

绿原酸的药理作用及机制研究进展

王庆华, 杜婷婷, 张智慧, 季 鸣, 胡海宇, 陈晓光*

(中国医学科学院、北京协和医学院药物研究所, 中国医学科学院小分子肿瘤免疫治疗药物研究重点实验室, 北京 100050)

摘要: 近年来, 随着绿原酸研究的不断拓展和深入, 其在医药、化工和食品等领域被广泛应用, 具有潜在的、广阔的应用前景。绿原酸作为一种水溶性酚类化合物, 在植物界中分布广泛, 展现出较强的生物活性和多方面的药理作用。本文拟从抗菌、抗病毒、抗肿瘤、抗氧化和抗炎以及治疗代谢类疾病等几个方面对绿原酸的药理作用及机制作一综述, 旨在为绿原酸作用机制的深入研究以及作用靶点的确证提供重要的理论依据。

关键词: 绿原酸; 抗菌活性; 抗病毒活性; 抗肿瘤活性; 抗氧化活性; 抗炎活性; 代谢调节活性; 作用机制

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Advances in research on the pharmacological effects and mechanism of action of chlorogenic acid

WANG Qing-hua, DU Ting-ting, ZHANG Zhi-hui, JI Ming, HU Hai-yu, CHEN Xiao-guang*

(Small-molecule Immuno-oncology Drug Discovery Key Laboratory of Chinese Academy of Medical Sciences, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China)

Abstract: In recent years, with rapid advances in chlorogenic acid (CGA) research, it has become widely used in medicine, the chemical industry and health care products, with the potential for broad application. CGA is a water-soluble phenolic natural product and is extensively distributed in a variety of plants. Numerous studies have shown that CGA has strong biological activities including antibacterial, antiviral, antitumor, antioxidative, anti-inflammatory and metabolic regulatory activities. This review summarizes the pharmacological effects as well as the underlying mechanisms of CGA, providing an important theoretical basis for further research on CGA drug targets and potential mechanisms.

Key words: chlorogenic acid; antibacterial activity; antiviral activity; antitumor activity; antioxidative activity; anti-inflammatory activity; metabolic regulatory activity; mechanism

绿原酸 (chlorogenic acid, CGA) 是由咖啡酸的羧基和奎尼酸的羟基缩合而成的缩酚酸, 是植物细胞通过莽草酸途径合成的一种苯丙素类物质, 其分子结构中有酯键、不饱和双键、多元酚和邻二酚羟基^[1,2]。绿原酸在杜仲、金银花、绿咖啡豆、土豆、苹果以及茶叶等

多种植物中含量很高^[3-5]。具有抗氧化、抗菌、抗病毒、抗肿瘤、降血脂、降血糖和免疫调节等多方面的药理作用^[6-8], 在食品、医药和化工等领域都有广泛的应用^[9] (图1)。随着科技的进步和研究的深入, 绿原酸药理作用机制逐渐被重视。现对绿原酸的药理作用和作用机制进行综述, 以为绿原酸的药用研究与开发提供参考。

1 绿原酸的药理作用及机制

1.1 抗菌 大量研究证明, 绿原酸具有广谱抗真菌和细菌的作用^[7]。Martínez 等^[10]在2017年报道了绿原酸对多种植物致病真菌有抗真菌活性, 具有生物杀真菌

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*通讯作者 Tel: 86-10-63165207, E-mail: chxg@imm.ac.cn

