insensitive internal control in all test vials. One tester strain (mus208B1 mus210B1 mus211B2) was successfully used in identifying 11 of 12 chemical carcinogens as genotoxic and two noncarcinogens tested as nongenotoxic [93].

Chromosomal abnormalities detection test

Both structural and numerical changes occurring in chromosomes can be identified *in vitro* in metaphase spread preparations from mutagen/antimutagen exposed mammalian cells. Common *in vitro* chromosomal damage tests include the mammalian chromosome aberration test and the micronucleus test. For chromosome aberration test, mammalian metaphase cells are analyzed for the presence of structural chromosome aberrations, whereas in the micronucleus test, micronuclei in the cytoplasm of cultured mammalian cells during interphase are detected. The micronucleus test is a procedure for the detection of both aneuploidy and clastogenicity in cultured mammalian cells [57, 58]. The *in vivo* chromosome aberration test in mammals allows the identification of structural chromosome changes induced by a substance in the bone marrow cells of animals, whereas the *in vivo* micronucleus assay is used for the identification of genetic changes induced by the tested compound to the chromosomes or the mitotic apparatus of cells by the analysis of erythrocytes as sampled in the bone marrow and/or peripheral blood cells of animals.

Fruits and Vegetables as Antimutagens

There are documented scientific evidences endorsing beneficial role of fruits and vegetables in the prevention as well as treatment of different diseases due to their biologically active substances, such as vitamins and secondary metabolites (polyphenols, carotenoids, sterols, glucosinolates, and saponins [94-103]. Hence, the consumption of fruits and vegetables, which is well below the recommended level, should be

encouraged. It has been shown that individuals who daily eat five servings or more of fruits and vegetables have approximately half the risk of developing many diseases including a wide variety of cancers, particularly those related to gastrointestinal tract [104]. In some studies, dietary fiber and polyphenols of fruits have been reported to improve lipid metabolism and prevent the oxidation of low density lipoprotein cholesterol (LDL-C), which hinder the development of atherosclerosis [105-107].

Antimutagenic potential of Fruits

Current research in author's laboratory and many other laboratories in the world has focused on health protective properties including antimutagenic potential of different fruit types and their cultivars. A brief outline on the antimutagenic potential of some common as well as exotic fruits found across the world is reported.

Apple *(Malus domestica)*

Worldwide, apple is cultivated as a fruit tree for its sweet and pomaceous fruit. Besides, it is the most widely grown species in the genus *Malus*. Apple fruits are low in calories and notable for impressive list of phytochemicals, and anti-oxidants. Studies suggest that its components are essential for optimal growth, development, and overall wellness. In a recent study different apple cultivars were evaluated for their potential health protective attributes such as antimutagenic and antioxidant properties [54]. These functional prophylactic attributes of apples displayed cultivar specificity as cv. 'Granny Smith' displayed significantly higher and broad spectrum antimutagenicity in *E. coli rpoB*/Rif^R assay, whereas, cultivars 'Ambri Kashmiri', 'Kinnaur' and 'Red Delicious' exhibited strong antioxidant activity. As compared to antimutagenicity, the antioxidant and radio protective properties were found to be better correlated than antimutagenicity. Suppression of error-prone DNA repair pathway (such as *E. coli* SOS response) was found to be one of the possible mechanisms contributing to its antimutagenicity. The phenolic extract of 'Granny Smith' was purified (through HPLC) and the antimutagenic bioactive was identified as procyanidin dimer. Besides, the purified compound also displayed significant antimutagenicity in thymidine kinase locus of human lymphoblast cell line (TK$^-$) against ethyl methanesulfonate induced mutagenesis.

In an another study, aqueous extract of Apple (*Malus domestica* 'Golden Delicious') strongly inhibited the mutagenicity of NPYR (N-nitrosopyrrolidone) by 54% in Ames test at a concentration of ≤ 250 µg/plate [108].

Aronia melanocczrpa

Aronia melanocczrpa is commonly called as 'Black chokeberry'. The anthocyanins isolated from this fruit displayed significant antimutagenicity against benzo[a]pyrene and 2-amino fluorene in the Ames test [109]. Besides, in the sister chromatid exchange test with human blood derived lymphocytes cultured *in vitro*, a significant decrease of SCEs frequency induced by benzo[a]pyrene was observed in the presence of anthocyanins.

Copaiba (*Copaifera langsdorffii*)

Copaifera langsdorffii (copaiba) is an exotic Brazilian fruit. In a recent study, the antimutagenic potential of this fruit was elucidated and copaiba powder (dose of 100 mg/ kg) showed great reduction of micronuclei [110].

Dillenia indica

Dillenia indica (Elephant apple, chulta/chalta or ouu) is a species of Dillenia native to southeastern Asia and produces a large hard fruit. Fruit extract *Dillenia indica* displayed moderate antimutagenic activity at 1000 µg/plate concentration and strong at 1500 and 2000 µg/plate concentrations against sodium azide induced mutation in *Salmonella* tester strain (TA 1531) [111].

Date fruit

Fruits of the date palm (*Phoenix dactylifera* L. Arecaceae) are very commonly consumed in many parts of the world and are a vital component of the diet in most of the Arabian countries. Date fruit extract produced a dose-dependent inhibition of benzo(a)pyrene-induced mutagenicity in *Salmonella* tester strains TA98 and TA100 (with metabolic activation). Extract from 3.6 mg/plate and 4.3 mg/plate was required for 50% inhibition of His+ revertant formation in TA98 and TA100, respectively. Antimutagenic activity in date fruit is quite potent and implicates the presence of compounds with potent free-radical-scavenging activity [112].

