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Licorice (*Glycyrrhiza glabra* L.): Chemical Composition and Biological Impacts

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ABSTRACT

This study was conducted to investigate the chemical composition and biological impacts of licorice drink in both infusion and tea forms. The chemical composition was determined by quantifying protein, fats, moisture, ash, fiber, carbohydrates and minerals. The most abundant minerals detected in licorice in both tea and infusion forms were calcium, sodium, phosphorus, potassium and iron, respectively. Zinc and copper were detected as trace elements. Essential and non essential amino acids were present in all licorice forms. HPLC analysis of the organic acids in licorice forms showed the presence of several aliphatic acids such as butyric acids, tartaric acid and acetic acids. The methanolic extract of licorice showed cytotoxic activities against intestinal carcinoma cell line (Caco-2) and prostate carcinoma cell line (PC-3) with IC_{50} values of 40 and 40.6 $\mu\text{g/ml}$, respectively. The effects of licorice in infusion and tea forms on body weight gain, white and red blood cells, hemoglobin and blood platelets, liver enzymes, kidney functions, total cholesterol and triglyceride levels, and some serum mineral levels were studied *in vivo*.

Keywords: licorice tea; licorice infusion; chemical composition; antitumor activity; *in vivo* biological impacts.

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INTRODUCTION

The root of licorice (*Glycyrrhiza glabra*) is one of the most frequently used natural medicines in the world, and has been described as 'the grandfather of herbs' (Asl and Hosseinzadeh, 2008). It has been used medicinally in both Western and Eastern countries for more than 4000 years (Ross, 2001; Saleem et al., 2011). It has been traditionally used for respiratory, gastrointestinal, cardiovascular, and skin disorders. The biological and pharmacological activities of *Glycyrrhiza glabra* have been widely studied as its long history and biologically active constituents are still interesting to many research groups (Hatano et al., 2000). A large number of clinical and experimental studies reported its useful biological properties such as antioxidant, immunomodulatory, cardioprotective, anti-inflammatory, anti-viral and anticancer effects (Hardy et al., 2012; Kobayashi et al., 2002). Recently, *Glycyrrhiza glabra* is reported to have neurological properties such as antidepressant, anxiolytic, and anticonvulsant effects (Cho et al., 2012). Chemical analysis of *Glycyrrhiza glabra* root extract showed the existence of triterpenes (glycyrrhizin, glycyrrhetic acid and liquiritic acid), flavonoids (liquiritin and formononetin) and various other substances (Farang et al., 2012). In Egypt, licorice in infusion form has been a popular sweet drink since the time of the pharaohs. The aim of this study is to: (i) identify the chemical composition of licorice infusion and tea; and (ii) evaluate their biological activities and impacts *in vitro* and *in vivo*.

MATERIALS AND METHOD

Collection of samples and extraction

Licorice herbs were purchased and collected from different Egyptian markets at Cairo, Giza, El Kalubia, Helwan, 6-October, Alexandria, El Beheira, Dakahlia, Mersa Matruh, Beni Suef, Assiut, Suez and Damietta. The selected samples were free from other herbal materials with fine texture. The licorice samples (100 g) were air dried, grinded and soaked four times in methanol (4 × 300 ml). The extract was then dried under reduced pressure at 45 °C to give 11.4 g of reddish orange extract. The licorice tea was prepared by boiling 15.0 g of dried herbs in 1000 ml of distilled water for 1 hour and filtrated by gauze. The filtrate was collected and the residue was further extracted with 1000 ml of distilled water. The tea extract was then evaporated to dryness under reduced pressure in a rotary evaporator at 45°C. The licorice infusion was prepared by infusing 15.0 g of dried herb with 1000 ml of distilled water. We poured on water that's just off the boil and leaved it for 10-15 minutes and filtrated by gauze.

Chemical composition

The samples were analyzed for chemical composition (protein, fat, moisture content, fibers and ash) according to the reported method (AOAC, 1995). Carbohydrate content was estimated by difference (Sara et al., 2008). Estimation of minerals (calcium, phosphorus, sodium, potassium, iron, zinc and copper) in licorice herbs was done by inductive coupled

plasma ICP "optima 2000" (Iva et al., 2003). Amino acid profiles of the raw herb, methanolic extract, tea and infusion forms of licorice were determined by AOAC protocol (AOAC, 2006).

HPLC analysis of organic acids and aflatoxins

The methanolic extract, tea and infusion of licorice herb were subjected to estimate the more predominant organic acids via HPLC investigations. Reversed phase HPLC was Dionex ultimate 3000 coupled with UV- Vis 170 μ dionex detector using C₁₈ stationary phase column, 5 μ m 150 \times 4.6 mm. This method uses 100% aqueous mobile phase at UV/Vis 226 nm. Determination of aflatoxins was carried out by mixing 50.0 g of grinded licorice herb with 2.5 g NaCl and 200 ml of 80% (v/v) solution of methanol in water. The mixture was blended in a blender for 5 minutes. The extract was filtrated and evaporated to dryness under reduced pressure at 45 °C. Total aflatoxins and ochratoxin A standards were purchased from Sigma (St. Louis, MO, USA). Stock solutions of each mycotoxin were prepared by dissolving solid commercial toxin. The presence of aflatoxins was detected by high performance liquid chromatography (HPLC, Agilent 1200) using C₁₈ column of LiChrospher RP-18 (5 μ m \times 25cm). The mobile phase consisted of water: methanol: acetonitrile (54:29:17, v/v/v) at flow rate of 1ml/min. The excitation and emission wave lengths for all aflatoxins were 362 and 460 nm.