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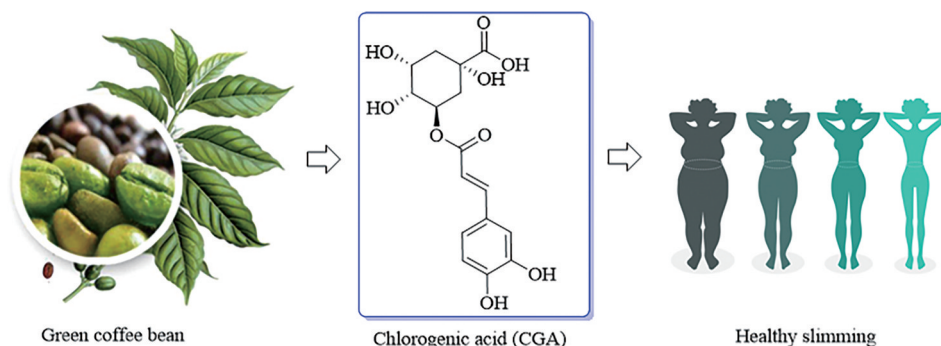


Figure 1 The application of chlorogenic acid from green coffee beans in weight loss

剂的潜力,其机制是通过抑制真菌孢子的早期透膜化来控制不同的植物病原真菌的生长。而绿原酸的抗菌作用研究,已有大量文献报道,其主要是通过破坏肺炎链球菌 (*Streptococcus pneumoniae*)、金葡球菌 (*Staphylococcus aureus*) 和痢疾志贺氏菌 (*Shigella dysenteriae*) 的细胞膜,增加外膜和质膜通透性,导致细菌的屏障功能丧失,进而发挥其抗菌活性^[11,12]。近些年,关于绿原酸新的抗菌机制被报道,通过降低细菌细胞壁的硬度,减慢细菌的迁移,影响细菌细胞膜的稳定性和诱导活性氧 (reactive oxygen species, ROS) 的产生^[13,14]。由此可见,随着绿原酸的抗菌机制深入研究将有助于新型的抗菌药物的研发。

1.2 抗病毒 已有多篇文献指出,绿原酸及其衍生物作为天然化合物,对多种类型的病毒有很好的抗病毒活性,其中包括艾滋病病毒 (human immunodeficiency virus, HIV)、甲型流感病毒、单纯疱疹病毒 (herpes simplex virus, HSVs) 和乙型肝炎病毒 (hepatitis B virus, HBV) 等^[15,16]。1997年,Robinson等^[17]报道了绿原酸可以抑制 HIV-1 整合酶。随后,Tamura等^[18]在人淋巴瘤细胞株 MT-2 上验证了绿原酸可以有效地抑制 HIV 病毒。近年来,研究发现绿原酸及其衍生物可有效抑制甲型流感病毒 (H1N1/H3N2) 的感染,其机制是下调了病毒核蛋白的表达和抑制神经氨酸酶活性^[19,20]。绿原酸还可以通过有效地降低炎症反应和抑制病毒感染产生来治疗病毒感染。Zhao 课题组^[21]实验结果表明,加入绿原酸显著提高 HSV-1 感染的小胶质细胞 (BV2) 的存活率,其机制是抑制感染细胞中 Toll 样受体 2、Toll 样受体 9 和髓样分化因子 88 的增加,降低炎症因子肿瘤坏死因子- α (tumor necrosis factor- α , TNF- α) 和白细胞介素-6 (interleukin-6, IL-6) 释放。Wang 等^[22]以 HepG2.2.15 细胞和鸭乙型肝炎病毒感染模型证明了绿原酸可以抑制 HBV,其机制是通过抑制 HBV-DNA 复制以及乙型肝炎表面蛋白抗原的产生。综上所述,绿原酸有望成为一个潜在的广谱抗

