Research Journal of Medicinal Plant

ISSN 1819-3455

Academic Journals Inc.

www.academicjournals.com

Research Journal of Medicinal Plant 9 (7): 354-360, 2015
ISSN 1819-3455 / DOI: 10.3923/rjmp.2015.354.360

Acute, Sub-Acute and Cell Toxicity of Verbascoside

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ABSTRACT

Verbascoside or acteoside is the most abundant phenylethanoid glycoside that possesses health beneficial pharmacological activities, including anti-nociceptive, anti-inflammatory and anti-cancer. Due to the wide range of pharmacological activities of verbascoside and insufficient data on the safety profile, the acute, subacute and cellular toxicity of verbascoside was determined. The acute and subacute toxicity of verbascoside was evaluated in mice after single intraperitoneal injection at the dose range of 0, 1, 2 and 5 g kg^{-1} b.wt. (acute model) and 21 days administration at the dose range of 0, 10, 30 and 60 mg kg^{-1} b.wt. (subacute model). In MTT assay, HepG2 and NIH cells were exposed to different concentrations of verbascoside. According to result, the LD_{50} value of verbascoside was found to be greater than 5 g kg^{-1}. In subacute toxicity study, no statistically significant differences were observed in the values of hematological, biochemical and pathological parameters in comparison with control group. The cytotoxicity assay revealed that the viability in all groups were greater than the IC_{50} value. In conclusion, the results from the present study elucidate that verbascoside is well tolerated for both single and chronic administration and does not produce any toxic effects or deaths in animals.

Key words: Acute toxicity, sub-acute toxicity, cell toxicity, verbascoside

INTRODUCTION

Verbascoside or acteoside is a the most abundant phenylethanoid glycoside that was isolated from mullein in 1963 (*Verbascum sinuatum* L., Scrophulariaceae) for the first time (Scarpati and Monache, 1963). To date, verbascoside has also been detected in more than 200 plant species that classified within the family verbenaceae. Historically, plants with high concentrations of verbascoside have been used in folk remedy to treat inflammation and microbial infections (Georgiev *et al*., 2012; Quirantes-Pine *et al*., 2013). Most pharmacological properties of verbenaceae family plants have been attributed to the essential oil rich in phenolic compounds, namely phenyl

propanoids and glycosylated flavones (Moradi *et al*., 2014) . Among these constituents, verbascoside has attracted a good deal of attention. Verbascoside, a disaccharide ester, contains a rhamnose unit linked to glucose, where the glucose acts as a bridge (Pastorelli *et al*., 2012). It can be produced in both underground and above-ground organs but at widely different levels as well as *in vitro* plant culture systems, cost-effective technology (Kirmizibekmez *et al*., 2012; Lim and Bowles, 2012). Verbascoside has health beneficial pharmacological activities, including anti-nociceptive, anti-inflammatory and anti-cancer, cytotoxic and anti-metastatic properties in addition to neuroprotective properties (Akdemir *et al*., 2011; Alipieva *et al*., 2014). Recent studies have revealed a significant antioxidant effect of verbascoside in comparison with numerous natural antioxidants (Aleo *et al*., 2005; Dell'Aquila *et al*., 2 014). Verbascoside offers additional skin-protection capacity against harmful ultraviolet (UV) radiation and inflammatory insults (Potapovich *et al*., 2013).

Due to the wide range of pharmacological activities and biological of verbascoside and insufficient data on the safety profile, it may be needed for assessing toxicity profiles. In this work, the acute, subacute and cellular toxicity of main constituent of verbascoside was determined.

MATERIALS AND METHODS

Material and animals: Male BALB/c Mice(25-30 g) and male Wistar rats (200-250 g) obtained from Animal Center, School of Pharmacy, Mashhad University of Medical Sciences, were maintained in an environmentally controlled room (18-22°C) with a 12-h light/12-h dark cycle. Laboratory animal chow and tap water were provided *ad libitum*. Verbascoside was purchased from Xian Aladdin Biological Technology.

