

## Acute Oral Toxicity Evaluation and Median Lethal Dose Determination of Ethanolic Extract of *Quercusinfectoria* galls (*Fagaceae*) in Experimental Rats

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**Abstract:** Medicinal plants, either as an extract, pure compound or as a derivative, offer limitless opportunities for the discovery of new drugs. It is popularly believed that medicinal plants are safer than pharmaceuticals because they are of natural origin. However, recent scientific reports have demonstrated that several medicinal plants used in phytomedicine are potentially toxic, and some are even mutagenic and/or carcinogenic. Therefore emphasis should be given to elucidate the safe use of any plant for medicinal purposes. The gall of *Quercus infectoria* (Family *Fagaceae*) is described in detail in ethnobotanical and literature to possess various pharmacological actions. In this study, *Q. infectoria* galls were selected for evaluation from a wide collection of examined plants (data not shown), traditional uses, folk medicine and literature survey. It was recorded that *Q. infectoria* galls contains many bioactive constituents, the majority of these compounds have notable antimicrobial activity. Tannins, especially those obtained from plants, have been found to possess strong antimicrobial activity. Tannins which constituted for almost 50-70% of *Q. infectoria* galls were reported to demonstrate most of the anti-inflammatory, antibacterial, and antifungal activities. Apart from that, a small amount of gallic acid and ellagic acid were also present in the gall extracts. Despite the increasing number of reports on the medicinal benefits of the *Q. infectoria* galls, the *in vivotoxicological* effect of the plant extract has yet to be reported. It is therefore deemed necessary to evaluate the acute oral toxicity of the *Q. infectoria* galls extract in a rat model. The present study was aimed to determine LD50 and to establish the safety of ethanolic extract of *Q. infectoriagalls* by acute oral toxicity study in female rats as per Organization for Economic Cooperation and Development (OECD) guideline 425. From the result of the acute toxicity study of ethanol extract of *Q. infectoriagalls* in rats, no mortality was recorded in any all groups of treated rats at 175, 550, 1750 and 5000 mg/Kg. In this study, all groups of treated rats did not show any toxic signs through the observation period. In light of these findings, the *Q. infectoria* galls extract is nontoxic in all the doses studied herein and did not produce any toxic signs or evident symptoms in acute oral toxicity study. The experimental animals did not showed any drug related changes in behavior, water consumption, impairment in food intake and temperature, body weight and wellness parameters used for evaluation of toxicity. Therefore, the extract seems to be safe at a dose level of 5000 mg/kg, and the LD50 was considered be is greater than 5000 mg / kg. Therefore, this study indicated that *Q. infectoria* galls ethanolic extract seem to be safe in use as material source for herbal products development. However, the repeated dose toxicity evaluation of the extract is still necessary in further study. In addition, it would be useful to investigate *Q. infectoria* galls ethanolic extract toxicity in pregnant animals. The use of other animal models to evaluate toxicity such as rabbits and guinea pigs may provide greater reassurance about the safety of this product in humans.

**Keywords:** *Q. infectoria* galls, acute oral toxicity, *in vivotoxicological* effect, mortality, wellness parameters, medicinal plants, phytomedicine.

## INTRODUCTION

Medicinal plants, either as an extract, pure compound or as a derivative, offer limitless opportunities for the discovery of new drugs. Most of the naturally occurring products used in folk medicine have solid scientific support in favor of their different biological properties. However, there is very less information available about the possible toxicity that medicinal plants may cause to the consumers [1]. So much has been done in screening medicinal plants for efficacy based on traditional claims while less emphasis is placed on the issue of safety, as reports of efficacy far outnumber those of toxicity, probably as a result of the greater demands for resources and time such exercise warrant. Pharmacological and toxicological evaluations of medicinal plants are essential for drug development [2]. It is popularly believed that medicinal plants are safer than pharmaceuticals because they are of natural origin. However, recent scientific reports have demonstrated that several medicinal plants used in phytomedicine are potentially toxic, and some are even mutagenic and/or carcinogenic [3]. The latest surveys have indicated that many medicinal plants used as traditional medicine showed undesirable effects [4, 5]. Therefore emphasis should be given to elucidate the safe use of any plant for medicinal purposes. The possible toxic effects resulting from the short term and long term use of such medicinal plants have raised an alarm. To increase the confidence in their safety to the consumers especially for use in the development of pharmaceuticals, the data of the acute and sub-acute toxicity studies on medicinal plants should be obtained [6]. The most critical part of any medicinal plant extract used for animal or human is the evaluation of its toxic effects. Hence a systemic approach in evaluating the efficacy and safety profile in such plants is needed.

