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1 **Dose-dependent functionality and toxicity of green tea**
2 **polyphenols in experimental rodents**

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1 **Abstract**

2 A large number of physiologically functional foods are comprised of plant polyphenols.
3 Their antioxidative activities have been intensively studied for a long period and proposed to
4 be one of the major mechanisms of action accounting for their health promotional and
5 disease preventive effects. Green tea polyphenols (GTPs) are considered to possess marked
6 anti-oxidative properties and versatile beneficial functions, including anti-inflammation and
7 cancer prevention. On the other hand, some investigators, including us, have uncovered their
8 toxicity at high doses presumably due to pro-oxidative properties. For instance, both
9 experimental animal studies and epidemiological surveys have demonstrated that GTPs may
10 cause hepatotoxicity. We also recently showed that diets containing high doses (0.5-1%) of a
11 GTP deteriorated dextran sodium sulfate (DSS)-induced intestinal inflammation and
12 carcinogenesis. In addition, colitis mode mice fed a 1% GTP exhibited symptoms of
13 nephrotoxicity, as indicated by marked elevation of serum creatinine level. This diet also
14 increased thiobarbituric acid-reactive substances, a reliable marker of oxidative damage, in
15 both kidneys and livers even in normal mice, while the expression levels of antioxidant
16 enzymes and heat shock proteins (HSPs) were diminished in colitis and normal mice.
17 Intriguingly, GTPs at 0.01% and 0.1% showed hepato-protective activities, *i.e.*, they
18 significantly suppressed DSS-increased serum aspartate aminotransferase and alanine

1 aminotransferase levels. Moreover, those diets remarkably restored DSS-down-regulated
2 expressions of heme oxygenase-1 and HSP70 in livers and kidneys. Taken together, while
3 low and medium doses of GTPs are beneficial in colitis model mice, unwanted side-effects
4 occasionally emerge with high doses. This dose-dependent functionality and toxicity of
5 GTPs are in accordance with the concept of hormesis, in which mild, but not severe, stress
6 activates defense systems for adaptation and survival.

7
8 **Keywords:** green tea polyphenol; colitis; nephrotoxicity; heat shock protein; molecular
9 chaperone; pro-oxidation

10
11 **Abbreviations:** ABC, ATP-binding cassette; ACR, acrolein; ALS, amyotrophic lateral
12 sclerosis; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DMH,
13 dimethylhydrazine; DSS, dextran sulfate sodium; EGCG, (-)-epigallocatechin-3-gallate; ERK,
14 extracellular signal-regulated kinase; GTP, green tea polyphenols; GSH, glutathione; GST,
15 GSH S-transferase; HNE, 4-hydroxy-2-nonenal; HO-1, heme oxygenase-1; Hsf, heat shock
16 factor; HS, heat shock; HSP, heat shock protein; Keap1, Kelch-like ECH-associated protein;
17 MDA, malondialdehyde; MMP, matrix metalloproteinase; Nrf2, nuclear factor
18 (erythroid-derived 2)-like 2; NQO1, NAD(P)H dehydrogenase; PQC, protein quality control;

1 ROS, reactive oxygen species; SOD, superoxide dismutase; TBARS, thiobarbituric
2 acid-reactive substances; Ub, ubiquitin

3 **Stress-induced biomolecule modifications**

4 Homeostatic regulation of biological functions of macromolecules, such as
5 membrane lipids, enzymes, proteins, and DNA, is a key determinant for health promotion,
6 disease prevention, and longevity. Reactive oxygen species (ROS) have long been
7 demonstrated to play numerous roles in both physiological and pathological conditions *via*
8 oxidative modification of biomolecules present in cells and tissues. Accumulation of
9 oxidative damage footprints in those biological molecules has been described to be involved
10 in the onset of numerous diseases and aging [1]. In particular, biochemical modifications of
11 cellular DNA [2] and proteins [3] by ROS and lipid peroxidation products, such as acrolein
12 [4], malondialdehyde [5], and 4-hydroxy-2-nonenal [6] lead to either loss or undesired gain
13 of their functions for homeostasis disruption (Figure 1). In addition, mitochondrial DNA
14 mutations caused by ROS play significant roles in dysfunctions of cellular metabolism, and
15 defense and other systems [7], though Laquoue and Larsson have proposed that those
16 mutations may be generated by replication errors rather than accumulated oxidative damage
17 [8]. Recently, Akatsuka *et al.* uncovered a novel base modification, *i.e.*,
18 1,*N*⁶-propanoadenine (acrolein-adenine), in a ferric nitrilotriacetate-treated mouse model, in

1 which oxidative stress plays an essential role in the development of renal carcinogenesis
2 [9]. Also, mutations of the *Cu,Zn-sod* gene were reported to partially truncate
3 anti-oxidative systems, thereby increasing the risk of familial amyotrophic lateral sclerosis
4 (ALS) [10], a neurodegenerative disease that exhibits dysfunctions of muscle movement and
5 strength. On the other hand, proteo-stresses have been shown to be characterized to be
6 inducible by physical, chemical, and biological stimuli, and interfere with and disable
7 protein folding for denaturation [11]. Other recent studies have demonstrated that
8 accumulation of un- and mis-folded proteins may promote the formation of their aggregates,
9 the hallmark of neurodegenerative diseases, such as Alzheimer's, Parkinson's
10 and Huntington's diseases [12]. In addition, the status of cellular junk proteins is associated
11 with adiposity and thus the development of metabolic syndrome [13]. Furthermore, it should
12 be pointed out that concerted and tight regulation of the biological protein quality may
13 contribute to longevity, as demonstrated in experiments using the naked mole rats, which
14 have marked protein stability and a long life-span [14]. Thus, it is highly conceivable that
15 quality maintenance of biological proteins greatly affects health status and longevity.
16 Among a number of constitutive and inducible mechanisms that function to protect
17 biological macromolecules from oxidative stress and proteo-stress, 2 representative
18 biochemical defense systems are highlighted (Figure 2) and described below.

