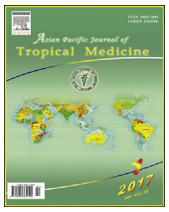
HOSTED BY
ELSEVIER

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage: http://ees.elsevier.com/apjtm



Original research http://dx.doi.org/10.1016/j.apjtm.2016.10.011

Evaluating the safety of forsythin from *Forsythia suspensa* leaves by acute and sub-chronic oral administration in rodent models

Zhong Han[1,2,3], Xia-Ling Lei[2,3], Hong Zhang[2,3], Lu Liu[2,3], Zhi-Sen Chen[2,3], Wei Yang[2,3✉], Zhao-Rong Lun[1✉]

[1]*School of Life Sciences, Sun Yat-sen University, Guangzhou 510275, China*

[2]*Guangdong Key Laboratory of Animal Conservation and Resource Utilization, Guangdong Public Laboratory of Wild Animal Conservation and Utilization, Guangdong Institute of Applied Biological Resources, China*

[3]*Guangdong Lewwin Pharmaceutical Research Institute Co., Ltd., Guangzhou 510990, China*

ARTICLE INFO

Article history:
Received 1 Oct 2016
Received in revised form 12 Oct 2016
Accepted 29 Oct 2016
Available online 28 Dec 2016

Keywords:
Forsythin
Acute toxicity
Sub-chronic toxicity
Safety

ABSTRACT

Objective: To access the toxicity of forsythin from *Forsythia suspensa* leaves and evaluate its safety.

Methods: Acute toxicity was determined by oral administration of a single dose of 18 100 mg/kg forsythin in NIH mice. Sub-chronic toxicity was evaluated by oral administration of several doses of forsythin for 30 days at does of 0, 540, 1 620, and 6 480 mg/kg in SD rats.

Results: In the acute toxicity study, mortality was not observed after 14 days. In addition, clinically relevant adverse effects, or variations in body weight or food consumption were not observed. Similarly, after 30 days in the sub-chronic toxicity study, no mortality or significant toxicological effects such as decreased food consumption, body weight, biochemical parameters and vital organs etc. were noticed.

Conclusion: The results revealed that the forsythin from *Forsythia suspensa* leaves has low or no toxicity via oral administration, and therefore is suitable for further development and applications.

1. Introduction

Forsythin belongs to a class of natural glycosidic lignan compounds, and was recorded in the China Pharmacopoeia [1], as one of the medicinal compounds found in *Forsythia suspense* (*F. suspense*) Lianqiao in Chinese [2]. Forsythin is a state first class new drugz in China [3].

Modern pharmacological studies have shown that forsythin may perform a number of biological functions such as antioxidant [4], anti-inflammatory [5], anti-hyperlipidemia [6,7], anti-virus [8] and antipyretic activities [9]. *F. suspense* fruits, as traditional Chinese medicine (TCM), have been often used to treat infectious diseases such as gonorrhea, erysipelas inflammation, pyrexia, ulcer, etc [10]. However, there are a number of factors that limit the wide use of these fruits. For instance, the period of fruit harvest, plant cultivation techniques and climatic conditions during plant growth (usually 7–9 months) are known to influence the properties of *F. suspense* fruits [11]. An alternative to the use of *F. suspense* fruits is the use of their leaves, which have been used in recent years [12]. Tea infusions prepared from.

F. suspense leaves have been commonly consumed as medicinal beverages in China for centuries due to their versatile health benefits such as modulating blood lipids, anti-fatigue, anti-senile and anti-influenza activities [13]. Studies have shown that *F. suspense* leaves have forsythin, the same components present in fruits, but in significantly higher amounts [2,14]. Besides, compared to the traditional methods of using *F. suspense* fruits, modern uses of *F. suspense* as a new drug increase the medicinal resources thus promoting the economic value of *F. suspense*.

First author: Zhong Han, School of Life Sciences, Sun Yat-sen University, Guangzhou 510275, China.

✉Corresponding authors: Wei Yang, Guangdong Lewwin Pharmaceutical Research Institute Co., Ltd., No. 18, Gaohu Road, Conghua Economic development zone High-tech industrial Park, Guangzhou, China.

Tel: +86 020 22698382.

E-mail: yangwei0719@163.com

Zhao-Rong Lun, School of Life Sciences, Sun Yat-sen University, No. 135, Xingang Xi Road, Guangzhou 510275, China.