Eugenia stipitata

Eugenia stipitata ssp. Sorroria Mcvaugh belongs to *Myrtaceae* family and is predominantly found in the Amazonian rain forest of Brazil, Colombia and Ecuador. The edible fruit is considered as a rich source of various phytochemicals including terpenes, volatile compounds, fiber, and vitamin C and is widely known for its functional (high antioxidant activity) and potential health benefits to humans. Antimutagenic and anti-genotoxic activities of the fruit were assessed employing micronucleus test and comet assay in mice, respectively. The ethanolic extract displayed significantly higher antimutagenic and antigenotoxic potential (at concentration of 300 mg/kg of body weight) thus apparently highlighting its potential preventive effects against cancer [113].

Grape (*Vitis vinifera*)

A grape is a fruiting berry of the deciduous woody vines of the botanical genus Vitis. Recently, the strong beneficial health effects of grape flavonoids have been directly connected to the so called "French Paradox". This term refers to the epidemiological observation of comparatively low incidence of coronary heart diseases in the population of the Mediterranean region, despite the presence of a local diet rich in saturated fats. Concord grapes are rich in polyphenolic chemicals and anthocyanin pigments that may have health protective biological properties. In an earlier study it was shown that grape juice consumption could significantly inhibit the initiation stage of 7, 12-dimethylbenz[a]anthracene (DMBA) induced rat (female Sprague–Dawley rats) mammary tumorigenesis. Rats fed with grape juice phenolics displayed significantly lower levels of *in vivo* mammary DMBA-DNA adduct formation [114]. In another study, the antimutagenic and antigenotoxic potential of grape juice concentrate in rodent organs exposed to cadmium chloride (cadmium chloride at 1.2

mg/kg body weight) when assayed by single cell gel (comet) and micronucleus assays indicated decreased genotoxic effects in peripheral blood and liver cells [115]. Also a decrease in anti-8-hydroxy-20-deoxyguanosine (8-OHdG) expression level in hepatocytes was observed.

Guava

Psidium guajava (common guava, lemon guava) is a small tree in the Myrtle family (*Myrtaceae*) and guavas are common tropical fruits cultivated and enjoyed in many tropical and subtropical regions of the world. Fruit is considered to be a rich source of phytonutrients having health benefits. In a study, the water and chloroform extracts of guava were tested for their antimutagenicity [116]. The water extract was effective in inactivating the mutagenicity of direct-acting mutagens, e.g., 4-nitro-O-phenylenediamine, sodium azide, and the S9-dependent mutagen, 2-aminofluorene, in the tester strains of *S. typhimurium*. The chloroform extract was found inactive. The enhanced inhibitory activity of the extracts on pre-incubation suggests the possibility of desmutagens in the extracts. Major constituents were found to be ascorbic and citric acid, however, the role of other antimutagenic factors in the extracts cannot be ruled out.

Jackfruit (*Artocarpus heterophyllus* Lam)

Jackfruit (*Artocarpus heterophyllus* Lam) is a rich source of several high-value compounds with potential beneficial physiological activities. Due to its reported health benefits the consumption of Jackfruit pulp has increased. In a recent study, the pulp of Jackfruit extract was evaluated for its antimutagenic and antiproliferative properties, using *S. typhimurium* tester strains TA98 and TA100 with metabolic activation (S9) and a cancer cell line M12.C3.F6 (murine B-cell lymphoma), respectively [117]. The Jackfruit extract purified fractions were reported to reduce the number of aflatoxin B1 revertants as well as proliferation of M12.C3.F6 cells in a concentration dependent manner, which indicated its health protective and antimutagenic potential.

Java plum (*Syzygium cumini*)

Syzygium cumini (Java Plum) belongs to the family *Myrtaceae* and is believed to have originated in Indian subcontinent. *S. cumini* fruits have been reported to be rich in flavonoids and anthocyanins. In a study carried out at Food Technology Division, Bhabha Atomic Research Centre, Mumbai, India the antimutagenic potential of 11 commonly consumed fruits in India was analyzed wherein fruits displayed significant variation in antimutagenicity when assayed by *E. coli* rifampicin resistance (Rif^R) assay [52]. Among them, Java plum displayed highest antimutagenicity. Java plum was further selected and characterized for its bioactive compound(s). Anthocyanins were found to be responsible for the observed antimutagenicity and the anthocyanins were further purified by HPTLC. All bands visible on TLC plate showed antioxidant activity whereas, only one band at R_f 0.22 was most antimutagenic and resolved into two peaks in HPLC. The second peak (t_R 3.8 min) displayed a strong and broad spectrum antimutagenicity which was identified as petunidin-3, 5-diglucoside [52].

Mangaba

Mangaba (*Hancornia speciosa* Gomes) is the fruit obtained from mangabeira tree (family *Apocynaceae*) which is predominantly found in the tropical areas of Brazil. The shape of the fruit is ellipsoidal or spherical berry having sweet and acidic, and the viscous texture. Mangaba fruit pulp has been reported to display protection against doxorubicin and dimethyl hydrazine-induced mutagenicity in male Swiss mice in bone marrow and gut micronucleus test and also apoptosis index [118]. The *in vivo* tests revealed that mangaba fruit pulp showed no genotoxic effects in any of the assays performed.

Murici (*Byrsonima crassifolia*)

The fruit known as murici (*Byrsonima crassifolia* L., Malpighiaceae) grows on small trees (at the most 5 m tall) and is found both in the Amazonian region as well as some Northeastern states of Brazil. When mature, it is yellow, has a diameter of 1.5 to 2 cm, and a strong odor resembling a fruity, rancid cheese. Murici, at 400 mg extract/kg body weight was found as an effective treatment to protect against genotoxicity and induced mutagenicity. The extract effects of these fruits on the cell, as well as other beneficial roles that they could have on cell metabolism still remains to be elucidated [119].

