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Progress of the antioxidation of the Chinese herbal medicine Gan-Cao (Licorice)

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ABSTRACT

Gan-Cao, or licorice, is a popular Chinese herbal medicine derived from the dried roots and rhizomes of Glycyrrhiza uralensis, G. glabra, and G. inflata. The review describes the progress from four aspects including the history of the research on licorice's antioxidation, the main chemical anti-oxidative constituents, the mechanism of Glycyrrhizic antioxidation, the relation between the pharmacological function and the mechanism. We also give a prospect in the fields such as research on the total-herb activity and improvement of the anti-oxidative effect by chemical modifying.

KEYWORDS

Licorice;
Antioxidation.

INTRODUCTION

In China, licorice is called Gan-Cao, which means "sweet weed". Gan-Cao is extensively used in the Traditional Chinese Medicine (TCM). It is called "the king of the herbs" because it appears as a component herb in about 60% of all TCM prescriptions. Licorice is derived from the dried roots and rhizomes of Glycyrrhiza species (Leguminosae family). According to the Chinese Pharmacopoeia, three species, Glycyrrhiza uralensis, G.glabra and G. inflata, are officially used as Gan-Cao[1].

With the development of pharmacy and its related disciplines, as well as the research equipment, people learn more about licorice than before. It has a variety of pharmacological activities, including antiulceric, antiinflammatory, antispasmodic, anti-oxidative, antiallergic, antiviral, antidiabetic, anticancer, antidepressive, hepatoprotective activitie[2]. These bioactivities are attributed to the chemical constituents of licorice. Glycyrrhizic ingredients' anti-oxidative activity has been paid more and more attention as Gao-Cao has been applied to medicine, health care products, cosmetics and many other fields.

In this review, we summarize the progress in the anti-oxidative effects of licorice and mainly focus on the active ingredients of Glycyrrhiza, its anti-oxidative mechanism, the relation between the pharmacologica function and the mechanism. We also give a prospect in the fields such as research on the total-herb activity and improvement of the anti-oxidative effect by chemical modifying.

THE HISTORY OF THE RESEARCH ON LICORICE'S ANTIOXIDATION

More than 200 kinds of chemical substances have

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been extracted, isolated and identificated from Glycyrrhiza. The biologically active ingredients are mainly flavonoids, triterpenoids, saponins, coumarins, polysaccharides, alkaloids, amino acids.

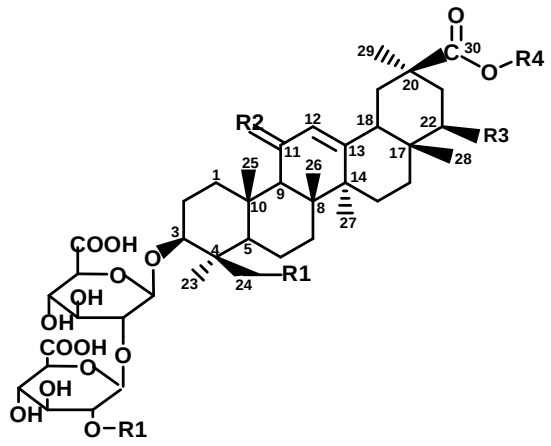
It has long been conceived that glycyrrhizic acid is the only constituent underwriting the pharmacological effects of licorice. The pharmacological studies of licorice saponins mainly focus on the constituents glycyrrhizic acid and its aglycone glycyrrhetic acid. These two compounds exhibit extensive biological activities, including anti-oxidative activities.

The anti-gastric ulcer effect of licorice extract was ascribed to glycyrrhizic acid-free fractions abounding in flavonoids[3]. This finding verified that the flavonoids also made up part of the pharmacological activities of licorice. In 1978, Japanese researchers found licorice ingredients extracted from the polar solvent have a good anti-oxidation effect at linoleic acid methyl ester substrate, which pioneered a research on Glycyrrhizic Antioxidation. In 1993, Wang et al.[4] studied the antioxidant ingrediants and its cooperated function with other antioxidants and found Glycyrrhizic antioxidants' main active ingredients are neutral lipophilic ingredients (polyphenols), organic acidic ingredients and flavonoids containing phenolic hydroxyl group. Michael H. Gordo et al. used column chromatography and thin-layer chromatography (TCL) to purify eight flavonoids and found that a single has a lower anti-oxidative effect than the compound containing all flavonoids. Liang et al.[5] identified the most effective anti-oxidant component in ether extract of licorice by thin-layer TLC separation. Her research indicated the flavonoids in the liquorice were actually the ingredients playing the role of anti-oxidation. Now, the main components of flavonoids mixtures have been used in edible oils on the food as the natural antioxidants.

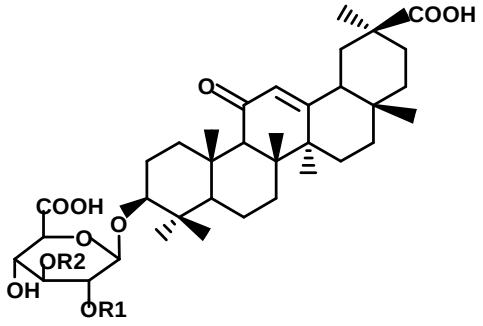
At present, licorice anti-oxidative constituents mainly concentrated in the flavonoids and triterpenoids. Glycyrrhiza Polysaccharides' anti-oxidative effect also has been reported, but the number of the reports is much fewer than the two ingredients mentioned above.

THE MAIN ANTI-OXIDATIVE CHEMICAL CONSTITUENTS

Triterpene saponins are the major characteristic constituents of Gan-Cao, and they are responsible for the sweet taste. So far, at least 18 saponins have been obtained from the three official species of Gan-Cao. The chemical structures of these saponins (1–18) are given in Figure 1. Most licorice saponins are present as glucuronides. The aglycones are oleanane type pentacyclic triterpenes with 11-oxo-12-ene, 12-ene, 11, 13(18)-diene or 9(11), 12-diene skeletons, and 3β-OH, 24-OH, 22β-acetoxy, 30-COOH or 29-COOH as functional groups. Compounds 14 and 15 contain unique lactone rings between 30-COOH and 22-OH. In addition, a series of methyl, ethyl or butyl esters (19–24) (Figure 2) have also been isolated from Gan-Cao. However, they might be esterified artifacts formed during the extraction and purification process.



glycyrrhizic acid (1) R1=R3=R4=R5=H, R2=O
licorice-saponin A3 (2) R1=R3=R4=R5=H, R2=O, R4=glu
licorice-saponin B2 (3) R1=R3=R4=R5=H, R2=H2
licorice-saponin D3 (4) R1=R4=H, R2=H2, R3=OCOCH3, R5=rha



licorice-saponin G2 (5) R1=OH, R2=O, R3= R4=R5=H
licorice-saponin J2 (6) R1=OH, R2=H2, R3= R4=R5=H
licorice-saponin L3 (7) R1=OH, R2=H2, R3=OCOCH3, R4= H, R5= rha
22β-acetoxyglycyrrhizin (8) R1=R4=R5=H, R2=O, R3=OCOCH3
uralsaponin B(9) R1=H, R2=gluA
apioglycyrrhizin (10) R1=api, R2=H
araboglycyrrhizin (11) R1=ara, R2=H