Tel: +86 020 84115079

E-mail:lsslzr@mail.sysu.edu.cn

Peer review under responsibility of Hainan Medical University.

Foundation project: This work was supported by the Basic Science Research Program of Guangdong Province Science and Technology Plan Projects Fund [grant numbers: 2016A010119136] and the High-level Leading Talent Introduction Program of GDAS [grant numbers: 2016GDASRC-0104].

Although pharmacological studies have been performed using forsythin and *F. suspense*, which is used widely in China, little is known about its toxicity. Therefore, it is necessary to evaluate its safety using standardized experimental protocols approved by the regulatory agencies. In this study, the safety of orally administered forsythin was assayed by performing an acute toxicity experiment in NIH mice and a sub-chronic toxicity experiment in SD rats. The results of this study will be an important reference to better understand the biosafety of forsythin for further development and applications.

2. Materials and methods

2.1. Test substance

Forsythin (batch No. 20141202) was provided by Dalian Fusheng Pharmaceutical Co., Ltd. The purity of forsythin was >90.2% determined by HPLC using an external standard.

2.2. Animals

NIH mice (18–22 g) and SD rats (150–200 g) were purchased from Slack Hunan Laboratory Animal Co., Ltd. (Hunan, China; license number SCXK (XIANG) 2011-0003). All animals were certified pathogen-free, and were maintained in plastic cages at 20–26 °C, 40–70% relative humidity, 12 h light/dark cycles, and an air change of 10–20 cycles/h. Animals were provided an unlimited supply of food and water. All experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of Guangzhou General Pharmaceutical Research Institute Co., Ltd. Experiments also followed the regulations of the American Association for Accreditation of Laboratory Animal Care (AAALAC) Guide for the Care and Use of Laboratory Animals (Eighth Edition) [15].

2.3. Acute toxicity study in NIH mice

The acute oral toxicity of forsythin was evaluated in NIH mice using the maximum-tolerated dose (MTD) method. Forty mice were separated into two groups: normal control group and forsythin group with 10 males and 10 females in each group. Normal control group were orally administered with 0.5% CMC-Na (40 mL/kg). The mice of forsythin group were orally administered with forsythin at a single dose of 18 100 g/kg after 3-day quarantine, acclimatization, and an overnight fast. The mice were monitored 4 h after treatment and every 24 h for 14 days. At the end of the experiment, all animals were sacrificed, and gross pathological changes in vital organs (liver, kidneys, brain, heart, lung, spleen, thymus, ovaries, and testes) were observed. Histopathological examinations were performed when any gross pathological changes were detected in any organ [16–19].

2.4. Sub-chronic toxicity study in SD rats

SD rats were randomly separated into 4 groups: normal control group, low, medium and high dosage of forsythin groups. There were 26 rats in each group (13 males and 13 females, 8 animals/group/sex were killed at the end of 30th day of administration, five animals/group/sex were killed at the end of recovery period). Normal rats were orally administered with 0.5% CMC-Na (15 mL/kg). Rats of three dosage of forsythin groups were orally administered with 540, 1 620, and 6 480 mg/kg forsythin, once a day for consecutive 30 days. Clinical examinations were observed daily. Body weights and mean daily food consumption were recorded once in 2 days. In the following days, these parameters were recorded only once a week. After 30 days treatment and recovery period, all animals were sedated using propofol injection (11 mg/kg body weight, intravenous injection, Guangdong Jiabo Pharmaceutical Co., Ltd.) and then dissected out to obtain blood samples from the abdominal aorta. The blood samples transferred into EDTA or heparin sodium containing tubes for haematological and biochemical analyses, and were centrifuged at 3 500 r/min for 15 min. For haematological analyses, the full blood cell count were determined [i.e. erythrocyte count (RBC), hemoglobin concentration (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cell count (WBC) and differential, platelet count (PLT), prothrombin time (PT), fibrinogen (Fbg), and activated partial thromboplastin time (APTT)], using an automated analyzer (Sysmex, Japan). Serum biochemistry tests were performed such as: aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), creatine kinase (CK), alkaline phosphatase (ALP), urea nitrogen (UREA), total serum protein (TP), albumin (ALB), blood glucose (GLU), total bilirubin (TBIL), creatinine (Crea), triglycerides (TG), sodium (Na), potassium (K), and chloride (Cl), using an automated analyzer (Hitachi, Japan).

