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Safety assessment of the *Quercus brantii* gall hydroalcoholic extract: Single and repeated oral dose toxicity studies

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ABSTRACT

Objective(s): *Quercus brantii* galls (QBGs) are well-known in Iranian traditional medicine for treating various diseases. The aim of study was to assess the acute and repeated oral toxicity of the hydroalcoholic extract of QBG in female rats.

Materials and Methods: The ethanolic extract of QBG was administered in rats by gavage in both acute and repeated dose models. In the acute section of the study, a single oral dose of 2000 mg/kg was administered to female rat which were observed for physical symptoms and behavioral changes for 14 days. In the repeated dose toxicity study, the QBG extract (50, 500, and 1000 mg/kg/day) was administered for a period of 28 days to rats. On 28th day of experiment, blood sampling of animals was done for hematological and biochemical analysis and then sacrificed for histopathological examination of the harvested tissues (liver, heart, kidney, lung, spleen, stomach, ovary and uterus).

Results: A single oral administration of the QBG extract (2000 mg/kg) did not produce mortality or significant behavioral changes during 14 days of observation. In repeated oral toxicity models, the extract significantly increased (P<0.05) the levels of mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), thyroid-stimulating hormone (TSH) and significantly decreased the levels of triiodothyronine (T3) and thyroxin (T4) in 500 and 1000 mg/kg dosage. The histopathological studies showed the absence of toxic effects of QBG (50 mg/kg dosage) and revealed evidence of microscopic lesions in the liver, kidney, stomach, heart, spleen, lung, uterus, and ovary in the 500- and 1000-mg/kg groups.

Conclusion: The results indicate that the oral acute toxicity of QBG extract was of a low order with LD50 being more than 2000 mg/kg in rats. In addition, slight tissue damage was observed in some tissues in the 500 and 1000 mg/kg groups. It was found that prolonged use at higher doses i.e. 500 mg/kg/day of QBG extract should be avoided.

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Introduction

Herbal medicines are one of the most widely used medicinal classes with a wide range of applications, including hepatoprotective, antibacterial, anti-diabetic, anti-inflammatory, and anticancer properties. The oaks belong to the family Fagaceae, the *Quercus* genus. They are deciduous trees and shrubs with leaves of varying sizes and irregularly shaped fruits and acorns. Various oak species can be found across the Zagros, Arasbaran, and Hyrcanian forests (1). The Genus *Quercus* exhibits great morphological variety in these woodlands. All of the oak species of Iran are categorized into one subgenus *Quercus*, and two sections, *Quercus* and *Cerris* (2). *Quercus*, which grows in the country's central forest, is one of the most important oak genera with 45 species, the most common of which is *Quercus brantii*. In addition to their ecological value, these tree species are very important for human use. For a long time, *Quercus* species have been used due to the commercial value of their barks, woods, and galls, as well as for medicinal and nutritional purposes since ancient times. In particular, the use of oak galls for tanning, dyeing, and as a component of ink has made these trees very important throughout history. This genus is a subject of intense research due to its unique range of uses for humans (3, 4). Galls are an abnormal swelling of plant tissue, which can be caused by mites, insects, nematodes, bacteria, or fungi. Galls are most commonly found on leaves and stems, although they can also be found on other parts of the plant. The gall surrounds the insect and protects it from predators and the elements. Until the wasp is fully mature, the gall will also provide nourishment to the wasp (5). *Quercus brantii* galls (QBGs; locally called "mazoo") are caused by abnormal growth of plant tissues due to living factors such as bees. It was reported that *Quercus* galls were produced by the asexual activity of the Cynipidae family (bee), called Cynipstinctoria. Iranian traditional medicine uses QBG to cure chronic diarrhea and a variety of ailments, both with and without inflammatory etiology. The following pharmacological activities of QBG have been reported: astringent, anti-pyretic, anti-parkinsonism, anti-tremorine, local anesthetic, central nervous system (CNS) depressant, analgesic. Galls contain 5%-11% water, 1%-2% minerals, and a small amount of starch, and the

