Licorice root, fluid, extract and powder

Ingredient synonym names

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

IDENTIFIER DETAILS

Ingredient chemical structure

CAS Number	FEMA Number	Additive Number	Ingredient EC Number
68916-91-6	2630	-	272-837-1

CAS Additional Number	FL Number	CoE Number
- 1405-86-3	-	218

Chemical formula | A complex mixture of various compounds |

Ingredient CLP Classification

Ingredient REACH Registration Number

-

Acute Oral Toxicity	Eye Damage/Irritation	Carcinogenity
0	0	0

Acute Dermal Toxicity	Respiratory Sensitisation	Reproductive Toxicity
0	0	0

Acute Inhalation Toxicity	Skin Sensitisation	Aspiration Toxicity
0	0	0

Skin Corrosive/Irritant	Mutagenicity/ Genotoxicity	Specific Target Organ Toxicity
0	0	0

SPECIFICATIONS

Melting Point | No data provided | Boiling Point | No data provided |

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STATUS IN FOOD AND DRUG LAWS

Acceptable Daily Intake (ADI, mg/kg)	Glycyrrhizinic acid Not established
Acceptable Daily Intake (ADI) comments	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 JECFA (2005)
FDA Status	184.1408: Generally recognised as safe (GRAS). Liquorice and liquorice derivatives (0.05 % - 16 %).

CoE limits - Beverages (mg/kg)	CoE limits - Food (mg/kg)	CoE limits - Exceptions (mg/kg)
No data provided	No data provided	No data provided

HUMAN EXPOSURE

Ingredient Natural Occurence (if applicable)

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].

References - Ingredient Natural Occurence

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

Ingredient 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]

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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].

References - Ingredient Reported Uses

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

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.

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

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

TOXICITY DATA

In Vivo Data

Acute Toxicity Data

LD50, Mice, >7500 mg/kg (EMA, 2013)
LD50, Rats, 14,200 mg/kg (EMA, 2013)

EMA (2013). European Medicines Agency. Committee on Herbal Medicinal Products (HMPC). Final community herbal monograph on Glycyrrhiza glabra L. and/or Glycyrrhiza inflata Bat. and/or Glycyrrhiza uralensis Fisch, radix. 12 March 2013. EMA/HMPC/571122/2010.
http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/herbal/medicines/herbal_med_000132.jsp&mid=WC0b01ac058001fa1d

In Vivo Carcinogenicity/Mutagenicity

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].

Glycyrrhizin, a major constituent of liquorice extract, did not significantly affect the incidence of tumours, or types or distribution of tumours in mice treated for 96 weeks at 71, 166 or 229 mg/kg bw/day (males) and 117, 217 or 407 mg/kg bw/day (females). The investigators considered the results of this study to show that glycyrrhizin lacked the potential to induce tumours [no further details in citing source] (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].

An EFSA Expert Panel concluded that despite the chromosome aberrations detected in mammalian cells, in vivo data do not provide any evidence of genotoxicity. As such, the Expert Group had no concerns with regards to the genotoxic potential of the liquorice extract, Glavonoid® (EFSA, 2011).

Concentrated liquorice flavonoid oil was reported to lack tumour promoting potential for liver carcinogenesis in a study in which rats were injected with diethylnitrosamine (a known liver carcinogen) two weeks prior to treatment with the oil (EFSA, 2011).

Male rats (5/group) were administered Keneka glavonoid™ (a product containing licorice flavanoid oil solution comprising 30% liquorice ethanol extract and 70% medium chain triglycerides) at up to 5000 mg/kg bw by oral gavage on two consecutive days. Bone marrow was examined 24 hours after final treatment, and no significant increase in micronuclei was observed. Similarly, no increase in micronuclei was seen in the peripheral blood and liver cells of F344 rats (4/group) orally administered Keneka glavonoid at 5000 mg/kg bw/day for 3 days (Nakagawa et al., 2008b).