病毒药物。

1.3 抗肿瘤 20 世纪 80 年代,绿原酸被发现具有抑制肿瘤的作用,从而引起了人们的广泛关注^[23]。2017 年本课题组首次报道了绿原酸通过介导巨噬细胞的 M1/M2 极化,即促进 M1 型巨噬细胞和抑制 M2 表型巨噬细胞,进而抑制脑胶质瘤生长^[24]。Jiang 课题组^[25]发现绿原酸对肝癌、肺癌和胶质瘤都有明显抑制作用,其机制不是通过直接的肿瘤细胞杀伤作用,而是通过诱导其分化来抑制肿瘤,并指出绿原酸有望成为一种安全有效的肿瘤分化诱导剂。Sapio 等^[26]报道绿原酸可有效抑制骨肉瘤其机制是抑制磷酸化的信号传导及转录激活蛋白 3,同时指出当绿原酸与细胞外信号调节激酶 1/2 的抑制剂 PD98059 联用时可显著增强抑瘤效果。然而,一些文献报道绿原酸是通过诱导 ROS 的产生进而抑制结肠癌和通过影响细胞凋亡相关基因的表达,从而发挥抑制肺癌细胞生长的作用^[27,28]。尽管大量文献报道了绿原酸抗肿瘤作用机制,但看法不尽一致。因此,潜在靶点的寻找和作用的机制有待进一步探索。

1.4 抗氧化和抗炎 绿原酸是一种广泛存在植物中的多酚类次生代谢产物,具有很强抗氧化和抗炎特性。儿茶酚结构的存在为自由基提供了结合位点^[29,30],绿原酸能够螯合金属离子和清除自由基 [超氧阴离子 (O_2^-)、过氧化氢 (H_2O_2)、羟基自由基 ($\cdot OH$)、次氯酸 ($HOCl$)、过氧亚硝酸盐阴离子 ($ONOO^-$) 和一氧化氮 (NO)]^[31-33]。Lu 等^[34]研究显示绿原酸具有很强的抗氧化活性,其清除 1,1-二苯基-2-三硝基苯肼 (DPPH) 自由基的活性是维生素 C 和 E 的 2~3 倍,清除超氧阴离子自由基的活性是维生素 C 和 E 的 10~30 倍。近年来,绿原酸的抗炎作用也备受关注。2006 年,Dos Santos 等^[35]在角叉菜胶致大鼠足跖肿胀炎症模型上首次报道了绿原酸具有抗炎活性。随后,有很多文献对其抗炎机制进行了深入地探讨。绿原酸及其异构体可通过清除细胞内 ROS,抑制 p38 级联磷酸化和上调核因子 κB