Noni

Morinda citrifolia L., "Noni" (*Rubiaceae*) is an evergreen plant indigenous to Southeast Asia and in traditional Polynesian medicine, Noni has been used to treat variety of diseases for more than 2000 years. Currently, Noni products such as juices and encapsulated powders are popular functional foods in Asia, Europe, and North America. Its juice is already a significant player in the growing functional beverage market and has been accepted as a new food in the European Union, and has been found to be acceptable for human consumption after official safety evaluations. In a recent study, a commercial noni juice was evaluated for its protective activities against the lesions induced by mitomycin C (MMC) and doxorubicin (DXR) using the Somatic Mutation and Recombination Test (SMART) in *Drosophila melanogaster*. Three-day-old larvae, trans-heterozygous for two genetic markers (*mwh* and *flr3*), were cotreated with TNJ plus MMC or DXR. A significant reduction in genotoxic effects of MMC and DXR caused by the juice was observed which highlighted its strong antimutagenic potential.

Pomegranate

Pomegranate is one of the common fruits that is available worldwide and has been used for centuries for the treatment of various ailments. Pomegranate is a rich source of many phenolic compounds including flavonoids and hydrolyzable tannins. Pomegranate seeds are rich in sugars, polyunsaturated fatty acids, vitamins, polysaccharides, polyphenols and minerals and have high antioxidant activity. When crushed and dried, the seeds produce oil with 80% punicic acid, the 18-carbon fatty acid, along with the isoflavone genistein, the phytoestrogen coumestrol and the sex steroid estrone. The antimutagenic effect of the bioactive pomegranate compounds has been demonstrated by a decrease in the frequency of genotoxicant induced chromosomal aberrations in bone marrow cells of mice and rats [120]. Recent findings suggest that agents

derived from pomegranate fruit can effectively interfere with multiple pathways critically involved in different stages of the development and progression of tumors.

Randia echinocarpa

Randia echinocarpa belongs to *Rubiaceae* plant predominant in Mexico. The edible fruit is known for its immense ethno pharmacological relevance under various diseased conditions. Its hexane fraction displayed significantly strong antimutagenic activity in *S. typhimurium* YG1024 through micro suspension assay when 1-nitropyrene was used as mutagen [121]. In this study highest antimutagenicity was attributed to the presence of sterols (predominantly campesterol and β-sitosterol) and fatty acids (palmitic and linoleic). The samples evaluated did not show any toxicity or mutagenicity.

Vegetables as antimutagen

Antimutagenic activity of many vegetable juices were earlier studied against mutagenicity induced by 2-amino-3-methyl[4,5-f]-quinoline (IQ), 2-amino-3,4-dimethylimidazo[4,5-f] quinoline (MeIQ) or 2-amino3,8-dimethylimidazo[4,5-f] quinoxaline (MeIQx) in *S. typhimurium* TA98 and TA100 [122]. Strong antimutagenic activity was displayed by beets, chives, horseradish, onions, rhubarb and spinach. All cruciferous vegetables showed strong to moderate antimutagenic activities, except Chinese cabbage, which displayed weak activity. Moderate antimutagenicity was found with green beans and tomatoes, whereas weak activities in eggplant, garden cress, many lettuces, leeks, mangold, cucumber, pumpkin, radish and summer squash. However, some vegetables such as *Asparagus*, carrots, fennel leaves, parsley, green pepper and radishe were not found to display any antimutagenicity.

Effect of variety/cultivar

In a comprehensive study, forty one vegetables were screened along with their common varieties employing Rif^R assay in wild type *E. coli* MG1655 and Ames test at Food Technology Division, Bhabha Atomic Research Centre, Mumbai [50]. Most antimutagenic vegetables were cauliflower, cabbage, pepper (bell-red, hot-red Jalapino, and hot Arbol), eggplant (This, small-violet and green-yellow-striped), garlic, onian (red), Zucchini, Bean (lima, clustered and yardlong), squash, gourd (bottle), cucumber (Madras), pea (green), drumstick, and Indian gooseberry against ultraviolet induced mutagenesis. Effect of cultivar difference in antimutagenic activity was also studied with traditional vegetables cultivated in limited areas near Kyoto [123]. Among those traditional vegetables, Kamo eggplant and Katsura oriental pickling melon, Shishigatani pumpkin showed higher antimutagenicity against UV-induced mutation of *E. coli* B/r WP2 than their corresponding common vegetable. Five different types of *Capsicum* spp. ('Chilaca', 'Poblano', 'Serrano', 'Jalapeno' and 'Pimiento') were analyzed for antimutagenicity using *S. typhimurium* tester strain YG1024 against 1nitropyrene (1-NP), 1,6-dinitropyrine (1,6-DNP), and 1,8-dinitropyrine (1,8-DNP). In that study too, varietal effect on antimutagenicity was observed and varieties such 'Chilaca' and 'Pimiento' were found to be comparatively more potent [124].

Mutagen specificity

Mutagen based variation in antimutagenicity of vegetables has been reported in numerous studies. Hexane and chloroform extracts of Chinese radish strongly inhibited the mutagenicity of both direct acting mutagens (2-(2-furyl)-3-(5-nitro-2- furyl) acrylamide and sodium azide) as well as indirect mutagens (aflatoxin B1) but was not found to inhibit the mutagenicity of an indirect acting benzo[a]pyrene [125].