References - In Vivo Carcinogenicity/Mutagenicity

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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.

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

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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.

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.

EFSA (2011). European Food Safety Authority. Panel on Dietetic Products, Nutrition and Allergies (NDA). Scientific opinion on the safety of 'Glavonoid®', an extract derived from the roots or rootstock of Glycyrrhiza glabra L., as a novel food ingredient. EFSA Journal 2011, 9(7), 2287.

Nakagawa K, Hidaka T, Kitano M, Asakura M, Kamigaito T, Noguchi T and Hosoe,K (2008b). Genotoxicity studies on licorice flavonoid oil (LFO). Food and Chemical Toxicology 46, 2525-2532 [cited in CCRIS, 2010; EFSA, 2011].

Dermal Toxicity

A 76-year old Japanese woman who suffered an allergic response to a cosmetic cream was patch tested with the ingredients of the cream. Oil-soluble liquorice extract (tested at 1% in aqueous solution) was the only test material to produce a positive response indicative of skin sensitisation (Numata et al., 2015).

A similar case of skin sensitisation was reported in a 39-year old Japanese woman, patch tested with extracts of liquorice after suffering allergic responses to facial cosmetics (Kanoh et al., 2016).

References - Dermal Toxicity

Numata T, Kobayashi Y, Ito T, Harada K, Tsuboi R and Okubo Y (2015). Two cases of allergic contact dermatitis due to skin-whitening cosmetics. Letter to the Editor. Allergology International 64, 194-195.

Kanoh H, Banno Y, Nakamura M and Seishima M (2016). Contact allergy to liquorice flavonoids: analysis with liquid chromatography–mass spectrometry. Contact Dermatitis 74, 181-182.

Reproductive/ 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].

According to the EMA, the main constituent of liquorice extract (glycyrrhizin) exhibited some embryotoxicity to the developing rat foetus in developmental toxicity studies, but the foetal effects were considered as minor (EMA, 2013).

Pregnant mice, rats, hamsters and rabbits were administered the ammonium salt of glycyrrhizin (the main constituent of liquorice extract) at 0, 27, 90, 300 or 1000 mg/kg bw/day, commencing on day 6 of gestation. Mice and rats were dosed for 10 consecutive days, whereas rabbits and hamsters were dosed for 5 and 13 days, respectively. There were no adverse effects on implantation or on maternal or foetal survival in any species. Gross and histological examination revealed no abnormalities in either the soft or skeletal tissues of offspring (EMA, 2013).

Pregnant Wistar rats were administered disodium glycyrrhizin in the diet during days 0-20 of gestation. There were no significant effects on food intake, number of implants, number of corpora lutea, incidence of intrauterine deaths, number of live foetuses, sex ratios, foetal body weights, placental weights, degrees of ossification, live birth index or body weight gain after birth. The investigators concluded that disodium glycyrrhizin is not a developmental toxin in rats under the conditions of this study (EMA, 2013).

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When pregnant Sprague-Dawley rats were administered ammonium glycyrrhizin in the drinking water from gestation day 7-20, the embryotoxicity score was "significantly dose related", although there were no significant differences in number of corpora lutea, implants, or live foetuses. Skeletal abnormalities were significantly elevated in the two highest dose groups, however, the investigators noted that these abnormalities (including misaligned, asymmetric and bipartic sternebrae and hemisternebrae) were common in control litters. In addition, the occurrence of soft-tissue abnormalities was significantly elevated in the two highest dose groups (mostly renal), and external haemorrhages were also observed in some foetuses. The investigators concluded that ammonium glycyrrhizin exhibited some embryotoxicity to the developing rat foetus, but no toxicity to the mother, and that the foetal effects were minor (EMA, 2013; Mantovani et al., 1988).

