International Journal of Enhanced Research in Medicines & Dental Care (IJERMDC),
ISSN: 2349-1590, Vol. 13 Issue 9, September 2026

# To Investigate Sesamol and its Chosen Derivative in Vitro for Hepatoprotection against Paracetamol-Induced Damage in Hepg2 Cell Lines

Km Kajal Sachan[1], Dr Sushila Kaure[2]

[1]Research Scholar, Dept of Pharmacy, Jayoti Vidyapeeth University
[2]Associate Professor, Dept of Pharmacy, Jayoti Vidyapeeth University

**ABSTRACT**

**Depending on the duration of injury and the histological location of damage, drug-induced liver injury (DILI) is categorized as acute or chronic, and either as hepatitis, cholestatic, or a mixed pattern of injury. The hepatitis pattern is characterized by hepatocyte necrosis and is associated with a poor prognosis. There are three types of acute cholestatic drug-induced injury: bland cholestasis is the result of abnormal biliary secretion, and is not accompanied by significant hepatocellular damage; cholestatic hepatitis (mixed type) refers to cholestasis with concomitant hepatic parenchymal damage; and the third form of acute cholestasis is defined by the presence of bile duct injury or cholangiolitis. Medications may cause chronic cholestasis through two additional mechanisms: through the obliteration of bile ducts, also known as the vanishing bile duct syndrome, or by extrahepatic biliary obstruction, known as secondary sclerosing cholangitis. Hepatotoxicity is a potential complication of nearly all classes of medication. Most cases of DILI are benign, and improve after drug withdrawal. It is important to recognize and remove the offending agent as quickly as possible to prevent the progression to chronic liver disease and/or fulminant hepatic failure. There are no definite risk factors for DILI, but pre-existing liver disease and genetic susceptibility may predispose certain individuals. Although most patients have clinical symptoms that are identical to other liver diseases, some patients may present with symptoms of systemic hypersensitivity. Treatment of drug-and herbal-induced liver injury consists of rapid drug discontinuation and supportive care targeted to alleviate unwanted symptoms.**

***Keyword: Medications, Hypersensitivity, Hepatitis, Cholestatic, Drug***

## INTRODUCTION

The majority of medications are broken down in the liver before being eliminated in urine or bile. Phase-I and phase-II processes, each of which has its own enzymes that cooperate for metabolism and excretion, are involved in drug metabolism. Phase-I enzymes are involved in the first stage of drug metabolism; they react with the medication to create an intermediate product. In the phase-II reaction, the intermediate product created in the phase-I reaction was rendered inactive and eliminated. Toxic metabolites build up as a result of any variation in the way enzymes function during phase II. These harmful metabolites may interact with various cellular organelles, such as mitochondria, to produce reactive oxygen species (ROS), which exacerbates OS and can cause immune-mediated liver injury as well as direct damage to enzymes, proteins, and DNA within cells and tissues [9,10]. OS is brought on by interference with the production and accumulation of ROS as well as the biological system's ability to neutralise the generated ROS. ROS can be produced by external stimuli such as xenobiotics or as a byproduct of oxygen metabolism within cells. It is essential to several physiological processes, such as cell differentiation, signalling, and cell death, when it is present in small amounts. Because it activates the inflammatory pathways, increased concentration causes a number of disorders to progress. Oxidative stress-related cell and tissue damage can result from an imbalance caused by external variables such as xenobiotics (e.g., anticancer medicines) and environmental factors (e.g., pollutants & radiation) that enhance ROS production (15–17). The body uses nuclear factor erythroid 2–related factor 2 (NRF2) activation as one of its immunological responses to combat this ROS-related OS.