(nuclear factor kappa-B, NF- κ B) 信号通路来抑制白介素-8 (interleukin-8, IL-8) 的产生, 从而起到抗炎作用^[36,37]。然而在硫酸葡聚糖诱导的小鼠溃疡性结肠炎模型中, 发现绿原酸是通过调控丝裂原活化蛋白激酶/ERK/c-Jun 氨基末端激酶信号通路来降低组织的炎症反应^[38]。文献报道, 绿原酸有望成为治疗类风湿关节炎的新型治疗剂, 其机制是通过降低了 NF- κ B 与 B 细胞活化因子启动子区域的 DNA 结合能力, 进而抑制了通过 NF- κ B 途径介导的 B 细胞活化因子表达^[39]。绿原酸具有较强的肝功能保护作用, 研究发现绿原酸可以有效降低 CCl₄ 诱导的急性肝损伤产生 TNF- α 、IL-6 和 IL-1 β 炎症因子, 其机制是通过核因子 E-2 相关因子 2 (nuclear factor erythroid 2-related factor 2, Nrf2) 介导的抗氧化和抑制 Nod 样受体家族热蛋白结构域 3 炎症小体的激活来进行急性肝损伤的保护作用^[40]。绿原酸也可以通过增加 B 淋巴细胞瘤-2 (B-cell lymphoma-2, Bcl2) 表达并抑制环氧化酶-2、诱导型一氧化氮合酶、Bax (Bcl2 associated X, Bax) 和 caspases 3、9 介导的炎症反应来降低甲氨蝶呤 (methotrexate, MTX) 诱导的肝毒性^[41]。绿原酸还可以通过影响肝星状 LX2 细胞系中 IL-13/microRNA-21 (miR-21)/Smad7 信号传导相互作用, 抑制血吸虫病诱导的肝纤维化, 因此, 绿原酸有望成为用于治疗血吸虫病肝纤维化的抗肝纤维化类药物^[42]。神经退行性疾病阿尔茨海默病和帕金森病的病理研究表明, 慢性氧化应激和炎症反应会导致神经元受损^[43]。基于绿原酸具有较强的抗氧化和抗炎作用, 很多学者发现它具有很好的神经系统保护作用^[44]。一些临床和临床前研究表明, 咖啡提取物 (主要成分绿原酸) 对阿尔茨海默病和帕金森病展现出很好的治疗效果^[45,46]。Hermawati 等^[47]发现绿原酸可以改善记忆力减退和海马细胞短暂性全脑缺血后死亡, 其机制是通过增加 Bcl2、超氧化物歧化酶 2 和血小板-内皮细胞黏附分子 CD31 表达, 并降低内皮素-1 表达来改善空间记忆, 防止双侧颈总动脉闭塞后 CA1 锥体细胞死亡。由于绿原酸作为一种有效的自由基清除剂, 可以显著保护 PC12 细胞免受氧化损伤。其机制包括直接的 ROS 淬灭活性和通过激活 Nrf2 诱导内源性抗氧化酶^[48]。因此, 绿原酸可以作为潜在的神经保护药物。近年来, 文献报道绿原酸还可以通过调节线粒体的功能进而发挥其抗氧化和抗炎的功能。Tsai 等^[49]在用绿原酸预处理的情况下, 用氧化型低密度脂蛋白 (oxidized low density, oxLDL) 处理 HUVEC 细胞。结果表明, 绿原酸预处理可提高 NAD-依赖性去乙酰化酶 (sirtuin 1, SIRT1) 活性水平, 逆转了由 oxLDL 刺激造成 SIRT1 的损伤和 AMP 激活的蛋白激酶 (AMP-acti-

vated protein kinase, AMPK)/过氧化物酶体增殖物受体 γ 共激活因子 1 的活性, 并减轻了 oxLDL 诱导的氧化应激和线粒体生物发生的功能障碍。其机制是通过调节 SIRT1 和 AMPK/过氧化物酶体增殖物受体 γ 共激活因子 1 功能来抑制 oxLDL 诱导的内皮细胞凋亡。Yang 等^[50]报道了绿原酸通过激活 SIRT1 调节的线粒体功能来预防饱和和游离脂肪酸 (free fatty acid, FFA) 诱导的脂毒性。其机制是通过减少 ROS 的产生以及增加线粒体质量和线粒体膜电位, 减轻了氧化应激和线粒体功能障碍; 显著降低促细胞凋亡蛋白 Bax 表达, 从而减少线粒体介导的 caspase 依赖性细胞凋亡。

1.5 治疗代谢性疾病 随着社会的进步和人类物质生活水平的提高, 代谢相关性疾病已成为全球影响人类健康的重要问题。其中包括血脂异常、高血压、高空腹血糖水平、胰岛素抵抗、慢性炎症和血栓形成等^[51]。随着天然产物在治疗代谢性疾病优势的逐渐展现, 人们又重新燃起了对天然产物的兴趣。许多研究已经评估绿原酸对代谢类疾病的影响, 其中包括肥胖、血脂异常、糖尿病、高血压、代谢综合征和保护心血管等^[52]。

