Journal of Medicinal Plants Research Vol. 6(14), pp. 2857-2863, 16 April, 2012
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR011.1476
ISSN 1996-0875 ©2012 Academic Journals

Full Length Research Paper

Toxicological evaluation of the petroleum ether extract of *Leonurus japonicus* on liver, kidney and testis in rats

Yi Luo[1], Ben-Hong Zhou[1], Gang Liu[1], Hong-Yan Liu[1] and Xian-Ming Hu[2*]

[1]Department of Pharmacy, Renmin Hospital of Wuhan University, Wuhan 430060, PR China.
[2]College of Pharmacy, Wuhan University, Wuhan 430072, PR China.

Accepted 1 February, 2012

The sub-acute toxicity of the petroleum ether extract of *Leonurus japonicus* (PELJ) in rats was investigated, and as a comparison, that of the boiled decoction of *L. japonicus* (DLJ) was also investigated. In rats which received a once-daily administration of PELJ and DLJ at the dose of 60 g/kg by intragastric gavage for 15 days, there was a significant decrease in the body weight gain and an increase in the relative weight of testis and levels of serum blood urea nitrogen (BUN) as well as in the amount of creatinine, glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), alkaline phosphatase (ALP), lactic dehydrogenase (LDH) and γ-glutamyltransferase (GGT), urinary protein concentration and urinary NAG. Also, histopathological changes of liver, kidney and testis were observed. The altered levels of body weight, relative organ weight and the biochemical parameters except for urinary protein concentration tended to be restored, and the histopathological changes of liver, kidney and testis were ameliorated in rats that had been withdrawn from the treatment for another 15 days recovery period. PELJ and DLJ exhibited similar toxic effects and the toxic effects of DLJ were lower than those of PELJ. The results demonstrated that PELJ and DLJ at the given dose produced apparent toxicity in rats and that permitting rats to recover for 15 days resulted in partial recovery. Besides, the compositions of PELJ were analyzed by GC–MS. The major components identified in PELJ were 17b-hydroxy-1a, 17-dimethyl-5a-androstan-3-one (21.47%), stigmast-5-en-3-ol (11.86%), E-phytol (9.94%), and caryophyllene oxide (8.69%).

Key words: *Leonurus japonicus*, petroleum ether extract, GC-MS, toxicity, liver, kidney, testis.

INTRODUCTION

Aerial part of *Leonurus japonicus* Houtt (syn *L. heterophyllus* Sweet), a traditional Chinese herbal medicine known as “Yi-Mu-Cao”, has been used widely for several thousand years in China. By invigorating blood circulation, regulating menstrual disturbance and relieving edema, *L. japonicus* is usually used for blood stasis syndrome with menoxenia, dysmenorrhea, metrorrhagia, postpartum lochiorrhea, coronary heart disease, cerebral infarction, blood hyperviscosity, myocardial ischemia etc. and for the treatment of various kinds of nephritis with edema (Pharmacopoeia Committee, 2005; Yan and Chen, 2000; Xu et al., 2002; Zou et al., 1989; Wang et al., 1988; Tao et al., 2003; Yin et al., 2010). Besides, *in vitro* anticancer activities of *L. japonicus* have also been shown (Chinwala et al., 2003). In practice, some herbalists often use *L. japonicus* at doses 30 to 60 g up to 120 g per day for hypertension, acute nephrotic edema and central retinochoroiditis (Bian and Lv, 1998). In recent years, a limited number of studies have provided evidence for the nephrotoxicity of *L. japonicus* at high dose (Cai et al., 2000; Sun et al., 2005), and some traditional Chinese physicians recommend that administration of *L. japonicus* should be contraindicated for those patients once suffered kidney injury. So the clinical application of *L. japonicus* is restricted unavoidably. Nevertheless, to the best of our knowledge, the toxicity of *L. japonicus* has not been studied intensively and systematically so far, and little research on the toxic effects of various fractions of ethanol extract of *L. japonicus*, prepared by using solvent

*Corresponding author. E-mail: xmh_yi@yahoo.cn.

of varying polarity, has been reported. Therefore, the present study was designed to evaluate the subacute toxicity of petroleum ether extract fraction of *L. japonicus* (PELJ) on Sprague Dawley rats and the reversibility of its toxic effects, and to identify the target organs. Meanwhile, the toxicity of the boiled decoction of *L. japonicus* (DLJ) was also been investigated as a comparison, and the chemical composition of PELJ was analyzed by GC-MS.