Measurement of potential cytotoxicity by SRB assay

Potential cytotoxicity of the methanol extract of licorice herb was tested at the National Cancer Institute, Egypt on Caco-2 (Intestinal carcinoma cell line) and PC3 (Prostate carcinoma cell line) (Skehan et al., 1990). Cells were plated in a 96-well plate (10⁴ cells/well) for 24 h before treatment to allow the attachment of cells to the wall of the plate. Different concentrations of the materials under investigation (0, 12.5, 25, 50 and 100 μ g/ml) were added to the cell monolayer. Triplicate wells were prepared for each individual dose and they were incubated for 48 h at 37 °C in 5% CO₂. After 48 h cells were fixed, washed and stained with sulforhodamine B stain. Excess stain was washed with acetic acid and attached stain was recovered with Tris-EDTA buffer and the color intensity was measured in an ELISA reader. The average of the transformed cells was recorded. The survival curve of the tumor cell line was plotted for each tested fraction.

Antimicrobial activity

The three forms of licorice (methanolic extract, infusion and tea) were individually tested against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* and *Saccharomyces cerevisiae* using the broth dilution method (Jones et al., 1985). These species were supplied by the food safety laboratory, central laboratory for food and feed, agriculture research center, ministry of agriculture, Egypt. The antibacterial activities were examined after incubation at 37°C for 18 h. Three replicas were prepared in each case.

In vivo experiment

Animals and Diets

Male Sprague-Dawley outbred Albino rats (5 weeks old) were divided into three groups, caged individually in stainless steel cages and maintained at 22-24 °C with a relative humidity of 45-55%. Diets and water as well as licorice in tea and infusion forms were provided fresh daily *ad libitum*. All the utensils used in providing the diets were either stainless steel or acid washed. Diets were prepared according to the composition of basal diet (Zhi et al., 1992). Ingredient analysis of diet for the three subjected rats groups are carbohydrates (51.4%), proteins (22.4%), fat (6.8%), fiber (6.1%), ash (7.4%) and moisture (5.9%). The mineral amounts include Ca (0.28 %), P (0.21 %), K (0.14 %), Na (0.065%), Fe (42.7 ppm), Zn (14.0 ppm), and Cu (5.8 ppm). All nutrients were added according to the recommended dietary allowance [RDA] for each rat and according to National Research Council method (NRC, 1995).

Experimental designs

Experiments were initially designed to evaluate the biological impacts of licorice in its tea and infusion forms *in vivo*. The duration of these experiments was eight weeks divided into two intervals (2 × 4 weeks for each). Rats in these experiments were housed individually in stainless steel cages and randomly divided into three groups. Each group consisted of six rats and assigned as tap water rats group "control rats group" (A), licorice tea rats group (B) and licorice infusion rats group (C). All groups were fed the same diet and *ad libitum*. Additionally, all groups were eaten barley for three days before starting the experimental period in purpose of to be adapted. The rats of both zero time and each interval were anaesthetized with CO₂, whereas blood samples were collected *via* the retro-orbital plexus (Saka, et al., 2012). Serum was obtained by centrifuging at 3000 rpm for 15 min as (Akhigbe et al., 2008). The biochemical analyses of liver enzymes, urea, creatinine (Moss et al., 1999; Friedmann and Young, 1997; Newmann et al., 1999), triglycerides and total cholesterol (Guder et al., 2001) were carried out according the reported methods. Complete Blood Count "CBC" was estimated (ABC Vet, 1996); where the Animal Blood Counter Veterinary was a fully automated (Microprocessor controlled) hematology analyzer used for the *in vitro* diagnostic testing of whole blood specimens.

Analysis & Investigation

All the estimations were carried out in the Regional Center for Food and Feed. These estimations were taken place at the 3- interval periods "zero time, the end of the first interval and at the end of the experimental period", except diet analysis which were conducted only after complete homogenization of their ingredients. Diets analysis for determination of moisture content, crude protein, fiber and fat were carried out (AOAC, 2000).

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

Chemical Composition

The chemical composition of raw, tea and infusion forms of licorice is summarized in Table 1. Percentage of protein, fat, moisture, ash, fiber, carbohydrates, silica and minerals is calculated based on dry weight of raw herb. The results showed that raw herb of licorice is rich with carbohydrate (47.11%), fiber (24.48%), protein (9.15%), silica (3.56%) and low in fat content (0.53%). Additionally, the moisture and ash content of the licorice root were found to be 6.80 % and 7.70, respectively. These data are in a good agreement with literature (Duke et al., 1985).

The contents of Ca, P, Na, K, Fe, Zn and Cu in the licorice forms were measured by inductivity coupled plasma-optical emission spectrometry ICP optima 2000 as shown in Table 1. Calcium, sodium, phosphorus, potassium and iron seem to be predominant elements in the all investigated samples. The level of sodium is the highest in both tea and infusion forms. As described in the literature, sodium is important for chemical reaction within the cells and regulates the transfer of nutrients to the cells (Okaka et al., 2001). But excessive consumption of licorice in both forms can lead to the classic symptoms of hypertension due to increasing the absorption of sodium and water in kidneys (Miettinen et al., 2010). Calcium helps in forming and maintaining bone, blood clotting and muscle contraction. Other less abundant elements turned out to be zinc and copper in the raw material, tea and infusion forms of licorice. The presence of copper and zinc may play an important role in the functioning of various enzymes (e.g., copper is incorporated into metalloenzymes involved in hemoglobin formation, drug/xenobiotic metabolism, carbohydrate metabolism, catecholamine biosynthesis and cross-linking of collagen, as well as in the antioxidant defense mechanism (Isidoros et al., 2011). The presence of these mineral elements could thus indicate that drinking of licorice tea and infusion could be useful in the management of diseases where deficiencies of these metal ions are an important mechanism for the disease pathogenesis and progression.