In addition, the vital organs such as brain, heart, liver, spleen, lungs, kidneys, adrenal glands, thymus, testis, epididymides, prostates, ovaries, and uterus were collected, weighed and histopathologic examinationed individually. Finally, the organ-to-final-body-weight ratios were calculated [20–23].

2.5. Statistical analysis

The statistical significance of the differences among the treatments and control was verified using one-way analysis of variance (ANOVA) and Kruskal–Wallis test. Significance level was set at $\alpha = 0.05$ (two sided) [24,25].

3. Results

3.1. Acute toxicity test

Mortality was not detected in animals despite those receiving a high dose of forsythin (18 100 mg/kg). The forsythin administered group was similar to the control in skin appearance, breathing, postural properties, body weight, and food consumption. Although some animals showed adverse symptoms such as reduced activity, eye closure and piloerection within 1 h after forsythin administration, these were short-lived and the animals recovered quickly. In addition, the vital organs including the liver, kidney, brain, heart, lung, spleen and thymus did not exhibit any significant pathological changes in the colors and textures.

3.2. Sub-chronic toxicity test

During the study, two of male animals in the high dose group, experienced transient loose stools on treatment days 3

and 16. These animals recovered the next day and were isolated cases.

3.2.1. Body weight changes and food consumption

Compared to the controls, the forsythin treated females showed no differences in body weights. In contrast, the males showed a different trend; their body weights were significantly lower than the control on the 11th day and from week 2–4 post-forsythin administration. However, the food consumption showed a different trend with both females and males treated with 6 480 mg/kg forsythin consuming significantly lesser in week 5 (recovery phase) than the control. But the males consumed significantly higher than the control in week 3 (data not shown).

3.2.2. Hematology and serum biochemistry

Only one hematological parameter was significantly different between the control and forsythin treated rats; MCH was higher in rats that received 1 620 mg/kg forsythin (Table 1). However, the other hematological parameters analyzed did not show any significant change even 30 days post-treatment (data not shown). On the other hand, biochemical analysis of sera showed that urea was significantly lower in females that received a high dose of forsythin compared to the control (Table 2). The TBIL levels were significantly higher in males that received higher doses of forsythin. In both males and females, the levels of Na^+ and Cl^- ions were significantly higher only in the high dose groups whereas K^+ level was significantly higher in all three groups treated with forsythin when compared to the control. All other parameters analyzed remained the same between the control and forsythin treated groups even 30 days post-treatment (data not shown).

3.2.3. Organ/body weight ratios of the rats

As shown in Table 3, the relative organ weights (ROWs) of some groups treated with forsythin were significantly higher than the control group. For example, ROWs were significantly elevated in females that received higher doses of forsythin (1 620 and 6 480 mg/kg), and in the males that were treated with 1 620 mg/kg forsythin. No significant changes were observed among the ROWs of other organs even 30 days post-treatment.

3.3. Histopathological analyses of vital organs

Histopathological examinations revealed mild adipose degeneration only in the liver cells of one rat that received high doses of forsythin (Figure 1). On the other hand, forsythin treatment did not induce lesions and pathological changes in the brain, heart, spleen, lungs, kidneys, uterus, testis and so on (photograph not shown).

Table 1

Hematological values of rats treated orally with forsythin for 30 days.

Mean corpuscular hemoglobin (pg)	Control	Dosage (mg/kg/day)		
		540	1 620	6 480
Female	18.5 ± 0.3	18.6 ± 0.7	19.1 ± 0.4*	18.6 ± 0.2
Male	19.2 ± 1.0	19.4 ± 0.2	18.9 ± 0.3	19.0 ± 0.5

Values are means ± SD deviation (16 rats in each group). Compared with control group.
*$P < 0.05$, **$P < 0.01$.

Table 2

Serum biochemistry of rats treated orally with forsythin for the 30 days (mg/kg/day).