1

2 **The Keap1/Nrf2-dependent defense system**

3 Anti-oxidation can be defined as a self-defense mechanism ubiquitously distributed
4 among organisms. Anti-oxidative modes of actions can be classified into at least the
5 following 3 categories: (1) scavenging and quenching ROS [as seen with vitamin C, vitamin
6 E, glutathione (GSH), *etc.*], (2) attenuation of ROS-generating enzymes (NADPH oxidase,
7 xanthine oxidase, *etc.*), and (3) up-regulation of anti-oxidant enzymes [superoxide dismutase
8 (SOD), catalase, *etc.*]. The Kelch-like ECH-associated protein 1/nuclear factor
9 (erythroid-derived 2)-like 2 (Keap1/Nrf2) system, belonging to the category (3), adaptively
10 functions to protect cells from endogenous and exogenous oxidative and electrophilic
11 damages [15] (Figure 3). In a normal state, the transcription factor Nrf2 is continuously
12 ubiquitinated by the Cul3-Keap1 ubiquitin E3 ligase complex and thereby rapidly transported
13 to degradation systems in proteasomes. ROS and electrophilic chemicals oxidize the reactive
14 cysteine residues of Keap1 in both direct and indirect manners. This critical step stabilizes
15 Nrf2, thereby inducing robust expressions of a battery of cytoprotective genes, including
16 those related to anti-oxidation [Cu/Zn-SOD, Mn-SOD, extracellular SOD,
17 γ -glutamylcysteine synthetase, *etc.*], xenobiotic detoxification [GSH *S*-transferase (GST),
18 glucuronidase, sulfatase, *etc.*], and protein quality control (PQC) (molecular chaperones,

1 ubiquitin/proteasome systems, *etc.*) for adaptation [15]. In addition, prior to translocation of
2 Nrf2 into the nucleus, its transcription activity is modulated by several protein kinases, which
3 are simultaneously activated by stressors. Feng *et al.* disclosed that activation of Akt and
4 extracellular signal-regulated kinase (ERK)1/2 is required for activation of Nrf2, leading to
5 up-regulation of the expression of heme oxygenase (HO)-1, one of the major inducible
6 anti-oxidant enzymes [16]. On the other hand, Nrf2 activity is continuously repressed in a
7 normal state by Bach1, which constitutively binds to the Maf recognition element [17].

8

9 **Heat shock proteins as molecular chaperones**

10 Various types of stresses toward biological proteins critically disrupt their
11 conformation and folding state, which often results in abolishment of their biological
12 functions. A number of recent studies have indicated that several distinct PQC systems play
13 key roles in counteraction against proteo-stress. Heat shock proteins (HSPs), highly
14 conserved families of proteins ubiquitously expressed in most types of cells, are molecular
15 chaperones that allow misfolded and unfolded proteins to achieve functionally active
16 conformation (Figure 4). The expression and functional status of HSPs are considered to be
17 critical determinants of PQC systems and thus essentially associated with homeostasis,
18 health and longevity. In fact, expression of HSPs at high levels substantially contributes to

1 extending the lifespan of many experimental animals [18]. HSPs are comprised of numerous
2 family proteins. Constitutive HSPs, sharing approximately 1% of cytosolic proteins, are
3 essential for maintaining PQC systems under a non-stressed condition. On the other hand,
4 various types of stressors are known to up-regulate inducible HSPs. In a normal state,
5 HSP90 β , a major constitutive isoform, is bound to the transcription factor heat shock factor1
6 (Hsf1), thereby forcing it to be biologically dormant [19]. Heat shock and some other stimuli
7 are capable of dissociating this heterodimer complex. Thereafter, the resultant free Hsf1
8 forms a trimer complex and is also phosphorylated at multiple sites, finally translocating
9 into the nucleus to induce a number of HSP genes to amplify defense capacity [19]. For
10 example, HSP70, a major inducible isoform, protects neurons from protein aggregation and
11 apoptosis, which may contribute to regulate Parkinson's, Alzheimer's and polyglutamine
12 diseases, and ALS [20]. Additionally, Hsf1 has been reported to up-regulate ABC
13 transporters [21] and Bcl-2 [22], both of which potentiate cellular defense capacity against
14 foreign chemicals and other stress stimuli. Meanwhile, it is interesting to note that there are
15 mechanistic interactions between oxidative stress- and proteo-stress-triggered signaling
16 pathways. In this regard, Back *et al.* showed that oxidative stress chemically cleaves HSP90
17 protein, resulting in dissociation with and subsequent degradation of its client proteins,
18 including Bcr-Abl, RIP, and Akt, for cancer cell death [23]. Also, oxidative stress-triggered

1 Keap1/Nrf2 system activation was reported to up-regulate the expression of p62, a
2 multifunctional adapter protein implicated in autophagy against proteo-stress [24]. The
3 crosstalk between oxidative stress and proteo-stress-triggered signaling pathways resembles
4 that between the complement system and toll-like receptors in innate immunity [25], and is
5 critical for first-line host defense.

6

7 **Potential side-effects of green tea polyphenols**

8 *Introduction*

9 Tea (*Camellia sinensis*, Theaceae) is a popular beverage and consumed by over
10 two-thirds of the world population [26]. Green tea polyphenols (GTPs) account for 30-42 %
11 of the dry weight of the solids in brewed green tea, while (-)-epigallocatechin-3-gallate
12 (EGCG) (Figure 5A) is estimated to account for 50-80% of the total catechins in tea [26].
13 There is scant evidence that oral consumption of green tea, but not supplements, even at
14 large amounts has side-effects, and is actually beneficial. For example, an epidemiological
15 survey, of over 8500 people followed up for 9 years showed that the age-standardized
16 average annual incidence rate of cancer was significantly lower among females who daily
17 consumed 10 or more cups of green tea [45]. In addition to the health benefit effects of
18 GTPs [27-29], many independent studies have also suggested their harmful aspects, which