Acute toxicity: Total 24 mice randomly divided to 4 groups (10 per each group) and received single intraperitoneal injection of 0, 1, 2 and 5 g kg^{-1} b.wt. verbascoside. The same volume of normal saline was injected to control group. The general behavioral changes, signs of toxicity and mortality were monitored for 24 h after treatment.

Subacute toxicity: Subacute safety study was performed on 24 male Wistar rat divided in four groups (n = 6). The animals were injected verbascoside i.p. with 0, 10, 30 and 60 mg kg^{-1} b.wt. Body weights of the rats were recorded every week during the treatment period. Food and water consumption was also recorded on every day. All the animals were observed for mortality, behavioral and clinical signs. At the completion of the experiment on day 21, rats were anaesthetized by chloroform and blood samples were collected by cardiac puncture and immediately submitted for hematological and biochemical analysis.

Hematological and biochemical parameters: Heparinized samples were used for the estimation of hematological parameters such as red blood cell count, hemoglobin, hematocrit, platelet count and white blood cell count by automated hematology analyzer. Biochemical parameters such as level of ALP, LDH, total bilirubin, total cholesterol, triglyceride, albumin, urea and creatinine in serum were assayed using commercial colorimetric kits.

Histopathology: Autopsy was performed immediately after sacrifice and the tissues from vital organ liver, kidney, lung, brain, heart and spleen were fixed in 10% formalin and embedded in paraffin. At the end of process, specimens were sectioned at 6 μm thickness and stained with hematoxylin and eosin for histopathological study.

***In vitro* cytotoxicity study:** The *in vitro* cytotoxicity was determined in HepG2 cells (National Cell Bank, Pasteur Institute, Tehran, Iran) using MTT methods. Briefly, the cells were cultivated in 96 well plate and exposed to different concentrations of verbascoside (0, 100, 200 and 400 μM) incubated for 24 and 72 h. Then MTT solution was added to each well and left for 2 h in dark conditions. Subsequence, the purple formazan crystals were dissolved in DMSO. Finally, the optical density was read at a wavelength of 570 nm a plate reader (Bio-Tek, ELX 800, USA).

Statistical analysis: Data was expressed as Mean±SEM. All data was analyzed using analysis of variance (ANOVA) followed by Tukey-Kramer. Statistical significance was considered as p values less than 0.05 ($p<0.05$).

RESULTS

Acute toxicity: In the toxicity assay, single i.p. injection of verbascoside at 1, 2 and 5 g kg^{-1} b.wt. did not produce any deaths and adverse effects in mice. So, LD_{50} value of verbascoside was found to be greater than 5 g kg^{-1}. According to classification, chemical substance with a LD_{50} in the range of 1-5 g kg^{-1} is considered as practically low-toxic (Loomis and Hayes, 1996). Therefore, it seems verbascoside should be regarded as practically low toxic in acute i.p. treatment.

Subacute toxicity: In subacute study, no mortality and symptoms of adverse effects were recorded in rats treated at 10, 30 and 60 mg kg^{-1} i.p. during 21 days of treatment (Table 1). No significant differences in mean body weight food and water intake were observed between control and treated groups (data not shown).

Hematological and biochemical parameters: The hematological profile of experimental and control group were summarized in Table 2. Verbascoside at different doses could not alter significantly the hemoglobin, hematocrit, platelet count, white blood cell count and red blood cell count. No statistically significant differences were observed in the values of different biological parameters in comparison with control group and values obtained were within normal biological and laboratory limits (Table 3).

Histopathological study: The results did not reveal any significant changes in color or texture of all vital organs (kidney, spleen, heart, liver, lung and brain) when compared with control

Table 1: Effect of verbascoside on animal mortality and behavior

Dose (g kg^{-1} b.wt.)	Toxic symptoms	Mortality
0	None	None
1	None	None
2	None	None
5	None	None

Table 2: Effect of verbascoside on hematological parameters after 21 days treatment

