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Research Article

# Toxicology Assessment of Licorice Root Tea Using Experimental Histological Studies Validated by *In Silico* Physicochemical Properties and ADMET Analysis

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## Abstract

**Introduction:** This research was aimed at investigating the dosage at which licorice root tea could exhibit toxicity through histological studies as well as validation of the result using in silico physicochemical properties and ADMET (adsorption, distribution, metabolism, excretion and toxicity) analysis.

**Method:** HPLC-DAD was used for quantitative and qualitative characterization of licorice tea compounds. Liver tissues were obtained after 30 days of tea infusion and immediately fixed in 10% formaldehyde for histological examination. The predictions of ADMET properties were estimated using OSIRIS Property Explorer and Swiss ADME on characterized compounds.

**Result:** HPLC-DAD results revealed that gallic acid, glycyrrhetinic acid, luteolin, caffeic acid, rutin and quercetin are the phenolics and flavonoids compounds found in licorice root tea. The quantitative estimates of the identified phenolic compounds showed that the most abundant phenolic compound in licorice root tea is quercetin (4.19 mg/g) while the least phenolic compound is rutin (0.41 mg/g). The licorice tea infusions have no adverse effect on the liver at 10, 30 mg/kg body weight, however, high dose 50mg/kg body weight result showed an increase in the porosity of the cytoplasm in subcapsular hepatocytes under the light microscopic level. Quercetin being the most abundant compound found in licorice root tea together with caffeic acids showed high mutagenic, tumorigenic toxicity risk and high GI absorption which could responsible for the toxicity seen at high dose 50 mg/kg (L5) of licorice tea upon administration.

**Conclusion:** Licorice root tea at dose 50mg/kg body weight showed damage to the liver cells and this could be as a results of high toxicity exhibited by quercetin, the most abundant compounds found in the tea.

***Keywords:*** *Toxicology; Licorice; Quercetin; Histology; ADMET*

## Abbreviations

HPLC-DAD: High-Performance Liquid Chromatography; ADMET: Adsorption, Distribution, Metabolism, Excretion and Toxicity; GI: Gastro-Intestinal

## Introduction

Herbal teas have been in use for many years not only in Africa but also globally for human wellbeing to withstand stress without altering the physiological functions of the body. Organic products are receiving significant attention in the healthcare system and their diagnostic and curative use are gradually rising [11]. Since many inorganic medications have lethal side effects, a beneficial approach is to look for organic or herbal products with good therapeutic action and less toxicity. In the last few years, much attention has been channeled towards the potential health promoting properties of phenolic phytochemicals [9].

*Glycyrrhiza glabra* (Licorice) is one of the most extensively used medicinal herbs from the ancient medical history of Ayurveda [24]. It is useful as a flavoring herb. Licorice root extracts have been reported to possess diverse therapeutic activities [8]. The active components such as phenolic compounds in the tea are likely be responsible for most of its functional properties [10]. The liver, as a specialized tissues is made up of cells called hepatocytes which control diverse highly functional biological reactions including digestion, production and harmful substances removal detoxification which are important for necessary surviving functions [17]. Likewise, because of these numerous benefits of liver, it was used for the histological studies to observe change in the hepatocytes at different doses of tea infusion administered for 30 days.

The different levels of pharmacokinetics exhibited by each or combination of compounds in a plant make it impossible to generalize the safety of all natural products [20]. Such information is seen to be crucial as several people are consuming licorice root teas.

## Aim of the Study

The aim of this study is to investigate the dosage at which licorice root tea could exhibit toxicity through histological studies and validation using in silico physicochemical properties and ADMET analysis.

## Materials and Methods

### Quantification of phenolic and flavonoid compounds

Quantification and qualitative analysis was carried out following the method described by Ademosun., *et al*. [1] and Boligon., *et al*. [2] with slight modifications.

### Preparation of tea extracts

Licorice tea was obtained from the Traditional medical centre, in Ibadan, Oyo state, Nigeria. An infusion of hot water was used to prepare the tea extract. The tea sample worth 15g was infused in hot water estimated as 1.2Litres, the resulting mixtures were then filtered and the filtrate kept prior analysis.

