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PROTECTIVE EFFECT OF NATURALLY-DERIVED ANTIOXIDANTS AGAINST ACETAMINOPHEN-INDUCED HEPATOTOXICITY: A REVIEW

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Abstract: Acetaminophen (APAP) is a commonly used over-the-counter (OTC) drug known to induce hepatotoxicity when consumed in excess. Formation of reactive oxygen species (ROS) and oxidation of cellular proteins and enzymes are directly involved in its toxic mechanisms. However, antioxidants can be helpful to inhibit or restrict the oxidative damage. Besides synthetic antioxidants, naturally-derived substances can be used to serve the purpose. In this paper, a thorough literature review revealed that APAP combined with food-derived natural antioxidants exhibit a protective effect against APAP-induced hepatotoxicity.

Keywords: acetaminophen, chemoprevention, hepatotoxicity, free radical, natural antioxidants, redox homeostasis.

Introduction

Acetaminophen (APAP) is a well-known and frequently used antipyretic and analgesic drug by people all over the world (Bessems et al., 2001; Dargan & Jones, 2002; James et al., 2003; Yang et al., 2013). It is normally prescribed to relieve conditions such as mild to moderate pain from headaches, muscle aches, toothaches, backaches, menstrual cramp, common colds, sore throats, reaction to vaccines and fever reduction (Medline Plus drug information). At therapeutic dose, acetaminophen is conjugated to glucuronic acid and eliminated in bile as part of metabolism. Nevertheless, 7.0 mg/day for adults and 150 mg/kg for children are considered to be toxic for the liver (Hazai et al., 2002; Kon et al., 2007).

The liver is one of the most vital organs of the body, carrying out over 500 functions including metabolism of ingested substances and detoxification of toxic substances (Almeer et al., 2018). Extensive use of acetaminophen in thousands of prescriptions and over-the-counter drugs has escalated the risk of hepatotoxicity (Larson, 2007; Hinson et al., 2010).

Though the exact mechanism of APAP-induced liver injury is yet unclear, the idea is that hepatotoxicity starts from the moment its metabolic activation is set in motion. About 95% of the therapeutic dose is converted into inactive metabolites however, CYP1A2, CYP2E1 coverts the rest of the dose into a toxic metabolite named N-acetyl-*p*-benzo-quinone imine (NAPQI) (Corcoran et al., 1985; Esterline et al., 1989; Nelson, 1990; James et al., 2003) (**Fig. 1.**). In some cases involving APAP overdose, alcohol abuse, hepatic impairment, and starvation, a low reduced glutathione (GSH) level is seen which exponentially multiplies the pernicious effect of NAPQI. As the GSH level is always low, the NAPQI targets cytosolic and mitochondrial proteins and lipids and disrupts the function of several pro (Bcl-Xs, Bad, Bax, Bid, BAK, BIM) and anti-cell death (Bcl-2, Bcl-XL) genes. This series of incidences gradually leads to mitochondrial dysfunction and results in hepatotoxicity and hepatocellular death (Ray & Corcoran, 2009; Ghosh et al., 2010; Jaeschke et al., 2012; McGill et al., 2012). NAPQI on the other hand can also increase the formation of reactive oxygen species (ROS) that includes-superoxide ions, hydrogen peroxide, and hydroxyl radical leading to lipid peroxidation and decreased antioxidant enzymes (Michael et al., 1999; Hinson et al., 2002; Hinson 2004).