MATERIALS AND METHODS

Plant material

Aerial part of *L. japonicus* was collected from Enshi, Hubei Province, in late May 2005. The plant was identified to conform with the Chinese Pharmacopoeia criterion by Prof. H. Zhang, Wuhan University, China. A voucher specimen was deposited in the herbarium of the College of Pharmacy, Wuhan University, China. The plant material was then dried in open air and kept for use.

Preparation of the extract

PELJ

The dried plant material was ground to pass 20 mesh sieves before extraction. The powder was refluxed with 95% ethanol twice, each for 1.5 h. The filtrate was evaporated at low pressure to obtain nearly dried extract. The extract was suspended in distilled water and then was partitioned with petroleum ether (60 to 90°C). PELJ, a brown yellowish viscous paste, was obtained after removal of the solvent *in vacuo* (yield: 1.21%, w/w). PELJ was dispersed in distilled water and diluted to make up the concentration equivalent to original herb 4 g/ml for testing, and stored at 4°C until used. The mixture was subjected to ultrasonic waves for 15 min in order to disperse uniformly before administration. Based on the yield of PELJ, 12.1 mg PELJ was equal to 1 g original herb.

DLJ

The dried plant material was soaked in water for 30 min, and then extracted by water boiling method for 2 times, each for 1.5 h. The filtrate was combined and then concentrated to make up the concentration equivalent to original herb 4 g/ml, and was stored at 4°C until used.

GC-MS analysis of PELJ

The GC-MS analysis of PELJ was performed using a Thermo-Finngan trace GC-MS equipped with a DB-5 fused silica capillary column (30 m × 0.25 mm i.d.; coating thickness 0.25 μm). The GC oven temperature was programmed to increase from 60°C, held 1 min, to 290°C at a rate of 6°C /min, and finally held at 290°C for 10 min. Total run time was 63 min. The injector temperature was set at 250°C; and the carrier gas was helium at a flow rate of 1.0 ml/min; 1:40 split ratio. The MS operating parameters were as follows: Ionization energy, 70 eV; scan mode, EI; ion source temperature, 250°C; scanning mass range, 40 to 650 atomic mass units.

Qualitative identification of the components was performed by comparison of their mass spectra with those stored in the database of NIST/WILEY of GC-MS system and those reported previously.

Percentages of the components were calculated from the GC peak areas using the normalization method.

Animals

Male adult Sprague Dawley (SD) rats weighing 120 to 150 g were obtained from the Laboratory Animal Center, Tongji Medical College of Huazhong University of Science and Technology after the approval for the study. All animals were housed in the animal facility of the experimental center of the Renmin Hospital of Wuhan University with a 12 h dark/light cycle; the temperature was kept at 22±1°C with 60±5% relative humidity. The animals were allowed to have free access to food and clean water and were acclimatized for a week prior to experimentation. This study was performed based on the guidelines for the care and use of animals established by Wuhan University. The experimental protocol of the study was approved by the Ethics Committee, Wuhan University.

Subacute toxicity study

The animals were randomly divided into three groups of 12 animals respectively. Two served as experimental group, namely PELJ group and DLJ group, in which rats received PELJ, DLJ by intragastric gavage daily at a dose of 60 g/kg body weight for a period of consecutive 15 days, respectively. The other served as normal control group in which rats received water. All of the test solutions were administered in a volume of 15 ml/kg body weight per day.

Body weights of the rats were recorded before the beginning of treatment, every day and at the end of treatment. The rats were observed for signs of abnormalities throughout the study. On day 15 (at the end of administration period), six rats randomly selected from each group were housed individually in stainless-steel metabolic cages that allowed for separate collection of urine and faeces and for the next day, 24-h collections of urine were done. On day 16, these rats were anesthetized with ether after the collection of urine samples, and then were sacrificed by dislocating cervical past after blood samples were obtained by cardiac puncture technique. These rats' main organs were observed, collected, and weighted individually. The specimens of liver, kidney and testis of them were also collected for pathological examination. The remaining rats in the control and experimental groups, served as recovery animals observed for reversibility, persistence and delayed occurrence of toxic effects, were further cared without treatment for another 15 days and their urine, blood and organ samples were similarly collected and examined.