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rest is gallic acid and free ellagic acid (6). Tannic acid is the most important active ingredient, accounting for 31%-51% of the active substance in galls (7). *Quercus* galls have approximately 52% more tannins than other galls. Tannin is a spherical, inflated mass or a spongy, yellowish mass that is readily broken and crushed, has a distinct odor, and darkens gradually when exposed to air and light (8). Tannins have unique chemical compositions and a phenolic structure that allows them to bind to proteins and boost their enzyme resistance. Tannins have anti-bacterial, anti-cancer, anti-nutritional, and cardioprotective characteristics, as well as antioxidant and free radical scavenging action. They also appear to have a protective impact against metabolic abnormalities and the beginning of a number of oxidative stress-related diseases (9). The high percentage of tannins in galls has attracted the attention of many researchers. Despite observing the wonderful properties of *Quercus* galls, not much has been done about their safety and toxicity. It is essential to conduct further research on its toxicity for future preclinical and clinical studies. The present study uses the Organization for Economic Co-operation and Development (OECD) guidelines to investigate the possible toxic effects of hydroalcoholic extracts of QBG at different doses to determine safe levels of the *Quercus* gall extract in rats. Biochemical parameters and histopathological and hematological examinations were used to assess its toxicity.

Materials and Methods

Plant collection

The galls of *Q. brantii* were gathered from Lorestan Province in 2021, and the Botanists of Lorestan Province's Agricultural Research, Education, and Extension Organization (AREEO610-PMP/A) verified the authenticity using applicable criteria.

Extraction and preparation

The dried galls of *Q. brantii* were carefully powdered in a laboratory electric mill. The extract of galls was provided by the method of maceration using a 70% ethanol solvent. Ethanol and water solvent (70:30) were used to extract the gall extract using the cold solvent method. The combined and solvent galls were blended in a 1:20 ratio and shaken for 24 hr at room temperature in a shaker. The extract was then filtered through Whatman filter paper grade 3, and the resultant solution was concentrated using a rotary (50 °C) before being dried at room temperature. A desiccator was a vacuum-ventilated oven at 25 °C, where extracts were extracted from the glass plates by a metal blade and then scraped off the surface of the glass. A stock solution at a concentration of 10 mg/mL of QBG was added to distilled water and sonicated for more than 5 minutes to obtain a homogeneous solution.

Experimental animals and housing conditions

Thirty normal female Wistar rats, aged 10-12 weeks and weighing an average of 150 ± 30 g, were obtained from the Pasteur Institute of Iran. The Ethics Committee of Islamic Azad University, Tehran Medical Branch approved this study (code: IR.IAU.PS.REC.1400.003). In acute and repeated dose experiments, they were randomly caged in 6 groups (n = 5) under conventional laboratory indoor conditions, including a 12-hr light/dark cycle, room temperature (23.2 °C), and relative humidity (20%); they had free access to tap water and a standard pellet diet *ad libitum*. However, daily food and water intakes were recorded until the study's conclusion. The cage cleaning schedule, air filtration and recirculation, health checks, and facility maintenance were all done according to standard operating procedures (SOPs) and recorded daily. The Ministry of Health and Medical Education of Iran's Guidelines for the care and use of laboratory animals and the Canadian Council on Animal Care (CCAC) guidelines for the care and use of experimental animals were utilized to house and care for the animals.

Acute toxicity

An acute oral toxicity study was carried out in accordance with OECD guideline 420 for chemical testing. Ten Wistar rats were randomly divided into 2 groups of 5 cases (control group vs. treatment group). The control group received only distilled water, and the treatment group received the QBG extract orally by gavage feeding. To determine the lethal dose (LD_{50}), all abnormal signs and mortality in rats exposed to a high dose of the extract (2000 mg/kg) in the first 24 hr were monitored and compared to the control group. All animals were fasted for 1-2 hr before receiving the gall extract and had free access to water. Following the gavage feeding, all 5 animals were closely examined, and their behavior was recorded, particularly in the first 30 minutes to 4 hr after extract administration. After 14 days of observation, no fatalities or problems were noted in the rats. All animals underwent surgery at the end of 14 days, and the tissues of various organs, such as the lung, spleen, liver, stomach, heart, uterus, and ovaries, were assessed for appearance and weight in comparison to 5 female Wistar rats in the control group.

