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Recent advances in chemistry, therapeutic properties and sources of polydatin.

Recent advances in chemistry, therapeutic properties and sources of polydatin

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Abstract

Polydatin (PLD), the 3-O-β-glucopyranoside of the well-known stilbenoid compound resveratrol, is a major compound of *Fallopia japonica* (Houtt.) R. Decr. (Japanese knotweed), which is widely used in traditional Chinese medicine to treat infection, inflammatory diseases and circulatory problems. It has shown a wide range of biological activities including anti-inflammatory, anti-oxidant, anti-cancer, neuroprotective, hepatoprotective, nephroprotective and immunostimulatory effects. Although resveratrol has similar beneficial effects, its low bioavailability has remained a problem. Glycosylation increases solubility of resveratrol in an aqueous environment, thus improving its bioavailability. This has led to a growing interest in PLD. Promising results obtained from bioactivity studies have boosted an intense research on this compound. The aim of this review is to give a comprehensive overview of the botanical sources, pharmacology, biosynthesis, biotechnological production, and bioactivities of PLD, and to discuss clinical studies on this compound.

Keywords: Polydatin, piceid, stilbene, trans-resveratrol 3-O-glucoside


Abbreviations

Aβ  Amyloid β
ABTS  2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid
AMPK  AMP-activated protein kinase
ALT  alanine aminotransferase
AST  aspartate aminotransferase
Bax  Bcl-2-associated X protein
Bcl-2  B-cell lymphoma-2
BUN  blood urea nitrogen
CAT  Catalase
CC  column chromatography
COX-2  Cyclooxygenase-2
CPC  centrifugal partition chromatography
DPPH  2,2-Diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl
EC  Endothelial cell
ERK  extracellular signal regulated kinase
GC  gas chromatography
GSH-Px  glutathione peroxidase
GSH  Glutathione
GR  glutathione reductase
HO-1  Heme oxygenase 1
HSCCC  high-speed counter-current chromatography
HSP  Heat shock protein
IBS  irritable bowel syndrome
ICAM  Intercellular Adhesion Molecule 1
ICH  The International Council for Harmonisation
Ig  Immunoglobulin
iNOS  Inducible nitric oxide synthase
I/R  Ischemia/Reperfusion
JNK  Jun N-terminal kinase
IL  Interleukin
IRAK  interleukin-1 receptor-associated kinase
LDH  lactate dehydrogenase
LPS  Lipopolysaccharide
MAO  Monoamine oxidases
MC  Myocardial cell
MCAO  Middle cerebral artery occlusion
MAPK  Mitogen activated protein kinase
MDA  Malondialdehyde
MI  myocardial infarction
NF-κB  The Nuclear Factor κB
NO  Nitric oxide
Nrf2  The nuclear factor erythroid 2–related factor 2
OAT  m organic anion transporter
PLDGF  platelet-derived growth factor
PI3K  Phosphoinositide 3-kinase
PG  Prostaglandin
PGC-1α  peroxisome proliferator-activated receptor-gamma coactivator-1α
PPAR  peroxisome proliferator-activated receptors
ROS  Reactive oxygen species
SIRT  Siruin
SMA  Smooth muscle actin
SOD  superoxide dismutase
TAK1  Transforming growth factor beta-activated kinase 1
TBARS  thiobarbituric acid reactive substances
TGF-β1  Transforming growth factor beta 1
Th  T helper
TLC  Thin layer chromatography
TLR  toll like receptor
TNF  Tumor necrosis factor
TXA  Thromboxane
UHPLC  ultra-high-performance liquid chromatography
URAT  urate anion transporter
VCAM-1  vascular cell adhesion molecules-1
VEC  vascular endothelial cells
VLC  Vacuum liquid chromatography
VSMC  Vascular smooth muscle cells
WBC  White Blood Cell
Introduction

Stilbenoids are natural defense polyphenols that occur in many plant species. Resveratrol (3,5,4′-trihydroxy-\textit{trans}-stilbene) is possibly the best-known polyphenolic phytoalexin, and has attracted extensive scientific attention due to its potential health benefits. It is prominently present in red wine, and believed to contribute to the so-called ‘French paradox’, where moderate consumption of wine is correlated with a low incidence of coronary heart disease.

Polydatin (PLD, formal IUPAC name: \((2S,3R,4S,5S,6R)-2-[3\text{-hydroxy-5-[(E)-2-(4-hydroxyphenyl)ethenyl]phenoxy}]6-(hydroxymethyl)oxane-3,4,5-triol\), also known as piceid, \((E)\)-piceid, \((E)\)-polydatin, \textit{trans}-polydatin, 3, 5, 4′-trihydroxystilbene-3-\textit{β}-glucopyranoside, \textit{trans}-resveratrol-3-\textit{O-β}-glucopyranoside) is a glucoside of resveratrol in which a glucose is transferred to the C-3 hydroxyl group (Figure 1). Two isomeric forms exist in nature, \((\text{cis}-\text{and } \textit{trans}-\text{)}\)-polydatin. \textit{Cis}-resveratrol and \textit{cis}-polydatin are typically found at lower concentrations and are often less biologically active than their \textit{trans} forms (Ribeiro de Lima et al., 1999). We review the recent studies on \textit{trans}-polydatin and refer to it as PLD in this review.

Resveratrol is well-known for its chemopreventive, antidiabetic, and neuroprotective properties (Rivière et al., 2012). However, recent studies highlighted that polydatin (PLD) may have higher bioavailability and possess better antioxidant activity than resveratrol (Wang et al., 2015). In addition, other bioactivities may be altered, e.g. whereas resveratrol inhibited tyrosinase with an \(\text{IC}_{50}\) value of 565 μM, PLD inhibited with an \(\text{IC}_{50}\) value of 14 μM, indicating that the \textit{glycosylation} of resveratrol improved its tyrosinase inhibitory activity (Uesugi et al., 2017).
PLD was first isolated from Japanese Knotweed (*Fallopia japonica*), but it also exists in a variety of other sources including dietary plants such as grape, peanut and berries. It is probably the most abundant form of resveratrol in nature. Glucose substitution gives PLD a more hydrophilic character than resveratrol and makes it more resistant to enzymatic oxidation. Further, the glucose groups make that PLD is more easily absorbed in the intestinal tract than resveratrol (Regev-Shoshani et al., 2003; Cao et al., 2016), resulting in a significantly increased bioavailability. The Chinese FDA has approved PLD for multiple phase II clinical trials mainly for anti-shock applications (Chen et al., 2015). It exhibits various biological activities such as anti-shock, anti-inflammatory, anti-oxidant, anti-cancer, anti-microbial, neuroprotective, lung protective and hepatoprotective effects (Du et al., 2013).

In this paper, recent studies are reviewed on the natural occurrence of PLD, methods of analysis, biological effects, its bioavailability and metabolism, and the clinical studies conducted with this compound.

**Natural sources**

PLD was first reported in the peel of grapes. PLD is mainly present in red and white wines and grape juice; *cis*- PLD is the predominant form in rose and sparkling wines whereas *trans*
<table>
<thead>
<tr>
<th>Species</th>
<th>Parts</th>
<th>Family</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vateria indica L.</td>
<td>leaves</td>
<td>Dipterocarpaceae</td>
<td>Ito et al., 2012</td>
</tr>
<tr>
<td>Vatica rassak (Korth.) Blume</td>
<td>stem bark</td>
<td>Dipterocarpaceae</td>
<td>Tanaka et al., 2000</td>
</tr>
<tr>
<td>Upuna borneensis Symington</td>
<td>leaves</td>
<td>Dipterocarpaceae</td>
<td>Ito et al., 2017</td>
</tr>
<tr>
<td>Arachis hypogaea L.</td>
<td>stems, roots</td>
<td>Fabaceae</td>
<td>Iber-Gómez et al., 2000</td>
</tr>
<tr>
<td>Lysidice brevicalyx C.F.Wei</td>
<td>bark</td>
<td>Fabaceae</td>
<td>Hu et al., 2008</td>
</tr>
<tr>
<td>L. rhodostegia Hance</td>
<td>seeds</td>
<td>Fabaceae</td>
<td>Orsini et al., 1997</td>
</tr>
<tr>
<td>Quercus coccifera L.</td>
<td>stems, roots</td>
<td>Fagaceae</td>
<td>Sohretoglu et al., 2014</td>
</tr>
<tr>
<td>Iris tingitana Boiss. &amp; Reut.</td>
<td>bulb</td>
<td>Iridaceae</td>
<td>Farag et al., 2009</td>
</tr>
<tr>
<td>Smilax glabra Rxb.</td>
<td>rhizome</td>
<td>Liliaceae</td>
<td>Zhang et al., 2009</td>
</tr>
<tr>
<td>Theobroma cacao L.</td>
<td>seeds</td>
<td>Malvaceae</td>
<td>Hurst et al., 2009</td>
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<tr>
<td>Morus atropurpurea Rxb.</td>
<td>fruit</td>
<td>Moraceae</td>
<td>Yang J et al., 2017</td>
</tr>
<tr>
<td>Abies koreana E.H.Wilson</td>
<td>leaf</td>
<td>Pinaceae</td>
<td>Jeon et al., 2015</td>
</tr>
<tr>
<td>Picea abies (L.) H. Karst., P. jezoensis (Siebold &amp; Zucc.) Carrière</td>
<td>phloem root bark, stumps, needles</td>
<td>Pinaceae</td>
<td>Kiselev et al., 2016</td>
</tr>
<tr>
<td>Polygonum cuspidatum Siebold &amp; Zucc.</td>
<td>roots, radix</td>
<td>Polygonaceae</td>
<td>Ma et al., 2013; Kimura et al. 1983</td>
</tr>
<tr>
<td>P. multiflorum Thunb, P. reynoutria Makino</td>
<td>roots</td>
<td>Polygonaceae</td>
<td>Feng et al., 2016</td>
</tr>
<tr>
<td>Rumex bucephalophorus L., R. aquaticus L.</td>
<td>roots, aerial parts</td>
<td>Polygonaceae</td>
<td>Orbán-Gyapai et al., 2017</td>
</tr>
<tr>
<td>Rheum australe D. Don</td>
<td>roots</td>
<td>Polygonaceae</td>
<td>Hu et al., 2014</td>
</tr>
<tr>
<td>Malus sp.</td>
<td>fruit</td>
<td>Rosaceae</td>
<td>Farneti et al., 2015</td>
</tr>
<tr>
<td>Rosa laevigata Tausch</td>
<td>roots</td>
<td>Rosaceae</td>
<td>Yan et al., 2014</td>
</tr>
<tr>
<td>Vitis vinifera L.</td>
<td>cell culture, seeds</td>
<td>Vitaceae</td>
<td>Sák et al., 2014</td>
</tr>
<tr>
<td>Parthenocissus tricuspidata (Siebold &amp; Zucc.) Planch.</td>
<td>stems</td>
<td>Vitaceae</td>
<td>Jeon and Kim, 2013</td>
</tr>
<tr>
<td>Humulus lupulus L.</td>
<td>cone</td>
<td>Cannabaceae</td>
<td>Jerkovic and Collin, 2007; Jerkovic et al., 2008; Ares et al., 2015</td>
</tr>
<tr>
<td>Beeswax</td>
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</table>
resveratrol is more abundant in grapes, berries, peanuts and pistachios (Zamora-Ros et al., 2008). PLD can also be detected in many vegetable food and fruit, hop cones, hop pellets, beer, cocoa-containing products and chocolate products (Chiva-Blanch et al., 2011; Peng et al., 2015; Regev-Shoshani et al., 2003).

The predominant source of PLD are roots and rhizomes of *Fallopia japonica* (syn. *Polygonum cuspidatum* Siebold & Zucc., Polygonaceae), which have a long history of use in traditional Chinese (referred to as Huzhang) and Japanese Medicine (known as Itadori) as an analgesic, anti-pyretic, diuretic, anticancer and expectorant agent, and in the treatment of atherosclerosis (Jensen et al., 2010). However, the compound is present in several other genera like *Rosa, Rumex, Picea, Malus* and *Quercus* species (Table 1).

