Review Article

นิพนธ์ปริทรรศน์ของกลไกการเหนี่ยวนำภาวะเครียดออกซิเดชั่นในตับโดยสารพิษ

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บทคัดย่อ

นิพนธ์ปริทรรศน์ของกลไกการเหนี่ยวนำภาวะเครียดออกซิเดชั่นในตับโดยสารพิษ

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ว. เภสัชศาสตร์อีสาน 2561; 14(1) : 1-12

รับบทความ : 15 กันยายน 2560 **ตอบรับ :** 14 มกราคม 2561

ภาวะเครียดออกซิเดชั่นเป็นผลจากความไม่สมดุลระหว่างสารต้านออกซิแดนท์และอนุมูลอิสระออกซิแดนท์ โดยเฉพาะอนุมูลอิสระออกซิเจนว่องไว (reactive oxygen species, ROS) ซึ่งเป็นโมเลกุลที่มีความว่องไวสูงสามารถถูกสร้างจากปัจจัยทั้งภายในและภายนอกร่างกาย ตับเป็นอวัยวะเป้าหมายของการสร้าง ROS เนื่องจากเป็นอวัยวะหลักที่ต้องสัมผัสกับสารต่างๆ ในกระบวนการเมแทบอลิซึมและการแปลงรูปทางชีวภาพที่เป็นผลให้เกิดการสร้าง ROS ดังนั้นการได้รับสารพิษจึงเป็นสาเหตุภายนอกที่ทำให้เกิดการสร้าง ROS มากที่สุด ความเข้าใจกลไกการเกิดภาวะเครียดออกซิเดชั่นจึงมีความสำคัญต่อการสนับสนุนแนวคิดเกี่ยวกับความสัมพันธ์ของ ROS และการเกิดโรค เอธานอล โซเดียมเซเลไนต์ (Na_2SeO_3) และเทิร์ต-บิวทิลไฮโดรเปอร์ออกไซด์ (TBHP) มักถูกนำมาใช้เป็นสารเหนี่ยวนำภาวะเครียดออกซิเดชั่น การศึกษาก่อนหน้าแสดงให้เห็นถึงศักยภาพของสารทั้งสามชนิดนี้ในการเหนี่ยวนำภาวะเครียด ออกซิเดชั่นทั้งในหลอดทดลองและในสัตว์ทดลอง ซึ่งมีกลไกเกี่ยวข้องกับกระบวนการเมแทบอลิซึมในตับ เอธานอลถูกเมแทบอลิซึมผ่านเอนไซม์แอลกอฮอล์ดีไฮโดรจีเนส ไซโตโครม P450 2E1 และคะตาเลส ได้ผลิตภัณฑ์ ROS และ อะซิทัลดีไฮด์ ส่วนซูเปอร์ออกไซด์แอนไอออนถูกสร้างจากกระบวนการเมแทบอลิซึมของโซเดียมเซเลไนต์โดยผ่านระบบกลูตาไธโอน ขณะที่กระบวนการเมแทบอลิซึมของ TBHP ผ่านเอนไซม์ไซโตโครม P450 2E1 และระบบเอนไซม์กลูตาไธโอนเปอร์ออกซิเดส-กลูตาไธโอนรีดักเทส ก่อให้เกิดอนุมูลเปอร์ออกซิลและอนุมูลอัลคอกซิล รวมทั้งเกิดเทิร์ต-บิวทิลแอลกอฮอล์ ยิ่งไปกว่านั้นการศึกษาก่อนหน้ายังแสดงให้เห็นว่า TBHP เป็นสารเหนี่ยวนำภาวะเครียดออกซิเดชั่นที่มีความแรงจากการใช้ความเข้มข้นที่ต่ำและเวลาน้อยที่สุดในการเหนี่ยวนำภาวะเครียดออกซิเดชั่นได้ ทั้งในหลอดทดลองและสัตว์ทดลอง ดังนั้นความรู้ความเข้าใจเกี่ยวกับกลไกของภาวะเครียดออกซิเดชั่นและคุณสมบัติเฉพาะของสารเหนี่ยวนำการสร้าง ROS จึงมีคุณค่าต่อการพัฒนาโมเดลความเครียดออกซิเดชั่นที่เหมาะสมสำหรับการศึกษาเพื่อประเมินผลกระทบของสารต้านอนุมูลอิสระต่อกลไกภายใต้ภาวะเครียดออกซิเดชั่น

คำสำคัญ: ภาวะเครียดออกซิเดชั่น, เอธานอล, โซเดียมเซเลไนต์, เทิร์ต-บิวทิลไฮโดรเปอร์ออกไซด์, ตับ

A Review on the Mechanism of Oxidative Stress-Induction in the Liver by Xenobiotics

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Abstract

A Review on the Mechanism of Oxidative Stress-Induction in the Liver by Xenobiotics