Repeated oral dose toxicity

To evaluate the repeated oral dosetoxicity of the gall extract, 20 healthy Wistar rats were randomly divided into 4 groups of 5 cases. Three groups of animals were administered different doses of 50 mg/kg (low dosage level), 500 mg/kg (medium dosage level), and 1000 mg/kg (high dosage level) of the extract suspended in water at a volume not exceeding 1 mL/100 g BW/ rat, for 28 consecutive days. The control group received water by gavage feeding in the same volume as the treatment group. The animals' general behavior was monitored daily, and the female rats were weighed according to the same method every day before any daily administration. Once a day, water intake, food consumption, and body weight were measured. After 28 days of study, total body weights, organ weights, macroscopic organ evaluations, hematology, serum biochemistry, and organ histopathology were examined according to the OECD 407 toxicity assessment guideline (OECD guidelines, TG 407, 2008)with a little modifications. On the 28th day,the rats were fasted overnight and subsequently sacrificed after being anesthetized in a chloroform-filled chamber. Blood was collected from the hepatic portal vein or thoracic aorta using a 5-mL syringe and emptied into a 5-mL heparinized specimen vial through the thoracic and abdominal regions. The blood was then centrifuged for 15 minutes at 3,500 rpm to obtain a clear supernatant (plasma), which was stored at -18 °C until biochemical assays were necessary (which were completed within a few days).

Necropsy and histopathological examinations

All the rats were subjected to a complete postmortem examination, which included a thorough evaluation of all organs, external surfaces, and orifices. The target organs, including the liver, kidneys, and spleen, were separated, cut into small pieces, and fixed in a 10% buffered formalin solution after weighing the important organs. The samples

were then dehydrated in an ethanol series before being embedded in paraffin. On 5-µm-thick sections, hematoxylin and eosin (H&E) or periodic acid–Schiff (PAS) staining was performed. The slides were examined blinded using optical microscopy (Olympus, Tokyo, Japan) at a magnification of 40 (in 5 fields/each section) by 2 experts (a pathologist and a histologist). A grading method was used to examine the liver, kidney, spleen, heart, lung, stomach, and uterus sections for semi-quantitative analysis. The liver sections were graded as 0 (normal), 1 (mild), 2 (moderate), and 3 (severe) based on the level of damage, sinusoidal dilatation, inflammatory cell infiltration, congestion, degeneration, and cytoplasmic vacuolization. In addition, the kidney sections were graded on a 4-point scale depending on the amount of epithelial necrosis in the cortical tubules (0: normal, 1: 25% damage, 2: 25%–50% damage, and 3: >75% damage). In addition, the spleen sections were rated on a 4-point scale based on the amount of fibrotic lymphoid and red pulp growth (0: normal, 1: mild, 2: moderate, and 3: severe).

Statistical analysis

In this study, a comparison between the control and treatment groups was performed by the unpaired Student's *t* test. The sample means that were significantly different from each other were identified using a stepwise multiple comparison technique. When analysis of variance (ANOVA) revealed a significant difference between 3 or more sample means, we employed a *post hoc* test. Means and SEM were used to express the data. *P*-values less than 0.05 were considered statistically significant for all tests.

Results

Single dose toxicity of the hydroalcoholic extract of QBG Within the first 24 hr and 14 days of follow-up, none of the female rats showed any behavioral or visible alterations. Until day 14, all the animals remained healthy and free of any harmful effects. The LD_{50} of the QBG extract was found to be greater than 2000 mg/kg in this investigation.

Repeated-dose 28-day oral toxicity of the QBG extract

Daily oral administration of *QBG* ethanolic extract at the doses of 50, 100 and 500 mg/kg for 28 days induced no obvious symptom of toxicity in rats. Neither group experienced any deaths or clear clinical symptoms during the experiment. The treated rats' skin, fur, eyes, and mucus membranes showed no symptoms of toxicity throughout the research, according to physical examination. In addition, no behavioral abnormalities, diarrhea, tremors, salivation, drowsiness, or coma were seen.