The collected urine sample was centrifuged at 3000 rpm for 5 min, and the supernatant was stored at -20°C until the level of urinary protein content ($U_{protein}$) and the activity of urinary N-acetyl-β-D-glucosaminidase (NAG) were measured. Determination of the level of $U_{protein}$ by the Bradford coomassie brilliant blue method (Bradford, 1976) was carried out by using commercial kit (Nanjing Jiancheng Bioengineering Institute, P.R. China). Urinary NAG activity was determined with commercial kit (Shanghai Taiyang Biology Technology Co. Ltd., P.R. China) by using p-nitrophenyl- N-acetyl-β-D-glucosaminide as substrate (Pedraza-Chaverri et al., 2000).

Blood sample was allowed to clot at room temperature for 30 min, and then was centrifuged at 4500 rpm for 15 min to separate the serum. Serum sample was stored at -20°C until analyzed. The contents of blood urea nitrogen (BUN), creatinine and the activities of glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), alkaline phosphatase (ALP), lactic dehydrogenase (LDH) and γ-glutamyltransferase (GGT) in the serum were determined using commercial spectrophotometric diagnostic kits (Nanjing Jiancheng Bioengineering Institute, P.R. China) according to the manufacturer's directions. BUN was

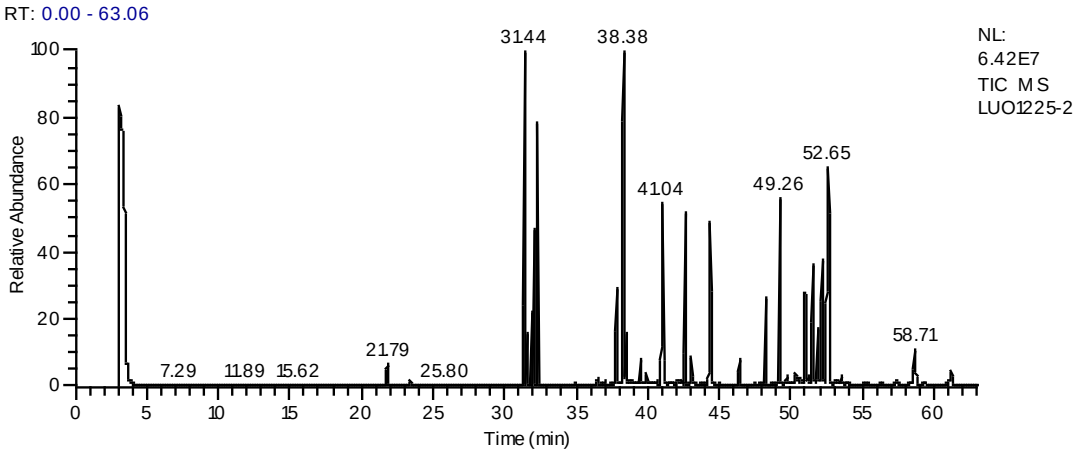

Figure 1. GC-MS chromatogram of PELJ.

assayed by the Fearon method and serum creatinine by the Jaffe method (Tietz et al., 1994). Estimation of GOT activities and GPT activities were done using Reitman-Frankel method (Reitman and Frankel, 1957). The ALP activity was determined by Kind and King's method (Kind and King, 1954). The activity of GGT was measured using the colorimetric method based on diazo reaction (Liu et al., 2009). The activity of LDH was detected with a colorimetric assay based on the conversion of pyruvic acid to phenylhydrazone (Wang et al., 2005).

The shapes, sizes and colors of internal organs, namely, heart, liver, spleen, lungs, kidneys, and testes in rats were visually observed for any signs of gross lesions. The relative organ weight of each animal was then calculated using the following formula: Relative organ weigh = (absolute organ weight (g)/body weight of rat on day of sacrifice (g)) ×100. Small pieces of tissue samples (liver, kidney and testis) were fixed immediately in 10% phosphate-buffered formalin. The fixed tissues were processed routinely and tissue slides were stained with hematoxylin and eosin (H & E) using standard techniques for histological examination by optical microscope.

Statistical analysis

Quantitative data are expressed as mean ± standard difference (SD). All analyses were performed with the standard statistics software SPSS, version 13.0. One-way analysis of variance ANOVA was used. The data were firstly tested for homogeneity-of-variance by Levene's test. Student-Newman-keuls test was used for analysis of means when equal variances assumed, whereas Dunnett's T3 test was used for comparisons of means when equal variances not assumed. Differences were considered to be statistically significant when $P < 0.05$ or 0.01.