Pregnant Wistar rats were fed a daily diet delivering 0, 10, 100, or 1000 mg/kg bw of 18β-glycyrrhetinic acid, a major constituent of liquorice extract, commencing on the day 13 of gestation. Foetuses were examined on days 17, 19 and 21 of gestation as well as on the day 1 post-partum. Foetal lung 11β-HSD activity was moderately, but significantly, reduced in the highest dosed group as compared to controls. There was a significant decrease in the foetal lung surfactant protein A mRNA levels in the 1000 mg/kg group. Histological examination of foetal lungs in this dose group showed a significant reduction in lamellar body content and a reduced number alveolar lamellar body and surfactant clusters as compared to controls. Despite these effects, there was no apparent increase in malformation or foetal death rate associated with 18β-glycyrrhetinic acid exposure; neither was there any abnormal behaviour observed in the neonatal rats (Hundertmark et al., 2002).

Effects of liquorice extract on foetal abnormalities induced by cyclophosphamide were investigated in rats. Pregnant Sprague–Dawley rats were orally administered liquorice extract (100 mg/kg bw) for 7 days, from days 6 to 12 of gestation, and intraperitoneally exposed to cyclophosphamide (11 mg/kg) 1 hour after the final treatment. Cyclophosphamide reduced foetal and placental weights and induced malformations in live foetuses (94.6%, 41.5% and 100% of external, visceral and skeletal defects, respectively). Liquorice extract further decreased the foetal body weights and markedly enhanced foetal defects (resulting in 76.4% of cranial defect and exencephaly, 22.7% of micrognathia and tongue extrusion, 85.5% of vertebral defects, 85.5% of costal defects, and 100% of delayed skeletal ossification) (Jeon et al., 2007).

Earlier puberty, especially in girls, is associated with physical and mental disorders. Prenatal glucocorticoid exposure influences the timing of puberty in animal models, but the human relevance of those findings is unknown. We studied whether voluntary consumption of licorice, which contains glycyrrhizin (a potent inhibitor of placental 11β-hydroxysteroid dehydrogenase type 2, the "barrier" to maternal glucocorticoids), by pregnant women was associated with pubertal maturation (height, weight, body mass index for age, difference between current and expected adult height, Tanner staging, score on the Pubertal Development Scale), neuroendocrine function (diurnal salivary cortisol, dexamethasone suppression), cognition (neuropsychological tests), and psychiatric problems (as measured by the Child Behavior Checklist) in their offspring. The children were born in 1998 in Helsinki, Finland, and examined during 2009-2011 (mean age = 12.5 (standard deviation (SD), 0.4) years; n = 378). Girls exposed to high maternal glycyrrhizin consumption (≥500 mg/week) were taller (mean difference (MD) = 0.4 SD, 95% confidence interval (CI): 0.1, 0.8), were heavier (MD = 0.6 SD, 95% CI: 0.2, 1.9), and had higher body mass index for age (MD = 0.6 SD, 95% CI: 0.2, 0.9). They were also 0.5 standard deviations (95% CI: 0.2, 0.8) closer to adult height and reported more advanced pubertal development (P < 0.04). Girls and boys exposed to high maternal glycyrrhizin consumption scored 7 (95% CI: 3.1, 11.2) points lower on tests of intelligence quotient, had poorer memory (P < 0.04), and had 3.3-fold (95% CI: 1.4, 7.7) higher odds of attention deficit/hyperactivity disorder problems compared with children whose mothers consumed little to no glycyrrhizin (≤249 mg/week). No differences in cortisol levels were found. Licorice consumption during pregnancy may be associated with harm for the developing offspring [Raikkonen, 2017].

References - Reproductive/ Developmental Toxicity

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Inhalation Toxicity

Liquorice extract reportedly has broncho-dilating properties (DKFZ, 2012; SCHEER, 2016).

Thirty workers exposed to liquorice root dust at a liquorice producing plant in Iran were assessed for adverse effects, and matched to 30 healthy male employees at a telecommunications company (without prior or current workplace exposure to liquorice). High blood pressure was reported in exposed workers, whereas blood concentrations of potassium and platelet counts were lower than the controls. Self-reported symptoms such as headache, lethargy and vertigo were more commonly reported in the liquorice group (Neghab and Choobineh, 2008).

