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Minireview

Pyrogallol-mediated toxicity and natural antioxidants: Triumphs and pitfalls of preclinical findings and their translational limitations

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ABSTRACT

Pyrogallol, a potent anti-psoriatic drug, produces toxicity due to its ability to generate free radicals, besides its beneficial effects. Oxidative stress is implicated in pyrogallol-mediated toxicity in general and hepatotoxicity in particular. Naturally occurring antioxidants including, resveratrol and silymarin have been proposed as potential supplements to counteract pyrogallol-mediated toxicity, without reducing its efficacy. Due to increase in the popularity of natural antioxidants in combating pyrogallol-mediated toxicity, a literature-based survey was performed to assess their role in experimental studies and possible implications in real life situations. Although preclinical studies revealed the boons of naturally occurring antioxidants in attenuating/abolishing the undesirable effects of pyrogallol exposure, limited studies have been conducted to evaluate their role in clinics. In this review, an update on the recent development in assessing the potential of natural antioxidants in pyrogallol-mediated toxicity in preclinical interventions, triumphs and pitfalls of such investigations, their translational challenges and future possibilities are discussed.

Contents

1. Introduction

Pyrogallol (benzene-1,2,3-triol) is a powerful reducing agent that absorbs oxygen from the air in alkaline solution. Due to its oxygen radical generating property, pyrogallol is commonly used as a photographic developing agent, in hair dying industry, as an antiseptic and to calculate the amount of oxygen in the air [1]. Despite beneficial properties, the practical applications of pyrogallol for meaningful purposes are rare due to its toxicity. Pyrogallol exerts toxicity in almost all vital organs but liver, lung, kidney and gastrointestinal tract are its major target organs [1–4]. Pyrogallol-mediated toxicity has been a major concern for the individuals exposed to it. Several studies have been performed to understand the molecular and cellular effects of pyrogallol-mediated toxicity

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[2–6], however, the complete molecular mechanism underlying its toxicity is not yet completely understood.

The increasing popularity of Ayurveda for the treatment of various ailments prompted investigators to look into the mechanistic aspects of herbal antioxidants in combating pyrogallol-mediated toxicity. Some naturally occurring antioxidants, such as silymarin, lack toxicity even at high doses and offer protection without any changes in their efficacy [2,5]. Although pyrogallol-induced hepatotoxicity in experimental animals has been widely used to evaluate the hepatoprotective potential of naturally occurring antioxidants such as silymarin and resveratrol [5,6], protective potential of these antioxidants in combating pyrogallol-mediated toxicity in clinics remains elusive. This review summarizes the harmful effects of pyrogallol in vital systems, the known mechanism of pyrogallol-mediated hepatotoxicity and protective potential of naturally occurring antioxidants along with their triumphs, pitfalls and translational challenges.

2. Pyrogallol

Pyrogallol is naturally present in oak, eucalyptus and other hardwood plants, as a decomposition product of hydrolysable tannins and possesses anti-fungal and anti-psoriatic properties [1,7]. Pyrogallol is widely distributed in nature, as it is commonly used in many industries and consumer products [8]. In natural conditions, pyrogallol is present as a contaminant in tannins, anthocyanins, flavones and alkaloids and released into environment during its isolation, disposal and industrial use [1]. Resorcinol, its metabolite, is released as a thermal breakdown product in wastewater effluents during coal-conversion processes. Humans are mainly exposed to pyrogallol through hair coloring formulations and consumption of tea in addition to water derived from aquifers and wells in geographical regions enriched with coal and shale [1,5]. Humans ingest smoke released during cooking fish, mutton and other materials, thereby get exposed to pyrogallol. A variety of toxic end points representing both environmental and human health hazards following pyrogallol exposure have been reported [9,10]. Pyrogallol causes oxidative damage and induces mutagenesis, carcinogenesis and hepatotoxicity [2,5,6,11,12]. It impairs immune responses, inhibits phagocytosis and suppresses the proliferation of mouse lymphocytes in a concentration dependent manner due to its strong free radical generating potential [13]. Several factors, such as age, nutritional status and health of exposed person, environmental factors, life style, dose, route and duration of exposure and interaction with other drugs could regulate pyrogallol-mediated toxicity (Fig. 1). The pro-oxidant action of pyrogallol is reported to be due to its auto-oxidation property [12]. Pyrogallol auto-oxidizes between pH 3.5 and 4.5, generates free radicals and constitutes a major source of hydrogen peroxide [12,14]. Auto-oxidation of pyrogallol is greatly affected by metallic ions, as Fe^{3+}, Cu^{2+} and Mn^{2+} increase while Co^{2+} decreases the rate of auto-oxidation in a concentration dependant manner [14].