在治疗肥胖方面, 研究表明绿原酸主要通过调控糖代谢和脂代谢来控制肥胖。Wang 等^[53]喂食高脂饮食 (high fat diet, HFD) 小鼠用绿原酸治疗 6 周。结果表明, 给予绿原酸可显著降低小鼠的体重, 降低血浆中的脂质水平, 并改变脂肪组织中脂肪生成和脂肪分解相关基因的 mRNA 表达。此外, 绿原酸改善 HFD 诱发的肠道菌群失调, 也有助于改善 HFD 诱发的肥胖。同时, 绿原酸可以通过促进葡萄糖的摄取和线粒体的功能来刺激褐色脂肪细胞的生热。其机制增强了棕色脂肪细胞的生热和质子泄漏, 上调葡萄糖转运蛋白 2 和磷酸果糖激酶来促进葡萄糖的吸收, 增加线粒体的数量和功能^[54]。绿原酸也可以通过激活 AMPK, 抑制 3-羟基 3-甲基戊二酰辅酶 A 还原酶, 增强肉碱棕榈酰转移酶的活性控制肥胖^[55,56]。Cho 等^[57]研究了绿原酸对高脂饮食诱导肥胖小鼠的体重、体脂和肥胖相关激素的影响。与高脂饮食对照组相比, 绿原酸和咖啡酸均显著降低体重、内脏脂肪量、血浆瘦素和胰岛素水平、肝脏和心脏中的甘油三酸酯以及脂肪组织和心脏中的胆固醇。在大鼠实验中获得了相似的结果, 绿原酸以剂量依赖性方式抑制体内和内脏脂肪的增加和高脂饮食诱导的游离脂肪酸。同样临床研究也显示, 富含绿原酸的食物具有抗肥胖作用^[58]。给予 30 名超重受试者每天饮用 5 杯普通速溶咖啡或 Coffee Slender® (富含绿原酸)。饮用 Coffee Slender® 参与者明显减少了体重, 其中 80% 的减少是体内脂肪, 而普通速溶咖啡的参与者减少体重和体内脂肪的作用并不显著^[59]。此

外, Soga 等^[60]研究了绿原酸对人体能量代谢的影响。18 名健康男性受试者每天食用 185 mL 含或不含绿原酸的测试饮料 (329 mg), 持续 4 周, 与对照饮料相比, 含有绿原酸的饮料可显著提高餐后能量的利用, 并且饮用含绿原酸饮料的受试者餐后脂肪利用率更高。

在控制血脂异常方面, 绿原酸和咖啡酸均显著降低小鼠血浆中的游离脂肪酸、甘油三酸酯、胆固醇和血清脂质水平, 显著提高高密度脂蛋白胆固醇/总胆固醇的比率^[57,58]。同时在大鼠高胆固醇血症模型中, 发现绿原酸可显著降低总胆固醇和低密度脂蛋白胆固醇, 并增加高密度脂蛋白胆固醇; 此外, 还改善了动脉粥样硬化指数和心脏危险因素^[61]。

在治疗糖尿病方面, 在动物水平上, 研究发现绿原酸可有效抑制糖尿病小鼠的肝中葡萄糖 6-磷酸酶表达和活性, 减少肝脂肪变性, 改善脂质分布和骨骼肌葡萄糖摄取, 从而改善空腹血糖水平、葡萄糖耐量、胰岛素敏感性和血脂异常^[62]。在链脲霉素诱导糖尿病大鼠模型中, 研究发现白桑树的叶提取物、绿原酸和芦丁的非空腹血糖水平呈剂量依赖性降低, 而异槲皮素则未见^[63]。Jin 等^[64]研究了绿原酸对晚期糖尿病小鼠葡萄糖和脂质代谢的影响。与对照组相比, 绿原酸组的体脂、空腹血糖和糖基化血红蛋白百分比显著降低。Kim 等^[65]通过建立大鼠半乳糖性白内障模型来确定绿原酸对糖性白内障的影响。实验显示, 持续 2 周口服绿原酸可以有效阻止糖性白内障的发展。同时在研究绿原酸对大鼠糖尿病伤口愈合的影响时, 发现绿原酸加速伤口愈合^[66]。在临床研究中, 通过对咖啡中绿原酸抗糖尿病作用的评估, 实验结果表明, 绿原酸可以显著降低葡萄糖依赖性促胰岛素多肽分泌, 显著增加胰高血糖素样肽 1 的分泌, 有效降低肠道葡萄糖吸收率, 对葡萄糖转运具有拮抗作用^[67]。同样 Iwai 等^[68]通过对 45 位受试者评估了富含绿原酸的不含咖啡因的绿咖啡豆提取物的降血糖作用。发现摄入含有绿原酸饮料后血浆葡萄糖显著降低, 但未观察到血浆胰岛素谱有显著变化。Ahrens 等^[69]研究了 Emulin™ (绿原酸、杨梅素和槲皮素的专利混合物) 的抗糖尿病作用。对 40 位 2 型糖尿病患者进行治疗, 结果显示, 如果定期食用 Emulin™, 不仅具有降低餐后血糖的作用, 而且可以长期降低 2 型糖尿病患者的血糖水平。基于绿原酸对糖代谢障碍具有调节作用, Gao 等^[70]通过绿原酸修饰的功能化磁微球蛋白发现绿原酸的靶点为蛋白激酶 B。并通过绿原酸分子探针的免疫荧光进一步证明了绿原酸与 AKT 的共定位。同时阐明了其作用机制是通过直接靶向 AKT 的 PH 结构域, 激活 AKT 在 Ser-473 上的磷酸化, 诱导下游分子糖原合酶激酶 3 和叉头框转录