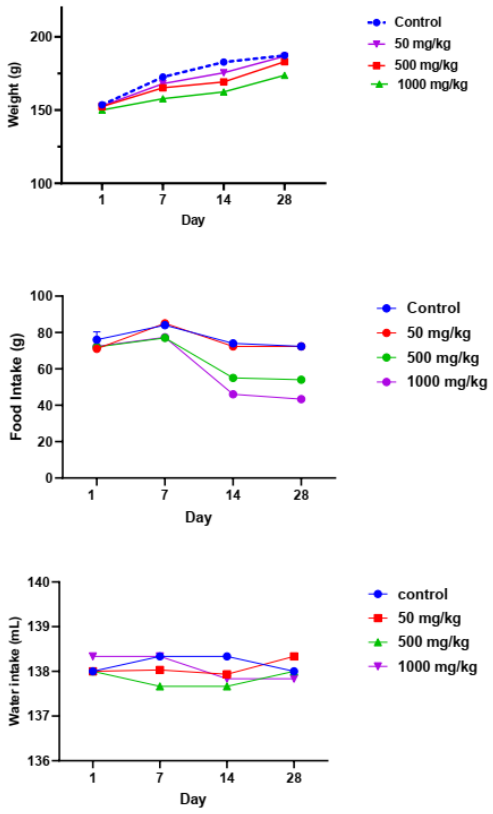

Figure 1. Effects of the repeated oral dosage levels (50, 500 and 1000 mg/kg) of *Quercus brantii* galls (QBG) hydro alcoholic extract on the changes of bodyweight (A) food intake (B) and water intake (C) in the rats

Effects of the QBG extract on the body weight and feed and water consumption of rats

A significant decrease ($P \leq 0.05$) was noticed in body weight by 14 and 28 days of treatment in rats receiving 500 and 1000 mg/kg of the QBG extract compared to the control group (Figure 1A), which is in line with a significant decrease ($P \leq 0.01$) in food intake recorded at the same doses in these rats. Body weight decreased in all treated animals. No significant changes ($P \geq 0.05$) were found in body weight, body weight loss, and food consumption between the groups receiving 50 mg/kg dosage level of the QBG extract and the control group (Figures 1B and 1C). The absolute weights of all vital organs, including the kidneys, liver, lungs, spleen, heart, stomach, ovaries and uterus, were measured and shown in Figure 2. In this study, reduced organ weights in the liver, stomach, and kidney were correlated with body

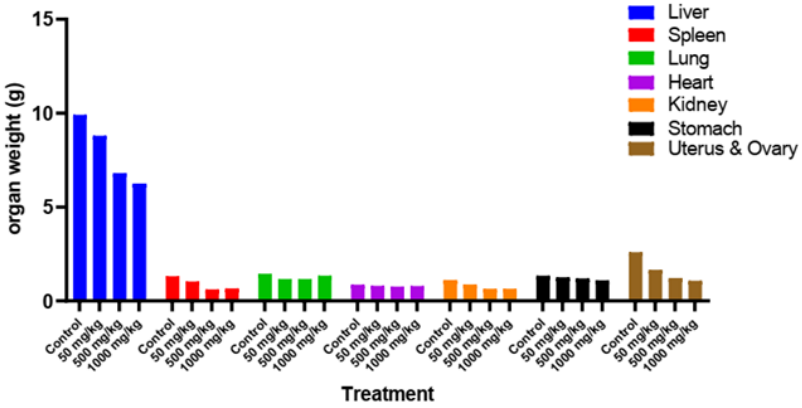

Figure 2. Effects of repeated oraldose toxicity of QBGethanolic extract on the organ weight to body weight ratio of female Wistar rats in the repeated oral dose toxicity

Table 1. Effects of *Quercus brantii* galls extract on some serum biochemical parameters in the repeated oral dose toxicity in female Wistar rats (Data are expressed as mean±SEM; n=5)