Parameters	Control		540		1 620		6 480	
	Female	Male	Female	Male	Female	Male	Female	Male
Total bilirubin (μmol/L)	2.00 ± 0.20	1.70 ± 0.20	1.90 ± 0.30	1.90 ± 0.20	2.00 ± 0.30	1.80 ± 0.20	2.10 ± 0.20	2.10 ± 0.10**
Urea(mol/L)	5.50 ± 0.68	6.31 ± 0.92	5.65 ± 1.36	6.84 ± 1.15	5.90 ± 0.95	6.26 ± 1.16	4.37 ± 0.59**	7.34 ± 1.41
Na^+ (mmol/L)	141.80 ± 2.30	141.9 ± 0.8	142.00 ± 1.70	142.80 ± 1.40	141.80 ± 2.20	142.10 ± 1.40	149.80 ± 1.80*	144.50 ± 1.50**
K^+ (mmol/L)	4.42 ± 0.38	4.37 ± 0.15	4.59 ± 0.53	4.72 ± 0.15**	4.81 ± 0.57	4.77 ± 0.30**	5.04 ± 0.58*	4.78 ± 0.30**
Cl^- (mmol/L)	106.40 ± 2.20	105.6 ± 0.7	105.80 ± 1.90	105.50 ± 1.70	106.00 ± 2.20	105.60 ± 2.10	114.60 ± 1.80*	108.80 ± 1.00**

Values are means ± SD deviation (16 rats in each group). Compared with control group, *$P < 0.05$, **$P < 0.01$.

Table 3

Organ/body weight ratios of rats treated orally with forsythin for 30 days.

Organ	Control		540		1 620		6 480	
	Female	Male	Female	Male	Female	Male	Female	Male
Brain	0.806 ± 0.094	0.476 ± 0.023	0.819 ± 0.067	0.423 ± 0.044	0.787 ± 0.031	0.446 ± 0.047	0.757 ± 0.048	0.496 ± 0.023
Heart	0.316 ± 0.021	0.295 ± 0.018	0.306 ± 0.023	0.303 ± 0.025	0.309 ± 0.015	0.306 ± 0.012	0.310 ± 0.011	0.299 ± 0.018
Liver	2.735 ± 0.153	2.664 ± 0.131	2.824 ± 0.141	2.746 ± 0.095	2.906 ± 0.136*	2.966 ± 0.147**	2.970 ± 0.124**	2.788 ± 0.153
Spleen	0.188 ± 0.026	0.180 ± 0.025	0.186 ± 0.021	0.176 ± 0.015	0.196 ± 0.023	0.184 ± 0.017	0.188 ± 0.016	0.186 ± 0.025
Lungs	0.467 ± 0.065	0.322 ± 0.040	0.442 ± 0.027	0.332 ± 0.027	0.433 ± 0.052	0.343 ± 0.025	0.441 ± 0.044	0.333 ± 0.050
Kidneys	0.170 ± 0.030	0.637 ± 0.021	0.161 ± 0.028	0.658 ± 0.031	0.183 ± 0.043	0.639 ± 0.019	0.176 ± 0.035	0.647 ± 0.024
Ovaries	0.035 ± 0.006	0.807 ± 0.067	0.034 ± 0.005	0.863 ± 0.060	0.031 ± 0.006	0.856 ± 0.071	0.032 ± 0.006	0.875 ± 0.089
Uterus	0.206 ± 0.048	0.476 ± 0.023	0.214 ± 0.049	0.423 ± 0.044	0.235 ± 0.057	0.446 ± 0.047	0.198 ± 0.058	0.496 ± 0.023

Values are means ± SD deviation (16 rats in each group). Compared with control group, *$P < 0.05$, **$P < 0.01$.