Toxicology is the important aspect of pharmacology that deals with the adverse effects of bioactive substance on living organisms prior to the use as drug or chemical in clinical use [7]. According to the OECD guidelines, in order to establish the safety and efficiency of a new drug, toxicological studies are very essential in animals like rat, mice, guinea, pigs, rabbits and monkeys. Toxicological studies help to make decision whether a new drug should be adopted for clinical use. Depending on the duration of drug exposure to animals, there are three types of toxicological studies namely; acute, sub-acute and chronic toxicological studies. In acute toxicity studies, a single dose of a large quantity of the drug is given to determine immediate toxic effects. Acute toxicity studies are commonly used to determine LD50 of drug or chemicals and natural products. In sub-acute toxicity studies, repeated doses of drug are given in sub-lethal quantity for a period of 15-20 days. Sub-acute toxicity studies are used to determine effect of drug on biochemical and pathological parameters of organs and tissues. In chronic toxicity studies drug is given in different doses for a period of 90 days to over an year to

determine carcinogenic and mutagenic potentials of a drug [8].

Limit test and main tests are types of acute oral toxicity tests. The limit test is primarily used in situations where the experimenter has information indicating that the test material is likely to be nontoxic that is, having toxicity only above regulatory limit doses. Information about the toxicity of the test material can be gained from knowledge about similar tested compounds or similar tested mixtures or products, taking into consideration the identity and percentage of components known to be of toxicological significance. However, in situations where there is little or no information about its toxicity, or in which the test material is expected to be toxic, the main test should be performed [9].

The galls of *Quercusinfectoria* (Family *Fagaceae*) is described in detail in ethnobotanical and literature to possess various pharmacological actions such as analgesic, antidote, anti-inflammatory, antipyretic, antiseptic, antistomatitis, deodorant, derivative, desiccant, expectorant, germicidal, hypnotic, hypoglycaemic, powerful astringent, sedative, styptic, tonic, tonic to teeth and gum, and wound healing [10-15]. In this study, *Q. infectoria* galls were selected for evaluation from a wide collection of examined plants (data not shown), traditional uses, folk medicine and literature survey. It was recorded that *Q. infectoria* galls contains many bioactive constituents, for e.g., a large amount of tannins and significant percentages of gallic, ellagic and syringic acids,  $\beta$ -sitosterol, hexamethyl ether, isocryptomerin, amento  $\beta$  avone, methyl betulate, and hexagalloyl glucose [16,17], the majority of these compounds have notable antimicrobial activity. Tannins, especially those obtained from plants, have been found to possess strong antimicrobial activity [18]. Tannins which constituted for almost 50-70% of *Q. infectoria* galls were reported to demonstrate most of the anti-inflammatory, antibacterial, and antifungal activities. [19, 20] Apart from that, a small amount of gallic acid and ellagic acid were also present in the gall extracts [21]. However, the high tannin content in *Q. infectoria* galls could be considered the main responsible for their antimycotic activity.

Despite the increasing number of reports on the medicinal benefits of the *Q. infectoria* galls, the *in vivo* toxicological effect of the plant extract has yet to be reported. It is therefore deemed necessary to evaluate the acute oral toxicity of the *Q. infectoria* galls extract in a rat model. The toxicity study would serve as a very important baseline for further studies in developing this plant as an herbal medicine.

The present study was aimed to determine LD50 and to establish the safety of ethanolic extract of *Q. infectoria* galls by acute oral toxicity study in female rats as per Organization for Economic Cooperation and

Development (OECD) guideline 425. The test procedure described in this guideline uses pre-defined doses and the results allow a substance to be ranked and classified according to the Globally Harmonized System for the classification of chemicals that cause acute toxicity. Also, this method is of value in minimizing the number of animals required to estimate the acute oral toxicity of a chemical. In addition to the estimation of LD50 and confidence intervals, the test allows the observation of signs of toxicity.

## MATERIALS AND METHODS

### Plant Materials Collection and Identification

The of *Quercusinfectoria* galls were collected. The plant was identified by a taxonomist at Medicinal and Aromatic Plants Institute, National Center for Research - Khartoum, Sudan. The tested plant part then ground into powder and was used for the subsequent experimentation.