PLD has received similar attention to resveratrol because, in red wine and other grape products, the concentration of the glucoside usually exceeds that of the aglycone. The exact ratio of glycosylated to aglycone forms in wine depends on a number of factors such as method of fermentation and ecological conditions in the vineyards. Red wine contains up to 14.3 mg/l, and 29.2 mg/l of trans-resveratrol and PLD respectively, i.e. roughly equimolar amounts (60 - 70 µM) of the aglycone and glucosylated forms; white wines contain a factor 100 less (Galeano-Díaz et al., 2007). The higher concentrations in red wines are mostly due to the winemaking process: in the preparation of white wine, grapes are pressed and only the juice is fermented, whereas for red wine grape skins and seeds are removed only after the wine is fermented. On average, red grape skins are slightly richer in PLD and resveratrol than white ones, but differences between varieties or vintages of the same variety can be large (ranging from 50 - 200 mg/kg DW). Grape skins exhibit higher PLD contents than the seeds (Kammerer et al., 2004).
PLD was detected in 19 top selling commercially available cocoa-containing and chocolate products from the U.S. market. The cocoa-containing and chocolate products have about 3-5 times more PLD than free trans-resveratrol. Levels of PLD were highest in the cocoa powders (7.14 μg/g), followed by unsweetened baking chocolates (4.04 μg/g), semisweet chocolate baking chips (2.01 μg/g), dark chocolates (1.8 μg/g), milk chocolates (0.44 μg/g), and chocolate syrups (0.35 μg/g) (Hurst et al., 2009). Resveratrol and PLD contents in natural peanut butters were found to be significantly higher than those in blended peanut butter (Ibern-Gómez et al., 2000). Moreover, PLD was detected as the major stilbene derivative (4–8.8 mg/kg) in hop pellets (Jerkovic et al., 2005).

**Biosynthesis, Biotransformation and Chemical Synthesis**

Stilbenoids are synthesized via the phenylpropanoid and polyketide pathways. Resveratrol biosynthesis begins with the deamination of phenylalanine through phenylalanine ammonia lyase (PAL) to produce cinnamic acid, which is then hydroxylated by cinnamate-4-hydroxylase (C4H) to form p-coumaric acid. This product is attached to coenzyme A (CoA) via 4-coumarate-CoA ligase. Next, stilbene synthase (STS) condenses 4-coumaroyl-CoA with three molecules of malonyl-CoA to form the resveratrol (Figure 2), (Choi et al., 2014).

Subsequently, trans-resveratrol, may be further metabolized to form other stilbenoids, such as; polydatin via resveratrol glycosylation by glucosyltransferases (Kiselev et al., 2016).

PLD has also been generated on preparative scale through the microbial transformation of resveratrol by of *Bacillus cereus* strain UI 1477 (Cichewicz and Kouzi, 1998).

Cell suspension cultures of American pokeweed (*Phytolacca americana* L., Phytolaccaceae) could glucosylate trans-resveratrol to synthesize PLD (Ozaki et al., 2012).
A glucosyltransferase (GT) of *P. americana* (PaGT3) was expressed in *E. coli* and purified for use in the synthesis of two *O*-β-glucoside products of trans-resveratrol. Resveratrol has been produced in engineered *Escherichia coli* expressing an artificial gene cluster that contains transgenes coding for tyrosine ammonia lyase, cinnamate/4-coumarate:coenzyme A ligase, caffeic acid *O*-methyltransferase, and stilbene synthase (Choi et al., 2011). Further addition of a UDP-glucosyltransferase transgene to the gene cluster resulted in *E. coli* that can produce PLD from simple carbon sources (Choi et al., 2014).

A range of resveratrol derivatives, including PLD, has been synthesized by means of Wittig reactions, followed by glucosylation under phase transfer catalysis (Orsini et al., 1997).
An alternative synthetic route to produce polydatin involves dropwise addition of $R$-bromotetra-$O$-acetyl-D-glucose (2.1 g, 5 mmol) in anhydrous MeOH (20 ml) to a stirred solution of NaOMe (230 mg, 10 mmol) and $\text{(E)}$-resveratrol (2.28 g, 10 mmol) in anhydrous MeOH (80 ml), at room temperature under argon atmosphere. The resulting solution was heated to 40 °C, under argon atmosphere, for 1 h. The dark brown solution was evaporated to dryness and the residue extracted with Et$_2$O. The suspension was filtered, and the filtrate evaporated to dryness. The crude residue was further purified by silica gel column chromatography, eluted with CH$_2$Cl$_2$/MeOH 8.5:1.5, to afford 170 mg of PLD (yield 25 %).

The synthetic strategy proposed in this study represented an improvement over previously known procedures for PLD preparation, because large scale production of PLD is now achievable through a one-step reaction, starting from commercially available materials (Brandolini et al., 2002).

**Extraction methods**

Various used extraction methods to obtain PLD are given in **Table 2**.

Supercritical fluid extraction, with acetonitrile as modifier at 40 MPa, 100 °C has been used for the extraction of PLD from *F. japonica* and gave 0.22 mg/g yield (Beňová et al., 2010).

Microwave-assisted extraction of resveratrol from the same plant species, using 1-butyl-3-methylimidazolium bromide solution has been reported to afford 2.65 mg/g yield of PLD (Du et al., 2007). Due to being simple, inexpensive and suitable for industrial purposes, ultrasound-assisted extraction has get more interest compared to other extraction methods.

Recently, multiple Response Surface Methodology and Box-Behnken designs were employed to investigate the affinities between the extraction variables (solvent concentration, extraction temperature and ultrasonic power) and response (extraction yield %), and to obtain the optimal conditions for extraction of PLD from *P. cuspidatum* (Kuo et al., 2014). Based on the
Table 2. Extraction methods of polydatin

<table>
<thead>
<tr>
<th>Sample</th>
<th>Methods</th>
<th>Conditions</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Polygonum cuspidatum</em> Siebold &amp; Zucc. (roots)</td>
<td>Solvent extraction, Heated reflux extraction, Ultrasonic extraction, Microwave- and ultramicrowave assisted extraction, Supercritical fluid extraction</td>
<td>60% aqueous ethanol 2 h reflux at 80 °C, room temperature, 1 h 10 min at 80 °C, acetonitrile</td>
<td>Benova et al., 2008, Kuo et al., 2014, Gao F et al., 2016</td>
</tr>
<tr>
<td><em>Rheum australe</em> D. Don (roots)</td>
<td>Solvent extraction</td>
<td>EtOH/H₂O (75:25), room temperature</td>
<td>Hu et al., 2014</td>
</tr>
<tr>
<td><em>Rosa laevigata</em> Tausch (roots)</td>
<td>Solvent extraction under reflux</td>
<td>80% EtOH partitioned with petroleum ether, EtOAc and n-BuOH</td>
<td>Yan et al., 2014</td>
</tr>
<tr>
<td><em>Rumex bucephalophorus</em> L. (roots and leaves)</td>
<td>Solvent extraction, acidified ethyl acetate (0.1% formic acid), room temperature</td>
<td></td>
<td>Kerem et al., 2006</td>
</tr>
<tr>
<td><em>R. aquaticus</em> L. (aerial parts)</td>
<td>Percolation</td>
<td>MeOH, room temperature 50% aqueous MeOH partitioned with n-hexane, CHCl₃ and EtOAc</td>
<td>Orbán-Gyapai et al., 2017</td>
</tr>
<tr>
<td><em>Upuna borneensis</em> Symington (leaves)</td>
<td>Solvent extraction</td>
<td>acetone</td>
<td>Ito et al., 2017</td>
</tr>
<tr>
<td><em>Vitis</em> sp. (berry skins and leaves)</td>
<td>Solvent extraction</td>
<td>Methanol: ethyl acetate (50:50), 24h, 25 °C, darkness</td>
<td>Liu et al., 2013</td>
</tr>
</tbody>
</table>
model, and the consequent selection of appropriate extraction parameters, the extraction yields were improved. Under optimal conditions, the extraction yield of PLD from *P. cuspidatum* matched the predicted yield of 10.74 mg/g.

**Isolation and Identification methods**

The conventional methods of isolation PLD from crude plant extracts are based on liquid–liquid partition and silica gel column chromatography. These methods require a relatively long separation time, high consumption of organic solvents, and unsatisfactory resolution. There are various methods to obtain PLD from biological sources given in Table 3. Recently, high-speed counter-current chromatography (HSCCC) was used for fast and simple separation and high purification of PLD, and compared favourably to traditional column liquid chromatography (Bai et al., 2014; Chen et al., 2001; Chu et al., 2005).

A variety of analytical methods have been used to identify PLD. Organic solvent extraction, solid phase extraction, and direct injection techniques, have been used prior to resolution of resveratrol isomers by gas chromatography (GC) or high-performance liquid chromatography (HPLC). Most GC methods require derivatization with bis-[trimethylsilyl]-trifluoroacetamide (BSTFA) prior to injection into the GC, and are detected by flame ionization or mass-spectrometry (MS). Among the HPLC procedures, detection has been based upon UV-VIS, fluorescence, electrochemistry, chemiluminescence, diode array, MS, MS/MS (Cui et al., 2015; Glavnik et al., 2017; Soleas et al., 1997). Reversed phase columns, packed with n-octadecylsilane (ODS or C18) bonded phase are commonly used for separation of PLD by HPLC. Details about the eluent systems, HPLC columns, and detection methods are summarized in Table 4.
<table>
<thead>
<tr>
<th>Sample</th>
<th>Methods</th>
<th>Conditions</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Abies koreana</em> E.H.Wilson</td>
<td>CPC DiaionHP resin C</td>
<td>ethyl acetate/isopropanol/water (9:1:10)</td>
<td>Jeon et al., 2015</td>
</tr>
<tr>
<td>(<em>leaves</em>)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Lysidice brevicalyx</em> C.F.Wei</td>
<td>Polyamide CC</td>
<td>H₂O:EtOH</td>
<td>Hu et al., 2008</td>
</tr>
<tr>
<td>(<em>bark</em>)</td>
<td>Sephadex LH-20</td>
<td>MeOH</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ODS CC</td>
<td>MeOH-H₂O (15:85-90:10)</td>
<td></td>
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<tr>
<td><em>Rheum australe</em> D. Don</td>
<td>Si gel CC</td>
<td>Petroleum ether/acetone gradient CHCl₃/MeOH (90:10)</td>
<td>Hu et al., 2014</td>
</tr>
<tr>
<td>(<em>roots</em>)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Rosa laevigata</em> Tausch</td>
<td>Si gel CC</td>
<td>CHCl₃/MeOH (40:1-1:5)</td>
<td>Yan et al., 2014</td>
</tr>
<tr>
<td>(<em>roots</em>)</td>
<td></td>
<td>CHCl₃/MeOH (10:1-3:1)</td>
<td></td>
</tr>
<tr>
<td><em>R. aquaticus</em> L. (aerial parts)</td>
<td>Si gel VLC</td>
<td>CHCl₃/MeOH (99:1-1:1)</td>
<td>Orbán-Gyapai et al., 2017</td>
</tr>
<tr>
<td></td>
<td>Prep. TLC</td>
<td>CH₂Cl₂/MeOH (8:2)</td>
<td></td>
</tr>
<tr>
<td><em>Upuna borneensis</em> Symington (leaves)</td>
<td>RP CC</td>
<td>MeOH/H₂O, MeOH, acetone, CHCl₃ EtOAc/CHCl₃/MeOH/H₂O (15:8:4:1)</td>
<td>Ito et al., 2017</td>
</tr>
<tr>
<td></td>
<td>Prep. TLC</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Polygonum cuspidatum</em> Siebold &amp; Zucc.</td>
<td>HSCCC</td>
<td>chloroform/methanol/water (4:3:2). EtOAc/EtOH/H₂O (10:1:10, 70:1:10, 70:1:70)</td>
<td>Chen et al., 2001; Chu et al., 2005</td>
</tr>
<tr>
<td></td>
<td></td>
<td>light petroleum/ethyl acetate/methanol/water (2:5:4:6), light petroleum/ethyl acetate/water (1:5:5)</td>
<td></td>
</tr>
<tr>
<td>Sample</td>
<td>Methods</td>
<td>Conditions</td>
<td>Detection</td>
</tr>
<tr>
<td>---------------------------------------------</td>
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</tr>
<tr>
<td><em>Rumex bucephalophorus</em> L</td>
<td>RP HPLC</td>
<td>C18 (25x4.6 mm), 30 °C, flow rate 1 ml/min A linear gradient using water and methanol, both acidified with 0.01% formic acid, following 2 min at 40% methanol and reaching 55% methanol in 8 min</td>
<td>DAD</td>
</tr>
<tr>
<td><em>Lysidice brevicalyx C.F.Wei</em></td>
<td>RP HPLC</td>
<td>XDB-C18 (2.1 × 150 mm), 25 °C, flow rate, 0.8 mL/min MeOH:H2O gradient isocratic</td>
<td>DAD</td>
</tr>
<tr>
<td>Red wine</td>
<td>normal phase HPLC</td>
<td></td>
<td>ESIMS</td>
</tr>
<tr>
<td><em>Polygonum cuspidatum</em> Siebold &amp; Zucc.</td>
<td>RP HPLC</td>
<td>LiChrospher C18, Ammonium acetate (pH 3) and ACN in gradient</td>
<td>multichannel CoulArray electrochemical detector 200–900 mV</td>
</tr>
<tr>
<td>Wine and grapes, Peanuts and Peanut Butter, <em>Polygonum cuspidatum</em> Siebold &amp; Zucc.</td>
<td>RP HPLC</td>
<td>ODS Hypersil (250x4.6 mm), flow rate 1 ml/min 25% acetonitrile in 0.5% aqueous formic acid</td>
<td>DAD 307 nm fluorimeter operating 298 nm, 385nm</td>
</tr>
<tr>
<td><em>P. multiflorum</em> Thunb</td>
<td>UHPLC</td>
<td>C18 (1.7 μm, 2.1×50 mm), flow rate 0.3 ml/min 0.1% formic acid aqueous solution (A) and acetonitrile (B) with a gradient</td>
<td>DAD</td>
</tr>
<tr>
<td>Foods</td>
<td>RP HPLC</td>
<td>Kromasil 100 C18 (150 mm x40 mm), flow rate 0.8 ml/min. water/acetonitrile/acetic acid (67:32:1) = B, acetonitrile = C, gradient, 0 min, 20% B; 18 min, 100% B; 28 min, 100% C; 33 min, 100% B and 37 min, 20% B.</td>
<td>DAD 280 nm Fluorometric</td>
</tr>
</tbody>
</table>
UV, MS, $^1$H- and $^{13}$C-NMR data which can be used to identify PLD are available through literature (Chu et al., 2005; Cichewicz and Kouzi, 1998; Jeon and Kim, 2013; Moreno-Labanda et al., 2004).