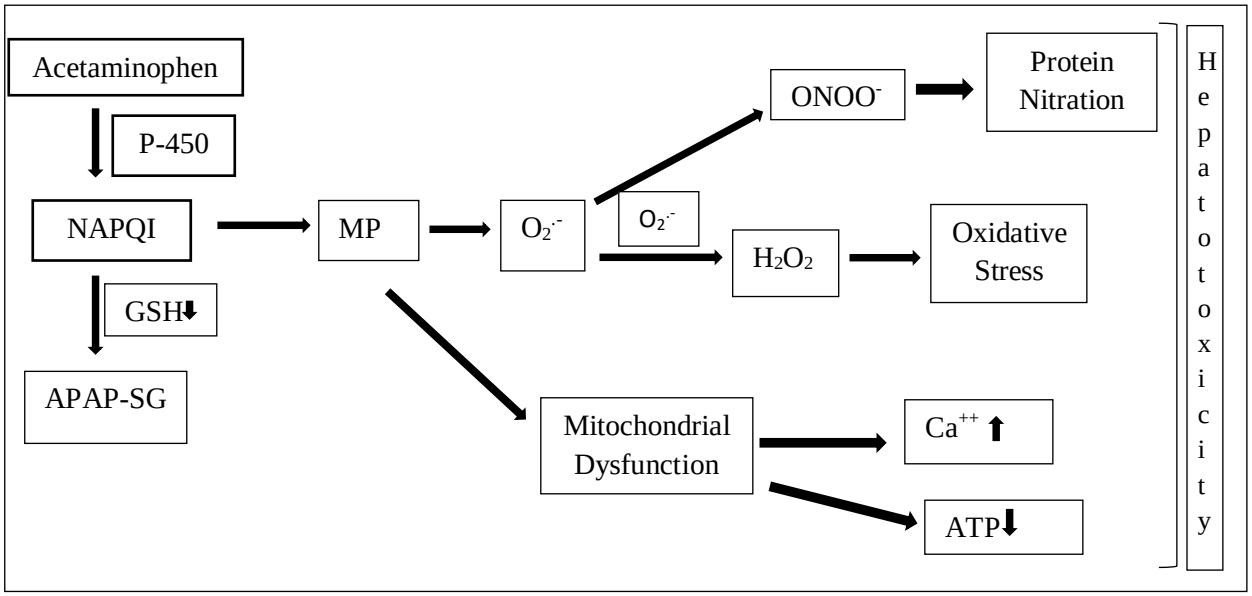

Fig. 1. Acetaminophen (APAP) metabolism and induction of hepatotoxicity

As the redox reaction is directly involved with hepatotoxicity, antioxidants application could be a potent alternative. Antioxidants are generally known as substances or compounds that have free radical scavenging capacity while inhibiting oxidative progression (Antioxidants: In Depth. NCCIH. 2010). Different naturally-derived compounds like organosulfur compounds, triterpenoids, sulphoraphae, resveratrol, saponins, lipids and different acids are more likely to act as potent antioxidants (Adeyemi et al., 2018; Atolani et al., 2019; 2020; Wang et al., 1996; Kumari & Kakkar, 2012; Noh et al., 2015; Du et al., 2015; Xu et al., 2017; Pang et al., 2016; Elshazly et al., 2014). These compounds generally inhibit the acetaminophen-induced oxidative reaction in the liver and confer hepatoprotection. This review aims to enlighten the capacity of

naturally-derived antioxidants against APAP-induced hepatotoxicity.

Hepatoprotective activity of naturally-derived antioxidants:

Several naturally derived substances showed great antioxidant activity when tested on animals with APAP-induced liver toxicity. Kumari & Kakkar (2012) claimed in their study that, lupeol (150 mg/kg), a naturally occurring triterpenoid derived from olive, mango, crataeva, strawberry, and fig reduced oxidative damage by scavenging free radicals and prevented alteration in the antioxidant defense, inhibited depolarization of the mitochondria, prevented down-regulation of Bcl-2, up-regulation of Bax and activation of caspases, prevented DNA damage and cell death in rats (**Fig. 2.**). Honey is another natural substance derived from the floral nectar that prevented an increase of the serum levels of hepatic enzyme markers, reduce both oxidative stress and inflammatory cytokines thus confirming hepatoprotection (Galal et al., 2012). Different acids derived from natural sources also have great antioxidant activity when tested against APAP-induced hepatotoxicity. Arjunolic acid (AA) found in the bark of *Terminalia arjuna*potentially inhibited P450-mediated APAP bio-activation, and c-Jun N-terminal kinase (JNK)-mediated activation of mitochondrial permeabilization (Ghosh et al., 2010). Alpha-Lipoic acid another naturally derived compound is found to have hepatoprotective activity at a dose of 20 or 100 mg/kg (Elshazly et al., 2014). Caffeic acid generally found in coffee decreases Kcap 1 expression, inhibits binding of Kcap 1 to nuclear factor erythroid 2-related factor 2 (Nrf2) which activates Nrf2, increases expression of anti-oxidative signals including heme oxygenase-1 (HO-1), NAD(P)H Quinone Dehydrogenase 1 (NQO1), thus protects the liver from APAP-induced toxicity (Pang et al., 2016).