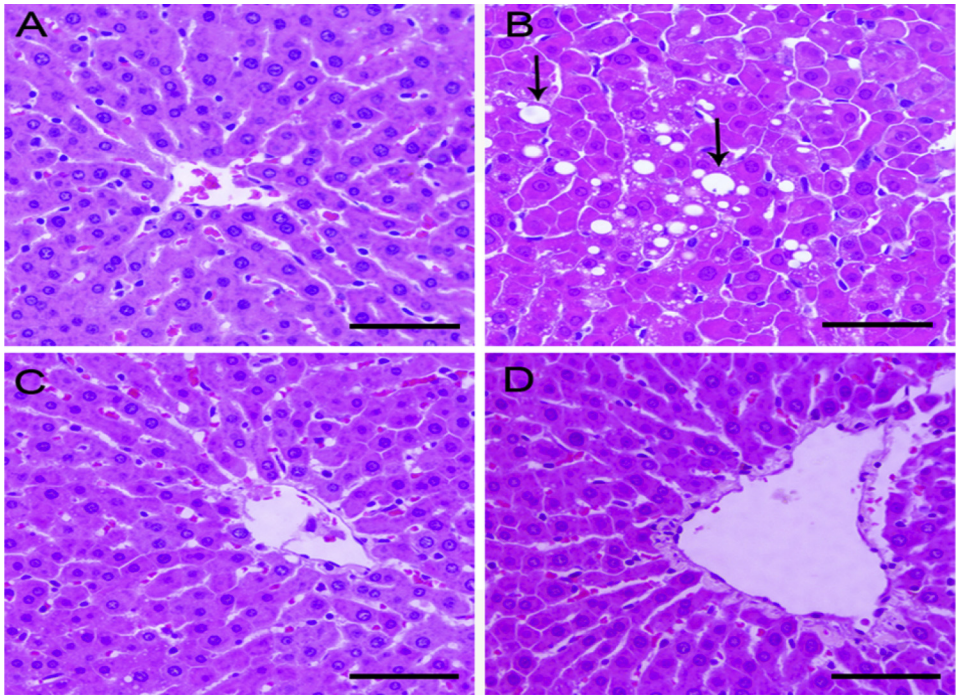


Figure 1. H&E staining of the liver (×400, Scale bar: 50 μm) from rats used in the sub-chronic toxicity test. The different treatments of animals are shown in (A) Control; (B) 6 480 mg/kg; (C) 1 620 mg/kg; (D) 540 mg/kg. Liver cells adipose degeneration were marked with arrows.

4. Discussion

Forsythin is one of the compounds extracted from *F. suspensa* that has been used as TCM preparations such as Shuanghuanglian Granules and Yinqiao Powders, and also as a health-tea in China [26]. Therefore, studies on the safety of forsythin from *F. suspensa* leaves for human consumption are critical to promote drug development.

Consistent with the results of pre-tests where animal mortality was not observed at the maximum intragastric dose, in the single-dose study, we found that the maximum forsythin dose at 18 100 mg/kg by a single intragastric administration reduced the activity level in NIH mice immediately after administration but all mice recovered within 1 h. Based on these results, the maximum tolerated dose (MTD) for a single intragastric administration of forsythin is presumed to be greater than 18 100 mg/kg in NIH mice. According to the GHS and Gosselin scale [27,28], forsythin could be assessed as relatively non-toxic compound.

In the repeated-dose toxicity study, neither death nor marked toxicities were observed in rats in any of the treatment group. In general observations, male animals in the high dose group experienced transient loose stools on treatment days 3 and 16, which were only isolated cases and all animals recovered the next day. No other abnormalities were observed indicating no significant pathological conditions. Generally, body weight changes and food consumption are commonly monitored indicators of toxicity after giving to drugs and chemicals [29]. In this study, we observed decreases in the body weight in the high dose male group, but become normal in recovery phase. The anti-obesity activity of forsythin may be considered as a possible explanation of these alterations in body weight [30]. However, the food consumption changes were not associated with dose and should be considered toxicologically irrelevant effects.

Hematological indicators are important to determine drug toxicity [31]. During the treatment period, increases were observed in MCH in the medium dose group indicating enhancement in medullary hematopoiesis (erythroid hyperplasia) in the animals. However, no abnormality was observed in the hematological indicators or bone pathology indicating the absence of pathological events.

Serum biochemistry is an indication of drug toxicity in the liver and kidney [14]. In this study, we observed increases in total bilirubin (TBil) in the high dose group although this was within the normal range [32], and no abnormality was observed in histopathological examinations. In addition, in all group treated with low, medium and high dose, forsythin also increased the potassium levels in blood; and increased chlorine in the high dose group. However, all these changes were within the normal range and therefore did not induce significant toxicological changes [32].

Results of the organ weight and visceral coefficient in rats showed that in the late treatment period, liver enlargement was observed in the high dose group, but this change was within the normal reference laboratory range. No abnormality was observed in liver histopathology or liver function, and all changes disappeared following treatment withdrawal. These changes were hence considered as compensatory changes without pathological significance.

Histopathological results showed slight changes in adipose degeneration in the liver cells of one experimental animals without presenting significant pattern across groups or positive correlation with forsythin dose. Therefore, it was assessed as spontaneous changes taking into account the background pathological data in animals. Together, these results reveal no pathological changes or delayed toxicities related to the oral administration of forsythin at the tested doses in the experimental animals.

In conclusion, the results of acute toxicity tests in mice revealed that the maximum-tolerated dose (MTD) of forsythin was greater than 18 100 mg/kg. In the sub-chronic toxicity tests in rats, the no-observed-adverse-effect-level (NOAEL) was observed after 30 days at the concentration of 6 480 mg/kg

bodyweight. Take together, these results reveal that the use of forsythin is safe, and therefore is suitable for further development and applications.

