LICORICE ROOT, FLUID, EXTRACT AND POWDER

SYNONYMS

Glycyrrhiza glabra
Liquorice root or extract
Regaliz [Spain]
Reglisse [France]

CHEMICAL STRUCTURE

A complex mixture of various compounds: Its main ingredient is the triterpene glycoside glycyrrhizin [the potassium calcium magnesium salt of glycyrrhizin acid], commercial glycyrrhizin is the ammonium salt of the acid. The concentration of which has been reported to range from 1 to 24 % depending upon the sources and assay methodology. Glycyrrhizin acid is a glycoside that on hydrolysis yields glycyrrhetic acid and two molecules of glucuronic acid. In addition licorice is reported to contain flavonoids and isoflavonoids, triterpenoids, B vitamins, coumarins, 2 - 20 % starch, 3 - 14 % sugar [glucose and sucrose] lignin, amino acids, gums, and a volatile oil consisting of many aroma compounds [Fenaroli 1995; Leung *et al.,* 1996].

IDENTIFIER DETAILS

CAS Number	:	8008-94-4 or 68916-91-6 (1405-86-3 Glycrrhizic acid)
CoE Number	:	218
FEMA	:	2630
EINECS Number	:	272-837-1
E Number	:	

CLP CLASSIFICATION

Ingredient CLP Classification: No

Endpoint	Classification	Category
Acute Oral Toxicity	-	-
Acute Dermal Toxicity	-	-
Acute Inhalation Toxicity	-	-
Skin Corrosive/irritant	-	-
Eye Damage/Irritation	-	-
Respiratory Sensitisation	-	-
Skin Sensitisation	-	-
Mutagenicity/Genotoxicity	-	-
Carcinogenicity	-	-
Reproductive Toxicity	-	-
Specific Target Organ Toxicity	-	-
Aspiration Toxicity	-	-

SPECIFICATIONS

Melting Point:-

Boiling point: -

PURPOSE

Flavouring substance.

STATUS IN FOOD AND DRUG LAWS

CoE limits:

Beverages (mg/kg)	Food (mg/kg)	Exceptions (mg/kg)
-	-	-

Acceptable Daily Intake:

ADI (mg/kg)	ADI Set by	Date Set	Comments
Glycyrrhizinic acid Not established	JECFA	2005	The Committee concluded that the critical effect of glycyrrhizinic acid is pseudohyperaldosteronism, which, when sustained, can lead to elevated blood pressure.The Committee concluded that the safety evaluation of glycyrrizinic acid should be based on the data from humans. As the available data from clinical studies did not allow the Committee to adequately characterize sensitive population subgroups, an ADI for glycyrrhizinic acid was not established. The available data suggest that an intake of 100 mg/day (about 2 mg/kg bw per day) would be unlikely to cause adverse effects in the majority of adults. The Committee recognized that, in certain highly susceptible

			individuals, physiological effects could occur at intakes somewhat below this figure. The intake data indicate that consumers with a high intake of liquorice confectionery or herbal tea containing liquorice may be exposed to glycyrrhizinic acid at more than 100 mg per day.

FDA Status: [CFR21]

Section Number	Comments
184.1408	Generally recognised as safe (GRAS). Liquorice and liquorice derivatives (0.05 % - 16 %).

HUMAN EXPOSURE

Natural Occurrence: Licorice is an herbaceous plant native to southern Europe; it grows wild in Eastern Europe. The plant is 1 to 2 m tall and has a large creeping root [the secondary roots are branched], erect stalk, alternate leaves, violet flowers, and kidney-shaped seeds. The parts of the plant which are used are the stolons and the roots (at least 2 years old). Most commercial liquorice is obtained from G.glabra. The intense sweetness of liquorice which is 50 times sweeter than sucrose comes from the glycyrrhizin [Fenaroli, 2005].

Reported Uses: Licorice (extract) is reportedly used in baked goods at 630.7 ppm, frozen dairy at 553.20 ppm, meat products at 800.00 ppm, non-alcoholic beverages at 196.7 ppm, gelatins and puddings at 209.8 ppm, sweet sauce at 15.55 ppm and alcoholic beverages at 1424.00 ppm. Individual consumption has been reported to be 0.04943 mg/kg/day (liquorice), 0.03192 mg/kg/day (extract) and 0.03997 mg/kg/day (extract powder) [Fenaroli, 2005].

Estimated consumption of liquorice and glycyrrhizin in the US is 0.027 - 3.6 mg/glycyrrhizin/kg/day [Isbrucker & Burdock., 2006]

Sources other than foods: Licorice has been reported to have various uses among those are pharmaceutical applications including, detoxification, anti-inflammatory, anti viral, anti-ulcer, anti-atherogenic and anti carcinogenic [Wang *et al.,* 2001]. Licorice has been used extensively in cough drops and syrups, laxative and tonics and reportedly been used to treat respiratory tract catarrhs and for the treatment of gastric duodenal ulcers [Leung *et al.,* 1996].

TOXICITY DATA

Carmines (2002), Rustemeier *et al*., (2002), Roemer *et al*., (2002) and Vanscheeuwijck *et al.,* (2002) reported on a testing program designed to evaluate the potential effects of 333 ingredients added to typical commercial blended test cigarettes on selected biological and chemical endpoints. The studies performed included a bacterial mutagenicity screen [Ames assay] a mammalian cell cytotoxicity assay [neutral red uptake], determination of smoke chemical constituents and a 90-day rat inhalation study. Based on the

findings of these studies, the authors concluded that the addition of the combined ingredients, including licorice at levels up to 18802 ppm, "did not increase the overall toxicity of cigarette smoke" [Carmines, 2002].

In Vivo Toxicity Status

Test Type	Species	Route	Reported Dosage
LD_{50}	rat	oral	14,200 mg/kg
LD_{lo}	rat	oral	114 mg/kg/day
LD_{50}	rat	i.p.	1420 mg/kg
LD_{50}	rat	s.c.	4200 mg/kg
LD_{50}	mouse	i.p.	1500 mg/kg
LD_{50}	mouse	s.c.	4000 mg/kg

[Lewis, 2000]

$B6C3F_1$ mice were dosed with licorice root extract for 30 or 90 days at 0, 8 and 25 % of the diet, the treated mice showed a range of treatment related clinical signs including, poor weight gain with 30 % mortality in rats dosed liquorice root at 25 % of the diet. Lesions were noted in the liver spleen and thymus. A modest increase in the liver activity of UDP glucoronyl transferase activity and a marked increase in 7-ethoxycoumarin 0-dethylase activity were reported [Mirsalis *et al.,* 1993]. Wang *et al.,* (1991) reported that β-glycyrrhetinic acid markedly inhibited rat epidermal aryl hydrocarbon hydroxylase, 7-ethoxy coumarin-0-dethylase, and 7-ethoxyresorufin 0-dethylase in a dosage dependent manner. α-Glycyrrhetinic acid had a similar action but was less affective [Wang *et al.,* 1991].

A no observed effect level [NOEL] was determined for glycyrrhizic acid in human female volunteers [females were chosen as they were found to be more susceptible then males]. Glycyrrhizic acid was given to 39 females at doses of 1, 2 and 4 mg/kg/day for 8 weeks. They suggest a no effect level of 2 mg/kg from the results of the study and an ADI of 0.2 mg/kg [Van Gelderen *et al.,* 2000].

Carcinogenicity and mutagenicity

A recent mouse skin painting study investigated the carcinogenicity of condensate prepared from cigarettes containing a number of additives in combination, including licorice extract at 18001 ppm. The authors concluded that the study "did not indicate any substantive effect of these ingredients on the tumourigenicity of cigarette smoke condensate" [Gaworski *et al.*, 1999].
[It should be noted that the cigarettes contained a typical American blend humectant and sugar component (*i.e.* glycerine ≈ 20,000 ppm, propylene glycol at ≈ 24,000 ppm, and brown invert sugar at ≈ 24,000 ppm)] [Gaworski *et al.*, 1999].

Maser (2004) stated that as less than 20 % of habitual smokers develop lung cancer has stated that genetic, nutritional and environmental factors may be responsible. Glycyrrhetinic acid (a major component of licorice) inhibits four

pathways for the detoxification of nicotine derived nitrosamine ketone (NNK) [Maser 2004].

Oral administration of disodium glycyrrhizinate to male and female B6C3F1 mice at 0.1 % [Male] and 0.3 % [female] for 96 weeks failed to induce any evidence of tumourigenicity or chronic toxicity when compared to control mice [Kobuke *et al.,* 1985].

In a two stage model for skin tumorigenesis in Sencar mice, administration of 0.05 % glycyrrhizin in the drinking water prior to administration of 7,12 dimethyl benz[a]anthracene (DMBA) and 12-O-tetradeconyl phorbol-13-acetate [TPA] for 1 group and administration after 112 days after DMBA application for group 2, gave protection against the initiation of skin tumours [Argawal *et al.,* 1991].

