

## Acute Oral Toxicity Study of Ivy-Thyme Syrup in Albino Rats

*Ruba Tarawneh<sup>1</sup>, Rana AbuFarha<sup>1</sup>, Mohammad Hudaib<sup>1</sup>, Khaled Tawaha<sup>1</sup>, Khaled Aieda<sup>1</sup>, Yasser Bustanji<sup>1</sup>, Mohammad Mohammad<sup>1</sup>✉*

<sup>1</sup> Faculty of Pharmacy, University of Jordan, Amman, Jordan

### ABSTRACT

This study was designed to assess the acute oral toxicity of Ivy-Thyme syrup in rats. The tested product was administered at a dose level of 3, 6 and 12 mL/kg. All animals were examined for clinical signs of ill-health or mortality at 1, 2, 4 and 8 hours after oral administration, and twice daily thereafter for 14 days. At the end of the study, all rats were alive with normal appearance and showed body weight gain during the study. The kidneys and livers of the sacrificed animals appeared normal.

Ivy-Thyme syrup having 0.75% Ivy leaf dry extract and 5% thyme fluid extract was found to be non-toxic by the oral route at a dose level of 3, 6 and 12 ml/kg in female rats under the conditions of this study.

**Keywords:** Acute oral toxicity, Ivy, Hedera, Thyme, Albino rats, Herbal medicine, Antitussive.

### INTRODUCTION

Plants have been utilized as medicines for thousands of years. These medicines were initially taken in the form of crude drugs such as tinctures, elixirs, poultices, powders, and other herbal formulations. These natural products have played an important role in treating and preventing human diseases<sup>1</sup>. An analysis of the origin of the drugs that were launched in the last twenty-five years showed that both natural products and semi-synthetic compounds, derived from natural origin, comprised 34% of all new chemical entities, while 18% of them were synthetic mimics of natural compounds<sup>2</sup>. Furthermore, according to the WHO, 80% of the world's population, primarily those of developing countries, rely on plant-derived medicines for their healthcare<sup>3</sup>.

However, many of these herbs and natural supplements not have been thoroughly evaluated and their safety and effectiveness may have not been proven.

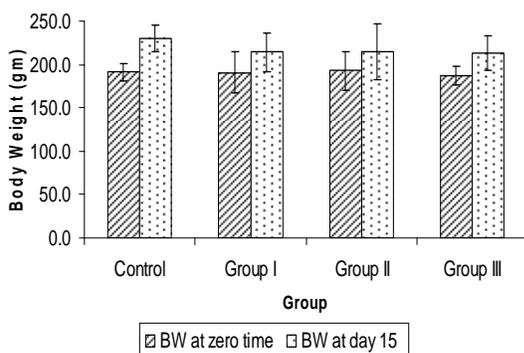
The clinical and pharmacological interest of the efficacy and safety of herbal remedies has grown during the past ten years because of the realization that many people are self-medicated using these agents<sup>4,5</sup>. However, the use of herbal products should be based on scientific origin; otherwise they would be useless and unsafe. Furthermore, the irrational use of these herbal products may cause serious toxicity for humans. Unfortunately, many people underestimate the toxicity of natural products and do not realize that these agents could be as toxic or more than synthetic drugs. A typical example for a toxic herbal product are the leaves of *Atropa Belladonna*<sup>6</sup> and *Digitalis purpurea*<sup>7</sup>, which show severe systemic toxicity if taken orally.

One method for the evaluation of herbal toxicity is the acute oral toxicity test<sup>8</sup> in which the herbal preparation is given orally as a single and very high dose to laboratory animals like rats. The tested animals are then observed for 14 days for activity, behavior and indications of toxicity or illness. At the end of the experiment, all animals are sacrificed and their major organs (liver, spleen, kidney and lungs) are examined for any abnormal changes compared to the control groups.

---

Received on 16/2/2010 and Accepted for Publication on 16/5/2010.

✉ E-mail: mkmohammad@ju.edu.jo



**Figure 1. Body weight changes during the study. All treated and control groups showed normal weight gain. There was no significant difference in average body weights between the treated groups and the control ( $p > 0.05$ ). Values represent the mean weight of 5 rats  $\pm$  standard deviation.**

Ivy-Thyme extract syrup is a herbal preparation used to alleviate cough as a result of common cold, bronchitis or respiratory tract disorders.

Thyme is a genus of about 350 species of aromatic perennial herbaceous plants, the most common *Thymus vulgaris* in the family Lamiaceae and native to many worldwide regions. Thyme has been used medicinally for thousands of years. Beyond its common culinary application, it has been recommended for numerous indications; antimicrobial, antitussive, spasmolytic and antioxidant activity<sup>9</sup>.

Hedera (English name ivy), is a genus of 15 species of climbing or ground-creeping evergreen woody plants in the family Araliaceae, native to the Atlantic Islands, western, central and southern Europe, northwestern Africa and across central-southern Asia east to Japan. On suitable surfaces (trees and rock faces), they are able to climb to at least 25–30 meters above the basal ground level<sup>10</sup>. Ivy is an ingredient of many herbal preparations intended for respiratory tract infections and for the treatment of cough associated with the illness.