**Biological properties**

PLD shows no apparent toxicity; it did not reduce viability of human keratinocyte HaCaT cells up to 250 µM (He et al., 2012), and did not cause any cell death in primary hippocampal neuron cells up to 125 µM (Zhang Y et al., 2015). The acute toxicology of PLD was evaluated in mice by oral administration of 5000 mg/kg; no behavioral changes or deaths were observed over a period of 72 hours (Ni et al., 2017). However, the glucoside does have a wide range of potential therapeutic properties.

**Figure 3.** Biological properties of PLD
Anti-inflammatory effect

PLD modulates expression of some transcription factors and pro-inflammatory mediators both in vivo and in vitro in different test models. Anti-inflammatory action is one of the mechanisms underlying different beneficial effects of this compound.

In stimulated peripheral blood mononuclear cells, PLD inhibited Interleukin (IL)-17 production through downregulating of IL-17 mRNA expression in a dose dependent manner. At 20 µM, it inhibited IL-17 production 98 % whereas resveratrol caused 50% inhibition at this concentration (Lanzilli et al., 2012). In human epidermal keratinocytes (HaCat) that were induced with transforming growth factor-α (TGF-α), PLD at concentrations of 10-50 µM downregulated monocyte chemotactic protein-1 and interferon γ-produced protein of 10 kDa transcriptions, reduced tumor necrosis factor-α (TNFα), and IL-6 levels in normal human epidermal keratinocytes (HaCat) and significantly inhibited extracellular signal regulated kinase (ERK) and p65 phosphorylation (Pastore et al., 2012; Potapovich et al., 2011), but significantly enhanced IL-8 release.

Application of 44 µM PLD to heat-stressed HaCat cells, reduced IL-6, IL-8 and TNF-α levels, but upregulated toll like receptor (TLR)-2 expression. Simultaneously, it showed anti-inflammatory and cytoprotective effects by inducing the human β-defensin 2 and heat shock protein HSP70B’ expression (Ravagnan et al., 2013).

IL-1β (10 ng/ml) was used to induce apoptosis and ROS production in rat articular chondrocytes. Co-treatment with 20, 30 and 40 µg/ml PLD attenuated the IL-1β-induced processes, and increased chondrocyte viability. It led to reduced IL-1β-induced release of lactate dehydrogenase (LDH), superoxide dismutase (SOD), and NO levels, and it reduced the levels of TNF-α, IL-1β, IL-8 and COX-2. PLD decreased the levels of Bax, p-p38, and matrix metalloproteinase 13 and increased level of Bcl-2, while reducing the activity of caspase-3,
and the levels of pro-inflammatory cytokines (Yang G et al., 2017). From these experiments, it was concluded that PLD may protect against osteoarthritis, by acting on the p38 MAPK signaling pathway.

Histamine release from compound 48/80-induced rat peritoneal mast cells was inhibited by 29% after application of 1 µM PLD, and the effect was stronger than that of the aglycone resveratrol which inhibited by 15% (Shimoda et al., 2015).

Anti-inflammatory activity was also tested in vivo, using a variety of mouse or rat models where inflammation was artificially induced in a variety of ways, e.g. collagen- induced arthritis (Li and Wang, 2016), D-galactose induced brain damage (Xu LQ et al., 2016), sepsis induced by puncture of the cecum (Zeng et al., 2015a), ligature-induced vascular damage (Gugliandolo et al., 2017), Staphylococcus aureus-induced mastitis (Jiang et al., 2017), surgically induced endometriosis (Di Paola et al., 2016), UVB-induced erythema in the epidermis of BALB/c-nu mice (He et al., 2012), or ov-albumin-induced hyperactivity in the small intestine of allergic rats (Yang et al., 2013). The in vivo experiments broadly confirm the in vitro assays: doses of 30 -100 mg/kg PLD decreased the artificially-induced overexpression of TLR2, and consequently inhibited TLR2-mediated activation of the p38 MAPK/NF-κB signaling pathway (Jiang et al., 2017). This in turn resulted in a decreased phosphorylation of NF-κB p65, and IκBα (Di Paola et al., 2016; Gugliandolo et al., 2017; Jiang et al., 2017; Zhao G et al., 2017), and attenuated a rise in TNFα, IL-1β, and IL-6 levels (Li and Wang, 2016; Xu LQ et al., 2016; Zeng et al., 2015a; Zhao G et al., 2017). PLD thus inhibited the iNOS production and generation of reactive oxygen species, apoptosis and activities of caspase 3 and 9 (Gugliandolo et al., 2017; Zeng et al.; Zhao G et al., 2017).
In mice with collagen induced-arthritis, 30 mg/kg PLD attenuated a rise in TNFα, and IL-6 levels; it was thought to act via activation of the expression of matrix metalloproteinase-9 (Li and Wang, 2016). It also attenuated D-galactose induced brain damage in mice by reducing TNF-α, IL-1β and IL-6 expression \textit{in vivo} at 100 mg/kg (p.o) (Xu LQ et al., 2016).

45 mg/kg intravenous PLD also improved histopathological alterations in multiple-organ dysfunction syndrome induced by hemorrhage and ligation, and puncture of cecum-induced sepsis. It also reversed the increase in serum of advanced oxidative protein products, reduced TNF-α, IL-1β and IL-6 levels, and led to a decrease in Bax levels and caspase-3 activity and a concomitant increase in Bcl-2 levels in the kidney and liver in rats (Zeng et al., 2015a).

It inhibited nitric oxide (NO) and prostaglandin E2 (PGE2) production, and reduced inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) expression at protein and transcriptional levels. It also inhibited lipopolysaccharide (LPS)-induced activation of ERK1/2, Jun N-terminal kinase (JNK), and p38 in Mouse monocyte macrophage (RAW) 264.7 cells. Inhibition of NO and PGE2 production is thought to be a result of the downregulation of phosphorylation of Nuclear Factor κB (NF-κB) and Mitogen-activated protein kinases (MAPK) (Lou et al., 2015). Also \textit{in vivo}, in mice, PLD suppressed lipoteichoic acid-induced injury by attenuating ROS generation. PLD downregulated the lipoteichoic acid-induced NF-κB p65, and IκBα phosphorylation, resulting in a decrease in the transcription of TNF-α, IL-1β and IL-6. It thus inhibited the generation of reactive oxygen species (ROS), apoptosis and activities of caspase 3 and 9 (Zhao G et al., 2017).

PLD also protects from osteoarthritis, most likely by acting on the p38 MAPK signaling pathway. IL-1β (10 ng/ml) was used to induce apoptosis and ROS production in rat articular chondrocytes. Co-treatment with 20, 30 and 40 μg/ml PLD attenuated the IL-1β-induced processes, and increased chondrocyte viability. It led to reduced IL-1β-induced release of lactate dehydrogenase (LDH), superoxide dismutase (SOD), and NO levels, and it reduced the
levels of TNF-α, IL-1β, IL-8 and COX-2. PLD decreased the levels of Bax, p-p38, and matrix metalloproteinase 13 and increased level of Bcl-2, while reducing the activity of caspase-3, and the levels of pro-inflammatory cytokines (Yang G et al., 2017).

Experimentally induced vascular damage in mice was reduced by a palmitoylethanolamide: PLD (10:1) mixture, given by gavage at 30mg/kg. Treatment inhibited expression of intercellular and vascular adhesion molecules (ICAM-1 and VCAM-1), and suppressed inflammation by reducing pro-inflammatory factors TNF-α and IL-1β, iNOS production, and NFκB expression which in turn led to inhibition of Poly ADP-ribose polymerase formation, and inhibition of apoptosis via downregulation of Bax and Fas-ligand activation (Gugliandolo et al., 2017).

In mice with Staphylococcus aureus-induced mastitis, intraperitoneal administration of PLD at 15, 30, 45 mg/kg, down-regulated pro-inflammatory cytokine levels as TNF-α, IL-1β, IL-6 and IL-8, and decreased the expression of TNF receptor-associated factor-6, myeloid differentiation factor 88, IL-1 receptor-associated kinases (IRAK)-1 and IRAK4, and TLR2. Consequently, TLR2-mediated activation of the p38 MAPK/NF-κB signaling pathway was inhibited, which resulted in a decreased phosphorylation of Transforming growth factor β-activated kinase 1 (TAK1), M KK3/6, p38 MAPK, IκB-α and NF-κB (Jiang et al., 2017).

Treatment with palmitoylethanolamine: PLD 10 mg/kg (10:1) reduced the level and severity of the macroscopic and histological markers of experimentally induced endometriosis in rats. This treatment decreased angiogenesis, nerve growth factor, ICAM-1, matrix metalloproteinase 9 expression, and lymphocyte accumulation. It also reduced NFκB translocation and decreased the phosphorylation of Iκβα (Di Paola et al., 2016).

PLD suppressed UV-B induced expression of COX-2 and ROS production dose dependently by downregulating activation of p38, JNK and ERK1/2 in UVB-irradiated HaCaT cells, and 10 mg/kg treatment reduced UVB-induced erythema, desquamation, epidermal proliferation...
and the expression of COX-2 and CD45 in the epidermis of BALB/c-nu mice (He et al., 2012).

PLD administration (orally, 150 mg/each) for 52 days after ov-albumin-induced hyperactivity in the small intestine of allergic rats, inhibited IgE production, secretion of Th2-type cytokine, IL-4, and decreased histamine levels in both serum and intestinal mucosa homogenates. It also decreased mast cell degranulation due to reduced Ca^{2+} influx through store-operated calcium channels (Yang et al., 2013). 1 µM PLD inhibited histamine release from rat peritoneal mast cells by 29%, and its effect was stronger than that of the aglycone resveratrol which inhibited by 15% (Shimoda et al., 2015).

