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Acute and subchronic oral toxicity of *Galega officinalis* in rats

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Abstract

Galega officinalis L. (Papilionaceae) is widely used in folk medicine as antidiabetic or for increasing lactation. There is a little information about its possible toxicity. In this study, acute and subchronic toxicity of aerial parts of *Galega officinalis* in Wistar rats have been evaluated. For the acute toxicity study, the animals received orally four different single dose of plant suspension and were kept under observation for 14 days. The results indicated that LD50 of *Galega officinalis* is higher than 5 g/kg. In the subchronic study, 48 rats were divided into four groups and were fed a diet containing 0%, 0.15%, 1.5% and 3% (w/w) of *Galega officinalis*. After 90 days blood and tissue samples were taken for hematological, biochemical and histopathological determinations. An increase in serum levels of cholesterol, creatine phosphokinase, lactate dehydrogenase and total and conjugated bilirubin was observed. Some parameters such as calcium, albumin, albumin/globulin ratio, hematocrit, WBC and platelet counts were decreased. In microscopic examination, sinusoidal congestion in liver and alveolar hemorrhage was observed. Other parameters showed non-significant difference between treatment and control groups. Present data suggest that liver and lung could serve as target organs in oral toxicity of this plant.

Keywords: *Galega officinalis*; Acute toxicity; Subchronic toxicity

1. Introduction

Galega officinalis (Papilionaceae) is a native plant from southeastern Europe and was used as a treatment for diabetes in medieval times (Oubre et al., 1997). The discovery of the plant's active hypoglycemic agent led to the development of metformin, a biguanide which has been used to treat type 2 diabetes mellitus (Oubre et al., 1997; Palit et al., 1999). In fact, the only example of an approved antidiabetic drug that was developed from an herbal source with a long history of use for diabetes is metformin from *Galega officinalis* (Vuksan and Sievenpiper, 2005).

Also in traditional medicine, *Galega officinalis* (syn.: galega) is well known as a galactagoguing plant to improve milk secretion, both in human and in animals (Uncini Manganelli et al., 2001; Leporatti and Ivancheva, 2003). Recent experimental investigations indicate that crude aqueous extracts and gel-filtered fractions of the plant suppress platelet aggregation (Atanasov, 1994; Atanasov and Spasov, 2000). Moreover, it was found that active platelet disaggregating fraction of the extract appeared to be a polysaccharide–protein complex (Atanasov and Tchorbanov, 2002). Alcoholic extract of *Galega officinalis* was tested on gram-positive and gram-negative bacteria as the plant was claimed to hasten skin healing after surgery. This ethanolic (60%) extract exhibited significant inhibition on growth of both gram-positive and gram-negative bacteria (Pundarikakshudu et al., 2001). In addition to above established effects, the plant has weight reducing action, the mechanism of which is unclear but involves loss of body fat (Palit et al., 1999).

Previous studies found the plant *Galega officinalis* to be toxic to sheep and are capable of causing death. The animals usually presented with hydrothorax, accumulation of a frothy fibrinous fluid in airways, and pulmonary congestion and edema (Puyt et al., 1981; Keeler et al., 1986, 1988). In some cases, subendocardial hemorrhage was present and the most consistent microscopic changes were found in the lungs with congestion of alveolar capillaries and eosinophilic protein-rich edema fluid. It is believed that galegine is the major compound in the plant that causes injury (Keeler et al., 1992).

Considering both the ethnobotanical and pharmacological applications of the plant, the aim of this study was to investigate the possible toxic effects of the aerial parts of plant in rats

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using histopathological and hematological examinations as well as biochemical parameters.

2. Materials and methods

2.1. Plant material

Galega officinalis has been harvested in flowering period by a pharmaceutical company (Zard Band Co., Tehran, Iran). The company has supplied the dried aerial part of plant in powdered form. The plant was authenticated by M. Kamalinejad, Department of Pharmacognosy and a voucher specimen (Registration Number 94) has been deposited in the Herbarium of the School of Pharmacy (Shaheed Beheshti Medical University, Tehran, Iran).