Amino acid composition

In order to obtain more information about the chemical composition of licorice forms, the amino acids were determined and presented in Table 2. From the results, proline was found to be the major free amino acid in the raw herb, licorice tea and infusion with concentrations of 1.02 %, 7.60 mg/100 ml and 6.80 mg/100 ml, respectively. Following in the order is aspartic acid, glutamic, valine and the other amino acids. The major amino acids in the methanolic extract were aspartic (91.96 mg/100 ml) followed by proline (77.14 mg/100 ml) and glutamic

acid (27.05). These results are in agreement with the literature (Fenwick et al., 1990). From the literature, proline is the immediate precursors for polyamine synthesis, which is essential to proliferation, differentiation and repair of intestinal epithelial cells (Reeds et al., 1997).

Table 1: Chemical composition of licorice forms

Components (%)	Raw herb	Tea form	Infusion form
Protein	9.15	1.55	1.81
Fat	0.53	free	free
Moisture	6.80	-	-
Ash	7.70	0.02	0.15
Fiber	24.48	Free	Free
Carbohydrate	47.11	-	-
Silica	3.56	Free	Free
Elements	mg/100 g	mg/100 ml	mg/100 ml
Calcium (Ca)	1720	30	80
Phosphorus (P)	78	1	4
Elements (ppm)			
Sodium (Na)	18580	455.2	550
Potassium (K)	7276	178.4	215.1
Iron (Fe)	1224	4.189	2.28
Zinc (Zn)	17.08	0.118	L
Copper (Cu)	11.01	0.076	L

L=lower than instrument sensitivity

Table 2: Amino acid composition of licorice forms.

Amino acids	Raw herb %	Methanolic extract (mg/100 ml)	Tea form (mg/100 ml)	Infusion form (mg/100 ml)
Aspartic	0.88	91.96	4.87	4.17
Glutamic	0.50	27.05	2.06	2.87
Threonine	0.32	5.40	1.20	1.54
Serine	0.41	7.41	1.44	1.81
Proline	1.02	77.14	7.60	6.80
Glycine	0.25	5.64	1.18	1.56
Alanine	0.51	16.05	0.86	1.34
Valine	0.44	8.02	2.55	3.47
Isoleucine	0.21	3.12	0.55	0.92
Leucine	0.38	3.42	1.24	2.04
Tyrosine	0.31	-	-	1.55
Phenylalanine	0.33	-	-	-
Histidine	0.22	2.59	0.71	0.84
Lysine	0.36	3.98	1.49	1.53
Arginine	0.30	6.37	1.14	0.93

HPLC analysis of organic acids and aflatoxins

HPLC analysis of the organic acids in the licorice methanolic extract, tea and infusion forms (Table 3) showed the presence of 3~5 organic acids. Tartaric acid was the predominant acid in the licorice methanolic extract (3.5%), followed by butyric acid, malic acid, propanoic acid and citric acid. The tea form had butyric acid (38.4 %) as a main acid followed by propanoic acid (0.33 %) and tartaric acid (0.22 %). The infusion form had acetic acid (19.9 %) as a major organic acid.

Table 3. HPLC analysis of organic acids in licorice forms

Licorice forms	Organic acids (%)							
	Tartaric acid	Citric acid	Malic acid	Propanoic acid	Butyric acid	Formic acid	Fumaric acid	Acetic acid
Methanolic extract	3.50	0.18	0.29	0.26	2.93	-	-	-
Infusion form	1.90	-	-	-	-	0.97	0.16	19.9
Tea form	0.22	-	-	0.33	38.4	-	-	-

On the other hand, **the raw herb was tested for the presence of aflatoxins. Sample was extracted by the procedure of the Association of Official Analytical Chemists International (AOAC, 2006) and then analyzed by HPLC. The results showed the absence of aflatoxins contamination in the raw herb of licorice.**

Antitumor and antimicrobial activities

In this study, we investigated the antitumor activity of licorice methanolic extract against intestinal carcinoma cell line (Caco-2) and prostate carcinoma cell line (PC-3). The tested concentrations for each cell line were 0, 12.5, 25, 50 and 100 µg/ml. The assay results in Figure 1 showed that licorice methanolic extract had a growth inhibitory action against Caco-2 and PC-3 with IC₅₀ values of 40 and 40.6 µg/ml, respectively. It was reported that administration of the licorice extract significantly inhibited tumor growth in BALB/C mice inoculated with CT-26 colon cancer cells (Lee et al., 2007).

The antimicrobial activities of licorice tea and infusion were determined in a primary screen by broth dilution method. The tested pathogens were *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* and *Saccharomyces cerevisiae*. The used concentrations were 0.05%, 0.1%, 0.2%, 0.4%, 0.6% and 0.8%. The results showed no effect against the above pathogens. It was reported that acetone extract of licorice has shown antibacterial effect against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* (Nitalikar, et al., 2010). On the other hand, It was reported that (Shirazi et al., 2007) licorice had no susceptibility to different medically important bacteria with concentrations lower than 7.5%. Therefore, further studies are needed to characterize the active principles in accordance.