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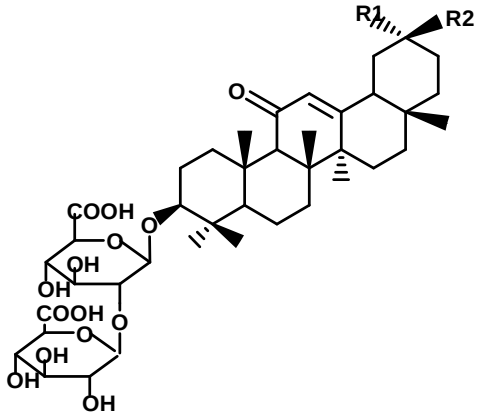
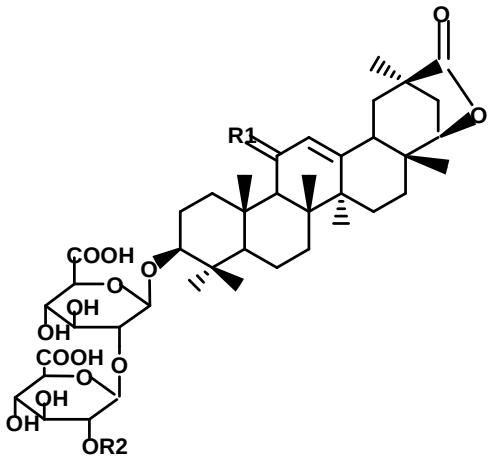
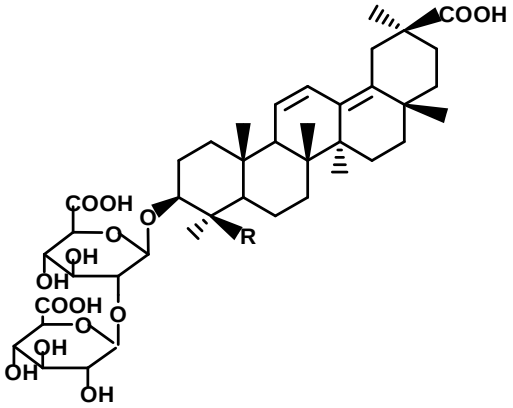


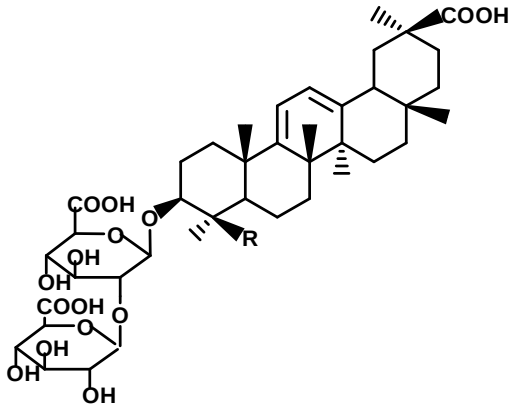
licorice-saponin H2
(12) R1=COOH, R2=CH
(13) R1=CH3, R2=CH2OH



licorice-saponin E2(14) R1=O, R2=H
licorice-saponin F3(14) R1=H2, R2=rha

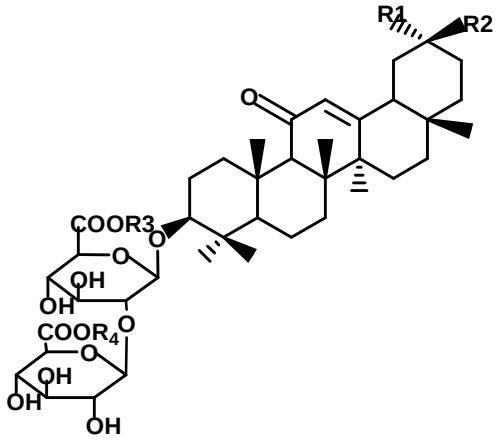


licorice-saponin C2 (16) R=CH3
licorice-saponin K2 (17) R=CH2OH

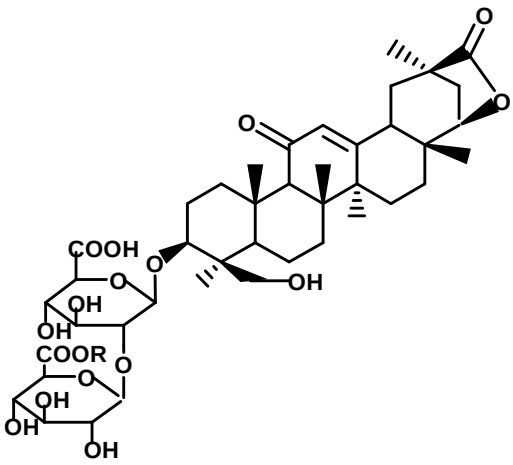


(18)

Figure 1 : Saponins from licorice. ara: α-L-arabinopyranosyl; api: β-D-apiofuranosyl; glu: β-D-glucopyronosyl; gluA: β-D-glucuronopyronosyl; rha: α-L-rhamnopyronosyl



(19) R1= CH3, R2=COOH, R3= n-Bu, R4=CH3
(20) R1= CH3, R2=COOH, R3= n-Bu, R4=CH2CH3
(21) R1=CH3, R2 =COOH, R3= n-Bu, R4=n-Bu



(22) R1=COOH, R2=CH3, R3=CH2CH3, R4= n-Bu
(23) R1=COOH, R2=CH3, R3= n-Bu, R4= n-Bu
(24) R= CH3

Figure 2 : Saponin esters from licorice. n-Bu: –CH2CH2CH2CH3

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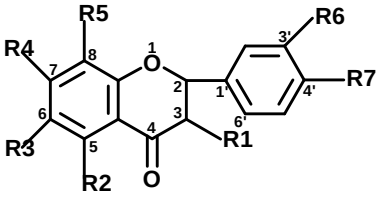
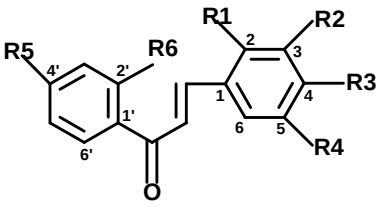
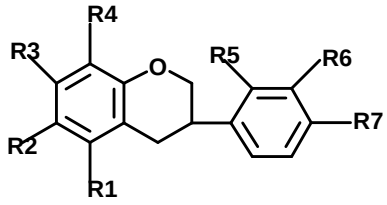
Flavonoids are naturally organic compounds in the composition of tricyclic C6-C3-C6 way. More than 300 flavonoids have been isolated from Glycyrrhiza species[6]. These flavonoids belong to various types, including flavanones or flavanonols (25–32), chalcones (33–39), isoflavans (40–46), isoflavenes (47), flavones or flavonols (48–52), isoflavones (53–59) and isoflavanones (60–63). A number of licorice flavonoids are substituted with isoprenyl groups, which may form a pyran ring with adjacent hydroxyl groups (Figure 3).