The biological and genotoxic reactions of pyrogallol have been extensively studied in animals and cell lines. Pyrogallol has been employed as a superoxide generator and is commonly used to investigate the role of free radicals in the biological system [15]. Usually, free radicals modulate nuclear erythroid factor 2-related factor 2 (Nrf2) pathways, which in turn modulate phase II detoxifying enzymes [16–18]. The activation of antioxidant response element takes place by the activation of Nrf2, a transcription factor, which is tightly coupled with Kelch-like ECH-associated protein 1 (Keap 1) during normal physiological state. After activation, Nrf2 is released from Keap 1 and translocates into the nucleus where it binds to antioxidant response element after heterodimerizing with another leucine zipper protein and transciptionally activates the downstream genes (Fig. 2) [16–18].

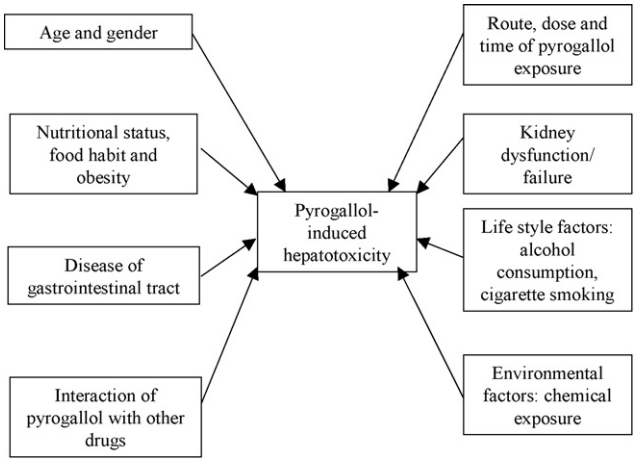

Fig. 1. Factors that influence pyrogallol-induced toxicity: age, gender, food habit, life style factors, genetic variables, route, dose, composition and duration of pyrogallol exposure and its interaction with other drugs could be responsible for individual susceptibility to pyrogallol-induced toxicity.

Pyrogallol is possibly, therefore, used to explore the mechanisms of apoptosis, carcinogenesis and genotoxicity in various model systems [15]. Pyrogallol chelates magnesium ions, and reversibly and non-competitively inhibits the activity of the hepatitis C RNA-dependent RNA polymerase. It is involved in the transfer of phosphate residue and provides a measure for therapeutic intervention [19].

2.1. Pyrogallol and pulmonary research

Pyrogallol alters intracellular calcium ion (Ca^{2+}) concentration, cyclic guanosine monophosphate (cGMP) production and nitric oxide (NO) biosynthesis and regulates signal transduction machinery of the respiratory system. Pyrogallol-mediated modulation in intracellular Ca^{2+} concentration is achieved due to its potential to impair Ca^{2+} mobilization, activate Ca^{2+} entry and adenosine-5′-triphosphate-induced Ca^{2+} release [20]. Pyrogallol also inhibits stress-mediated increase in intracellular calcium concentration by regulating Ca^{2+} dependent cGMP production [21,22]. Similarly, pyrogallol increases NO generation in cells by the activation of nuclear factor-κB and inducible NO synthase (iNOS) mRNA expressions [23]. In addition to these, pyrogallol regulates an early relaxation of lung pericytes through a signaling cascade [4], generates biphasic responses in basal and 5-hydroxytryptamine enhanced muscle tone in airways [24], causes cell cycle arrest and depletes glutathione level [25,26] (Table 1).