### Oral toxicity test

There were 5 albino rats each of the 4 groups. Tap water (control), 10 mg, 30 mg and 50 mg of the tea infusion were orally administered to the rats for 30 days respectively. The research was carried out according to the methods described by Li., *et al*. [12] with slight modifications:

- Group 1: Control; group without treatment; normal diet and 0% of the tea samples.
- Group 2: Aqueous extract of Licorice Tea; 10mg/kg b.wt.

- Group 3: Aqueous extract of Licorice Tea; 30mg/kg b.wt.
- Group 4: Aqueous extract of Licorice tea; 50mg/kg b.wt.

### Histological analysis

The albino rats were sacrificed by dislocating their cervix and liver tissues were obtained and fixed in the formaldehyde with 10% concentration for histological analysis.

### *In silico* drug-likeness and toxicity predictions

The prediction which determine whether a specific therapeutic agent has qualities consistent with being an orally active drug is called drug-likeness ([6,19]). Lipinski rule of five is a concept already created by Lipinski., *et al*. [13] on which this prediction was based. The rule predicts that there is likely to be poor absorption or permeation when a compound possesses more than 5H-bond donors, 10H-bond acceptors, molecular weight greater than 500 and the calculated LogP (CLogP) greater than 5.37 [19]. The selection of compounds as drug candidates is also determined by a parameter called drug score [3]. The higher the drug score value, the higher the chance of the compound being considered as a drug candidate [3]. The in silico drug-likeness and toxicity predictions of the designed ligands were carried out using OSIRIS Property Explorer [21] and Swiss ADME predictor ([4,14]). OSIRIS Property Explorer programme estimates the mutagenic, tumorigenic, irritant and reproductive risks, and also provides information on the compound's hydrophilicity (LogP), solubility (LogS), molecular weight, drug-likeness and drug score [16]. Meanwhile, SwissADME predictor provides information on the numbers of hydrogen donors, hydrogen acceptors and rotatable bonds, total polar surface area, the synthetic accessibility and gastrointestinal absorption of the compounds. The phenolic compounds were also subjected to Lipinski., *et al*. [13], Muegge., *et al*. [18], Ghose., *et al*. [7], Egan., *et al*. [5] and Veber., *et al*. [22] screenings using SwissADME predictor [19].

## Results and Discussion

### Quantification and qualitative analysis of compounds

The qualitative and quantitative estimates of phenolic compounds of the licorice root tea extracts obtained with the aid of HPLC-DAD is as presented in table 1 and figure 1 respectively. Qualitatively, the result revealed the presence gallic acid, glycyrrhetinic acid, luteolin, caffeic acid, rutin and quercetin are the phenolics and flavonoids compounds found in licorice root tea. The quantitative estimates of the identified phenolic compounds (Table 1) showed that the most abundant phenolic compound in licorice root tea is quercetin (4.19 mg/g) while the least phenolic compound is rutin with values of 0.75 mg/g and 0.41 mg/g in licorice root tea. There is significant ($p < 0.05$) difference in the phenolics composition of tea samples.

| Compounds | Glycyrrhza glabra (mg/g) | LOD µg/mL | LOS µg/mL |
|---|---|---|---|
| Caffeic acid | $1.13 \pm 0.01^{b}$ | 0.008 | 0.043 |
| Rutin | $0.41 \pm 0.04^{a}$ | 0.009 | 0.030 |
| Quercetin | $4.19 \pm 0.03^{d}$ | 0.017 | 0.084 |
| Gallic acid | $0.45 \pm 0.02^{a}$ | 0.025 | 0.082 |
| Glycyrrhetinic acid | $3.72 \pm 0.01^{c}$ | 0.008 | 0.026 |
| Luteolin | $1.08 \pm 0.01^{b}$ | 0.014 | 0.046 |

***Table 1**: Phenolic composition of Glycyrrhza glabra.*
*Results are expressed as mean ± standard deviations (SD). Turkey test at $p < 0.05$.*
*Abbreviation: LOD= Limit of detection, LOQ= Limit of qualification.*