**Anti-oxidant effect**

Like other polyphenols, PLD exhibits high antioxidant activity through its ability to scavenge ROS and enhance the endogenous antioxidant defense system. It possesses significant antioxidant properties, due to its molecular structure of long-conjugated systems and polyphenol functions in the molecule. Studies have shown that PLD is more resistant to enzymatic oxidation than resveratrol. It seems that antioxidant mechanisms involving to most of biological effects of this compound. PLD scavenged DPPH (2,2-Diphenyl-1-(2,4,6-trinitrophenyl)-hydrazyl), ABTS (2,2'-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) and •O_{2}^{-} radicals with IC_{50} values of 87, 20, 125 µg/ml, respectively *in vitro* (Xu LQ et al., 2016). Oxygen radical absorbance capacity values of PLD and resveratrol are 4.89 and 5.26 Trolox equivalents/µM respectively (Uesugi et al., 2017). PLD dose-dependently (0.05-2.00 mM) scavenges radical species and, in phenanthroline-Fe^{2+} system, possessed stronger hydroxyl radical scavenging capacity than resveratrol or vitamin C. Moreover, PLD dose-dependently (25-100 µM) protected human umbilical vein endothelial cells from H_{2}O_{2} induced cell death (Su et al., 2013). PLD at 7.5 µM improved the cellular adhesion rate and enhanced the
migratory ability of human umbilical vein endothelial cells (HUVECs) injured by H$_2$O$_2$. At the same concentration, it also induced a remarkable decrease in the level of LDH by more than 50% and ROS by 18% and significantly attenuated the changes in the content of glutathione peroxidase (GSH-Px) by 33% and SOD by 60% compared to the H$_2$O$_2$ group. Further, it reduced H$_2$O$_2$ induced apoptosis, decreased caspase-3 activity and proapoptotic protein Bax level, upregulated antiapoptotic protein Bcl-2 level. PD was concluded to suppress apoptosis in H$_2$O$_2$-injured HUVECs through inhibition of the protein kinase C signaling pathway. (Qiao et al., 2016). 240 µM PLD alone or in combination with 100 µM resveratrol synergistically suppressed oxidative stress by affecting the mitochondrial superoxide anions, extracellular NO production, and the antioxidant enzymes in Caco-2 cells, a colon cancer cell line (De Maria et al., 2013).

In mice and rats, oxidative stress was induced by single injection of 7 mg/kg cisplatin (Ince et al., 2014), weekly i.p. injections of 5-15 mg/kg doxorubicin (Wang et al., 2015), chronic exposure to 100 mg/l arsenic through drinking water for 60 days (Ince et al., 2016), or daily subcutaneous injection with 200 mg/kg D-galactose for 8 weeks (Xu LQ et al., 2016). The markers used to determine the levels of oxidative stress are an increase in the level of malondialdehyde (MDA), and a concomitant decrease in activities of the enzymes superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px); reduced GSH-Px activity results a decrease in glutathione (GSH) levels. Oral administration of PLD, at doses ranging from 25 - 100 mg/kg/day was shown to dose-dependently ameliorate or even reverse the damaging effects of oxidative stress injury (Ince et al., 2014, 2016; Wang et al., 2015; Xu et al., 2016).

Moreover, 50-200 mg/kg PLD treatment reduced the activity of several markers of tissue damage, i.e. aspartate aminotransferase (AST) (45 % lower at 200 mg/kg PLD), alanine aminotransferase (ALT) (33 % lower at 200 mg/kg PLD), in a dose dependent manner.
compared to control (Xu LQ et al., 2016). Oral administration of PLD administration (50-100 mg/kg) for 8 weeks ameliorated D-Gal induced symptoms of aging, such as dullness, decreased activity, and shed hair in mice compared to control group (Xu LQ et al., 2016).

**Anti-cancer effects**

As for resveratrol, effects of PLD on tumour development have been investigated excessively using a variety of cell cultures and in vivo animal tumor models. An overview of the signaling pathways involved in the induction of cell death or inhibition of cell proliferation by PLD is given in Figure 4. Cytotoxicity of PLD for various cancer cell lines are given in Table 5.

PLD showed significant cytotoxicity at 50 µM and higher concentrations for HepG2, MCF-7 and MDA-MB-231 cells in a concentration- and time-dependent manner. It caused significant apoptosis in MDA-MB-231 cells at 100 µM and slight G1 cell cycle arrest (Su et al., 2013). PLD inhibited the proliferation of C2C12 mouse myoblast and PC3 human prostate cancer cell lines, and increased stress resistance via mechanism involving the Estrogen receptor β-mediated induction of Mn-superoxide dismutase in mitochondria (Robb and Stuart, 2014). 1–50 µM PLD showed concentration dependent growth inhibition on Caco-2 intestinal epithelial cell line through arresting the cell cycle in G0/G1 (10-25 µM) phase and inducing apoptosis. While 50 µM PLD treatment induced DNA fragmentation, resveratrol showed apoptosis at 100 µM concentration in Caco-2 cells (Storniolo et al., 2014). PLD inhibited the proliferation of RPMI 8226 multiple myeloma cells via mTOR/p70s6k signaling pathway. IC$_{50}$ values of PLD are 131 µM and 93 µM in RPMI 8226 cells at 24 h and 48 h, respectively. PLD also induced apoptosis by downregulating Bcl-2 and increasing the caspase-3 and caspase-9, Bax levels in a dose-time dependent manner starting from 50 µM. It also induced autophagy by upregulating expressions of Beclin 1, Atg5 and LC3II at also same doses. It decreased the
Table 5. Cytotoxicity of polydatin on various cell lines

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Determining Method</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; value (48h, µM)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPMI 8226 Human Multiple Myeloma cells</td>
<td>Cell Counting Kit 8</td>
<td>93</td>
<td>Yang and Zhao, 2017</td>
</tr>
<tr>
<td>CCRF-CEM Human T Cell Leukemia</td>
<td>Resazurin reduction</td>
<td>25.08 ± 2.48</td>
<td>Kuete et al., 2015</td>
</tr>
<tr>
<td>CEM/ADR5000 Doxorubicin-Resistant Leukaemia</td>
<td>Resazurin reduction</td>
<td>39.87 ± 2.91</td>
<td>Kuete et al., 2015</td>
</tr>
<tr>
<td>Cells</td>
<td>assay</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDA-MB231 Human Breast Cancer Cells</td>
<td>Resazurin reduction</td>
<td>97.64 ± 7.60</td>
<td>Kuete et al., 2015</td>
</tr>
<tr>
<td>MDA-MB231/BCRP Human Breast Cancer Cells with Breast Cancer Resistance Protein</td>
<td>Resazurin reduction</td>
<td>95.59 ± 8.17</td>
<td>Kuete et al., 2015</td>
</tr>
<tr>
<td>HCT116 (p53+/+) p53 Resistance-Human Colon</td>
<td>Resazurin reduction</td>
<td>63.77 ± 4.61</td>
<td>Kuete et al., 2015</td>
</tr>
<tr>
<td>Carcinoma Cells</td>
<td>assay</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCT116 (p53−/−) Human Colon Carcinoma Cells</td>
<td>Resazurin reduction</td>
<td>47.03 ± 4.94</td>
<td>Kuete et al., 2015</td>
</tr>
<tr>
<td>U87MG Human Glioblastoma Cells</td>
<td>Resazurin reduction</td>
<td>55.64 ± 3.73</td>
<td>Kuete et al., 2015</td>
</tr>
<tr>
<td>U87MG.ΔEGFR Luciferase expressing Human</td>
<td>Resazurin reduction</td>
<td>47.59 ± 3.29</td>
<td>Kuete et al., 2015</td>
</tr>
<tr>
<td>Glioblastoma Cells</td>
<td>assay</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A549 Human Lung Adenocarcinoma Cells</td>
<td>MTT assay</td>
<td>2.95±0.37</td>
<td>Zhang et al., 2014</td>
</tr>
<tr>
<td>NCI-H1975 Non-Small Cell Lung Carcinoma Cells</td>
<td>MTT assay</td>
<td>3.23±0.46</td>
<td>Zhang et al., 2014</td>
</tr>
<tr>
<td>MDA-MB-231 Human Breast Adenocarcinoma Cells</td>
<td>MTT assay</td>
<td>2.66±0.73</td>
<td>Zhang et al., 2014</td>
</tr>
<tr>
<td>MCF-7 Human Breast Cancer Cells</td>
<td>MTT assay</td>
<td>1.49±0.26</td>
<td>Zhang et al., 2014</td>
</tr>
<tr>
<td>HeLa Human Cervical Cancer Cells</td>
<td>MTT assay</td>
<td>2.13±0.52</td>
<td>Zhang et al., 2014</td>
</tr>
<tr>
<td>SKOV-3 Human Ovarian Carcinoma Cells</td>
<td>MTT assay</td>
<td>4.44±0.89</td>
<td>Zhang et al., 2014</td>
</tr>
<tr>
<td>SMMC-7721 Human Hepatocarcinoma Cells</td>
<td>MTT assay</td>
<td>2.43±0.27</td>
<td>Zhang et al., 2014</td>
</tr>
<tr>
<td>CNE-1 Human Nasopharyngeal Carcinoma Cells</td>
<td>MTT assay</td>
<td>5.62±1.28</td>
<td>Zhang et al., 2014</td>
</tr>
<tr>
<td>HL-60 Human Promyeloctic Leukemia Cells</td>
<td>MTT assay</td>
<td>1.63±0.91</td>
<td>Zhang et al., 2014</td>
</tr>
<tr>
<td>K562 Human Leukemia Cells</td>
<td>MTT assay</td>
<td>1.91±0.37</td>
<td>Zhang et al., 2014</td>
</tr>
</tbody>
</table>
phosphorylation of mTOR and p70s6k compared with the control (Yang and Zhao, 2017). PLD showed significant antiproliferative effects on different cancer cell lines (Table 5).

0-6 µM PLD decreased the Bcl-2 and cyclin D1 level in a lung cancer cell, but increased the Bax level and induced S phase cell cycle arrest in a dose-dependent manner. Interestingly, PLD showed less cytotoxicity on HBE non-cancerous human nasopharyngeal cell line (Zhang et al., 2014). 0-100 PLD µM dose-dependently induced apoptosis and inhibited the proliferation of 143B and MG63 osteosarcoma cells and induced apoptosis by suppressing β-catenin signaling and the increasing expression of Bax/Bcl-2 and caspase-3 activity (Xu G et al., 2016). PLD showed antiproliferative effect and induced apoptosis in laryngeal cancer cell lines (Hep-2 and AMC-HN-8), and HeLa cervix cancer cell line via platelet-derived growth factor (PLDGF)/Akt pathway in time- and dose-dependent manner. It decreased PLDGF-B and p-Akt levels without altering total Akt level. PLD affected cell differentiation and showed

**Figure 4.** Signaling pathways involved in the induction of cell death or inhibition of cell proliferation by PLD
cytotoxic effect on CaCo-2 human colon adenocarcinoma cells by reducing the expression of 
Hsp27 and vimentin (IC$_{50}$ values are 72 and 192 μM for exponentially growing and post-
confluent cells, respectively). 240 μM PLD treatment caused increase of the cleaved form of 
poly-(ADP-ribose) polymerase and G1 phase arrest. It also reduced total and phosphorylated 
forms of Akt. It upregulated p21 level and ERK1/2 phosphorylation in CaCo-2 cell line (De 
Maria et al., 2013). PLD reduced the growth of MDA-MB-231 and MCF-7 breast cancer cell 
lines by leading to cell S phase arrest in both cell lines. Molecular mechanism studies showed 
that it down-regulated the phosphorylation of Creb proteins and reduced the cyclin D1 
expression dose dependently. It upregulated phosphorylation of Akt but the (Phosphoinositide 
3-kinase) PI3K inhibitor wortmannin exhibited an insignificant effect on the cell survival, cell 
cycle, and apoptosis of PLD-treated breast cancer cells. Similarly, 2.5 μM PLD increased 
phosphorylations of p38 and ERK. However, cell growth inhibition, cell cycle arrest, and 
apoptosis induced by PLD were not affected by ERK inhibitor PLD98059 or p38 inhibitor 
SB203580. These results indicated that neither PI3K pathway nor MAPK pathway were 
involved in cell proliferation inhibitor effect of PLD (Chen et al., 2017). 0-20 μM PLD 
impeded the proliferation of MOLT-4 leukemia cells in a dose dependent manner. It induced 
apoptosis and blocked the cell cycle in S phase by increasing the Bcl-2/Bax ratio, and 
downregulated the cyclin B1 and D1 levels. It showed synergistic effect with Janus kinase 2 
inhibitors, indicating JAK2-STAT3 pathway is involved in PLD induced cell cycle arrest and 
apoptosis in MOLT-4 cell line (Cao et al., 2016). 0-20 μM PLD induced apoptosis also in 
nasopharyngeal carcinoma cell line (CNE) through increasing the cytosolic cytochrome c, and 
decreasing the mitochondrial cytochrome c as compared with the drug-untreated group. It 
caused cleavage fragment of caspase-9 at 10 μM, but not cleavage fragment of caspase-8, 
suggesting that mitochondria-mediated intrinsic apoptosis pathway but not death receptor-
mediated extrinsic apoptosis pathway might be involved in PLD-induced apoptosis.

