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Utilization of Glycyrrhizin and Licorice Extract as Natural Sweetener in Some Food Products and Biological Impacts

Abd El-Lahot, M. S.[1]; Amal M. Abd El-Razek[1]; Mona I. Massoud[2] and E. G. Gomaa[1]
[1]Food Science &Technology Dept., Fac. of Agric. El-Shatby,21545, Alexandria Univ., Alexandria, Egypt.
[2]Sugar Crops Research Institute, Agric. Res. Center, El Sabahia, Alexandria., Egypt

ABSTRACT

Replacing sucrose with safe natural intense sweeteners has importance in health concerns as a way to produce healthy foods with low caloric value. The present study has been carried out to utilize glycyrrhizin and licorice extract (LE) from *Glycyrrhiza glabra L* as a substitute of sucrose for sweetening toffee and cake preparation. Sensory attributes of the produced products (toffee and cakes) were evaluated and its biological effects on the body weight, organ relative weight, hematological and biochemical parameters and enzyme activities were studied *in vivo*. The results indicated that toffee prepared from replacing sugar with glycyrrhizin at 50:50 ratio (750 mg/100 g) showed the highest sensory over all acceptability scores. Replacing 25% glucose syrup with licorice extract (1.37 g /100 g) gave the higher score of sweetness, flavour, overall acceptability and absence of bitterness comparable to those prepared using increase the level of licorice extract. Cakes sweetened with 250 mg glycyrrhizin as 50% sugar replacer had no significant effect on the organoleptic properties but reduced their caloric value. We investigated biological impacts of young male rats fed with low and high doses of glycyrrhizin or licorice extract for 60 days. The results showed glycyrrhizin treated group significantly ($P< 0.05$) decreased feed intake with insignificant reduction in body weights gain (%) and insignificant increases in feed efficiency ratio as compared to control group but licorice extract treated group showed insignificant increase in body weight gain and feed efficiency ratio compared with the control group. Treatment with glycyrrhizin or licorice extract caused significant reduction in triglycerides (TG), with insignificant increase in high-density lipoprotein (HDL) and decrease alanine transaminase (ALT), aspartate transaminase (AST), and acid phosphatase (ACP) values compared to control groups. Histopathological investigation of liver and kidney tissues of glycyrrhizin treated rats showed completely normal structure with regular arrangement of cell when compared with control group.

Keywords: Glycyrrhizin, organoleptic properties, biological effects, biochemical parameters

INTRODUCTION

Emerging technologies applied in food science includes the incorporation of natural extracts in order to improve the food quality properties. The licorice (*Glycyrrhiza glabra L.)* and its derivatives have been used as additive in food industry to eliminate undesirable tastes and intensify and extend sweetness and flavours, as well as a foaming agent in alcoholic and non-alcoholic beverages. Licorice is a therapeutic plant that has been used from ancient times in different ways, mainly in food as a sweetener and as a source of bioactive compounds, including glycyrrhizin, volatile compounds, flavonoids, saponins, and others with pharmacological activities (Omar *et al*., 2012).

Glycyrrhizin is a triterpene saponin, and it is a conjugate of two molecules of glucuronic acid and glycyrrhetinic acid and as an active component in licorice is responsible for the sweet-tasting properties of licorice. The glycyrrhizin sweet taste is considered to be 50 to 200 times higher than sucrose and this important characteristic makes it a powerful natural sweetener, flavouring agent and is commonly used as an emulsifier and gel-forming agent in foodstuff (Hayashi and Sudo, 2009 and Tohma and Gulçin, 2010).

Licorice extract is used as sweetener agent to mask the bitter taste in the confectionery and tobacco industry (Carmines *et al.*, 2005; Hayashi and Sudo, 2009). It is used as food additive could provide foods with health benefits in preparing some specified products for persons suffering from low blood pressure. Currently, Licorice extract has antiviral, antiinflamatory, antihepatotoxic, and antimutagenic effects (Kaur *et al*., 2013).

Nowadays, licorice confectionery is widely available, and large quantities of licorice oil are used in this kind of industry. Therefore, glycyrrhizin and licorice extracts are commonly used in sweet foods such as sweet snacks, ice cream and sherbets to enhance their sweetness. Snacks like sticks and toffee bars, instant noodles and sauces are others examples of foods containing licorice. Furthermore, licorice oil has been used in foods as an antioxidant and antimicrobial agent to increase food quality (Al-Turki *et al*., 2008 and Jiang *et al*., 2013). Mocanu *et al*. (2009) obtained a novel probiotic product named ROSALACT®, prepared from pasteurized milk with rosehip and licorice extract, using a mixed culture of probiotic bacteria.

Biological studies have demonstrated that, No observed adverse-effect level (NOAEL) was in the range of 15 - 229 mg/kg/day for purified glycyrrhizin (Isbrucker and Burdock 2006). The US Food and Drug Administration (21 CFR 184.1408) FDA, (2011) stated that licorice and ammoniated glycyrrhizin can be considered GRAS (generally recognized as safe). The Joint Food and Agriculture Organization/ World Organization Expert Committee on Food Additives (JECFA) indicated that consumption of glycyrrhizic acid (GA), up to 100 mg/day would be cause adverse effects in the adults.

The present study is aimed to utilize glycyrrhizin and licorice extract from *Glycyrrhiza glabra* as substitute of sucrose for sweetening toffee and cake preparation and to investigate its effects on some toxicological parameters such as body weight, organ relative weight, hematological, biochemical parameters and enzyme activities on young male rats.

MATERIALS AND METHODS

Chemicals and Reagents

Glycyrrhizic acid ammonium salt (95% pure) was obtained from Pharco Pharmaceuticals Company, Alexandria, Egypt. Roots of *Glycyrrhiza glabra*, were

purchased from Herbal Store in Alexandria, Egypt. All other chemicals were of analytical grade.

Toffee and cake ingredients: Namely lecithin, commercial glucose, flour, butter, corn oil, eggs, milk powder, sugar, baking powder and vanilla were all purchased from Alexandria markets, Egypt.