Similarly, aqueous extract of some vegetables such as onion (500 µg/plate) showed 60% antimutagenicity in Ames test using *S. typhimurium* TA100 against N-nitrosodimethylamine (NDMA); carrot (250 µg/plate) showed 49% against N-nitrosodibutylamine (NDBA), and garlic extract (2000 µg/plate) showed 65% against N-nitrosopiperidine (NPIP) [108].

Effect of mode of cultivation

In a study, juices of organically cultivated vegetables (using a water-soluble chitosan and leaf surface spray) were compared with generally cultivated vegetables in forward mutation test with *S. typhimurium* TM677 and 8-azaguanine as a detection agent [126]. Organically cultivated Chinese cabbage, carrot, Welsh onion, and Qing-gen-cai displayed 37–93% antimutagenicity against 4-nitroquinoline oxide (4NQO), while the generally cultivated ones displayed only 11–65%. Antimutagenicity of organically cultivated and GC spinach was 78 and 49%, respectively against 3-amino-1-methyl-5H-pyrido[4,3b]indole acetate (Trp-P-2). Similarly, the antimutagenicity of methanol extract from the spring baechu cabbage (*Brassica campestris* L. ssp pekinensis [Lour.] Rupr.) particularly cultivated by Tunnel method was higher than Noji against mutagenicity induced by MNNG and AFB1 in Ames test [127] and thus indicated effect of cultivation on this bioactivity.

Solvent based variation

Type of solvent used for extraction of vegetables has been reported to affect the extent of reduction in induced mutagenesis [128]. Interestingly, around 96% of the n-hexane extracts, 64% of the dichloromethane extracts, 44% of the acetone extracts, and 36% of the 2-propanol extracts of different vegetables displayed antimutagenic activities [128]. In other study, vegetables such as Brussels sprouts, carrot, and yellow-red peppers were sequentially solvent extracted with n-hexane, dichloromethane, acetone, and 2-propanol and tested for the inhibition of induced mutagenesis by aflatoxin B1 (AFB1), benzo[a]pyrene (BaP), 2-amino-3-methylimidazo[4,5-f] quinoline (IQ), and cyclophosphamide (CP) using Ames test in *S. typhimurium* strains [129]. This activity was found in different extracts, but most prevalent in the n-hexane extracts. In another study, aqueous dialysates of most of the vegetables were reported to reduce the mutagenicity of Trp-P-2 in *S. typhimurium* TA100, whereas only some dialysates of burdock, eggplant, and spinach also inhibited the mutagenicity of Trp-P-l, benzo[a]pyrene, sterigmatocystin, aflatoxin Bl, 2-(2-furyl)-3-(5-nitro-2-furyl)-acrylamide and N-methyl-N'-nitroso-N-nitrosoguanidine [130].

Antimutagenicity evaluation in higher systems

In a study protective effect of vegetables was assessed against

the genotoxicity of 2-amino-3methylimidazo[4,5-f]quinoline (IQ) and 2-amino-1-methyl-6- phenylimidazo[4,5-b] pyridine (PhIP) in genetically engineered V79 Chinese hamster fibroblasts using comet assay [131]. Among vegetable juices, spinach and onion juices (IC_{50} = 0.42-0.54%) displayed strong inhibition of IQ genotoxicity whereas broccoli, cauliflower, beet root, sweet pepper, tomato, chard, and red-cabbage juices suppressed IQ genotoxicity only moderately, whereas cucumber juice was not effective.

In other study, the mice (C57Bl) were placed on diet (AIN-93G) supplemented to dry weight (20%) with grains or freeze-dried fruits or vegetables and the frequency of micronuclei in the peripheral blood measured [132]. Among foods (~26) tested, flaxseed was most effective in reducing the incidence of micronuclei by 30 and 11% in the reticulocyte and normochromatic erythrocyte cell populations, respectively. The antimutagenicity of lettuce and chard extracts against Benzo[a] pyrene was studied in male Balb/C mice [133]. The mutagenic activity of the urine samples from only B[a]P groups treated was high than the group treated also with vegetable extracts in Ames test.

Extract of the poblano pepper (*Capsicum* spp.) was assessed for antimutagenicity against the nitrosation process in wing cells of *Drosophila melanogaster* using the somatic mutation and recombination test [134]. The poblano juice decreased the mutations per wing by 40 and 80% as compared to methyl urea (MU) and sodium nitrite (SN), respectively. Some important vegetables having high antimutagenicity have been displayed in Table 3.

Besides certain fruit juices and tea were also analyzed for protective effects using Chinese hamster lung fibroblasts, genetically engineered for the expression of rat cytochrome P450 dependent monooxygenase 1A2 and rat sulfotransferase 1C1 (V79-rCYP1A2-rSULT1C1 cells) against genotoxicity induced by 2-acetylaminofluorene (AAF) or 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) [135]. Genotoxic activity of PhIP/AAF was strongly reduced by green, black, and rooibos tea, and juices of blueberries, blackberries, red grapes, kiwi, watermelon, parsley, spinach, morellos, and black-currants.

Contributing biochemical(s)

Studies have shown involvement of non-polar, mid-polar or polar group of compounds in observed antimutagenicity depending upon the vegetables.