Glycyrrhetinic acid was administered to backs of ICR mice and was found to protect against the rapid DNA damage caused by benz[alpha]pyrene [BaP]. At concentrations of 5 and 20 µg/ml this corresponded to 70 and 80 % protection. In ICR mice exposed to croton oil on the back for 5 hours, the administration of 50 - 200 mg/kg/day of glycyrrhetinic acid lead to a 20 - 80% reduction in the induced elevation of ornithine decarboxylase [ODC]. The authors suggest that glycyrrhetinic acid has both effective anti-promoting and anti-initiating properties [Chen *et al.,* 1994].

1 % Licorice water extract treated female A/J mice were exposed to either BaP [100 mg/kg per occasion] every 2 weeks for 8 weeks or administered *N*-nitroso diethylamine [20 mg/kg p.o.] once weekly for 8 weeks. Oral administration of 1% licorice water extract in the drinking water was reported to significantly reduce the multiplicity and tumor incidence of BaP or *N*-nitroso diethylamine in both the mouse lung and fore stomach [Wang *et al.,* 1992].

Glycyrrhizin has been reported to inhibit mouse lung and liver tumourigenesis induced by 4-nitroquinoline-1-oxide in ddY mice [Watari *et al.,* 1976; Nishino 1992]. The multiplicity of the natural incidence of hepatomas was reported to be reduced by 50 % with the addition of glycyrrhizin [5 mg/100 ml] of drinking water. The administration of glycyrrhizin at 5 mg/100 ml in the drinking water again was found to reduce by 75 % the lung tumor promoting effect of glycerol initiated by 4-nitroquinoline-1-oxide in ddY mice [Nishino, 1992].

Reproductive and Developmental toxicity

The increased cancer risk associated with hormone therapies has encouraged many women to seek non-hormonal alternatives including botanical supplements such as hops (Humulus lupulus) and licorice (Glycyrrhiza spec.) to manage menopausal symptoms. Previous studies have shown oestrogenic properties for hops, likely due to the presence of 8-prenylnarigenin, and chemopreventive effects mainly attributed to xanthohumol. Similarly, a combination of oestrogenic and chemopreventive properties has been reported for various Glycyrrhiza species. The major goal of the current study was to evaluate the potential oestrogenic effects of three licorice species

(Glycyrrhiza glabra, G. uralensis, and G. inflata) in comparison with hops. Extracts of Glycyrrhiza species and spent hops induced oestrogen responsive alkaline phosphatase activity in endometrial cancer cells, oestrogen responsive element (ERE)-luciferase in MCF-7 cells, and Tff1 mRNA in T47D cells. The oestrogenic activity decreased in the order H. lupulus > G. uralensis > G. inflata > G. glabra. Liquiritigenin was found to be the principle phytoestrogen of the licorice extracts; however, it exhibited lower oestrogenic effects compared to 8-prenylnaringenin in functional assays. Isoliquiritigenin, the precursor chalcone of liquiritigenin, demonstrated significant estrogenic activities while xanthohumol, a metabolic precursor of 8-prenylnaringenin, was not estrogenic. Liquiritigenin showed ERβ selectivity in competitive binding assay and isoliquiritigenin was equipotent for ER subtypes. The estrogenic activity of isoliquiritigenin could be the result of its cyclization to liquiritigenin under physiological conditions. 8-Prenylnaringenin had nanomolar estrogenic potency without ER selectivity while xanthohumol did not bind ERs. These data demonstrated that Glycyrrhiza species with different contents of liquiritigenin have various levels of oestrogenic activities, suggesting the importance of precise labeling of botanical supplements. Although hops shows strong estrogenic properties via ERα, licorice might have different estrogenic activities due to its ERβ selectivity, partial estrogen agonist activity, and non-enzymatic conversion of isoliquiritigenin to liquiritigenin [Hajirahimkhan et al., 2013].

Administration of glycyrrhizic acid at 21.3 - 679.9 mg/kg/body weight to female Sprague Dawley rats on days 7-17 of gestation, led to a dosage-related increase in embryo lethality. Renal ectopy increased significantly at the highest dosage level. There was also a dosage-related increase in minor abnormalities, especially sternebral variants. The authors conclude that there may be a possible embryo toxicity which should be considered [Mantovani *et al.,* 1988].

A recent study (of Finnish women) revealed that heavy licorice consumption (≥ 500 mg/week) is associated with shorter gestation times (greater risk of preterm delivery < 37 weeks) [Strandberg *et al*., 2002].

Licorice extract (water extract of *Glycyrrhiza uralensis*) was orally administered at doses of 500, 1,000 or 2,000 mg/kg, the upper-limit dose (2,000 mg/kg) recommended in the Toxicity Test guideline of the Korea Food and Drug Administration, to 6-week-old male rats for 9 weeks, neither induced clinical signs, nor affected the daily feed consumption and body weight gain. There were no significant changes in testicular weights, gross and microscopic findings, and daily sperm production between vehicle- and licorice-treated animals, in spite of slight decreases in prostate weight and daily sperm production at the high dose (2,000 mg/kg). In addition, licorice did not affect the motility and morphology of sperm, although the serum testosterone level tended to decrease without significant difference, showing a 28.6% reduction in the high-dose (2,000 mg/kg) group. The results suggest that the no observed adverse-effect level of licorice extract is higher than 2,000 mg/kg, the upper-limit dose, and that long-term exposure to licorice might not cause profound adverse effects [Shin et al., 2008].

Shin *et al*. (2005) assessed if water extract of licorice (*Glycyrrhiza glabra*) caused developmental toxicity in rats. Licorice extract (500, 1,000 or 2,000 mg/kg) was dissolved in drinking water and orally administered to male rats from 9 weeks before mating to the day of copulation, and to females from 2 weeks before mating to gestational day 19. On gestational day 20, the animals were sacrificed for Caesarean section, and maternal and foetal abnormalities were examined. Licorice extract neither induced clinical signs, nor affect the body weight gain, feed and water intake, oestrous cycle, copulation and fertility rates, blood 17beta-estradiol level and organ weights of dams. Also, the implantation and development .including body weights, absorption and death of embryos and foetuses were not influenced by *in utero* exposure to licorice. In addition, there were no increases in external, visceral and skeletal abnormalities of foetuses. The authors conclude that the no observed adverse effect level of licorice extract is higher than 2,000 mg/kg, and that long-term *in utero* exposure to licorice might not cause developmental toxicities of embryos and fetuses [Shin *et al.* 2005].

Inhalation studies

A recent study investigated the effect of cigarettes, containing various additives in three combinations, in a 90-day nose-only smoke inhalation study in rats. These ingredients included licorice extract at 18802 ppm, a level described as a multiple of its typical use in a US cigarette. The data from this study along with that from a number of other biological and chemical studies indicate that the addition of the combined ingredients "did not increase the inhalation toxicity of the smoke, even at the exaggerated levels used" [Vanscheeuwijck *et al.*, 2002].

When tested at 20000 ppm in cigarettes, in a 13-week inhalation study, the presence of licorice extract "…had no discernible effect on the character of extent of the biologic responses normally associated with inhalation of mainstream cigarette smoke in rats" [Gaworski *et al.*, 1998]. [However, it should be noted that the cigarettes had been spiked with a number of flavour ingredients in combination prior to smoking, and they contained a typical American blend humectant and sugar component (*i.e.* glycerine ≈ 20,000 ppm, propylene glycol at ≈ 24,000 ppm, and brown invert sugar at ≈ 24,000 ppm)] [Gaworski *et al.*, 1998].

The addition of licorice extract at 20,000 ppm to reference cigarettes, used in a 90 day-sub-chronic inhalation exposure in rats, led to a series of pathological changes to smoke exposure that were indistinguishable from those changes caused by the control cigarettes. This indicated that addition of licorice extract to a reference cigarette had no discernable effect upon the type or severity of the treatment related pathological changes associated with tobacco smoke exposure [Baker *et al.,* 2004].

Carmines *et al.,* (2005) demonstrated an increase in incidence and severity of nasal epithelial hyperplasia in female rats exposed to mainstream smoke from cigarettes containing 12.5 % block licorice for 90 days. No respiratory tract

changes were apparent in animals of either sex exposed to smoke from cigarettes containing 8% licorice powder. At 5% licorice extract (block and/or powder) no significant respiratory changes were apparent in animals exposed for 90 days compared to controls [Carmines *et al.*, 2005].

Roemer (2014) and Schramke (2014) reported on a testing program designed to evaluate the potential effects of 350 ingredients added to an experimental kretek cigarette on selected biological and chemical endpoints. The studies performed included a bacterial mutagenicity screen [Ames assay] a mammalian cell cytotoxicity assay [neutral red uptake], Mouse Lymphoma assay, determination of smoke chemical constituents, a 4-day in vivo micronucleus assay and a 90-day rat inhalation study. Based on the results of these studies, the authors concluded that the addition of ingredients commonly used in the manufacture of kretek cigarettes, including licorice extract at levels up to 9610 ppm, did not change the overall in vivo/vitro toxicity profile of the mainstream smoke.