Preparation of licorice root extract:

Licorice root extract was prepared from the dried roots according to the method described by Mukhopadhyay and Panja (2008). Briefly, fifty grams of powdered licorice root was mixed with 2 L ammonia solution (0.01w/v) and autoclaved at 110˚C for 90 min. The solution was filtered, and supernatant was kept at 4˚C until further uses.

Preparation of glycyrrhizin:

The crude extract of licorice roots was concentrated by rotary evaporator and ethanol (70%) was added at a ratio of 3:1(v/v). The ethanol layer was separated, concentrated and acidified by sulfuric acid to pH 1–2, then filtrated and washed with ethanol. White crystalls obtained of mono-ammonium glycyrrhizate (MAG) were dehydrated under vacuum then kept in polyethylene bags until analysis (Mukhopadhyay and Panja, 2008).

Toffee and cakes preparation

Preparation of Toffee: It was prepared as stated by El-Iraki *et al.* (1987) with some modification for ingredients as the follows: 31.4% sucrose, 55% glucose, 6.3% hydrogenated corn oils, 0.79% milk powder, 6.3% water, 0.08% lecithin and 0.08% vanillin flavour. Different trials were made to select the best blend to prepare low-calorie toffee with glycyrrhizin or licorice extract as sweetener. These trials were as follows: Replacing 25% (T_1), 50% (T_2) and 75% (T_3) of sucrose by glycyrrhizin, replacing 25% (T_4), 50% (T_5), 75% (T_6) and 100% (T_7) of glucose syrup by licorice root extract and replacing 50% of sucrose, 50% of glucose syrup by blend of glycyrrhizin and licorice extract, respectively (T_8). The amount of sweetener was calculated according to its sweetness equivalent.

Cakes preparation: The formula of cake manufacture was prepared as described by Nor Aini *et al.* (1992) with some modification for ingredients as the follows: 37.12% flour, 24.75% sugar, 12.37% milk, 12.37% fresh whole eggs, 12.37% butter, 0.99% baking powder and 0.03% vanilla. The sucrose was substituted by the glycyrrhizin at ratio 25 and 50%. The amount of sweetener was calculated according to its sweetness equivalence.

Physical properties of prepared products: Cake volume was measured by the rapeseed displacement method. The specific volumes of the respective cakes were calculated after measuring their weight according to the AACC (2000). The colour values of all samples were evaluated by a Hunter Lab Ultra Scan VIS model, colorimeter (USA). The lightness (L*), redness (a*) and yellowness (b*) were calculated from the colour primaries (Santipanichwong & Suphantharika, 2007). Total colour difference (ΔE) between reference and samples containing glycyrrhizin was calculated from the respective L*, a* and b* differences by $\Delta E = [(\Delta L^*)2 + (\Delta a^*)2 + (\Delta b^*)2]1/2$. Compression measurements were performed with a Texture Analyzer (Texture Pro CT3 V1.2, Brookfield, Middleboro, USA) equipped with a 5 kg load cell at a speed of 1 mm/s to a distance of 20 mm with a 4 g trigger load for 30 s.

Sensory evaluation: Colour, taste, flavour, texture, and overall acceptability of prepared products were assessed via 20 panelists of the Department of Food Science and Technology, Alexandria University using hedonic scale as proposed by Iwe (2002).

Determination of biological effects of aqueous extract of Glycyrrhizin and licorice extract

Feeding experiments: Feeding experiments were done in the animal house, Home economics Department, University of Alex. Male Wistar rats with average body weight of 60±5 g were used. Animals were housed individually in cages at a controlled temperature (22 ± 2 ◦C) and relative humidity (60 ± 10%) with a photoperiod of 12 h light/12 h dark . All animals were given *ad libitum* access to distilled water and a standard diet that meets the nutrient requirements for growing rats. The diet consisted of 44% soybean flour, 12 % fiber, 13.2% starch, 9.8% maize, 3.5% oil, 5% minerals, 2% salt and 0.5% vitamins mixture. After a 7 days acclimation, animals were divided into five groups.

Experimental groups were organized as five groups that included six animals each. The first group was orally given tap water (control group). The second group (NOAEL-GL) included the animals which were orally given solution of glycyrrhizic acid ammonium salt at dose 229 mg/day that no observed adverse-effect level. The other groups included the animals which administered a low and high dose of glycyrrhizin (low-GLY, high-GLY) and licorice extract (LE) which was chosen based on the results of organoleptic properties of the different products which accepted by the panelist. Solutions concentrations were adjusted weekly based on animal average weight and current fluid consumption. Fluid intake and feed intake were recorded daily and all tested materials intakes (mg kg-1 per day) were calculated from the mean fluids consumed (ml kg-1 per day) and the appropriate target concentration of test article in the solutions (mg kg-1). Body weights and feed intake were measured weekly. Feed efficiency ratio (FER) of the different diets was calculated as the gain in body weight (g)/ feed intake (g) Chapman *et al*. (1959). At the end of the experiment, after 60 days of receiving the extract, the rats were fasted overnight, then they were ether-anesthetized, sacrificed and blood samples were collected. Serum samples were obtained by centrifugation at 3000 rpm (800 xg) for 20 min and stored at -20 °C until assay. Liver, kidney, brain, lung, heart, testicular, and spleen were immediately removed; weighed and washed using chilled saline solution; relative organ weights were calculated on the basis of the animal weight at the time of killing then stored at -70 °C to be used for further studies. Immediately, the liver and kidney were stored in 10% neutralized formalin for histopathological examination.

Hematological parameters

The hematological analysis like red blood cells (RBC), white blood cells (WBC), haemoglobin level (HGB), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and packed cell volume (PCV) were carried out based on standard methods.