1 are largely due to pro-oxidative properties when consumed at high doses. These
2 pro-oxidative activities are broadly attributable to their catechol structures (*ortho* diphenols,
3 Figure 5B). This functional moiety has a chemical characteristic to generate superoxide anion
4 radical (O_2^-) from molecular oxygen (3O_2) through formation of an electrophilic *o*-quinone
5 counterpart. In addition, Kajiya *et al.* hypothesized that an ester group in EGCG plays a
6 crucial role in biochemical interactions with the lipid bilayer, providing it with a high affinity
7 to the cell membrane [30]. Of note, GTPs covalently bind protein thiols through their
8 conversion to an *o*-quinone counterpart [31, 32], while Elbling and colleagues showed that
9 EGCG exhibited a pro-oxidative, but not anti-oxidative, property in cellular models [33].
10 Similarly, EGCG induced injury in mouse blastocysts through intrinsic apoptotic signaling
11 processes to impair sequent embryonic development [34]. We also found that GTPs
12 increased expression of the tumor metastatic protein, pro-matrix metalloproteinase-7
13 (pro-MMP-7), in human colon adenocarcinoma cells *via* a pro-oxidation-dependent
14 mechanism since both SOD and catalase suppressed the production of pro-MMP-7 [35].
15 However, the pro-oxidative activity of GTPs may not be recognized as not necessarily
16 harmful because it executes induction of cancer cell apoptosis [36]. Moreover, as indicated
17 above, pro-oxidative stimuli may activate the Keap1/Nrf2 systems for exhibiting
18 anti-oxidative, anti-inflammatory, and chemopreventive activities [37]. In fact, Na *et al.*

1 showed that EGCG acted as a pro-oxidant to activate Nrf2-dependent gene expressions of
2 some representative antioxidant enzymes [38, 39]. Thus, it can be argued that the
3 pro-oxidative nature of GTPs cannot be simply defined as harmful, and the risks and
4 benefits depend on the situation. Meanwhile, the anti- and pro-oxidative properties of GTPs
5 as well as many other polyphenols are determined by their doses, the presence or absence of
6 oxidants, the responsiveness of adaptation systems, and other factors.

7

8 *Intestinal toxicity*

9 Ulcerative colitis encompasses several chronic inflammatory disorders that cause
10 damage to the gastrointestinal tract and affect individuals of both sexes throughout life [46].
11 Dextran sulfate sodium (DSS)-induced colitis animal models are most frequently used in
12 studies of inflammatory bowel disease. Importantly, a DSS-induced model exhibited many
13 symptoms similar to those seen in human ulcerative colitis, including diarrhea, bloody feces,
14 body weight loss, mucosal ulcerations, and shortening of the colorectum [47]. We previously
15 examined the effects of the commercially available GTPs (Figure 5C) (0.01%-1% in the
16 basal diet) on dimethylhydrazine (DMH)-initiated DSS-promoted mouse colorectal
17 carcinogenesis, and found that concentrations of 0.1%, 0.5% and 1%, but not 0.01%, tended
18 to increase tumor formation [48]. Furthermore, 1% GTP treatment following the DMH

1 exposure tended to increase histological tumor formation as compared to DMH alone group.
2 Also, the expressions of interleukin-1 β and macrophage-migration inhibitory factor, both of
3 which play essential roles in early pro-inflammatory processes, were significantly increased
4 in the DSS + 0.5% GTP and DSS + 1% GTP groups when compared with the DSS alone
5 group. These findings are in line with those reported by Hirose *et al.*, who demonstrated that
6 dietary GTPs enhanced colon carcinogenesis in rats treated with DMH [49]. Moreover,
7 consumption of a 0.36% GTP diet resulted in a significant increase in multiplicity of colonic
8 tumors as compared to the carcinogen azoxymethane alone [50].

9

10 *Hepatic and renal toxicity*

11 A recent study found that repeated administrations of high-dose GTPs (750 mg/kg)
12 increased hepatic injuries, which were augmented under febrile conditions induced by
13 endotoxin [40]. Likewise, a single high dose of EGCG (1500 mg/kg) dramatically increased
14 plasma alanine aminotransferase (ALT) and reduced survival in male CF-1 mice [41]. In
15 addition, a green tea extract potentiated acetaminophen-induced hepatotoxicity in mice at
16 doses of 500 and 1000 mg/kg [42]. To support those experimental findings with cultured
17 cells and rodents, an epidemiological survey based on an extensive publication analysis
18 between 1999 and 2008 by Mazzanti *et al.* showed a causal association between green tea

1 supplement intake and liver damage [43]. These authors speculated that this hepatotoxicity is
2 probably due to EGCG or its metabolites which can induce oxidative stress in the liver under
3 conditions related to the metabolism of patient [43]. Furthermore, Yellapu *et al.* implied the
4 side-effects of ‘fat burners’ and dietary supplements, including green tea constituents, which
5 may be hepatotoxic and may not be free of adverse effects [44]. Collectively, ingestion of
6 high doses of GTPs and other related supplements may increase the risk of hepatic injury
7 because of their pro-oxidative properties, though precise mechanisms underlying that
8 toxicity have not been fully elucidated.

9 We also assessed the potential toxicity of GTP toward liver and kidney functions
10 using a DSS-induced colitis model because, as mentioned above, high-dose GTPs may show
11 harmful effects in those organs. Importantly, oral feeding of 1% GTPs, caused kidney
12 dysfunctions in DSS-treated ICR mice, as revealed by increases in serum creatinine, the
13 most reliable biomarker of nephropathy [51]. In addition, we observed that the amounts of
14 thiobarbituric acid-reactive substances (TBARS, a potential indicator of lipid peroxidation)
15 were increased in both the livers and kidneys of the 1% GTP group. Previous
16 pharmacological studies have shown that EGCG is metabolized through methylation,
17 glucuronidation, and sulfation under normal physiological conditions, and then subsequently
18 excreted in urine [52]. It is important to note that, most, if not all, metabolites are biologically

1 inactivated and thus much less toxic than the intact form of EGCG. Furthermore, these
2 bioactive electrophiles have a potential to bind to nucleophilic amino acid residues (*e.g.*,
3 cysteine) for inducing proteo-stress [53, 54], and those stress-generating properties may be
4 associated with their toxicity in the liver and kidneys. Meanwhile, in other studies high-dose
5 EGCG reduced the expressions of Nrf2-dependent antioxidant enzymes, including HO-1,
6 SOD, NAD(P)H quinone oxidoreductase 1, and catalase [48, 55], and some of those findings
7 showing positive correlations with our results [48, 51]. Importantly, Nrf2 activity is a
8 primary contributor to hepatoprotection [56]. Also, Lim and colleagues have found a
9 correlation between decreased SOD activity and aggravated diabetic nephropathy [57],
10 while HO-1 was reported to attenuate the progression of chronic kidney disease [58].
11 Therefore, we speculate that high-dose GTPs not only act in a pro-oxidant manner, but also
12 down-regulate Nrf2-dependent antioxidant enzymes for disordering homeostasis in the liver
13 and kidney. Downregulation of HSP70 in the colonic mucosa of DSS-exposed mice has been
14 found to have an association with intestinal inflammation [59]. It should also be noted that
15 renal and hepatic levels of HSP27 were remarkably down-regulated by 1% GTP
16 administration as compared with the DSS group [51], while more strikingly, HSP90 in the
17 kidneys and livers of the non-treated mice was notably down-regulated in mice that received
18 the 1% and even by 0.1% GTP diets. These results are consistent with those of Tran *et al.*,