2.2. Pyrogallol and cardiovascular research

Pyrogallol modulates cardiovascular functions and induces both cellular and organ toxicity by multiple ways. Pyrogallol not only reduces resting cell length and inhibits cardiac contraction but also delays potassium channel opening and stimulates basal calcium current. The decrease in resting ventricular myocytes length and direct inhibition of cardiac contraction are achieved by pyrogallol through superoxide-mediated changes in the p38 mitogen activated protein kinases [27]. Similarly, pyrogallol pre-conditioning offers cardioprotection in isolated rat heart via the activation of protein kinase C during ischaemia-reperfusion-mediated injury [28]. Pyrogallol stimulates basal calcium current in ventricular myocytes and blocks respiration in a partially reversible manner in heart cardiac muscles [29,30]. Epigallocatechin–gallate contains a pyrogallol moiety that is responsible for delaying

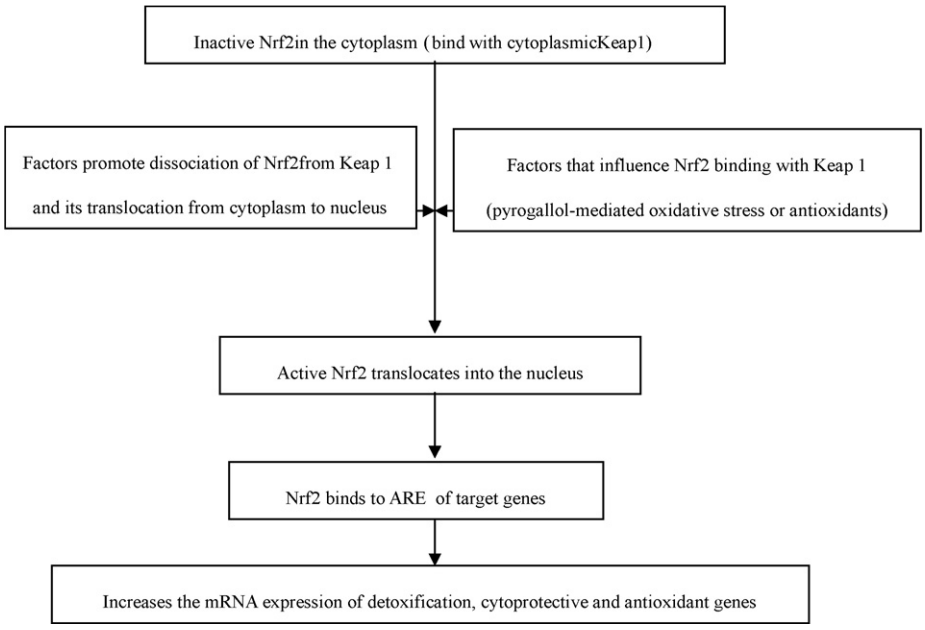

Fig. 2. Nrf2-mediated activation of phase II xenobiotic metabolizing genes during oxidative stress: in normal condition, Nrf2 binds with its negative regulator Keap 1 and remains inactive. Oxidative stress and other factors sensitize and catalyze the dissociation of Keap 1 from Nrf2–Keap 1 complex leaving active Nrf2, which further binds to target gene through antioxidant responsive element and enhances its expression.

the opening of dinitrophenol-induced adenosine-5′-triphosphate-sensitive potassium channel effectively in cardiac myocytes [31]. Additionally, pyrogallol is found to increase the duration but not the magnitude of chemically-induced sympathetic responses [32]. Pyrogallol is also shown to delay NO-dependent auricular–ventricular nodal conduction thereby reduces blood supply and increases oxidative stress in isolated heart [33] (Table 2).

2.3. Pyrogallol and gastrointestinal research

The gastrointestinal toxicity of pyrogallol is mainly based on its inhibitory potential on the relaxation of gastrointestinal muscles and sphincters. Because of its ability to generate superoxide, pyrogallol inhibits non-adrenergic non-cholinergic lower oesophageal sphincter relaxation and relaxation of intact and denuded mucosa of the fundus [34,35]. The pyrogallol exerts its

Table 1
Biological effects of pyrogallol on various pulmonary functions as evidenced by the experiments performed in cultured cell lines and animal model systems.

Systems	Biological effects	Refs.
Rat lung pericyte	Produces concentration dependent effect on muscular contraction and relaxation, thereby affecting muscular tone. Long-term exposure may lead to membrane damage	[4]
Cultured human nasal epithelial cell	Modulates intracellular Ca^{2+} concentration therefore alters Ca^{2+} dependent activities of pulmonary system	[20]
Bovine tracheal smooth muscle	Inhibits cGMP production in a concentration dependent manner and affects calcium signaling	[21,22]
Cultured A549 epithelial cell	Induces cell death by activating nuclear factor-kB and iNOS mRNA expressions in concentration and time of exposure dependent manner	[23]
Cat airways	Modulates basal and 5-hydroxytryptamine-induced muscle tone in a concentration dependent manner	[24]
Human lung cancer Calu-6 cell	Induces apoptosis in isolated pulmonary cells through multiple molecular events i.e., by inducing G2-specific cell cycle arrest, reducing the expression of cyclin-dependent-kinase (CDK) inhibitors, increasing the expression of cyclins, reducing intracellular antioxidants and causing loss of mitochondrial trans-membrane potential	[25,26]

Table 2
Biological effects of pyrogallol on cardiovascular functions as evidenced by the experiments performed in cultured cell lines and animal model systems.