All the chemicals used were of analytical grade. Chloroform (SD Fine India), Ferric Chloride (BDH England), Acetic anhydride (SD Fine England), Sulphuric acid (SD Fine India), Hydrochloric acid (Romile EU), Aluminium Chloride (BDH England), Potassium Hydroxide (Sharlau Spain), Hydrogen Peroxide (Sharlau Spain), Ammonium Hydroxide (SD Fine India), Benzene (Sharlau Spain), Sodium Chloride (Sharlau Spain), Gelatin salt (Sharlau Spain), Potassium chloride (BDH England), Mercuric iodide (BHD England), Ethanol ( National Distillation Company).

$$\text{Weight of extract / weight of sample} * 100$$

**Table-1: *Quercusinfectoria* galls ethanol and water Extractive yield**

Solvent	Weight of sample	Weight of extract	Extraction yield %
Ethanol	500 gm	76.3 g	15.26 %

## METHODOLOGY

Paragraph 22 of OECD Guideline 425 suggests two types of acute oral toxicity tests i.e. limit test and main test. The limit test is primarily used in situations where the experimenter has information indicating that the test material is likely to be nontoxic, i.e., having toxicity below regulatory limit doses. However, in this situation where there is little or no information about *Q. infectoria* galls toxicity, only the main test should be performed.

### Procedure for Main Test

Prior to dosing, animals were fasted overnight before being weighed, and the *Q. infectoria* galls ethanolic extract were orally administered in a single dose. The volume given was not more than 2 ml/100 gm body weight (body wt.). Following the period of fasting, the fasted body weight of each animal was determined and the dose was calculated according to the body weight. After the extract was administered, food was withheld for a further 3-4 hours. Control animals

## Animals

Healthy young adult albino rats (Wistar strain), weighing 100-120 g (7-9 weeks old) gm were used for the study. They were obtained from animal house Medicinal and Aromatic Plants Research Institute, National Center for Research, Khartoum, Sudan. Female rats were selected because literature surveys of conventional LD50 tests show that usually there is little difference in sensitivity between sexes, but in those cases where differences are observed, females are generally slightly more sensitive [22]. The animals were randomly selected and kept in their cages for 5 days prior to dosing to allow for acclimatization to the laboratory conditions. These animals were fed on standard diet and water. The animals were maintained under standard conditions of relative humidity, twelve hours light-dark cycle, adequate ventilation and ambient room temperature.

## Preparation of Extract

Extraction was carried out according to method described by [23]: 500 g of the plant sample was extracted by soaking in 2500 ml 80 % ethanol for about seventy two hours with daily filtration and evaporation. Solvent was evaporated under reduced pressure to dryness using rotary evaporator apparatus, In order to obtain a completely dry extract, the resultant extract were transferred to glass dishes. The yield percentages were calculated as followed.

were administered with calculated amount of water for injection. Single animals were dosed in sequence usually at 48 h intervals. Using the default progression factor, doses were selected from the sequence 1.75, 5.5, 17.5, 55, 175, 550, and 2000 (or 1.75, 5.5, 17.5, 55, 175, 550, 1750, 5000 for specific regulatory needs). Because no estimate of the *Q. infectoria* galls lethality was available, dosing was initiated at 175 mg/kg till 5000 mg/kg as recommended in OECD Guidelines 425 [24].

## Observations

Wellness Parameters: Animals were observed continuously during the first 30 min after dosing and observed periodically (with special attention given during the first 4 hours) for the next 24 hours and then daily thereafter, for 14 days. All observations were systematically recorded with individual records being maintained for each animal. Observations included

changes in skin and fur, eyes and mucous membranes and behavioral pattern. Attention was given for observations of tremors, convulsions, salivation, diarrhea, lethargy, sleep, coma and mortality. Changes in wellness parameters were compared with that of control animals (Table 2).

**Body Weight:** Individual weights of animals were recorded before the administration of *Q. infectoria* galls ethanolic extract on 1st day of the study and thereafter on the 7th and 14th day of the experiment. Changes in the weight of individual animals were calculated and compared with that of the control animals.

**Statistical Analysis**

The LD50 was calculated using the software program-AOT425statpgm.

**RESULTS**

Acute toxicity determination is a method for assessing acute oral toxicity that involves the recognition of a dose level that causes mortality. The

dose limits were selected on the basis of oral acute toxicity studies in rats according to OECD guidelines.

**Observation of Wellness Parameters and Body Weight**

No significant changes were observed in body weight indicates that the administration of the *Q. infectoria* galls extract does not affect the growth of the animals, and wellness parameters used for evaluation of toxicity. Skin, fur, eyes, mucous membrane, behavioral pattern, salivation, sleep of the treated as well as the control animals were found to be normal. Tremors, lethargy, diarrhea and coma did not occur in any of the animal (Table 2).

**Mortality**

In accordance to the OECD Guidelines 425, the results revealed that *Q. infectoria* galls ethanolic extract was found to be nontoxic at all levels, no mortality was observed at 175, 550, 1750 and 5000 mg/kg body weight of experimental animals as shown in table 2 and figure 1.