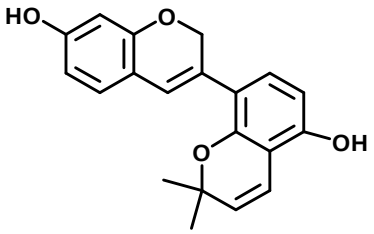
liquiritigenin (25) R1=R2=R3=R5=R6=H, R4=R7=OH
liquiritin (26) R1=R2=R3=R5=R6=H, R4=OH, R7=-O-glc
liquiritigenin-7-4-diglucoside (27) R1=R2=R3=R5=R6=H, R4=R7=-O-glc
rhamnoliquirin (28) R1=R2=R3= R5=R6=H, R4=OH, R7=-O-rha
liquiritin apioside (29) R1=R2=R3=R5=R6=H R4=OH, R7=-O-glc- (2-1) -api
glabranin (30) R1=R3= R6= R7= H, R2= R4=OH, R5=-CH2CH=C(CH3)2
glabrol (31) R1=R2=R3=H, R4=R7=OH R5=R6=-CH2CH=C(CH3)2



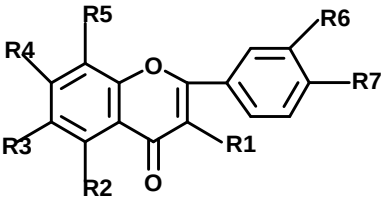
licoflavanone (32) R1=R3=R5=H, R2= R4= R7=OH, R6=-CH2CH=C(CH3)2
isoliquiritigenin (33) R1=R2=R4=H, R3=R5=R6=OH
isoliquiritin (34) R1=R2=R4=H, R3=-O-glc, R5=R6=OH
neoisoliquiritin (35) R1=R2=R4=H R3= R6=OH, R5=-O-glc
rhamnoisoliquiritin (36) R1=R2=R4=H R5=R6=OH, R3=-O-glc- (2-1) -rha
licuraside (37) R1=R2=R4=H, R3= R6=OH, R5=-O-glc- (2-1) -api
licochalcone A (38) R1=OCH3, R2=R6=H, R3=R5=OH, R4=-C(CH3)2CH=CH2
licochalcone B(39) R1=OCH3, R4=R6=H, R2=R3=R5=OH



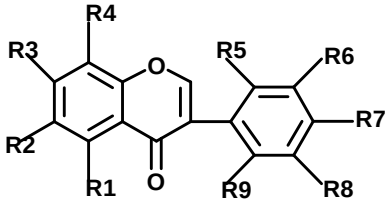
glabridin (40) R1=R2=R6=H R3, R4=7-O-C(CH3)2CH=CH-8, R5=R7=OH
4'-O-methylglabridin (41) R1=R2=R6=H R3, R4=7-O-C(CH3)2CH=CH-8, R5=R7=OHR7=OCH3
3'-O-methylglabridin (42) R1=R2=R7=H R3, R4=7-O-C(CH3)2CH=CH-8, R5=R7=OHR6=OCH3
licoricidin (43) R1=OCH3, R2=R6=-CH2-CH=C(CH3), R4=H, R3=R5=R7=OH



7'-methylglabridin (44) R1=R3=OCH3, R2=R6=-CH2CH=C(CH3)2, R4=H, R5=R7=OH
hispaglabridinA (45) R1=R2=HR3, R4=7-O-C(CH3)2CH=CH-8, R5=R7=OH, R6=-CH2CH=C(CH)2
hispaglabridinB (46) R1=R2=HR3, R4=7-O-C(CH3)2CH=CH-8, R5=OH, R6, R7=3-CH=CHC(CH3)2-O-4
glabrene (47)

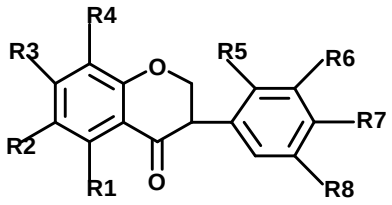


licoflavone A (48) R1=R2=R5=HR3=R6= -CH2CH=C(CH3)2, R4=R7=OH
licoflavone B (49) R1=R3=R6=HR2=R4= R7=OH, R5=-CH2CH= C(CH3)2
licoflavone C (50) R1=R2=R5=R6=H, R4= R7=OH, R3=-CH2CH= C(CH3)2



licoflavonol (51) R1=R2=R4=R5= R7= OH, R3=-CH2CH= C(CH3)2, R6=H
isolicoflavonol (52) R1=R2=R4= R7=OH, R3=R5=H, R6=-CH2CH= C(CH3)2
glabrone (53) R1=R2=R4=R5=R6=H, R3=R9=OH R7, R8=3'-CH=CHC(CH3)2-O-4'

glycyrrhisoflavone (54) R1=R3=R6=R7=OH, R2= R4=R5=R9=H, R8=-CH2CH= C(CH3)2
licoisoflavone A (55) R1=R3=R5=R7=OH, R2= R4=R8=R9=H, R6=-CH2CH= C(CH3)2
licoisoflavone B (56) R1=R3=R5=R7=OH, R2= R4=R8=R9=HR6, R7=3'-CH=CHC(CH3)2-O-4'
licoricone (57) R1=R2=R4=R6= R8= OH, R7= R9=OCH3, R8=-CH2CH= C(CH3)2
gancaoninH (58) R1=R3=R6=OH, R2=-CH2CH= C(CH3)2, R4=R5=R9=H, R7, R8=3'-CH=CHC(CH3)2-O-4'
gancaoninG (59) R1=R7=OH, R2=-CH2CH= C(CH3)2, R3=OCH3, R4=R5=R6=R8=R9=H



glyzaglabrin (60) R1=R2=R4=R8=H, R3=R5=OH, R6, R7=-O-CH2-O-
licoisoflavanone (61) R1=R3=R5=OH, R2= R4=R8=H, R6, R7=3'-CH=CHC(CH3)2-O-4'
glabroisoflavanone (62) A R1=R2=R6=R8=H, R3, R4=7-O-C(CH3)2CH=CH-8, R5=R7=OH
glabroisoflavanone (63) A R1=R2=R6=R8=H, R3, R4=7-O-C(CH3)2CH=CH-8, R5=OH, R7=OCH3

Figure 3 : Characteristic flavonoids from licorice. api: β-D-apiofuranosyl; glc: β-D-glucopyronosyl; rha: α-L-rhamnopyronosyl.

Flavonoids, as well as the triterpenoids, are polyhydroxy compounds. The hydroxies guarantee a strong ability to provide protons, thus giving a solid foundation of the ingrediants' antioxidant role. Some researches indicate that Flavonoids B o-ring phenolic hydroxyl group can contribute to its antioxidant activity. Researchers found Licochalcone B has the strongest ability of removing superoxide anion among Licorice flavonoids. The structure of Licochalcone B proves the rule[7].