References - Inhalation Toxicity

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SCHEER (2016). Scientific Committee on Health, Environment and Emerging Risks. Preliminary opinion on additives used in tobacco products (opinion 2). Tobacco Additives II. http://ec.europa.eu/health/scientific_committees/scheer/docs/scheer_o_001.pdf

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Cardiac Toxicity

The excessive consumption of liquorice (1020 g) over 3 days was considered the cause of pulmonary oedema seen in a 64-year old man (Chamberlain and Abolnik, 1997).

Liquorice has been reported to cause hypokalaemia and induce hypertension in humans (Celik et al., 2012; Koster and David, 1968; Neghab and Choobineh, 2008; Ottenbacher and Blehm, 2015; Pelttari and Välimäki, 2015; Schulze zur Wiesch et al., 2011; Sigurjónsdóttir et al., 2001; Tanaka, 2015) and its consumption has been associated with cases of cardiac arrest and other cardiac abnormalities (Bannister et al., 1977; Montoliu, 1977; Robinson et al., 1971). Indeed, the SCHEER Expert Committee found that hypokalemia and hypernatremia are primary problems associated with chronic liquorice ingestion, and cardiomyopathy, pulmonary oedema have also been reported in patients following chronic, excessive liquorice ingestion (SCHEER, 2016).

A significant decrease in erythrocyte counts (with a decrease in haematocrit in males) was observed in both sexes of rats orally administered liquorice extract at doses of 1250 and 2500 mg/kg bw/day for 90 days (EMA, 2013).

The intraperitoneal administration of 2000 mg/kg bw of 18α-glycyrrhetic acid (a major constituent of liquorice extract) to rats led to the progressive impairment of cardiac function, and histopathological examination revealed lung oedema. Focal changes of the papillary muscles as well as swollen cardiomyocytes were also noted (EMA, 2013).

In a sub-chronic oral toxicity study rats (10/sex/group) were administered a concentrated form of liquorice flavonoid oil (LFO) by gavage for 90 days at 400, 600, 800 or 1600 mg/kg bw/day. Blood coagulation parameters were affected, evidenced in a prolongation of prothrombin time (PT) and activated partial thromboplastin time (APTT), which caused haemorrhage in several organs and tissues and the death of several animals in the highest dose group. A prolonged PT was statistically significant in males receiving the lowest dose level (Nakagawa et al., 2008a).

References - Cardiac Toxicity

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Addictive Data

Liquorice can enhance the addictiveness of nicotine-products in a few different ways. Firstly it functions as a bronchodilator. This potentially enables the consumer to inhale a larger volume, which may increase the bioavailability of nicotine. Secondly, liquorice extract has been reported to exhibit a "smoothing" effect (possibly via interaction with transient receptor potential channel receptors). By creating the perception of soothing the oral and throat mucosa, it may (partially) mask any respiratory tract irritant effects caused by other agents present in the product. Thirdly, liquorice reduces dryness in the mouth and throat, and lastly, the extract provides a pleasant, sweet taste. These final three effects enhance the attractiveness of the product and stimulate the use of nicotine dosing (DKFZ, 2012; EMA, 2013; SCHEER, 2016).

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Licorice root, fluid, extract and powder

Behavioral 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].

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In Vivo - 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 [H3] BaP and [H3] 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 [H3] BaP or a 30 % or 48 % inhibition of in vivo binding of [H3] 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 11βHSD [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 CCl4 [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 T1/2 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 (prolongation 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] LD50 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].

A 33-year-old woman, who had been a herbalist for 13 years, was exposed to a number of natural products in powder form including liquorice root. She developed asthma 4-5 years after starting her job. Symptoms were brought on when she handled several natural products in powdered form, including liquorice root. Skin prick tests with liquorice extract and inhalation challenge tests with liquorice root powder confirmed her allergy (Cartier et al., 2002).