Biochemical and enzymes activities assays

Serum was used to determine total lipids (TL) by the method of Zollner and Kirsch (1962), triglycerides (TG) as described by Fassati and Prencipe (1982) and total cholesterol (TC) by the method of Allain *et al*. (1974). High-density lipoprotein (HDL) and low-density lipoprotein (LDL) were determined according to the methods of Warnick *et al*. (1983) and Wieland and Seidel (1983), respectively. Also in serum, the concentrations of glucose were determined with kits from Biosystems Co according to (Trinder, 1969), whereas urea, uric acid, creatinine and bilirubin levels were determined by the methods of Fawcett and Soctt (1960), Barham, and Trinder (1972), Larsen (1972) and Young (2001), respectively. Total protein was also assayed by the Biuret method according to Gornal *et al*. (1949) and for albumin concentration by the method of Doumas *et al*. (1971). Serum alanine aminotransferase (ALT; EC 2.6.1.2) and aspartate aminotransferase (AST; EC 2.6.1.1) activities were assayed by the method of Reitman and Frankel (1957). Serum alkaline phosphatase (ALP; EC 3.1.3.1) activity was measured at 405 nm by the formation of para-nitrophenol from para-nitrophenylphosphate as a substrate (Belfield and Goldberg, 1971). Acid phosphatase (ACP; EC 3.1.3.2) activity was measured using the method of Kind and King (1954).

Histopathological examination

Sections measuring approximately 2 µm thickness were taken from the livers and kidney of each animal which were perfused in 10% neutral-buffered formalin were mounted on a microscope slide, stained with haematoxylin–eosin (HE) and examined under light microscopy.

Statistical analysis

Analysis of variance for a completely randomized design was done according to the literature (Gomez and Gomez, 1984) by using SPSS software program. The level of significant difference was determined at $p \leq 0.05$.

RESULTS AND DISCUSSION

Physical and organoleptic properties of prepared products

1. Sensory evaluation of toffee

In the sensory evaluation, the data presented in Table (1) indicated that the toffee prepared from replacing sugar with glycyrrhizin at 50:50 ratio (T_2) resulted into highest score in all the sensory quality parameters. Meanwhile, toffee prepared by replacing 25% glucose syrup with licorice extract (T_4) had the higher score of sweetness, flavour, overall acceptability and absence of bitterness compared with those which sweetened with high concentration of licorice extract as glucose syrup replaces. Toffee prepared with 100% licorice extract (T_7) had lower mean scores for colour, sweetness, texture and overall acceptability as compared to other sample. The flavour and texture scores of toffee prepared with glycyrrhizin were comparatively higher than the toffees prepared from any of the blends. This may probably be due to synergistic effect of glycyrrhizin as a sweetener. The toffee prepared with licorice extract showed that the increase in licorice extract (above 50%) decreased colour scores. However, bitterness score was significant increases by increasing the level of replacer with glycyrrhizin or licorice extract. The general appearances of toffee containing glycyrrhizin, licorice extract and blend are shown in Fig.1 (a, b and c). Panelists accepted all the toffee prepared from 50% replacing sugar by 750 mg glycyrrhizin and 25% replacing glucose syrup with 1.37 g licorice extract. Maas (2000) reported that the mean content of glycyrrhizinic acid in licorice confectionery in the Netherlands was 0.17%, this amount of would correspond to a daily intake of 19 mg glycyrrhizinic acid. Glycyrrhizinic acid in the form of licorice extract have been used in a variety of food products and confectionery, including chewing gum, chewing tobacco (up to 4%) and ice cream (0.5 or 1.5%) (Rosseel and Schoors, 1993; Blakey, 1998; and Barakat, 2009)

2. Physicochemical and organoleptic properties of cake containing glycyrrhizin

Changes in cake characteristics with glycyrrhizin are shown in Table (2). A significant decrease in cake volume was noted with an increase in the glycyrrhizin level. Specific volume is an important parameter, as it measures the cake density and quality, especially when it is linked with acceptance, appearance, crumb texture and grain. The specific volumes of cake containing glycyrrhizin showed a higher value as compared to the control indicating a higher amount of air remained in the cake. A higher gas retention and higher expansion of the product may be lead to a higher specific volume (Gomez *et al.* 2008).

The colour of samples was affected by the replacement of cake sugar with glycyrrhizin (Table 2). For crumb colour, as the level of glycyrrhizin increased, the L, a and b values decreased, indicating that a darker, less redder and less yellow crumb was obtained as a result of glycyrrhizin substitution. It was observed that baked cakes made by glycyrrhizin were darker than the control. The ΔE parameters were higher than 3 in cake containing glycyrrhizin which are obvious for the human eye in comparison to the control and agree with the results of colour in sensory evaluation (Fig 2).

Table 1. Mean sensory score values of toffee with different ratio of glycyrrhizin or licorice extract substitution

Organoleptic property	100% sugar	Replacing sugar with glycyrrhizin			Replacing glucose syrup With licorice extract				Replacing glucose syrup and sugar With licorice extract and glycyrrhizin
	Control	T_1 25%	T_2 50%	T_3 75%	T_4 25%	T_5 50%	T_6 75%	T_7 100%	T_8 50:50
Colour	8.7±0.64^{a}	8.18±1.07^{ab}	8.64±0.5^{a}	7.82±0.6^{ab}	8.36±0.5^{ab}	8±0.89^{ab}	7.18±1.47^{b}	3.9±2.98^{c}	7.55±0.82^{ab}
Sweetness	8.6±0.49^{a}	8.45±0.52^{a}	8.73±0.64^{a}	7.91±0.94^{ab}	8.45±0.68^{a}	8.18±0.87^{a}	6.45±2.99^{b}	3.36±3.35^{c}	7.82±1.53^{ab}
Bitterness	0.0^{c}	0.0^{c}	0.0^{c}	2.82±2.6^{b}	0.0^{c}	0.0^{c}	2.45±2.84^{b}	4.45±3.56^{a}	2.18±2.44^{b}
Flavour	8.3±0.78^{ab}	8.45±0.68^{a}	8.64±0.67^{a}	7.73±1.49^{ab}	8.09±1.37^{ab}	7.82±1.77^{ab}	6.64±2.29^{b}	4.82±3.15^{c}	7.36±1.91^{ab}
Texture	8±0.44^{abc}	8.27±0.46^{ab}	8.91±0.3^{a}	8.18±1.07^{ab}	7.82±0.4^{abc}	6.73±0.9^{c}	4.91±2.21^{d}	1.82±2.27^{e}	6.91±2.8^{bc}
Overall acceptability	8.4±0.49^{ab}	8.27±0.46^{ab}	8.82±0.4^{a}	7.55±1.12^{abc}	7.82±0.75^{abc}	7.27±0.9^{bc}	5.55±2.2^{d}	2±2.44^{e}	6.73±1.95^{cd}

Means in a row not sharing the same letter are significantly different at $P \leq 0.05$.