Systems	Biological effects	Refs.
Adult rat ventricular myocyte	Reduces cardiac muscle contraction via superoxide and p38 mitogen activated protein kinase following long-term exposure	[27]
Isolated rat heart	Pyrogallol pre-conditioning reduces the size of myocardial infarct, reduces lactate dehydrogenase and creatine kinase release in coronary effluents during ischemia-reperfusion injury and offers protein kinase-C-dependent cardioprotection	[28]
Guinea pig ventricular myocyte	Stimulates basal calcium current and thereby reduces resting cell length	[29]
Cardiac muscle from bovine calf heart	Causes an irreversible inhibition of cardiac contractile function leading to respiratory failure	[30]
Cloned beta cell-type potassium channel	Delays adenosine-5′-triphosphate-sensitive potassium channel opening thereby alters membrane potential	[31]
Rabbit cardiac myocyte	Impairs interaction between phosphatidylinositol polyphosphate and potassium channel and alters membrane potential	[31]
Pithed rat	Increases the duration but not the amplitude of chemically-induced sympathetic stimulation	[32]
Guinea pig isolated heart	Delays NO-dependent auricular–ventricular nodal conduction thereby reduces blood supply and increases oxidative stress	[33]

Table 3
Biological effects of pyrogallol on digestive system as evidenced from the studies conducted in cultured cell lines and animal model systems.

Systems	Biological effects	Refs.
Mouse gastric fundus	Inhibits ultra-violet light irradiation, electric field stimulation and NO-induced relaxations of intact and denuded fundus mucosa	[34]
Japanese white rabbit	Inhibits NO-mediated non-adrenergic and non-cholinergic lower oesophageal sphincter relaxation due to its ability to generate superoxide	[35]
Mouse/rat duodenum	Offers dose-dependent inhibition of nitrergic nerve stimulation and gastric emptying and induces oxidative stress by inducing lipid peroxides and 5-hydroxytryptamine biosynthesis	[3,36,37]
Rat gastric fundus	Inhibits NO donors and/or metal sulphate-mediated relaxation of gastric fundus	[38,39]
Isolated circular muscle	Attenuates NO-dependent relaxation of isolated circular muscles	[40]
Canine ileocolonic junction/opossum lower esophageal sphincter	Reduces NO-induced relaxation of ileocolonic junction/opossum lower esophageal sphincter	[41,42]
Rabbit gastric epithelial monolayer	Inhibits cell migration, proliferation and induces apoptosis of epithelial cells	[43]
Cultured intestinal epithelial monolayer	Augments chemically-induced hyper-permeability of epithelial cells	[44]

modulatory activity in the gastrointestinal tract by NO dependent manner. Pyrogallol also produces a dose-dependent inhibition of duodenal relaxation by attenuating the NO donors-mediated inhibitory effect [36,37]. Although at lower doses it inhibits NO-mediated relaxations, at higher doses pyrogallol responds differently because of excessive production of malonaldehyde and 5-hydroxytryptamine [3]. The inhibitory effects of pyrogallol on gastrointestinal tract via NO-dependent mechanism are confirmed by the studies that have shown its modulatory potential on NO donors and their derivatives alone or in combination with metallic salt-mediated non-adrenergic non-cholinergic stimulation [38,39]. Although pyrogallol is reported to inhibit mainly NO-dependent relaxations of gastrointestinal muscles and sphincters, but NO-independent effects are also reported [40–42]. Some *in vitro* studies have also shown pyrogallol-mediated inhibition of cell migration and proliferation and augmentation of hyper-permeability and apoptosis of epithelial cells [43,44] (Table 3).