A 32-year-old man, who had been working in an anis liqueur factory for 8 years, handled a number of plants at his workplace, including liquorice. The subject developed occupational rhinitis and asthma, particularly when handling liquorice, about 4 years after starting his job. Skin-prick and immunological tests confirmed his allergy (González-Glutiérrez et al., 2000).

Liquorice and liquorice derivatives, are considered as 'Generally Recognized as Safe' (GRAS) for use in foods by the US FDA. The Council of Europe (CoE) and the UK Food Additive and Contaminants Committee (FACC) consider liquorice as a natural plant product for use (in small quantities) as a food additive with the intention that its consumption is limited by the glycyrrhizin levels (<50 ppm glycyrrhizin) (EMA, 2013).

Licorice root, fluid, extract and powder

Rats were orally administered liquorice extract at 310, 630, 1250 or 2500 mg/kg bw/day for 90 days. Body weight gain was "slightly inhibited", and total protein, albumin, cholesterol, AST and ALT levels were modulated in animals at 2500 mg/kg bw/day. A significant decrease in erythrocyte counts (with a decrease in haematocrit in males) was observed at the two highest tested doses. Average liver and kidney weights were increased from 1250 mg/kg bw/day, however, there were no significant histological changes observed in these organs. The investigators considered the no-observed-adverse-effect level (NOAEL) to be "310-630" mg/kg bw/day (EMA, 2013).

According to the European Commission Expert Committee (SCHEER), hypokalemia, hypernatremia, and water retention are primary problems associated with chronic liquorice ingestion. Furthermore, changes in the sodium/potassium ratios may result in pH changes; and cardiomyopathy, pulmonary oedema, myoglobinuria, ptosis, myopathy, tetany, cramping, seizures, and rhabdomyolysis have also been reported in patients following chronic, excessive liquorice ingestion (SCHEER, 2016).

Six studies were described by EFSA (2011), in which subjects received doses of the liquorice extract Glavonoid from 100 - 600 mg/day for several weeks. No significant changes in haematology, coagulation, clinical-chemistry or urinalysis parameters were observed after administration (EFSA, 2011).

In a sub-chronic oral study submitted to EFSA, to evaluate the potential toxicity of Glavonoid®, Sprague-Dawley rats (10/sex/group) were administered a concentrated form of liquorice flavonoid oil (LFO) by gavage for 90 days at 400, 600, 800 or 1600 mg/kg bw/day. Most of the males (8/10) died at the top dose. Blood coagulation parameters were affected, evidenced in a prolongation of prothrombin time (PT) and activated partial thromboplastin time (APTT), which caused haemorrhage in several organs and tissues and the death of several animals in the highest dose group. A prolonged PT was statistically significant in males receiving the lowest dose level. In reviewing the data, EFSA (2011) concluded a NOAEL could not be identified and the dose of 400 mg/kg bw/day can be considered the study LOAEL. However, a BMDL05 value of 167 mg/kg bw/day was derived from the APTT data of the male rats (which was lower than the value calculated for PT) (Nakagawa et al., 2008a).

Glycyrrhizin, a major constituent of liquorice extract, did not significantly affect average body weights, cumulative mortality rates or mean time-to-death in mice treated for 96 weeks at 71, 166 or 229 mg/kg bw/day (males) and 117, 217 or 407 mg/kg bw/day (females). The investigators considered the results of this study to show that glycyrrhizin lacked the potential to cause chronic toxicity (Kobuke et al., 1985).

Hypertension is one of the most common problems encountered in the primary care setting. Numerous secondary causes of hypertension exist and are potentially reversible. The ability to screen for such causes and manage them effectively may spare patients from prolonged medical therapy and hypertensive complications. Licorice can cause hypertension and hypokalemia due its effects on cortisol metabolism. We report a case of jelly bean ingestion that highlights the presentation, pathophysiology and management of licorice-induced hypertension [Foster et al, 2017].