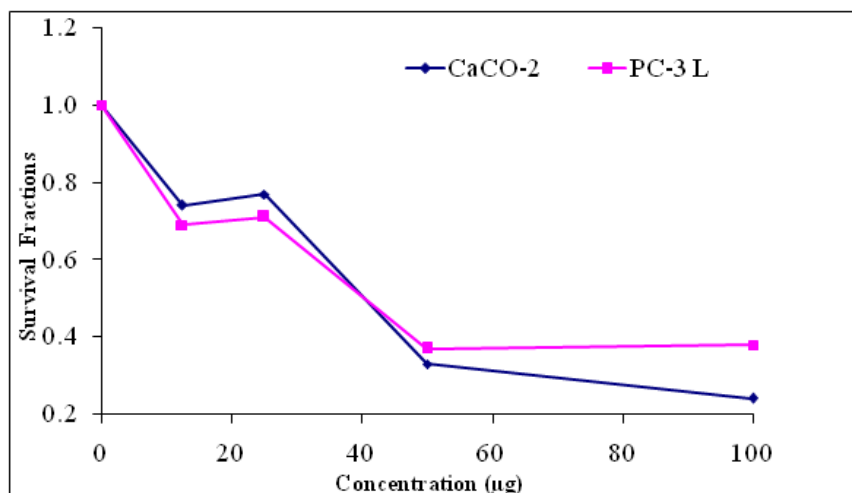


Figure 1. The cytotoxic activity of licorice methanolic extract against Caco-2 and PC-3 cell lines (IC_{50} of Caco-2 and PC-3 are 40.0 and 40.6 $\mu\text{g/ml}$, respectively).

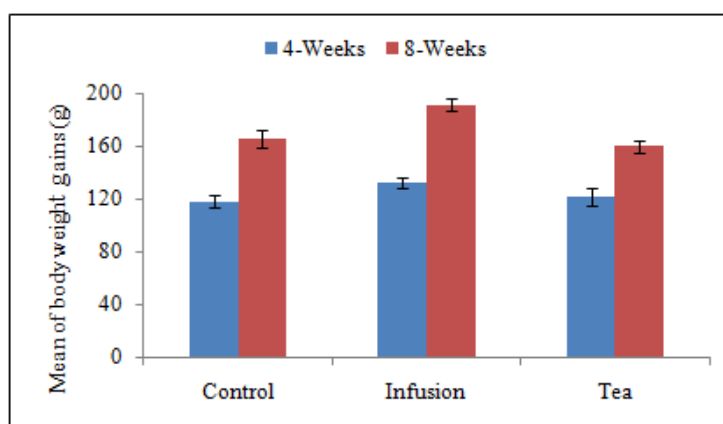


Figure 2. Effects of drinking licorice infusion and tea on body weight gains after four and eight weeks.

In vivo biological impacts

Effect of licorice on body weight gains

Figure 2 showed the body weight changes of rats pre-treated with licorice in infusion and tea forms. After 4-weeks, the mean values of body weight gains ($p < 0.01$) for control and pre-treated rats group with licorice infusion and tea are 118.5, 132.6 and 121.7 gm, respectively. After 8-weeks, the group of male rats drank licorice infusion were increased in weight than that of the control. The results may be explained by infusion process. It might be excellent for extracting the glycosides of licorice and also good for releasing the volatile oils. It was reported that licorice taken in excessive amounts or for long run can cause metabolic disturbances leading to oedema and weight gain (IDMA, 2002). Figure 2 also revealed that male rats group drank licorice tea did not lose weight during the first 4- weeks. From the fifth week onward, a

gradual loss of body weight occurred. The results indicated that licorice tea can be used by people who suffer from obesity to depress their body weights. It was reported that, licorice flavonoid oil can be used as a feed additive to reduce abdominal fat accumulation in domestic animals (Honda et al., 2003).

Effect of licorice on white and red blood cells

The effect of licorice infusion and tea on white and red blood cells of rats groups is shown in Figures 3a and 3b. After interval of 4-weeks, the mean values of white blood cells for the three subjected rats groups were 9.3, 10.9 and 12.1 " $10^3/mm^3$ " with probability significance $p < 0.05$. At the end of eighth week of the experimental period, the leukocytes or WBC's of licorice tea drank rats group released with highly significant difference ($p < 0.01$) rather than that of infusion and control rats group. Concerning the effects on red blood cells, the mean values for the subjected rats groups after 4-weeks were 8.4, 8.5 and 9.4 " $10^6/mm^3$ ", respectively. However, erythrocytes or RBC's of drank licorice tea rats group were a little bit more than that of drank infusion and control groups with significance level of ($p < 0.05$). After 8-weeks, drinking of licorice tea showed mean values of RBC's 13.4 " $10^6/mm^3$ " with significant difference ($p < 0.05$). This means licorice tea can improve WBC's and RBC's counts. From the literatures, glycyrrhizic acid can enhance the total white blood cells (WBC) and red blood cell (RBC) counts (Raphael and Kuttan, 2003).