Variables	[A]Control	[B]Low dose	[C]Medium dose	High dose	*P*-value
FBS	185.6 ± 23.26	166.2 ± 17.66	153.8 ± 3.03	144 ± 9.76	ns
Urea	49.8 ± 6.79	42.4 ± 3.64	63.2 ± 0.83	48.6 ± 14.53	ns
Uric acid	0.46 ± 0.18	0.34 ± 0.054	0.5± 0.2 8	0.56 ± 0.27	ns
creatinine	0.5 ± 0.27	0.7	0.5	0.72 ± 0.04	ns
ALT	77.2 ± 14.4	70.2 ± 2.48	47.5 ± 0.7	86.25 ± 20.69	ns
AST	108.6 ± 24.08	85.4 ± 9.89	128.5 ± 23.33	112.7 ± 25.1	ns
Total bilirubin	0.1	0.1	0.1	0.104	ns
Direct bilirubin	0.1	0.1	0.1	0.1	ns
calcium	11.26 ± 0.39	11.26 ± 0.82	11.3 ± 0.14	10.92 ± 0.93	ns
phosphorus	5.24 ± 1.30	5 ± 0.59	5.1± 0.23	5.32 ± 0.47	ns
T3	1.78 ± 0.54	1.61 ± 0.23	1.01 ± 0.3	1.02 ± 0.14	[A]:0.19
					[B]0.022
					[C]0.0098
T4	3.48 ± 0.46	3.44 ± 0.64	3.26 ± 0.09	2.92 ± 0.24	ns
TSH	0.046 ± 0.04	0.05	0.1	0.16 ± 0.054	[A]: 0.14
					[B]: 0.04
					[C]: 0.002
cholesterol	61.4 ± 6.99	54.2 ± 4.96	54.3 ± 5.25	55.75 ± 4.27	ns
triglyceride	170.6 ± 5.03	117 ± 6.1	99.9 ± 15.9	89 ± 13	ns

FBS: Fasting blood sugar; ALT: Alanine transaminase; ALS: Aspartate aminotransferase; T3: Triiodothyronine; T4: Thyroxin; TSH: Thyroid-stimulating hormone

weight loss at dosage levels of 500 and 1000 mg/kg of QBG.

Effects of the QBG extract on the hematological and biochemical parameters

The 28-day treatment with the QBG extract showed no abnormalities in hematological parameters, i.e., white blood cells (WBC), red blood cells (RBC), hemoglobin (HB), hematocrit (HCT), mean cell volume (MCV), and platelet (PLT) counts, compared to the control group. However, there was a significant increase ($P<0.05$) in mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) levels after the administration of the highest QBG dosage level (1000 mg/kg).

Most biochemical indicators, such as glucose, urea, creatinine, total cholesterol, aspartate aminotransferase (AST), alanine transaminase (ALT), calcium total, direct bilirubin, phosphorus, cholesterol, triglyceride, did not change significantly when 50, 500, and 1,000 mg/kg dosage of the QBG extract were given compared to the control group (Table 2). However, a significant decrease in triiodothyronine (T3; 1.6 fold) and a non-significant decrease in thyroxine (T4; 1.19 fold) were observed at 500

Table 2. Effects of *Quercus brantii* galls extract on some serum hematological parameters in the repeated oral dose toxicity in female Wistar rats (Data are expressed as mean±SEM; n=5)

Variables	Vehicle	[A]Low dose 50 mg/kg	[B]Medium dose	[C]High dose	*P-value*
WBC (10^3/µl)	10.93 ± 2.64	10.4 ± 2.5	11.26 ± 1.77	10.1 ± 3.6	ns
RBC (10^6/µl)	6.95 ± 0.28	7.02 ± 0.57	7.99 ± 0.87	6.6 ± 0.55	ns
Hb (g/dl)	13.3 ± 0.63	13.57 ± 0.68	14 ± 0.8	13.93 ± 0.34	ns
HCT (%)	38.95 ± 2.21	44.9 ± 1.2	36.7 ± 2.8	46.13 ± 1.45	ns
MCV (fl)	55.95 ± 1.19	53.47 ± 1.34	56.2 ±1.1	55.6 ± 1.26	ns
MCH (pg)	19.1 ± 0.29	19.325 ± 0.84	17.5 ± 1.4	21.1 ± 0.81	[A]=0.94
					[B]= 0.16
					[C]= 0.013
MCHC (g/dl)	34.15 ± 0.72	36.15± 1.31	31.2 ± 1.2	37.9 ± 1.4	[A]=0.1
					[B]=0.12
					[C]=0.007
PLT (10^3/µl)	819 ± 258.2	748.5 ± 125	1030 ± 1.9	753 ± 199	ns

WBC: White blood cells ; RBC: Red blood cells; Hb: Hemoglobin; HCT: Hematocrit; MCV: Mean cell volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; PLT: Platelet