NRF2 is a member of the cap'n'collar basic leucine zipper family and is located in the cytoplasm of the cell (18, 19). It is 605 amino acids long and has seven domains (Neh1-Neh7). Under OS, this transcription factor triggers the activation of cellular antioxidant enzymes. NRF2 is normally located in the cytoplasm, which is surrounded by Kelch-like ECH-

associated Protein (KEAP1). The two motifs that bind NRF2 to the Kelch-like ECH-associated protein 1 (KEAP1) protein are "Aspartic acid-leucine-glycine (DLG)" and "Glutamic acid-threonine-glycine-glutamic acid (ETGE)." The NRF2 DLG motif binds to the amino acid residues of SER (363, 508, 555 & 602), ARG (380, 415 & 483), ASN 382, GLN 530, and TYR 525, while the ETGE motif binds to the KEAP1 protein's SER (363, 508, 555 & 602), ARG (380, 415 & 483), ASN 382, GLN 530, and TYR 525) (20). NRF2 is bound by a KEAP1 under normal circumstances, and this bound NRF2 is ubiquitinated and degraded by proteases. The link between KEAP1 and NRF2's ETGE and DLG motif breaks under stress. Within the nucleus, the free NRF2 translocates. Under OS, NRF2 dissociates from KEAP1 and enters the nucleus, where it forms a heterodimer with Maf protein and binds in the antioxidant response element's regulatory region. This increases the transcription of genes related to detoxification and antioxidant enzymes, such as glutamate-cysteine modifier subunits, heme-oxygenase-1, and glutamate-cysteine ligase catalytic (21,22), which reduces sensitivity to OS (23,24). Research has recently been conducted to find compounds that can disrupt the KEAP1-NRF2 protein-protein connection, releasing NRF2 to protect against OS (20,25). NRF2 activation is a possible target for more investigation since it may prevent liver damage from DILI brought on by OS.

Researchers have long been interested in natural antioxidants because of their potential to reduce a number of illnesses, including inflammation (9,13), hepatotoxicity (33,34), diabetes (31,32), cancer (29,30), cardiovascular disorders (26,27), and gastric ulcers (28). Since inflammation and OS are known to contribute to the pathophysiology of metabolic and chronic illnesses, it is imperative that natural compounds have the ability to control many targets (35). Research has documented the involvement of OS and inflammatory response in DILI (36, 37). Antioxidants have either no effect or negative effects in clinical trials. The antioxidant conundrum refers to this discrepancy between experimental research and clinical results (38–41). Additionally, reactive species are released by inflammatory cells at the site of inflammation, exacerbating OS. Proinflammatory genes can also be triggered by a number of nitrogen or oxygen species (42–44). We can therefore conclude that OS and inflammation are related. The inability to use both anti-inflammatory and antioxidant agents simultaneously, the failure to choose agents that directly target OS and inflammation, or the use of nonselective agents that block either oxidative or inflammatory pathways but exacerbate the other condition could all be contributing factors to the failure of antioxidant agents during clinical trials.

Sesamol, a member of the Pedaliaceae family, is a naturally occurring antioxidant that was extracted from the Sesamum indicum L. plant seed oil, also referred to as sesame. For many years, India and other eastern Asian nations have utilised sesame for its nutritional and therapeutic properties.

## REVIEW OF LITERATURE

It has been discovered that polydatin can shield cardiac cells from a variety of damage caused by oxygen deprivation, cardiotoxic substances, or ischaemia reperfusion. Lactate dehydrogenase (LDH) and creatine kinase, two serum markers of cardiac stress, were found to be lowered by it, hence mitigating the cardiac injury (Zhang et al., 2006). Concurrently, it was shown that polydatin reduced the rate of apoptosis in ischaemia reperfusion injury by controlling the expression of Bax and Bcl-2 proteins (Zhang et al., 2009). Additionally, it was discovered to protect heart tissue by suppressing oxidative stress and inflammation, down-regulating L-type calcium channels, and activating protein kinase C-ATP-sensitive potassium channel-dependent signalling (Deng et al., 2012; Gao et al., 2010).