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Furthermore, PLD activated caspase-4, indicating of endoplasmic reticulum stress involved in PLD’s effect on apoptosis. PLD induced endoplasmic reticulum stress by increasing ATF4, XBP-1S, and CHOP proteins (Liu et al., 2011). 30 µM PLD enhanced bone marrow stromal cells migration by activating of ERK 1/2, which can be beneficial for stem cell therapy (Chen et al., 2016).

In a tumor xenograft experiment using nude Male–female athymic BALB/c mice, PLD treatment (50mg/kg) led to a 40% decrease in tumor weight compared with the 0.9% saline-treated control group (Li H et al., 2017).

**Anti-diabetic effect**

*Reduction of blood glucose level*

PLD partly restored glucose and lipid metabolism in high fat and sugar-induced diabetic rats. Orally administered PLD, taken for 8 weeks at 75 mg/kg reduced the levels of fasting blood–glucose (from 26 mM down to 23 mM), glycosylated hemoglobin glucose or HbA1c (from 9% to 6%), glycosylated serum protein (from 2.1 mM to 1.2 mM ), total cholesterol (from 3.6 mM to 1.4 mM), triglyceride (from 9.2 mM to 1.1 mM), and low-density lipoprotein cholesterol (from 1.6 mM to 0.2 mM) in diabetic rats. Furthermore, PLD markedly increased serum insulin levels in diabetic rats, and obviously activated the Akt signaling pathway in diabetic rat livers (Hao et al., 2014). PLD reduced altered lipid and glucose metabolisms in type 2 diabetes as well as 10 or 100 mg/kg PLD administered for 4 weeks (Wang et al., 2016).

*Improvement in insulin action*

Experiments done in vitro, with insulin-resistant HepG2 cells give an indication of the pathways that are involved in amelioration of type 2 diabetes. PLD regulated partly restored glucose and lipid metabolism in insulin resistant HepG2 cells. Furthermore, 20 µM PLD
significantly elevated glucose uptake and utilization, and diminished lipid accumulation in insulin resistant HepG2 cells. PLD markedly activated the Akt signaling pathway in insulin resistant HepG2 cells (Hao et al., 2014). At a concentration of 5 µM, PLD upregulated the protein levels of low density lipoprotein receptor and glucokinase by inhibiting proprotein convertase subtilisin/kexin type 9 in insulin-resistant type HepG2 cells (Wang et al., 2016).

**Amelioration of diabetes-induced secondary complications**

Various further pathways are involved in the anti-diabetic effects of PLD. PLD reduces renal fibrosis and thus, inhibits diabetic nephropathy dose dependently. It reversed the advanced glycation-end products (AGE)-induced downregulation of Sirtuin 1 (Sirt1) at 10-20 µM. Further, it increased the nuclear content, antioxidant response element-binding activity, and transcriptional activity of the nuclear factor erythroid 2–related factor 2 (Nrf2) as well as Heme oxygenase 1 (HO-1) and SOD1 expression, leading to a reduction of ROS production. Thus, PLD decreased the expression of fibronectin (FN) and TGF-β1, and reduction of renal fibrosis mechanisms was explained by activation of Sirt1-Nrf2/ARE anti-oxidative pathway (Huang et al., 2015). 10- 30 µM PLD supplementation reactivated production of Casein kinase 2 interacting protein-1 (CKIP1) in diabetic mice. CKIP1 is another activator of the Nrf2/ARE anti-oxidative stress signaling pathway, again leading to a reduction of renal fibrosis in diabetic nephropathy (Gong et al., 2017).

Oral 100 mg/kg PLD administration for 8 weeks stabilized the mitochondrial morphology and attenuated mitochondrial malfunction in KKAy mice (mice with type 2 diabetes) (Ni et al., 2017). PLD at 25 mM attenuated mitochondrial malfunction in hyperglycemia-induced podocyte MPC5 cells that were cultured in vitro. It also reversed podocyte hyperglycemia-induced apoptosis by downregulating dynamin-related protein 1 (Ni et al., 2017).
High glucose level damages endothelial cells (EC), and impairs vascular endothelial cells (VECs)-mediated vasodilatation in isolated rat aorta rings that were mounted in organ chambers. PLD at 1, 3, 10 μM improved the histological damage to endothelial cells in rat thoracic aorta tissue. At the same doses, it significantly ameliorated the impaired relaxation response to acetylcholine in high glucose group, in a dose dependent manner, the maximal drug relaxation effect of acetylcholine % (E_{max}) increased by 2.1, 2.6, and 3.5 folds, respectively. It restored the relaxation under acetylcholine in high glucose group to that of normal glucose group the control and, restored endothelium-dependent relaxation. This effect is probably mediated through activation of the peroxisome proliferator-activated receptors (PPAR)-β signaling pathway, and acts by upregulating the expression of endothelial NO synthase (eNOS), enhancing eNOS activity, and downregulating the iNOS level, in turn resulting in increased release in the blood vessels of nitric oxide (NO, also known as endothelium-derived relaxing Factor - EDRF). Thus, PLD effective to restored endothelial functions in high glucose conditions by PPARβ-NO signaling pathways (Wu Y et al., 2015).

**Amelioration of renal injury and renoprotective effect**

PLD reduced alterations in renal injury and also exhibited renoprotective effects in mice. PLD reduced hyperuremia by regulating ion transporter proteins. PLD given orally at 20 and 40 mg/kg significantly decreased serum uric acid, serum creatinine, and blood urea nitrogen (BUN) levels to their normal values, and increased clearance rate of urine creatinine and BUN to normal values in potassium oxonate-induced hyperuricemic mice. It showed antihyperuricemic and nephroprotective effects by regulating of expressions of some renal ion transporters, murine urate anion transporter (mURAT)1, mGLUT9, mAGCG2, murine
Figure 5. Mechanisms involved in amelioration of renal injury and renoprotective effect of PLD

organic anion transporter (mOAT)1, mOCT1, mOCTN1, mOCTN2, and mUMOD, in hyperuricemic mice (Shi et al., 2012). 5, 10, 20 mg/kg PLD treatment for 7 days also reduced serum uric acid level through promoting uric acid excretion and regulating mURAT1, mOAT1 and mOAT3 (Wu et al., 2014).

Fructose-induced urate nephropathy in mice is marked by increased xanthine oxidase (XOD) activity. XOD catalyzes the oxidation of hypoxanthine to xanthine further to uric acid, in the process the enzyme generates reactive oxygen species. Oral PLD treatment at 25 mg/kg for 4 weeks decreased the serum urate level and reduced nephropathy effect by antioxidant and anti-inflammatory mechanisms in mice. It inhibited xanthine oxidase in vitro, with an IC$_{50}$ value of 65.2 µM, which is 8 times less than the known XOD inhibitor allopurinol. However, this inhibition was competitive with Ki value of 47.2 µM. Moreover, treatment of fructose-induced nephropatic mice with 25 mg/kg PLD for 4 weeks enhanced the activity of SOD (going from 32.48 U/mg to 42.37 U/mg), GSH level (from 26 µg/g protein to, 31.46 µg/g
protein), and reversed MDA level (from 26 nmol/mg protein to 20 nmol/mg protein). Oral administration of PLD at 25 - 50 mg/kg decreased expression of several markers of inflammation, i.e. NF-κB, p65, COX-2 and iNOS, and TNF-α, PGE2 and IL-1β. The anti-inflammatory effect of PLD was stronger than that of the XOD inhibitor allopurinol (Chen et al., 2013). PLD also showed antioxidative, anti-inflammatory, and nephroprotective effects in infusion/reperfusion (I/R) injury model through the PI3K/Akt pathway. It improved the renal function, accelerated the mitogenic response and reduced cell apoptosis in renal I/R models. Especially at 40 mg/kg intraperitoneal treatment, PLD significantly inhibited apoptosis in kidneys of mice after I/R, compared with vehicle control at 1 d, 3 d, 5 d, and 7 d after I/R in vivo and completely inhibited in HK-2 cells in vitro. 40mg/kg PLD strongly suppressed the renal I/R injury induced upregulation of the expression of THF-α, IL-1β, COX-2, iNOS, PGE-2, and NO levels after 3 days of renal I/R. PLD at 20 - 40 mg/kg also increased deteriorated activities of SOD, GST, GSH-Px and CAT, and the level of GSH. PLD dose-dependently upregulated the phosphorylation of Akt in I/R-injured mouse kidneys. It did not exhibit these effects when the PI3K/Akt pathway was inhibited (Liu et al., 2015). In continuation of this work, it was shown that 20 and 40 µM PLD exerted nephroprotective effects effecting cell apoptosis and oxidative stress against renal ischemia/reperfusion injury induced the secretion of sonic hedgehog (Shh), upregulated Ptch 1 and Smo - two key elements in Shh signaling, and enhanced the nuclear translocation and target gene transcription of Glioblastoma 1 (Gli-1) (Meng et al., 2016). PLD’s protective effect in the renal I/R injury model also involves downregulation of I/R-induced expression of TLR4, and subsequent decreased expression of proinflammatory proteins NF-κB, TNF-α and IL-1β (Li Y et al., 2014). PLD suppressed high glucose-induced fibronectin production at 20 and 40 µM, it also inhibited NF-κB nuclear translocation, reduced the DNA-binding activity of NF-κB, and
decreased the protein expression of ICAM-1 and TGF-β in rat glomerular mesangial cells at 150 mg/kg (Xie et al., 2012).

**Effects on the reproductive system**

PLD exhibited protective effect against testicular torsion and detorsion injuries, at the histological, antioxidant stress and antiapoptotic levels. PLD treatment (intraperitoneal, 20 mg/kg) ameliorated the morphological damage, lowered the Cosentino histological score and increased the mean number of germ cell layers and Johnsen’s testicular biopsy score in rats with testicular torsion and detorsion injury compared to control group. It reduced MDA level by 34.2% and enhanced CAT, GSH-Px and SOD activity by 53.0%, 66.0% and 26.7% respectively. It also decreased testicular torsion/detorsion induced germ cell-specific apoptosis 51.5 % by downregulating the activation of caspase-3, 8, and 9 and, poly(ADP-ribose) polymerase and upregulating the Bcl-2/Bax ratio (Qiao et al., 2017). PLD administration (oral, 200 mg/kg) almost completely prevented the arsenic-associated deterioration sperm morphology and reversed the arsenic-induced alteration of sperm motility (85.71 control, 58.57 arsenic, 71.42 50 mg/kg, 74.28 100 mg/kg PLD, 80.00 200 mg/kg PLD) and plasma membrane integrity. It reduced lipid peroxidation (MDA levels: 0.88 nmol/g control, 8.30 nmol/g arsenic, 4.52 nmol/g 50 mg/kg PLD, 2.09 nmol/g 100 mg/kg PLD, 1.25 nmol/g 200 mg/kg PLD) and increased antioxidant enzyme activities (SOD activities: 5.69 U/µg protein control, 2.43 U/µg protein arsenic, 3.45 U/µg protein 50 mg/kg PLD, 4.07 U/µg protein 100 mg/kg PLD, 4.38 U/µg protein 200 mg/kg PLD; CAT activities 44.65 k/ µg protein control, 15.52 k/µg protein arsenic, 15.16 k/µg protein 50 mg/kg PLD, 26.14 k/µg protein 100 mg/kg PLD, 31.92 k/µg protein 200 mg/kg) and regenerated tissue damage in testis of rats (Ince et al., 2016).
10 mg/kg/day palmitoylethanolamine: PLD (10:1) mixture was orally administered to rats with testosterone-induced benign hyperplasia for 14 days. This treatment decreased prostate weight and dihydrotestosterone production in testosterone-induced rats. It also reduced PGE2 and dihydrotestosterone levels, and downregulated expression of 5α reductase 1 and 5α reductase 2. This treatment also inhibited NF-κB translocation, and IκBα degradation reduced NOS and COX-2 levels, improved Nrf-2, HO-1 and Mn-SOD expressions, and decreased MDA level compared to control (Cordaro et al., 2017).