RESULTS

Chemical composition of PELJ

The results of GC–MS analysis on PELJ were shown by Figure 1 and Table 1. A total of forty-two compounds, accounting for 89.87%, were identified in PELJ. The major components were 17b-hydroxy-1a, 17-dimethyl-5a-androstan-3-one (21.47%), stigmast-5-en-3-ol (11.86%), E-phytol (9.94%), caryophyllene oxide (8.69%), spathulenol (6.55%), hexadecadienoic acid methyl ester (6.50%) and cis-pinane (5.37%).

Effects on the general conditions

All animals survived to scheduled sacrifice and showed normal behavior and activity. After 15 days treatment, PELJ and DLJ decreased the body weight gains of the rats significantly when compared to the control. The shapes, sizes and colors of internal organs (heart, liver, spleen, lungs, kidneys, testes) in experimental rats were normal. For the relative organ weight, only the relative weights of testes of the rats in PELJ and DLJ groups were significantly higher than that of control rats (Table 2). After 15 days recovery period, decreased body weight gains of rats in PELJ and DLJ groups were reversed to normal levels, the relative organ weights of heart, liver, spleen, lung and kidney remained normal whereas significant increases were still observed in the relative weight of testes of rats in PELJ and DLJ groups (Table 2).

Effects on biochemical parameters

After 15 days treatment period, as shown in Table 3, the levels of GPT, GOT, ALP, LDH, GGT, BUN, creatinine, $U_{protein}$ and NAG in PELJ group and DLJ group were increased significantly when compared with those of the control group ($P<0.05$, $P<0.01$). The levels of all biochemical parameters tested in PELJ group were significantly higher than those of DLJ group ($P<0.01$).

After being kept for recovery period of 15 days, as shown in Table 3, the levels of GPT, GOT, ALP, LDH, GGT, BUN, creatinine, and NAG were decreased in PELJ

Table 1. Chemical composition of PELJ (GC–MS analysis).

Main compounds*	Retention time (min)	Percentage (%)	Molecular mass	Molecular formula
E-Phytol	31.44	9.94	296	$C_{20}H_{40}O$
Linoleic acid	31.90	0.88	280	$C_{18}H_{32}O_2$
1,2-Benzenedicarboxylicacid, 1,2-dibutyl ester	32.13	2.84	278	$C_{16}H_{22}O_4$
Cis-pinane	32.32	5.37	138	$C_{10}H_{18}$
7-Methyl-8-tetredecenyl acetate	37.69	0.72	268	$C_{17}H_{32}O_2$
Cis-(Z)-bisabolene epoxide	37.82	2.01	220	$C_{15}H_{24}O$
17b-Hydroxy-1a, 17-dimethyl-5a-androstan-3-one	38.38	21.47	318	$C_{21}H_{34}O_2$
7,11-Hexadecadienal	39.52	0.53	236	$C_{16}H_{28}O$
Caryophyllene oxide	41.04	8.69	220	$C_{15}H_{24}O$
β-Cedrene	42.65	3.77	204	$C_{15}H_{24}$
Hexadecadienoic acid methyl ester	44.36	6.50	266	$C_{17}H_{30}O_2$
Glyceryl linolenate	48.28	1.37	352	$C_{21}H_{36}O_4$
3-ethyl-5-(2-ethylbutyl)-octadecane	49.26	3.78	238	$C_{15}H_{26}O_2$
Retinal	51.01	2.61	412	$C_{29}H_{48}O$
Spathulenol	52.17	6.55	220	$C_{15}H_{24}O$
Stigmast-5-en-3-ol	52.65	11.86	414	$C_{29}H_{50}O$
(+)-Ledene	58.71	1.08	204	$C_{15}H_{24}$

The identified compounds are listed in the order of their elution on the column. *****Only the percentages over 1% are indicated in this table.

Table 2. Body weight gains and relative organ weights of rats treated with PELJ, DLJ after 15 days treatment and 15 days recovery period.