(a): Glycyrrhizin as replacement for sucrose.

(b): Licorice extract as replacement for glucose syrup.

(c): Glycyrrhizin and licorice extract

Fig. 1. (a, b, c) General appearance of toffee prepared with different ratio of glycyrrhizin or licorice extract substitution

Textural parameters of the studied samples are tabulated and summarized in Table (2). It can be noted that no differences were shown in deformation of hardness among all treatments comparing with the control cakes. Hardness and total work cycle values decreased in cake containing glycyrrhizin compared with the control. Where Khattab *et al*. (2017) found that the cake made with 100% steviolbioside as a sugar replacer had the lower values of hardness, hardness work cycle and total work cycle values than in case of the sample with sucrose. No differences were shown in deformation of hardness among all treatments compared with the control samples.

Also data in Table (2) show the organoleptic attributes of cake containing glycyrrhizin as a sugar replacer. In the light of data presented here, it can be concluded that substituting sucrose with glycyrrhizin at 25% did not affect the acceptability of cake as evaluated for its quality attributes including colour, taste, flavour, texture and overall acceptability. Increasing the ratio of glycyrrhizin more than 25% negatively affected the sensorial properties of tested cakes. These data are agreement with Khattab *et al*. (2017) who found that there was no significant differences between the organoleptic properties of cakes prepared using sugar and those prepared replacing sugar with 50% steviolbioside.

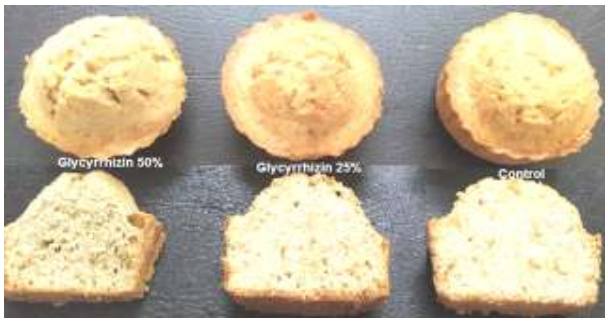

Fig. 2. Cakes prepared with glycyrrhizin as replacement for sucrose.

From the aforementioned results, toffee replacing sugar with glycyrrhizin at 50% (750 mg / 100 g) and toffee prepared with licorice extract 25% (1.37 g / 100 g) as glucose syrup replacer had the highest score of sweetness, flavour and overall acceptability. Also cakes sweetened with 250 mg glycyrrhizin as sugar replacer had no significant effect on the organoleptic properties.

Therefore, it can be selected to investigate the effects of glycyrrhizin low dose (250 mg / 100 g food equal to 3.846 mg / kg / day), glycyrrhizin high dose (750 mg / 100 g food equal to 11.538 mg / kg / day) and Licorice extract (1.37 g / 100 g food equal to 21.08 mg / kg / day) on some toxicological parameters on young growing male rats.

Table 2. Physicochemical and organoleptic properties of cake containing glycyrrhizin

Product Character	**Control**	**25% Glycyrrhizin**	**50% Glycyrrhizin**
Cake volume (Cm3)	140±0.0^{a}	135±5.0^{ab}	127.5±7.5^{b}
Specific volume	0.458±0.0^{b}	0.471±0.004^{ab}	0.497±0.025^{a}
Colour values			
L*	66.31	59.61	61.25
a*	11.33	11.06	9.69
b*	39.14	36.38	33.70
ΔE	———	7.25	7.61
Textural analysis			
Load (g) at 10 min	2697	2036	2015
Hardness cycle (g)	5098	3737	3620
Deformation at Hardness (mm)	19.98	19.95	19.97
Total work cycle (MJ)	721.8	518.7	514.6
Sensory evaluation			
Colour	8.615±0.5^{a}	8.069±0.44^{a}	6.846±1.21^{b}
Taste	8.385±0.65^{a}	8.308±0.63^{a}	7.769±1.16^{a}
Flavour	8.538±0.66^{a}	8.462±0.66^{a}	7.769±0.75^{a}
Texture	8.462±0.66^{a}	7.846±0.8^{ab}	7.538±1.45^{b}
Overall acceptability	8.538±0.66^{a}	8.038±0.59^{ab}	7.385±1.38^{b}

Means in a row not sharing the same letter are significantly different at $P \leq 0.05$.

L, lightness; a, redness; b, yellowness. $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$

Biological evaluation

1. **Body Weight and Food Consumption:** Data in Table (3) shows the mean changes in body weight, feed intake and feed efficiency ratio (FER). There was insignificant difference in the average initial weight of different groups of animals. At 60 days, the mean gain in body weight was 86.6, 79.67, 88.33, 86.00 and 96.67 g for control, NOEl-GL, low-GLY, high-GLY and LE, respectively. Groups received all glycyrrhizin doses had insignificant decrease in body weight gain as a percent (BWG, %) compared to control group .Oral administration of glycyrrhizin in doses 229 mg/day decreased the body weight gain by 8.00%, when compared to control group while rats drank licorice extract showed increase in body weight by 11.63% compared that of the control. This decrease in the body weight was related to glycyrrhizin which might be cause in loss of appetite of rats. However, the non-caloric sweetener group had a decrease in body mass index compared to an increase in body mass index in the sucrose group (Wiebe *et al*., 2011). Glycyrrhizin or licorice extract treated group significantly ($P \leq 0.05$) decreased feed intake as compared to control group but no significant ($P \leq 0.05$) increases in FER were detected in the glycyrrhizin treated group compared with the control group. FER revealed that licorice extract showed the highest FER value as compared as the other doses of glycyrrhizin studied. The increase in body weight gain by licorice root extract is in accordance with that reported by Miller (1998) and Shalaby *et al*. (2004) who found that administration 200, 400 and 800 mg *Glycyrrhiza glabra* extract/Kg for 4 weeks caused decrease in daily feed intake and increase in FER compared to the control. The increase of body weight gain might have associated with *Glycyrrhiza glabra* administration that inhibits 11 β-hydroxysteroid dehydrogenase and induces excess release of mineralocorticoids, which causes retention of sodium and water. Also, Snow (1996) demonstrated that the reduction in food intake produced by licorice extract could be possibly attributed to the presence of either an intensively sweet glycoside glycyrrhizin or bitter taste character.