因子 O 亚族 1 的磷酸化, 从而发挥降低血糖的作用。

在治疗代谢综合征方面, 研究发现绿原酸的预防和治疗对肥胖症及与肥胖有关的小鼠肝脏脂肪变性和胰岛素抵抗有很好的疗效。绿原酸有效防止体重增加, 抑制肝脂肪变性的发展, 并减低高脂饮食诱导的胰岛素抵抗^[71]。通过评价一个含有绿原酸的天然补品对代谢综合征患者的影响, 发现 78 名患有代谢综合征的患者持续服用 4 个月, 其体重、体重指数、腰围、空腹血糖和体重显著降低, 也观察到总胆固醇得到有效的降低^[52]。

在治疗心血管系统和血栓栓塞性疾病方面, 绿原酸显现出很好的疗效。在细胞水平, 用 200 mmol·L⁻¹ 绿原酸处理 3T3-L1 脂肪细胞后, 细胞内钙浓度相对于对照增加了 9 倍, 同时也促进了胰岛素的分泌, 显著提高了过氧化物酶体增殖剂激活受体 γ (150%)、GLUT4 (220%)、过氧化物酶体增殖剂激活受体 α (40%) 和脂肪酸转运蛋白 (25%) 的 mRNA 表达。因此, 可作为刺激胰岛素的增敏剂和降脂剂^[72]。在整体动物中, 绿原酸可有效减低自发性高血压大鼠的血压和改善血管功能。其机制通过抑制血管系统中 ROS 过量的产生来降低氧化应激和提高一氧化氮的生物利用度, 进而减轻了自发性高血压大鼠的内皮功能障碍、血管肥大和高血压^[73]。绿原酸可用于预防糖尿病引发的心血管系统损害^[74]。研究表明绿原酸也作为抗凝剂, 当糖尿病大鼠的血小板在激动剂二磷酸腺苷刺激下, 绿原酸治疗 30 天后血小板聚集可明显减少^[75]。此外, 绿原酸还可以通过激活血小板 A2A 受体/腺苷酸环化酶/环磷酸腺苷/蛋白激酶 A 信号通路进而抑制小鼠动脉血栓形成^[76]。在临床研究中, 纯绿原酸和绿原酸含量很高的食物可以对血压产生积极影响。Kozuma 等^[77]针对 117 名轻度的高血压健康男性进行研究, 分组给予 28 天不同浓度的绿原酸, 发现绿原酸可以有效降低收缩压和舒张压, 其原因为绿原酸具有抗氧化特性, 改善内皮功能障碍进而降低了血压。同时研究表明, 绿原酸有效降低患有高血压患者的血压^[78]。Mubarak 等^[79]研究了绿原酸对一氧化氮状态、内皮功能和血压的急性影响。结果显示, 给予绿原酸的受试者的收缩压和舒张压均明显降低, 一氧化氮的状态和内皮功能未受到明显影响。综上所述, 现对绿原酸治疗代谢性疾病的作用机制进行总结 (图 2)。