Parameter	After treatment period			After recovery period		
	Control group	PELJ group	DLJ group	Control group	PELJ group	DLJ group
Body weights gains (g)	116.08±12.82	78.58±7.24**	89.67±11.71**	181.33±15.08	176.08±17.54	178.00±20.64
Relative heart weight	0.55±0.09	0.50±0.06	0.51±0.06	0.50±0.05	0.51±0.04	0.51±0.05
Relative liver weight	4.47±0.57	4.11±0.23	4.03±0.54	3.20±0.22	3.18±0.10	3.19±0.29
Relative spleen weight	0.33±0.03	0.31±0.03	0.30±0.02	0.18±0.03	0.22±0.03	0.21±0.04
Relative lung weight	0.62±0.05	0.60±0.03	0.60±0.02	0.42±0.02	0.44±0.04	0.44±0.03
Relative kidney weight	0.49±0.02	0.46±0.02	0.48±0.03	0.38±0.02	0.40±0.01	0.39±0.02
Relative testis weight	0.57±0.03	0.65±0.02**	0.62±0.03*	0.41±0.02	0.49±0.04**	0.48±0.03**

Values are expressed as mean ± S.D. (n=6). Significantly different from control group, **P<0.01, *P<0.05.

group and DLJ group, whereas, the level of $U_{protein}$ did not demonstrate a tendency to decrease. Significant increases in the levels of all biochemical parameters tested in PELJ group were still observed as compared with the corresponding control group (P<0.05, P<0.01). When compared to the control group, there were significant increases in the levels of GOT, ALP, LDH, GGT and $U_{protein}$ (P<0.05, P<0.01) in DLJ group, but no significant differences in the levels of GPT, BUN, creatinine and NAG were found. The levels of all biochemical parameters tested in PELJ group were significantly higher than those of DLJ group (P<0.01), with the exception of GGT.

Histopathological examination of liver, kidney and testis

The liver sections of control rats showed normal histopathological feature (Figures 2.A). Histopathological examination of rats treated with PELJ for 15 days showed granular degeneration and fatty degeneration of hepatocytes and mild inflammatory cell infiltrations. Individual cell necrosis was also present (Figure 2B).

In the kidney, histopathological examination of PELJ treated rats revealed slight tissue damage (Figure 3B) when compared with the control (Figure 3A). The changes included granular degeneration of tubule

Table 3. Mean values of biochemical parameters in rats treated with PELJ, DLJ after 15 days treatment and 15 days recovery period.

	After treatment period			After recovery period		
Parameter	**Control group**	**PELJ group**	**DLJ group**	**Control group**	**PELJ group**	**DLJ group**
GPT(IU/L)	7.78±1.16	56.25±2.43**$^{\#\#}$	32.13±2.99**	7.29±1.16	16.54±2.47**$^{\#\#}$	9.07±2.03
GOT(IU/L)	9.37±1.87	118.89±2.76**$^{\#\#}$	85.48±4.94**	9.46±1.78	56.31±2.99**$^{\#\#}$	34.18±2.93**
ALP(IU/L)	51.18±3.18	392.30±40.64**$^{\#\#}$	153.94±7.66**	53.35±4.88	201.62±2.32**$^{\#\#}$	92.94±4.69**
LDH(IU/L)	8774.69±489.17	21719.14±792.58**$^{\#\#}$	13222.22±127.23**	8378.21±489.17	13503.21±195.90**$^{\#\#}$	9548.08±145.32**
GGT(IU/L)	25.60±6.90	262.50±24.09**$^{\#\#}$	123.21±12.73**	22.22±10.47	80.00±14.14**	68.33±10.27**
BUN(mmol/L)	4.29±0.53	14.54±0.39**$^{\#\#}$	9.29±0.66**	5.17±0.74	6.47±1.04*$^{\#\#}$	4.48±0.52
Creatinine(μmol/ L)	91.67±24.64	381.20±27.40**$^{\#\#}$	200.63±8.06**	81.30±5.66	128.73±15.96**$^{\#\#}$	79.71±6.53
$U_{protein}$(mg/L)	50.53±6.19	163.30±16.94**$^{\#\#}$	66.49±9.56*	47.57±8.57	237.28±16.76**$^{\#\#}$	66.92±6.77*
NAG(IU/L)	16.83±1.72	38.86±2.67**$^{\#\#}$	32.56±2.00**	17.53±1.01	22.91±0.84**$^{\#\#}$	18.22±0.83

Values are expressed as mean±S.D. (n=6). *, P<0.05, **, P<0.01, significantly different from control group; $^{\#\#}$, P<0.01 significantly different compared with DLJ group.