2.4. Pyrogallol and brain research

Parkinson's disease, second most common progressive neurodegenerative disorder, is mainly characterized by the loss of dopaminergic neurons of the nigrostriatal pathway. Selective degeneration of dopaminergic neurons results into reduced dopamine level in striatum. However, higher dopamine concentration results in auto-oxidation-mediated free radical generation. The dopamine level in the striatum is regulated by dopamine metabolizing enzymes. Catechol-*O*-methyl-transferase (COMT) is an enzyme that degrades dopamine into its end product homovanillic acid in presence of aldehyde dehydrogenase and monoamine oxidase. Pyrogallol acts as a non-competitive inhibitor of COMT in time of exposure dependent manner due to its ability to generate hydrogen peroxide and hydroxyl radicals via Haber–Weiss reaction [45,46]. At higher concentration, pyrogallol alone or in combination with pargyline and methamphetamine leads to oxidative stress due to its own ability to generate free radicals and 6-hydroxydopamine or enhanced dopamine auto-oxidation [47–50]. Additionally, pyrogallol causes lipid peroxidation, reduces S-adenosylmethionine and inhibits stereo-specific binding of dopamine agonists to neostriatal membranes [51–53] (Table 4). It is also observed that pyrogallol releases iron from ferritin, generates aldehydes leading to bleomycin-dependent DNA degradation that results into single strand nicks in pUC18 DNA during lipid peroxidation [54]. It is now well established that pyrogallol induces apoptosis directly or indirectly by inducing free radicals generation, lipid peroxides biosynthesis and DNA damage [47–52,54] (Table 4).

2.5. Pyrogallol and hepatic damage

Hepatotoxicity is characterized by the damage of hepatic tissues leading to an enhanced level of transaminases and bilirubin in the blood stream of rodents and humans. Pyrogallol is well established hepatotoxic chemical, as it increases transaminases and bilirubin levels in circulating blood, and causes histopathological aberrations in liver [2,5,6]. Pyrogallol-mediated hepatotoxicity is caused by altered mRNA and/or protein expressions of cytochrome P450s (CYPs), glutathione reductase, glutathione-S-transferases (GSTs) and glutathione peroxidase [2,5,6,11]. Pyrogallol reduces antioxidant enzymes, induces oxidative stress due to an altered iron biosynthesis and increases phase I enzymes in liver, thereby shifts the dynamic homeostasis towards the enhanced biosynthesis and accumulation of free radicals, which ultimately leads to lipid peroxidation, DNA damage and membrane dysfunction [5,6,54] (Fig. 3).

3. Herbal antioxidants

Herbal antioxidants have become a vital area of research due to their therapeutic potential against diseases and efficacy to counteract pyrogallol-induced toxicity [5,6,11,55]. Many plant products, such as flavonoids, triterpenes and polyphenols are used as hepatoprotective agents to ameliorate toxicant-induced hep-

Table 4
Biological effects of pyrogallol on nervous system particularly in brain as evidenced by the studies conducted in cultured cell lines and animal model systems.

Systems	Biological effects	Refs.
PC12 cell	Induces programmed cell death in isolated cultured cells	[47,48]
Rat brain	Depletes dopamine level and increases accumulation of 6-hydroxydopamine leading to oxidative stress and neurodegenerative changes in brain	[49]
Mouse brain	Induces accumulation of 6-hydroxydopamine in mouse brain leading to free radical generation through auto-oxidation	[50]
Rat brain	Enhanced generation of lipid peroxides and free radicals leading to oxidative stress and neurodegenerative changes	[51]
Rat brain	Attenuates methylation and augments demethylation of methionine more potentially in the cortex than the striatum leading to reduced chemotaxic responses and enhanced neuronal susceptibility	[52]
Rat neo-striatal membrane preparations	Weakly inhibits specific binding of dopamine agonists to neo-striatal membranes leading to degenerative effects	[53]