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IJPS, 2018; 14(1) : 1-12

Received : 15 September 2017 **Accepted :** 14 January 2018

Oxidative stress results from an imbalance between antioxidant and free radical oxidants, especially reactive oxygen species (ROS). ROS are highly reactive molecules that can be generated by endogenous and exogenous factors. Liver is the target organ for ROS generation because it mainly exposes to various substances in metabolism and biotransformation to result in ROS production. Thus, xenobiotic exposure is the most external factor which causes ROS formation. The understanding of oxidative stress mechanism is essential in order to support the notion that ROS relate with illnesses. Ethanol, sodium selenite, and *tert*-butyl hydroperoxide (TBHP) are commonly used as oxidative stressors. The previous studies revealed the potentials of these compounds to induce oxidative stress both *in vitro* and *in vivo*. The oxidative stress-mechanism of ethanol, sodium selenite, and TBHP associate with metabolic processes in the liver. Ethanol is metabolized via alcohol dehydrogenase, cytochrome P450 2E1 (CYP2E1), and catalase to produce ROS and acetaldehyde. Superoxide anion is produced by the metabolism of sodium selenite via glutathione system while the metabolism of TBHP via CYP2E1 and glutathione peroxidase–glutathione reductase system generates peroxyl and alkoxyl including *tert*-butylalcohol. Furthermore, the prior studies suggested that TBHP was a strong oxidative stressor because of using the least concentration and time for induction of oxidative stress both *in vitro* and *in vivo* studies. Therefore, the knowledge of oxidative stress mechanism and specific properties of compounds for ROS generation are worth for development of an appropriate oxidative stress model for a study on the impact of an antioxidant to investigate its effect on the mechanism behind oxidative stress.

Keywords: oxidative stress, ethanol, sodium selenite, *tert*-butyl hydroperoxide, liver

Introduction

Oxidative stress which is a harmful process that results in damage of cells, tissues, and organs, occurs from over level of free radicals, leading to disturbance of antioxidant homeostasis (Birben *et al*., 2012). Metabolism of toxic or chemical substances is one of the most pivotal causes of oxidative stress because biotransformation of these substances in the liver, the main organ of metabolism, resulted in generation of free radical products (Jaeschke *et al*., 2002). Numerous substances such as ethanol, sodium selenite, and *tert*-butyl hydroperoxide (TBHP), have been revealed to induce oxidative stress (Jaeschke *et al*., 2002; Rahman *et al*., 2012).

In the liver, ethanol is metabolized by several pathways consisting alcohol dehydrogenase, microsomal heme-monooxygenase, and catalase (Zakhari, 2013) while sodium selenite and TBHP are metabolized via glutathione system and cytochrome P450 pathway, respectively (Shen *et al*., 1999a; Liu *et al*., 2002). Ethanol metabolism produces both reactive oxygen species (ROS) and a major product-mediated oxidative stress, namely acetaldehyde. The most products from sodium selenite metabolism are superoxide anion ($O_2^{\bullet-}$) whereas peroxyl ($ROO^{\bullet}$) and alkoxyl ($RO^{\bullet}$) are products from TBHP metabolism. ROS associate to the risk of many diseases such as diabetes, cardiovascular disorder, liver disease, and cancer (Rahman *et al*., 2012).

To better understanding oxidative stress-induced mechanisms of these substances, the process of oxidative stress which results from the metabolism of ethanol, sodium selenite, and TBHP, and their consequences are reviewed. Furthermore, this review provides a worth information for further intensive study to develop a suitable model of oxidative stress.

Oxidative stress

Reactive oxygen species (ROS) such as hydroxyl ($OH^{\bullet}$), hydroperoxyl ($HO_2^{\bullet}$), $O_2^{\bullet-}$, $ROO^{\bullet}$, and $RO^{\bullet}$ and reactive nitrogen species (RNS) such as nitric oxide ($NO^{\bullet}$), nitrogen dioxide ($NO_2^{\bullet}$), and peroxynitrite ($ONOO^{\bullet}$), are occurred by cellular redox process and generation of energy in mitochondria. In addition, external factors (xenobiotics, cigarette smoke, and heavy metal ions exposure) are causes of ROS/RNS generation, in which ROS are mostly found in human body (Pham-Huy *et al*., 2008).

Under the normal condition, antioxidant system eliminates free radicals/ ROS for maintenance of homeostasis. The disturbance of antioxidant homeostasis results from ROS, leading to oxidative stress. The extreme production of ROS leads to cellular injury and damage to biological molecules such as protein, nucleic acid, and lipid. Furthermore, ROS can induce lipid peroxidation which is a harmful process and lead to a by-product, namely malondialdehyde (MDA). This process causes cell membrane rupture and loss of selective membrane permeability (Niki *et al*., 2005; Ray *et al*., 2012).