1 who previously found that HSP90 was repressed by EGCG in MCF-7 human breast cancer
2 cells [60]. As noted above, HSP90 is the most abundant molecular chaperone and plays
3 pivotal roles in maintaining organ homeostasis [61]. Therefore, a 1% GTP diet might induce
4 hepatic and renal toxicity by attenuating, at least in part, the expressions of anti-oxidant
5 enzymes (SOD, HO-1, *etc.*) and molecular chaperones (HSP90, HSP27, *etc.*).

6

7 **Beneficial effects of GTPs at low and medium doses**

8 In contrast to high doses, medium- and low-dose GTP diets (0.01-0.1%)
9 significantly inhibited rat colon carcinogenesis [62], which correlated in part with our
10 previous findings [48], *i.e.*, a diet of 0.01%, but not 0.5% or 1%, GTP tended to decrease
11 tumor multiplicity or frequency. Thus, administration of GTPs exhibits both beneficial and
12 harmful effects on colon carcinogenesis, depending on the dose. In parallel, Kohlmeier *et al.*
13 reported that it is difficult to define the inhibitory effects of green tea consumption on colon
14 cancer development in humans [63]. Interestingly, medium and low doses of GTPs also
15 exhibited beneficial effects on hepatic and kidney functions, as GTPs at 0.01% and 0.1%
16 significantly suppressed DSS-induced elevation of serum aspartate aminotransferase and
17 ALT levels [64]. Similarly, GTPs at both 0.01% and 0.1%, but not 1%, remarkably
18 up-regulated the expressions of HO-1 and HSP70 in livers and kidneys of DSS-exposed mice.

1 Collectively, low- and medium-doses of GTPs have beneficial effects on DSS-induced
2 intestinal inflammation and carcinogenesis as well as hepatotoxicity and nephrotoxicity *via*
3 up-regulation of self-protective enzymes, while those are abolished with a high dose (Figure
4 6).

5

6 **GTPs are potential phytoalexins (plant toxins)**

7 In nature, plants have a critical disadvantage for survival as compared with animals,
8 as they are unable to move to avoid biological enemies and stress stimuli, such as invading
9 microorganisms and insects, herbivorous animals, and intense sunlight. Thus, they have
10 developed specific biological systems that can adapt to and counteract against various
11 stresses. In particular, phytochemicals, biosynthesized in both constitutive and inducible
12 manners, function as a central group to fight against and mitigate exogenous stresses. This
13 raises an important question as to why phytochemicals, biosynthesized for their adaptation,
14 exhibit health benefit functions in animals, including humans. It is reasonable to speculate
15 that those chemicals have no nutritional value and are xenobiotics substances in animals.
16 Although mammals efficiently and actively absorb and utilize primary products, including
17 sugars, protein, and lipids, as essential nutrients, the bioavailability of phytochemicals in
18 experimental rodents and humans has been consistently reported to be fairly poor in several

1 studies. For example, a bioavailability study showed that administration of EGCG resulted
2 in its substantial biotransformation, *e.g.*, glucuronidation, sulfation, and *O*-methylation, and
3 its blood concentrations were quite limited [65, 66]. Therefore, most, if not all,
4 phytochemicals are substantially foreign chemicals to mammals, and it is not surprising that
5 they are actively subjected to detoxification and excretion systems. Based on those findings, it
6 is reasonable that phytochemicals up-regulate anti-oxidant enzymes, xenobiotic
7 detoxification proteins, and molecular chaperones, all of which are recognized as defense
8 molecules against oxidative- and proteo-stresses induced by those chemicals. Of paramount
9 importance, some of those adaptive responses have mechanistic links to their already-known,
10 beneficial bioactivities [67, 68]. Exposure levels to those foreign chemicals must essentially
11 remain at mild ranges to gain beneficial effects, otherwise, no positive effects or catastrophic
12 outcomes would become dominant. This notion is in line with the concept of hormesis
13 [69-71], in which mild biological or physical stresses at mild levels activate defense systems
14 for adaptation (Figure 7).

15

16 **Conclusion**

17 High doses, but not low and medium doses, of dietary GTPs deteriorated colitis and
18 colorectal cancer, and also induced liver and kidney dysfunctions in mice. The fundamental

1 reason why phytochemicals trigger activation of self-defense systems is probably because
2 they are essentially foreign chemicals with no nutritional value mammals. Accumulated
3 evidence regarding the functionality and toxicity of phytochemicals reminds us of the saying
4 'More than enough is too much'.
5

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12

13 **Disclosure**

14 There are no financial or contractual agreements that might cause conflicts of interest
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16

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11

12 **Figure legends**

13 **Figure 1**

14 Modifications of cellular membrane lipids, proteins, and DNA by reactive oxygen species
15 and electrophilic aldehyde compounds, the latter of which are degradation products of lipid
16 peroxide. ROS, reactive oxygen species; ACR, acrolein; MDA, malondialdehyde; HNE,
17 4-hydroxy-2-nonenal; HS, heat shock

18

19 **Figure 2**

20 Adaptive defense systems for oxidative stress and proteo-stress, which are specifically
21 sensed by Keap1 and HSP90 β , respectively, for activating the key transcription factors, Nrf2
22 and HSF1 to induce robust expressions of numerous genes for adaptation. Nrf2, nuclear
23 factor (erythroid-derived 2)-like 2; SOD, superoxide dismutase; HO-1, heme oxygenase-1;