Non-polar components

Possible involvement of non-polar compounds of vegetables was indicated in several studies [125, 129, 136]. In those studies, antimutagenicity was reported mainly in n-hexane or chloroform fraction(s). The n-hexane fraction has been reported to contain carotenoids, xanthophylls or carotenol esters in tomato and carrot [129]. Involvement of these phytochemicals (β-carotene and xanthophylls) has also been reported in green peppers using *S. typhimurium* tester strain YG1024 against 1-nitropyrene (1-NP), 1,6-dinitropyrine (1,6-DNP), and 1,8-dinitropyrine (1,8-DNP) [124]. Antimutagenicity of aqueous and acetone extracts of vegetables against 3-methylcholanthrene and benzo[a]pyrene in the Ames test (gene reversion mutagenesis/mammalian microsomal activation assay) has also been reported to be correlated with the chlorophyll content [137]. This inhibitory activity of chlorophyll was comparable to the sodium copper chlorophyll in level. In other study too, dietary chlorophyll derivatives showed a dose-dependent inhibitory activity against B[a]P induced mutagenesis in bacterial reverse mutagenicity assay using *S. typhimurium* TA100 [138].

Mid-polar/polar components

In a study, thirteen flavonoids and related compounds were reported from spinach which acted as antimutagen against the dietary carcinogen 2-amino-3-methylimidazo [4,5-f] quinoline in *S. typhimurium* TA 98 [139]. Interestingly, only 5,6,3',4'-tetrahydroxy-7-methoxyflavonol 3-O-disaccharide was reported as potent antimutagen. Several other studies have also demonstrated role of polyphenols in antimutagenicity of vegetables. The antimutagenic profiles and the total soluble phenolic content of the vegetables were reported to be strongly correlated [50]. Polyphenolic from French bean was found to display antimutagenicity against 1-NP and B[a]P and aflatoxin B1 [140, 141]. Anthocyanin-rich water fraction and ethyl acetate fraction from Andean purple corn were analyzed against Trp-P-1 in Ames test [142]. Further, ethyl acetate fraction was more potent and its sub-fraction that contained quercetin derivative displayed highest antimutagenic activity.

Components of varying polarity

In a study with various extracts of eggplant, lutein, pheophorbide or chlorophyllide, and tannins containing sugar-moieties from the 84% methanol (methanol/water, v/v), 70% methanol and water, respectively, were reported as possible antimutagens against Trp-P-2 in Ames test [143]. In the same study bioactives such as pheophytin a and b, Mg-free derivatives of chlorophyll a and b were isolated from the petroleum ether layer.

In a recent study, spinach, lettuce, iceberg lettuce, cabbage, broccoli and French bean were comprehensively analyzed for antimutagenicity against ethyl methanesulfonate (EMS) in Rif^R assay in *E. coli* MG1655 (Kumar et al., unpublished data). In most of these vegetables, phenolics were found to be responsible for antimutagenic activity (Table 4). However, in case of spinach quinone rich extract displayed higher activity than phenolic rich extract (Table 4). The content (yield) of phenolic or quinone rich extract was not found to be correlated to the antimutagenic activity. This indicated that antimutagenic activity of vegetables mainly depends upon the quality of phenolics or quinones.

Possible mechanism of antimutagenicity

Many mechanisms have been proposed to explain antimutagenicity of vegetables. In Thai vegetables antimutagenic activity against indirect acting mutagens was proposed to be related to the inhibition of the activity of metabolic-activating enzymes in rat liver homogenates [136]. A similar mechanism was proposed for indirect acting mutagens in case of non-polar fraction of Chinese radish where significant inhibition of rat liver aniline hydroxylase and aminopyrine demethylase was observed [125]. In Andean purple corn, anthocyanin-rich water fraction displayed blocking effect on S-9 mix activation

Table 3: List of vegetables having high antimutagenic activity in various models.