Other relevant studies

One of the most common adverse effects of traditional Japanese kampo and traditional Chinese medicine is pseudoaldosteronism caused by licorice. In this review, the authors describe the mechanisms of licorice-induced pseudoaldosteronism by the pharmacokinetics of chemical constituents and its metabolites containing licorice. Glycyrrhizin (GL), the main constituent of licorice, is absorbed as glycyrrhetinic acid (GA), which is a metabolite of GL produced by enterobacteria before its release into the circulation. Circulating GA is metabolized in the liver to become 3-monoglucuronyl-glycyrrhetinic acid (3MGA), which is excreted into the bile via multidrug resistance protein 2 (Mrp2). If Mrp2 function is damaged for some reason, 3MGA is secreted from the liver into the circulation, and excreted into the urine via organic anion transporters expressed at the basolateral side of tubular epithelial cells. Circulating GA cannot be excreted into the urine since GA binds highly to serum albumin and thus does not pass through glomerular filtration and is not a substrate of transporters expressed on tubular epithelial cells. Licorice-induced pseudoaldosteronism develops due to the inhibition of type 2 11β-hydrosteroid dehydrogenase (11β-HSD2) which results in the accumulation of cortisol in tubular epithelial cells that activate mineral corticoid receptors to stimulate the excretion of potassium that results in hypokalemia. GA, unlike 3MGA, cannot pass through tubular epithelial cells and cannot inhibit the enzyme in the cells. The authors conclude that, 3MGA may be a genuine causative agent for licorice-induced pseudoaldosteronism [Makino, 2014].

Glycyrrhizin, the major bioactive component in licorice root extract, exists as 2 isomers, α and β-glycyrrhizin, and is associated with causing pseudoaldosteronism due to its principal metabolites, glycyrrhetinic acid and 3-monoglucuronyl-glycyrrhetinic acid. The aim of the study reported here was to compare (a) the pharmacokinetics of glycyrrhizin and its metabolites in rat after the first and last intravenous doses of either α- or β-glycyrrhizin administered once a day over 6 days, (b) kidney levels of the metabolites at 24 h after the last dose and (c) the urinary cortisol:cortisone ratio (as a

biomarker of pseudoaldosteronism) in total urine collected for 24 h after the last dose.After the first dose, the clearance of glycyrrhizin in rats given α-isomer was significantly higher than in those given β-isomer and the AUC0-24 h values of glycyrrhizin and the metabolites were all significantly higher in β group than in α group. After the last dose, the AUC0-24 h values of glycyrrhizin and its metabolites were again significantly higher in rats given β-isomer than those given α-isomer and were all higher than the corresponding values after the first dose. Moreover, only kidney levels of glycyrrhetinic acid were detected in β group. The urinary cortisol:cortisone ratio was higher in rats given β-isomer and the correlation coefficients of the ratios with the AUC0-24 h values of 2 metabolites were 0.81 and 0.89 respectively. The authors concluded that the results of the present study indicate that α-glycyrrhizin is a safer drug than β- glycyrrhizin probably due to a lower systemic exposure to the 2 metabolites [Xu *et al*., 2013].

A previously healthy 49-year old female was presented with peripheral oedema of the legs, muscle aching, increased bowel movement and weight gain, which was due to the daily consumption of licorice candy cigars over a 2-week period. On physical examination it was noted that her blood pressure was higher than normal. Glycyrrhizic acid is present in natural licorice (NB. most licorice in North American candy contains artificial licorice flavouring rather than glycyrrhizic acid). The patient had been consuming between 4 and 7 licorice candy cigars each day, each cigar weighed 19 g and contained 0.45 g of glycyrrhizic acid in each cigar. Therefore, the patient was consuming 1.8 - 3.2 g glycyrrhizic acid per day. Several days after examination the patient's weight and blood pressure returned to normal, and the muscle aching and the oedema had subsided. Physicians were advised to enquire about licorice consumption when patients are presented with unexplained hypotension, hypokalemia, oedema, rhabdomyolysis or myoglobinuria [Johns, 2009].

Repeated administration of licorice extract or glycyrrhizin was tested on the liver drug metabolism of Swiss albino CD1 mice. Licorice root at 3138 or 6726 mg/kg or glycyrrhizin at 240 or 480 mg/kg was administered to different groups for 1, 4 or 10 consecutive days. Whilst a single dose of each material had no effect, multiple doses of either compound were able to induce specific cytochrome p450 isoforms of the CYP2 and CYP3 families. This may lead to the accelerated metabolism of co-administered drugs [Paolini *et al.,* 1998].
JECFA (2006), recently re-reviewed liquorice as a food additive, it was concluded that the safety evaluation must be conducted using human data currently suggesting an intake of 100 mg/day which would be unlikely to cause adverse effects in the majority of adults. However, it was recognised that in certain susceptible individuals who showed signs of pseudohyperaldosteronism, physiological effects could occur at levels below 10 mg/day [JECFA, 2006].

Isbrucker & Burdock., (2006) proposed an acceptable daily intake of 0.015 - 0.229 mg glycyrrhizin/kg bw/day based on current *in vivo* and clinical evidence.

The administration of licorice root extract (0.1 g/day) to moderately hypercholesterolemic patients for a period of 1 month (followed by the administration of placebo) was reported to produce a reduction in systolic blood pressure (10 %) which was sustainable throughout placebo treatment. The authors concluded that dietary licorice-root extract acts as a moderate hypocloesterolemic nutrient and antioxidant and hence has an effect in cardiovascular disease in hypercholesterolemic patients [Fuhrman *et al*., 2002].

In a human study conducted by the National Cancer Institute, a Phase 1 clinical trial for glycyrrhizic acid patients with previous breast cancer received 0.02 - 0.04 mmol/kg without significant signs of toxicity. Serum potassium levels were decreased at 4 - 6 hours after dosing, but had returned to similar predose values at 8 hours after dose administration. In the multiple dose wing of the study 6 females received 0.02 - 0.03 mg/kg four times a day, the women experienced hypertension or hypokaleamia, this lead to the need for dosage reduction or removal from treatment with only 2 women completing the 16 week study [Kellof *et al,.* 1994].

Stormer *et al.,* [1993] stated that it was impossible to determine the minimum required levels of glycyrrhizin required to produce the symptoms of hypertension or hypokalaemia due to the large individual variation in the susceptibility to glycyrrhizic acid. They stated that in the most sensitive individuals a regular intake of no more than approximately 100 mg of glycyrrhizin, approximately 50 g of licorice [assuming that the content of licorice is 0.2 % glycyrrhizin] was enough to produce adverse affects [Stormer *et al.,* 1993].

The ingestion of licorice and/or its active metabolites can sometimes cause a form of acquired mineralocorticoid excess [AME] syndrome expressed as the loss of potassium, the retention of sodium, and the loss and suppression of the renin-angiotensin–aldosterone system. Other reported findings have included oedema and increased blood pressure [Olukoga *et al.,* 2000].

A case of hypokalemic paralysis was reported in a patient who had a history of licorice consumption (in the form of a tea sweetener) in association with long-term consumption of licorice as a sweet. The treatment of this patient with fluid and potassium produced a complete and long-lasting recovery. This was the first reported case of hypokalemic paralysis due to exposure to licorice as a tea sweetener (which is reported to be a custom in the Arab population) [Elinav and Chajek-Shaul T, 2003].

Licorice has also been reported to have many endocrine effects. The ‘inappropriate use’ of licorice is associated with pseudoaldosteronism (reported to be caused by the inactivation of 11-beta-hydroxysteroiod-dehydrogenase and the binding to mineralocorticoid receptors). Liquorice is also reported to potentate the actions of cortisol reduce testosterone synthesis and exert an estrogen-like activity to reduce body fat mass, [Armanini *et al*., 2002]. The oral administration of freeze-dried extract of licorice at 100, 250 and 500 mg/kg in rats is also reported to produce a concentration-dependant

reduction in cortisol, ACTH, aldosterone and K, with a concurrent reduction in rennin and Na. This was reported to suggest a dose dependent suppression of the adrenal-pituitary axis (with a stimulation of rennin production in the kidney) [Al-Qarawi *et al*., 2002].

Licorice extract (using a menthol extract from the roots) was tested for it antioxidant properties in comparison with the antioxidants sodium metabisulfite and BHT. At the end of the study, the authors suggested the use of licorice extract at 0.5 and 1 % as an effective natural antioxidant [Morteza-Semnani *et al.,* 2003].

In an immunotoxicity assay licorice extract failed to modulate the cell mediated or humoral immune response of female CD1 mice, when it was administered intragastrically for five days at doses up to 5000 mg/kg/day [Gaworski *et al.*, 1994].

Argawal *et al.,* (1991) found that the addition of 0.05 % glycyrrhizin in the drinking water lead to a 36 % and 41 % inhibition of in *vivo* binding of [H_3] BaP and [H_3] DMBA to epidermal DNA in Sencar mice [Argawal *et al.,* 1991].

Topical application of α-glycyrrhizic acid or β-glycyrrhizic acid to the backs of sencar mice lead to a 41 or 55 % reduction in, in *vivo* binding of [H_3] BaP or a 30 % or 48 % inhibition of *in vivo* binding of [H_3] DMBA to epidermal DNA [Wang *et al.,* 1991].