Antioxidant system is divided into two-component systems. The first, enzymatic antioxidant system includes the main biochemical defense as superoxide dismutase (SOD) and catalase (CAT). SOD is the first defense against free radicals, mainly superoxide anion that is transformed into hydrogen peroxide (H_2O_2), and then turned to water (H_2O) and oxygen (O_2) by CAT (Limón-Pacheco and Gonsebatt, 2009). The other system, the glutathione (GSH) system is non-enzymatic antioxidant which is mostly synthesized in liver. The predominant mechanism of GSH against free radicals is a hydrogen or electron donor involving detoxification (Cacciatore *et al*., 2010). Hence, imbalance between antioxidant system and free radicals is an indicator for the occurrence of oxidative stress. Xenobiotic is claimed as the most common cause of this event.

The types of oxidants

Endogenous source of ROS. The main source of ROS generation is mitochondria. Its major role is to produce energy for cells, known as adenosine triphosphate (ATP) which is used for cellular respiration. The formation of ROS is occurred during the synthesis of ATP, especially superoxide anion (Zorov *et al*., 2014). Normally, oxygen is

produced during this process and then it is converted to water by electron transfer of the mitochondrial electron transport chain. However, superoxide anion is generated by 1-3% of all electron leak from this process (Birben *et al*., 2012). Furthermore, the natural response to offending agents in the body produces ROS. This phenomenon is called as oxidative burst; for example, macrophage and leukocyte are stimulated by pathogen that they generate superoxide anion via nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. Then superoxide anion is changed to hydrogen peroxide and hypochlorite which are highly oxidative potentials (Pham-Huy *et al*., 2008).

Exogenous source of ROS. Exogenous source is considered for the most factor of ROS generation. In particular, xenobiotic exposure induces oxidative stress via metabolic reaction in the liver and it can also damage biomolecules such as lipid, protein, and DNA (Simeonova *et al*., 2014). Additionally, ionizing radiation (e.g. UV, X-ray, or γ- ray) change hydroxyl radical and superoxide to hydrogen peroxide. Heavy metal ions (e.g. copper, iron, cadmium, or mercury) result in lipid peroxidation and nuclear protein damage. Besides, cigarette smoking and inhalation of smoke stimulate neutrophils and macrophages, leading to oxidant-induced cell injury (Birben *et al*., 2012).

Oxidative stress results from ROS overproduction which is formed by endogenous and exogenous sources. The structure of ROS has unpaired electron, leading to a high reactive molecules which can react with biological molecules. Therefore, oxidative stress disturbs homeostasis and damages cells such as increasing mitochondria stress, DNA damage, and apoptosis including the impairment of organ function (Fig. 1).