THE MECHANISM OF GLYCYRRHIZIC ANTIOXIDATION

Glycyrrhizic ingredient act on the enzymes related to the free radicals

It is known that the free radicals of the body are in the balance state between the generation system and the protection system, both can be regulated by the enzymes[8]. Licorice plays a dual role in achieving antioxidation, that is, on the one hand, enhance the enzyme activity scavenging in vivo free radicals, on the other hand, inhibit enzyme activity generating free radicals.

The inhibition of oxidase

A lot of Oxidase in vivo are related to the generation of free radicals. For example, xanthine-oxidation enzymes (XO): ATP gradually degrades to ADP, AMP, adenosine, and finally the hypoxanthin in the state of ischemia. In addition, xanthine dehydrogenase transforms into xanthine oxidase by Ca_{2+}-dependent protease, uric acid and O_2- followed by[9]. Electron spin resonance (ESR) indicated that the eruption of free radicals result from this process in ischemia reperfusion[10].

In addition, P-450 enzymes, myeloperoxidase, lipoxygenase and cyclooxygenase enzymes in vivo can catalyze the generation of free radicals[11]. Some ingrediants in liquorice can inhibit the ability of XO, induce uric acid and clear out peroxide. Haraguchi.H et al.[12] found Licochalcone B and O can inhibit the creation of superoxide anions in XO systems, clear out 1,1-Diphenyl-2-picrylhydrazyl radical (DPPH radical) and elimnate superoxide anions in microsome. A study[13] showed Yunnan licorice saponins strongly clear the peroxide in xanthine / xanthine oxidase system, thus having obvious inhibitory effect on lipid peroxides of in vitro rat cardiomyocytes.

There are studies about Glycyrrhizic inhibition of P450 enzymes. Jeong, et al.[14] found 18β-glycyrrhetinic acid can effectively inhibit the expression of CYP4502E1 to stop the drug into harmful metabolites, thereby reducing the damaging effects on the mice's liver by carbon tetrachloride. Hoitak Chen et al.[15] found that glycyrrhizin (GL) can reduce the CYP1A1, elevate the activity of glutathione s-transferase, thereby inhibite hepatic metabolic activation of toxic substances.

Mononitrogen (NO) is a molecule with special effects, the excessive NO can react with free radicals to create strong oxides, thus damaging the body[16]. Study on another natural antioxidants -Theaflavins showed it can inhibit the mRNA expression of inducible nitric oxide synthase (NOS), thus reducing the contents of NOS and NO[17]. Using the model of mice, study also showed Glycyrrhizic flavononids can inhibit the increase of NO'content caused by ischemia reperfusion, but Various doses of licorice flavonoids had no significant ef-

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fect on nitric oxide synthase (NOS), its mechanism is not clear[18].

Activate the antioxidase

The antioxidases include Superoxide dismutase (SOD), GSH and Catalase (CAT), they can clear away free radicals.

These enzymes not only coordinate to prevent the damaging effects of reactive oxygen species, but also protect each other[11]: O2- can inactivate CAT and GSH-Px, so SOD protect CAT and GSH-Px by scavenging O2- H2O2 can inactivate Cu-Zn-SOD, so CAT and GSH-Px protect Cu-Zn-SOD by scavenging H2O2. Once a number of the mutual protection system decreases unnormally, the entire enzyme system will collapse, leading to the irreversible damage of the cell. Therefore, exogenous antioxidants has become essential to protective roles.

Song et al.[19] used the mice to establish the intestine model of ischemia reperfusion by lighating the superior mesenteric artery. The three different doses of FG were given before intestinal ischemia reperfusion, the changes of malonaldehyde (MDA), superoxide dismutase (SOD). Nitric oxide (NO). Nitric oxide synthase (NOS) in serum and intestine were observed after reperfusion of 24 hours following intestinal ischemia of 2 hours. They found that as compared with the ischemia reperfusion group, the level of MDA. NO in serum and intestinal issue were decreased significantly in the FG treated group (medium and high dose), Whereas, the level of SOD increased significantly in the FG treated group, and there was significant difference ($P < 0.05$). There was no significant difference in the level of NOS between the different doses of FG treated groups and intestinal reperfusion group ($t=2.15$, $P>0.05$)

Yu et al.[20] observed the protective effects of Diammonii Glycyrrhizinatis (DG) on lesion caused by myocardial ischemia in rats. Rusults showed that DG exerted protective effects on the ischemic myocardium, which was related to the inhibition of lipid peroxidation and the enhancement of SOD and ATP-enzyme activity. Chen et al.[21] also proved in the oxidative stress induced by Fe-NTA, magnesium isoglycyrrhizinate, within a certain strength range, obviously enhanced the activity of SOD and decreased the contents of MDA in supernates of rat hepatic stellate cells (HSCs) culture media.

Licorice not only protect the in vivo antioxidant enzymes, but also promote and mobilize the activity of antioxidant enzymes. Wang et al.[22] measured the activities of CAT, GSH-Px of brain tissue and LPO level in plasma after adminstration of liquorice decoctum in different time (15, 30, 45days) The result showed that the liquorice had an affect on senile rat by raising the activity of antioxide (CAT, GSH-Px) and dropping LPO level, indicating that liquorice had effect on antisenium, its further study had an important value.

The current study only stays at the impact of Licorice acting on the activity of antioxidant enzymes, there was no direct evidences about the regulation of Glycyrrhizic ingredients acting on the synthesis of antioxidant enzymes. It was known Fructus Schisandrae can induce the in vivo synthesis of antioxidant enzymes[23], so we think the Liquorice is worthy of study in this direction.

On the other hand, in the body's antioxidant system, there is a class of antioxidants - vitamin c, vitamin E and GSH, they are separately located in ECM, cell membranes and cytosol, acting as the first, second and third anti-oxidation line of defense, and maintaining the normal function of cells jointly. Vitamin c, vitamin E and GSH play an important role of antioxidant in the body, compared to SOD and other enzymes, they are referred to as non-enzymatic antioxidants[11]. Studies showed that Tea polyphenols and other natural antioxidants can make these three categories of anti-oxidants renewable[24], but this anti-oxidative mechanism has not been reported in the study of licorice.

Act on the free radicals directly

Free radicals are atoms or radicles with unpaired electron. They are easy to lose electron (oxidation) or to seize electron (reduction), especially for its strong oxidation, leading to lipid peroxidation strongly. Free Radicals including two types: inorganic free radicals and organic free radicals. The organic free radicals include polyunsaturated fatty acids oxidation products of ROO-, RO-, etc. The inorganic ones mainly refers to O2 -, • OH, H2O2 and other reactive oxygen species[25]. Glycyrrhizic ingredients play dual effects scavenging both free radicals: preventative antioxidation and

denial-of-chain antioxidation.