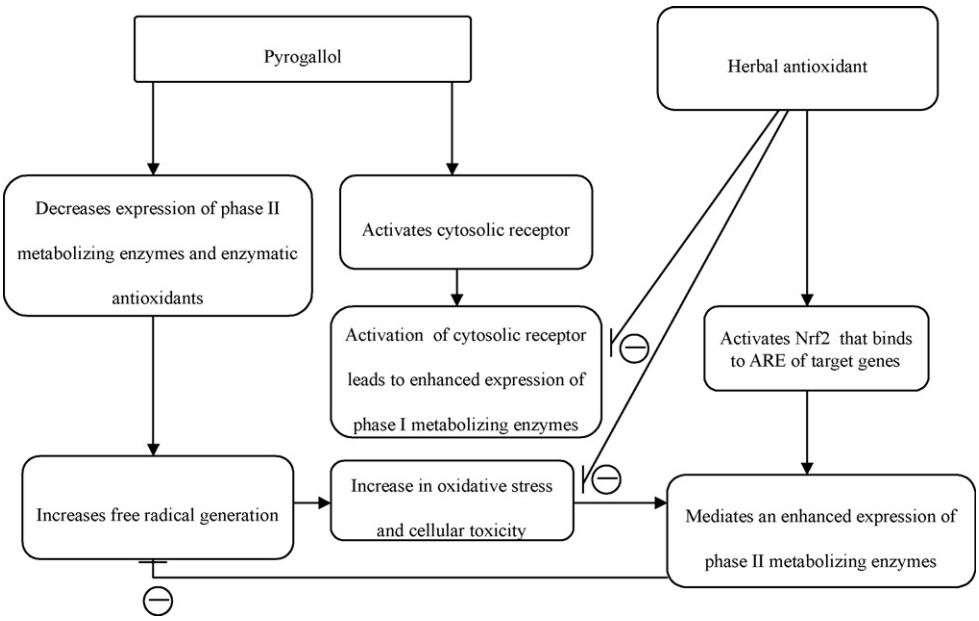

Fig. 3. Mechanism of pyrogallol-induced toxicity and the effects of herbal antioxidant: pyrogallol increases the level of phase I xenobiotic enzymes, such as CYP1A2 and CYP2E1 and decreases the level of phase II xenobiotic and antioxidant defense enzymes, however, herbal antioxidant restores pyrogallol-mediated changes.

atic damage in the experimental animal models [2,5,11]. As the pyrogallol-mediated toxicity response is mediated due to an imbalance between cellular oxidants and antioxidants, its attenuation is reported because of antioxidant potential of plant products [2,11]. Although the potential of these herbal components for protection against pyrogallol-induced toxicity at biochemical level began several decades ago (Table 5), studies to understand the mechanism of their action at molecular level started recently. Plant products either scavenge free radicals or enhance the expression and catalytic activities of antioxidant defense enzymes [2,6,11].

New Livfit, a polyherbal formulation of 11 medicinal plants, attenuates pyrogallol-mediated hepatotoxicity and hepatitis C [11]. *Ganoderma lucidum*, a medicinal mushroom, has been widely used for the treatment of liver diseases [56]. Its aqueous extract also prevents pyrogallol-mediated free radical chain reaction and erythrocyte membrane damage [57]. Curcumin [1,7-bis (4-hydroxyl-3-methoxyphenyl)-1,6-heptadiene-3,5-dione], one of the most widely used herbal formulations, is also used against pyrogallol-induced hepatotoxicity [58]. Curcumin inhibits the expression of cyclooxygenase-2 and interferes in the activation of nuclear factor-κB [59]. Rubiadin, isolated from the roots of *Rubia cordifolia*, also attenuates pyrogallol-mediated changes of immunological parameters and oxidative stress [13]. Ginger is commonly used to ameliorate pyrogallol-mediated symptoms of abdominal discomfort and bloating [60]. *Fagonia cretica* and *Tinospora cordifolia* are also shown to attenuate pyrogallol-mediated oxidative stress by restoring intracellular antioxidants and scavenging free radicals [61].

Table 5
Summary of the biological effects of some herbal antioxidants on pyrogallol-induced toxicity using *in vivo* and *in vitro* approaches.

Systems	Antioxidants	Biological effects	Refs.
Rat liver	Silymarin and new livfit	Attenuate pyrogallol-mediated increase of transaminases and lipid peroxides in circulating blood and check white blood cells infiltration thereby offering hepatoprotection	[2,11]
Rat gastro-intestine	Vitamins C and E	Pyrogallol-induced gastric emptying is effectively restored by vitamins C and E	[3]
Mouse liver	Silymarin and resveratrol	Reduce pyrogallol-induced liver toxicity by reducing the circulating level of transaminases and bilirubin. Reduce generation of lipid peroxides and free radicals due to altered phase I and phase II enzyme expressions or catalytic activity	[5,6]
Rat immune cell	*Rubia cordifolia*	Significantly restores pyrogallol-mediated changes in immunity and oxidative stress	[13]
Rat ventricular myocyte	Anisodamine and tetramethylpyrazine	Effectively encounter pyrogallol-mediated cardiovascular changes	[27]
PC12 cell	Curcumin	Attenuates apoptosis in PC12 cells induced by pyrogallol exposure	[48]
Rat erythrocyte membrane	*Ganoderma lucidum*	Pyrogallol-induced oxidative stress and erythrocyte membrane damage leading to red blood cell loss is effectively encountered by *Ganoderma lucidum*	[57]
Rat	Ginger	Ameliorates pyrogallol-induced discomfort and bloating of the gastrointestinal tract	[60]
Rat hippocampal slices	*Rubia cardifolia*, *Fagonia cretica*, *Tinospora cordifolia*	Ameliorate pyrogallol-mediated increase in oxidative stress by restoring antioxidants level and scavenging free radicals	[61]