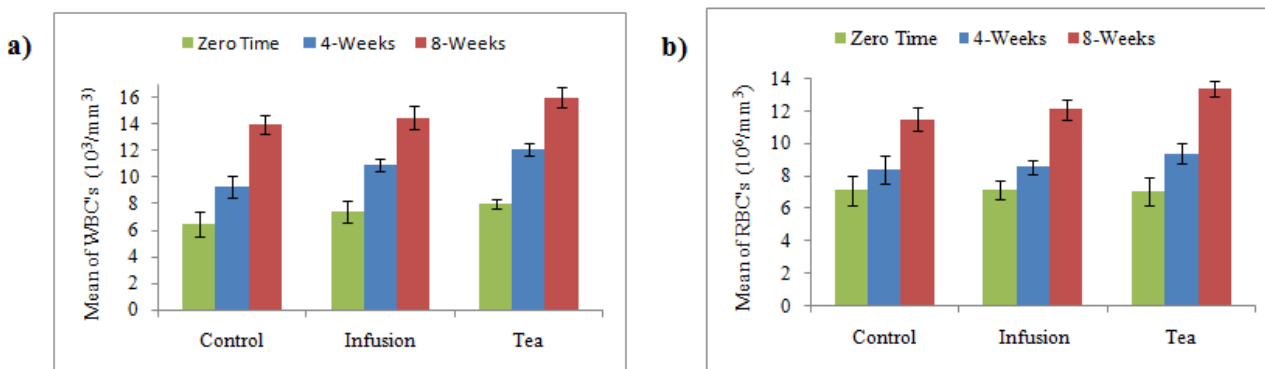


Figure 3. Histogram reporting the effects of drinking licorice infusion and tea after zero, four & eight-weeks on: (a) white blood cells histogram and (b) red blood cells histogram.

Effect of licorice on hemoglobin and blood platelets

The mean values of hemoglobin for the subjected three rats groups after 4-weeks of experiment (Figure 4a) were 15.1, 14.7 and 15.6 g/dl. After 8-weeks, drinking of licorice tea increased the hemoglobin in a small range. In contrast, drinking of licorice infusion ($p > 0.05$) for 8-weeks was negatively affected on hemoglobin levels as compared with control. Therefore, overabundance in drinking of licorice infusion may be lead to anemia. With regard to the effect of licorice forms on blood platelets (Figure 4b), the mean value of platelets after drinking of licorice infusion for 4 weeks was 342.6 " $10^3/mm^3$ " ($p > 0.05$). Additionally, drinking of licorice tea for 4-weeks increased the mean value of blood platelets to 406.5 " $10^3/mm^3$ " with highly

significance level ($p < 0.01$). Thus, licorice tea is a profitable source for formation of blood clots. After passing 8-weeks, the mean value of platelets in blood for rats group drank licorice infusion was $636.6 \times 10^3/\text{mm}^3$ which was highly significance ($p < 0.01$) than that obtained in licorice tea and control 542.8 and $525.6 \times 10^3/\text{mm}^3$, respectively. Therefore, licorice infusion on the long run may give rise to thrombocytosis (Campbell, 2008).

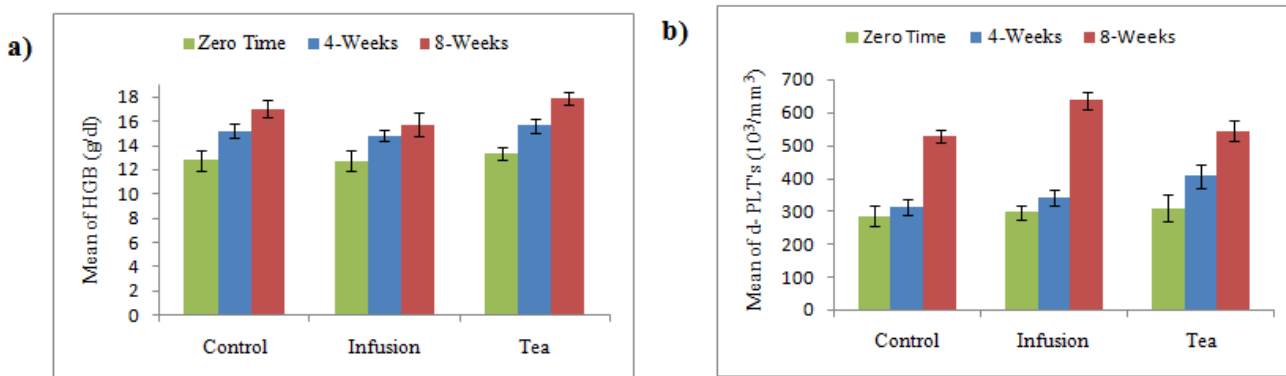


Figure 4. Histogram reporting the effects of drinking licorice infusion and tea after zero, four and eight weeks on: (a) hemoglobin and (b) blood platelets.