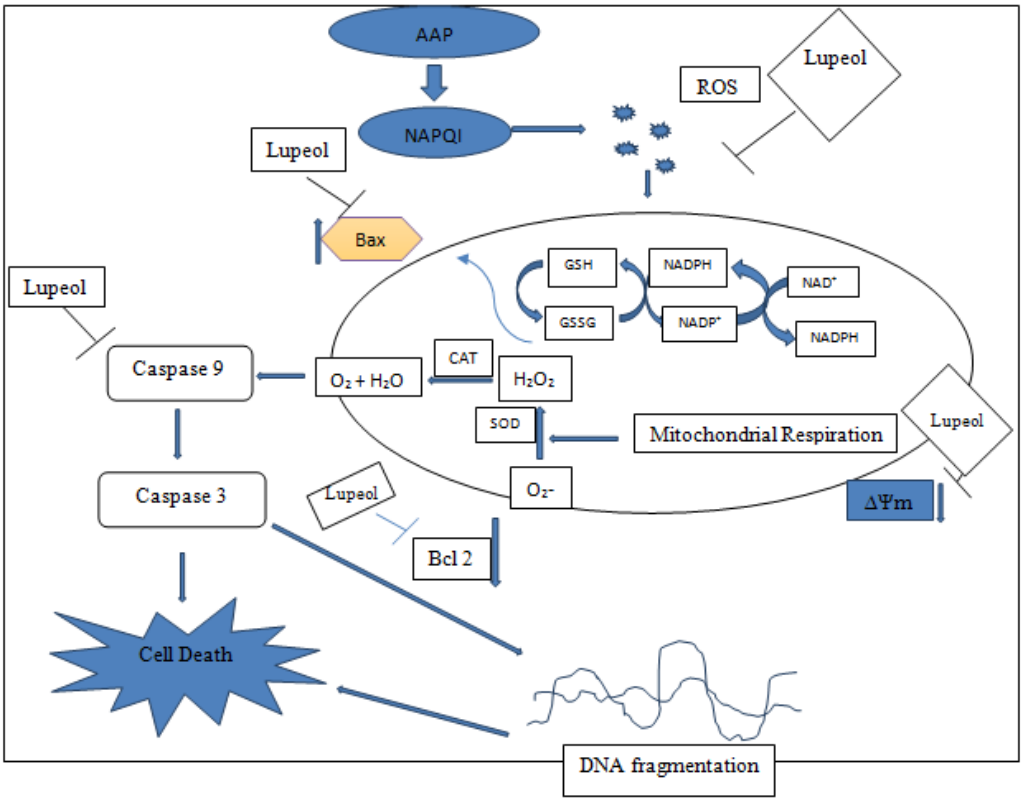

Fig. 2. Protective effect of lupeol in acetaminophen induced hepatotoxicity

Extracts of different fruits and plants are reported to have free-radical scavenging capacity. Yen et al (2008), reported that ethanolic extract of *Cuscuta chinensis* (CE) and nanoparticles are potential antioxidants as they enhanced antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and diminished lipid peroxidation (MDA) that resulted in hepatoprotective and antioxidant activity. Polyphenol extract of *Hibiscus sabdariffa* L. (HPE) at a dose of 100, 200, or 300 mg/kg reduced APAP-induced death of BABL/c in normal liver cells (BNLs), restored lost mitochondrial potency, and improved anti-oxidative status (Lee et al., 2012). Polyphenol enriched fraction from *Folium Microcos* (FM) also acts as a great antioxidant and safeguards the liver (Wu et al., 2017). A detailed summary of the hepatoprotective tendencies of some naturally derived molecules is presented in **Table 1**.

Table 1. Some reported hepatoprotective potencies of naturally-derived antioxidants