1 GST, glutathione *S*-transferase; NQO1, NAD(P)H dehydrogenase 1; HSP, heat shock
2 protein; HSF1, heat shock factor1; ABC, ATP-binding cassette

3

4 **Figure 3**

5 Mechanistic scheme for Nrf2 activation and resultant expressions of self-defense genes. Ub,
6 ubiquitin; ERK, extracellular signal-related kinase

7

8 **Figure 4**

9 Proteo-stress-induced HSF1-dependent activation of HSP40 and HSP70 protein expressions
10 for increased proteo-stress resistance. HSP, heat shock protein; Hsf1, heat shock factor1;
11 HSE, heat shock element

12

13 **Figure 5**

14 (A) Chemical structure of EGCG, (B) formation of *o*-quinone counterpart and its
15 reaction with protein thiols to form a covalent bond, (C) composition of GTPs used in our
16 experiments

17

18 **Figure 6**

1 Summary of results of experiments on effects of low medium, and high doses of GTPs on
2 large intestine, liver, and kidneys of ICR mice. Summarized from ref. [50] and [63]. TRABS,
3 thiobarbituric acid reactive substances; AST, aspartate aminotransferase; ALT, alanine
4 aminotransferase; HSP, heat shock protein; HO-1, heme oxygenase-1

5

6 **Figure 7**

7 Hormetic curve illustrating the relationship between the stress intensity (dose) and
8 homeostatic capacity. The hormetic zone areas differ among the various stressors, with a
9 more frequent chance of adaptation with a larger zone