Family	Common Name	Scientific Name	Antimutagenicity in various models	Ref. no.
Brassicaceae	Cauliflower	*Brassica oleracea* subsp. *Botrytis*	*S. typhimurium* TA98 and TA100; *E. coli* MG1655 based Rifampicin resistance (Rif^R) assay; V79 Chinese hamster fibroblasts	[50, 122, 131]
	Cabbage (Savoy, red, white, Chinese and spring baechu etc)	*Brassica oleracea* subsp. *Capitata*	*S. typhimurium* TA98, TA100, and TM677; *E. coli* MG1655 based Rifampicin resistance (Rif^R) assay; V79 Chinese hamster fibroblasts	[50, 122, 126, 127]
	Broccoli	*Brassica oleracea* var. *italica*	*S. typhimurium* TA98 and TA100; V79 Chinese hamster fibroblasts	[122, 131]
Solanaceae	Pepper (hot Arbol, bell-red, hot-red Jalapino, sweet, poblano, green etc)	*Capsicum annum* var. *grossum*	*E. coli* MG1655 based Rifampicin resistance (Rif^R) assay, *S. typhimurium* (histidine-deficient) strains, *S. typhimurium* strain YG1024, V79 Chinese hamster fibroblasts, somatic mutation and recombination test in wing cells of *Drosophila melanogaster*,	[50, 124, 129, 131, 134]
	Eggplant (Thai, small-violet, green-yellow-striped, Kamo etc)	*Solanum melongena*	*S. typhimurium* TA98, TA100, *E. coli* MG1655 based Rifampicin resistance (Rif^R) assay, *E. coli* B/r WP2	[122, 123, 130, 143]
	Tomato	*Lycopersicon esculentum*	*S. typhimurium* TA98 and TA100, V79 Chinese hamster fibroblasts	[122, 129, 131]
Alliaceae	Garlic	*Allium sativum*	*S. typhimurium* TA100, *E. coli* MG1655 based Rifampicin resistance (Rif^R) assay	[50, 108]
	Onion (red etc)	*Allium cepa*	*S. typhimurium* TA98, TA100, TM677, *E. coli* MG1655 based Rifampicin resistance (Rif^R) assay, V79 Chinese hamster fibroblasts	[50, 108, 122, 126, 131]
Cucurbitaceae	Gourd (snake)	*Trichosanthes cucumerina* var. *anguina*	*E. coli* MG1655 based Rifampicin resistance (Rif^R) assay	[50]
	Pumpkin (Shishigatani etc)	*Cucurbita maxima*	*S. typhimurium* TA98 and TA100, *E. coli* B/r WP2	[50, 122, 123]
	Gourd (bottle)	*Lageneria siceraria*	*E. coli* MG1655 based Rifampicin resistance (Rif^R) assay	[50]
	Gourd (bitter)	*Momordica charantia*	*S. typhimurium* TA100	[160]
	Cucumber (Madras etc)	*Cucumis sativus*	*E. coli* MG1655 based Rifampicin resistance (Rif^R) assay	[50]
	Squash	*Sechium edule*	*E. coli* MG1655 based Rifampicin resistance (Rif^R) assay	[50]
Fabaceae	Bean (French, lima, clustered, yardlong)	*Phaseolus vulgaris*, *Phaseolus lanatus*, *Cyamopsis tetragonolobus*, *Vigna ungiculata*	*E. coli* MG1655 based Rifampicin resistance (Rif^R) assay, *S. typhimurium* strains YG1024, *S. typhimurium* strains TA98 and TA100	[50, 124, 141]
	Pea (green)	*Pisum sativum*	*E. coli* MG1655 based Rifampicin resistance (Rif^R) assay	[50]
Amaranthaceae	Spinach	*Spinacea oleracea*	*S. typhimurium* strains TA98, TA100, TM677, Chinese hamster lung fibroblasts; V79 Chinese hamster fibroblasts	[122, 126, 131, 135, 139]
	Chard	*Beta vulgaris*	Male Balb/C mice	[133]
Asteraceae	Lettuce	*Lactuca sativa*	Male Balb/C mice	[133]
Euphorbiaceae	Gooseberry (Indian)	*Phyllanthus emblica*	*E. coli* MG1655 based Rifampicin resistance (Rif^R) assay	[50]
Zingiberaceae	Ginger	*Zingiber officinale*	*E. coli* MG1655 based Rifampicin resistance (Rif^R) assay	[50]
Apiaceae	Carrot (red etc)	*Daucus carrota*	*E. coli* MG1655 based Rifampicin resistance (Rif^R) assay; *S. typhimurium* TA100; *S. typhimurium* TM677	[50, 108, 122, 126, 129, 131]
Chenopodiaceae	Beet root	*Beta vulgaris*	*S. typhimurium* TA98 and TA100; V79 Chinese hamster fibroblasts	[122, 131]
Moringaceae	Drumstick	*Moringa oleifera*	*E. coli* MG1655 based Rifampicin resistance (Rif^R) assay	[50]

system of the mutagen, whereas ethyl acetate fraction also displayed Trp-P-1 electrophiles scavenging action [142]. In one study, co-incubation of phenolic extract from Fresh bean and aflatoxin B1 was reported to significantly reduce the mutagenicity and thus the possibility of chemical complex formation was proposed as a mechanism [141]. Antimutagenic effects of vegetable matrices on the activity of pesticides were observed in Ames test and SOS Chromotest [144]. In that study, the antimutagenicity of vegetables was proposed to be related to the antioxidant activity. Antimutagenic activity in broccoli, cauliflower, green beans and tomatoes was proposed to be due to the presence of peroxidase activity in these vegetables [122]. The extract of mixed cruciferous and legume sprouts was reported to reduce H_2O_2 induced DNA damage in HT29 cells when analyzed using comet (single cell microgelelectrophoresis) [145].

Antioxidant and antimutagenic activities of vegetables were not found to be well correlated in other study [50, 146]. However, suppression of SOS repair was proposed as possible mechanism for antimutagenicity of common vegetables as reduced cell-filamentation, cleavage of LexA in wild type *E. coli* cells, and decreased phage induction frequency in an *E. coli*

Table 4: Antimutagenic activity of different (aqueous, methanolic, total soluble phenolics; and quinones) extracts from some vegetables, and yield of total soluble phenolics and quinones.

Vegetables	Plant part	Antimutagenic activity*%				Yield (mg/g dry wt.)	
		Aqueous extract	Methanol extract	Extract rich in total soluble phenolics	Extract rich in quinones	Extract rich in total soluble phenolics	Extract rich in quinones
Spinach	Leaf	66 ± 3	61 ± 6	35 ± 4	72 ± 6	3.0 ± 1.1	2.5 ± 0.6
Lettuce	Leaf	63 ± 3	65 ± 5	78 ± 8	2 ± 1	1.5 ± 0.9	2.4 ± 0.9
Iceberg lettuce	Leaf	62 ± 4	48 ± 3	65 ± 3	21 ± 3	0.5 ± 0.2	2.6 ± 0.8
Cabbage	Leaf	61 ± 3	41 ± 4	68 ± 5	5 ± 2	2.0 ± 0.7	1.5 ± 0.5
Broccoli	Inflorescence	58 ± 5	78 ± 5	70 ± 4	11 ± 3	5.0 ± 2.1	3.4 ± 1.0
French bean	Fruit	73 ± 6	88 ± 6	63 ± 6	1 ± 1	1.0 ± 0.4	1.8 ± 0.7

*against ethyl methanesulfonate (EMS) induced mutagenicity at 2 mg/ml concentration of test extract using *E. coli* based rifampicin resistance (Rif^R) assay

strain carrying a defective lambdoid phage (SIVET assay) was observed [51].