Remove the inorganic free radicals

Fu et al.[26] studied scavenging effects of 14 kinds of licorice flavonoids and three kinds of triterpenoid compounds on reactive oxygen species. The results showed that Glycyrrhizicthe coumarin had the most significant scavenging effects on superoxide anion radicals. Licochalcone A and 4 - methoxy-4-hydroxy-chalcone had the most significant scavenging effects on the hydroxyl radical.

Ju et al.[27] studied the antiperoxidant activity of glycyrrhiza flavonoid (F G) by using colorimetric estimation of lipid peroxide (MDA) formation. The scavenging effects of FG on O2- and OH were investigated by using chemiluminescence method and spin trapping technique in different Systems. The results were as follows: FG 0.26-26.5μg/ml or 2.58-258μg/ml was shown to markedly scavenge O^{2-} in alkaline/DMSO or xanthine/xanthine oxidase systems respectively, in a concentration-dependent manner. FG 144μg/ml or 258μg/ml also significantly scavenged the active oxygen radicals produced by PMN stimulation with Phorbol-12-myristate-13-acetate (PMA) or OH^- produced in Fenton's reaction respectively (Figure 4 and Figure 5). Ju[28] also proved sodium 18beta-glycyrrhctinic acid (SGA) has the similar effect and can trap and clear away active oxygen radicals.

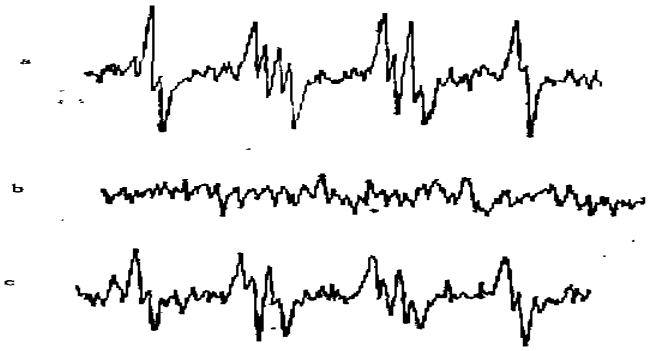

Figure 4 : Electron spin resonance spectra of DMPO spin trapped free radicals in the respiratory burst of PMA-stimulated human polymorphonuclear leukocytes incubated with (a) control, (b) FG 144μg/ml and (c) Vit E144μg/ml.

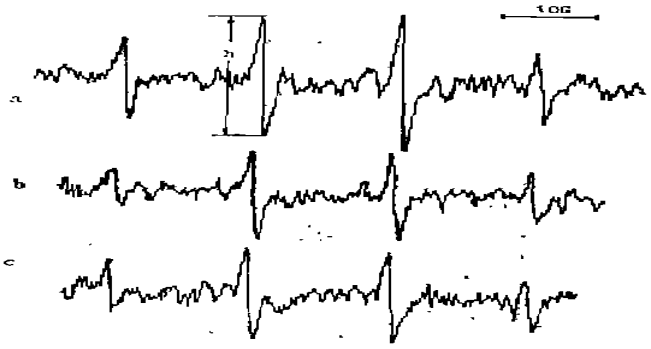

Figure 5 : The hydroxyl radical spin adduct spectrum of DMPO produced by Fenton's reaction with (a) control, (b) FG 258μg/ml and (c) VitE 258μg/ml

Wu et al.[29] used spectrophotometry to investigate the oxygen free radical scavenging effect of glycyrrhizae total flavonoids (GTF) and found that GTF in xanthine/xanthine oxidase system cound scavenge effectively the superoxide anion (IC50 = 226mg/L) dose-dependently, the IC value lower than Vitamin E (IC50 = 770mg/L); Flavonoids of Glycyrrhiza processed significantly scavenging effect on hydroxyl radical (OH) produced by Fention reaction, the effect was more patent than that of manna, with the OH –scavenging IC 50 and the OH^- inhibiting IC50 were 1/25 and 1/139 respectively as compared with that of manna.

There were researches about the antioxidation of Glycyrrhiza Polysaccharides. Yang et al.[30] tested the scavenging quality of polysaccharides on DPPH free radical, hydroxyl free radical (·OH) and super oxide free radical (O^{2-}) by spectrophotometry. The result showed that polysaccharides has good scavenging effect on ·OH DPPH· and O^{2-}. At a certain concentration range, the scavenging effect of polysaccharide was positively correlated to its concentration; liquorice polysaccharide has a good hydroxyl radical scavenging activity, and the removal ability of hydroxyl radical is better than that of superoxide anion.

Eliminate lipid free radicals and inhibit lipid peroxidation

Lipid will produce free radicals in the reactive oxygen species and radiation conditions, thus causing lipid free radical chain reaction[31]. (Figure 6)

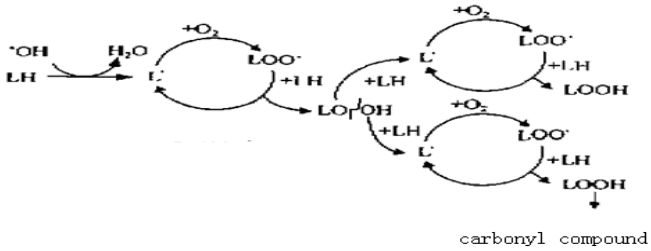

Figure 6 : Lipid free radical chain reaction

Certain components of liquorice can react with the

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intermediate production of the chain reaction, thus leading to the termination of the lipid oxidation.

Grease and oil-rich foods will go decay due to the oxidation during storage . In order to maintain the quality of edible oils and fats, people use synthetic antioxidants, for example BHA and BHT, since 1950s. In the past decade, people constantly have doubts about synthetic antioxidants' safety in food, so the study, exploitation and utilization has become an important task. Cui et al.[32] studied the antioxidation activity of the total flavonoids of Glycyrrhiza on edible oils, using peroxide value (POV) by Schaal oven-storage test. The results showed that the total flavonoids are of good antioxidant effects on four edible oils. Furthermore, the activity on lard is the strongest and dose-dependent. The total flavonoids of Glycyrrhiza added with vitamin C, citric acid or tartaric acid exhibit remarkablely synergistic antioxidation activity in lard. The antioxidation activity of the total flavonoids synergized with synthetic antioxidants such as BHA, BHT or PG is proved to be better than single antioxidant used.

For organisms, the cell membrane lipid peroxidation can cause serious damage to membrane systems, even leading to cell death (10). Research[33] has shown that licorice flavonoids can prevent the lipid peroxidation of low density lipoprotein (LDL), reduce the susceptibility coefficient of LDL in the patients' plasma, and improve the antioxidation and anticoagulation of LDL in plasma. Chen et al.[34] studied the effect of 18beta-sodium glycyrrhetate (18beta-SG) on the uptake of oxidized low density lipoprotein (oxLDL) in petoneal macrophage of mice cultured in vitro. The result showed the contents of total cholesterol (TC) and cholesterol ester (CE) were increased significantly by oxLDL, however, which were inhibited dose-depently in macrophage in oxLDL group pre-treated with -SG; As compared with oxLDL group, oxLDL plus -SG group decreased MDA content and increased the activities of SOD and GSH-PX. From the conclusion above, -SG can significantly inhibit mice peripheral macrophages' uptake of oxLDL, this can be associated with its action in enhancing of peripheral macrophages' antioxidation.