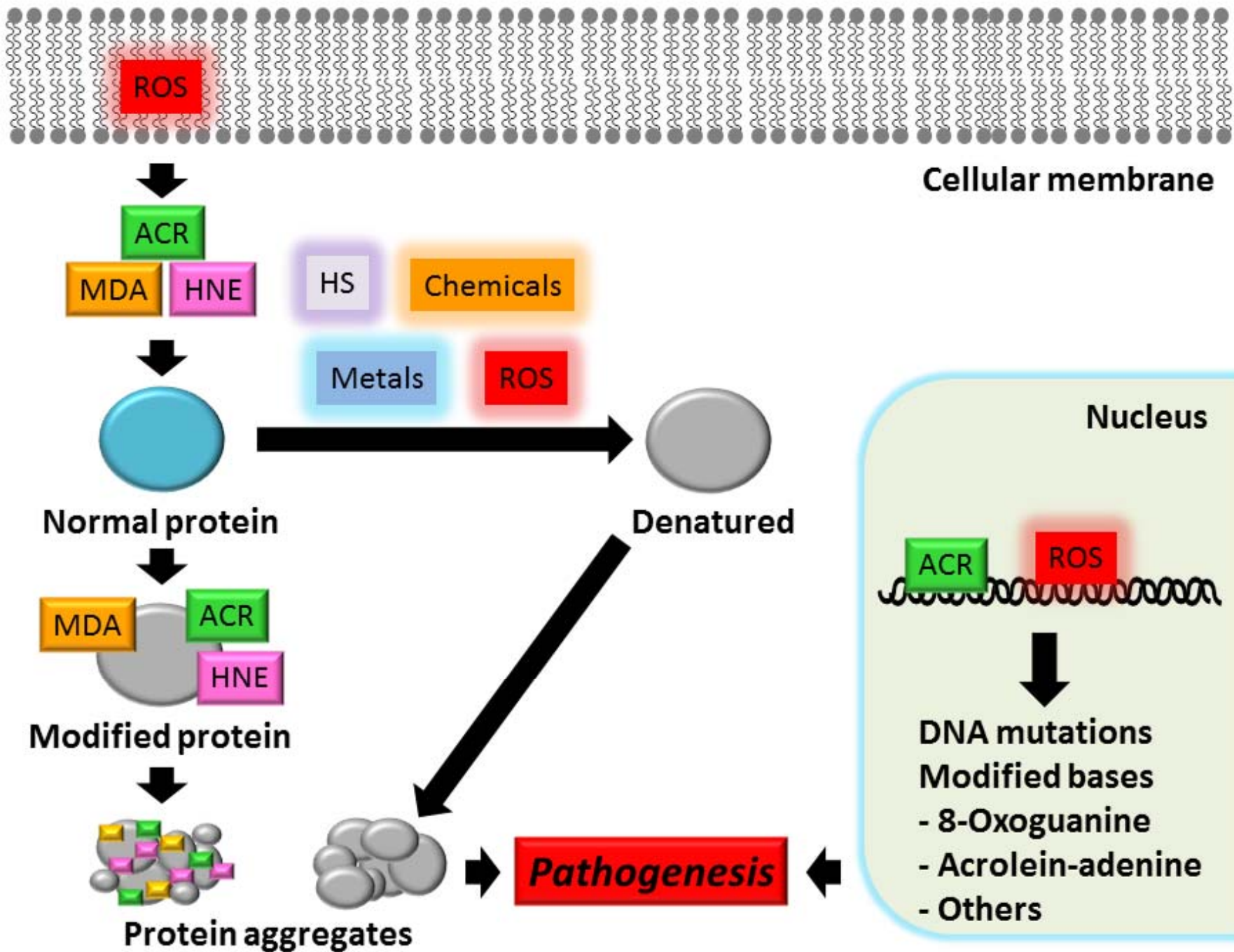


Figure 1

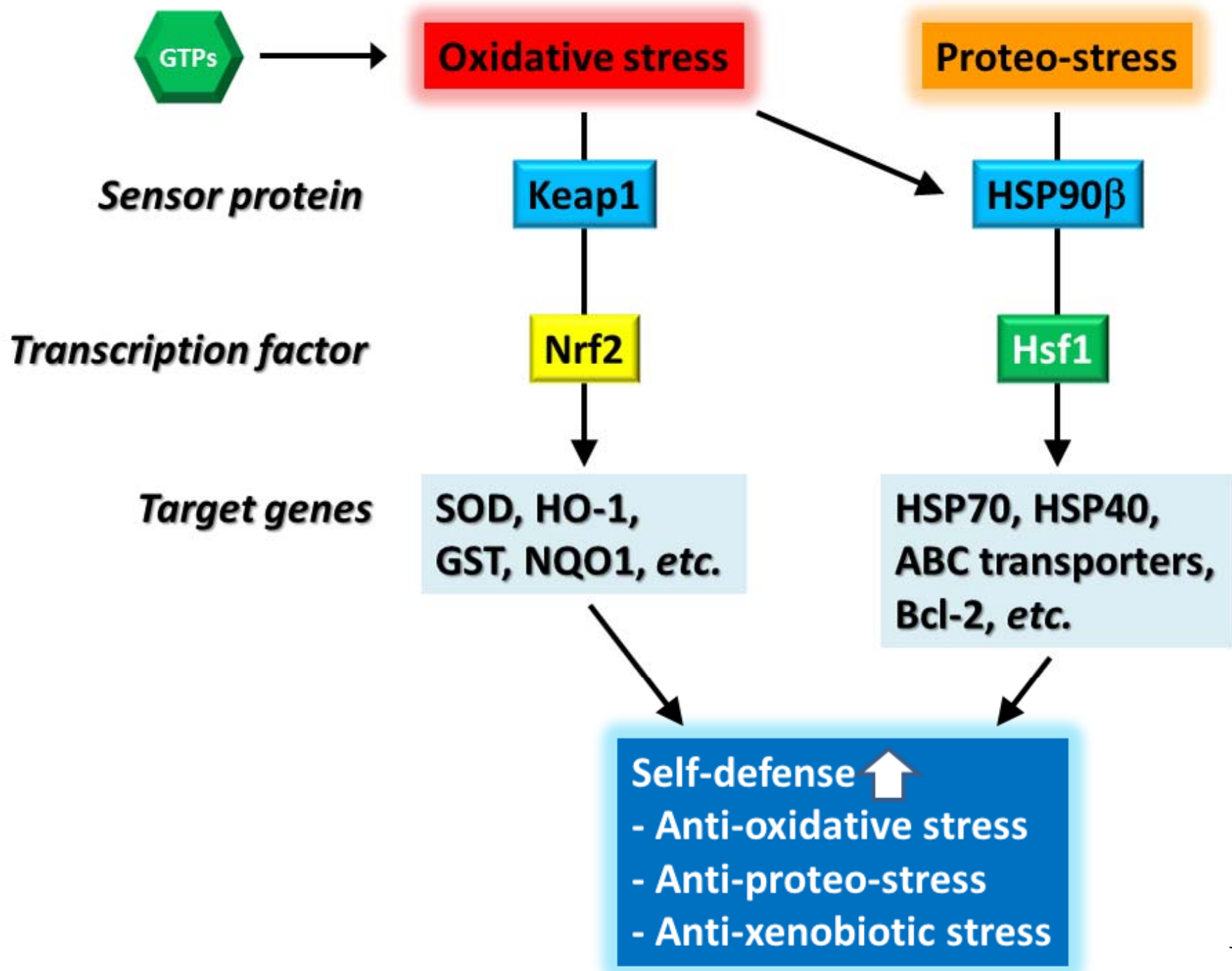


Figure 2