By blocking the ICAM-1, VCAM-1, and NF-κB pathways, polydatin can lessen inflammation in endothelial cells (Deng et al., 2011). By reducing the expression of TLR4, NF-κB, and ICAM-1, polydatin can lessen lung damage. Concurrently, the pulmonary tissue shows a rise in the cellular antioxidant state (Jin et al., 2009). Similarly, polydatin increases the ratio of high density lipids to total cholesterol while lowering serum cholesterol, triglycerides, low density lipids, and apolipo protein-1 levels when taken orally for approximately four weeks (Zhu and Jin, 2006).

Previous research demonstrated polydatin's neuroprotective potential, particularly in cases of cerebral ischaemic reperfusion injury. Polydatin protects the brain from harm by increasing the neurotissue's antioxidant capacity and reducing inflammatory pathways (Ji et al., 2012). Additionally, it was discovered to improve memory and learning, hence reducing cognitive deficiencies (Xu et al., 2012). Polydatin counteracts dementia caused by cerebral hypoperfusion by reducing MDA generation, which is followed by increased antioxidant activities (Li et al., 2012). Additionally, polydatin was discovered to prevent β-amyloid plaques from forming, protecting the brain from Alzheimer's disease (Riviere et al., 2009).

Numerous studies show that polydatin is cytotoxic to a variety of human tumour cell lines, suggesting that it has anticancer properties. Cervical, liver, skin, lung, and pancreatic tumours were found to be effectively treated with polydatin. It was discovered that polydatin's anti-inflammatory, anti-oxidant, and anti-apoptotic properties contributed to its anticancer effectiveness (su et al., 2013).

According to Ban et al. (2010), polydatin was also found to have antimicrobial action against Streptococcus mutans and Streptococcus sobrinus. In addition to the aforementioned positive effects, polydatin has been shown to have immunomodulatory, anti-thrombin, antioxidant, anti-inflammatory, anti-shock, anti-atherosclerotic, and

hepatoprotective properties (Du et al., 2013).The current study was conducted to investigate the protective nature of polydatin against alcohol-induced hepatotoxicity and to investigate the processes involved, based on its many promising therapeutic qualities.

**Objectives Of The Study**

- To investigate sesamol and its chosen derivative in vitro for hepatoprotection against paracetamol-induced damage in HepG2 cell lines

## RESEARCH METHODOLOGY

Masson's trichrome stain is used to distinguish between collagen and smooth muscle in cancer cells as well as to investigate the deposition of collagen in tissues. Muscle, erythrocytes, and collagen fibres were stained using three different dyes. The idea behind Masson's trichrome staining is that the least permeable tissues are coloured by the smallest dye molecules. The staining process is first treating the sections with Biebrich Scarlet, an acid dye that binds to acidophilic tissue components. Next, the sections are treated with phospho acids, which stain the less permeability components red while also connecting the collagen to bind with aniline blue.

As mentioned in the section above, the liver sections were prepared. The typical lab process is followed for staining with Masson's trichrome. In short, the sections are subjected to Bouin's solution (15 ml of saturated picric acid, 5 ml of formaldehyde, and 1 ml of glacial acetic acid) for one hour, and any surplus picric acid is then rinsed for five minutes under running tap water. The sections were then submerged for approximately ten minutes in Weigert's iron haematoxylin working solution, which consists of equal parts of stock solution A (haematoxylin, 1 g and 95% alcohol, 100 ml) and stock solution B (29% ferric chloride in water, 4 ml; distilled water, 95 ml; and hydrochloric acid, 1 ml). Washing under running tap water for around five minutes eliminated the surplus reagent.

After that, the sections were dyed for five minutes using a Biebrich scarlet--acid fuchsine solution (1% Biebrich scarlet, 90 ml; 1% acid fuchsine, 10 ml, and glacial acetic acid, 1 ml). The sections were rinsed with distilled water, stained for ten minutes with equal parts 5% phosphotungstic and 5% phosphomolybdic acid, and then immediately placed in aniline blue solution for five minutes. After rinsing the sections in distilled water, they were submerged in 1% glacial acetic acid for one minute before being rinsed again in distilled water. After being dehydrated as mentioned in the previous section, the stained sections were mounted using DPX, covered with a cover slip, and examined under a Zeiss light microscope (Axioplan 2 Imaging, Axiovision software).