**Table 1. Weights of both kidneys for each rat in control and treated groups. The last row shows the average weight of each group  $\pm$  standard deviation. There was no statistical difference between treated groups and control group ( $p > 0.05$ ).**

Control (gm)	Group I: 7X (gm)	Group II: 14X (gm)	Group III: 28X (gm)
1.6	1.95	3	1.4
1.9	1.97	2.1	1.8
2.4	2.5	2.6	2.7
1.7	2.08	2.2	2.9
1.7	2.1	1.5	2.2
<b>1.86 <math>\pm</math> 0.32</b>	<b>2.12 <math>\pm</math> 0.22</b>	<b>2.28 <math>\pm</math> .56</b>	<b>2.2 <math>\pm</math> .62</b>

The syrup made from the mixture of these two herbs has been clinically tested against acute bronchitis patients with good results<sup>11</sup>.

This study was designed to assess the acute oral toxicity produced when the test material, Ivy-Thyme Syrup, was administered by oral intubation to rats.

The study was conducted in accordance with US EPA/OPPTS 870.1100 and OECD Guidelines for Testing Chemicals, No. 401.

#### MATERIALS AND METHODS:

The test material, Ivy-Thyme Syrup was received from the supplier and stored at ambient temperature in an amber glass bottle. It appeared as a brownish concentrated fine aqueous dispersion (suspension).

The product contains the following components in 100 mL: 0.75 g Ivy leaf dry extract (10% Hederacoside C), 5.0 g Thyme fluid extract (non alcoholic) and other excipients (sorbitol, propylene glycol, potassium sorbate, citric acid and honey flavor). The supplier assumed responsibility for the purity and stability determinations.

#### Animals:

The animal experiments conform with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication no. 85-

23, revised 1996).

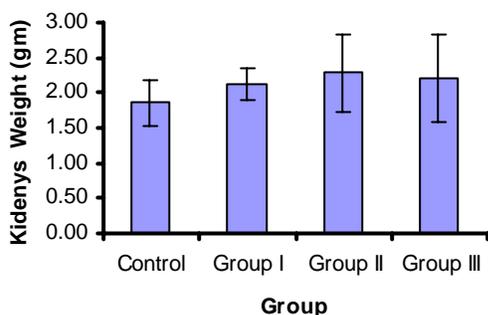
Young, adult female albino rats were purchased from the Applied Sciences University animal house. The animals were acclimated to laboratory conditions. After thirteen days of acclimation without showing any abnormality or pathological change, the animals were used in the study. Animals used in this study were allocated from all animals available and were within the protocol-specified weight range (173 to 230 g).

The animals were fed *ad libitum* with a commercial rodent diet (locally supplied by Hammoda Dairy Product Inc.) and water except when fasting was needed in the course of the study (12 hours before dosing).

After randomization, the animals were assigned into this study and to dose groups (Figure 1). The animals were separated into a total of four groups each containing five female rats.

**Experimental Design:**

Doses given to the animals were calculated as 3 ml/kg, 6 ml/kg and 12 ml/kg. The highest dose administered to the test animals was limited by the maximum allowable volume that can be given to rats (1 ml/100 gm). A control group of animals was given 12 ml/kg normal saline.



**Figure 2. Kidneys weight of the rats for each group. Results show there is no significant difference between treated groups and control group ( $p > 0.05$ ). Values represent the mean weight of both kidneys (left and right) of 5 rats  $\pm$  standard deviation.**

These dose levels are equivalent to 7x, 14x and 28x of the total daily dose for humans based on the manufacturers recommendation, 0.42 ml /kg, (2 teaspoons every 8 hours as needed equivalent to a maximum of 30 ml/Day/70 kg).

After fasting for 12 hours, all animals received a single dose of the test product administered by oral intubation. Individual doses were based on a dose volume of 3 ml/kg, 6 ml/kg and 12 ml/kg and were calculated based upon the fasted body weight.

Clinical observations were performed 1, 2, 4 and 8 hours after dose administration (Day 1) and twice daily thereafter for at least 14 days. Animals were observed in the cage for activity, behavior and indications of toxicity or ill-health. Body weights were determined prior to test product administration (Day 1), and again on day 14.

At the end of the study, the animals were sacrificed and their major organs (liver, spleen, kidney and lungs) were examined for any abnormal change compared to the control groups. Kidneys and liver weights for each rat were documented.

**Statistical Analysis**

All data of rat body, liver and kidney weights were analyzed by one-way analysis of variance (Minitab 14, Minitab Inc). Differences were considered significant at  $p < 0.05$ , and data were presented as means  $\pm$  SD (Standard Deviation).