2.2. Evaluation of toxicity following single dose administration

2.2.1. Animals

Four to 5 weeks old, Wistar rats of both sexes were purchased from Razi Research Institute (Hesarak, Karaj, Iran) and acclimated to holding facilities for 2 weeks prior to dosing. Animals were randomly assigned to control and four treatment groups (5 rats/(sex group)) and were housed in clear plastic cages containing wood shavings for bedding. Each cage contained five rats of the same sex and were fed on normal laboratory chow (Pars Co., Tehran, Iran) and given tap water *ad libitum* throughout the study. Environmental conditions were maintained at a temperature of $23 \pm 2\,^{\circ}C$ and a relative humidity of $40 \pm 10\%$ with 12 h light/dark cycle. At the onset of dosing, males weighed 172 ± 23 g and females weighed 135 ± 11 g. The research was conducted in accordance with the internationally accepted principles for laboratory animals use and care as found in the US guidelines (NIH Publication no. 85–23, revised in 1985).

2.2.2. Administration

Animals were fasted for 12 h prior to dosing on day 0. Treatment rats were dosed by oral gavage, using a curved, ball-tipped stainless steel feeding needle, with aqueous suspensions of ground very fine powder of *Galega officinalis*. The dosages delivered were 0.5, 1, 2.5, and 5 g/kg body weight galega herb in the constant volume of 10 ml/kg body weight. The controls received tap water by gavage in the same volume.

2.2.3. Observations

All rats were monitored continuously for 10 h after dosing for signs of toxicity. For the remainder of the 14 days study period, animals were monitored daily for mortality and any changes in food and water consumption, and any additional behavioral or clinical signs of toxicity. Animals body weight were measured prior to dosing and on days 7 and 14. On day 14, all animals were killed and at the end of the study the number of dead animals was expressed in percentage and, if possible, the LD_{50} value was established using Probits method (Angelis Pereira et al., 2003).

2.3. Evaluation of toxicity following subchronic treatment

2.3.1. Animals

Four weeks old Wistar rats of both sexes were obtained from Razi Research Institute and acclimated for 4 weeks prior to the start of study. They were randomly divided into control and three treatment groups (6 rats/(sex group)). Animals were caged in pairs of the same sex in clear plastic cages containing wood shavings for bedding. At the time when dosing was initiated, rats were 8 weeks old, and males weighed 190 ± 14 g and females weighed 153 ± 16 g.

2.3.2. Treatment

For the purposes of this study, the ground plant material was incorporated into the standard laboratory rat diet (Pars Co., Tehran) at constant concentrations of 0.1%, 1% and 2% (w/w), by mixing with powdered standard diet. Our pilot study indicated that based on daily food consumption of animals, these concentration would achieve daily doses of 0.1, 1, and 2 g/kg body weight, respectively. Finally, the galega containing diet was repelleted before being supplied to rat. Control diet was prepared similarly but without the addition of plant material.

The prepared pellets were given to rats as their daily diet (instead of standard diet) for 13 weeks, to determine the adverse effects of *Galega officinalis*.

Six weeks after the onset of treatment, due to a reduction in food consumption by animals, the concentration of plant material in diet was increased to reach 0.15%, 1.5% and 3% (w/w) of the prepared diet (Keyler et al., 2002). The animals had free access to food and tap water *ad libitum* throughout the test.

2.3.3. In life evaluations

Observations of mortality and toxicological signs were made daily for 13 weeks. The time of onset, intensity, and duration of these symptoms, if any, was recorded. The weight of each rat was recorded on day 0 and at weekly intervals throughout the course of the study. Food and water consumption were measured three times a week.

2.3.4. Biochemical and hematological analyses

At the end of the study, all animals were fasted for 12 h and then anaesthetized with an ip injection of a mixture containing ketamine (40 mg/kg) and xylazine (10 mg/kg). The jugular vein was exposed, and blood samples were taken by jugular vein puncture (Wilson et al., 2001).

Blood samples for biochemical analyses were centrifuged at $3000 \times g$ for 5 min and the plasma collected and analyzed for glucose, blood urea nitrogen, creatinine, total protein, albumin, albumin/globulin ratio, phosphorus, calcium, sodium, potassium, chloride, bilirubin (total and conjugated), cholesterol, triglycerides, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH) and creatin phosphokinase (CPK). These levels were determined by COBAS Mira S chemistry analyzer (Roche Diagnostic Systems).