Hepatoprotective effect

![Diagram showing the mechanisms of PLD](image)

**Figure 6.** Mechanisms involved in amelioration of hepatoprotective effect of PLD

PLD exhibited strong hepatoprotective effects which are closely linked with anti-inflammatory and anti-oxidant actions. Oral 50 or 100 mg/kg PLD pre-treatment for 8 days significantly alleviated the alcohol-induced (10 ml 50% ethanol/kg) hepatic injury in mice, by reducing the serum liver injury markers, ALT and AST and decreasing relative liver weight and morphological deteriorations. It also ameliorated deteriorations in the hepatic antioxidant
enzyme levels as in the levels of reduced GSH, GST, glutathione reductase (GR), CAT, SOD and hepatic thiobarbituric acid reactive substances (TBARS), nitrite and carbonyl content. When 50 mg/kg PLD was compared with 25 mg/kg silymarin, PLD restored changes in GSH, GSH levels and SOD, NQO1 activities at the similar level with silymarin. PLD, more than silymarin, led to increased CAT activity, and decreased carbonyl and nitrites. However, silymarin reduced TBARS level and enhanced activity of GR more than PLD. It also reduced alcohol-induced increase expression of HO-1; prevented the rise in matrix metalloproteinase 2 and 9 activities and attenuated the increase in the NF-κB (p65) (Koneru et al., 2017).

After artificially induced hepatic failure in mice (e.g. by lipopolysaccharide-galactosamine, or carbon tetrachloride), 10-100 mg/kg PLD treatment diminished histopathological injury in liver, reduced ALT, AST levels, and lowered mortality (20 % 10 mg/kg PLD) in a dose-dependent manner. Moreover, it suppressed LPS-induced TNF-α production, myeloperoxidase activity, ICAM-1 and endothelial cell adhesion molecule-1 (ECAM-1) expression, caspase-3 activation, and transcription of NF-κB activity (Wu et al., 2012). Pretreatment with 25, 50 and 100 mg/kg oral PLD for 5 days made that the levels of ALT, AST, MDA, activities of the antioxidant enzymes SOD and CAT activities, and expressions of TNF-α, IL-1β, COX-2, iNOS and NF-κB returned back to normal levels, whereas it upregulated mRNA and protein expression levels of hepatic TGF-β1 (Zhang H et al., 2012). Compared to the untreated mice with induced hepatic failure, levels of 25 - 100 mg/kg oral PLD dose dependently reduced ALT AST levels by up to 3-fold. Treatment decreased MDA level up to 1.4 fold, and increased activities of SOD, CAT, and GPx 1.5- fold, and GST levels up to 1.8 fold, respectively. The same treatment protocol reduced mRNA expression of TNF-α: 1.4, 2.2 and 2.3 fold, IL-1β: 1.2, 2.4 and 2.3 fold, COX-2: 1.4, 1.7 and 2.6, iNOS: 1.1, 1.4 and 1.6 fold, respectively, compared to CCl₄. Furthermore, 5 mg/kg intraperitoneal PLD suppressed level of a macrophage activation marker, CD68, and hepatic mRNA levels of
some inflammatory genes, TNF-α and monocyte chemoattractant protein-1, were also markedly increased in CCl₄-induced mice. PLD suppressed hepatic 4-hydroxynonenal (4-HNE) production, a sign of lipid oxidation, and nicotinamide adenine dinucleotide phosphate oxidase-4 expression, an inducer of profibrotic genes. In addition, PLD suppressed liver fibrosis by inhibiting collagen deposition (Zhao X et al., 2017).

*Ameliorative effect on hepatic steatosis*

PLD treatment (orally, 30 and 90 mg/kg) decreased hepatic steatosis and reduced plasma and liver triglyceride, total cholesterol and free fatty acid levels in high-fat diet fed rats. Moreover, it decreased TNF-α and MDA levels in the liver. PLD also downregulated the expression of some genes involved in lipogenesis, sterol-regulatory element binding protein1c, fatty acid synthase and stearoyl-CoA desaturase 1. Thus, PLD’s effect in hepatic steatosis might partly associated with reduced TNF-α expression, lipid peroxidation level and SREBP-1c-mediated lipogenesis (Zhang J et al., 2012). Furthermore, PLD reduced the fat tissue and accumulation of lipids in the liver (liver index: 3.12 control, 2.97 control+PLD, 5.04 high-fat, 3.68 high-fat+PLD groups; epididymal fat 1.43 control, 1.39 PLD, 2.25 high-fat, 1.78 high-fat+PLD groups) induced in high-fat diet fed rats. It also reduced insulin resistance, decreased high-fat supplementation induced insulin and leptin levels. PLD treatment was also rescued high-fat diet decreased insulin receptor substrate 2 and phosphorylated Akt protein expression in the liver (Zhang Q et al., 2015).

**Effects on Neurodegenerative diseases**

PLD exerted neuroprotective effects and improved learning and memory deficits in different experimental models.
Effects on memory function

Oral administration of 12.5, 25, 50 mg/kg PLD for 30 days attenuated cognitive deficits compared to the control in a rat model of vascular dementia induced by chronic cerebral hypoperfusion. 50 mg/kg PLD exhibited this effects in the similar manner with 25 mg/kg Ginkgo biloba extract. It also ameliorated deterioration of MDA level and SOD and CAT activities (Li et al., 2012). PLD treatment (intraperitoneally, 10 mg/kg) for 10 days enhanced long-term learning and memory in rats with perinatal hypoxic-ischemic brain injury through upregulating the expression of hippocampal brain-derived neurotrophic factor (Sun et al., 2014). PLD (intragastrically, 12.5 and 50 µg/ml) reduced ethanol-induced cell death rate and CDK5 expression in primary hippocampal neuron culture. It also reversed the performance impairments in chronic ethanol treated rats in Morris water maze test, especially 50 mg/kg PLD completely turned escape latency to normal level and rescued chronic ethanol administration-induced CDK5 expression (Zhang Y et al., 2015). 25 and 50 mg/kg PLD treatment improved memory acquisition and retention in permanent bilateral common carotid artery occlusion induced vascular disease and focal multiple infarction using homologous blood emboli induced vascular dementia models in the water maze tests (Xiao K et al., 2015).

Prevention and protection against cerebral ischemic injury

PLD showed neuroprotective effect by inhibiting both oxidative stress and mitochondria-dependent apoptosis in a middle cerebral artery occlusion (MCAO) experimental model. PLD injection (30 mg/kg) to caudal vein 10 min prior to I/R injury reduced modified neurological severity scores and infarct volume after 24 h following I/R injury, in the PLD treated group. PLD treatment, significantly increased intracellular ATP levels, and decreased mitochondrial apoptosis through upregulation of Bcl-2 and downregulation of Bax, compared to MCAO group. PLD also lowered MCAO induced
activation of caspase-3 and caspase-9, and reduced MCAO induced-ROS production (Gao Y et al., 2016). PLD protected the brain from damage caused by permanent MCAO. 50 mg/kg intraperitoneal PLD reduced body asymmetry in a Longa’s neurological abnormality scores (4.1 at 24 hours, 4.2 at 72 h in vehicle and 2.6 at 24 hours, 2.1 at 72 hours in PLD after MCAO) and promoted the functional recovery of rats compared to control group. It also reduced MCAO-induced brain edema (84.4 % in vehicle, 80.0 % in PLD after 24 hours; 85.1 % in vehicle, 78.1 % in PLD after 72 hours) and volume of infarcts (42.5 % in vehicle, 37.3 % in PLD after 24 hours; 42.9 % in vehicle, 36.3 % in PLD after 72 hours). It up-regulated the expression of Gli-1, Ptc1, and SOD1, and downregulated expression of NF-κB. PLD promoted the expression of claudin-5, a protein involved in regulation of blood-brain barrier permeability (Ji et al., 2012).

Effects on the aetiology of Parkinson’s disease

20-100 mg/kg orally administered PLD dose-dependently attenuated rotenone-induced Parkinson’s disease type defects like catalepsy and motor coordination disorder in male Sprague Dawley rats. Particularly, 80 mg/kg PLD group performed significantly better than the positive control group treated with 10 mg/kg L-dopa. PLD rescued rotenone-induced changes in the levels of GSH, ATP, MDA and manganese SOD in the striatum. PLD also alleviated the effects of chemically induced Parkinson’s disease in mice and rats. It reversed dopaminergic neurodegeneration in the substantia nigra pars compacta region. Furthermore, oral PLD treatment prevented or alleviated motor deficits, increased SOD activity, and reduced MDA levels (Chen et al., 2015).
**Effects on the aetiology of Alzheimer’s disease**

Alzheimer disease is characterized by the accumulation and polymerization of amyloid-β (Aβ) peptides. PLD inhibited Aβ25-35 fibril formation in vitro, with an EC\textsubscript{50} value of 4.7 µM probably via dynamic interaction between the PLD and the Aβ that could open the hydrophobic zipper and shift the reversible equilibrium “random coil ↔β-sheet” to the disordered structure (Rivière et al., 2007; 2009). PLD dose-dependently inhibited Aβ polymerization, 0.1 mg/ml PLD also reversed decreased expression of α3 and α7 nicotinic acetylcholine receptors, another prominent factor in the etiology of Alzheimer’s disease, in Aβ\textsubscript{1-42}-induced SH-SY5Y cells in vitro (Xiao H et al., 2015).

**Other neuroprotective effects**

PLD addition (30 and 120 µM) to cultured cerebral cortical neurons reduced oxygen-glucose deprivation induced cell body damage, swelling, and cellular debris in a dose dependent manner (Zhang Y et al., 2015). Neuroglobin has neuroprotective role in stroke and neurodegenerative diseases. PLD upregulated both mouse and human neuroglobin promoter activity and increased neuroglobin mRNA expression in primary neurons (Liu et al., 2016). Monoamine oxidases (MAO) are neurotransmitters, while MAO-A inhibitors are used as antidepressants, MAO-B inhibitors, are relevant tools in the therapy of Alzheimer’s and Parkinson’s diseases. MAO inhibitors are also important with regard to their neuroprotective effects. PLD inhibited MAO mixed type with IC\textsubscript{50} value of 85 µM, MAO-A 87 µM, and MAO-B 96 µM (Wei et al., 2016).

**Effects on Pulmonary System**

PLD exerted beneficial actions on pulmonary system underlying mechanisms generally associated with its antioxidant and anti-inflammatory effect.
PLD (intraperitoneal injection, 15, 45, and 100mg/kg) ameliorated sepsis-induced lung injury in a dose dependent manner by decreasing the TNF-α and IL-6 production, lung COX-2, and reducing the expression of iNOS and NF-κB activity in septic mice. Additionally, PLD increased the levels and activity of the new target of septic lung injury, HO-1 and abolished the effects of ZnPP IX (40 mg/kg), a specific HO-1 inhibitor (Li et al., 2013).

Due to its anti-inflammatory and antioxidant capacity, PLD showed protective effect in thoracic radiotherapy caused injuries. In an in vivo double controlled study on female C57BL/6 mice, treatment with PLD (100mg/kg/day by intraperitoneal injection, from 3 days prior to irradiation to 4 weeks after irradiation), reduced hyperemia and edema in lung tissues, alleviated structural damages and collagen deposit after irradiation. It also inhibited radiation-induced epithelial-mesenchymal transition by reversing upregulation of the interstitial markers vimentin and α-smooth muscle actin (α-SMA), and increased downregulated E-cadherin levels. It also alleviated increased acute inflammation and late fibrosis through inhibiting TGF-β1-Smad3 signaling pathway. Further, PLD regulated the balance of Th1- and Th2-type cytokines in acute lung injury. In cell culture studies, treatment with PLD (200-600 µM concentration range) increased the expression of Sirt3 and promoted Nrf2 and PGC1α levels on human bronchial epithelial cell line (BEAS-2B) and these findings suggested that Sirt3 may be a new potential target for PLD (Cao et al., 2017).

PLD, prevented the lung function impairment caused by airborne particles in polluted air through decreasing the oxidative potential in vitro and reducing the level of oxidative damage in vivo. Treating PM2.5-exposed rats (an inhalation injury animal model) with PLD (50 mg/kg, oral) for 8 weeks resulted in a notable increase in tidal volume, expiratory volume and minute ventilation volume comparing with the control group. Furthermore, PLD showed anti-inflammatory effect by reducing alveolar wall thickening and downregulating lymphocyte, monocyte, neutrophil, eosinophil and basophil and white blood cells in bronchoalveolar
lavage fluid, and inflammation-related lipids and pro-inflammatory cytokines, such as TNF-α and IL-1β in lung tissue (Yan et al., 2017).