Antioxidants	**Sources**	**Dose/ concentration (R/A)**	**Protective effect (possible mechanism of action)**	**Reference**
Fresh garlic homogenates (FGH) and related organosulfur compounds	Garlic bulbs	2.5 or 5.0 g/kg	Inhibited P450 2E1-mediated APAP bio-activation displays protective activity.	Wang et al., 1996
Cuscuta chinensis ethanolic extract (CE) &*Cuscuta chinensis* nanoparticles (CN)	Seeds of *Cuscuta chinensis* Lam. (Convolvulaceae)	125 & 250 mg/kg for CE, 25 & 50 mg/kg for CN	Enhanced antioxidant enzymes (SOD, CAT, GPx), and diminished lipid peroxidation (MDA) results in hepato-protective and antioxidant activity.	Yen et al., 2008
Sesamol	Sesame oil	10 mg/kg, i.p.	Maintained mitochondrial aconitase activity in the liver, ferrous ions (Fe^{2+}), hydrogen peroxide levels, and inhibited hydroxyl-radical-associated lipid peroxidation and hepatic injury.	Chandrasekan et al., 2009
Spirulina fusiformis	*Spirulina fusiformis*	800 mg/kg/b. wt	Decreased liver marker enzymes activity, tumor necrosis factor-alpha (TNF-α), and lipid peroxidation level with increased antioxidant status.	Sabina et al., 2009
Anthocyanin fraction (AF)	Purple fleshed sweet potato	800 mg/kg	Blocked bio-activation of APAP by inhibiting CYP2E1 activity, up-regulated GSH level, and glutathione (GST)	Choi et al., 2009

			activity which increases free radical scavenging capacity and inhibits lipid peroxidation.	
Arjunolic acid (AA)	Bark of *Terminalia arjuna*	80 mg/kg	Inhibited P450-mediated APAP bio-activation and JNK-mediated activation inhibition of mitochondrial permeabilization.	Ghosh et al., 2010
Curcumin (CUR; diferuloylmethane)	*Curcuma longa*	17 mg/kg	Blockage of APAP induced oxidative stress by decreasing the several pro-injury parameters (alanine aminotransferase (ALT), nitrate/ nitrite levels, lipid peroxidation, DNA fragmentation), and several protective parameters (GSH content, SOD activity).	Bulku et al., 2012
Honey	Honey	5, 10 and 20 g/kg	Prevention of increase in the serum levels of hepatic enzyme markers, reduction in both oxidative stress and inflammatory cytokines.	Galal et al., 2012
Polyphenol extract of *Hibiscus sabdariffa* L. (HPE)	*Hibiscus sabdariffa L.*	100, 200 or 300 mg/kg	Reduced APAP-induced death of BABL/c in normal liver cells (BNLs), restored lost mitochondrial potency, and improved anti-oxidative status.	Lee et al., 2012
Lupeol, a naturally occurring triterpenoid	Olive, mango, crataeva, strawberry, and fig.	150 mg/kg	Reduced oxidative damage by scavenging free radicals and preventing alteration in the antioxidant defense, inhibited depolarization of the mitochondria, prevented down-regulation of Bcl-2, up-regulation of Bax and activation of caspases, prevented DNA damage and cell death.	Kumari & Kakkar, 2012

Red ginseng extract	Roots of *Panax ginseng* C. A Meyer	10, 30, 100, 300, 500 mg/kg	Suppressed hepatotoxicity, suppressed hepatic CYP2E1 leading to high retention of intact APAP in plasma and GSTA2 gene induction with transcriptional activation of Nrf2 and/ or C/ EBPb downstream of multiple signaling pathways that facilitate conjugation of GSH with NAPQI.	Gum & Cho, 2013
Silymarin	*Silybum marianum* (milk thistle)	100 mg/kg	Inhibition of migrating neutrophils, protection against GSH depletion that elevates nitric oxide (NO) levels in tissue along with antioxidant and free radical scavenging properties.	Bektur et al., 2013
Ginger	*Zingiber officinale* Roscoe	100 mg/kg	Reduced hepatic marker enzymes (aspartate aminotransferase, serum alanine aminotransferase, and arginase) and total bilirubin in plasma, improved paracetamol (PARA)-induced oxidative stress by inhibiting lipid peroxidation malondialdehyde (MDA), restored triacylglycerols (TAGs), and total protein levels.	Abdel-Azeem et al., 2013
Apigenin	Parsley, onions, oranges, tea, and chamomile	100 and 200 mg/kg	Increased hepatic glutathione reductase (GR) activity, reduced GSH content, and decreased hepatic malondialdehyde content.	Yang et al., 2013
Glossogyne tenuifolia (GT) Cassini	*Glossogyne tenuifolia*	100 or 300 mg/kg	Decreased ALT, aspartate aminotransferase (AST) in serum, alleviated GSH depletion, and	Tien et al., 2014