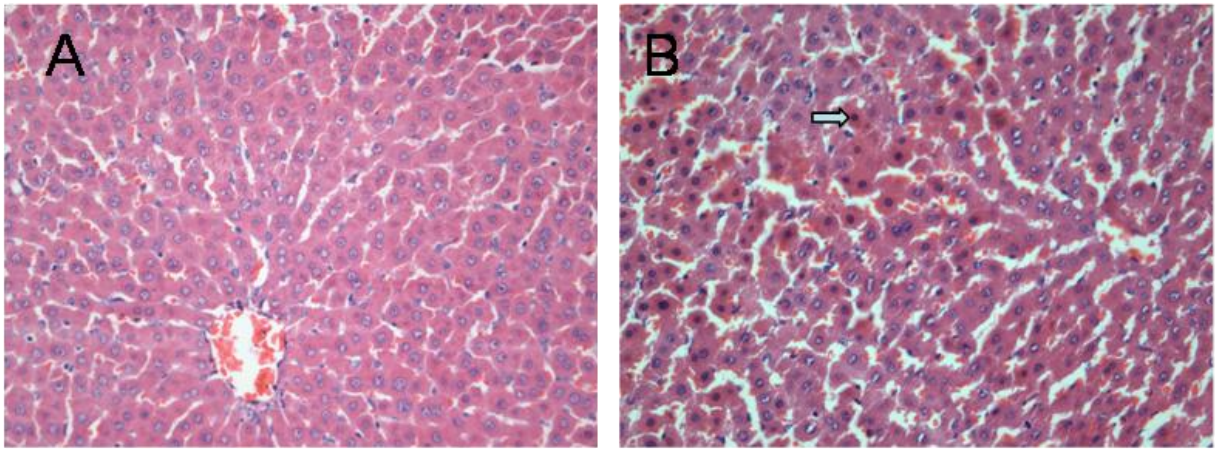

Figure 2. The histological morphology of rat livers from the control group (A) and PELJ group (B) treated for 15 days (H&E×400). Individual hepatocyte necrosis (arrow) was present in PELJ group.

epithelial cells and mild interstitial inflammatory cell infiltrations.

On histopathological examination of testis, control rats exhibited well-developed cytoarchitecture, indicated by the presence of normal seminiferous tubules, undergoing normal progression of spermatogenesis, from spermatogonia to primary spermatocytes and secondary spermatocytes to spermatids (Figure

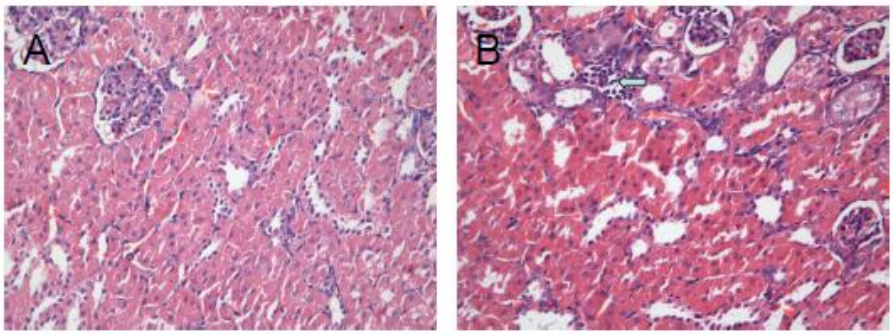

Figure 3. The histological morphology of rat kidneys from the control group (A) and PELJ group (B) treated for 15 days (H&E×400). Interstitial inflammatory cell infiltrations (arrow) were observed in PELJ group.

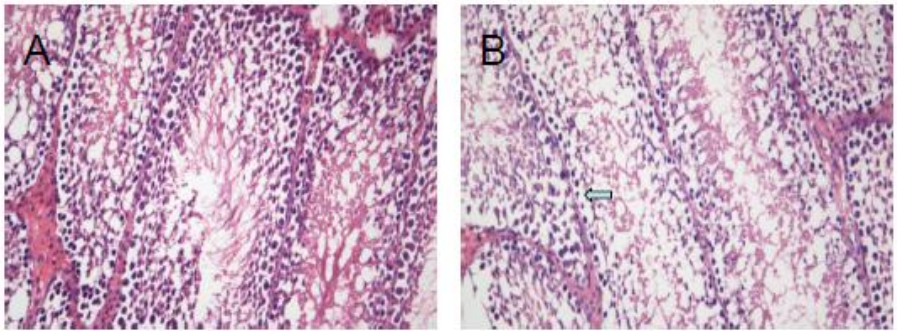

Figure 4. The histological morphology of rat Testes from the control group (A) and PELJ group (B) treated for 15 days (H&E×400). Mild reduction in the number of primary spermatocytes and secondary spermatocytes (arrow) was observed in PELJ group.