PLD demonstrated protective effect on acute lung injury by acting on the anti-inflammatory TLR4-MyD88-NF-κB pathway in vivo and in vitro. PLD (80 mg/kg, intraperitoneal) treatment on LPS-induced acute lung injury models resulted in decreased levels of lung edema, polymorphonuclear, neutrophil numbers, TNF-α, IL-6, IL-1β, and a reversed activation of the TLR4-MyD88-NF-κB signaling pathway. Besides, PLD treatment at 2, 4 and 8 µM concentrations reduced the inflammatory cytokines levels (IL-1β, IL-6, IL-8 and TNF-α) and downregulated the expressions of TLR4, MyD88, NF-κB in LPS stimulated human bronchial epithelial BEAS-2B cells (Jiang et al., 2015).

PLD relieved the effects of burn-induced lung injuries and LPS-induced acute lung injuries due to its anti-inflammatory and anti-apoptotic effects. PLD treatment (intravenously, 45 mg/kg) on burn-injured rats resulted in a decrease of lung polymorphonuclear activity and polymorphonuclear leukocytes in bronchoalveolar lavage fluid, and levels of proinflammatory factors as TNF-α, IL-1 and IL-6 and pulmonary microvascular hyperpermeability. Also PLD decreased the number of apoptotic cells, by inhibition of caspase-3 activity and Bax/Bcl-2 ratio (Li et al., 2014a; Li et al., 2014b)

50 nM PLD ameliorated mitochondrial biogenesis and function of human pulmonary arteriolar smooth muscle cells in vitro by increasing the levels of Sirt1, mitochondrial DNA content, mitochondrial transcription factor A and cellular ATP. This may present another mechanism through which PLD can attenuate the effects of ischemic pulmonary injury (Li P et al., 2017).
**Effect on Cardiovascular system**

PLD exhibited various beneficial effects including post-myocardial infarction protective, vascular smooth muscle dilation, inhibition of platelet aggregation and thrombosis, blood pressure lowering and atherosclerosis prevention cardiomyocyte protective effects.

*Post-myocardial infarction protective effect*

PLD reduced hypertrophy of cardiomyocytes and heart failure in different test models. Reduction of mitochondrial biogenesis and function, and deterioration through autophagy and apoptosis in cardiomyocytes are related with heart failure. PLD (7.5 mg/kg by intraperitoneal injection for 10 days) showed protective effect in post-myocardial infarction (MI) cardiomyocytes via Sirt3 activation. PLD relieved mitochondrial dysfunction by amplifying ATP level, citrate synthase activity and complexes I/II/III/IV/V activities. Also 10 µM PLD suppressed apoptosis and upregulated autophagy by increasing GFP-LC3 puncta, and reducing the protein aggresomes and p62 in cultured neonatal mouse ventricular myocytes subjected to hypoxia for 6 h to simulate MI injury (Zhang et al., 2017). PLD (intraperitoneally 7.5 mg/kg) showed protective effects on post-myocardial I/R injury. It increased autophagy by promoting the degradation of autolysosome and decreased apoptosis in post-MI C57BL/6 mice experiment model and cultured neonatal rat cardiomyocytes. PLD treatment minimized the MI in size and increased the left ventricular fractional shortening and ejection fraction. Administration of 10 µM PLD to neonatal rat cardiomyocytes reduced mitochondrial membrane potential and cellular ROS, which were contributed the cardioprotective activity (Ling et al., 2016). PLD (30 - 50 µM) increased the cell viability and reduced apoptosis in cardiomyocytes subjected to simulated IR. It also reduced the infarct size and increased the cardiac function in IR mice related to the renin–angiotensin system and RhoA kinase (ROCK) pathway. PLD significantly eliminated the simulated IR –induced angiotensin I–II enrichment
and inhibited the activity of angiotensin-converting enzyme. Moreover, it inhibited ROCK activity, especially the angiotensin I receptor-activated ROCK pathway (Ming et al., 2017).

**Effect on the aetiology of atherosclerosis**

PLD (23 µM) inhibited the formation of foam cells derived from peritoneal macrophages. Macrophage cholesterol accumulation-induced foam cell formation is one of the hallmarks of atherosclerosis, PLD increased cholesterol efflux from macrophage and decreased the uptake of oxidized LDL, resulting in the inhibition of cholesterol accumulation in macrophage. Its mechanism may be regulated by PPAR-γ pathway (Wu M et al., 2015). PLD (25 - 100 mg/kg/day, oral) reduced low-density lipoprotein cholesterol/ high-density lipoprotein cholesterol and total cholesterol / high-density lipoprotein cholesterol ratios compared with the values obtained from high-fat/cholesterol hamsters. Further, the treatment decreased levels of serum total cholesterol, triglyceride, and low-density lipoprotein cholesterol, and treatment with 50 and 100 mg/kg doses of PLD also reduced hepatic triglyceride concentrations in high-fat/cholesterol hamsters. (Du et al., 2009).

**Antihypertensive effect**

PLD (100 mg/kg/day, 200 mg/kg/day intragastric) decreased ventricular collagen content, hypertrophy of cardiomyocytes, and heart mass index in ventricular remodeling induced by isoproterenol in mice statistically significant compared to positive control, metoprolol (60 mg/kg/day intragastric). Also treatment with PLD (60 mg/kg/day, 120 mg/kg/day intragastric) significantly reduced angiotensin II, endothelin-1 in left ventricular tissue, and aldosterone and TNF-α concentrations in serum comparing with captopril (40 mg/kg/day intragastric compared to positive control) leading to reduced blood pressure in rats (Gao et al., 2010). PLD also caused vascular dilation and microcirculation promotion by reducing intracellular
Ca concentration, activating vascular K\(^+\) ATP channel and enhancing vascular activity through promoting extra-cellular sodium ion influx to cause cell depolarization (Liu et al., 2012).

*Other cardioprotective effects*

PLD (20–75 µM) attenuated phenylephrine-induced hypertrophy in cultured neonatal rat ventricular myocytes, and in a pressure overload-induced hypertrophic mouse model. PLD treatment inhibited the development of cardiac hypertrophy in cultured neonatal rat ventricular myocytes by increasing the cell surface area and decrease atrial natriuretic peptide and β- major histocompatibility complex levels. Also PLD acted as a barrier against Ca\(^{2+}\) - calcineurin- nuclear factor of activated T-cells pathway while keeping the cardiac contractility intact (Ding et al., 2014). PLD application (30 - 50 µM) reduced phenylephrine-induced oxidative stress and withheld Rho Kinase (ROCK) activation in cardiomyocytes. PLD also showed a similar effect in transverse aortic constriction applied mice as it suppressed the increased ROCK activity and decreased hypertrophic response and improved cardiac function (Dong et al., 2015).

PLD (intravenously, 10 mg/kg) improved cardiac dysfunction by correcting sarcoplasmatic reticulum Ca\(^{2+}\) leak that, after burn-injury, was mediated by hyperactive cardiac ryanodine receptors (RyR2) in an adult Sprague–Dawley rat experimental model. PLD reinstated Ca\(^{2+}\) load and Ca\(^{2+}\) levels in the sarcoplasmic reticulum. PLD treatment increased free thiol levels in RyR2 and reduced ROS (Jiang et al., 2013). Doxorubicin causes cardiotoxicity in patients who undergo chemotherapy. In order to evaluate the cardioprotective effect of PLD as well as its combination with vitamin C, male Sprague-Dawley rats divided into 4 groups and treated with doxorubicin, doxorubicin+PLD, doxorubicin+vitamin C and doxorubicin+PLD+Vitamin C for six weeks. PLD exhibited cardioprotective effect, however, combination therapy with
PLD (gavage, 100 µg/kg) and vitamin C (100 µg/kg) exhibited the best recovering effects on arterial pressure, heart rate, interval of QRS, GSH-Px activity. Both treatment ameliorated antioxidant parameters by enhancing activities of GSH-Px and SOD. PLD alone or combination with vitamin C also increased the mRNA expression levels of AMPK-α2 and PPAR-α without statistical difference in between each other in rats. Consistent with these results, PLD and vitamin C might relieve the myocardial toxicity caused by doxorubicin treatment (Wang et al., 2017). PLD has potential cardioprotective effects against Ca\(^{2+}\) mishandling related heart diseases. Treatment with 1,10 and 100 µM PLD caused down-regulation of L-type Ca\(^{2+}\) channel activity and upregulation of ryanodine receptor activity on rat ventricular myocytes and thus moderate decrease in Ca\(^{2+}\) transient. Moreover, it increased myofilament Ca\(^{2+}\) sensitivity in turn, increased myocyte contractility. It also modulated β-adrenergic receptor regulation of excitation–contraction coupling by alleviating β-adrenergic receptor stimulation-induced enhancement of Ca\(^{2+}\) signaling without inhibiting β-adrenergic receptor mediated inotropic effect (Deng et al., 2012).

50 µM PLD inhibited the TNF-α stimulated endothelial cell–monocyte adhesion. 50 µM PLD could reduce the mRNA transcription and the protein levels of Intercellular Adhesion Molecule 1 (ICAM-1) and VCAM-1 in human umbilical vein endothelial cell by inhibiting of NF-kB pathway (Deng et al., 2011).

**Anti-shock**

PLD exhibits anti-shock effect, and the use of PLD in shock treatment approved by Chinese FDA (Chen et al., 2015). Administration of PLD (30 mg/kg) and norepinephrine (10 µg/kg) to 120 min shock subjected rats ameliorated hemorrhagic shock induced mitochondrial swelling, decreased mitochondrial membrane potential, and reduced intracellular ATP levels. It also maintained lysosomal stability, reduced activation of K\(_{ATP}\) channels, arteriolar smooth muscle
cells hyperpolarization, and reduced vasoresponsiveness to norepinephrine (Wang et al., 2012).

120 minutes after hemorrhagic shock, PLD (intravenous, 30 mg/kg) increased SIRT1 protein expression and activity in the intestine homogenate of normal control rats and restored SIRT1 and peroxisome proliferator-activated receptor-gamma coactivator-1α (PGC-1α) activity in small intestine during severe shock. The increased SIRT1 activity lead to increased SOD2 activity; in turn, attenuated oxidative stress levels and reduced apoptosis. PLD possess beneficial effects through SIRT1-PGC-1α-SOD2 axis in hemorrhagic shock (Zeng et al., 2015b). It also alleviated mitochondrial dysfunction occurred after hemorrhagic shock in kidney cells via the SIRT1-p53 pathway (Zeng et al., 2016). PLD showed beneficial effects on the heart function and microcirculatory perfusion in shock by causing various effects on vascular smooth muscle cells (VSMC), myocardial cells (MC), endothelial cells (EC), and white blood cell (WBC). PLD suppressed the ICAM-1 expression in LPS-induced EC, attenuated the adherence of WBC-EC and increased the Ca^{2+} levels in MC. Also PLD activated K^+ ATP and decreased the pH value and Ca^{2+} levels of VSMC in shock (Zhao et al., 2003).

**Anti-microbial activity**

PLD has shown weak to moderate anti-microbial activity against a range of micro-organisms. It inhibited growth of *Helicobacter pylori*, with an MIC of 12.5 µM which is in the same order of magnitude as resveratrol (6.25 µM) (Khalil et al., 2016). In addition, PLD exhibited antibacterial activity against *Enterococcus faecalis, Staphylococcus aureus, S. saprophyticus, S. epidermidis, Escherichia coli, Klebsiella pneumoniae, and Salmonella typhimurium* with MIC values of 100, 400, 200, 200, 50, 25, 400 µg/ml, respectively. It possessed antifungal
activity against *Candida albicans* and *Trichophyton rubrum* with MIC values of 50 and 90 µg/ml, respectively. However, it only possessed a good antimicrobial activity against Gram negative bacteria, not to Gram positive bacteria or fungi, compared to positive control gentamycin for bacteria and clotrimazole for fungi (Mbossos et al., 2015). PLD further exhibited antibacterial activity against *Bacillus subtilis, B. epidermidis, Proteus vulgaris, Pseudomonas aeruginosa, Shigella dysenteriae* with MIC values of 20, 16, 24, 24, and 23 µg/ml. The MIC values found for PLD were about twice as high as those of positive controls, streptomycin, gentamycin or ciprofloxacin (Dar et al., 2016).

