In Vivo Acute Toxicity Study of Thyme – Primulae Syrup

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ABSTRACT

The current study is an acute toxicity study of Thyme – Primulae syrup in Albino male and female rats. The syrup was administered in three escalating doses: 3, 6 and 12 ml/kg body weight. The weight-based doses were given to the rats by intra gastric to minimize loss and variability among them. The treated groups were compared to the untreated control in regards to their body weight gain, hepatic and kidney appearances and their weight. Even at the highest dose, the syrup was found to be non-toxic under the conditions of this study.

Keywords: Acute Toxicity, Thyme-Primulae Syrup, Plant Extract, Medicinal Plants, Anti-Cough Syrup.

INTRODUCTION

Plants have been an endless source of conventional and folkloric medicines for thousands of years. These plant-based medicines were formulated into a wide range of pharmaceutical preparations such as tinctures, elixirs, poultices, powders, and several herbal formulations. Historically, natural medicines did indeed play an important role in treating and preventing a vast number of human diseases [1]. Currently, a recent analysis of the origin of the drugs, that were launched to the market in the last twenty-five years, showed that both natural products and semi-synthetic compounds, derived from natural origin, are comprised of 34% of all new chemical entities. The report also showed that about 18% of the drugs were synthetic mimics of natural compounds[2]. In fact, according to the WHO, 80% of the world's population, primarily those in developing countries, rely on plant-derived medicines as a main source of pharmacologic intervention[3].

It should be emphasized, however, that many of the popular herbal and natural supplements have not been thoroughly evaluated, and their safety and effectiveness have not been established. On the other hand, product consistency has been a chronic dilemma with herbal and dietary supplements. The FDA does not require any product validation or standardization for dietary supplements as long as the manufacturers follow GMPs. Brand names may vary tremendously, even within the same brand, despite of the claims on the labels and packages.

Nonetheless, the shortcomings in product consistencies failed to halt the clinical and pharmacological interests in the efficacy and safety of herbal remedies in the past ten years. Those interests were driven by the realization that many people are self-medicated on a plethora of complementary and alternative medicines especially when conventional pharmacologic agents fail to cure diseases such as cancer, diabetes, autoimmune diseases and resistant infections [4-5]. Unfortunately, the majority of the general public base their choice of the herbal supplements on unsubstantiated manufacturer's claims and the lack of an evidence-based approach. A common misconception among people seeking alternative medicines is that nature is synonymous with safe.

Received on 16/8/2010 and Accepted for Publication on 13/10/2010.
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It is currently well-established among the scientific community that the improper use of these herbal products could result in serious toxicities and adverse reactions in humans. One typical example for toxic herbal product is the leaves of *Atropa Belladonna*[6] and *Digitalis purpurea*[7] , which cause severe cardiovascular toxicities if taken indiscriminately.

In the current study, we followed the acute oral toxicity test in evaluating the toxicity of the Thyme-Primulae syrup as described previously [8]. In this test the herbal preparation under investigation is given to laboratory animals like rats as single large dose based on body weight. The tested animals were then monitored for 14 days for any signs of pathologic and behavioral toxicities. At the end of the experiment, all animals were sacrificed and their major organs (liver, spleen, kidney and lungs) were examined for any abnormal histological or pathological changes.

Thyme – Primulae syrup is a highly prescribed herbal preparation that has been used traditionally in the management cough, bronchitis, bronchial edema and mucous exacerbations in COPDs. This herbal medicine consists of aqueous extracts of thyme (aerial part) and primula root extract [9].

Thyme is a genus of about 350 species of aromatic perennial herbaceous plants, and 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 reported to have antimicrobial, anti-tussive, spasmolytic and antioxidant activities [10].

Primula veris is a flowering plant in the genus Primula. The species is native throughout most of temperate Europe and Asia. It is used medicinally as a diuretic, an expectorant, and an antispasmodic as well as for the treatment of headaches, whooping cough and tremors [10].

The main objective of the current study is to evaluate any potential toxicities of the Thyme – Primulae syrup in using the single dose acute oral toxicity method in albino rats. This product was developed in SANA Pharmaceutical research, and it is intended to be used as anti-tussive preparation.

Materials and Methods

The test material, Thyme–Primulae Syrup, was received from the SANA Pharmaceuticals and stored at ambient temperature in amber glass. Upon receipt, the test material was described as brown, clear to slightly turbid, slightly viscous liquid with a characteristic taste and odor. The product contains the following components per 10 mLs: 500 mg of thyme fluid extract (non alcoholic), 32.5 mg primulae dried extract and 16.5 mg potassium sorbate.