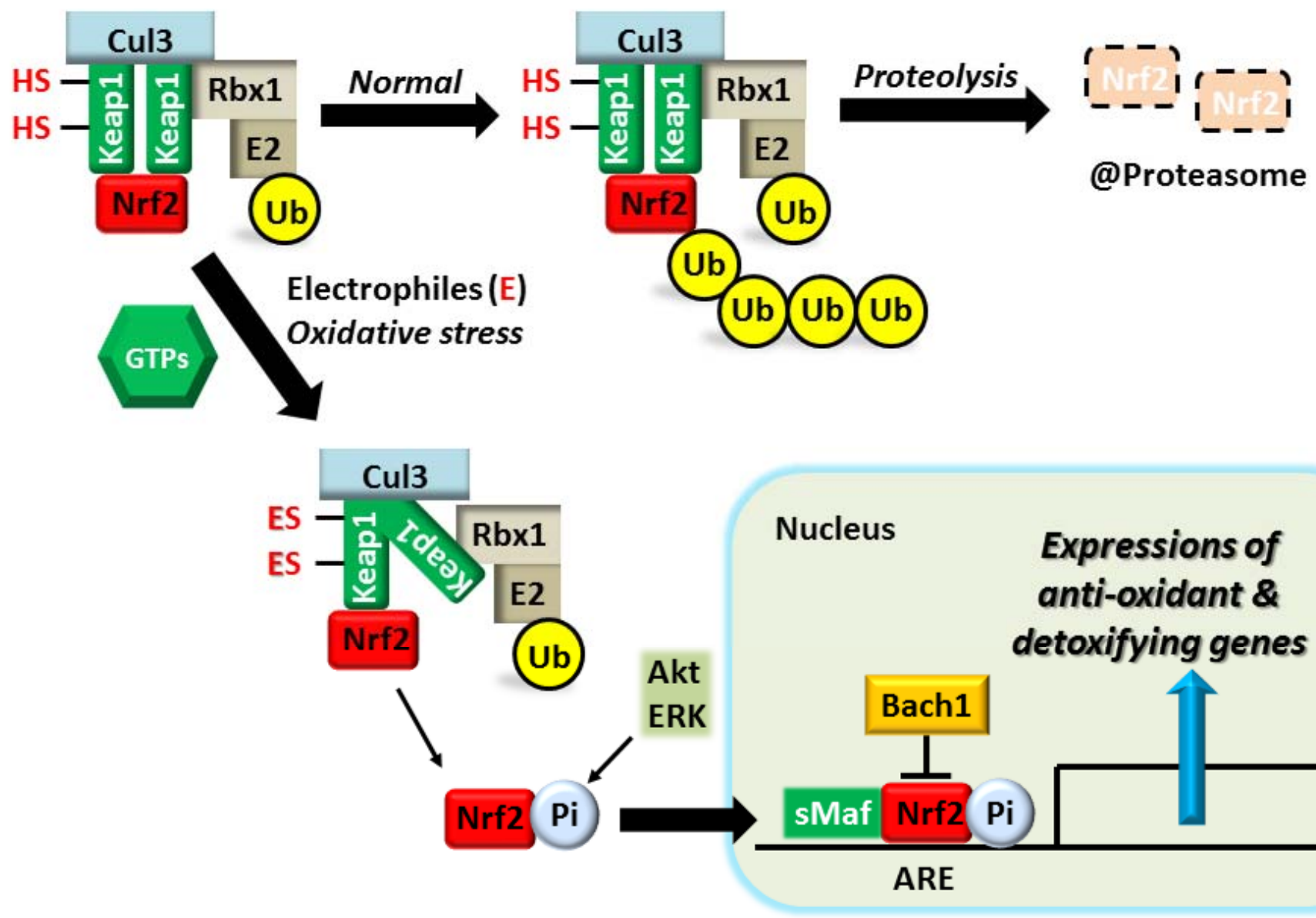


Figure 3

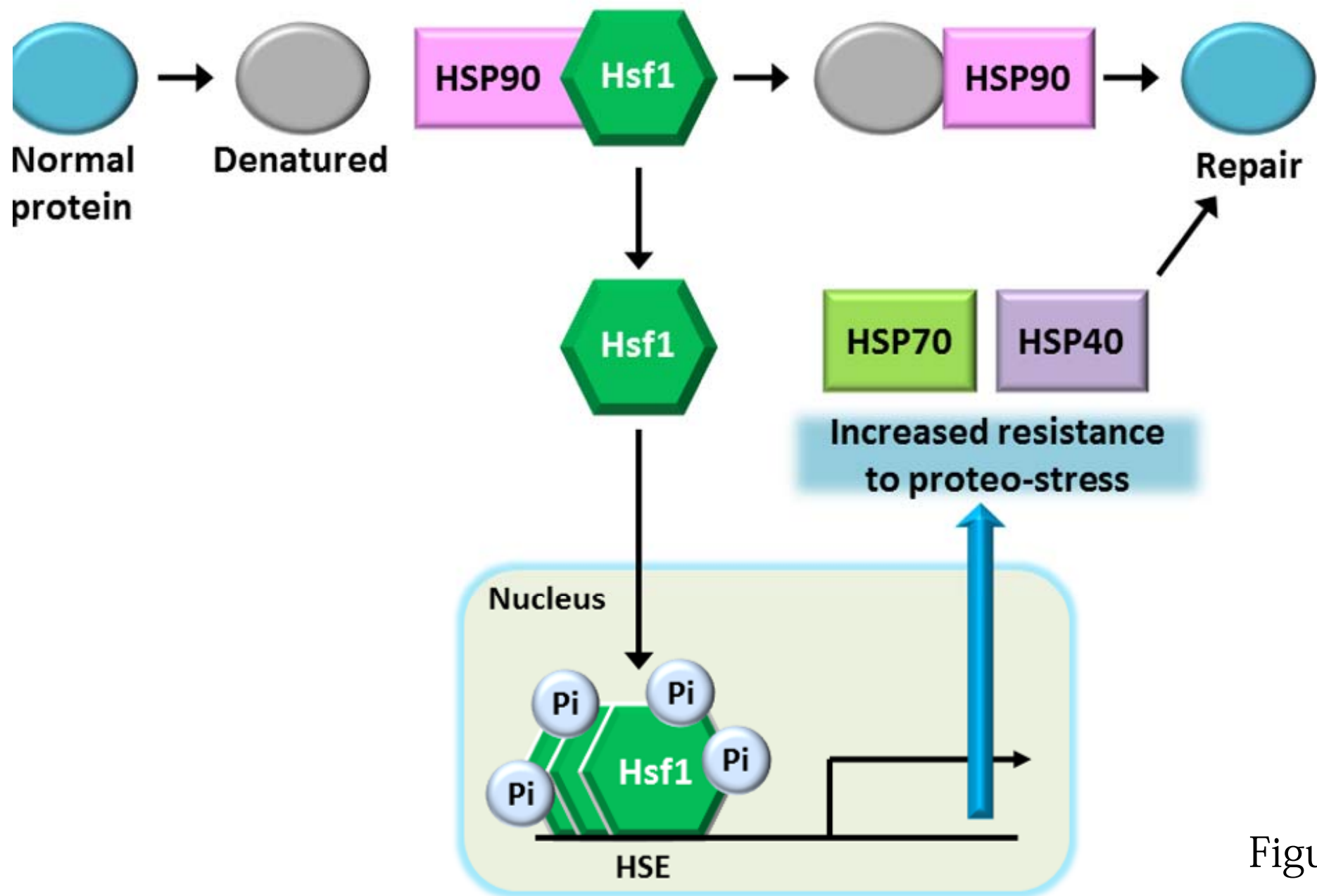
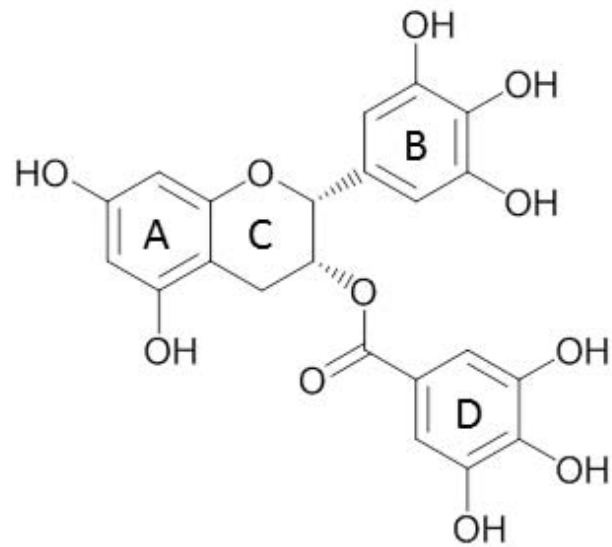