In the periportal and vascular areas, the degree of fibrosis was rated from 1 to 12. The fibrous septa's length and width were measured in three distinct locations and graded as follows: 90–125 mm received a score of 6, 50–70 mm received a score of 4, and 30–40 mm received a score of 2 (Jung et al., 2000).

**Statistical assessment**

Every experimental value is expressed as mean ± SEM (n = 8). GraphPad Prism software (version 5.0) was used to examine the statistical significance of the data using one-way analysis of variance (ANOVA), and Dunnett's Multiple Comparison Test was used to compare the group averages. At the $p < 0.05$ level, a difference was deemed significant.

## RESULT AND DATA INTERPRETATION

The findings of measuring several antioxidant enzymes in mouse liver tissues are shown in Table 2. When compared to the vehicle control group, the mice in the alcohol control group displayed notable alterations in their antioxidant levels. Catalase ($p < 0.001$), SOD ($p < 0.001$), GSH ($p < 0.05$), GST ($p < 0.001$), GR ($p < 0.01$), vitamin C ($p < 0.05$), and NQO1 ($p < 0.001$) were all significantly lower in the alcohol control group than in the vehicle control. Fisetin pretreatment at doses of 5 and 10 mg/kg, however, brought the antioxidant levels back to normal. Catalase ($p < 0.001$), SOD ($p < 0.001$), GSH ($p < 0.05$ at fisetin 10 mg/kg dose and $p > 0.05$ at fisetin 5 mg/kg), GST ($p < 0.05$), GR ($p < 0.01$), vitamin C ($p < 0.01$ at fisetin 10 mg/kg dose and $p > 0.05$ at fisetin 5 mg/kg), and NQO1 ($p < 0.001$). Similarly, endogenous antioxidant levels such as catalase ($p < 0.01$), SOD ($p < 0.001$), GSH ($p < 0.01$), GR ($p < 0.001$), vitamin C ($p < 0.01$), and NQO1 ($p < 0.001$) activities were similarly recovered by pre-treatment with silymarin (25 mg/kg) (Fig. 17). However, when compared to the alcohol control group, the GST activity remained unchanged ($p > 0.05$).