Singletary *et al.,* (2000), demonstrated that dibenzoyl methane, which is a minor constituent of licorice was found to be effective in inhibiting the production of BaP induced DNA adducts in MCF-10 human mammary cells.

It has also been stated that licorice products are suppressing agents that appear to block the carcinogenic process at the post initiation phase. Wang *et al.,* (1991) noted that α-glycyrrhizic acid and β-glycyrrhizic acid were able to inhibit TPA induced ornithine decarboxylase induction and inhibit lipoxygenase activity in sencar mice. Licorice and its derivatives have also been reported to possess preventative and suppressive effects on breast cancer in humans [Wang et al 1991; Kellof *et al.,* 1994; Hundertmark *et al.,* 1997; Tamir *et al.,* 2000].

Glycyrrhizin (10 mg/kg administered to mice 1, 3, 5 and 7 days after B16F10 melanoma cell inoculation (highly metastatic cell line) was reported to inhibit pulmonary metastases of B16 melanoma cells. This was reported to be through the regulation of tumour associated T-helper type 2 cells, [Kobayashi *et al*., 2002].

18beta-glycyrrhetinic acid (GA) the aglycone of glycyrrhizin (GL) derived from licorice was reported to protect against carbon tetrachloride-induced hepatotoxicity. This was reportedly due to its ability to block the bioactivation of carbon tetrachloride (by inhibiting the expression and subsequent activity of P450 2E1), and due to its free radical scavenging effects [Jeong *et al*., 2002].

The most widely observed pharmacological activity of the licorice triterpenoids has been reported to be a hydrocortisone-like anti-inflammatory effect. 11β-Hydroxysteroid dehydrogenase [11β HSD] is one of the major factors involved in the regulation of the levels of circulating cortisol under normal conditions [Wang *et al.,* 2001]. Licorice triterpenoids such as glycyrrhetinic acid have been shown to be specific inhibitors of the enzyme 11β HSD [Monder *et al.,* 1989; Zhang *et al.,* 1994]. The inhibition of this enzyme leads to a cortisol dependent increased sodium and water retention with an increased potassium (K+) excretion. when compensatory mechanisms are (i.e. suppression of the rennin-angiotensin-aldosterone axis) suppressed pseudohyper-aldosteronism may result, which is reflected in oedema hypokalaemia and increased blood pressure. These are not acute effects, but due to the repeated administration of glycyrrhizinic acid, clinical signs may develop in in a few days to weeks [SCF 2003]. The affinity of glycyrrhetinic acid has been shown to be between 3000 [human leukocytes] -10,000 [renal tissue of rats] times less than that of human aldosterone for the11β HSD enzyme [Armanini *et al.,* 1983, 1989].

Glycyrrhizic acid is widely applied as a sweetener in food products and chewing tobacco. To analyze the relationship between the pharmacokinetics of glycyrrhizic acid in its toxicity, the kinetics of glycyrrhizic acid and its biologically active metabolite glycyrrhetic acid were evaluated by Ploeger et al., (2001). Glycyrrhizic acid is mainly absorbed after presystemic hydrolysis as glycyrrhetic acid. Because glycyrrhetic acid is a 200 - 1000 times more potent inhibitor of 11-β-hydroxysteroid dehydrogenase compared to glycyrrhizic acid, the kinetics of glycyrrhetic acid are relevant in a toxicological perspective. Once absorbed, glycyrrhetic acid is transported, mainly taken up into the liver by capacity-limited carriers, where it is metabolized into glucuronide and sulfate conjugates. These conjugates are transported efficiently into the bile. The conjugates are hydrolyzed to glycyrrhetic acid by commensal bacteria; glycyrrhetic acid is subsequently reabsorbed, causing a pronounced delay in the terminal plasma clearance. Physiologically based pharmacokinetic modeling indicated that, in humans, the transit rate of gastrointestinal contents through the small and large intestines predominantly determines to what extent glycyrrhetic acid conjugates will be reabsorbed. The authors suggest that this parameter may serve as a useful risk estimator for glycyrrhizic-acid-induced adverse effects, because in subjects with prolonged gastrointestinal transit times, glycyrrhetic acid might accumulate after repeated intake [Ploeger *et al*., 2001].
Yokozawa *et al.,* (2000), demonstrated that administration of glycyrrhizin to rats for 30 days orally prior to ischemia reperfusion in rats, was found to increase endogenous activities of antioxidant enzymes which included glutathione peroxidase and catalase [Yokozawa *et al.,* 2000].

Approximately 300 polyphenols have been extracted from the dried licorice root accounting for between 1 and 5 % of the dried mass. These polyphenols have included phenolic acids, flavones, isoflavanoids, chalcones and flavans [Wang *et al.,* 2001]. It has been suggested that these polyphenols may be responsible for the anti-oxidant properties of licorice. Glabridin treated EO mice [20 μg/mouse/day] for 6 weeks were found to reduce the oxidation of low

density lipoprotein by up to 80 %, when compared to control placebo treated mice [Rosenblat *et al.,* 1999].

Mendes-Silva et al., (2003) reports on the *in vivo* effects of GL upon two experimental models of induced thrombosis in rats. Intravenous administration of GL caused a dose-dependent reduction in thrombus size on a venous thrombosis model that combines stasis and hypercoagulability. It was observed that GL doses of 180 mg/kg body weight produced 93 % decrease on thrombus weight. This effect showed a time-dependent pattern being significantly reduced when the thrombogenic stimulus was applied 60 min after drug administration. GL was also able to prevent thrombosis using an arteriovenous shunt model. GL doses of 180 and 360 mg/kg decreased the thrombus weight by 35 and 90 %, respectively. Accordingly, the APTT ex vivo was enhanced by 1.5- and 4.3-fold at GL doses of 180 and 360 mg/kg, respectively. In addition, GL doses above 90 mg/kg caused significant hemorrhagic effect. In contrast with heparin, GL did not potentiate the inhibitory activity of antithrombin III or heparin cofactor II towards thrombin. The authors conclude that altogether, the data suggest that GL is an effective thrombin inhibitor in vivo [Mendes-Silva et al., 2003].

Kiso *et al.,* [1984)] examined the effects of administration of glycyrrhizic acid to rat hepatocytes that had been pre-treated with 5 mM CCl_4 [a source of free radical production and lipid peroxidation]. Nakamura *et al.,* (1985) found that the release of lactate dehydrogenase [LDH], glutamic oxaloacetic aminotransferase [GOT] and glutamic-pyruvic aminotransferase [GPT] from hepatocyte cells was inhibited by the administration of glycyrrhizic acid. Kiso *et al.,* [1984] found that at 1mg/ml glycyrrhizic acid exhibited no significant antioxidant activity, but 18β-glycyrrhetinic acid did. The authors concluded that the *in vivo* hepatocyte protective activity of glycyrrhizic acid was actually due to the presence of 18β-glycyrrhetinic acid, which was able to inhibit both lipid peroxidation and free radical production [Kiso *et al.,* 1984].

Licorice flavonoid oil (LFO) is a new dietary ingredient containing licorice flavonoids dissolved in medium-chain triglycerides (MCT). Glabridin is one of the bioactive flavonoids included specifically in licorice. Glycyrrhiza glabra L. and is the most abundant flavonoid in LFO. Aoki *et al.,* [2007] assessed the safety of LFO in healthy humans and determined the plasma concentration profile of glabridin as a marker compound. A single-dose and two multiple-dose studies at low (300 mg), moderate (600 mg) and high (1200 mg) daily doses of LFO were carried out using a placebo-controlled single-blind design. In each study the safety of LFO and the pharmacokinetics of glabridin were assessed. Pharmacokinetic analysis in the single-dose study with healthy male subjects (n = 5) showed that glabridin was absorbed and reached the maximum concentration (Cmax) after approximately 4 h (Tmax), and then was eliminated relatively slowly in a single phase with a $T^{1/2}$ of approximately 10 h at all doses tested. The Cmax and area under the curve (AUC, 0 - 24 h) increased almost linearly with dose. The multiple-dose studies with healthy male and female subjects for 1 week and 4 weeks suggested that plasma glabridin reached steady state levels within 2 weeks with a single daily administration of 300 to 1200 mg/day LFO. In all studies there were no

clinically noteworthy changes in haematological or related biochemical parameters. All clinical events observed were mild and considered to be unrelated to LFO administration even after repeated administration for 4 weeks. The authors concluded that the studies demonstrated that LFO was safe when administered once daily at doses up to 1200 mg/day [Aoki *et al.,* 2007].