Effect of processing of vegetables on their antimutagenic activity

Heating caused a remarkable reduction in antimutagenicity of the juices of beets, cabbage (Chinese, Savoy, red and white), cauliflower, leafy lettuce, cucumber, onions, radish and rhubarb against 2-amino3-methyl [4,5-f]-quinoline (IQ). Brussels sprouts, chicory greens, eggplant, garden cress, mangold, pumpkin, lamb's lettuce and spinach were heat stable [122]. Partial reduction of antimutagenicity due to heating was reported for green beans, kohlrabi, horseradish, tomatoes and chives juices. Later, antimutagenicity of solvent extracts of vegetable was reported to be heat stable and heating caused an increase of antimutagenic potential of some solvent fractions such as of broccoli, white and red cabbage [128]. In other study, boiling was found to significantly affect the antimutagenicity of several vegetables against UV [50]. Apart from peppers (*Capsicum*) and carrot significant reduction in antimutagenic potential was noted in gourd (snake), pumpkin and cucumber (Madras) of family *Cucurbitaceae* which indicated heat sensitivity of these bioactive principle(s). Interestingly, heating was found to increase the antimutagenic potential of pepper (bell-red and bell-green), eggplant (long violet), gourds (pointed and Ivy), tomato, and beet. In one study, boiling of various dialysate of vegetables was not found to affect the antimutagenic activity against Trp-P-2 [130]. Sweet corn processed using chlorination, blanching, and gamma radiation was reported to display similar antimutagenic activity similar as of fresh control in Rif^R assay [78]. Thus, most studies have indicated high stability of antimutagenic principle toward different processing.

Antimutagenicity of other Dietary (Allied) Products

Apart of fruits and vegetables, certain (allied) products have also been reported for antimutagenic properties such as fruit/vegetable products (processed papaya cube), honey, beverages (tea), and specialized ready-to-eat cooked products.

Processed papaya cubes

Intermediate moisture (IM) papaya cubes (Figure 2) were developed using a novel combination technology which have shelf life of 60 days at ambient temperature whereas, the unprocessed freshly cut samples generally spoil within 2 days. These IM cubes were hygienized by exposing to gamma radiation (2 kGy). Interestingly, in the aqueous extract of the processed papaya, the antimutagenic activity was found to be higher (~41%) as compared to unprocessed control (~18%) when analyzed using *E. coli* based Rif^R assay against ethyl methanesulfonate [79].

Honey

Floral honey demonstrated strong antimutagenicity against physical (UV-C) as well as chemical (ethyl methanesulfonate) mutagens as ascertained by *E. coli* based Rif^R assay and Ames tests [20]. Irradiation (15 kGy) decontaminated the honey, however antimutagenicity of irradiated honey was found to be similar to the non-irradiated control. Honey phenolics contributed to the antimutagenicity of honey. The antimutagenicity of honey was found to be due to the suppression of error-prone repair pathway (as manifested by SOS response) in *E. coli*. Besides, honey also displayed strong antiproliferative property against different cancer cell lines (myeloid leukemia, breast and lung cancer) but did not affect normal cell line (Int-407, intestinal epithelial cell) indicating its differential and selective cytotoxicity [53]. The findings explained the mechanism of possible therapeutic and prophylactic action of honey against neoplastic changes caused by environmental mutagens and carcinogens.

Beverages from tea leaves and rose-petal

Green and black tea extracts were reported to have antimutagenicity against MNNG in *E. coli* B/r WP2 and *S. typhimurium* TA100 [147]. Both tea extracts were also reported to posses antimutagenicity against PhIP in *S. typhimurium* TA98 [148]. Aflatoxin B1 (AFB1)-induced chromosome aberrations (consisted mainly gaps and breaks) in rat bone marrow cells was found to be significantly inhibited by tea extract [149]. Tea polyphenols such as gallic acid, methyl gallate, catechins, theaflavins, tanic acid, epigallocatechin (EGC), and epigallocatechin gallate (EGCG), epicatechin gallate

(ECG) contributed significantly towards antimutagenicity [150]. The antimutagenicity of tea extracts against IQ and Glu-P-1 in *S. typhimurium* TA 100 showed a significant correlation to the contents of catechins and ascorbic acid, whereas against Trp-P-1 in TA98 or TA100 correlation was high with caffeine contents [151]. Antimutagenic activity of extracts of black tea and green tea was analyzed in *in vitro* gastrointestinal model, which simulates the conditions in the human digestive tract [152]. In this study, dialysate from the jejunal compartment due to introduction of black and green tea, inhibited the mutagenicity of the food mutagen MeIQx (max. at 2 h) in the direct plate assay with *S. typhimurium* (Ames test). Food matrices were reported to significantly influence the antimutagenic activity of these tea studied. In an antimutagenic study related to petals of rose (*Rosa centifolia*) cultivars ("passion," "pink noblesse," and "sphinx") by Rif^R assay, the red colored cultivar "passion" displayed highest antimutagenicity and preparation of tea beverage using its petals was not found to affect this activity [55].

Specialized ready to eat (RTE) cooked food

Food for immune-compromised patients

A nasogastric liquid feed formulation (NGLF) (Figure 2) was developed for immune compromised patients (vulnerable targets of pathogenic assault). NGLF consisted of cereals, pulses, vegetables, and milk powder to provide balanced nutrients. Due to its high water content and rich nutrients, the shelf life was limited up to a few hours only because of microbial contamination and subsequent spoilage. Gamma irradiation at 10 kGy reduced the microbial load of NGLF to non-detectable levels, and the packed and irradiated product could be stored even up to 1 month at 4 °C without any detectable increase in microbial load. The antimutagenic potential of irradiated NGLF was similar to unprocessed fresh control against ethyl methanesulphonate induced mutagenesis in *E. coli* cells as evaluated by Rif^R assay [76].