		Verbascosides (mg kg^{-1} day^{-1})		
Parameters	Control	10	30	60
WBC ($\times10^3$ μL^{-1})	25.08±2.14	17.27±1.88	9.73±0.94	12.18±0.64
RBC ($\times10^6$ μL^{-1})	6.68±0.83	7.18±0.96	7.16±0.18	7.24±0.39
Hemoglobin (g dL^{-1})	12.71±1.24	13.77±1.43	13.11±0.92	13.36±1.73
Hematocrit (%)	37.00±3.59	39.80±1.14	38.23±3.54	39.15±1.84
Platelets ($\times10^3$ μL^{-1})	507.66±29.32	509.25±8.52	671.33±16.66	521.83±7.16

WBC: White blood cells, RBC: Red blood cells, values are expressed as Mean±SEM (n = 6)

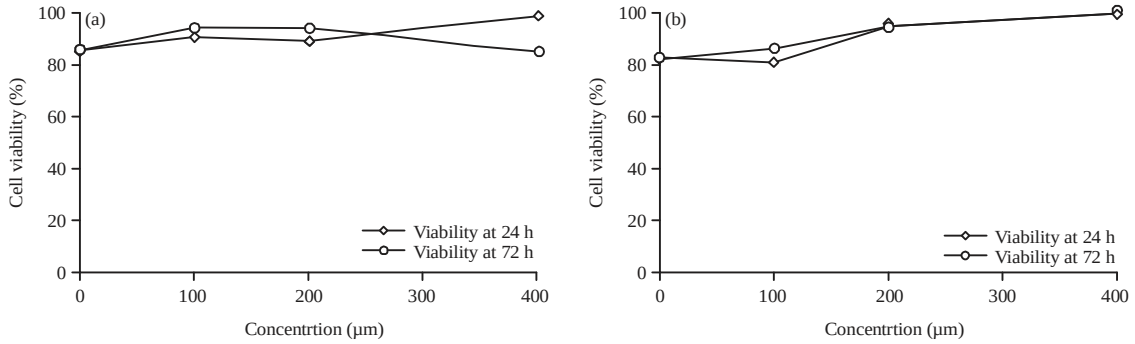

Fig. 1(a-b): Effect of verbascoside on (a) HepG2 and (b) NIH cell viability after 24 and 72 h exposure. Data is expressed as the Mean±SEM

Table 3: Effect of verbascoside on biochemical parameters after 21 days treatment

		Verbascosides (mg kg^{-1} day^{-1})		
Parameters	Control	10	30	60
Amylase (U L^{-1})	31.66±3.39	40.250±1.95	38.33±4.12	36.50±2.52
Lipase (U L^{-1})	36.16±3.48	38.500±2.91	42.33±2.17	41.50±1.73
CPK (IU L^{-1})	13801.67±1039	14887.500±845	12409.50±933	14720.17±1157
LDH (IU L^{-1})	1444.16±210	1405.000±91	1430.00±158	1903.33±103
Tg (mg dL^{-1})	96.33±12.4	115.000±17.08	76.83±8.91	74.16±9.58
Cholesterol (mg dL^{-1})	76.66±4.23	64.000±3.74	62.00±2.91	64.00±3.11
Creatinine (mg dL^{-1})	0.93±0.05	0.950±0.04	0.95±0.03	0.95±0.06
Urea (mg dL^{-1})	30.00±2.65	29.000±3.41	30.00±3.27	29.33±2.14
T. bilirubin (mg dL^{-1})	1.33±0.30	1.400±0.32	0.98±0.05	0.96±0.04
Albumin (g dL^{-1})	4.13±0.07	3.950±0.13	3.98±0.09	3.93±0.04
T. protein (g dL^{-1})	6.36±0.20	5.770±0.07	5.85±0.16	5.95±0.11
ALP IU L^{-1}	788.50±42.2	708.000±58.32	737.66±39	644.50±47.73

ALP: Alkaline phosphatase, LDH: Lactic acid dehydrogenase, Tg: Triglyceride, CRK: Creatinine phosphokinase, T: Total, values are expressed as Mean±SEM (n = 6)

animals. Congestion in spleen and fat vacuoles in liver specimens were observed in all groups. However, no differences were found between control and experimental groups.