RATIONALE:

Glycyrrhizin is the main active component of licorice. Licorice and glycyrrhizin induced hypertension has been widely reported, yet licorice and glycyrrhizin induced hypertensive crisis has been rarely known.

PATIENT CONCERNS:

The case of this report was a 47-year-old woman, who took 225 mg of glycyrrhizin daily for 3 years due to primary biliary cholangitis. She was found to have a dramatically elevated blood pressure of about 230/110 mmHg without a history of hypertension and was referred to the emergency department.
Hypokalemia, hypertensive retinopathy, and nephropathy were found during the following work-up. Since no other risk factors of hypertension were identified, she was suspected to have glycyrrhizin induced pseudo-

hyperaldosteronism. Glycyrrhizin was discontinued. Intravenous sodium nitroprusside was used during the first few days. Nifedipine and irbesartan were taken after discharge, and the dosage was reduced gradually under supervision. She stopped all the anti-hypertensive drugs 6 months since glycyrrhizin was stopped. Her blood pressure was about 110/60 mmHg after repetitive measurement. Her serum potassium and urine albumin/creatinine ratio were also normalized [Li et al, 2018].

Licorice (Glycyrrhiza glabra) has been considered as an herbal drug since ancient time. Nowadays, it is a well-known spice that possesses worth pharmacological effects. However, some relevant articles have revealed negative impacts of licorice in health. By considering the great wishes in using herbal medicine, it is important to show adverse effects of herbal medicine in health. At present, there are misunderstandings toward the safety of herbal medicines. Herein, we gathered scientific research projects on the toxicity effects of licorice and glycyrrhizin to highlight their safety. In this regards, we categorized our findings about the toxicity effects of licorice and glycyrrhizin in acute, sub-acute, sub-chronic, and chronic states. Besides, we discussed on the cytotoxicity, genotoxicity, mutagenicity, and carcinogenicity of licorice and glycyrrhizin as well as their developmental toxicity. This review disclosed that G. glabra and glycyrrhizin salts are moderately toxic. They need to be used with caution during pregnancy. G. glabra and glycyrrhizin possess selective cytotoxic effects on cancerous cells. The most important side effects of licorice and glycyrrhizin are hypertension and hypokalemic-induced secondary disorders. Licorice side effects are increased by hypokalemia, prolonged gastrointestinal transient time, decreased type 2 11-beta-hydroxysteroid dehydrogenase activities, hypertension, anorexia nervosa, old age, and female sex [Nazari et al, 2017].

There have been numerous case reports of severe adverse events including deaths following chronic licorice ingestion. The aim of the present study was to evaluate the effect of chronic ingestion of licorice on blood pressure, plasma potassium, plasma renin activity and plasma aldosterone. A search of MEDLINE, PubMed, EMBASE, CENTRAL, DARE, CINAHL and Current Contents Connect was performed from inception through to 26 April 2017. Trials that included a treatment group ingesting a product containing at least 100 mg of glycyrrhizic acid daily were selected. Pooled mean changes from baseline with 95% confidence intervals were calculated for diastolic blood pressure, systolic blood pressure, plasma potassium, plasma renin activity and plasma aldosterone using a random effects model. An assessment of dose-response was also undertaken. A total of 18 studies (n=337) were included in the meta-analysis. There was a statistically significant increase in mean systolic blood pressure (5.45 mm Hg, 95% CI 3.51-7.39) and diastolic blood pressure (3.19 mm Hg, 95% CI 0.10-6.29) after chronic ingestion of a product containing glycyrrhizic acid. Plasma potassium (-0.33 mmol l-1, 95% CI -0.42 to 0.23), plasma renin activity (-0.82 ngml-1 per hour, 95% CI -1.27 to -0.37) and plasma aldosterone (-173.24 pmol l-1, 95% CI -231.65 to -114.83) were all significantly decreased. A significant correlation was noted between daily dose of glycyrrhizic acid and systolic blood pressure (r2=0.55) and diastolic blood pressure (r2=0.65), but not for the other outcome measures. Hence, chronic licorice ingestion is associated with an increase in blood pressure and a drop in plasma potassium, even at modest doses. This is of particular relevance for individuals with existing cardiovascular disease [Penninkilampi et al, 2017].