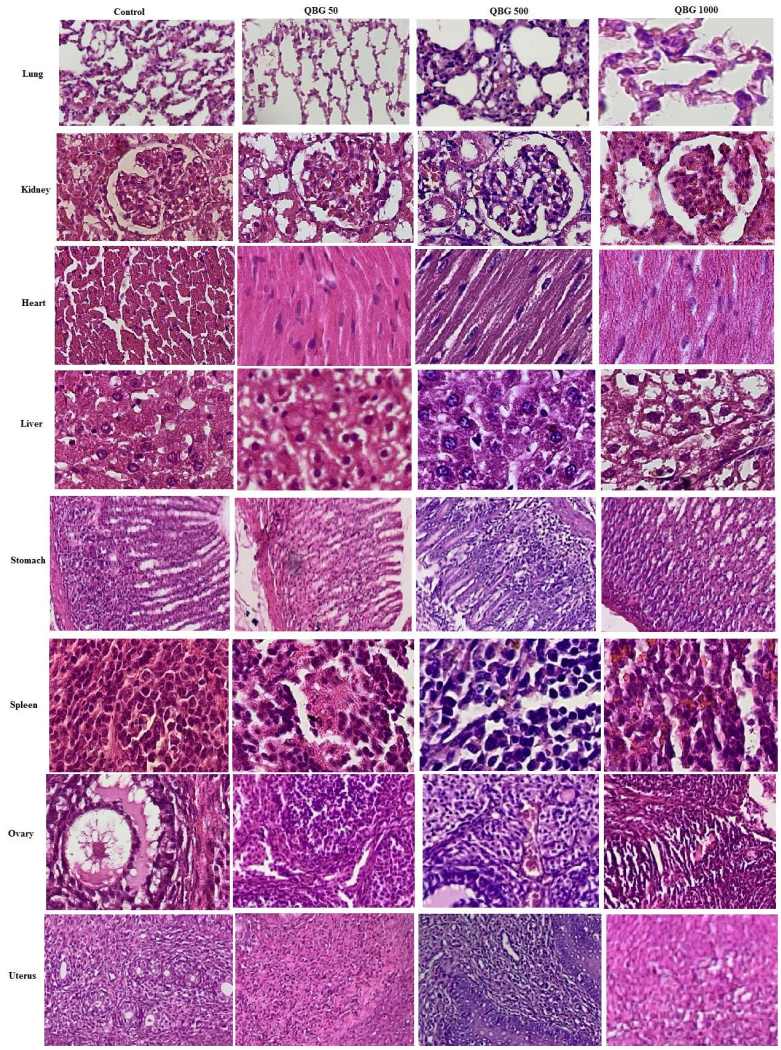

Figure 3. Microscopic photograph of different organs of female Wistar rats in the repeated oral dose toxicity (H&E stain (*40))

and 1000 mg/kg dosage of QBG. With 500 and 1000 mg/kg dosage levels of QBG, thyroid-stimulating hormone (TSH) increased 2.17 and 2.6 times compared to the control group.

Effects of the QBG extract on histopathological changes

Lung

The microscopic examinations of pulmonary tissues in rats given a 50-mg/kg dosage of the extract revealed no indication of proliferative or non-proliferative alterations. However, infiltration of eosinophils and inflammatory cells was observed in the mild- and high-dose groups that received 500 and 1000 mg/kg dosage of QBG.

Kidney

The renal histopathology results in the group that received 50 mg/kg dosage of the extract showed no evidence of necrosis or degeneration. The glomeruli and capillary rings inside the glomerulus looked completely normal, with no indications of necrosis, hypertrophy, or congestion. While bleeding and glomerular debris were obvious in the group that received 500 mg/kg of the extract; also, mild to moderate tubular cell degradation with chronic inflammatory cell infiltration was observed at a dosagelevel of 1000 mg/kg of QBG.

Heart

In the heart sections, a decrease in cell size, cellular degradation, and nuclear degeneration were observed at a dosage level of 50 mg/kg of QBG. Hemorrhage and cellular degradation were observed at a dosage level of 500 mg/kg of QBG. Extravasations of erythrocytes and severe degradation were observed at a dosage level of 1000 mg/kg of QBG.