Glycyrrhizic effect of inhibition of lipid peroxidation also includes inorganic free radical scavenging. Lipid peroxidation includes three steps: start-up, diffusion, the termination[25], so we think Glycyrrhizic play the role of anti-lipid peroxidation should include two parts: First, certain components of licorice participate in the initial reaction, an effective way to remove reactive oxygen species, preventing the start-up of lipid peroxidation; Second, reaction with lipid free radical reactions, resulting in the break-off of the chain. These two mechanism combine together, leading Glycyrrhizic significant anti-lipid oxidation.

Glycyrrhizic ingredients complex with metal ion inducing oxidation

Some transition metals such as copper, iron, can catalyze LDL oxidation or the formation of free radicals. Because flavonoids have 4-keto-5-hydroxy-molecular structure (see Figure 3), they can complex with metal ions. The B ring 3 'and 4' position OH group is also necessary to cu ion complexation. Hu et al.[7] inferred Flavonoids can complex the metal ions, thus inhibiting auto-oxidation of lard. The complex role of flavonoids depends on the formation manner of the phenolic hydroxyl group and the flavone carbonyl on the ring. This mechanism should become one of the reasons why Glycyrrhizic flavonoids inhibit LDL oxidation catalyzed by cu ion.

Glycyrrhizic acid molecules, containing the carboxyl and carbonyl, easy to form complex with the metal ions. Zhuo et al.[35] studied the Glycyrrhizic acid-Cu(II) and glycyrrhizic acid-Fe(1lI) complexe characterized by UV -Vis spectra analysis. From the ultraviolet spectrum we can see glycyrrhizic acid has the largest absorption peak at 267nm, which is the characteristic absorption of gly-

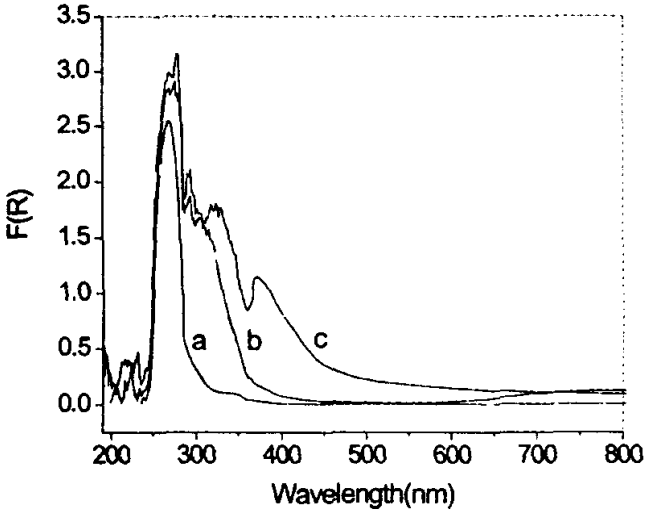

Figure 7 : UV patterns of glycyrrhizic acid (a), glycyrrhizic acid-Cu2+ (b) and glycyrrhizic acid-Fe3+ (c)

cyrrhizic acid's α, β unsaturated carbonyl conjugated bond; the copper (II) complexes appeared two new peaks at 290nm and 310nm, iron (III) complex appeared three new peaks at 290nm, 325nm and 375nm, while UV absorption did not change significantly at 267nm . All the above indicate glycyrrhizic acid formed complex with Copper (II) ion, Fe (III) ion respectively (Figure 7). The FT-IR spectra also showed glycyrrhizic acid formed a complex with Cu (II), Fe (III) ion based on the carboxyl (Figure 8).

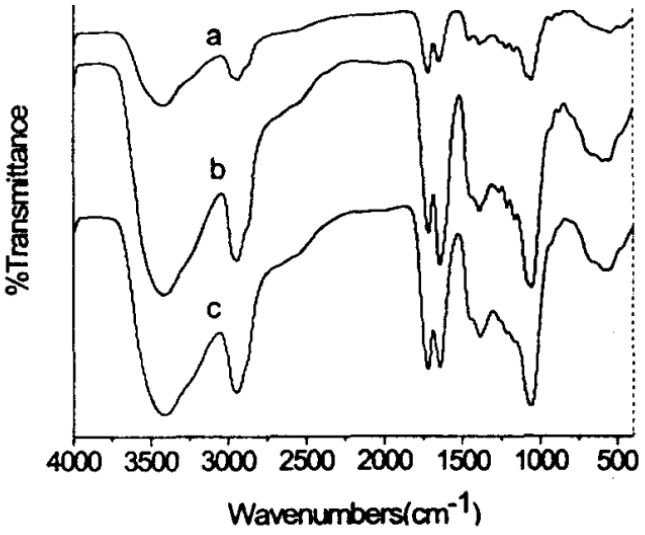

Figure 8 : FT-IR spectra of glycyrrhizic acid (a), glycyrrhizic acid-Cu2+ (b) and glycyrrhizic acid-Fe3+ (c)

Synergistic effect of Glycyrrhizic ingredients and other substances

Some natural substances are of weak anti-oxidative capacity, but this activity increased significantly adding some non-antioxidant. Flavonoid compounds play the efficient role of antioxidant activity in oils and fats, which is the result of the synergistic effect with phospholipid[36]. Researches have shown[37] Isoflavones' anti-oxidative activity can be significantly improved by phospholipids (especially phosphatidylethanolamine) in the edible oil, the mechanism can be the release protons of the synergistic agents, thus enabling the rapid decomposition of hydrogen peroxide and will not generate free radicals.

Wang et al.[4] studied the synergistic effect of Glycyrrhizic antioxidants with other antioxidants such as BHT, α-tocopherol and lecithin as well as organic acids (ascorbic acid, citric acid, DL-malic acid, fumaric acid and sorbic acid). They found that Glycyrrhizic antioxidants had synergistic effect with all the antioxidants mentioned above, α-tocopherol was the strongest among them. The synergistic effect between Glycyrrhizic antioxidants and organic carboxylic acids have the law as follows: citric acid > DL-malic acid > fumaric acid ≈ sorbic acid.

By analysis of Glycyrrhizic ingredients, we think the mechanism is that certain Glycyrrhizic components form large and stable hydrogen precursors with other substances such as citric acid by the hydrogen bonding association, which not only enhance the anti-oxidative activity of licorice, but also combine the free radicals generated by unsaturated fatty acids.

The immunoregulatory role of Glycyrrhizic ingredients

Many research proved a close relation between the immune ability and the anti-oxidative activity of the organism[11,38]. Liu et al.[39] found that the Glycyrrhizic flavonoids can increase the number of leukocytes, lymphocytes and the CD4 / CD8 cell ratio, thus improving the immune ability of the body.