As part of a safety evaluation of Licorice flavonoid oil (LFO), a 90-day oral toxicity study in rats was conducted using an LFO concentrate solution (2.90 % glabridin). Male and female CRj;CD(SD) rats were assigned to one of 12 groups (10 males or females per group) and received corn oil (negative control), MCT (vehicle control), or 400, 600, 800 or 1600 mg/kg of the LFO concentrate solution. LFO concentrate solution induced an anticoagulation effect in both sexes (prolonagation of APTT and PT times), although there was a clear sex difference. Changes in RBC, HCT, MCHC and MCH were seen for males at 1600 and less marked for those at 800 mg/kg/day. There was a significant increase in ALT for females at 1600 mg/kg and males at 800 and 1600 mg/kg, there was a decrease in K+ for males at 600, 800 and 1600 mg/kg. Changes in the absolute spleen and kidney weights were seen in females at 1600 mg/kg/day. Based on these findings, it is concluded that the no-observed-adverse-effect level (NOAEL) for the LFO concentrate solution was estimated to be 800 mg/kg/day for female rats, and approximately 400 mg/kg/day for male rats [Nakagawa *et al.,* 2008b].

Sakr *et al*., (2011) studied the effect of aqueous extract of licorice on metiram toxicity in mice. Treating mice with metiram at a dose level of [1/2] LD_{50} daily for 3 weeks induced many histological changes in the kidney cortex. The renal tubules lost their characteristic appearance and their lining epithelial cells were degenerated. The glomeruli were atrophied and the renal blood vessels were congested. The intertubular spaces infiltrated by inflammatory leukocytic cells. Metiram caused an increase in proliferating cell nuclear antigen (PCNA) expression in nuclei of tubular epithelial cells. Metiram also caused marked elevation in serum creatinine and blood urea nitrogen. Treating animals with metiram and licorice aqueous extract led to an improvement, in both biochemical and histopathological alterations. From these results the authors stated that licorice had an ameliorative effect against kidney injury induced by metiram and this effect may be attributed to its antioxidant activity [Sakr *et al*., 2011].

Ni *et al*., (2011) investigated the anti-inflammatory effect of Glycyrrhizin (GL) on lipopolysaccharide (LPS)-induced acute lung injury (ALI) in mice. ALI was induced in Balb/c mice by intratracheal instillation of LPS (1 mg/kg). Before 1 h of LPS administration, the mice received intraperitoneal injection of GL at varied doses (10, 25, and 50 mg/kg). The severity of pulmonary injury was evaluated 12 h after LPS administration. GL pretreatment led to significant attenuation of LPS induced evident lung histopathologic changes, alveolar hemorrhage, and neutrophil infiltration with evidence of reduced myeloperoxidase (MPO) activity. The lung wet/dry weight ratios, as an index of lung edema, were markedly reduced by GL pretreatment. The concentrations of pro-inflammatory cytokines interleukin (IL)-1β and tumor necrosis factor (TNF)-α were elevated in bronchoalveolar lavage fluid (BALF)

after LPS administration, which were significantly inhibited by GL pretreatment. GL pretreatment also reduced the concentrations of nitric oxide (NO) in lung tissues. Furthermore, the expression of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) was suppressed by GL pretreatment. In conclusion, the authors state that GL potently protected against LPS-induced ALI, and the protective effects of GL may attribute partly to the suppression of COX-2 and iNOS expression [Ni *et al*., 2011].

In this study, Shi *et al*., (2010) investigated the anti-inflammatory effects of Monoammonium glycyrrhizinate (MAG) on lipopolysaccharide (LPS)-induced acute lung injury (ALI) in mice and the possible mechanisms involved in this protection were investigated. Pretreatment with MAG prior to the administration of intratracheal LPS significantly induced a decrease in lung wet weight/dry weight ratio, in total leukocyte number and neutrophil percent in the BALF, and in myeloperoxidase (MPO) activity of lung in dose-dependent manners. At the same time, pretreatment with MAG also significantly improved the super oxide dismutase (SOD) activity and induced the malondialdehyde (MDA) content in the bronchoalveolar lavage fluid (BALF). Importantly, pretreatment with MAG prevented an increase in cyclic adenosine monophosphate-phosphodiesterase (cAMP-PDE) activity of lung in a dose-dependent manner. In addition, it can up-regulate the interleukin-10 (IL-10) level and down-regulate the tumor neurosis factor-α (TNF-α) level in the lung tissue of ALI mice. These results showed that anti-inflammatory effects of MAG against the LPS-induced ALI may be due to its ability of primary inhibition of cAMP-PDE activity, oxidative stress and its regulation of cytokine effects [Shi *et al*., 2010].

Qamar *et al*., (2012) investigated the protective effects of glycyrrhizic acid (GA) against B(a)P induced debilities in lungs of Wistar rats. Intratracheal instillation of B(a)P significantly suppressed NF-κB translocation, sEH, TrxR and catalase activities in lung tissue. A marked induction of H(2)O(2) levels along with caspases activation (caspases-2, -3, -6, -8, and -9) in lung tissue after B(a)P exposure was observed. Lung injury was assessed by measuring lactate dehydrogenase (LDH), alkaline phosphatase (ALP), total cell count, total protein, neutrophil elastase activity in bronchoalveolar lavage fluid (BALF). Reduction in phospholipid content further potentiated these parameters. GA oral administration (50 and 100mg/kg b.wt.) significantly showed protection of lung epithelium by suppression of caspases activities in lung tissue and reduction of total protein, total cells, elastase activity, LDH and ALP activities along with fortification of phospholipids in BALF. Histological observations also confirm the findings in above mentioned parameters. Results indicate a strong correlation between amelioration of sEH and TrxR activities, and NF-κB activation [Qamar *et al*., 2012].

Park *et al*., (2011) assessed the effects of licorice, a CYP2B inducer, on the fetal defects induced by cyclophosphamide. Pregnant Sprague-Dawley rats were daily administered with licorice (100 mg/kg) by gavage for 7 days, from the 6th to 12th day of gestation, and intraperitoneally administered with cyclophosphamide (11 mg/kg) 1 hr after the final licorice treatment. On the 20th day of gestation, maternal and fetal abnormalities were determined by

Cesarian section. Cyclophosphamide was found to reduce fetal and placental weights without increasing resorption or death. In addition, it induced malformations in live fetuses; 93.8, 41.1, and 100% of the external (skull and limb defects), visceral (cleft palate and ureteric dilatation), and skeletal (acrania, vertebral/costal malformations, and delayed ossification) abnormalities, respectively. When pre-treated with licorice, cyclophosphamide-induced body weight loss and abnormalities of fetuses were remarkably aggravated. Moreover, repeated treatment with licorice greatly increased mRNA expression and activity of hepatic CYP2B [Park *et al*., 2011].

Behavioural data

Licorice has traditionally been used for the treatment various conditions due to its antiulcer, expectorant, antimicrobial and anti-inflammatory properties. Three doses of 75, 150 or 300 mg/kg p.o. of an aqueous extract, the dose of 150 mg/kg was reported by the authors to significantly improve the learning and memory of mice. The dose was also reported to reverse the amnesiac effect of scopolamine (0.4 mg/kg i.p.) or diazepam (1mg/kg i.p.) [Dhingra *et al.,* 2004].

In Vitro Toxicity Status

Carcinogenicity and mutagenicity

Additional information concerning the *in vitro* mutagenicity of this material may be found in “An Interim report on data originating from Imperial Tobacco Limited’s Genotoxicity testing programme September 2003” or “An updated report on data originating from Imperial Tobacco Limited’s external Genotoxicity testing programme – Round 2 August 2007”.

Licorice flavanoid oil (LFO) is a new functional food ingredient. The genotoxicity of LFO was investigated using a test battery of three different methods (Ames, chromosomal aberration test and an in vivo micronucleus assay). In the Ames, LFO did not increase the number of revertant colonies in any tester strain with or without metabolic activation by rat liver S9 mix. In the chromosomal aberration test using Chinese hamster lung (CHL/IU) cells, LFO did not induce any chromosomal aberrations either in the short period test without rat liver S9 mix or in the continuous treatment (24 h or 48 h) test. However, in the short-period test with rat liver S9 mix, LFO induced structural chromosomal aberrations at concentrations higher than 0.6 mg/mL. in the in vivo micronucleus assay male F344 rats were dosed by oral gavage at doses up to 5000 mg/kg/day. No significant or dose-dependent increases in the frequency of micronucleated polychromatic erythrocytes (MNPCE) were observed and the high dose suppressed the ratio of polychromatic erythrocytes (PCE) to total erythrocytes was seen. Subsequently, a liver and peripheral blood micronucleus test using male F344 rats was conducted. No micronuclei induction either in hepatocytes or PCE was observed even at the highest dose of 5000 mg/kg/day. The authors concluded that the genotoxicity

assays performed it appears unlikely that dietary consumption of LFO will present any genotoxic hazard to humans [Nakagawa *et al.,* 2008a].

The 70 % methanol soluble fraction from a licorice acetone extract (containing the active component licocoumarone) was reported to inhibit cell proliferation in the human monoblastic leukaemia U937 cell line. The inhibitory action was reported to be due to the induction of apoptosis [Watanabe, 2002].