4A). In PELJ treated rats, reduction in the number of primary spermatocytes, secondary spermatocytes and spermatids was observed in some seminiferous tubules as compared with the control (Figure 4B).

Histopathological changes of the liver, kidney and testis sections of DLJ treated rats were similar to that of PELJ, whereas the alternations occurred in the lower incidence and severity.

At the end of 15 days recovery period, the histopathological changes induced by PELJ and DLJ were ameliorated. However, histopathological alterations were minor in DLJ treated rats and the recovery rats; these rats are not included in the figure for sake of simplicity.

DISCUSSION

PELJ was tried to be disperse in a known volume of 0.05% CMC (Féres et al., 2006), 10% DMSO (Nevin and Vijayammal, 2005) or 0.5% Tween 80 (Costa et al., 2006), but the homogeneity of the aforementioned suspension did not have more advantage than that in distilled water, so distilled water was selected to be used as vehicle for administration, and the mixture for administration was subjected to an ultrasonic wave treatment.

In the present study, the results of the changes in the body weight gains of experimental rats indicate that the adverse effects of PELJ and DLJ on the body weight are reversible. Regarding the toxic effects on liver and kidney in rats, the activities of GPT, GOT, ALP, LDH, GGT in serum were determined as markers of liver damage, as well as the levels of creatinine, BUN in serum and $U_{protein}$ and NAG in urine were tested as markers of kidney damage (Novelli et al., 1998; Nevin and Vijayammal, 2005; Trevor, et al., 2003).

The significant increases in the levels of all determined parameters in experimental rats treated for 15 days indicate the hepatic and renal toxicity of PELJ and DLJ, which were further proved by the histopathological observations of liver and kidney sections in rats. After 15 days recovery period, the increased levels of most of parameters decreased except for $U_{protein}$ in experimental rats.

The increased level of $U_{protein}$ did not display a tendency

to reverse, thus suggesting a lack of reversibility in this parameter. The recovery period of 15 days in this study may not have been long enough, and it is possible that the altered levels of all parameters including $U_{protein}$ may return to the control value and the histopathological changes be normalized after longer recovery period. However, these assertions need to be clarified. In addition, it was reported that high dose of DLJ given orally may cause renal interstitial fibrosis in rats (Cai et al., 2000; Sun et al., 2005).

But in our study, only slight renal pathologic changes without interstitial fibrosis were observed in DLJ treated rats. The discrepancy may be explained by the fact that variation may sometimes occur in toxic components in the same herb collected in different habitats. Differences in the experimental protocol could account for the discrepancy as well.

To investigate the toxic effects of PELJ and DLJ on testis, serum testosterone concentrations were measured by radioimmunoassay in the study. Because the used of commercially available kit is for human serum, the absolute level of testosterone in rats could not be qualified and the data were not listed in this paper. However, the comparative level of testosterone could be ascertained as follows: the levels of testosterone in experimental rats significantly descended when compared with the control and did not return to the control value after recovery.

As for the testis, testicular damages characterized by reduction of the number of spermatocytes and spermatids were observed in experimental rats, and the changes were not normalized after recovery. So it is evident that administration of PELJ and DLJ can induce testicular toxicity. Although it is difficult to extrapolate result from animal experiments to humans due to inter-species differences, and there have not been a clinical report on the adverse effect of *L. japonicus* on reproductive system till now; our results suggest that the high attention on the reproductive toxicity of *L. japonicus* at high dose should be aroused.

In conclusion, the results obtained in the present study demonstrated that PELJ has obvious toxic effects on the liver, kidney and testis in rats under the present experimental conditions, and showed partial recovery of the toxic effects in short period. Meanwhile, the present study revealed that PELJ exhibited a higher toxicity in rats than DLJ at the same dose. Further studies will focus on isolating the toxic compounds responsible for the toxicity of *L. japonica* on the basis of the results obtained herein and elucidating the mechanism of the toxic effects of *L. japonica*.