Glycyrrhizic acid (GA) has a non-specific immune regulatory role, its mainly enhancing the cellular immunity, for example the phagocytosis of MΨ cells. Researches have shown recently the total number of white blood cell (WBC) increased by 5 times in mouse peritoneal immune system after the use of GA. Using ursolic acid, oleanolic acid and other drugs on animal treatment, the largest number of WBC continuous for only 6 days, while treatment using could last for more than nine days. The increasing rate of the total number of WBC was 91.48 ± 4.6%, 135.75 ± 6.4%, using ursolic acid and oleanolic acid respectively. In contrast, the figure was 114.9 ± 18%. The results showed that GA can play the immunoregulatory role in the body[40]. Yoshikama et al[41] proved that glycyrrhizin could selectively enhance T-lymphocyte proliferation and activity, increase $CD4^+$ cells, decrease $CD8^+$ cells.

The study on Glycyrrhizic polysaccharide (GPS) showed that GPS can stimulate the activity of mouse spleen lymphocytes, dose-dependently. The main performance is GPS can remarkably elevate IL-2 release. IL-2 is an effective T cell growth factor, and is also one of the key factors that regulating Th1 and Th2 cells' function. Secretion of IL-2 can promote Th1cell prolif-

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eration and differentiation, thus promoting Th1 cell-mediated immune function[42].

THE RELATION BETWEEN THE PHARMACOLOGICAL FUNCTION AND THE ANTI-OXIDATIVE MECHANISM

The anti-atherogenic role

In vivo and *in vitro* experiments showed that LDL oxidation would lead to atherosclerosis (LDL is the cholesterol carrier in serum), Glycyrrhizic Flavonoid slow down atherosclerosis by confrontation to the lipoprotein lipid peroxidation[43]. Fuhrman et al.[44] found that Licorice extracts (the main component is Glabridin) could protect LDL, preventing its oxidation, through combination[45].

Israeli academics[46] separated Glycyrrhiza glabra (G.glabra), obtaining seven components such as Glycyrrhizin A, Glycyrrhizin B, Glabridin, isoprene-based chalcone etc. Glycyrrhizic flavonoids in G.glabra was the main composition (11.6%, w/w). They detected the inhibitory ability of the seven components to LDL oxidation and found Glabridin played the main role. They also studied the structure and inferred there was a great relation between the inhibition to LDL oxidation and the position of flavanone derivaties' hydroxy group. The results suggested the phenolic part in the flavanone must be connected with the lipophilic part to create the inhibitory effect while this effect was not sufficient with the phenolic part separately existed[47]. The same year, they also found[48] Glabridin could reduce the susceptibility of LDL oxidation, the mechanism might be the combination of LDL particles and Glabridin played a role in the protection of LDL, inhibiting the formation of lipid peroxide, protect the carotenoid binded with LDL.

Researches showed[49,50] that Glabridin could be gathered in macrophages, affecting macrophage-induced LDL oxidation. Its inhibitory rate to LDL oxidation was more than 80%, hindering rate to the release of peroxides in macrophage was 60%, reducing rate to protein kinase activity was 70%. Accordingly, Glabridin consumption can inhibit cell-induced LDL oxidation and atherosclerosis.

The liver-protection role

Researches found[51] that Glycyrrhizic flavonoids could alleviate the increasing activity of glutamic-pyruvic transaminase (GPT) and lactate dehydrogenase (LDH) in serum induced by CCl_4. CCl_4 could be activated by cytochrome P450 enzymes in liver microsomes to generate $\bullet CCl_3$. $\bullet CCl_3$. attacked on the liver cell membrane phospholipid molecules, causing lipid peroxidation in liver microsomes and the covalently conjugation of lipids and proteins. With the infiltration of cytoplasmic soluble enzymes into blood, the liver cell membrane's structure and function was damaged. Glycyrrhizic flavonoids inhibited the infiltration, thus reducing the unnormally increasing activity of GPT and LDH in the mouse serum poisoned by CCl_4. At the same time, licorice flavonoids could significantly inhibit the increase of malonydiadehyde (MDA) content in mouse liver poisoned by CCl_4.

When pre-treated with Glycyrrhizic flavonoids, the increase of MDA content and the exhaust of deoxidized- glutathione could be inhibited, dose-dependent. By electron microscope examination, Glycyrrhizic flavonoids did protect the ultrastructure of the liver cells induced by ethanol. The mechanism is the removal on the oxygen free radicals[52].

Diammonium glycyrrhizinate (DG) could protect the lipid peroxidative damage in liver. After treated with DG, the content of MDA decreased, the content of GSH increased and the activities of SOD, CAT and GSH-Px increased in liver of mice, compared with cadmium-exposed group. Pathological damage to tissue and cell ultrastructure was significantly recovered[53].

Glycyrrhetinic acid (GA) could inhibit liver microsomal lipid peroxidation induced by a variety of chemicals by in vivo study[54]. It had inhibitory effect on the generation of free radicals in rat liver microsome induced by CCl4. The same effect was found in mouse peritoneal macrophages stimulated by zymosan. The clinical use of tiopronin (a drug having effects as follows: free radical scavenging, protection of liver cell membrane, the alleviation of exogenous toxic substances) combined with GA (Ganlixin) had significantly curative effect[55].

The anti-hemolytic role

Fukaitoshio et al[56] found Licochalcone A could reduce the amount of urinary protein and increase the intensity of free radicals, in sodium salt of ascorbic acid.

Zou et al.[57] studied antioxidative activity of compounds identified in liquorice. 4'5'7'-trihydroxide-8- isoamylene flavonoid,

Glycyrrhizin, Glabrone showed significant activity in scavenging oxygen free radicals. Glabrone had a good inhibitory effect on the hemolysis induced by H_2O_2. Both of Glycyrrhizin and Glabrone could inhibit the (HPD + hv)-induced hemolysis, inhibitory effect better than some typical anti-oxidants.

The anti-tumor role and the protective effect for cancer chemotherapy

Oxygen free radicals played an important role in the pathogenesis of tumor. Oxygen free radicals could cause damage to the base, leading to gene mutation, resulting in cancer cells[11]. Using antioxidants to prevent occurrence of tumor has been focused.

G9315(extracted from G. inflate Bat.) are compounds containing six flavonoids, could significantly inhibit the chemiluminescence of croton oil-induced Wista rat neutrophils (PMN) and Balb / c newborn mouse skin epidermal cells, as well as liver mitochondrial lipid peroxidation. Its in anti-tumor role had relations with its anti-oxidative effect[58].

CYP450 is one member of cytochrome b protease superfamily, which is widely distributed in liver, brain, kidney, lung, small intestine and other parts, but the content is richest in the liver. Its main physiological function is to metabolize the endogenous and exogenous compounds. Exogenous carcinogenic substances were activated through CYP450, the cancer chemotherapy drugs were also metabolized by the enzymes. Researches have found[14] that glycyrrhizin (GL) could reduce the CYP1A1, elevate the activity of glutathione s-transferase, thereby inhibiting hepatic metabolic activation of toxic substances.