Table 3. Effect of glycyrrhizin and licorice extract on body weight, food intake, and feeding efficiency ratio of rats

Parameter	Control group	NOEL-GL group	Low- GLY group	High- GLY group	LE group
Initial body weight(g)	61.8±2.59^{a}	62±2.00^{a}	63.67±3.51^{a}	62.67±2.52^{a}	62.33±0.58^{a}
Final body weight (g)	148.4±6.98^{ab}	141.67±3.51^{b}	152±13.11^{ab}	148.67±9.02^{ab}	159±10.29^{a}
Body weight gain (g)	86.6 ±5.59^{ab}	79.67±1.53^{b}	88.33±9.61^{ab}	86.00±6.56^{ab}	96.67±9.93^{a}
Body weights gain (%)	140.13±8.52^{ab}	128.50±1.77^{b}	138.73±7.62^{ab}	137.23±5.23^{b}	155.09±18.34^{a}
Feed intake (g/ day)	36.9±1.01^{a}	32.38±0.95^{c}	32.98±0.99^{c}	32.86±0.97^{c}	34.28±1.00^{b}
Feeding efficiency ratio	0.039±0.003^{b}	0.041±0.001^{ab}	0.045±0.005^{ab}	0.044±0.003^{ab}	0.047±0.006^{a}

Values with different superscripts within each column are significantly different at P≤ 0.05.

2. **Relative organ weight:** Changes in relative organ weight are shown in Table (4) statistically significant increases in relative liver weights was observed in rats treated with NOEL-GL (229 mg/day) compared with control. On the other hand, significant ($P \leq 0.05$) decrease in heart and insignificant decrease in liver and lung relative weight were observed in rats treated with LE compared to control. Kuai *et al*. (2011) reported that the organ coefficient of lung in the glycyrrhizin-treated group (50 mg/kg) was significantly lower than in the model group.

Table 4. Relative organ weights of rats treated with a glycyrrhizin (NOEL-GL), low dose (Low-GLY), high dose (High-GLY), and licorice extract (LE) for 60 days (% body weight)

Parameter	Experimental groups				
	Control	NOEL-GL	Low-GLY	High-GLY	LE
Liver	3.06± 0.19^{bc}	3.43± 0.13^{a}	3.32± 0.25^{ab}	3.10± 0.35^{bc}	2.98±0.17^{c}
Brain	1.0 ± 0.09^{ab}	1.19 ± 0.29^{a}	0.91± 0.13^{b}	.90 ± 0.14^{b}	0.95±0.15^{b}
Kidney	0.80 ± 0.03^{a}	0.82± 0.14^{a}	0.83 ± 0.07^{a}	0.82± 0.09^{a}	0.83 ± 0.05^{a}
Heart	0.40 ± 0.04^{ab}	0.44 ± 0.03^{a}	0.39 ±0.04^{b}	0.35 ± 0.03^{bc}	0.33±0.02^{c}
Lung	0.76 ± 0.09^{ab}	0.86± 0.15^{a}	0.75 ± 0.09^{ab}	0.73±0.07^{ab}	0.68 ±0.05^{b}
Spleen	0.35 ± 0.07^{a}	0.46 ± 0.07^{a}	0.47 ± 0.1^{a}	0.42 ± 0.13^{a}	0.44±0.09^{a}
Testicular	1.50± 0.19^{a}	1.48± 0.19^{a}	1.53±0.12^{a}	1.52± 0.24^{a}	1.47± 0.29^{a}

Data are presented as means ±SD, (n = 6 for each group).
Values with different superscripts within each column are significantly different at *P*≤ 0.05.

3. **Hematology:** The results of the hematological examination are shown in Table (5). There was insignificant change in WBC, RBC, MCV, MCHC, MCH and PCV between the groups of rats compared with the control group. The NOEL-GL doses (229 mg/kg) of glycyrrhizic acid ammonium salt caused significant increase in HGB when compared to control and other groups as shown in Table (5). From the literatures, glycyrrhizic acid can enhance the total white blood cells (WBC) and red blood cell (RBC) counts (Raphael and Kuttan, 2003).Therefore, licorice infusion on the long run may give rise to thrombocytosis (Campbell, 2008).
4. **Lipid profile:** As shown in Table (6) the oral administration of glycyrrhizin at all tested doses to male rats for 60 day showed a significant reduction in

TL and TG with insignificant change in serum TC and HDL when compared to control group. Whereas, serum lipids of rats given glycyrrhizic acid ammonium salt at doses 229 mg /day (NOEL-GL) showed significant increase in serum concentration of TL (259.73 mg/dl), TC (82.02 mg/dl) and HDL (52.82 mg/dl). Rats given licorice extract showed significant change in LDL (24.35 mg/dl) when compared to the control group but within normal range. Results revealed that treatment with glycyrrhizin or licorice extract caused significant decrease of VLDL as compared to control group. These results may be due to the presence of phytosterols and saponins in the root of the plant could play an important role in affecting serum lipid profile parameters. These results are in agreement with Shalaby *et al.*, 2004 who reported that the watery licorice extract had greater effect on serum lipid profile. The hypocholesterlmic effect of licorice may be attributed to the presence of isoflavones as anti-oxidants which decrease of LDL-cholesterol oxidation and inhibit the local mechanism of atherosclerosis. (Nikitina *et al.*, 1995; and Saleem *et al.,* 2011). Abd El-Ghffar (2016) glabridin is a major active constituent of licorice root decrease total cholesterol and LDL level at dose 25 or 50 mg with insignificant reductions in VLDL concentrations and prevent accumulation of cholesterol in cells.