Conflict of interest statement

The authors report no conflict of interest.

Acknowledgements

This work was supported by the Basic Science Research Program of Guangdong Province Science and Technology Plan Projects Fund [grant numbers: 2016A010119136] and the High-level Leading Talent Introduction Program of GDAS [grant numbers: 2016GDASRC-0104].

References

[1] National Commission of Chinese Pharmacopoeia. *Pharmacopoeia of peoples Republic of China*. Beijing: Chemical Industry Press; 2010.
[2] Piao X, Jang MH, Cui J, Piao X. Lignans from the fruits of *Forsythia suspensa*. *Bioorg Med Chem Lett* 2008; **18**(6): 1980-1984.
[3] Provisions for Drug Registration (CFDA Order No. 28). The state food and drug administration of China. 2007. [in Chinese]. [Online] Available from: http://www.sda.gov.cn/WS01/CL0053/24529.html [Accessed on 17th August, 2016]
[4] Pan X, Cao X, Li N, Xu Y, Wu Q, Bai J, et al. Forsythin inhibits lipopolysaccharide-induced inflammation by suppressing JAK-STAT and p38 MAPK signalings and ROS production. *Inflamm Res* 2014; **63**(7): 597-608.
[5] Zhong W, Wu Y, Xie X, Zhou X, Wei MM, Soromou LW, et al. Phillyrin attenuates LPS-induced pulmonary inflammation via suppression of MAPK and NF-κB activation in acute lung injury mice. *Fitoterapia* 2013; **90**: 132-139.
[6] Kang HS, Lee JY, Kim CJ. Anti-inflammatory activity of arctigenin from *Forsythiae* Fructus. *J Ethnopharmacol* 2008; **116**(2): 305-312.
[7] Li Y, Jiang X, Huang L, Lu J, Zhou J. Determination of phillyrin in rat plasma by high-performance liquid chromatography and its application to pharmacokinetic studies. *J Pharm Biomed* 2006; **41**(2): 628-632.
[8] Qu X, Li Q, Zhang H, Zhang XJ, Shi PH, Zhang XJ, et al. Protective effects of phillyrin against influenza A virus in vivo. *Arch Pharm Res* 2016; **39**(7): 998-1005.
[9] Zhang Q, Wei X, Wang J. Phillyrin produced by *Colletotrichum gloeosporioides*, an endophytic fungus isolated from *Forsythia suspensa*. *Fitoterapia* 2012; **83**(8): 1500-1505.
[10] Li H, Chen F. Preparative isolation and purification of phillyrin from the medicinal plant *Forsythia suspensa* by high-speed counter-current chromatography. *J Chromatogr A* 2005; **1083**(1–2): 102-105.
[11] Bai Y, Li J, Liu W, Xiu-cheng Jiao, Jun He, Jiao Liu, et al. Pharmacokinetic of 5 components after oral administration of Fructus Forsythiae by HPLC-MS/MS and the effects of harvest time and administration times. *J Chromatogr B* 2015; **993–994**: 36-46.
[12] Jiao J, Gai Q, Luo M, Wei Wang, Cheng-Bo Gu, Chun-Jian Zhao, et al. Comparison of main bioactive compounds in tea infusions with different seasonal *Forsythia suspensa* leaves by liquid chromatography–tandem mass spectrometry and evaluation of antioxidant activity. *Food Res Int* 2013; **53**(2): 857-863.
[13] Qu H, Li B, Li X, Tu G, Lü J, Sun W. Qualitative and quantitative analyses of three bioactive compounds in different parts of *Forsythia suspensa* by high-performance liquid chromatography-electrospray ionization-mass spectrometry. *Microchem J* 2008; **89**(2): 159-164.
[14] Mukinda JT, Eagles PFK. Acute and sub-chronic oral toxicity profiles of the aqueous extract of *Polygala fruticosa* in female mice and rats. *J Ethnopharmacol* 2010; **128**(1): 236-240.
[15] American Association for Accreditation of Laboratory Animal Care. Guide for the care and use of laboratory animals. 2011. [Online] Available from: http://www.aaalac.org/resources/Guide_2011.pdf [Accessed on 17th August, 2016]
[16] Li P, Wang S, Guan X, Liub Bin, Wanga Yonggang, Xu Ke, et al. Acute and 13weeks subchronic toxicological evaluation of naringin in Sprague-Dawley rats. *Food Chem Toxicol* 2013; **60**: 1-9.