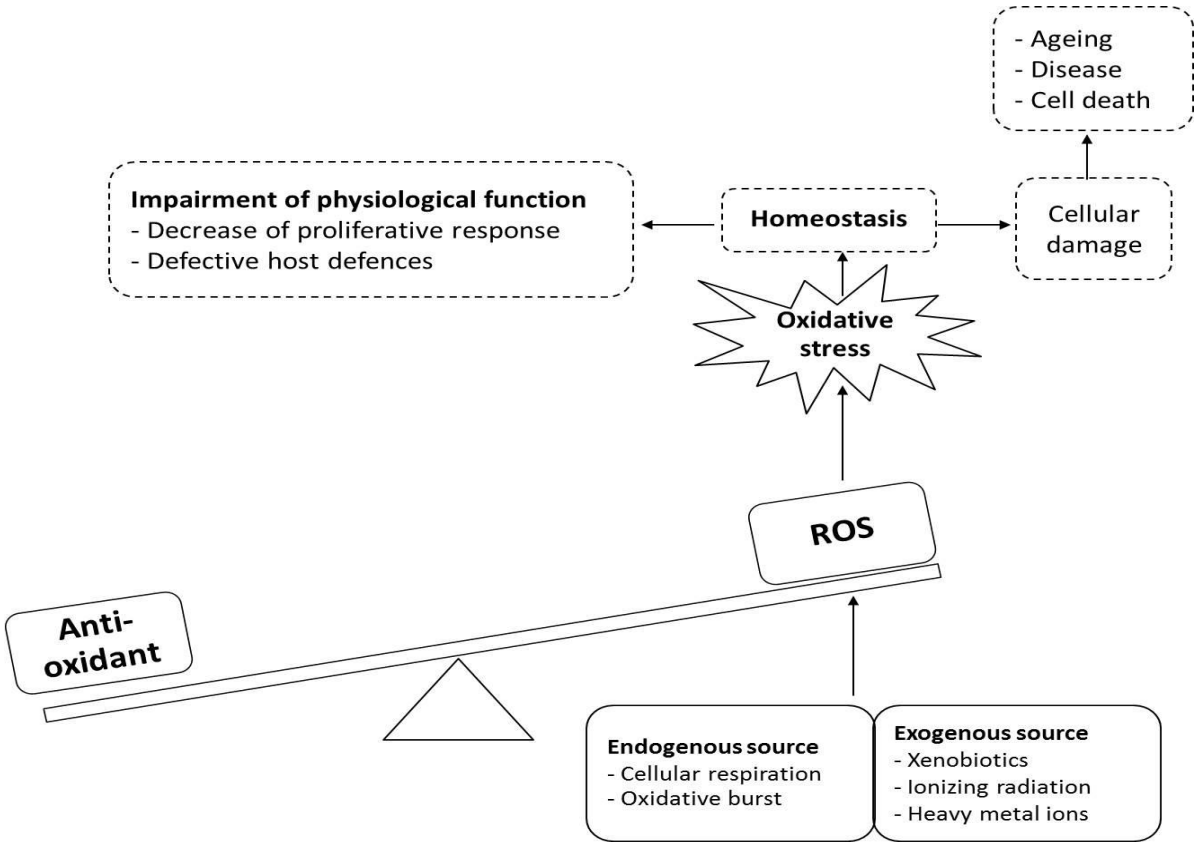

Fig. 1. The source of ROS and the imbalance of ROS and antioxidant

Mechanism of ethanol-induced oxidative stress

Ethanol, also called as alcohol, ethyl alcohol, and drinking alcohol, is volatile, flammable, and colorless liquid. It is widely used as a substance to stimulate oxidative stress and hepatotoxicity both *in vivo* and *in vitro* models (Simeonova *et al*., 2014).

Ethanol is metabolized to acetaldehyde by three pathways in the liver consisting alcohol dehydrogenase (ADH), microsomal ethanol oxidation system (MEOS), and CAT (Fig. 2) (Zakhari, 2013). ADH is the main pathway which metabolizes ethanol to acetaldehyde which is subsequently converted by aldehyde dehydrogenase (ALDH) to acetate which is toxic to organs and systemic circulation. Acetaldehyde is a toxic metabolite which increases nicotinamide adenine dinucleotide that inhibits β-oxidation in mitochondria, leading to the accumulation of intracellular lipid to encourage lipid peroxidation and further tissue damage (Sid *et al*., 2013; Zakhari, 2013). MEOS has the main component, namely cytochrome P450 2E1 (CYP2E1). Moreover, CYP2E1 is involved in ROS over-production (Sid *et al*., 2013). CAT contributes the metabolism of ethanol including generation of hydrogen peroxide which mediated oxidative stress (Bansal *et al*., 2010).

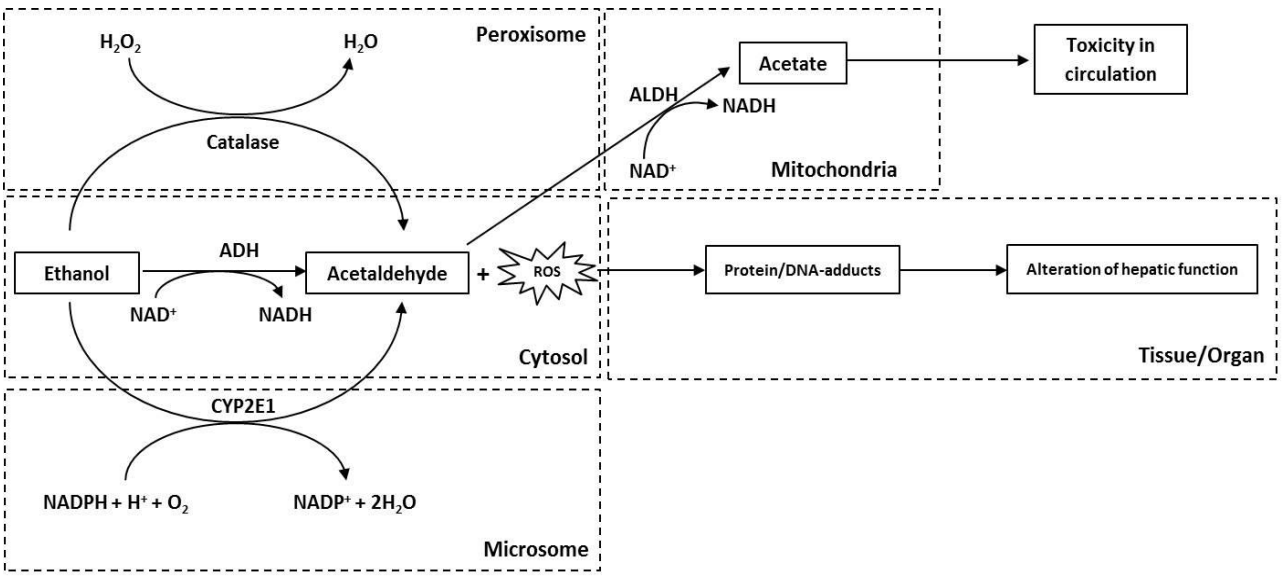

Fig. 2. Pathways of ethanol metabolism

Yao *et al* (2007) determined cellular damage from ethanol- induced oxidative stress in human primary hepatocyte. Ethanol exposure at a dose of 100 mM for 24 h increased the release of AST and LDH into the culture medium with a decrease in the GSH level and a parallel increasing of MDA level in human hepatocytes.