Table 5. The effect of (NOEL-GL), glycyrrhizin low dose (Low-GLY) and high dose (High-GLY), and licorice extract compared with control on hematology of treated rats.

Biochemistry	Experimental groups				
	Control	NOEL-GL	Low-GLY	High-GLY	Licorice extract
White blood cell (ml)	6166.67±1484^{a}	6050±1761^{a}	7800±1252^{a}	7666.67±995^{a}	7466.67±1723^{a}
Hemoglobin (g/dl)	13.87±0.78^{b}	14.82±0.62^{a}	13.58±0.84^{b}	13.87±0.85^{b}	13.75±0.66^{b}
Eerythrocyte count (10^{6}/ml)	8.23± 0.42^{ab}	8.55± 0.56^{a}	7.67±0.61^{b}	7.92± 0.46^{b}	7.7± 0.14^{b}
Mean corpuscular volume (mm^{2})	49.83±2.78^{a}	53.33±3.26^{a}	52±3.22^{a}	47.67± 5.78^{a}	48.83±6.05^{a}
Mean corpuscular hemoglobin (P g)	16.73±0.94^{a}	17.3±1.6^{a}	17.67±0.7^{a}	17.48±0.74^{a}	17.75±0.7^{a}
Mean corpuscular hemoglobin	33.62±1.8^{ab}	32.42±1.8^{b}	34.12±2.22^{ab}	37.05±3.96^{a}	36.7±4.33^{a}
Packed cell volume (%)	41.3±3.45^{ab}	45.8±2.67^{a}	40.12±4.64^{b}	37.98±5.88^{b}	37.83±4.76^{b}

***Significantly different from the control group at $P<0.05$ using student test.**

Table 6. Effect of oral administration of glycyrrhizin and licorice extract on serum lipid profile in rats.

Biochemistry		Experimental groups				
		Control	NOEL-GL	Low-GLY	High-GLY	licorice extract
TL	(mg/dl)	222.75±1.62^{b}	259.73±1.05^{a}	202.83±2.31^{c}	186.58±1.19^{d}	221.75±1.22^{b}
TC	(mg/dl)	75.95±3.01^{b}	82.02±2.27^{a}	75.35±3.96^{b}	76.35±3.44^{b}	76.25±4.66^{b}
TG	(mg/dl)	37.16±1.01^{a}	33.83±1.39^{b}	29.51±0.55^{c}	30.81±1.07^{c}	29.81±0.60^{c}
HDL	(mg/dl)	47.41±3.53^{b}	52.82±2.69^{a}	48.94±4.02^{b}	47.88±4.72^{b}	45.93±6.84^{b}
LDL	(mg/dl)	21.10±2.55^{b}	22.43±1.13^{b}	20.50±0.71^{b}	22.30±1.37^{b}	24.35±1.13^{a}
LDL/HDL(mg/dl)		0.44±0.06^{a}	0.42 ± 0.09^{a}	0.42 ±0.16^{a}	0.47±0.07^{a}	0.53±0.08^{a}
VLDL	(mg/dl)	7.43±0.40^{a}	6.78±0.80^{b}	5.90±0.71^{d}	6.16±0.39^{c}	5.96±0.68^{d}

Values are expressed as means±SD; n = 6 for each treatment group. Significant difference from the control group at $P\leq 0.05$

5. Kidney function and Serum biochemistry: The results of the examination of serum biochemical values are shown in Table (7). The levels of uric acid were significantly decreased in all groups when compared with control group. The NOEL-GL doses (229 mg/kg) of glycyrrhizic acid ammonium salt caused significant increase in total protein (7.79 g/dl)) when compared to control group. Groups of rats treated with low or high glycyrrhizin and licorice extract had insignificant ($P\leq 0.05$) decrease in the levels of urea, bilirubin, total protein and albumin with significant ($P\leq 0.05$) decrease in the levels of creatinine compared to the control group. Also, results showed that there was insignificant change in the level of glucose between all the groups of rats. Results of kidney function is similar to that reported by Barakat (2009) who found that treated rats with licorice extract at 1000 mg/kg improved significantly their kidney function parameters (uric acid, urea and creatinine). Saleem *et al*. (2011) reported that small concentrations of ethanolic extract of licorice significant decrease the concentration of urea and creatinine in albino male mice. On contrast, Rossi (1999) concluded that intake of large amounts of glycyrrhizin is sometime associated with renal insufficiency. The difference in results may be due to type, varieties, geographical origins, environmental condition or the method applied in the extraction transportation and storage of licorice.

6. Liver enzymes activities: The effect of drinking glycyrrhizin and licorice extract on the liver enzymes glutamic oxaloacetic transaminase (AST), glutamic pyruvate transaminase (ALT), alkaline phosphatase level (ALP) and acid phosphatase level (ACP) was shown in Fig (3). The present results showed that groups had significant decrease in AST, ALT, and ACP values compared to control groups. While, results of the ALP levels showed insignificant change in all groups compared to control groups. According to Eisenburg (1992) and Saleem *et al*. (2011), glycyrrhizin reduced the liver enzymes in rats and induced interferon production in patients with chronic hepatitis B and C. Lee *et al*. (2007) observed that pretreatment with licorice extract reduced ALT and AST values in serum of rats and decrease the necrosis and degenerative hepatic regions.

Table 7. Serum biochemical values of rats treated orally with glycyrrhizin and licorice extract for 8 weeks

Biochemistry	Experimental groups				
	Control	NOEL-GL	Low-GLY	High-GLY	licorice extract
Urea (mg/dl)	74.22±4.04^{a}	74.48±5.19^{a}	73.44±8.15^{a}	72.92±5.47^{a}	73.44±8.03^{a}
Uric acid (mg/dl)	1.81±0.16^{a}	1.41±0.24^{b}	1.30±0.20^{b}	1.26±0.18^{b}	1.22±0.18^{b}
Creatinine (mg/dl)	1.63±0.22^{a}	1.530±0.07^{ab}	1.43±0.11^{b}	1.40±0.06^{b}	1.41±0.09^{b}
Bilirubin (mg/dl)	0.170±0.07^{a}	0.140±0.08^{a}	0.150±0.07^{a}	0.140±0.03^{a}	0.150±0.09^{a}
Total protein (g/dl)	6.88±0.10^{b}	7.79±0.49^{a}	7.29±0.31^{ab}	7.23±0.99^{ab}	6.68±0.57^{b}
Albumin (g/dl)	3.78±0.38^{ab}	4.03±0.08^{a}	3.73±0.33^{ab}	3.68±0.16^{b}	3.55±0.07^{b}
Glucose (mg/dl)	109.18±9.73^{a}	111.10±8.26^{a}	110.98±7.38^{a}	112.08±6.21^{a}	108.59±6.56^{a}

Data are presented as means ± SD, (n = 6 for each group).Values with different superscripts within each column are significantly different at P≤ 0.05.