Silymarin, isolated from the seeds of milk thistle (*Silybum marianum*), is rich in silybinin, silydianin and silychristin. Two main mechanisms of action of silymarin have been proposed, based on its cell-regenerating and cytoprotective functions [62]. The action of silymarin involves various biochemical events, such as the stimulation of ribosomal RNA synthesis, protection against cell membrane damage and blockage of the uptake of toxins [63]. Silymarin offered liver regenerating property in many patients by direct interaction with cell membrane [62,64]. The inhibition of lipid peroxidation in *in vitro* models using erythrocytes, isolated and cultured hepatocytes and human mesangial cells has been accepted as one of the major protective mechanisms [62]. Silymarin possesses antioxidant, anti-inflammatory, anti-fibrosis and anti-proliferative properties [62]. Hepatoprotective potential of silymarin is reported due to its cellular regeneration and cytoprotection activities [2,6,11,63].

Resveratrol (trans-3,4′,5-trihydroxy-trans-stilbene), a natural polyphenol, is present in peanuts, grapes and red wine. Trans-resveratrol and its analogues are present in numerous plants used in Chinese and Japanese traditional medication. During the last decade, the potential health benefits of trans-resveratrol have been widely investigated [65]. The role of trans-resveratrol in cancer chemoprevention and in the prevention of cardiovascular and neurodegenerative diseases has been reported in preclinical studies [65,66].

Silymarin as well as resveratrol effectively diminishes the pyrogallol-mediated augmentation in mRNA and protein expression of CYP1A2 and CYP2E1, lipid peroxidation, pyrogallol-mediated attenuation in glutathione content [5,6]. In general, resveratrol and silymarin offer hepatoprotective potentials against pyrogallol-mediated toxicity due to their antioxidant and anti-lipid peroxidation activities and also because of their potential to alter toxicant metabolism [2,5,6,11].

4. Triumphs and pitfalls

Despite beneficial effects, pyrogallol elicits organ toxicity in individuals either residing in geographic regions rich in organic matters, such as coal and shale or working in dye, fur and chemical industries, as they are continuously exposed. The prevalence of pyrogallol-mediated organ toxicity demonstrates the need for its efficient and cost-effective treatment. The search for suitable antidotes to minimize its toxicity is inevitable [2,5]. As pyrogallol produces toxicity in many organs, therefore, its antidote must be completely or relatively non-toxic and safe [5,6,28,49]. Researchers are looking at naturally occurring antioxidants, as they are relatively non-toxic and are mainly used as dietary supplements [67]. Flavanoids have been a major component of traditional medication system in developing countries and are becoming complementary and alternative medicines [68].

Recent developments in molecular biology and knockout technology have revolutionized pharmacology and toxicology. The generation of classical and knockout animal models has made the evaluation of efficacy and toxicity of pyrogallol more reliable, simple, convenient and less time consuming. Development of synthetic chemistry has made it possible to synthesize naturally occurring chemicals in the laboratory and store them in their naturally occurring form. These studies provided basic mechanistic research to understand the mechanism of modulation of indices of oxidative stress and predict their possible use in clinics. The major advantages of naturally occurring antioxidants are lack of toxicity at biologically effective concentrations, edible plant origin, easy synthesis in the laboratory and more importantly, most of the scientific preclinical evidences for their usefulness are well understood [67].