[17] China Food and Drug Administration CFDA. Technical guideline for single dose acute toxicity testing. 2014. [Online] Available from: http://www.cde.org.cn/zdyz.do?method=largePage&id=189 [Accessed on 17th August, 2016]
[18] US FDA. Guidance for industry: single dose acute toxicity testing for pharmaceuticals (Final). 1996. [Online] Available from: http://101.110.118.23/www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm079270.pdf [Accessed on 17th August, 2016]
[19] Organization for Economic Co-operation and Development OECD. Acute oral toxicity: acute toxic class method, OECD Guideline No. 423. 2001. [Online] Available from: http://www.oecd-ilibrary.org/docserver/download/9742301e.pdf [Accessed on 17th August, 2016]
[20] Xu K, Lin Y, Zhang R, Lan M, Chen C, Li S, et al. Evaluation of safety of iridoids rich fraction from *Valeriana jatamansi* Jones: acute and sub-chronic toxicity study in mice and rats. *J Ethnopharmacol* 2015; **172**: 386-394.
[21] China Food and Drug Administration CFDA. Technical guideline for repeated dose toxicity testing. 2014. [Online] Available from: http://www.cde.org.cn/zdyz.do?method=largePage&id=192 [Accessed on 17th August, 2016]
[22] US FDA. Redbook Chapter IV.C.4. A sub-chronic toxicity studies with rodents. 2007. [Online] Available from: http://www.fda.gov/downloads/Food/GuidanceRegulation/UCM222779.pdf [Accessed on 17th August, 2016]
[23] Organization for Economic Co-operation and Development OECD. Repeated dose 28-day oral toxicity study in rodents, OECD Guideline No. 407. 2008. [Online] Available from: http://www.oecd-ilibrary.org/docserver/download/9740701e.pdf?expires=1483431261&id=id&accname=guest&checksum=C759FE2D0F5636B0FAF53AAD451BEDA2 [Accessed on 17th August, 2016]
[24] Yuan G, Dai S, Yin Z, Lu H, Jia R, Xu J, et al. Toxicological assessment of combined lead and cadmium: acute and sub-chronic toxicity study in rats. *Food Chem Toxicol* 2014; **65**: 260-268.
[25] Koumba Madingou NO, Traore A, Souza A, Mounanga Marlaine Michelle Boukandou, Reine Raissa, Samseny Aworet, et al. Preliminary studies of acute and sub-chronic toxicity of the aqueous extract of *Guibourtia tessmannii* (Harms) J. Leonard stem barks (Caesalpiniaceae) in mice and rats. *Asian Pac J Trop Biomed* 2016; **6**(6): 506-510.
[26] Lin Z, Lin Z, Mu Y, Yan D. Comparison of collision-induced dissociation and electron-induced dissociation of phillyrin using FT-ICR MS. *Spectrochim Acta A Mol Biomol Spectrosc* 2016; **167**: 84-88.
[27] Gosselin RE, Smith RP, Hodge HC. *Clinical toxicology of commercial products*. Baltimore, London: Williams and Wilkins; 1984.
[28] Scott TS. Clinical toxicology of commercial products. *Br J Ind Med* 1970; **27**(3): 295-296.
[29] Teo S, Stirling D, Thomas S, Hoberman A, Kiorpes A, Khetani V. A 90-day oral gavage toxicity study of D-methylphenidate and D,L-methylphenidate in Sprague-Dawley rats. *Toxicology* 2002; **179**(3): 183-196.
[30] Do MT, Kim HG, Choi JH, Khanal T, Park BH, Tran TP, et al. Phillyrin attenuates high glucose-induced lipid accumulation in human HepG2 hepatocytes through the activation of LKB1/AMP-activated protein kinase-dependent signalling. *Food Chem* 2013; **136**(2): 415-425.
[31] Wang X, Zhang W, Wang Y, Peng D, Ihsan A, Huang X, et al. Acute and sub-chronic oral toxicological evaluations of quinocetone in Wistar rats. *Regul Toxicol Pharm* 2010; **58**(3): 421-427.
[32] Petterino C, Argentino-Storino A. Clinical chemistry and haematology historical data in control Sprague-Dawley rats from pre-clinical toxicity studies. *Exp Toxicol Pathol* 2006; **57**(3): 213-219.