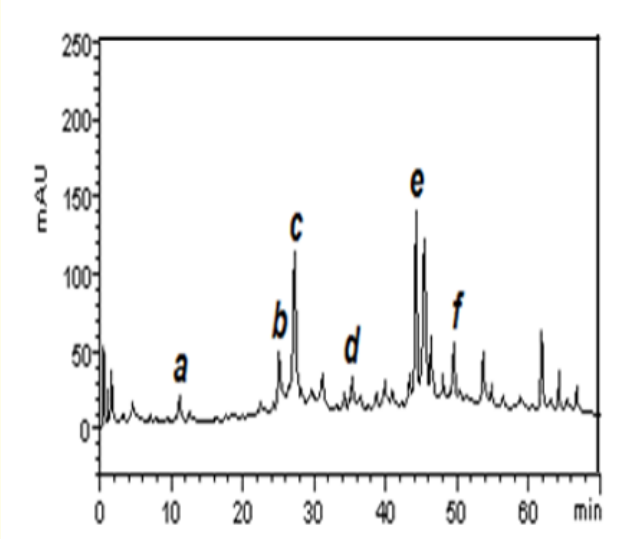


***Figure 1:*** *Representative high performance liquid chromatography profile of licorice tea. Gallic acid (peak a), caffeic acid (peak b), glycyrrhetinic acid (peak c), rutin (peak d), quercetin (peak e) and luteolin (peak f).*

## Histological analysis

In the licorice tea extract treated group at dose 10, 30 and 50 mg/kg b.wt, (L1, L3, L5), no lesions recorded for licorice at doses 10 mg/kg body weight and 30 mg/kg body weight, however, there is mild to moderate diffuse degeneration of hepatocytes at dose 50 mg/kg (L5) body weight (Figure 2).

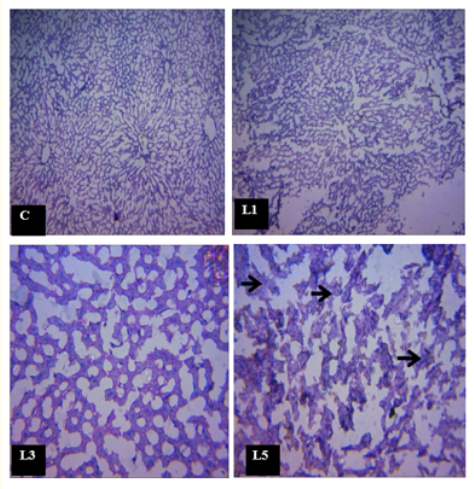


***Figure 2:*** *Histopathological images of liver sections of normal Control rats (C), Glycyrrhiza glabra treated rats at doses 10, 30 and 50 mg/kg b.wt (L1, L3 and L5). The architecture of hepatic parenchyma showed evidence of normal histology. L5 at dose 50 mg/kg b.wt showed mild to moderate diffuse degeneration of hepatocytes.*

The 50 mg/kg body weight dose of licorice implicated to cause degeneration of hepatocytes under the light microscopic level from this study, is in line with the reports of Luty., *et al.* [15], who reported an increase in the porosity of the cytoplasm in subcapsular hepatocytes under light microscopic level.

## Results of in silico drug-likeness and risk of the compounds

**Results of OSIRIS property explorer:** With the exception of gallic acid, caffeic acid and quercetin, all the predicted toxicity risk factors for the characterized compounds from licorice root tea were low (Table 1). Also, the 6 compounds possessed molecular weights less than 500 except rutin, which means that the compounds are possibly adsorbable and will get to the point of action when taken as drugs [23]. The entire compounds except glycyrrhetinic acid had LogP values not higher than 5, meaning that good adsorption and cell membranes permeability [23]. From the compounds, luteolin had drug score with the highest value (0.84).

Moreover, it was determined that glycyrrhetinic acid, luteolin and rutin possessed low tumorigenic, mutagenic, irritant and reproductive effective toxicity risks as shown in table 2 while caffeic acid, quercetin, and gallic showed varied toxicity. Quercetin being the most abundant compound found in licorice root tea with caffeic acids from table 2 showed high mutagenic and tumorigenic toxicity risk which could responsible for toxicity seen at high dose 50 mg/kg (L5) of licorice tea upon administration.