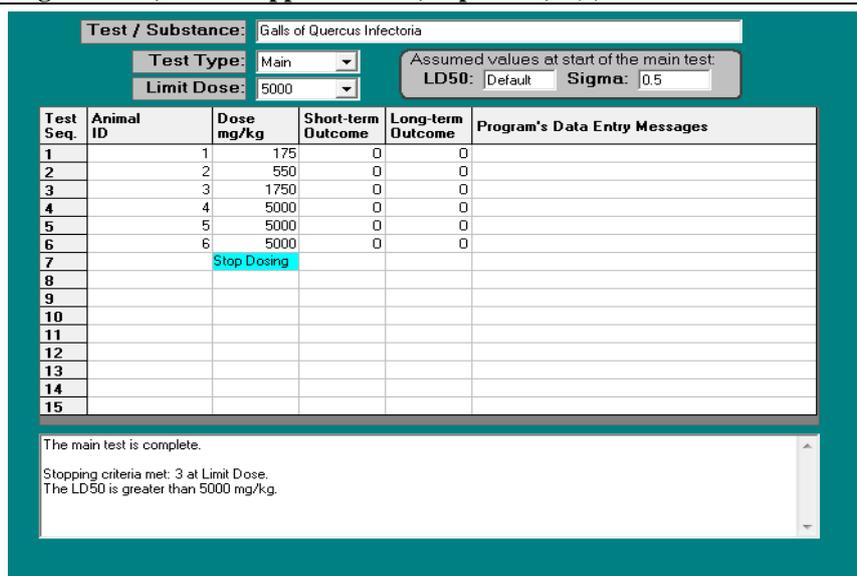
**Table-2: Effect of ethanolic extract of *Q. infectoria* galls on wellness parameters used for evaluation of acute oral toxicity test in Albino Wistar rats**

Response	Animals				
	Before treatment	After treatment (175mg/kg)	After treatment (550mg/kg)	After treatment (1750 mg/kg)	After treatment (5000 mg/kg)
Eyes	Normal	Normal	Normal	Normal	Normal
Mucous Membrane	Normal	Normal	Normal	Normal	Normal
Salivation	Normal	Normal	Normal	Normal	Normal
Skin & Fur	Normal	Normal	Normal	Normal	Normal
Lethargy	Absent	Absent	Absent	Absent	Absent
Sleep	Normal	Normal	Normal	Normal	Normal
Coma	Absent	Absent	Absent	Absent	Absent
Convulsion	Absent	Absent	Absent	Absent	Absent
Tremors	Absent	Absent	Absent	Absent	Absent
Diarrhea	Absent	Absent	Absent	Absent	Absent
Mortality	Not applicable	Nil	Nil	Nil	Nil

**Determination of LD50 Value**

The LD50 was calculated from Acute Oral Toxicity (Guideline 425) using the software program-AOT425statpgm [25], The LD50 for

*Quercusinfectoriagalls* ethanolic extract was found to be greater than 5000 mg / kg body weight (LD50 > 5000 mg/kg), (Figure 1).



**Fig-1: Determination of Median Lethal Dose (LD50) of *Quercusinfectoriagalls* ethanolic extract**

The aim to perform acute toxicity studies was for establishing the therapeutic index of a particular drug and to ensure the safety in-vivo.

Therefore, any dose can be selected up to 5000 mg/kg. The selection of dose was made based upon the

minimum concentration of *Q. infectoria* galls extract required for therapeutic action which will be economically fruitful for further research and formulation (Table 3, Figure 1).

**Table-3: LD50 value of *Q. infectoria* galls**

Dose (mg/kg)	Observation
175	No Death
550	No Death
1750	No Death
5000	No Death

**DISCUSSION**

Plant-derived medicines continue to be used throughout the world, and many major drugs have historically been extracted from plants. Herbal medicines are commonly used in alternative medical practice [26–28]. The therapeutic use of plant products is increasingly popular as more consumers have faith in their benefits and in their purported absence of adverse effects [29]. However, the rationale for the utilization of medical plants has rested largely on experiences of clinical practitioners with little or no scientific data on their efficacy and safety [30]. To determine the safety of drugs for human use, toxicological evaluation is always firstly carried out with experimental animals to assess potential toxicity and to provide guidance on safe doses for human being [31].