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In Vitro Data

In Vitro Carcinogenicity/Mutagenicity

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].

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].

Liquorice extract (GutGard™) did not show any clastogenic effects in chromosome aberration and micronucleus tests, conducted according to OECD Test Guidelines 473 and 487 respectively. In these assays, Chinese hamster ovary (CHO) K1 cells were treated for either 4 or 18 hours, with and without metabolic activation (S9) respectively (Chandrasekaran et al., 2011).

Liquorice extract (GutGard™) gave no evidence of mutagenicity in the Ames II™ test with S. typhimurium (strains TA98 and TAMix) when tested with and without S9. The extract was tested at up to 501 µg/mL (as cytotoxicity was observed at 1590 µg/mL) (Chandrasekaran et al., 2011).

Chinese hamster lung (CHL) cells were treated with Keneka glavonoid® at up to 0.7 mg/mL for 6 hours with and without S9, or for 24 or 48 hours without S9. The cells were then examined for structural chromosome aberrations and polyploidy. Structural aberrations were detected in the presence of S9 following 6-hour treatment at 0.6 mg/mL and above only (Nakagawa et al., 2008b).

In a bacterial reverse mutation (Ames) assay, Salmonella typhimurium strains TA98, TA100, TA1535, and TA1537, and Escherichia coli (WP2uvrA/pKM101), were treated with liquorice flavonoid oil in the presence and absence of S9. No increase in the frequency of revertant colonies was observed (Nakagawa et al., 2008b).

Glycyrrhiza glabra L. (licorice) is one of the most important medicinal plants, which is widely used throughout the

world both in traditional and contemporary medical industries. This study was undertaken to investigate the potential genotoxic activity of G. glabra methanolic root extract, and its possible antigenotoxic properties against mitomycin C (MMC)-induced DNA damage in in vitro chromosome aberrations (CAs) and cytokinesis-block micronucleus (CBMN) assays in human peripheral blood lymphocytes (PBLs). Lymphocytes were treated with 25, 50, and 100 microg/ml G. glabra methanolic root extract alone as well as in combination with MMC (0.1 microg/ml) for 24 and 48 h treatment periods. It was found that there were no statistically significant differences between the negative control and the groups treated with all concentrations of G. glabra root extract of alone (p > 0.05), demonstrating the absence of genotoxic effects at both 24 and 48 h treatment periods. Besides, the co-treatment of G. glabra methanolic root extract and MMC significantly decreased the percentage of structural CAs and MN formation when compared with the culture treated with MMC alone (p < 0.001). In addition, the negative interaction factor (IF) values obtained for all combinations represent an antagonistic effect of G. glabra versus MMC. We can state that this extract acts as an antagonist and markedly decreased MMC-induced cytogenotoxicity. In conclusion, the present results demonstrate that in the tested experimental conditions, G. glabra methanolic root extract is not genotoxic in cultured human PBLs and has also antigenotoxic activity against MMC, which is widely used in chemotherapy against cancer. (Yavuz et al, 2018).

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In Vitro - 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 bke 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).

Hypertension is one of the most common problems encountered in the primary care setting. Numerous secondary causes of hypertension exist and are potentially reversible. The ability to screen for such causes and manage them effectively may spare patients from prolonged medical therapy and hypertensive complications. Licorice can cause hypertension and hypokalemia due its effects on cortisol metabolism. We report a case of jelly bean ingestion that highlights the presentation, pathophysiology and management of licorice-induced hypertension.

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Emissions and Associated 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].

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].

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.

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].

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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 (Internal document R-009, 010).

References - Emissions and Associated Toxicity Data

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