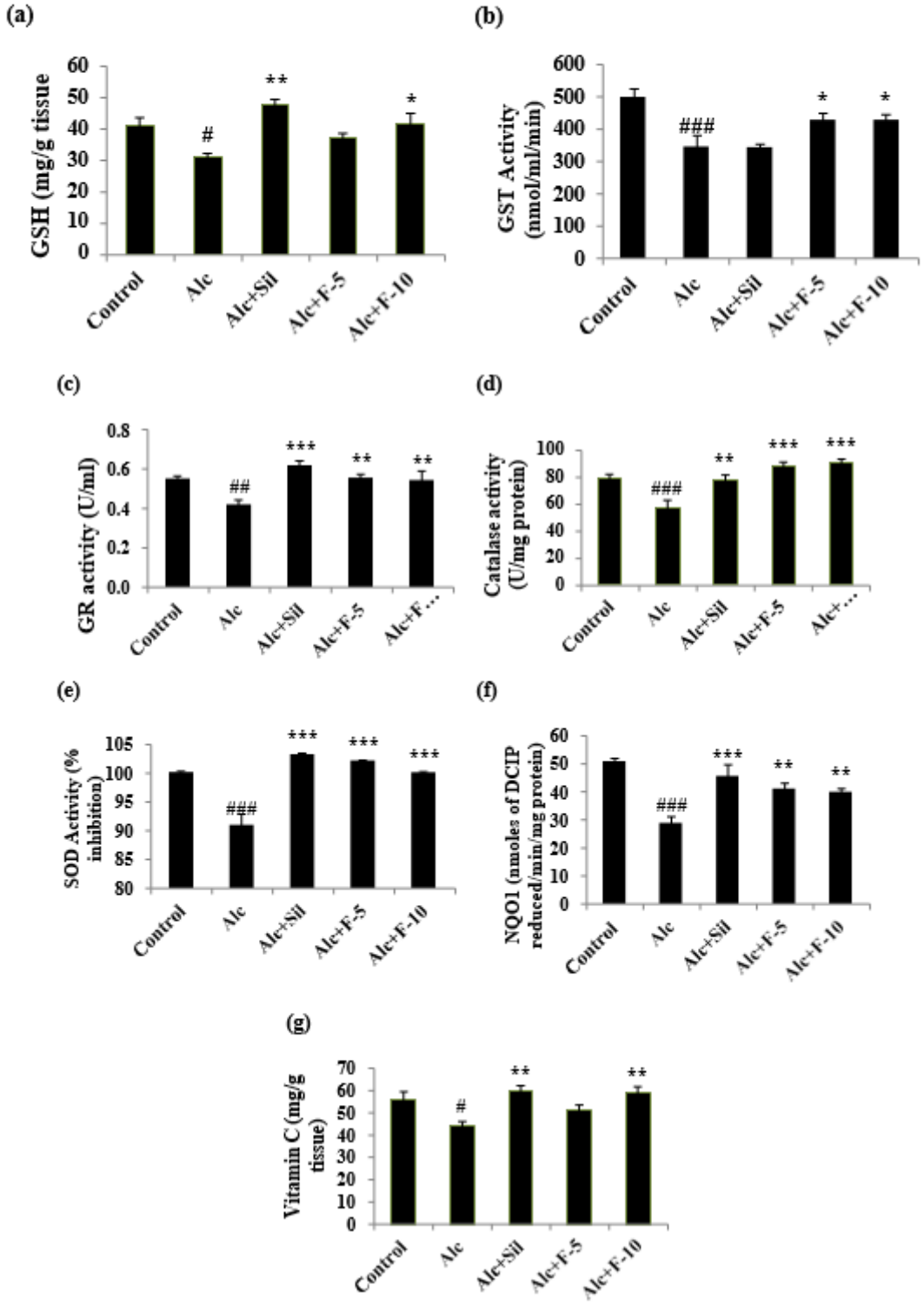


**Fig. 1: Effect of fisetin on alcohol induced changes in the hepatic antioxidants GSH, reduced glutathione (a); GST, glutathione S-transferase (b); GR, glutathione reductase (c); CAT, catalase (d); SOD, superoxide dismutase (e); NQO1, NAD (P) H: quinine oxidoreductase 1 (f); Vit C, vitamin C (g).**

All data was expressed as mean ± S.E.M (n=8). Where, Control, vehicle control mice; Alc, alcohol control mice; Alc + Sil, alcohol + silymarin (25 mg/kg) treated mice; Alc + F-5, alcohol + fisetin (5 mg/kg) treated mice; Alc + F-10, alcohol + fisetin (10 mg/kg) treated mice. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$vs. alcohol control group. #$p < 0.05$, ##$p < 0.01$, ###$p < 0.001$ vs. vehicle control group.