Hemeoxygenase-1 (HO-1) is an isoform of hemeoxygenases which catabolizes heme into biliverdin and then it is converted to bilirubin by biliverdin reductase. HO-1 is an endogenous antioxidative defense system against stress-associated physiological disorders (Szabo *et al*., 2004). The mechanism of HO-1 is regulated by signal transduction via mitogen-activated protein kinase (MAPK) in order to protect cells from oxidative stress. Extracellular signal regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38 are the three major pathways of MAPK. Therefore, HO-1 activity in hepatocyte was eliminated by measuring the conversion rate of heme into bilirubin. The result showed that the activity of HO-1 was increased in 100 mM ethanol treated-hepatocyte for 24 h, compared with untreated hepatocyte. While the hepatocytes were co-incubated with 100 mM ethanol, 100 μM quercetin, 15 μM

PD98059 (ERK inhibitor), and 15 μM SB203580 (p38 inhibitor), the decreasing of HO-1 activity were noted since the signaling pathways was blocked. These phenomenon reflected HO-1-activation under the stimulation of oxidative stress via MAPK signaling transduction (Yao *et al*., 2007).

Additionally, HepG2 cell treated with ethanol at a dose of 50 mM for 24 h resulted in a decrease in GSH content and activities of SOD, CAT, and GPx including an increase in MDA level in HepG2 cells (Gutierrez-Ruiz *et al*., 2001).

An acute ethanol-induced hepatotoxicity was studied in male ICR mice (Xing *et al*., 2011). The mice were received ethanol (56% w/v or 71%v/v, 15.2 mL/kg of body weight) by gavage for 7 days. The elevation of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in blood were observed. The MDA level was increased while the GSH content was decreased in the ethanol-treated mouse livers. Furthermore, hepatic microvesicular steatosis was observed by hematoxylin and eosin (H&E) staining. Production of hepatic tumor necrosis factor alpha (TNF-α), the major cytokine of acute ethanol condition, was enhanced, indicating the liver injury associated with oxidative stress (Ji *et al*., 2004).

A chronic ethanol-feeding mouse model was employed (Cui *et al*., 2014). Male Kunming mice were orally given 50% ethanol in water twice a day for 11 weeks. The GSH depletion and the reduction of SOD activity were observed along with an increase in the MDA content in liver of the ethanol-treated mice, followed by activation of Kupffer cells (KC), enlargement of hepatocyte and macro- and micro-vesicular steatosis.

Tang *et al* (2012) investigated the role of CYP2E1 and oxidative stress for impaired adiponectin secretion from adipocytes in response to ethanol. Adiponectin is an insulin sensitizer which possesses anti-diabetic, anti-atherogenic, anti-inflammatory, and cardioprotective properties (Wang *et al*., 2008). Male Wistar rats were fed with Lieber-DeCarli liquid diet containing ethanol as 36% of total calories for 4 weeks. The chronic ethanol feeding suppressed the secretion of adiponectin from isolated epididymal adipocytes while it induced the expression of CYP2E1 protein in adipocytes followed by an increase in oxidative stress markers, including 4-hydroxynonenal, and protein carbonyls (Tang *et al*., 2012).

Mechanism of sodium selenite-induced oxidative stress

Sodium selenite (Na_2SeO_3) is an inorganic compound. The hepatic GSH system converts sodium selenite to selenium (Se) which is an essential element. Interestingly, the previous study suggested Se had oxidative stress-related cytotoxicity in HepG2 cells that resulted from the metabolism of sodium selenite (Thompson *et al*., 1994). Sodium selenite is metabolized via glutathione system with the reduced glutathione (GSH) as the main compound involves in the production of ROS. GSH reacts with sodium selenite to become selenium and subsequently produces ROS, mainly superoxide anion and hydrogen peroxide (Fig. 3) (Shen *et al*., 1999a).

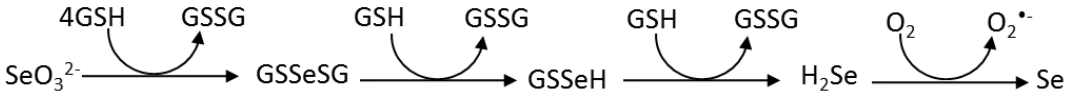

Fig. 3. The reaction of sodium selenite with GSH

Two different modes were proposed to demonstrate a dual role of glutathione in sodium selenite-induced oxidative stress in HepG2 cells and modulation of intracellular GSH content (Shen *et al*., 1999a). Mode A (pretreatment), cells were pretreated with N-acetylcysteine (5 mM, NAC), buthionine sulfoximine (2.5 mM, BSO), or 0.25 mM GSH prior to 10 μM Se exposure. Mode B (simultaneous treatment), cells were treated with Se and

NAC, BSO, or GSH simultaneously (the doses are same as mode A). Both mode A and B were studied for 24 h. The results showed that Se-induced oxidative stress was closely related to the intracellular level of GSH. Both the increase and decrease of GSH content supplemented Se-induced oxidative stress in HepG2 cells. This study demonstrated that the dual role of GSH against the effect of selenium on oxidative stress was pro-oxidant and antioxidant.