Effect of licorice on rat liver functions

The effect of drinking licorice in infusion and tea forms on the liver enzymes glutamic oxaloacetic transaminase (GOT), glutamic pyruvate transaminase (GPT) and alkaline phosphatase level (APL) is shown in Figures 5a-5c. After zero, four and eight weeks, the treated rats group with licorice in infusion form showed a significant increase in the mean values of GOT and GPT enzymes ($p < 0.05$ and < 0.01 , respectively) as shown in histograms 5a and 5b. Our results are in agreement with literature (Yin et al., 2011). On the other hand, licorice tea recorded a highly significant ($p < 0.01$) reduction in GOT and GPT enzymes than control (78.17 and 28.13, respectively). The mean levels of serum APL were significantly increased ($p < 0.01$) after drinking licorice in the tea form when compared to the normal control group. After 8-weeks, licorice tea drank rats group recorded a highly significant increase ($p < 0.01$) in alkaline phosphatase levels. Our results supported the use of licorice in tea form as a hepatoprotective agent (Saleem et al., 2011). It was reported that, glycyrrhizic acid showed hepatoprotective effect by preventing changes in cell membrane permeability and increasing survival rate of hepatocyte (Maurya et al., 2009). The results also showed that licorice infusion significantly increase the level of hepatic enzyme. From the literatures, one of the commonly reported side effects of highly concentration of glycyrrhizic acid is hepatic function abnormal. Usually, the extent of hepatic damage is assessed by the increased level of cytoplasmic enzymes, thus leads to leakage of large quantities of enzymes into the blood circulation. This was associated by massive centrilobular necrosis, ballooning degeneration and cellular infiltration of the liver (Gowri et al., 2008).

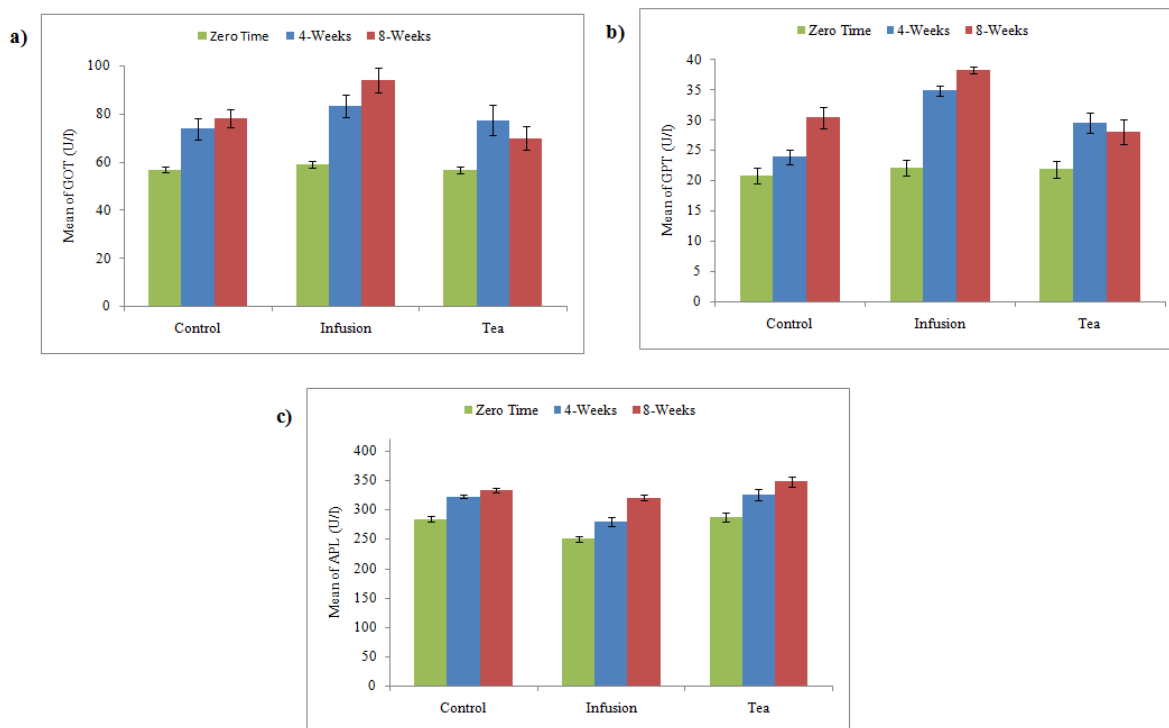


Figure 5: Histograms reporting the effect of drinking licorice infusion and tea after zero, four & eight-weeks on: (a) glutamic oxaloacetic transaminase (GOT), (b) glutamic pyruvate transaminase (GPT) and (c) alkaline phosphatase level (APL).

Effect of licorice on rats kidney functions

Figures 6a and 6b showed the results of kidney functions indicators (urea and creatinine) of rats administered with the licorice in infusion and tea forms. Along the experimental duration, after 4- weeks and 8-weeks, blood urea and creatinine of licorice infusion drank rats group were highly significant ($p < 0.01$) increased than control. Thus, licorice infusion possibly to cause excessive protein levels in the gastrointestinal tract and kidney failure. In the tea form, there were a significant reduction in the level of urea and creatinine ($p < 0.05$) compared to infusion form. The results of our study are similar to that reported in literature (Rossi et al., 1999). He found that intake of large amounts of glycyrrhizin was sometime associated with renal insufficiency. Therefore, in our study drinking of licorice in infusion and tea forms may be not useful in patients with renal insufficiency. On contrast to our study, Salem *et al.* reported that small concentrations of ethanolic extract of licorice significant decrease the concentration of urea and creatinine in albino male mice (Saleem et al., 2011). 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.