Licorice extract was found to be negative in the Ames *Salmonella typhimurium* assay with the tester strains TA98, TA100, TA1535, TA1537 and TA 1538 both with and without metabolic activation at concentrations up to 1000 μg/plate [Heck et al., 1989]. Glycyrrhizic acid and glycyrrhetinic acid were also found to be negative when tested in Ames strains TA98, TA100 at 200 μg/plate both with and with out metabolic activation [Yamaguchi *et al.,* 1984]. Glycyrrhiza glabra extract [25-100 μl/plate] and glycyrrhizic acid [100-2000 μl/plate] were again found to be negative in the Ames strain TA100 mutagenicity test [Zani *et al.,* 1993].

Mitscher *et al.,* (1986) found that *Glycyrrhiza glabra* [*G. glabra*] extract was highly effective in protecting the Ames strain TA100 against the mutagenic effect of ethyl methanesulfonate [EMS] This mutagen dose not require metabolic activation. The *G. glabra* extract was also found to be negative in the rec-assay with the M45 strain of *Bacillus subtilis* [Mitscher *et al.,* 1986].

Roemer *et al.,* (2002) reported on a study in which cigarettes containing various additives in three different combinations were produced. Smoke condensates prepared from these cigarettes were then tested in two different *in vitro* assays. The mutagenicity of the smoke condensate was assayed in the *Salmonella* plate incorporation [Ames] assay with tester strains TA98, TA100, TA102, TA1535 and TA1537 in the presence and absence of an S9 metabolic activation system. The cytotoxicity of the gas/vapour phase and the particulate phase was determined in the neutral red uptake assay with mouse embryo BALB/c 3T3 cells. The authors concluded that the *in vitro* mutagenicity and cytotoxicity of the cigarette smoke was not increased by the addition of the ingredients which included licorice extract at levels up to 18802 ppm [a multiple of its typical use in a US cigarette] [Roemer *et al.,* 2002]. These data are consistent with Carmines *et al.,* (2005), in which no relevant difference in the genotoxic or cytotoxic potential of mainstream smoke/smoke preparations from cigarettes with added licorice extracts (up to 12.5 %) compared to controls was observed.

When liquorice extract was tested at 669 μg/ml with rat hepatocytes for 18 - 20 hours, there was reported to be no unscheduled DNA synthesis of the rat hepatocytes [Heck *et al.,* 1989]. Liquorice extract was reported to be positive in the mouse lymphoma assay with the +/- L5178Y lymphoma cell line at concentrations of 1400 μg/ml without metabolic activation and at 800 μg/ml with metabolic activation [Heck *et al.,* 1989].

Baker *et al.,* [2004], examined the effects of the addition of 482 tobacco ingredients upon the biological activity and chemistry of mainstream smoke.

The ingredients, essentially different groups of flavourings and casings, were added in different combinations to reference cigarettes. The addition of licorice extract at 20,000 ppm was determined not to have affected the mutagenicity of the total particulate matter (TPM) of the smoke in either the Ames, *in vitro* micronucleus assay or the neutral red assay when compared with that of the control cigarettes [Baker *et al.,* 2004].

The mutagenicity of the smoke condensate was assayed in the *Salmonella* plate incorporation [Ames] assay with the tester strain TA98 in the presence of an S9 metabolic activation system. The cytotoxicity of the cigarette condensate was determined in the neutral red uptake assay and the (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H tetrazolium, inner salt assay (MTS assay) with the human hepatocellular liver carcinoma cell line, HEP-G2. It was concluded that the *in vitro* mutagenicity and cytotoxicity of the cigarette smoke was not increased by the addition of the ingredients, which included *Licorice* at levels up to 20, 000 ppm.

Roemer (2014) and Schramke (2014) reported on a testing program designed to evaluate the potential effects of 350 ingredients added to an experimental kretek cigarette on selected biological and chemical endpoints. The studies performed included a bacterial mutagenicity screen [Ames assay] a mammalian cell cytotoxicity assay [neutral red uptake], Mouse Lymphoma assay, determination of smoke chemical constituents, a 4-day in vivo micronucleus assay and a 90-day rat inhalation study. Based on the results of these studies, the authors concluded that the addition of ingredients commonly used in the manufacture of kretek cigarettes, including licorice extract at levels up to 9610 ppm, did not change the overall in vivo/vitro toxicity profile of the mainstream smoke.

Other relevant studies

Glabridin which is one of the major flavonoids in licorice extracts, and its isoflavan derivatives has been shown to have potent *in vitro* antioxidant activities against low density lipoprotein oxidation, and protect the liver mitochondrion against oxidative stress in male Wistar rats [Haraguchi *et al.,* 2000].

G. glabra has also been shown to be able to scavenge reactive molecules produced by the photo illumination of rose bengal, and is effective in preventing the cytotoxicity to *E. coli* (k12+ strain) in the presence of rose bengal in a dose related fashion [Kuo *et al.,* 1992].

Glycyrrhetinic acid and glycyrrhizic acid have been shown by Abe *et al.,* [1987] to be inhibitory to the growth of B16 melanoma cell line. Glycyrrhetinic acid was found to cause complete growth inhibition of the cell line at concentrations above 10 μg/ml and glycyrrhizic acid caused a 40 % reduction of growth at a concentration of 200 μg/ml [Abe *et al.,* 1987].

In a National Cancer Institute [NCI] sponsored *in vitro* assay β-glycyrrhetinic acid was reported to inhibit the anchorage dependent growth of human lung

tumour A427 cells [Kellof *et al.,* 1994]. In another study examining the effect of glucocorticosteroids on the inhibition of the MCF-7 and ZR-75-1 breast cancer cells, their effects were found to be weak. On co-administration with glycyrrhizic acid, their inhibitory actions were found to increase significantly [Hundermark *et al.,* 1997].

Glycyrrhizic acid (a major component of licorice) was reported to protect against aflatoxin induced oxidative stress in the human hepatoma cell line (HepG2), The protective effect was reported to be due to the ability to inhibit the metabolic activation of hepato-toxin which is reported to be a critical factor in the pathogenesis of chemically induced carcinogenicity [Chan *et al*., 2003].

Glycyrrhizic acid was also reported to actively inhibit the replication of the Epstein-Barr virus (*in vitro*) as well as being active in the inhibition of the replication of the SARS-associated virus (Lin, 2003). Cinatl *et al*., (2003) have suggested the use of glycyrrhizin in the treatment of SARS [Cinatl *et al*., 2003].

Glycyrrhizic acid (GA) and liquorice protected rat hepatocytes cultured in an in vitro monolayer culture from intracellular glutathione (GSH) depletion caused by exposure to 1 μM azathioprine (immunosuppressant used to prevent organ rejection in kidney transplant recipients). The author concluded that licorice or GA may be useful to provide protection against azathioprine hepatotoxicity [Wu *et al.,* 2006].

Badr *et al*., (2011) evaluated the cytotoxic effects of Liquorice as a root canal medicament and compared it's action to the commonly used root canal medicament calcium hydroxide $Ca(OH)_2$. Human periodontal ligament fibroblast tissue culture was used to assess the cytotoxicity of the preparations under investigation. The use of Liquorice extract followed by Liquorice/ $Ca(OH)_2$ mixture retained significantly more viable periodontal ligament cells than $Ca(OH)_2$, which had a strong lethal effect on the cells [Badr *et al*., 2011].

Cao *et al*. (2010) carried out a first-line screening of four herbal chemicals with reported antioxidative properties and capabilities to suppress endothelial cell growth and migration. One of these herbal chemicals included isoliquiritigenin (ISL) from licorice. Cytotoxicity was studied by MTT cell viability/proliferation assay on human retinal pigment epithelial cells (ARPE19). Effects on vascular endothelial growth factor (VEGF)-induced endothelial cell proliferation and migration were investigated by a scratch-wound model using human umbilical vein endothelial cells (HUVECs). The effects on VEGF signaling in HUVECs were analyzed by Western blotting. At sub-cytotoxic levels, ISL (10 μM) suppressed HUVEC proliferation and migration under VEGF (20 ng/mL) stimulation in our scratch-wound model. HUVEC migration was reduced more by ISL than bevacizumab, a humanized monoclonal antibody against VEGF.
ISL is highly effective and efficient in suppressing endothelial cell proliferation and migration, with low cytotoxicity on ARPE19 and HUVEC lines [Cao *et al*. 2010].

In a study by Yu *et al*., (2010), normal serum-free mouse embryo (SFME) and tumorigenic human c-Ha-ras and mouse c-myc cotransfected highly metastatic serum-free mouse embryo-1 (r/m HM-SFME-1) cells were treated with various concentrations of clinically available antitumor agents or glycyrrhetinic acid (GA), and the antiproliferative effects of these compounds were determined by the MTT assay. Western blotting analysis, RT-PCR, fluorescence staining and confocal laser scanning microscopic observation were adopted to analyze H-Ras regulation. GA exhibited the tumor cell-selective toxicity through H-Ras downregulation, and its selectivity was superior to those of all the clinically available antitumor agents examined. For the selective toxicity of tumor cells, GA was most effective at 10 microM. Interestingly, this concentration was the same as the previously reported maximum plasma GA level reached in humans ingesting licorice [Yu *et al*., 2010].