To confirm the previous study of Shen *et al* (1999a), sodium selenite exposure at a dose range of 5-25 μM for 24 h resulted in the increase of LDH leakage, the decrease of GSH content, and the enhancement of ROS generation in HepG2 cells. Additionally, changes of characteristic apoptotic morphology were examined by Wright-Giemsa staining under light microscope.Treatment of sodium selenite (10 μM) caused chromatin condensation, cell membrane blebbing, and formation of apoptotic body (Shen *et al*., 1999b).

Although the previous study on sodium selenite-induced oxidative stress model in liver had a few amount of scientific evidence, sodium selenite was studied in other cells in order to confirm the potentiality of sodium selenite as an oxidative stressor.

For example, Human colonic carcinoma cells (HT29) were induced by oxidative stress by various doses of sodium selenite (1, 5, and 10 μM) for 4 days (Stewart *et al*., 1997). The two higher doses of sodium selenite decreased the GSH content in HT29 cells. This phenomenon assured an enhancement of ROS level; moreover, it related to the induction of DNA-strand breaks that was also studied using gel electrophoresis. Treatment of sodium selenite (5 μM) for 24 h caused the DNA fragmentation which lead to a harmful event as apoptosis.

Human acute promyelocytic leukemia cell lines (NB4) was induced oxidative stress by sodium selenite at different doses (2, 5, 10, and 20 μM) and different times (6, 12, and 24 h) (Li *et al*., 2003). Treatment of three doses of sodium selenite (5, 10, and 20 μM) for 12 h decreased the GSH content in NB4 cells in a dose dependent manner. Similarly, NB4 cells were incubated by 5 μM sodium selenite for different times. Sodium selenite started to reduce the GSH content after 6 h of the treatments. Therefore, sodium selenite showed dose- and time-dependent pattern against the depletion of GSH. Correspondingly, morphological alterations of the NB4 cells by Wright-Giemsa staining were shown the nuclear fragmentation and chromatin condensation.

Mechanism of *tert*-butyl hydroperoxide (TBHP)-induced oxidative stress

TBHP is a short-chain organic hydroperoxide, composed of a tertiary butyl group and a hydridodioxygen group .It causes necrotic cell and induces mitochondrial reactive oxygen formation)Drahota *et al*., 2005 .(In the liver, TBHP is metabolized by CYP2E1 which can generate ROS, mainly peroxyl)$ROO^{\bullet}$ (and alkoxyl)$RO^{\bullet}$(, leading to an initiation of oxidative stress)Fig .4) (Liu *et al*., 2002 .(Alternatively, TBHP can be changed by glutathione peroxidase–glutathione reductase system to tert-butyl alcohol and glutathione disulfide)GSSG(, resulting in pyridine nucleotide oxidation)NADP (and the depletion of GSH which disturb homeostasis)Fig .4) (Lin *et al*., 2000 .(In addition, TBHP is a pro-oxidant which influences on cell metabolism such as alteration of calcium homeostasis)Nicotera *et al*., 1988(, increase of lipid peroxidation, and impairment of mitochondrial membrane potential)Kmonickova *et al*., 2001.(