Animals

The animal experiments were conducted in accordance 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 (6-week old) male and female albino rats were received from the Applied Sciences University Animal House. The animals were acclimated to our animal facility conditions for 7 days. During acclimation, animals that have shown any signs of abnormal physical or behavioral changes were excluded from the study. At the end of the 7 day period, all of the animals used in this study were within the protocol-specified weight range (250 to 300 g). The animals were assigned temporary numbers during acclimation and identified by cage label for the duration of the study. The animals were pair-housed during the acclimation period and singly housed during the study period in plastic cages.

The animals were fed *ad libitum* with standard chow and water except when fasting was needed in the course of the study (12 hours before dosing). After randomization, the rats were assigned into the control and different dose groups. The animals were separated into a total of 8 groups; 4 groups of five male rats each, and another 4 groups of five female rats each.

Dose levels and Animal Treatment:

The Thyme – Primulae syrup doses, given to the test animals, were calculated as 3mL/kg, 6 mL/kg and 12 mL/kg body weight. The highest dose administered to test animals was limited by the maximum allowable volume that can be forced intragastically to rats (1 mL/100 gm). These dose

levels represent 7x, 14x and 28x of the total daily dose for humans based on the manufacturers recommendation (5ml every 2-3 hours as needed equivalent to a maximum of 30 mL/Day/70 kg). A control group of each sex was given 12 mL/kg normal saline.

The first three groups of each sex received the previously calculated doses of the test product administered by oral intubation after fasting for 12 hours. Historically, the oral route has been one of the routes used to assess the acute toxicity of various materials. In addition, the product is intended for per-oral administration.

Clinical Observations:

Animals were observed in the cage for activity, behavior and any signs of toxicity at approximately 1, 2, 4 and 8 hours after dose administration in the first day and twice daily thereafter for 14 days. All of the observations were documented daily. The animals were weighed prior to test product administration and prior to sacrifice after 14 days.

At the end of the study, the animals were scarified, and their major organs (liver, spleen, kidney and lungs) were weighed and examined for any abnormal pathological changes was compared to the control groups.

Data analysis

Data are presented as means±S.E. Statistical comparisons were performed using one-way ANOVA or unpaired Student's T test. In all cases, $p<0.05$ is considered statistically significant.

Results and Discussion

This study was designed to assess the safety of a pharmaceutical product containing herbal ingredients; Thyme-Primulae syrup intended to be used in cough preparations.

This product was administered, by oral intubation, at three escalating doses; 3, 6 and 12 mL/kg of the syrup to a total of 5 healthy, male and female rats (5 animals/sex/dose) after normalization to animal weight. The highest dose was limited by the maximum allowable volume that can be given to rats (1 mL/100gm of rat body weight). All animals were examined for clinical signs of ill/health, changes in physical activity and mortality for 14 days.

During the study period, no mortality in any group was recorded. All the animals were noted as normal at all observation intervals and survived until the scheduled sacrifice. In fact, the studied animals gained weight normally throughout the study, and there was no significant difference in weight gain (Figure 1 and Figure 2), water and food consumption between control and treated groups in both sexes (See Table I and Table 2).

Table I: Acute oral toxicity study of thyme – Primulae syrup in male rats*

	Control group	**Group I 3mL/kg**	**Group II 6mL/kg**	**Group III 12mL/kg**
Number of treated animals	5	5	5	5
Number of dead animals by end of exp.	0	0	0	0
Activity	Normal	Normal	Normal	Normal
Food and water consumption	Normal	Normal	Normal	Normal
Signs of toxicity	None	None	None	None
Gross Anatomy	No sign of toxicity	No sign of toxicity	No sign of toxicity	No sign of toxicity

After fasting for 12 hours, all the 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 calculated based upon the animal's body weight. A control group was given 12 mL/kg normal saline.

Table 2: Acute oral toxicity study of thyme – Primulae syrup in Female rats*

	Control group	Group I 3mL/kg	Group II 6mL/kg	Group III 12mL/kg
Number of treated animals	5	5	5	5
Number of dead animals by end of exp.	0	0	0	0
Activity	Normal	Normal	Normal	Normal
Food and water consumption	No obvious change	No obvious change	No obvious change	No obvious change
Signs of toxicity	None	None	None	None
Gross Anatomy	No sign of toxicity	No sign of toxicity	No sign of toxicity	No sign of toxicity

*After fasting for 12 hours, all the 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 calculated based upon the animal's body weight. A control group was given 12 mL/kg normal saline.