Figure 4

A**C**

Component	%
Polyphenols	95
Catechins	70
EGCG	35
Caffeine	3
Water	5

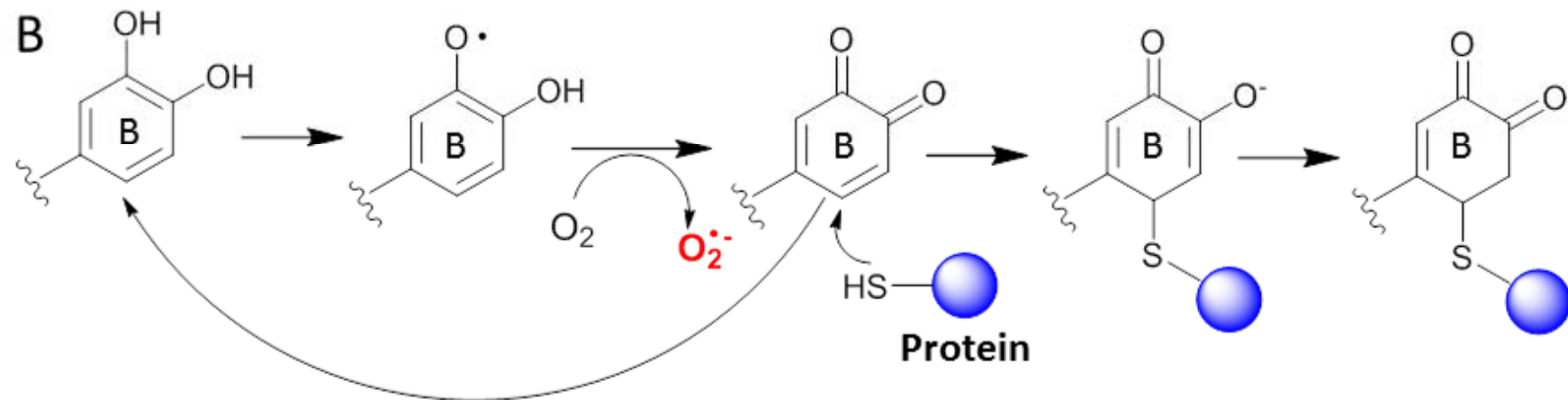
B

Figure 5

	Normal intestine			Inflamed intestine		
	0.01%	0.1%	1%	0.01%	0.1%	1%
Survival rate	Grey	Grey	Grey	Grey	Grey	Red
Kidney wt.	Grey	Grey	Grey	Grey	Grey	Red
Liver wt.	Grey	Grey	Grey	Grey	Grey	Red
Spleen wt.	Grey	Grey	Grey	Blue	Grey	Grey
TBARS	Grey	Grey	Red	Grey	Grey	Red
AST/ALT	Grey	Grey	Grey	Blue	Blue	Light Blue
Creatinine	Grey	Grey	Grey	Grey	Grey	Red
HSPs, HO-1, etc.	Grey	Red	Red	Blue	Blue	Red

■ Ameliorated
 ■ Aggravated
 ■ No significant change

Figure 6

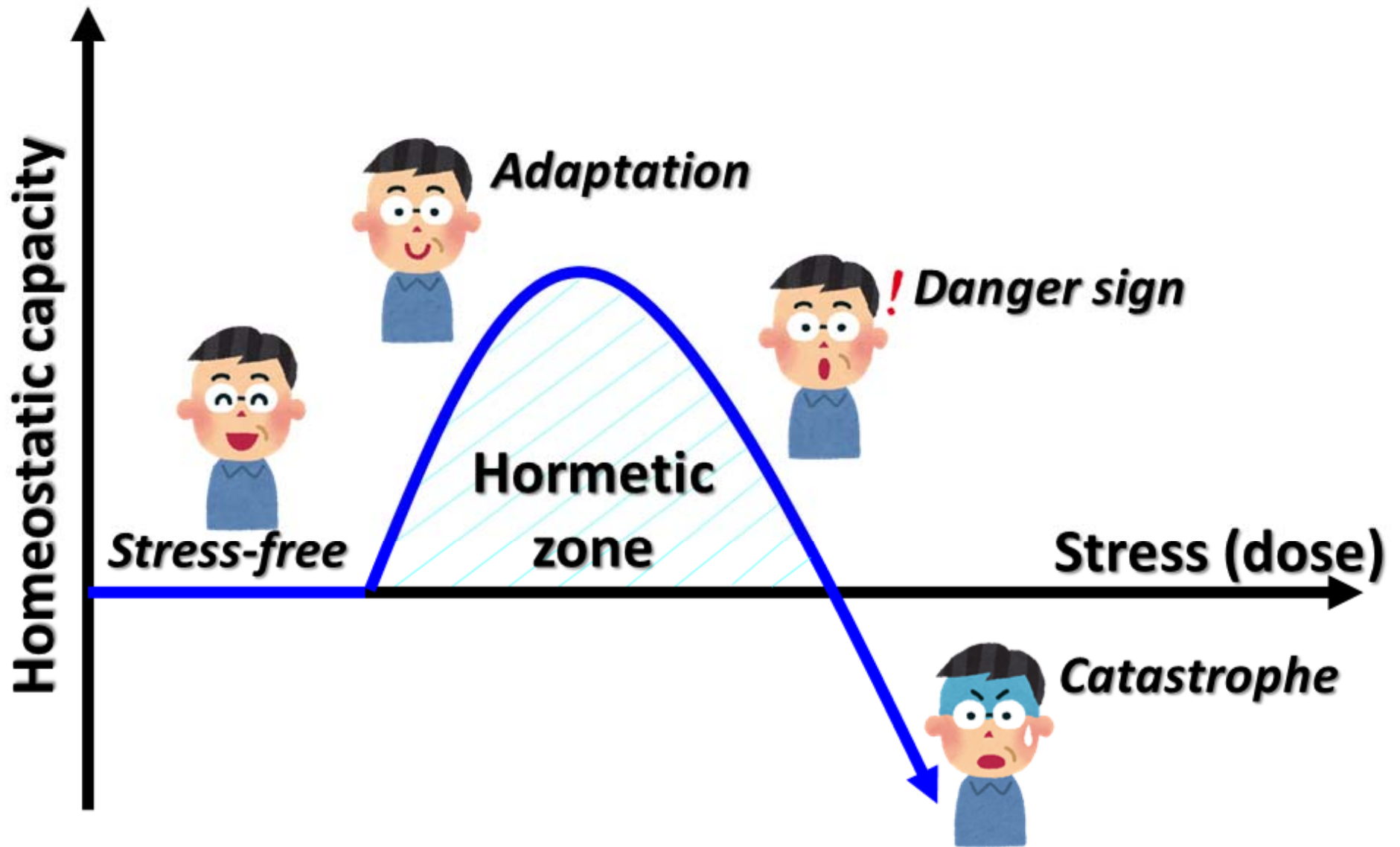


Figure 7