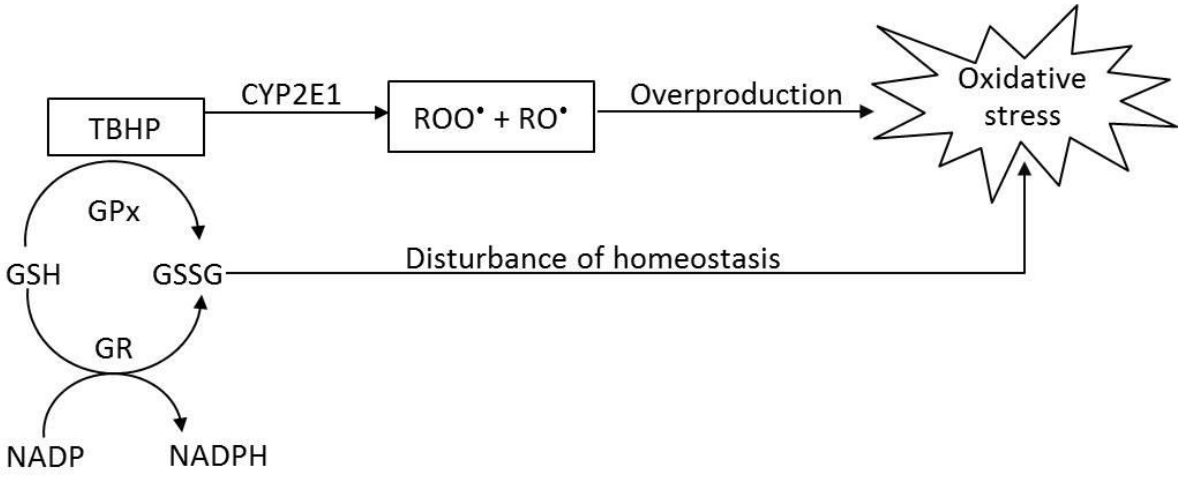

Fig. 4. Pathways of TBHP metabolism

Treatment of HepG2 cells with a dose above 50 μM of TBHP for 3 h elevated the level of MDA while decreased the activities of GR, SOD, GPx, and CAT (Alia *et al.*, 2005). Correspondingly, HepG2 cells exposed with 200 μM TBHP for 3 h noted a decrease in the GSH content with reduction of GPx, SOD, CAT, and GR activities, followed by an increase in MDA level and LDH release (Alia *et al*., 2006). In addition, an increase in ROS production was detected in HepG2 cells treated with TBHP (Alia *et al*., 2006).

According to a previous study, Sohn *et al* (2005) measured biomarkers of cellular oxidative stress after treatment of HepG2 cells with 250 μM of TBHP for 3 h. The results demonstrated that a viability of TBHP-treated HepG2 cells was 47.33%, indicating a suppression of cell growth. This phenomenon related with an enhancement in ROS production and MDA level along with a reduction in the GSH content in the TBHP-treated HepG2 cells.

Choi *et al* (2006) isolated hepatocytes from male Sprague-Dawley rats. Then the hepatocytes were incubated with 250 μM TBHP for 24 h. TBHP caused the depletion of GSH and increased the formation of MDA in the hepatocytes, followed by an elevation of ROS production in hepatocytes monitoring by dichlorodihydrofluorescein. Moreover, the impact of TBHP- induced oxidative DNA damage on hepatocytes was studied by Comet assay. The result showed that the DNA damage was increased in the TBHP- treated hepatocytes in accordance with an increase of ROS. These observations indicated that TBHP caused ROS overproduction, leading to oxidative stress through the pathway of DNA damage.

Additionally, male ICR mice were intraperitoneally given TBHP (20 mg/kg) for 24 h (Choi *et al.*, 2006). TBHP caused degeneration of the liver tissue and congestion of the central vein and sinusoids. Correspondingly, an increase in MDA level and a decrease in GSH content were presented along with an enhancement of serum levels of hepatic enzyme makers (AST and ALT).

Liu *et al*) 2002 (induced hepatotoxicity in Male Sprague-Dawley rats by intraperitoneal injection of TBHP)0.2 mmol/kg/day(for 5 consecutive days .TBHP increased the levels of LDH, ALT, and AST in blood, and elevated MDA, the oxidative stress marker in the livers, followed by reduction of GSH in the livers .In addition, histopathology of the rat livers)H&E staining (showed that TBHP caused the incidence of liver lesions, including hepatocyte swelling, leukocyte infiltration, and necrosis.

Table 1 The xenobiotic-induced oxidative stress models

Xenobiotics	Mechanism		Doses of study	Models	Effects	References
Ethanol	- Induce ADH - Disturbance of antioxidant system	*In vitro*	50-100 mM	- HepG2 cell - Primary human hepatocyte	- Increase AST, LDH, and MDA levels - Impairment of endogenous antioxidant system	Gutierrez-Ruiz *et al*., 2001 Yao *et al*., 2007
	- Induce CYP2E1	*In vivo*	- 71%v/v (Acute, 7 days) - 36-50%v/v (Chronic, 4-11 weeks)	- Male mice (ICR, Kunming) - Male Wistar rat	- Increase oxidative stress markers and inflammatory cytokine - Impairment of endogenous antioxidant system - Induce CYP2E1 protein - Hepatocyte damage	Xing *et al*., 2011 Tang *et al*., 2012 Cui *et al*., 2014
Sodium selenite	Disturbance of glutathione system	*In vitro*	5-25 μM	- HepG2 cell - HT29 cell - NB4 cell	- Increase oxidative stress markers - Depletion of GSH content - DNA damage - Apoptotic cell	Shen *et al*., 1999a Shen *et al*., 1999b Stewart *et al*., 1997 Li *et al*., 2003
TBHP	- Induce CYP2E1 - Disturbance of GPx-GR system	*In vitro*	50-250 μM	- HepG2 cell - Primary rat hepatocyte	- Increase oxidative stress markers - Impairment of endogenous antioxidant system - Inhibition of cell growth - DNA damage	Alia *et al*., 2005 Sohn *et al*., 2005 Alia *et al*., 2006 Choi *et al*., 2006
		In vivo	18-20 mg/kg (1-5 days)	- Male ICR mice - Male SD rat	- Increase oxidative stress markers - Depletion of GSH content - Disturbance of hepatic function	Liu *et al*., 2002 Choi *et al*., 2006

Conclusion

Oxidative stress is a harmful condition affecting homeostasis and relating with illnesses. The overproduction of ROS is a cause of these events. The sources of ROS generation are both endogenous and exogenous sources. In particular, an external factor as xenobiotic exposure is the most evidence to induce ROS formation in several organs, especially liver which is the main site of xenobiotic biotransformation. The previous studies employed several toxic compounds to create models for understanding the mechanism of oxidative stress and supporting the hypothesis that ROS associated with the chronic diseases.