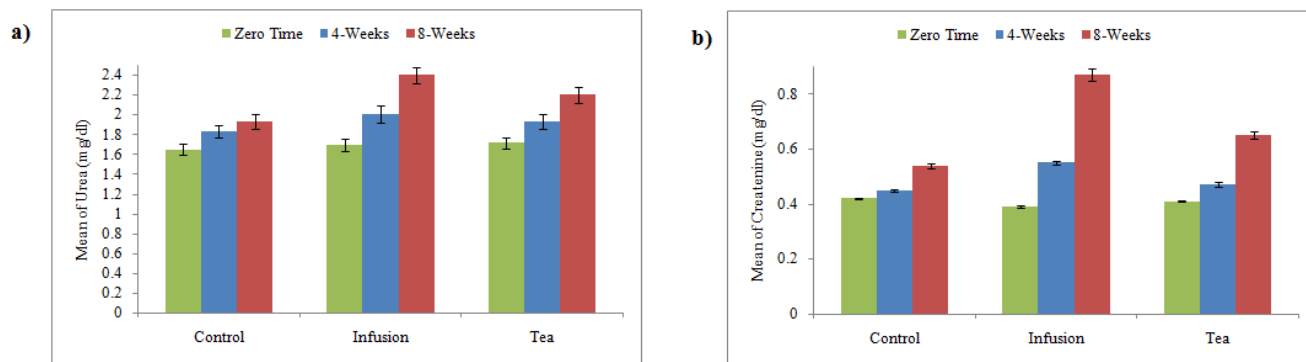


Figure 6: Histograms reporting the effect of drinking licorice in infusion and tea forms after zero, four and eight-weeks on: (a) rats blood urea and (b) rats blood creatinine.

Effect of licorice on total cholesterol and triglyceride levels

The effect of licorice forms on the total cholesterol (TC) level is shown in Figure 7a. After 4-weeks of experimental period, the blood cholesterol levels in rats groups drank tap water, licorice infusion and tea were 79.8, 108.4 and 88.4 mg/dl, respectively. The results indicated that drinking of licorice in both forms for 4-weeks increases TC levels with high significance ($p < 0.01$). These findings are matched with National Cholesterol Education Program (Grundy et al., 2004). After prescription 8-weeks, drinking of licorice infusion and tea decreased TC levels with high significance ($p < 0.05$). The hypocholesterlmic effect of licorice may be attributed to the presence of certain isoflavones, which act as anti-oxidants via inhibition of LDL-cholesterol oxidation and inhibit the local mechanism of atherosclerosis. Moreover, it was reported that the glycosides of licorice prevent accumulation of cholesterol in cells as well as human blood serum (Saleem et al., 2011). The results of the present study also revealed that drinking licorice infusion for four and eight weeks increased the triglycerides (TG) level ($p < 0.05$) than licorice tea and control (Figure 7b). Additionally, drinking of licorice tea decreased TG after 8-weeks. These results are in a good agreement with literature (Birari et al., 2011).

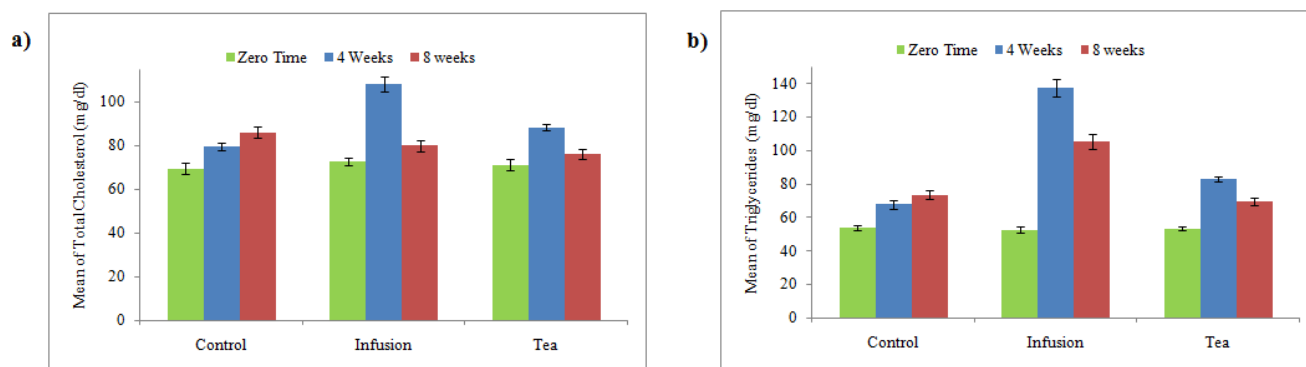


Figure 7: Histograms reporting the effect of drinking licorice in infusion and tea forms after zero, four and eight weeks on: (a) total cholesterol level and (b) triglyceride level.

Effect of licorice on some serum mineral levels in rats

Figures 8a-8d showed the effects of licorice infusion and tea on sodium, potassium, iron and zinc in the serum of rats after zero, 4 and 8 weeks administration. The concentrations of sodium and potassium (Figures 8a and 8b) were increased by drinking licorice in its infusion form when compared with control. Therefore, people suffering from hyponatremia (sodium deficiency) and hypokalemia (low potassium level) are recommended to drink licorice in infusion form. On the other hand, animals treated with licorice tea showed also an increase in sodium and potassium concentrations, but not much as in the infusion form. The effect of drinking licorice on iron levels in the serum of rats is shown in Figure 8c. After four and eight weeks, the iron level with the licorice tea was highly significant ($p < 0.01$) increased than the other two rats groups. Therefore, licorice tea can be considered as iron supplement drink.