Food for natural calamity victims and other targets

An ambient storable Stuffed Baked Food (SBF) (Figure 2) was developed in India as a ready to eat meal for natural calamity victims and other target groups under a Coordinated Research Project (CRP) of FAO/IAEA by gamma radiation (15 KGy) processing. Mutation analyses in models including human TK6 lymphoblast cell line at genes *tk*$^{+/-}$ and *hprt*$^+$; and bacterial systems [*E. coli* MG1655 cells (*rpoB* gene); and Ames strains (TA 100 and TA 102)] endorsed the genotoxic safety of the SBF product. The product displayed similar antimutagenicity as of fresh samples during Rif^R assay, Ames test or mutation assay using TK6 lymphoblast cell line against EMS ans 5-AZ [77]. In Ames test, no mutagenicity induction was observed in high dose (25 kGy) irradiated food 'Kimchi', a Korean food developed and certified as space food [153].

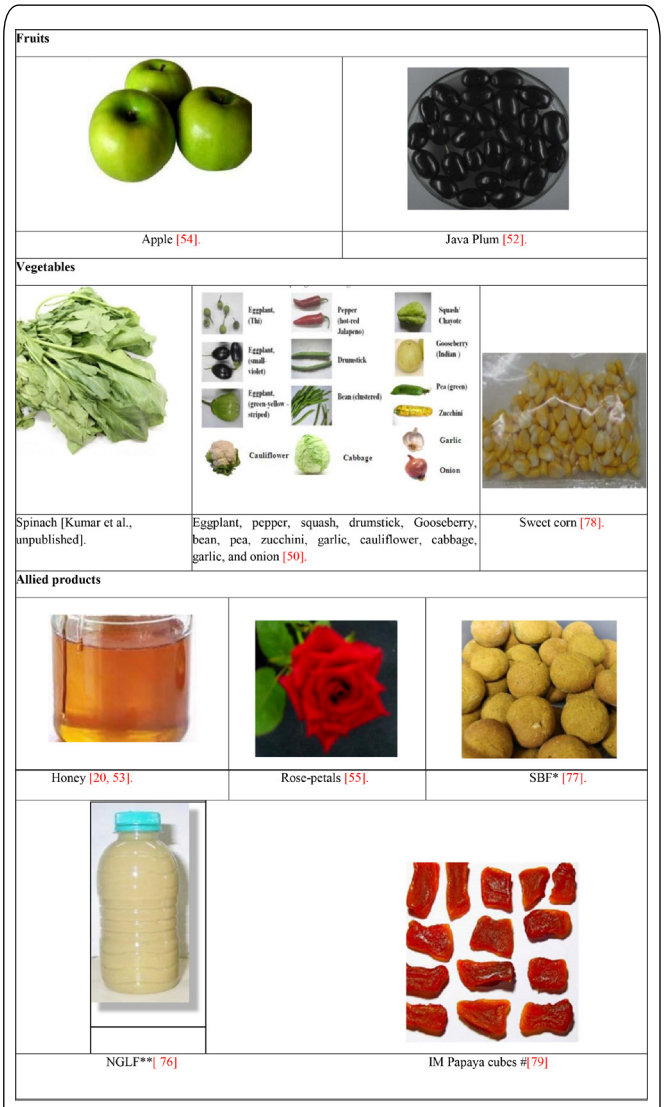

Figure 2: Recent studies reporting antimutageic potential of various fruits, vegetables, and allied products. SBF, (Stuffed Baked Food), a ready to eat meal for natural calamity victims and other target groups; NGLF** (Naso-Gastric liquid food) for immune compromised patient; and IM Papaya cubes# (Intermediate Moisture papaya cubes as a minimally processed product) developed at Food Technology Division, Bhabha Atomic Research Centre, Mumbai, India.

Conclusion and Future Perspectives

The findings discussed here suggest that there is an utmost need for regular healthy eating habits with plenty of fruits and vegetables to maintain good health, and counteract the unseen challenges caused due to mutagenic exposures from various unknown sources. A close interaction is very much needed amongst public-health agencies, state and local governments, schools, the food industry and the media to promote healthy food choices. Also the global demand for more affordable therapeutics and concerns about side effects of commonly used drugs has renewed interest in dietary phytochemicals and traditional complimentary medicines. Use of chemotherapeutic drugs to treat deadly disease like cancer further place challenges to search for the dietary phytochemicals having antimutagenic potency to take care of possible onset of secondary oncogenesis, which is quite frequently encountered possibly due to DNA damage in bystander cells. How to use this scientific knowledge for actual application needs to be worked out. Development of nutraceuticals is one such option where other molecular techniques related to plant tissue culture can be of use to produce the desired compound in sufficient quantity. If the product is a protein coded by a single gene, it can be even produced by genetic engineering approach using suitable host(s). The issue of bioavailability and also the evaluation of

concentration dependent cytotoxicity need to be addressed before actual application to achieve above said benefits. When such phytochemicals are considered for use within a defined chemotherapeutic framework, their antineoplastic effects need to be assessed in suitable animal models and ultimately in pharmacokinetic and pharmacodynamics pilot studies in human beings. The costs associated with the isolation and development of dietary phytochemicals based therapies could well be lower than those associated with the discovery and development of altogether new chemical entities. These phytochemicals have many molecular targets and are therefore non-specific. Thus, they are dissimilar to molecularly targeted chemotherapeutic agents that are designed to hit only one, or very few, specific targets. This pleiotropism might constitute an advantage, because a complicated disease, such as cancer, is sustained by many oncogenic (i.e., functionally deregulated) events. This review is intended to highlight a comparatively newer but quite relevant thought for researchers working in the area of nutraceuticals and functional foods to utilize the nature's precious gift for the societal health.

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