Ethanol, sodium selenite, and TBHP are commonly used as toxic compounds to induce oxidative stress via the ROS generation from different metabolism pathways. The metabolism of ethanol produces both ROS and the main product- mediated oxidative stress, namely acetaldehyde. The most product of sodium selenite metabolism is superoxide anion, whereas peroxyl and alkoxyl are products from TBHP metabolism (Fig. 5). These compounds are not equivalent potential to induce oxidative stress, depending on the concentration and time employed in the experiments (Table 1). In this review, both concentration and time of TBHP- induced oxidative stress *in vitro* and *in vivo* were the least. These observations suggested that TBHP strongly induced oxidative stress and was the most toxic agent to cells and tissues. The dose of sodium selenite was less than TBHP, while the time was longer than TBHP. Interestingly, sodium selenite has a dual role, both pro- oxidant and antioxidant. Therefore, sodium selenite may be not an appropriate choice for development of an oxidative stress-model because both ROS generation and ROS elimination are occurred simultaneously by sodium selenite. The dose and time of ethanol were employed more than either TBHP or sodium selenite, hence ethanol was a least potent oxidative stressor. Since the productions of ROS of these compounds are different, selection of a compound as an oxidative stressor should depend on a purpose of the study to investigate oxidative stress- related pharmacological activities of an antioxidant.

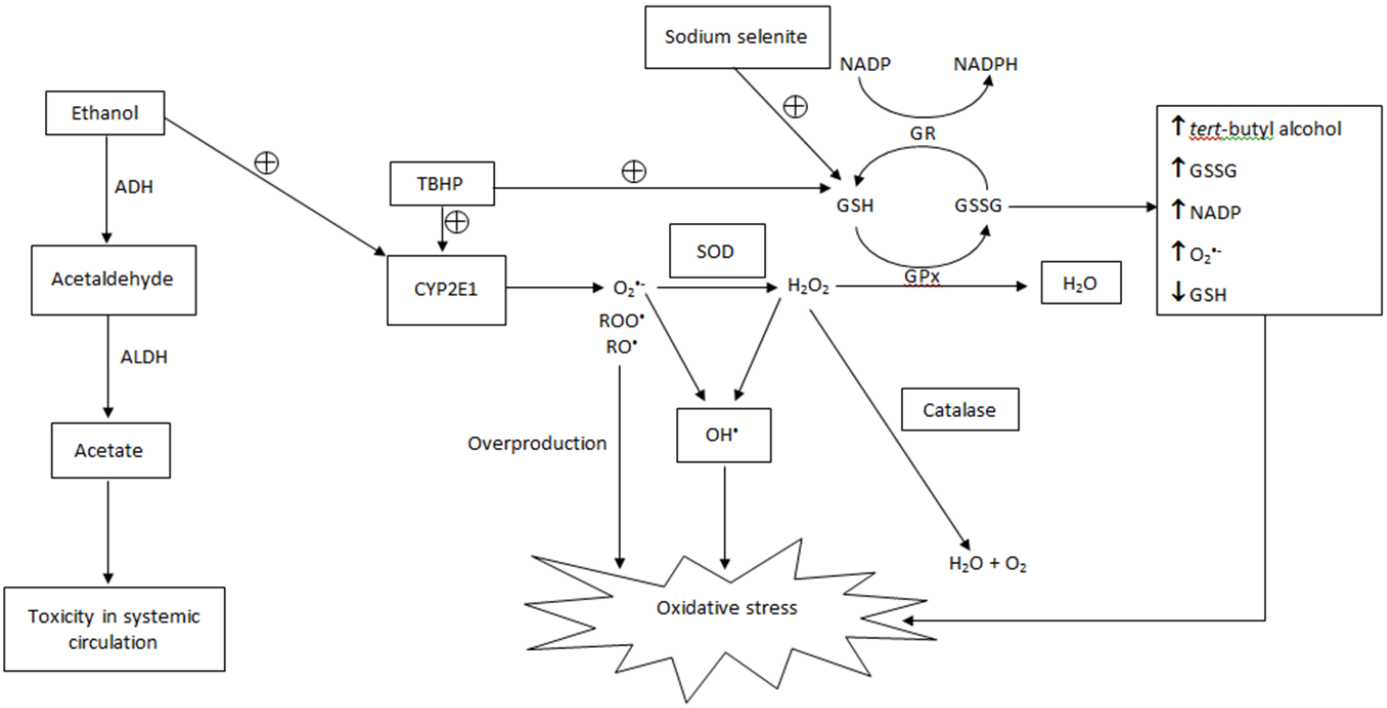

Fig. 5. Metabolic pathways-induced oxidative stress in the liver of ethanol, sodium selenite, and TBHP

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