Liver

At a dosage level of 50 mg/kg of QBG, there was no notable localized necrosis or cellular edema indicating cellular breakdown. Dilation of veins and lymphocytes and inflammation of the port space were observed at a dosage level of 500 mg/kg of QBG. Sinusoidal dilatation and slight congestion in the liver tissue, along with a few inflammatory foci and focal cellular necrosis, were observed at a dosage level of 1000 mg/kg of QBG.

Stomach

The results of stomach histopathology in the group receiving 50 mg/kg dosage level of QBG showed mild to acute inflammation and infiltration of the mucosa. Immune cells, inflammatory mediators, and smooth muscle were observed at a dose of 500 mg/kg dosage level of QBG. Moderate to acute inflammation, infiltration of deep muscle, and focal mucosal degradation all were histopathologically symptoms of QBG consumption at a dosage level of 1000 mg/kg.

Spleen

Increased white pulp cellularity and RBC cells were observed at a dosage level of 50 mg/kg of QBG. Inflammatory cells, hemorrhage, and moderate to severe white pulp activation were seen at a dosage level of 1000 mg/kg of QBG.

Ovary and uterus

Microscopic evaluation showed normal features in ovary and uterus sections in the 50-mg/kg groups. Hemorrhage, degeneration/inflammation of the ovary, and inflammation and increase of eosinophils in the uterus were histopathological changes at a dosage level of 500 mg/kg of QBG. At a dosage level of 1000 mg/kg of QBG, RBC extravasation, hemorrhage, and congestion were observed in both uterus and ovary tissues.

Discussion

Herbs have proven to be effective not only as treatments for a variety of human and animal ailments, but also as appropriate beginning points for the discovery of bioactive compounds for drug development (10). Identifying a wide range of substances that have been created as new medicines for cancer, hypertension, diabetes, and anti-infective has emerged from scientific exploitation of herbs used ethnomedicinally for pain relief, wound healing, and eliminating fevers (11). Galen, a Greek pharmacist and physician, was the first to write on the toxicity of plants, demonstrating that herbs can not only contain medicinally useful elements, but may also be comprised of harmful substances. To determine the safety of medications for human use, toxicological testing is always conducted first on experimental animals to assess potential toxicity and provide guidelines on safe human doses. In determining the total toxicity of these medications, the study of persistent adverse effects may be more important (12).

In this study, the toxicity of the hydroalcoholic extract of QBG was assessed in single and 28-day repeated dose models. Despite receiving the maximum dose (2000 mg/kg), no rats died during the acute toxicity testing. The animals' behavioral patterns were evaluated for the first 1 hr and then again for 24 hr after the administration, and the animals in both the vehicle-treated and extract-treated groups were normal and showed no significant behavioral changes. This proves that the extract could be safely administered for acute treatments up to the dose of 2000 mg/kg. This discovery leads us to believe that QBG is a practically harmless herb with little risk of death or organ damage, with no need for supportive treatment in cases of accidental or purposeful human poisoning.