			inhibited lipid peroxidation leads to free radical scavenging and antioxidant activity.	
Alpha-Lipoic acid	Naturally found in plants and animals.	20 or 100 mg/kg	Reduction in APAP-induced liver injury is seen by restoring the changes in ALT, total protein, GSH, MDA, GSH synthase, crystathionine β-synthase, NADPH oxidae, and nuclear factor kappa B (NF-κB) towards control value. Also improves the hepatic histopathology with an increase in the expression of HO-1, Nrf2.	Elshazly et al., 2014
Aloe vera	*Aloe vera*	150 mg/kg	Improved level of serum alanine aminotransferase, hepatic MDA, number of interleukin-12 (IL-12), and interleukin-18 (IL-18) positive-stained cells, and hepatic GSH along with improved liver histopathology.	Werawatgann et al., 2014
Sulphoraphae (SFN)	Cruciferous vegetables of the genus Brassica such as cauliflower, kale, broccoli, cole crops, cabbage, collards, brussels sprout, mustard, cress, and even radish.	5 mg/kg	Antioxidant activity against APAP-induced liver injuries by blocking ROS generation, GSH depletion, and lipid peroxidation followed by up-regulation of Nrf2-targeted cytoprotective genes such as HO-1.	Noh et al., 2015
Resveratrol	Skin of grape	50 mg/kg	Reduced hepatotoxicity by scavenging peroxynitrite and preventing apoptosis-inducing factor (AIF), EndoG release from mitochondria, and subsequent nuclear DNA fragmentation.	Du et al., 2015

Caffeic acid	Coffee, some fruits, and traditional Chinese medicines	10, 30 mg/kg	Decreased Kcap 1 expression, inhibited binding of Kcap 1 to Nrf2 which activates Nrf2, increased expression of anti-oxidative signals including HO-1, NQO1.	Pang et al., 2016
Black ginseng (BG)	Roots of *Panax ginseng* C. A Meyer	600 mg/kg	Decreased level of ALT, AST, decreased lipid peroxidation, increased hepatic antioxidants GSH, apoptotic pathway suppression by increasing Bcl-2, and decreasing Bax protein expression which resulted in inhibited APAP-induced necrosis and inflammatory infiltration in the liver tissue.	Hu et al., 2017
Saponins (ginsenosides)	Leaves of *Panax quinquefolius* (PQS)	150 and 300 mg/kg	Ameliorated oxidative stress via lipid peroxidation suppression, down-regulation of pro-inflammatory factors disrupted apoptotic signal pathway by Bcl-2 overexpression, and Bax low-expression, prevented caspase-3 release.	Xu et al., 2017
Polyphenol enriched fraction from *Folium Microcos* (FM)	Leaves of *Microcos paniculata* L.	100, 200 & 400 mg/kg body weight	Modified ROS/ mitogen-activated protein kinase (MAPK)/ apoptosis axis, and Nrf2-mediated antioxidant response by four phenolic compounds: narcissin, isorhamnetin-3-O-β-D-glucoside, isovitexin, and vitexin.	Wu et al., 2017
Nutmeg	Kernel extract of *Myristica fragrans*	300 mg/kg	Suppressed oxidative stress, inflammation, and apoptosis, promoted Nrf2/antioxidant responsive element	Dkhil et al., 2019

			(ARE) pathway which leads to hepatoprotection.	
Sonneratia apetala	*Sonneratia apetala*	100, 200 & 400 mg/kg	Decreased ALT, AST level in serum, reduced MDA in the liver, increased glutathione (GSH), glutathione peroxidase (GPx) activity, enhanced catalase and antioxidant capacity, and inhibited TNF-α, IL-6, myeloperoxidase (MPO) formation in liver inhibits APAP induced liver injury.	Liu et al., 2019

Conclusions

The liver is a major organ of our body that performs several functions including metabolism and detoxification of substances. Acetaminophen is a globally used drug for different health issues. Exceeding the therapeutic level, a higher dose may often induce severe toxicity in the liver. Researchers from time to time have discovered the direct connection of redox reaction with hepatotoxicity. However, this situation can be controlled by using antioxidants that scavenge free radicals produced during the oxidative reaction. As enumerated in this review, antioxidants derived from natural sources like saponins, acids, triterpenoids, polyphenols seem to have a significant effect against APAP-induced hepatotoxicity.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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