In the present study, the cytotoxicity of the methanol extracts of nine samples of the roots of G. glabra, collected from various geographical origins, was assessed against immortal human keratinocyte (HaCaT), lung adenocarcinoma (A549) and liver carcinoma (HepG2) cell lines using the in vitro 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazoliumbromide cell toxicity/viability assay. The authors reported a considerable variation in levels of cytotoxicity among various samples of G. glabra (Basar *et al.,* 2015).

REFERENCES

Abe *et al.,* (1987). The effects of glycyrrhizin and glycyrrhetinic acid on the growth and melanogenesis in cultured B16 melamnoma cells. *European Journal of Cancer and Clinical Oncology*. **23:** 1549-1555.

Agarwal *et al.,* (1991). Inhibition of mouse skin tumor-initiating activity of DMBA by chronic oral feeding of glycyrrhizin to in drinking water. *Nutrition & Cancer.* **15**: 187-193.

Al-Qarawi *et al*., (2002). Liquorice (Glycyrrhiza glabra) and the adrenal-kidney-pituitary axis in rats. *Food & Chemical Toxicology*. **40(10):**1525-7.

Aoki F *et al.,* (2007). Clinical safety of licorice flavanoid oil (LFO) and pharmacokinetics of glabridin in healthy humans. *J Am. Coll. Nutr.* **26(3):** 209-218.

Armanini *et al.,* (1983). Affinity of licorice derivatives for mineralocorticoid and glucocorticoid receptors. *Clinical Endocrinology.* **19**: 609-612.

Armanini *et al.,* (1989). The pathogenesis of pseudo hyperaldosteronism carbenoxolone. *Journal of Endocrine Investigation*. **12**: 337-341.

Baker RR, *et al.,* (2004). An overview of the effects of tobacco ingredients on smoke chemistry and toxicity. *Food & Chemical Toxicology*. **42** Suppl: S53-83.

Badr *et al*., (2011) A laboratory evaluation of the antibacterial and cytotoxic effect of Liquorice when used as root canal medicament. Int Endod J.; 44(1):51-8

Basar *et al.,* (2015). Comparative Cytotoxicity of Glycyrrhiza glabra Roots from Different Geographical Origins Against Immortal Human Keratinocyte (HaCaT), Lung Adenocarcinoma (A549) and Liver Carcinoma (HepG2) Cells. Phytother. Res. 29: 944–948

Carmines (2002). Evaluation of the potential effects of ingredients added to cigarettes. Part 1: Cigarette design, testing approach, and review of results. *Food & Chemical Toxicology*. **40**: 77-91.

Carmines, E. L., Lemus, R. and Gaworski, C. L (2005). Toxicologic evaluation of licorice extract as a cigarette ingredient. *Food & Chemical Toxicology*. **43(9):** 1303-22.

Cao *et al*. (2010), In vitro screening for angiostatic potential of herbal chemicals. Invest Ophthalmol Vis Sci.; **51**(12):6658-64

Chan *et al*., (2003). Inhibition of glycyrrhizic acid on aflatoxin B1-induced cytotoxicity in hepatoma cells. *Toxicology*. **188(2-3)**:211-7.

Chen *et al.,* (1994). Effect of glycyrrhetinic acid on DNA damage and unscheduled DNA synthesis induced by benzo(α)pyrene. *Yao Hsueh Hsueh Pao* **29(10):** 725-729.

Cinatl *et al*., (2003). Glycyrrhizin, an active component of liquorice roots, and replication of SARS-associated coronavirus. *Lancet*. **361(9374)**: 2045-6.

Dhingra D *et al.,* (2004). Memory enhancing activity of Glycyrrhiza glabra in mice. *Journal of Ethnopharmacology*. **91 (2-3):** 361-365.

Elinav and Chajek-Shaul T, (2003). Licorice consumption causing severe hypokalemic paralysis. *Mayo Clin Proc*. **78(6):**767-8.

Fenaroli (2005). Fenaroli's Handbook of Flavour Ingredients. 5th Edition. CRC Press London.

Fuhrman *et al*., (2002). Anti-atherosclerotic effects of licorice extract supplementation on hypercholesterolemic patients: increased resistance of LDL to atherogenic modifications, reduced plasma lipid levels, and decreased systolic blood pressure. *Nutrition*. **18(3):**268-73.

Gaworski *et al.*, (1994). An immunotoxicity assessment of food flavouring ingredients. *Food & Chemical Toxicology*. **32**: 409.

Gaworski *et al.*, (1998). Toxicologic evaluation of flavour ingredients added to cigarette tobacco: 13-week inhalation exposure in rats. *Inhalation Toxicology* **10:** 357-381.

Gaworski *et al.*, (1999). Toxicologic evaluation of flavour ingredients added to cigarette tobacco: skin painting bioassay of cigarette smoke condensate in SENCAR mice. *Toxicology* **139**: 1-17.

Hajirahimkhan A, Simmler C, Yuan Y, Anderson JR, Chen SN, Nikolić D, Dietz BM, Pauli GF, van Breemen RB, Bolton JL (2013). Evaluation of estrogenic activity of licorice species in comparison with hops used in botanicals for menopausal symptoms. PLoS One.**12**;8(7)

Haraguchi *et al.,* (2000). Protection of mitochondrion functions against oxidative stress by isoflavans from Glycyrrhiza glabra. *Journal of Pharmaceutical Pharmacology.* **52**: 219-223.

Heck *et al.,* (1989). An evaluation of food flavouring ingredients in a genetic toxicity screening battery. *The Toxicologist* **9(1):** 257.

Hundertmark *et al.,* (1997). V: Inhibition of 11β-hydroxysteroid dehydrogenase activity enhances the antiproliferative effect of glucocorticosteroids on MCF-7 and ZR-75-1 breast cancer cells. *Journal of Endocrinology*. **155**: 171-180.

In vitro toxicity testing of tobacco ingredients in burnt form (Internal document R-10).

Isbrucker RA & Burdock GA (2006). Risk and safety assessment on the consumption of Licorice root (Glycyrrhiza sp.), its extract and powder as a food ingredient, with emphasis on the pharmacology and toxicology of glycyrrhizin. *Regulatory Toxicology & Pharmacology*. **46(3):** 167-192.

JECFA (2006). Safety Evaluation of Certain Food Additives (54). Licorice.

JECFA (1978). *http://www.inchem.org/documents/pims/plant/pim249fr.htm*

Jeong *et al*., (2002). Hepatoprotective effects of 18beta-glycyrrhetinic acid on carbon tetrachloride-induced liver injury: inhibition of cytochrome P450 2E1 expression. *Pharmacological Research* **46(3)**: 221-7.

Johns C. Glycyrrhizic acid toxicity caused by consumption of licorice candy cigars. CJEM. 2009 Jan;11(1):94-6.

Kelloff *et al.,* (1994). National Cancer Institute, Clinical development plan 18β-glycyrrhetinic acid. *Journal of Cell Biochemistry*. **Suppl 20**: 166-175.

Kiso *et al.,* (1984). Mechanism of antihepatotoxic mechanism of action of glycyrrhizin. The effect on free radical generation and lipid peroxidation. *Plant Medicine.* **50(4)**: 298-302.

Kobayashi *et al*., (2002). Inhibitory effect of glycyrrhizin on experimental pulmonary metastasis in mice inoculated with B16 melanoma. *Anticancer Research* **22(6C)**: 4053-8.

Kobuke, *et al*., (1985). Tumourigenicity study of disodium glycyrrhizinate administered orally to rats. *Food & Chemical Toxicology*. **23(11):** 979-83.

Kuo *et al.,* (1992). Glycyrrhiza glabra as an effector of interception in Escherichia coli k12+. *Mutation Research.* **282(2)**: 93-98.

Leung and Foster (1996). Encyclopaedia of common natural ingredients used in food, drugs and cosmetics. Second Edition. John Wiley & Sons, Inc.

Lewis (2000). Sax's dangerous properties of industrial materials. Vol **2** 10th Edition. John Wiley & Sons New York.

Lin (2003). Mechanism of action of glycyrrhizic acid in inhibition of Epstein-Barr virus replication *in vitro*. *Antiviral Research*. **59(1)**: 41-7.

Makino T, 2014. 3-Monoglucuronyl glycyrrhretinic acid is a possible marker compound related to licorice-induced pseudoaldosteronism. Biol. Pharm. Bull. **37**(6):898-902.

Mantovani *et al.,* (1988). Teratogenicity of study of ammonium glycyrrhizinate in the Sprague Dawley rat. *Food & Chemical Toxicology* **26**: 435-440.

Maser E (2004). Significance of reductases in the detoxification of the tobacco-specific carcinogen NNK. *Trends in Pharmacological Science*. **25(5):** 235-237.

Mendes-Silva W *et al.,* (2003). Anti-thrombotic effect of Glycyrrhizin, a plant derived thrombin inhibitor. *Thrombosis Research*. **112(1-2):** 93-98.

Mirsalis *et al.,* (1993). Effects of soya bean flakes and licorice root extract on enzyme induction and toxicity to B6C3F1 mice. *Food & Chemical Toxicology*. **31(5):** 343-50.