**Table 1:** Effect of fisetin on alcohol induced changes in the hepatic antioxidant status

| Parameters estimated | Control | Alc | Alc+Sil | Alc+F-5 | Alc+F-10 |
|---|---|---|---|---|---|
| **GSH levels** | 41.34±2.66 | # 31.46±1.13 | ** 48.15±1.68 | 37.22±1.72 | * 42.09±3.30 |
| **GST activity** | 501.90±25.12 | ### 329.20±35.48 | 345.30±9.05 | * 430.90±19.48 | * 430.80±15.87 |
| **GR activity** | 0.5525±0.015 | ## 0.42±0.03 | *** 0.62±0.02 | ** 0.54±0.01 | ** 0.55±0.02 |
| **Catalase activity** | 79.59±1.99 | ### 57.64±5.16 | ** 77.74±3.53 | *** 88.01±2.35 | *** 90.25±2.36 |
| **SOD activity** | 100.00±0.51 | ### 90.81±2.91 | *** 103.10±0.56 | *** 102.00±0.46 | *** 99.95±0.52 |
| **NQO1 activity** | 50.83±1.38 | ### 28.80±2.37 | *** 45.57±4.32 | ** 41.13±2.13 | ** 39.96±1.30 |
| **Vit C levels** | 55.82±4.05 | # 44.70±1.34 | ** 59.98±2.18 | 51.75±1.66 | ** 59.04±3.09 |

The mean ± S.E.M. (n = 8) was used to express all data. Alc, alcohol control mice; Alc + Sil, alcohol + silymarin (25 mg/kg) treated mice; Alc + F-5, alcohol + fisetin (5 mg/kg) treated mice; and Alc + F-10, alcohol + fisetin (10 mg/kg) treated mice. GSH, reduced glutathione (µg/g of tissue); GST, glutathione S-transferase (nmoles of CDNB conjugated/min/ml); GR, glutathione reductase (U/mg protein); CAT, catalase (U/mg protein); SOD, superoxide dismutase (% of control); NQO1, NAD (P) H: quinine oxidoreductase 1 (nmoles of DCIP reduced/min/mg protein); Vit C, vitamin C (mg/g of tissue); TBARS, thiobarbituric acid reactive substances (nmoles of MDA eq./g of tissue); Nitrites, (µmol/g of tissue); carbonyl content (nmol/g tissue); CDNB, 1-chloro-2, 4-dinitrobenzene; DCIP, 2,6-dichlorophenolindophenol. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ in comparison to the alcohol control group; #$p < 0.05$, ##$p < 0.01$, and ###$p < 0.001$ in comparison to the vehicle control group.

## CONCLUSION

The goal of the current study is to show how certain phytochemicals can protect the liver against alcohol-induced acute liver injury in mice. Based on the existing scientific data, capsaicin, polydatin, and fisetin were selected for the investigation. The current study improves understanding of the alcohol-induced acute liver injury model and aids in the discovery of new therapeutic targets.

**The following are the main topics discussed in this work:**

- Hepatic antioxidant enzymes such as GSH, GST, GR, SOD, catalase, vitamin C, NQO1, and HO-1 were found to be out of balance after alcohol-induced hepatic damage was characterised by an increase in the production of AST and ALT enzymes in the serum. Additionally, alcohol consumption raised nitrite levels, protein carbonylation, and lipid peroxidation. Alcohol exposure significantly damaged the hepatic tissue, resulting in haemolysis, inflammation, fibrosis in the periportal area, and micro and macro vesicular damage. In addition to the aforementioned, alcohol significantly increased CYP2E1 and NF-κB levels and distorted the MMP/TIMP balance.

- Fisetin, a bioflavonoid found in foods including strawberries, apples, persimmons, grapes, and onions, was found to have a protective effect against alcohol-induced harm in a mouse model of acute alcohol-induced hepatotoxicity. Alcohol-induced changes in liver function, antioxidant defence, histological abnormalities, mitochondrial respiratory enzymes, and matrix metalloproteinase activities were reversed by pretreatment with fisetin (5 and 10 mg/kg). This study suggests that fisetin may be able to mitigate the liver damage caused by alcohol.

- The protective efficacy of polydatin, a stilbenoid glucoside, against an acute alcohol-induced liver injury model in mice was also evaluated. According to this study, acute alcohol exposure-induced liver damage was significantly reduced by polydatin at 50 mg/kg and 100 mg/kg. The hepatoprotective effect of polydatin was linked to the reduction of matrix metalloproteinases and the attenuation of oxidative stress, mitochondrial damage, and hepatic steatosis. The results of this investigation showed that polydatin's antioxidant and hepatoprotective properties made it a powerful inhibitor of acute alcohol-induced liver damage.