| | Physicochemical Properties | | | | | Toxicity | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Compounds** | **MW** | **cLOGP** | **Solubility Prediction** | **Drug Likeness** | **Drug Score** | **Mutagenic** | **Tumorigenic** | **Irritant** | **Reproductive Effective** |
| Gallic acid | 170 | 0.11 | -0.74 | 0.12 | 0.27 | High | Low | Low | High |
| Caffeic acid | 180 | 0.78 | 1.41 | -1.62 | 0.19 | High | High | Low | High |
| Glycyrrhetinic acid | 470 | 5.36 | -5.78 | -2.36 | 0.2 | Low | Low | Low | Low |
| Rutin | 610 | -1.26 | -2.4 | 3.31 | 0.57 | Low | Low | Low | Low |
| Quercetin | 302 | 1.49 | -2.49 | 1.6 | 0.3 | High | High | Low | Low |
| Luteolin | 286 | 1.99 | -2.56 | 1.9 | 0.84 | Low | Low | Low | Low |

***Table 2:*** *Physicochemical properties and toxicity risks of compounds as predicted using OSIRIS property explorer. MW: Molecular Weight.*

**Results from SwissADME prediction:** The numbers of hydrogen bond acceptors (NHA) and hydrogen bond donors (NHD) in characterized compounds (Table 3) are in accordance with the Lipinski., *et al.* [13] rule of five except quercetin and rutin which have higher NHA and NHD, hence break Lipinski rule. The LogS prediction of −5.11 to −3.81 indicated that all the compounds except glycyrrhetinic acid (-6.15) were moderately soluble.

| **Compounds** | **Formula** | **NHD** | **NHA** | **NRB** | **TPSA ($A^2$)** | **LOGP (ILOGP)** | **LogS (ESOL)** | **Synthetic accessibility** | **GI Absorption** |
|---|---|---|---|---|---|---|---|---|---|
| Gallic acid | $C_7H_6O_5$ | 4 | 5 | 1 | 97.99 | 0.21 | -1.64 | 1.22 | High |
| Caffeic acid | $C_9H_8O_4$ | 3 | 4 | 2 | 77.76 | 0.97 | -1.89 | 1.81 | High |
| Glycyrrhetinic acid | $C_{30}H_{46}O_4$ | 2 | 4 | 1 | 74.6 | 3.56 | -6.15 | 6.08 | High |
| Rutin | $C_{27}H_{30}O_{16}$ | 10 | 16 | 6 | 269.43 | 2.43 | -3.3 | 6.52 | Low |
| Quercetin | $C_{15}H_{10}O_7$ | 5 | 7 | 1 | 131.36 | 1.63 | -3.16 | 3.23 | High |
| Luteolin | $C_{15}H_{10}O_6$ | 4 | 6 | 1 | 111.13 | 1.86 | -3.71 | 3.02 | High |

***Table 3:*** *ADME prediction of compounds predicted by SwissADME.*
*Abbreviations: ADME: Absorption, Distribution, Metabolism, Excretion; NHA: No. of Hydrogen Bond Acceptors; NHD: No. of Hydrogen Bond Donors; NRB: No. of Rotatable Bonds; TPSA: Total Polar Surface Area; GI: Gastric Intestinal.*

In addition, rutin has the highest value (6.52) for synthetic accessibility, depicting that the compound will be the most difficult to synthesize from the library compound. Generally, the synthetic accessibility of most of the compounds (3.02-6.52) was not within the range of easy synthetic accessibility except gallic and caffeic acids which have 1.22 and 1.81 respectively. Moreover, all the compounds except rutin showed high gastrointestinal absorption value. Interestingly, only quercetin and luteolin compounds did not violated the Lipinski rule of five, Ghose filter, Veber rule, Egan rule and Muegge rule. This suggests that these compounds can be further refined as lead compounds for design drugs.

The role of Quercetin at the high dose at 50 mg/kg of licorice tea may generate Quercetin based derivatives as sirtuin inhibitors with effects on tumorigenic toxicity [25]. Sirtuin 1 is critical for liver cell function and the high doses of Quercetin and derivatives may act as a Sirtuin 1 inhibitor with relevance to liver programmed cell death [26].

## Conclusion

Based on the results obtained from histological analysis, the hot water infusion of licorice tea is safe for consumption at low dosage of 10 and 30 mg/kg body weight but could be toxic at high dosage of 50 mg/kg body weight. Quercetin is the most abundant compounds found in the tea and it was found to be readily soluble and high gastro-intestinal absorption. Its high mutagenic and tumorigenic toxicity risk together with caffeic acids could responsible for toxicity seen at high dose 50 mg/kg (L5) of licorice tea upon administration.

## Conflict of Interest

No conflict of interest.

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