Mitscher *et al*., (1986). Isolation and identification of higher plant agents active in antimutagenic assay systems: Glycyrrhiza glabra. *Basic Life Sciences*. **39**. 153-65.

Monder *et al.,* (1989). Licorice inhibits corticosteroid 11-β-dehydrogenase of rat kidney and liver *in vitro* and *in vivo* studies. *Endocrinology* **125**: 1046-1053.

Morteza-Semnani K *et al.,* (2003). Comparison of the antioxidant activity of extract from the roots of licorice (*Glycyrrhiza glabra* L.) to commercial antioxidants in 2% hydroquinone cream. *Journal of Cosmetic Science*. **54(6):** 551-558.

Nakagawa K *et al.,* (2008a) Genotoxicity studies on licorice flavanoid oil (LFO). *Food Chem Toxicol* **46(7):** 2525-2532.

Nakagawa K *et al.,* (2008b) 90 Day repeat dose toxicity study of licorice flavanoid oil (LFO) in rats. *Food Chem Toxicol* **46(7):** 2349-2357.

Nakamura *et al.,* (1985). Enzyme leakage due to change of membrane permeability of primary cultured rat hepatocytes treated with various hepatotoxins and its prevention by glycyrrhizin. *Cell & Biological Toxicology.* **1(4)**: 285-295.

Ni *et al*., (2011) Glycyrrhizin treatment is associated with attenuation of lipopolysaccharide-induced acute lung injury by inhibiting cyclooxygenase-2 and inducible nitric oxide synthase expression. J Surg Res.; 165(1):e29-35.

Nishino (1992). Anti-tumour promoting activity of glycyrrhizic acid and it related compounds. In Cancer Chemo prevention L Wattenburg *et al* EDS Boca Raton, CRC 457-469.

Normura *et al.,* (1998). Phenolic constituents of licorice (*Glycyrrhizin* species) *Forstr. Chem. Org. Naturst.* **73**: 1-158.

Olukoga *et al.,* (2000). Liquorice and its health implications. *J.R. Soc Health* **120(2)**:83-89.

Paolini *et al.,* (1998). Effect of licorice and glycyrrhizin on murine liver CYP-dependent monooxygenases. *Life Sciences.* **62(6)**: 571-582.

Park *et al*., (2011) Licorice extract increases cyclophosphamide teratogenicity by upregulating the expression of cytochrome P-450 2B mRNA. Birth Defects Res B Dev Reprod Toxicol.; **92**(6):553-9.

Ploeger *et al.,* (2001). The pharmacokinetcis of glycyrrhizic acid evaluated by physiologically based pharmacokinetic modeling. *Drug Metabolism Reviews.* **33(2)**: 125-147.

Qamar *et al*., (2012) Alleviation of lung injury by glycyrrhizic acid in benzo(a)pyrene exposed rats: Probable role of soluble epoxide hydrolase and thioredoxin reductase. Toxicology; **291**(1-3):25-31

Roemer *et al.,* (2002). Evaluation of the potential effects of ingredients added to cigarettes. Part 3: *In vitro* genotoxicity and cytotoxicity. *Food & Chemical Toxicology*. **40**: 105-111.

Roemer (2014) Toxicological assessment of kretek cigarettes: Part 1: background, assessment approach, and summary of findings. Regul Toxicol Pharmacol.; **70** Suppl 1: 2-14.

Roemer (2014) Toxicological assessment of kretek cigarettes Part 6: the impact of ingredients added to kretek cigarettes on smoke chemistry and in vitro toxicity. Regul Toxicol Pharmacol.; **70** Suppl 1: 66-80.

Rosenblat *et al.,* (1999). Macrophage enrichment for with the isoflavan glabridin inhibits NADPH oxidase induced cell mediated oxidation of low

density lipoprotein. A possible role for protein kinase C*. Journal of Biological Chemistry.* **274**: 13790-13799.

Rustemeier *et al.,* (2002). Evaluation of the potential effects of ingredients added to cigarettes. Part 2: Chemical composition of mainstream smoke. *Food & Chemical Toxicology* **40**: 93-104.

Sakr *et al*., (2011) Metiram-induced nephrotoxicity in albino mice: Effect of licorice aqueous extract. Environ Toxicol. [Epub ahead of print]

Schramke (2014) Toxicological assessment of kretek cigarettes. Part 7: the impact of ingredients added to kretek cigarettes on inhalation toxicity. Regul Toxicol Pharmacol; **70** Suppl 1: 81-9.

SCF (2003) Scientific Committee on Food Opinion on Glycyrrhizinic acid and its ammonium salt. http;//europa.eu.int/comm./food/fs/sc/scf

Shi *et al*., (2010) Monoammonium glycyrrhizinate inhibited the inflammation of LPS-induced acute lung injury in mice. Int Immunopharmacol; 10(10):1235-41.

Shin *et al.* (2005) Effects of Licorice on Embryonic and Fetal Development in Rats. *Journal of Toxicology and Public Health: an Official Journal of the Korean Society of Toxicology*; **21**:325-32
Shin S, Jang JY, Choi BI, Baek IJ, Yon JM, Hwang BY, Park D, Jeon JH, Nam SY, Yun YW, Kim YB (2008). Licorice extract does not impair the male reproductive function of rats. *Exp Anim*. 57(1):11-7.

Singletary *et al.,* (2000). Inhibition of benzo[α]pyrene and 1,6, dinitropyrene DNA adduct formation in human mammary epithelial cells by dibenzoylmetane sulphorafane. *Cancer Letters*. **155**: 47-54.

Strandberg *et al*., (2002). Preterm birth and licorice consumption during pregnancy. *American Journal of Epidemiology* **156(9):**803-5.

Stromer *et al.,* (1993). Glycyrrhizin acid in licorice – an evaluation of the health hazard. *Food & Chemical Toxicology.* **31**: 303-312.

Tamir *et al.,* (2000). Estrogenic and antiproliferative properties of glabridin from licorice in human breast cancer cell. *Cancer Research.* **06**: 5704-5709.

Van Gelderen *et al.,* (2000). Glycyrrhizic acid: the assessment of a no effect level. *Human & Experimental Toxicology.* **19(8)**: 434-439.

Vanscheeuwijck *et al.,* (2002). Evaluation of the potential effects of ingredients added to cigarettes. Part 4: Subchronic inhalation toxicity. *Food & Chemical Toxicology* **40**: 113-131.

Wang *et al.,* (1991). Inhibition of mutagenicity in Salmonella typhimurium and skin tumor initiating and and tumor promoting activiaties of in Sencar mice by

glycyrrhetinic acid: comparisons of the 18α and 18β-stereo isomers. *Carcinogenesis* **12**: 187-192.

Wang *et al.,* (1992). Protection against benzo[α]pyrene and N-nitrosodiethylamine-induced lung and fore stomach tumorogenesis in the A/J mice by water extracts of green tea and licorice. *Carcinogenesis* **13**: 1491-1494.

Wang *et al.,* (2001). Licorice and cancer. *Nutrition and cancer.* **39(1)**: 1-11.

Watanabe *et al*., (2002). Identification of licocoumarone as an apoptosis-inducing component in licorice. *Biological & Pharmaceutical Bulletin*. **25(10)**:1388-90.

Watari *et al.,* (1976). Ultrastructural studies on the on the protective effect of glycyrrhizin for liver injury induced by a carcinogen (3'-MeDab) (Second report). *Journal of Clinical Electron Microscopy* **9**: 394-395.

Wu YT *et al.,* (2006). Azathioprine hepatoxicity and the protective effect of licorice and glycyrrhizic. *Phytotherapy Research*. **20(8):** 640-645.

Xu R, Xiao Q, Cao Y, Yang J (2013). Comparison of the exposure of glycyrrhizin and its metabolites and the pseudoaldosteronism after intravenous administration of alpha- and beta-glycyrrhizin in rat. *Drug Res.* (Stuttg). **63**(12):620-4.

Yamaguchi *et al.,* (1984). Effects of triterpenes on the mutagenesis of various mutagens toward Salmonella. *Agricultural & Biological Chemistry*. **48(12)**: 3137-3139.

Yokozawa *et al.,* (2000). Protective effects of *Glycyrrhiza radix* extract and its compounds in a renal hypoxia (ischemia)-reoxygenation (reperfusion) model. *Phytomedia* **6**: 439-4445.

Yu *et al*., (2010) Selective cytotoxicity of glycyrrhetinic acid against tumorigenic r/m HM-SFME-1 cells: potential involvement of H-Ras downregulation. Toxicol Lett. Vol. 15; 192(3):425-30.

Zani *et al.,* (1993). Inhibition of the mutagenicity in *Salmonella typhimurium* by Glycyrrhiza glabra extract, glycyrrhizinic acid 18a and 18b-glycyrrhetinic acids. *Plant Medicine*. **59(6):** 502-507.

Zhang *et al.,* (1994). Inhibition of 11-β-hydroxysteroid dehydrogenase obtained from the guinea pig kidney by furosemide, naringenin and some other compounds. *Journal of Steroid Biochemistry & Molecular Biology*. **49**: 81-85.