We used clinical, hematological, biochemical, and histological evidence to assess the repeated oral toxicity of this medicinal herb in 3 dose groups compared to the control group. The repeated dosage levels of the QBG hydroalcoholic extract did not result in evident toxicity and mortality, similar to acute administration. Although certain changes in body weight and water and food consumption were seen, a significant decrease in body weight, food intake, and water intake was observed by 14 and 28 days of treatment in rats receiving 500 and 1000 mg/kg of the QBG extract compared to the control group. Except for the stomach, liver, and kidney ($P<0.05$) at dosage levels of 500 and 1000 mg/kg, there was no significant difference in organ weights compared to the control group. It has been noted that after exposure to a toxic substance, body and internal organ weights are considered sensitive markers of toxicity. Because of their sensitivity in predicting toxicity and their ability to correlate well with histological alterations, liver and kidney weights have been used in toxicity studies. Since there is little inter-animal variation, it is typically a toxicity target organ. Furthermore, the liver is the primary detoxifying organ. As a result, the liver and kidney could be considered target organs for the extract's sub-acute oral toxicity effect (13, 14). Hematological characteristics can be used to identify the extent to which a foreign component, such as a plant extract, has a negative effect on the blood. It can also be used to explain how a chemical compound/plant extract affects blood flow (15, 16). Repeated dose exposure of the rats to the highest dosage level (1000 mg/kg/day) of the QBG hydroalcoholic extract produced small changes in some biochemical and hematological parameters such as MCHC and MCH. Our results are not in line with Kwestan *et al.* study, in which *Q. brantii*decreased MCHC and MCH mean values (17). However, our result was consistent with Mubarak *et al.* study, showing that *Quercus infectoria* galls water extract increased MCHC in male rats during the repeated dosetoxicity test (18). Another finding in this study is the increased TSH and decreased T3 and T4 levels. The present data suggest that oral administration of QBG (at moderate and high dosage levels) exerts an anti-thyroid effect, reducing T3 and T4 concentrations, which in turn increases TSH secretion and, consequently, hypertrophic/hyperplasic changes in the thyroid follicles. This observation can be attributed to the possibility of the presence of flavonoids compounds in the extraction of QBG (19). Several investigators reported that flavonoids showed the potential to disrupt thyroid hormone metabolism. Since isolated or concentrated flavonoids are increasingly utilized as therapeutic interventions, more research on the potential influence of these substances on thyroid hormone metabolism is desirable (20). The histopathological results showed the absence of toxic effects of QBG at a dosage level of 50 mg/kg on the lung, kidney, liver, uterus, and ovary tissues and supported the toxic effect of QBG in moderate and high dosage levels on all organs.

Considering that tannic acid, ellagic acid and gallic acid are the main components of quercus plant, dollahite et al, investigated the toxicity of these compounds in *Quercus havardi* in the rabbit and concluded that tannic acid was absorbed from the gastrointestinal tract into the blood stream.This mild to acute inflammatory effect observed from QBG in low to high doses can be due to the high percentage of tannic acid in this extract (21).

In previous studies, following the delivery of Quercus Infectoria Gall (QIG) through rectum, mice and rabbits were used to assess the acute impact and mucosal irritation. Additionally, the long-term harmful effects of QIG on rats were investigated. Despite receiving a dose (10 g/kg) 300 times higher than what is often used in traditional Uyghur medicine, no mice died during the acute toxicity testing. Furthermore, no discernible negative impacts on animal behavior were found. Reduced levels of hepatic kupffer cell proliferation and intestinal mucosal hyperplasia were seen in exposed mice compared to controls, suggesting possible anti-inflammatory effects. In another study, toxicity of the QIG extracts after a 24-hr exposure was first screened using a brine shrimp lethality test. Brine shrimps were not harmed by QIG extracts that were made using polar solvents

and then diluted with pure dimethylsulfoxide (DMSO). By observing the hemolytic effect of the QIG extracts on regular human erythrocytes, the toxicological activity of the extracts was further investigated. The normal erythrocytes were not adversely affected by the extracts overall (22). All these studies and the present study indicate that this extract is safe in low doses.

Conclusion

The results of this study showed that the QBG extract was mildly hazardous, with an LD_{50} of more than 2000 mg/kg. However, repeated treatment of the QBG extract over a 28-day period and at moderate and high doses caused significant damage in rats. Some biochemical indicators and histological tests changed significantly when the dosage level was increased to 500 and 1000 mg/kg. To limit the undesirable effects of the QBG extract, it would be wise to use it at a maximum dosage level of 50 mg/kg. However, further pharmacological studies are required to elucidate the toxicity mechanism of QBG. In addition, more research is required to assess this gall's safety profile and use it in future pharmaceutical dosage forms. More research on its genotoxic potential/developmental and endocrine toxicity is required.

Acknowledgment

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Authors' Contributions

SZM and MSH Study conception, study design and data processing, collection, perform experiment; analysis and interpretation of results. SZM and MSH Draft manuscript preparation and critical revision of the paper. SZM Supervision of the research. MSH, JKH, PN, SZM Final approval of the version to be published.

Ethical Approval

This study and all procedures performed were approved by the Ethics Committee of Islamic Azad University, Tehran Medical Sciences University (IAUTMU) Tehran, Iran. IR.IAU.PS.REC.1400.003.

Conflicts of Interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

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