2 总结

近年来, 有关绿原酸的研究报道越来越多, 业已成为天然产物领域研究热点之一。绿原酸作为金银花、杜仲、咖啡豆、茵陈等许多中草药的主要有效成分之一, 随着其生物活性的不断深入研究, 它的应用愈加

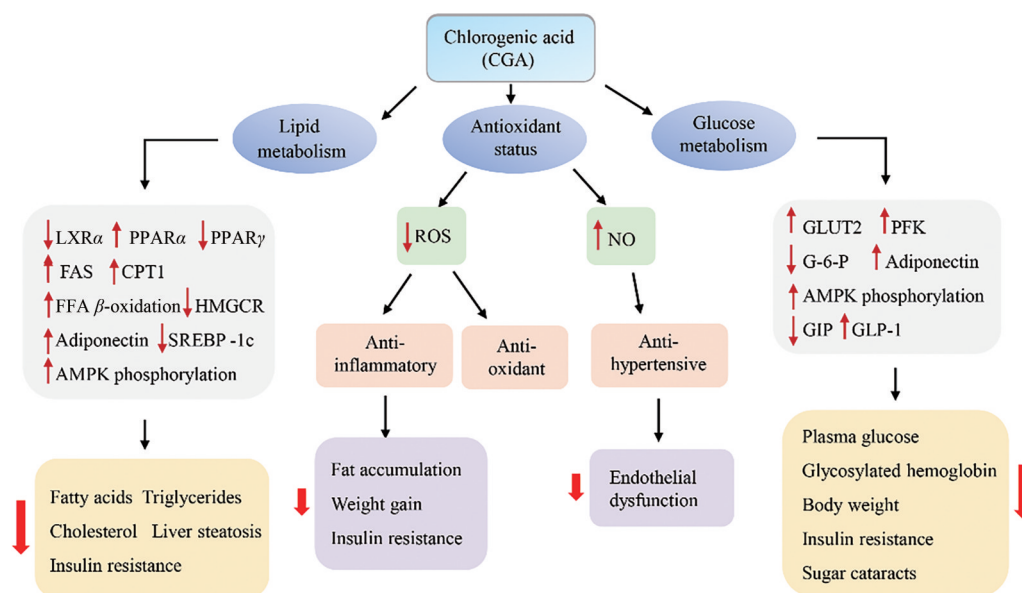


Figure 2 Mechanisms of CGA on improving metabolic diseases. AMPK: AMP-activated protein kinase; CPT1: Carnitine palmitoyltransferase 1; FAS: Fatty acid synthase; FFA: Free fatty acid; GLUT2: Glucose transporter type 2; G-6-P: Glucose-6-phosphatase; GIP: Glucose-dependent insulintropic polypeptide; GLP-1: Glucagon-like peptide-1; HMGCR: 3-Hydroxy-3-methylglutaryl CoA reductase; LXR: Liver X receptor; NO: Nitric oxide; PFK: Phosphofructokinase; PPAR: Peroxisome proliferator-activated receptor; ROS: Reactive oxygen species; SREBP-1c: Sterol regulatory element-binding protein 1c

广泛。在国外,已将绿原酸作为减肥的保健品出售。在我国,绿原酸也作为抗肿瘤药物进行晚期复发脑胶质母细胞瘤的Ⅱ期临床研究。但值得注意的是,虽然我国对含有绿原酸及其衍生物的中药的使用有着悠久的历史,但是研究者对绿原酸分子机制的探索和靶点验证仍然存在很大的不足,这也是今后研究着力解决的问题。

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