The failure of naturally occurring antioxidants is mainly based on the interventional clinical trials exploring the benefits of vitamin antioxidants in combating drug-induced toxicity that have shown mostly negative results in clinics [69]. The failure of vitamin antioxidants does not necessarily mean that herbal antioxidants should not be tried in clinics and would be unsuccessful as therapeutic agents. The negative findings of vitamin antioxidants can be explained in many ways. Firstly, the result of such studies was contributed by the use of different doses of antioxidants in separate clinical trials and secondly, due to differences in the baseline demographic characteristics of the participants. The inappropriate doses could be mainly responsible for the negative findings, as fat soluble vitamins are well known to produce toxicity, a condition called hyper-vitaminosis, since its inception [70]. Vitamin antioxidants are mainly chain-breaking antioxidants and exhibit their beneficial effects by prevention of free radical generation and not by re-storage of antioxidant activity or expression of antioxidant enzymes [71]. Silymarin and resveratrol do produce such effects but are not expected to produce any toxic effect at commonly prescribed doses [5,6]. Even minor decision making error in supplementation dose selection of naturally occurring antioxidants in combating drug-induced toxicity is not expected to produce negative effects, as already reported to resist ethanol-induced hepatotoxicity [72,73].

5. Translational challenges

Despite well known effects of pyrogallol and application of naturally occurring antioxidants to combat pyrogallol-induced toxicity in various model systems including rat, mouse and human cell lines, antioxidant therapy did not attract clinicians for clinical trials to evaluate their exact effects in real life situations [5,6,48,57,60]. The use of naturally occurring antioxidants in the treatment of liver ailments in clinics remains a controversial issue. Resveratrol and silymarin possess metabolic and cell regulating effects that include regulation of cell membrane permeability, inhibition of the 5-lipoxygenase pathway, scavenging of free radicals and suppression of nuclear factor-κB [74]. Although resveratrol and silymarin have good safety records and some positive results in patients with alcohol-induced liver toxicity have also been known [73,75], no definite conclusion is drawn regarding their potential in the attenuation of pyrogallol-induced toxicity in general and hepatotoxicity in particular. Before application of these antioxidants against pyrogallol-induced toxicity, it is essential to know the name and type of pro-oxidants produced during its exposure, details of other possible and probable sources of free radical generation in the target organ/liver in addition to pyrogallol exposure at that particular condition, full proof and well established role of antioxidants in attenuation of pyrogallol-induced hepatic injury and proper measurement tools to assess the sufficient concentration of antioxidants that is desirable to abolish the hepatotoxic effects in clinical trials. The translational limitations of naturally occurring antioxidants against pyrogallol-induced toxicity are due to a variety of other reasons as well, such as lack of knowledge to extrapolate the mechanism of action of drug and antioxidants altogether obtained from preclinical studies to clinical situation in humans and difficulty in optimization of concentration, dose and time of treatment in humans as compared with experimental models. The clinical interventional planning for the use of antioxidants needs a proper scrutiny of available preclinical data before counselling the patients. Although these are still the major concerns of clinicians, it is demanded to make a decision to evaluate their usefulness in real life situations.

A number of ways may be adopted to reach the final conclusion how to use naturally occurring antioxidants in clinics. Basic scientists and clinicians need to decide the dose, time and con-

centration of pyrogallol and flavonoid antioxidants in consultation with trained statisticians and toxicologists and cautiously extrapolate the data in humans. Despite the fact that naturally occurring antioxidants have potential in clinics in combating pyrogallol-induced toxicity, the indiscriminate use of these antioxidants should be avoided. The decision of their supplementation should be dependent on the nutritional status of the patient and severity of the toxic effects. It is also advisable to analyze the current evidences corroborating or rejecting the presumed protective role of antioxidants in clinics properly, before recommending their intake.

6. Future directions

Despite recent advancement in understanding the biology of antioxidants in pyrogallol-induced toxicity, only fewer tests have been conducted in clinics. Genomic and proteomic patterns and histopathological interventions of target as well as nontarget tissues and biological fluids of animals need to be analyzed to assess the actual impact of antioxidants against pyrogallol-induced toxicity, as biochemical and minor molecular parameters may not be sufficient to predict the practical consequences and long lasting effects of naturally occurring antioxidants. Preclinical interventions in animals need to be conducted using reproducible and novel molecular biology platforms. Lack of infrastructures and collaborative platforms, has contributed to marginal success in providing full proof evidences for potential benefits of antioxidants and has raised controversy among clinicians for clinical trials of these antioxidants. Establishing a network for sharing the experiences among researchers, clinicians and industrial organizations on assessing the efficacy of antioxidants against pyrogallol-induced toxicity may help in developing therapeutic strategy for recommending naturally occurring antioxidants in the clinics.

Conflict of interest

The authors declare that there are no conflicts of interest.

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