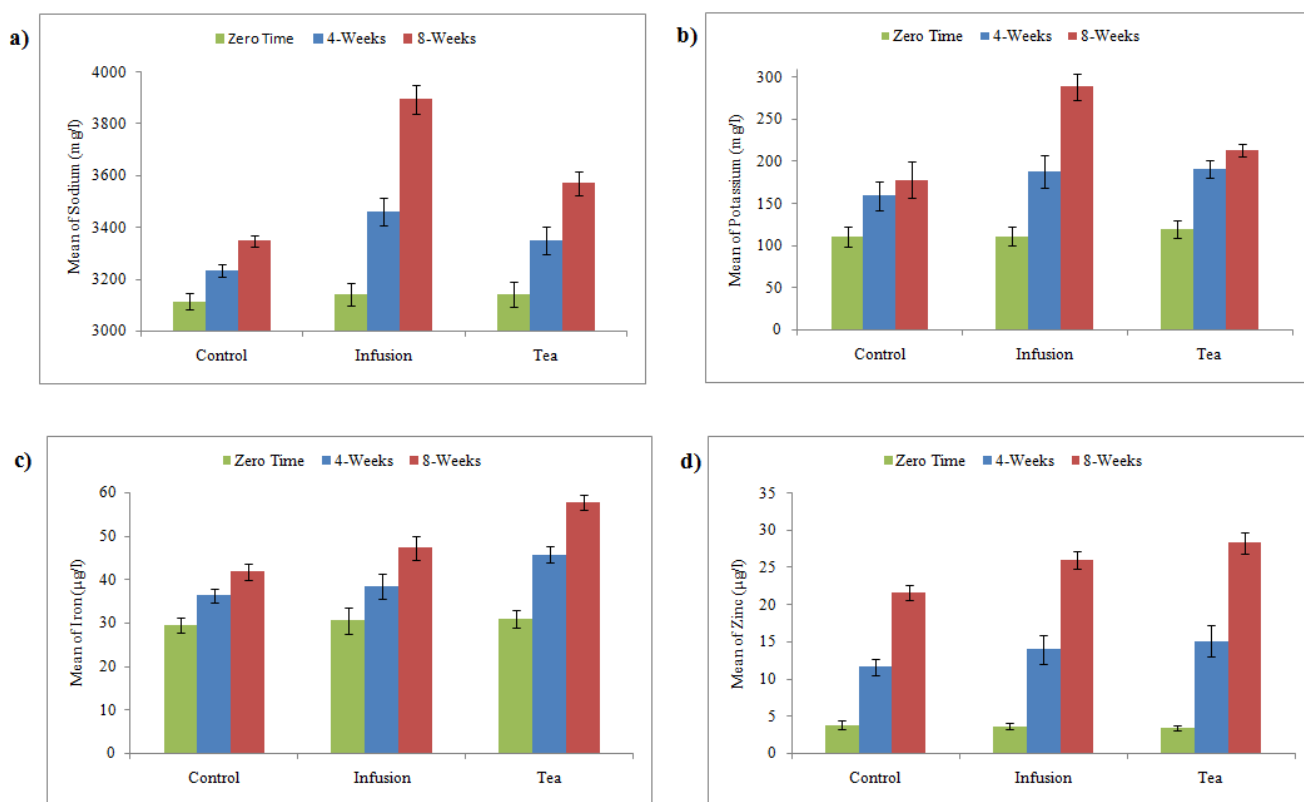


Figure 8: Minerals in rats blood serum after zero, four and eight-weeks of drinking licorice in infusion and tea forms. (a) sodium levels histogram, (b) potassium levels histogram, (c) iron levels histogram and (d) zinc levels histogram.

Figure 8a showed the zinc levels in rats blood serum after drinking licorice in both forms for 4 and 8-weeks. After 4-weeks, the zinc concentration after drinking of tap water, licorice infusion and tea were 11.6, 14.0 and 15.1 $\mu\text{gm/l}$, respectively. By increasing the duration of drinking, the corresponding zinc levels of subjected rats groups were increased. From literatures, it was found that zinc is an essential mineral required for the catalytic activity of approximately hundred enzymes. It plays an important role in immune function, protein

synthesis, wound healing and cell division (Lönnerdal et al., 2000). Therefore, our study reports that licorice tea is a good source of zinc.

CONCLUSION

In conclusion, licorice extracts were investigated for their chemical composition and biological impacts. The potential of licorice infusion and tea as a source of minerals (e.g. calcium, sodium and potassium) and amino acids (e.g. proline, aspartic acid, glutamic acid and other amino acids) is now being realized. The GC-MS and HPLC demonstrated the types of some chemical constituents in licorice infusion and tea. As observed, the methanolic extract of licorice has anticancer activities against intestinal carcinoma cell line (Caco-2) and prostate carcinoma cell line (PC-3) (IC₅₀ of Caco-2 and PC-3 are 40.0 and 40.6 µg/ml, respectively). Based on *in vivo* experiments, we can conclude that drinking of licorice infusion may lead to increase in body weight, anemia, thrombocytosis, increase in liver enzymes, decrease the total cholesterol, and increase blood urea and creatinine. Additionally, people suffering from hyponatremia (sodium deficiency) and hypokalemia (low potassium level) are recommended to drink licorice in infusion form. This study also showed that drinking licorice tea can decrease body weight, improve WBC's and RBC's counts, increase hemoglobin and blood platelets, decrease liver enzymes activities and not useful in patients with renal insufficiency. Our study also found that licorice tea is a good source of zinc and considered as iron supplement drink.

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