Ceriello et al.[59] found that glutathione could antagonize the toxicity (immuno-suppressive effects) of cyclophosphamide (an anti-tumor medicine) and acrolein (its metabolite) to mouse spleen cells, without affecting the anti-tumor activity. The study also found that combinative use of Glycyrrhizin (GL) and the chemotherapy drugs such as ADM, CP, TNF could increase the content of glutathione, thus reducing the damage of free radicals (produced by these drugs) to normal cells.

The anti-inflammatory role

Researches found[60] that Glycyrrhetic acid sodium salt could inhibit the formation of malondialdehyde (MDA) in the inflammatory part of the mice. This effect could be antagonized by exogenous arachidonic acid. In addition[61], it also had efficacy on chemical peritonitis in rats. The effects were as follows: inhibiting the migration of neutrophil (Neu), inhibiting the synthesis of prostaglandin E (PGE), reducing capillary permeability, scavenging oxygen free radicals.

The anti-inflammatory effects of 11-sodium deoxyglycyrrhetinic acid were observed by 3 models in Luo's experiment[62]: xylene-induced mice ear swelling, chronic granuloma by filter paper method, air sacsynovltis. The prostaglandin E2(PGE2) in serum and activity of NOS were detected. Results showed that 11-sodium deoxyglycyrrhetinic acid could inhibit mice ear swelling (xylene-induced) as well as chronic granulom a by filter paper method obviously, and decreased PGE2 level in serum. It also could decrease PGE2 level and reduce the activity of NOS and the leve1 of NO significantly. Accordingly, 11-sodium deoxyglycyrhetinic acid had an anti-inflammatory effect, and it may be related to the inhibitory effects of the synthesis of PGE2 and NO, besides the inhibitory effect of 1ipid peroxidation.

Liu et al.[63] studied the effect of diammonium glycyrrhizinate (DG) and possible mechanism on ulcerative colitis (UC) induced by trinitrobenzene sulfonic acid (TNBS) in rats. The result showed that colonic mucosa of UC rats showed hyperemia, ulcers and infiltrated inflammatory cells. DG dose-dependently attenuated the severity of gross lesions and reduced the histopathological scores MPO activity and MDA concentration in colon, and IL21β and TNF2α in serum were significantly increased; SOD activity was reduced in UC rats. Accordingly, Colon administration of DG exerts a protective effect on colon injury in TNBS2induced UC rats, which may be due to anti2oxidation and inhibition of inflammatory cytokines.

The anti-aging role

Free radical theory of aging explained that the oxygen free radical initiated chain reaction, producing more toxic hydroxyl radical and H_2O_2. These free radicals attack cell cytoplasm to generate the lipid peroxidation.

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Then LPO attack biological macromolecules, such as protein and nucleic acid, thus creating old age Lipofuscin. Lipofuscin content are increasing with age in general, and this will lead to decline in cell function, which manifests as aging body. Exogenous free radical scavenger can reduce LPO Organize content to play the role of anti-aging[64].

Researches have shown that rat plasma LPO levels increase with age, also reported that elderly rats GSH-Px (glutathione peroxidase) activity[65] and catalase (CAT) activity[66] lower than young people significantly, so LPO content and GSH-Px, CAT activity in animals are important biochemical markers of aging. Wang et al.[22] studied the effect of liquorice on senile rat, and found that lower antioxidation and higher lipid peroxidation were important cause for senility. The liquorice had an affect on senile rat by raising the activity of antioxide (CAT, GSH-Px) and dropping LPO level.

In recent years there are many studies on carbohydrate anti-aging or anti-fatigue effect. Yang[30] used ultrasonic-microwave synergistic extraction technique to extract polysaccharides in liquorice. Hydroxyl free radical (·OH) and super oxide free radicall (O^{2-}·) were detected by spectrophotometry. The result showed that polysaccharides has good scavenging effect on ·OH and O^{2-}, which can be the basis for health-care drugs using Glycyrrhiza polysaccharides.

THE PROSPECT

Liquorice is extensively used in the traditional Chinese medicine (TCM). In recent years, there are a lot of pharmacological research on licorice. But the vast majority concentrated in the Triterpene saponins and the Flavonoids.

As one of the major components, Glycyrrhiza Polysaccharides was studied much less. Flavonoids in licorice and glycyrrhizic acids have been developed into food additives, pharmaceuticals, health care and other skin care products, while the product of liquorice polysaccharides are still in primary stage. By reference of herbal polysaccharides' development recently, we treat liquorice polysaccharide as a good research direction.

On the other hand, the research ideas in general are as follows: separation and purification of licorice, and then conduct a study of its pharmacological effects. Although the source of the pharmacological effects can be precisely known, but after all, licorice is natural Chinese herbal, the interaction between the various components in the body can be playing more important role than the single component. Therefore, we suggest that on the basis of the study of the active ingredients, we should pay more attention to the total-herb activity and the interaction between different components.

It is necessary for drugs to arrive at the target site with a certain concentration. Licorice has such characteristics. Researches have shown[67] that glycyrrhetinic acid is a target to liver cells, can be used as therapeutic drug delivery (such as anti-liver cancer drugs and antiviral drugs) . The use of licorice antioxidation to develope health care new drugs has broad prospects.

The means of molecular chemical modification to improve the biological activity of antioxidants and expand the scope of application is a forward-looking research field. The researchers have transformed vitamin C into fat-soluble vitamin C ascorbyl palmitate, the tea polyphenol (TP) into a fat-soluble compounds[68]. Research showed that these chemical modification can significantly improve its antioxidative activity.

At present, the chemical modification of licorice antioxidative ingredients mainly concentrated in triterpenoids (glycyrrhizic acid and glycyrrhetinic acid). Glycyrrhizic acid contains hydroxyl and carboxyl groups, thus reacting with drugs containing hydroxyl, sulfo, carboxyl functional groups. The ester derivatives of glycyrrhizin can add, complement or even generate new pharmacological effects.

Russian scholars[69] produced glycyrrhetic acid through chemical semi-synthetic method. They found that the compounds have similar hypolipidemic and antioxidant activity with glycyrrhetinic acid, lower side effects. Another Russian scholar[70] successfully trasformed 18β-glycyrrhetinic acid into 18α-glycyrrhetinic acid through a more easy way. The method is of great significance in similar structural design and synthesis. Reasearcher in Xinjiang Medical University used structural modification principle, glycyrrhetinic acid as the lead compounds, to eliminate or reduce its side effects of false aldosterone. They synthesized new derivatives with antioxidant, anti-inflammatory and anti-atherosclerotic activity[71]. We trust that deep research

into Glycyrrhizic antioxidation will provide more detailed theoretical basis for the utilization of this traditional chinese herb.

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