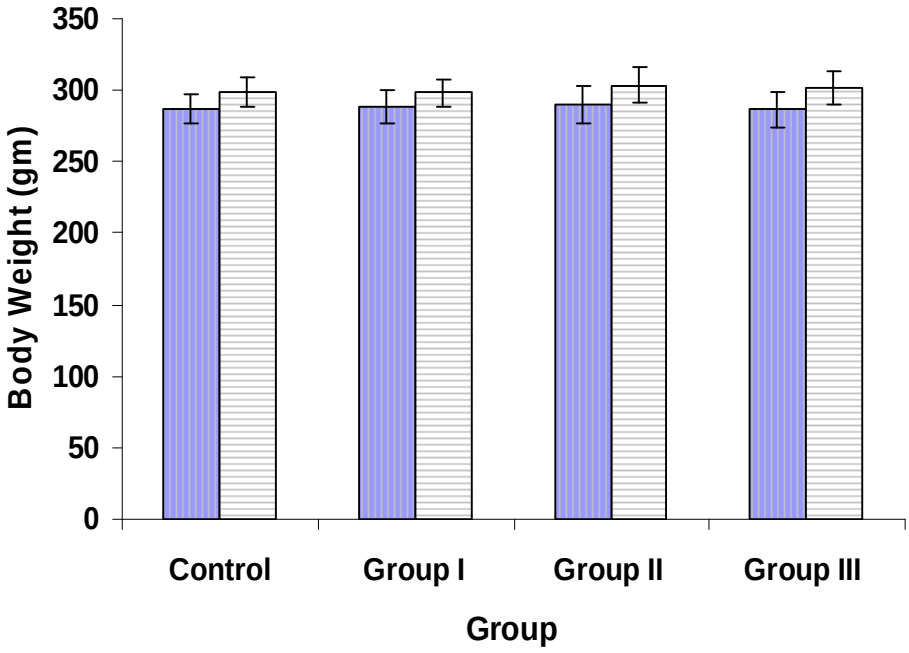

Figure 1: Weight change for male group: animal weights before treatment; (vertical lines) and 14 days after the treatment (horizontal lines). Data are represented as the mean weight of 5 rats ±SD.

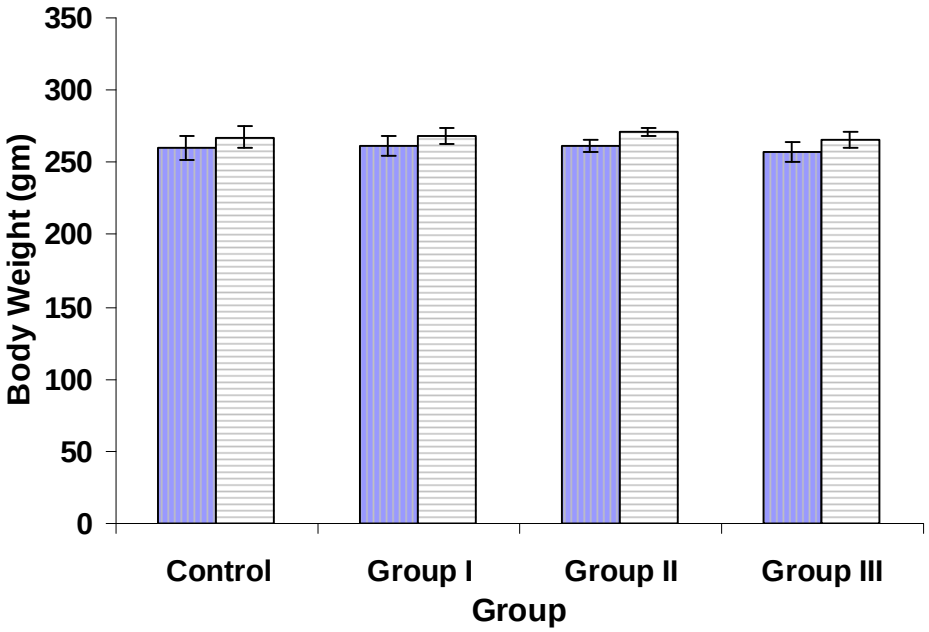

Figure 2: Weight change for Female group: animal weights before treatment; (vertical lines) and 14 days after the treatment (horizontal lines). Data are represented as the mean weight of 5 rats ±SD.