Cell viability: Figure 1 clearly revealed that 24 and 72 h treatment with verbascoside had no cytotoxic effects at the concentrations up to 400 µM on HepG2 and NIH cell. In overall, the cytotoxicity assay revealed that the viability in all groups were greater than the IC_{50} value.

DISCUSSION

The use of plants as natural sources in folk medicine has expanded sharply over the last few decades, but this does not mean plants are all safe or side-effect free. Different studies revealed the potential risks involved with such plants. Verbascoside, the major constituents of *L. citriodora,* with wide potential effects also needs to be evaluated for possible toxic effects.

The results of our study revealed that the LD_{50}value of verbascoside i.p. administration in mice is more than 5 g kg^{-1} b.wt. and may be considered practically nontoxic. A single oral dose of 2000 mg kg^{-1} b.wt. of lemon verbena extract (25% verbascoside) also did not cause death and alterations in the body weight between control and treated groups (Funes *et al*., 2009). The evaluation of acute toxicity of *L. citriodora* aqueous extract (containing verbascoside) also confirmed our results and did not produce any death at 6.4 g kg^{-1} b.wt. (El-Hawary *et al*., 2012). In the present study, the body weight gain, food and water consumption and behavioral parameters

were not affected during 21 days experiment. However, giving feed supplemented to pig with verbascoside rich diet (10 mg kg^{-1}) for 56 days result in improved growth performance including final weight, the average daily gain and the gain/feed ratio during the experimental period (Pastorelli *et al*., 2012). Our study did not confirm these results, may be due to the short duration feeding. Similar observations such as no mortality and no symptoms of adverse health effects were found in case of subacute toxicity studies during 21 days.

The changes in the blood parameters in animals have been considered useful indicator for predicting human toxicity (Olson *et al*., 2000). In the present study, hematological parameters in treated groups showed no significant changes in comparison with control group which indicate that the verbascoside did not affect the blood cellular components or their production. None of the hematological parameters in verbascoside treated groups showed any significant changes in comparison with control group. No significant difference in ALP, total bilirubin, albumin, protein, cholesterol and triglyceride levels between control and verbascoside treated groups, indicates the normal function and synthetic capacity of liver. The normal values of other biochemical parameters such as urea and creatinine proposed that subacute administration of verbascoside did not induce any renal damage. No histological abnormalities also showed the absence of toxic effects on vital organ examined and supported the biochemical and hematological observations. Pastorelli *et al*. (2012) found that verbascoside rich diet can decrease low-density lipoprotein cholesterol by 17% in high verbascoside group and other biochemical parameters such as urea, glucose, triglycerides, total cholesterol and high-density lipoprotein cholesterol serum concentrations were not changed.

Daily administration of olive oil with low, medium and high phenolic contents to large number of participants increased the level of HDL cholesterol and decreased the total cholesterol/HDL ratio, cholesterol and triglyceride levels (Covas *et al*., 2006). Therefore *L. citriodora* extract or verbascoside may be useful in treatment of hyperlipidemia.

In HepG2 and NIH cells no significant differences were observed in cell viability assay between groups. Verbascoside at concentration of 500 μM produced 50% growth inhibition in U937 mononuclear cells. Although at lower concentrations from 10-250 μM, it did not affect cell viability (Pesce *et al*., 2015). Therefore, verbascoside exerts relatively low cytotoxic effects on these cell lines.

CONCLUSION

Treatment with single oral doses of 5000 mg kg^{-1} did not result in any toxic signs or mortality in the acute toxicity studies. Daily oral administration of verbascoside at dose up to 60 mg kg^{-1} for a period of 21 days did not cause mortality, changes in body weight and body weight gain. Also, no significant changes in hematological, biochemical and histopathological parameters were observed at the end of the duration of the experiment. Overall, it can be concluded that the verbascoside was well tolerated for both single and chronic administration and can be used with little caution. However, further investigations are required to evaluate long term toxicities.

ACKNOWLEDGMENT

The authors are thankful to the Vice Chancellor of Research, Mashhad University of Medical Sciences for financial support. The results described in this article are part of an MSc thesis.

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