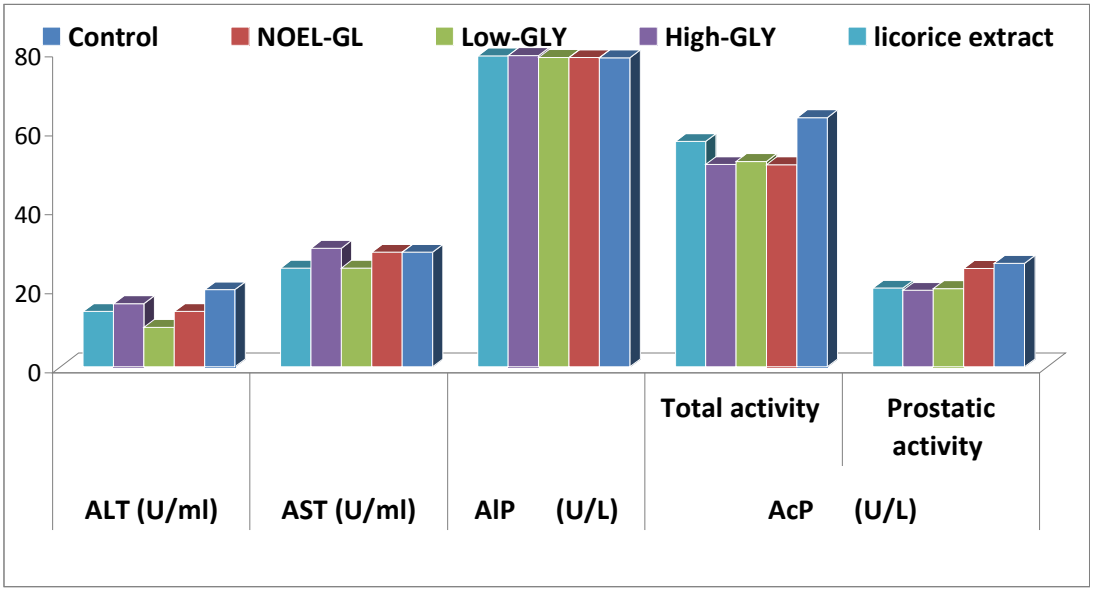

Fig. 3. Serum ALT, AST, ALP and ACP in control and rats treated with glycyrrhizic acid ammonium (NOEL-GL), low (Low-GLY), high (High-GLY) glycyrrhizin, and licorice extract

Histopathological Examination:

Liver:

Wistar rats fed with the glycyrrhizic acid ammonium salt (NOEL-GL) and glycyrrhizin at low dose (low-GLY) were not different from tissues from the control animals. All these sections were essentially normal without any evidence of active inflammation, no fibrosis, and the central vein lies at the center of the lobule surrounded by the hepatocytes with well-preserved cytoplasm, and distinct nuclei. In addition, no steatosis and no cholestasis were observed as shown in Fig. (4). The liver of rats treated with high dose of glycyrrhizin (high-GLY) showed that hepatic parenchyma with preserved architecture, portal tracks were enlarged and infiltrated with moderate spilling into the adjacent parenchyma and moderate with no confluent necrosis, no steatosis and no cholestasis. Moreover, sections of liver from LE group showed mild spilling into the adjacent parenchyma and moderate lytic with no confluent necrosis, no steatosis and no cholestasis. Glycyrrhizin might have a direct protective effect on the hepatocyte. It might stabilize the cell membrane of hepatoeytes by preventing penetration by viral particles or by reducing cell membrane permeability which is increased by immuno-activation during viral infection (Crance *et al.,* 1994)

Kidney

Histopathological examination photomicrographs of the kidney sections in the control, NOEL-GL and low-GLY groups showed normal morphology of renal parenchyma, with well-defined glomeruli and tubules and architectureas shown in Fig. (5). Sections of the kidney from high-GLY group showed mild hydropic changes in subcapsular renal tubules, no interstitial inflammation and no fibrosis. Similarly, the histopathological changes in kidneys tissue of LE group showed mild infiltration by polymorphs, no interstitial inflammation and no fibrosis in renal glomeruli In conclusion, glycyrrhizin as a natural alternative sugar can be consumed to concentration 750 mg / 100 g food with safety effect so that did not show any significant toxicological changes as observed by histopathological examination in the kidney and liver tissues and all the biochemical markers investigated were within normal range.

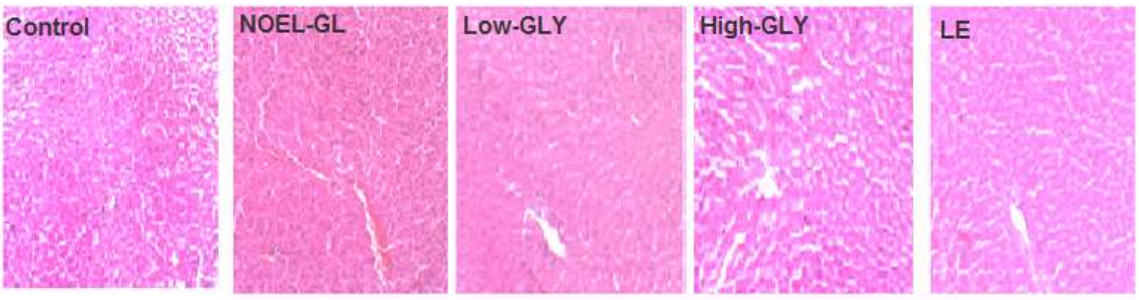

Fig. 4. Light micrograph of the liver sections. Control liver, NOEL-GL group liver sections treated with 229 mg glycyrrhizin /day, low-GLYgroup liver sections treated with 250 mg glycyrrhizin /day, high-GLY group liver sections treated with 750 mg glycyrrhizin/day and LE group liver sections treated with 1.37 g licorice extract /day for 60 day.