## REFERENCES

1. Chatterjee A, Bandini G, Motari E, Samuelson J. Ethanol and Isopropanol in Concentrations Present in Hand Sanitizers Sharply Reduce Excystation of Giardia and Entamoeba and Eliminate Oral Infectivity of Giardia Cysts in Gerbils. Antimicrobial Agents and Chemotherapy. 2015;59(11):6749-6754.
2. Chen CM, Hsieh YH, Hwang JM, Jan HJ, Hsieh SC, Lin SH, Lai CY. Fisetin suppresses ADAM9 expression and inhibits invasion of glioma cancer cells through increased phosphorylation of ERK1/2. Tumour Biol. 2015;36:3407–3415.
3. Chen J, Li L, Li Y, Liang X, Sun Q, Yu H, Zhong J, Ni Y, Chen J, Zhao Z. Activation of TRPV1 channel by dietary capsaicin improves visceral fat remodelling through connexin43-mediated Ca2+Influx. Cardiovasc Diabetol 2015;14:22.
4. Cichewicz RH, Kouzi SA. Biotransformation of resveratrol to piceid by Bacillus cereus. Journal of Natural Products. 1998;61(10):1313–1314.
5. Clark DB. Children at high risk for underage drinking and alcohol use disorders. Frontlines. Bethesda (MD): National Institute on Alcohol Abuse and Alcoholism. 2006.
6. Consolo M, Amoroso A, Spandidos DA, Mazzarino MC. Matrix metalloproteinases and their inhibitors as markers of inflammation and fibrosis in chronic liver disease (review) Int J Mol Med. 2009;24(2):143–152.
7. Cui R, Yan L, Luo Z, Guo X, Yan M. Blockade of store-operated calcium entry alleviates ethanol-induced hepatotoxicity via inhibiting apoptosis. Toxicol Appl Pharmacol. 2015;287:52–66.
8. Dalle-Donne I, Rossi R, Giustarini D, Milzani A, Colombo R. Protein carbonyl groups as biomarkers of oxidative stress. Clinica Chimica Acta. 2003;329(1-2):23–38.
9. Das K, Roychoudhury A. Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. Front Environ Sci. 2014;2:53.
10. Deng J, Liu W, Wang Y, Dong M, Zheng M, Liu J. Polydatin modulates Ca(2+) handling, excitation-contraction coupling and beta-adrenergic signaling in rat ventricular myocytes. J Mol Cell Cardiol. 2012;53:646–656.
11. Deng YH, Alex D, Huang HQ, Wang N, Yu N, Wang YT, Leung GP, Lee SM. Inhibition of TNF-alpha-mediated endothelial cell-monocyte cell adhesion and adhesion molecules expression by the resveratrol derivative, trans-3,5,4'-trimethoxystilbene. Phytother Res. 2011;25:451–457.
12. Dey A, Cederbaum AI. Alcohol and oxidative liver injury. Hepatology. 2006;43(2, supplement 1):S63–S74
13. Dinkova-Kostova AT, Talalay P. NAD(P)H:quinone acceptor oxidoreductase 1 (NQO1), a multifunctional antioxidant enzyme and exceptionally versatile cytoprotector. Arch Biochem Biophys. 2010;501:116–123.
14. Dou X, Li S, Wang Z, Gu D, Shen C, Yao T, Song, Z. Inhibition of NF-κB activation by 4-hydroxynonenal contributes to liver injury in a mouse model of alcoholic liver disease. The American Journal of Pathology. 2012;181(5):1702–1710.
15. Droge W. Free radicals in the physiological control of cell function. Review. Physiol. Rev. 2002;82:47-95.
16. Du QH, Peng C, Zhang H. Polydatin: a review of pharmacology and pharmacokinetics. Pharm Biol. 2013;51:1347–54.