PLD exhibited an anti-*Leishmania amazonensis* activity with an IC$_{50}$ values of 95 µM for promastigotes 29 µM for intracellular amastigotes. However, positive control amphotericin B IC$_{50}$ values were found to be 0.1 µM for promastigotes 8.8 nM for intracellular amastigotes in the same study, indicating that PLD was not effective against to this organism (Passos et al., 2015).

![Structure of PLD-glucoside isolated from Parthenocissus tricuspidata](image)

**Figure 7.** Structure of PLD-glucoside isolated from *Parthenocissus tricuspidata*
A PLD-related stilbenoid glycoside, PLD-(1→6)-\(\beta\)-D-glucopyranoside, isolated from Parthenocissus tricuspidata showed activity in vitro (IC\(_{50}\) 5.3 \(\mu\)M) and in vivo (orally, 5 mg/kg day) against Plasmodium berghei (Figure 7), (Son et al., 2007; Park et al., 2008).

**Anti-osteoporotic activity**

PLD administration (intraperitoneal, 10, 20 and 40 mg/kg/day) decreased body weight, and increased uterine index and dry weights of thigh-bones of ovariectomized mice compared to control. It increased the serum calcium, phosphorus, alkaline phosphatase and osteoprotegerin and of ovariectomized mice. It increased the serum calcium, phosphorus, alkaline phosphatase and osteoprotegerin and of ovariectomized mice. It upregulated the ratio of osteoprotegerin/receptor activators of nuclear factor-\(\kappa\)B ligand and \(\beta\)-catenin protein in ST2 (a bone marrow-stroma) cells. PLD possessed anti-osteoporotic activity via regulating osteoprotegerin, RANKL and \(\beta\)-catenin (Zhou et al., 2016).

**Antithrombotic Effect**

PLD concentration-dependently suppressed arachidonic acid-induced platelet aggregation in vitro and in vivo, and thus exhibited antithrombotic effect. The underlying activity mechanisms may be related to inhibition of platelet aggregation, which is related to a decrease of platelet cytosolic calcium and plasma thromboxane B2, and a concomitant increase in plasma 6-keto-PGF\(_{1\alpha}\) level and suppression of platelet-neutrophil interactions (Chen et al., 2011).

**Lifespan extension**

1 mM PLD extended the mean lifespan of Caenorhabditis elegans by up to 30.7% and 62.1% under normal and acute stress conditions, respectively. The lifespan-extension role of PLD is associated to its anti-oxidative activity by regulating the aging-associated genes daf-16 and
stress-resistance protein SOD-3 involved in insulin/insulin like growth factor 1 signaling pathway (Wen et al., 2014).

**Metabolism and pharmacokinetics of polydatin**

The metabolism and pharmacokinetics of PLD follow a pattern common to most polyphenolic compounds. The absorption and metabolism in humans of the most abundant dietary polyphenols are well understood (Williamson & Clifford, 2017). Glycosides of polyphenolic compounds are generally believed to be hydrolyzed in the digestive tract, after which the aglycones are absorbed by the intestine epithelium and subsequently transported into the blood stream, where they are conjugated with glucuronic acid or sulphates as part of the phase II detoxification process.

Lactase (also known as lactase-phlorizin hydrolase, or LPH) is an enzyme that is active in the brush border of the small intestine epithelium. The enzyme is best known as the catalyst in the hydrolysis of the disaccharide lactose to form glucose and galactose, but it does accept a variety β-glucosides as substrates. LPH from rat small intestine cell-free extracts catalyzed hydrolysis of PLD, resulting in the formation of resveratrol and glucose (Henry-Vitrac et al., 2006). Caco-2 cultures growing on a polycarbonate membrane filter were used as an *in vitro* model for enterocytes lining the small intestine in a study on uptake, transport and metabolism of PLD (Henry et al., 2005; Henry-Vitrac et al., 2006). The picture that arises from the *in vitro* model studies is that PLD is partially hydrolyzed by LPH, after which resveratrol can passively diffuse into the lumen of the epithelial cells. Since intact PLD was also found inside the epithelial cells, a second, active transport mechanism is proposed involving sodium-dependent glucose transporter 1 (SGLT1), as had been reported previously for several other polyphenol glucosides (Walle and Walle, 2003; Hollman, 2004).
Transport of PLD or other glycosides of polyphenolic compounds across the membrane by SGLT1 is a matter of some controversy; the main argument in favor is that transport is reduced in the presence of either glucose or the SGLT1 inhibitor phlorizin. However, an alternative explanation for this observation is that glucose and phlorizin inhibit LPH and thus prevent hydrolysis of PLD or other glycosides, which results in a reduced amount of aglycones which constitute the main transport form (Kottra and Daniel, 2007). In this context, it may be worth noting that glucosides of polyphenolic compounds (including PLD) are efficiently hydrolyzed by LPH, more or less independent of the aglycone part of the glucoside. Other glycosides of polyphenolic compounds, e.g. galactosides, arabinosides, xylosides, rhamnosides, and galactosides, are poor substrates for LPH, and show much lower bioavailability than glucosides (Hollman, 2004). SGLT1 may still play a role in the kinetics of polyphenol uptake; recent results showed that, in addition to passive diffusion, SGLT1 in vascular endothelial cells actively transport free resveratrol (Chen et al., 2013). In humans, orally administered resveratrol is effectively absorbed in the ileum, but then rapidly converted into sulfate and glucuronic acid conjugates. Thus, the bioavailability of free resveratrol in circulating plasma is very low, although high accumulation may occur in epithelial cells along the digestive tract (Walle et al., 2004). It may be argued that, apart from in the digestive tract, free resveratrol levels will rarely be high enough to have any meaningful pharmacological effect. However, this assumes that resveratrol conjugates are biologically inactive, which may not be the case; the biological activity of PLD in vitro seems to exceed that of free resveratrol. Alternatively, PLD or other resveratrol conjugates may serve as an inactive precursor pool but are hydrolyzed to give resveratrol once they reach the target tissues (Santner et al., 1984; Walle et al., 2004).
Any PLD that is not hydrolyzed and/or absorbed in the ileum will be transported toward the colon where it is metabolized by the bacterial microflora. The metabolic steps typically

**Figure 8.** Fermentation products of PLD
involve hydrolysis and dehydrogenation of the aglycone moiety. After the oral administration of *trans*-resveratrol to rats, dihydroresveratrol was the most abundant compound in urine and colon samples, followed by *trans*-resveratrol glucuronide- and sulfate-conjugates (Wang et al., 2005; Alfaras et al., 2010). Similar results were found in humans after oral consumption of red wine or grape extract tablets; dihydroresveratrol conjugates were the main metabolic products of resveratrol and PLD (Figure 8), (Rotches-Ribalta et al., 2012). An earlier attempt at profiling metabolic products of resveratrol failed to mention dihydroresveratrol conjugates (Burkon & Somoza, 2008), but this might be attributed to a lack of accurate analytical methods and absence of a commercially available pure standard at the time (Juan et al., 2010).

The high level of dihydroresveratrol conjugates as end metabolites after PLD consumption indicate that a substantial amount is not absorbed in the small intestine but passes unchanged into the large intestine. Several efforts have been made to increase the absorption of PLD.

PLD is poorly absorbed due to its large molecular size which prohibits passive diffusion, and is poorly lipid soluble. However, like other water soluble phytoconstituents, it can be bound to phospholipids, mostly phosphatidylcholine, resulting in a lipid compatible molecular complex, called a phytosome (Bhattacharya 2009). The molecular complex is held together by hydrogen bonds, and produces a little cell, whereby the plant extract is protected from destruction by gastric secretions owing to the gastroprotective property of phosphatidylcholine. A PLD phytosome was shown to double the oral bioavailability of polydatin in rats (Cheng et al., 2017).

**Clinical trials**

In a patient trial aimed at improving liver biochemical parameters, serum oxidative stress, and mental state, 20 chronic alcoholic patients that were hospitalized for rehabilitative therapy
were divided into two groups. A GSH and vitamin C (group 1), or a GSH, vitamin C and PLD (group 2) were treated for 2 weeks. GSH was administered twice a day (intravenously, 600 mg), 1 g vitamin C once per day (orally, 1 g), and 40 mg PLD twice a day (orally, 40 mg). Deteriorated ALT and AST levels were ameliorated compared to group 1, or to levels of these parameters at the beginning of treatment. Also, PLD treatment significantly reduced serum lipid peroxidation in alcoholic patients. Furthermore, Group 2 patient’s cognitive functions improved, compared to beginning of treatment, whereas the recovery of the cognitive functions in group 1 at the end of treatment was not significant (Pace et al., 2015).

A pilot, 12-week, randomized, double-blind, placebo-controlled, multicenter study conducted on 54 irritable bowel syndrome (IBS) patients and 12 healthy volunteers to assess effect of palmithoylethanolamide/PLD 200 mg/20 mg. IBS patients showed higher mucosal mast cell counts (3.2 ± 1.3 vs. 5.3 ± 2.7%, p = 0.013), reduced fatty acid amide oleylethanolamide (12.7 ± 9.8 vs. 45.8 ± 55.6 pmol/mg, p= 0.002) and increased expression of cannabinoid receptor 2 (0.7 ± 0.1 vs. 1.0 ± 0.8, p = 0.012) compared with controls. The treatment did not significantly modify IBS biological profile, but compared to placebo, palmithoylethanolamide/PLD significantly improved abdominal pain severity (p < 0.05) (Cremon et al., 2017).

A pilot, 6 months, randomized, open-labeled, three arm study was conducted to evaluate the efficacy of treatment with palmithoylethanolamide/PLD on chronic pelvic pain related to endometriosis on thirty patients at reproductive age. Group A was administered palmithoylethanolamide 400 mg + PLD 40 mg 2 tablets daily for 6 months; group B leuprolelin acetate 11.25 mg, one vial every 3 months during 6 months, group C ethinylestradiol 0.03 mg + drospirenon 3 mg 1 tablet for 6 months were administered. This study showed that palmithoylethanolamide/PLD treatment was as effective as hormonal
therapy for reducing pain related to endometriosis without effecting ovulation (Di Francesco & Pizzigallo, 2014).

A randomized, placebo-controlled study on 220 women aged 16 to 24 years with primary dysmenorrhea conducted to evaluate the effectiveness of the association between palmitoylethanolamine and PLD on pelvic pain in primary dysmenorrhea. Patients treated with 400 mg + 40 mg palmitoylethanolamine + PLD combination (1 tablet a day for 10 days from the 24th day of cycle) or placebo (1 tablet a day for 10 days from the 24th day of cycle). An improvement of pelvic pain was seen in 98% of cases in treatment group vs 56% in placebo group (p < 0.001), indicated effectiveness of palmitoylethanolamine + PLD combination (Tartaglia et al., 2015).

A randomized, double-blind, placebo-controlled trial on twenty women with vestibulodynia received oral 400 mg + 40 mg palmitoylethanolamine + PLD combination or placebo, twice daily for 60 days to assess whether to see that palmitoylethanolamide + PLD combination may contribute to a down-regulation of mast cell hyperactivity, which is believed to be responsible for the proliferation and sprouting of vestibular pain fibers and the associated hyperalgesia and allodynia. This treatment improved symptoms especially in cases with more recent disease onset, as compared with the placebo group (Murina et al., 2013).

Conclusion

PLD, represents a new promising and well-tolerated therapeutic agent for the management of different pathologies, especially degenerative diseases. However, some biological activities that were reported are only seen in vitro at very high doses of PLD. The levels are considered unrealistic in in vivo experiments, and therefore not applicable to human use. Many studies explored metabolism of PLD or resveratrol in the body, showing that PLD is likely to convert into resveratrol (Wang et al., 2015), so, it could be regarded as a prodrug of resveratrol. It
seems that the mechanisms that underlie most of PLD’s beneficial effects are related to cellular antioxidant and anti-inflammatory cascades. According to clinical trials, it seems a promising drug candidate especially for alleviation of chronic low-grade inflammation, and for pain management. Although PLD’s bioavailability is higher than that of resveratrol, it remains a limiting factor in clinical applications, and novel formulations need to be designed to overcome this hurdle; liposome carriers and phytosomes have shown a good promise in this respect.
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