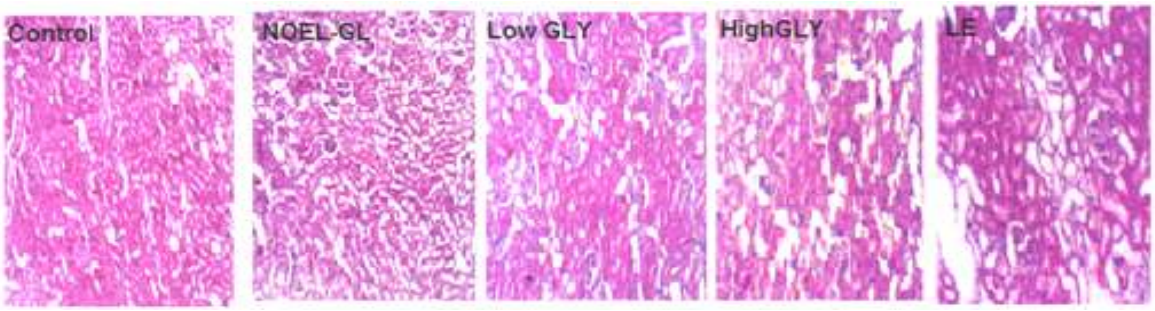

Fig. 5. Light micrographs of kidney. Control kidney, NOEL-GL group kidney sections treated with 229 mg glycyrrhizin /day, low-GLY group kidney sections treated with 250 mg glycyrrhizin /day, high-GLY group kidney sections treated with 750 mg glycyrrhizin /day and LE group kidney sections treated with 1.37 g licorice extract /day for 60 day.

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إستخدام الجليسرهيزين ومستخلص العرقسوس كمحليات طبيعية فى بعض المنتجات الغذائية و تأثيراتها البيولوجية

مينا سمير عبداللاهوت[١] ، أمل محمد عبد الرازق[١] ، منى إبراهيم مسعود[٢] و عماد الدين جمال جمعه[١]

[١] قسم علوم وتقنية إلاغذية- كلية الزراعة (الشاطبى) - جامعة الإسكندرية

[٢] معهد بحوث المحاصيل السكرية- مركز البحوث الزراعية - الصبحية- الإسكندرية

استبدال السكروز بالمحليات الطبيعية الآمنة ذات الفاعلية العالية له أهمية من الناحية الصحية كوسيلة لأنتاج غذاء صحي منخفّض السُعرات الحراريةَ. لذا أجريت هذه الدراسة للاستفادة من الجليسرهيزين ومستخلص العرقسوس المستخلص من نبات *Glycyrrhiza glabra L* كبديل للسكروز فى تحلية كل من التوفى و الكيك ودراسة تأثير الاحلال على الخواص العضوية الحسية لتلك المنتجات و التقييم البيولوجى لأستخدامها على وزن الجسم ، الوزن النسبى للاعضاء و بعض التحليلات لمكونات الدم والمقاييس الكيموحيوية و كذلك بعض الانشطة الانزيمية لحيوانات التجارب. وأوضحت نتائج الخواص الحسية أن التوفى المحلى بإستبدال السكروز بالجليسرهيزين بمعدل ٥٠:٥٠ (٧٥٠مجم /١٠٠جم) حاز على أعلى درجة تقبل حسى من قبل المحكمين. و كذلك التوفى المحلى باستبدال ٢٥% شراب الجلوكوز بمستخلص العرقسوس (١.٣٧جم/١٠٠جم) حاز على أعلى درجة فى الخواص الحسية ، المذاق (حلاوة) ، النكهة والتقبل العام مع غياب المرارة مقارنة مع العينات التى تحتوى نسب استبدال أعلى من مستخلص العرقسوس. كما أوضحت نتائج الخواص الحسية عدم وجود أى فرق معنوي في الخواص الحسية للكيك المستبدل بنسبة ٥٠ % جليسرهيزين (٢٥٠مجم/١٠٠جم) مقارنة مع العينات المرجعية مع خفض محتواها من السعرات الحرارية. وأوضحت نتائج الدراسة البيولوجية لتأثير إستخدام التركيز المنخفض و التركيزالعالى من الجليسرهيزين و مستخلص العرقسوس على ذكور الفئران الصغيرة لمدة ٦٠ يوم انخفاضا معنويا فى معدل استهلاك الغذاء و إنخفاضا غير معنوى فى معدل الزيادة فى وزن الجسم مع زيادة غير معنوية فى معدل كفاءة الغذاء في المجموعات التى تستخدم محلى الجليسرهيزين بالمقارنة مع المجموعة الضابطة. وان المستخلص المائى للعرقسوس ادى الى زيادة غير معنوية فى كلا من معدل الزيادة فى وزن الجسم و معدل كفاءة الغذاء مقارنة بالمجموعة الضابطة .كما اوضحت النتائج انخفاضا في محتوى الجليسريدات الثلاثية و زيادة غير معنوية في نسبة الليبوبروتينات عالية الكثافة إنخفاض فى كلا من انزيم ألانين أمينو ترانسفيريز (ALT) أو اسبرتات أمينو ترانسفيريز (AST) و الفوسفاتيز الحامضي (ACP) فى الفئران المغذاة بالمستخلص المائى للعرقسوس أو الجليسرهيزين بالمقارنة بالمجموعة الضابطة وكذلك قد أظهرالفحص الهستولوجي عدم وجود تغيرات مرضية في الخلايا الكبد والكلى .

الكلمات الدالة: الجليسرهيزين ، الخواص العضوية الحسية ، التاثيرات البيولوجية ، المقاييس الكيميائية الحيوية