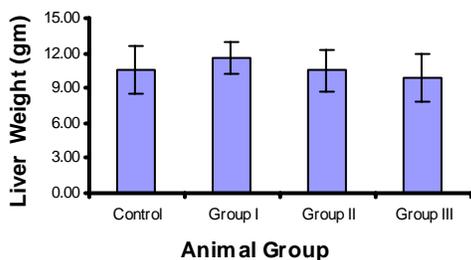
**Table 2. Liver weight of each rat for control and treated animals. The last row shows the average weight of each group  $\pm$  standard deviation. There was no statistical difference between treated groups and control group ( $p > 0.05$ ).**

Control (g)	Group I: 7X (g)	Group II: 14X (g)	Group III: 28X (g)
13.7	9.8	13.4	8.4
10	11.4	10.8	8.3
8.5	13.5	10.5	12.8
9.5	12	9.3	11.1
11.2	10.9	8.7	8.7
<b>10.6 <math>\pm</math> 2.0</b>	<b>11.5 <math>\pm</math> 1.37</b>	<b>10.5 <math>\pm</math> 1.81</b>	<b>9.9 <math>\pm</math> 2.01</b>

### Results and Discussion:

All rats were alive and appeared normal at the end of the experiment (Day14). There was no significant difference ( $p > 0.05$ ) in average body weights among the four groups. Moreover, a similar weight gain was observed for control and treated groups (Figure 1).

Furthermore, all the animals appeared normal at all observation intervals during the period of the study and without observing any clinical abnormalities. All clinical signs of the treated animals were similar to that of the control group.



**Figure 3. Liver weight of each rat for control and treated animals. Results showed that there was no significant difference in the liver weights between control and treated animals. Values represent the average of liver weight of 5 rats  $\pm$  standard deviation.**

After the sacrifice, gross anatomical examination of the major organs of treated and control groups showed no significant difference in their weight, volume and appearance. Kidneys and livers from all groups were removed and studied in terms of their weights and appearance. The results showed no sign of toxicity or significant difference ( $p > 0.05$ ) in the average weights between the kidneys of treated animals and control group (Table 1 and Figure 2). Similar results were observed regarding the weight gain in the livers of experimental animals (Table 2 and figure 3).

### CONCLUSION:

Ivy-Thyme syrup was found to be non-toxic by the oral route at a dose level of 3, 6 and 12 ml/kg in female rats. These dose levels are equivalent to 7X, 14X and 28X of the total daily dose for humans based on the manufacturer's recommendation.

### ACKNOWLEDGMENTS

This project was sponsored by both the Faculty for Factory Program and SANA Pharmaceutical Research Company supervised by Yasser Bustanji, University of Jordan. The authors wish to thank the Faculty for Factory Program, SANA Pharmaceutical Research Company and the Deanship of Academic Research for their generous funds.

### REFERENCES

- (1) Gullo Vincent, P., McAlpine, J., Lam Kin, S., Baker, D. Petersen, F. Drug discovery from natural products. *J. Ind. Microbiol. Biotechnol.* 2006; 33: 523-531.
- (2) Newman, D.J.Cragg, G.M. Natural Products as Sources of New Drugs over the Last 25 Years. *J. Nat. Prod.* 2007; 70: 461-477.
- (3) Gurib-Fakim, A. Medicinal plants: Traditions of yesterday and drugs of tomorrow. *Mol. Aspects of Medicine.* 2006; 27: 1-93.
- (4) Calixto. J.B. Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents). *Brazilian j. Med. Biol. Res.* 2000; 33: 179-189.
- (5) Firenzuoli F, Gori L. Herbal medicine today: clinical and research issues. *Evid Based Complement.* 2007; 4(Suppl 1):37-40.
- (6) Greenblatt DJ, Shader RI. Uses and toxicity of belladonna alkaloids and synthetic anticholinergics. *Semin Psychiatry.* 1971; 3:449-76.
- (7) Vaccari A, Furlani A. Cardiotoxicity in rats of two extracts of *Digitalis purpurea* after transplantation of the plants into different habitats. *Minerva Med.*

- 1967;58:3021-4.
- (8) Walum E. Acute oral toxicity. *Environ Health Perspect.* 1998; 106(Suppl 2): 497–503.
- (9) Trease and Evans pharmacognosy, by William Charles Evans (Author), George Edward Trease (Author) Saunders (W.B.) Co Ltd; 14Rev Ed edition (Mar 1996).
- (10) Mecalfe, D.J. *Hedera helix*. *J. Ecol.* 2005; 93:632-648.
- (11) Kemmerich B, Eberhardt R, Stammer H. Efficacy and tolerability of a fluid extract combination of thyme herb and ivy leaves and matched placebo in adults suffering from acute bronchitis with productive cough. A prospective, double-blind, placebo-controlled clinical trial. *Arzneimittelforschung.*

1 1 1 1 1 1

1

14

/ 12,6,3  
8,4,2,1

%5

%0.75  
/ 12,6,3

:

---

.2010/5/16

2010/2/16