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 3, and Table 4). Similar results were observed regarding the weight gain in the livers of experimental animals (Table V and table VI).

Table 3: Weights of both kidneys for each female rat in control and treated groups. The last row shows the average weight of each group ± standard deviation. There was no statistical difference between treated groups and control group (p > 0.05).

Control	**Group I:**	**Group II:**	**Group III**
(gm)	(gm)	(gm)	(gm)
1.9	2.1	2.96	1.35
1.8	2.03	2.4	1.75
2.3	2.3	2.5	2.45
1.8	2.01	2.1	2.35
1.7	1.95	1.7	2.4
1.90 ± 0.23	**2.08 ± 0.14**	**2.33 ± 0.47**	**2.06 ± 0.49**

Table 4: Weights of both kidneys for each male rat in control and treated groups. The last row shows the average weight of each group ± standard deviation. There was no statistical difference between treated groups and control group ($p > 0.05$).

Control	Group I:	Group II:	Group III:
(gm)	(gm)	(gm)	(gm)
2.3	2.5	3.1	2.35
2.1	3.1	2.5	2.41
2.4	2.1	2.9	2.68
2.4	2.4	2.1	3.2
2.92	3.2	2.4	2.9
2.42 ± 0.30	**2.66 ± 0.47**	**2.60 ± 0.40**	**2.71 ± 0.35**

Table 5: Liver weight of each female rat for control and treated animals. The last row shows the average weight of each group ± standard deviation. There was no statistical difference between treated groups and control group ($p > 0.05$).

Control	Group I: 7X	Group II: 14X	Group III: 28X
(g)	(g)	(g)	(g)
12.5	10.2	13.5	10.5
9.5	10.6	11.2	8.2
10.2	12.9	9.5	13.2
11.5	11.7	8.9	12.5
12.1	11.2	10.5	9.7
11.16 ± 1.27	**11.32 ± 1.05**	**10.72 ± 1.8**	**10.82 ± 2.04**

Table 6: Liver weight of each male rat for control and treated animals. The last row shows the average weight of each group ± standard deviation. There was no statistical difference between treated groups and control group ($p > 0.05$)

Control	Group I: 7X	Group II: 14X	Group III: 28X
(g)	(g)	(g)	(g)
13.52	11.6	10.6	12.3
12.6	13.6	13.6	11.9
10.6	12.5	14.1	13.4
13.6	13.4	12.9	13.5
14.5	10.9	13.1	10.1
12.96 ± 1.48	**12.40 ± 1.16**	**12.86 ± 1.35**	**12.24 ± 1.38**

CONCLUSION

The acute toxicity study of Thyme – Primulae syrup was evaluated in male and female rats. In this study, there was no mortality during the study period. The LD50 of Thyme – Primulae syrup was found herein to be greater than 12 mL/kg when administered orally to fasted male

and female albino rats. Moreover, there were no remarkable body weight changes or gross anatomical findings during the study.

ACKNOWLEDGEMENTS

This project was sponsored by the Deanship of Academic Research at the University of Jordan, the national program Faculty For Factory, and SANA pharmaceutical research under supervision of Dr. Bustanji. The authors wish to thank sponsors for their generous funds.

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دراسة للسمية الحادة لشراب زهرة الربيع (بريميولا) والزعتر في الجسم الحي.

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ملخـص

تمت دراسة السمية الحادة لشراب زهرة الربيع (بريميولا) والزعتر في إناث و ذكور الجرذان. أعطيت الجرذان الجرعات:12,6,3 مل/كغم داخل المعدة لتقليل ضياع الجرعات و تفاوت الجرعات بين الجرذان. قورنت المجموعات التي أخذت الشراب مع مجموعة لم تأخذ الشراب من حيث زيادة الوزن وكل من مظهر و أوزان الكليتين والكبد. وجد الشراب الخاضع للفحص غير سام حتى عند أعلى جرعة أعطيت للجرذان ضمن ظروف الدراسة.

الكلمات الدالة: السمية الحادة، شراب زهرة الربيع (بريميولا) والزعتر، مستخلصات النباتات، النباتات الطبية، شارب مضاد للسعال.

تاريخ استلام البحث2010/8/16 وتاريخ قبوله للنشر 2010/10/13.