Toxicology tests are used to observe products such as individual compounds, mixture of compounds, crude extract, pesticides, medications, food additives, packing materials or their chemical ingredients. World health organization (WHO) recommends that medicinal herbs would be the dominant source to obtain a range of drugs. Therefore, such medicinal plants must be investigated for better understanding of their medicinal

properties, safety and effectiveness [32]. Safety of plant extract is evaluated mostly by acute oral toxicity analysis. The primary aim of toxicological assessment of any herbal medicine is to identify adverse effects and to determine limits of exposure level at which such effects occur. Two important factors which are taken into consideration in evaluating the safety of any herbal drug are the nature and significance of the adverse effect and in addition, the exposure level where the effect is observed. Toxicity testing can reveal some of the risks that may be associated with use of herbs especially in sensitive populations. An equally important objective of toxicity testing is the detection of toxic plant extracts or compounds derived thereof in the early (pre-clinical) and late (clinical) stages of drug discovery and development from plant sources. This will facilitate the identification of toxicants which can be discarded or modified during the process and create an opportunity for extensive evaluation of safer, promising alternatives [33].

Acute toxicity is defined as the toxic effects produced by single exposure of drugs by any route for a short period of time [34]. Acute toxicity studies in animals are considered necessary for any

pharmaceutical intended for human use. The main objective of acute toxicity studies is to identify a single dose causing major adverse effects or life threatening toxicity, which often involves an estimation of the minimum dose causing lethality. The lethal dose (LD50) is defined as the dosage of a substance which kills 50 per cent of the animals in a particular group, usually determined in an acute, single exposure study. LD50 is the dose which has proved to be lethal to 50% of the tested group of animals. Determination of oral toxicity is an initial screening step in the assessment and evaluation of the toxic characteristics of all compounds [35].

From the result of the acute oral toxicity study of ethanol extract of *Q. infectoria* galls in rats, no mortality was recorded in any all groups of treated rats at 175, 550, 1750 and 5000 mg/Kg (Tables 2 and 3, Figure 1). In our earlier study, we have observed the presence of various phytochemicals e.g. tannins, flavonoids, saponins, triterpenes, anthraquinones and coumarines, occurring in various amounts in the plant. These phytochemicals elicit a wide array of pharmacological actions. Some of the phytochemicals produced by plants against herbivorous insects also end up being harmful to humans, because highly conserved biological similarities are shared between both taxa as seen in most pathways involving protein, nucleic acid carbohydrate and lipid metabolism [36]. Human neurochemicals, often with similar biological functions are also reportedly present in insects [36]. These include signaling molecules, neuropeptides, hormones and neurotransmitters [37-40]; whose functions can be mimicked or antagonized by phytochemicals like alkaloids, flavonoids, terpenoids and saponins. Some lipid soluble terpenes have shown inhibitory properties against mammalian cholinesterase [41], whilst some interact with the GABAergic system in vertebrates [42]. In addition to these, saponins are potent surfactants that can disrupt lipid-rich cellular membranes of human erythrocytes and microorganisms which explains the potent antimicrobial properties of this group of phytochemicals [43].

As shown in in this study, all groups of treated rats (175, 550, 1750 and 5000 mg/Kg) did not show any toxic sings through the observation period (Tables 2). In light of these findings, the *Q. infectoria* galls extract is nontoxic in all the doses studied herein and did not produce any toxic signs or evident symptoms in acute oral toxicity study. The experimental animals did not showed any drug related changes in behavior, water consumption, impairment in food intake and temperature, body weight and wellness parameters used for evaluation of toxicity. Therefore, the *Q. infectoria* galls extract seems to be safe at a dose level of 5000 mg/kg, and the LD50 (Figure 1) was considered be is greater than 5000 mg / kg (LD50 > 5000 mg/kg). Any compound with oral LD50 of 5000 mg/kg or more in rat should be considered as practically harmless [44].

These results are comparable to those of [45] who obtained an LD50 of 0.75 g / kg with aqueous extract of *Q. infectoriagalls* in mice by subcutaneous administration.

## CONCLUSION

In light of these findings, we may conclude that *Q. infectoriagallsextract* is nontoxic in all the doses studied herein and did not produce any toxic signs or evident symptoms in acute oral toxicity study. The experimental animals did not showed any drug related changes in behavior, water consumption, impairment in food intake and temperature, body weight and wellness parameters used for evaluation of toxicity. Therefore, the *Q. infectoria* galls ethanolic extract seems to be safe at a dose level of 5000 mg/kg, and the LD50 was considered be is greater than 5000 mg / kg.

This study indicated that *Q. infectoria* galls ethanolic extract seem to be safe in use as material source for herbal products development. However, the repeated dose toxicity evaluation of the extract is still necessary in further study. In addition, it would be useful to investigate *Q. infectoria* galls toxicity in pregnant animals. The use of other animal models to evaluate toxicity, such as rabbits and guinea pigs may provide greater reassurance about the safety of this product in humans [46].

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