REFERENCES

Bian ZK, Lv XS (1998). The new use of motherwort. J. Pharm. Pract., 16(5): 320.

Bradford MM (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72: 248-254.

Cai ZY, Zhou JM, Ge YR (2000). Influence of motherwort on renal function and tissues in rats. Shanghai J. Tradit. Chin. Med., 34(11):37-39.

Chinwala MG, Gao M, Dai J, Shao J (2003). In vitro anticancer activities *Leonurus heterophyllus* Sweet (Chinese motherwort herb). J. Altern. Complement. Med., 9(4): 511-518.

Costa EA, Rocha FF, Torres MLB, Souccar C, De Lima TCM, Lapa AJ, Lima-Landman MT (2006). Behavioral effects of a neurotoxic compound isolated from *Clibadium surinamense L* (*Asteraceae*). Neurotoxicol. Teratol., 28(3): 349-353.

Féres CAO, Madalosso RC, Rocha OA, Leite JPV, Guimarães TMDP, Toledo VPP, Tagliati CA (2006). Acute and chronic toxicological studies of Dimorphandra mollis in experimental animals. J. Ethnopharmacol., 108(3): 450-456.

Kind PRN, King EJ (1954). Estimation of plasma phosphatase by determination of hydrolyzed phenol with amino-antipyrene. J. Clin. Pathol., 7(4): 322-326.

Liu F, Liu JW, Cai Y, Wang LJ, Geng Y (2009). Sulfated derivative of polysaccharide from *Pinus massoniana* pollen induces differentiation of human liver cancer HepG2 cells. World Chin. J. Digest, 17(29): 2990-2995

Nevin KG, Vijayammal PL (2005). Effect of *Aerva lanata* against hepatotoxicity of carbon tetrachloride in rat. Environ. Toxicol. Pharmacol., 20(3): 471-477.

Novelli ELB, Vieira EP, Rodrigues NL, Ribas BO (1998). Risk assessment of cadmium toxicity on hepatic and renal tissues of rats. Environ. Res., 79(2): 102-105.

Pedraza-Chaverri J, Maldonado PD, Medina-Campos ON, Olivares-Corichi IM, Granados-Silvestre MA, Hernández-Pando R, Ibarra-Rubio ME (2000). Garlic ameliorates gentamicin nephrotoxicity: relation to antioxidant enzymes. Free Radic. Biol. Med., 29(7): 602-611.

Pharmacopoeia Committee of the Health Ministry of People's Republic of China (2005). Chinese Pharmacopoeia, part 1. Chemical Technologic Publisher, Beijing, P. 203.

Reitman S, Frankel S (1957). A colorimetric method for the determination of serum glutamic oxaloacetate aminotransferase. Am. J. Clin. Pathol., 28: 56-63.

Sun R, Sun LG, Wu XD, Lv LL (2005). The attenuation effect of compound compatibility to nephrotoxicity of rat caused by Leonurus. Chin. J. Pharmacovigilance, 2(3): 144-147.

Tao LH, Gao F, Li HJ (2003). The clinical application of motherwort in treating ischemic diseases. J. Tonghua Teach. Coll., 24(6): 29-31.

Tietz NW, Pruden EL, Siggaard-Anderson O (1994). In: Tietz textbook of Clinical Chemistry (Burtis CA, Ashwell ER eds.) WB Saunder Co. London. Pp. 1354-1374.

Trevor G, Jacky D, John F (2003). Increased formic acid excretion and the development of kidney toxicity in rats following chronic dosing with trichloroethanol, a major metabolite of trichloroethylene. Toxicol., 191(2-3): 109-119.

Wang G, Gong Y, Anderson J, Sun D, Minuk G, Roberts MS, Burczynski FJ (2005). Antioxidative function of L-FABP in L-FABP stably transfected Chang liver cells. Hepatol., 42(4): 871-9.

Wang ZS, Li DW, Xia WJ, Qiu HQ, Zhu LY (1988). The therapeutic effect of *Herba leonuri* in the treatment of coronary myocardial ischemia. J. Tradit. Chin. Med., 8(2): 103-106.

Xu CY, Chen SR, He WZ, Wu YB, Wu SX, Chen XG, Li SM (2002). Leonurus heterophyllus injection in treating acute cerebral infarction. Chin. J. New Drugs Clin. Rem,. 21(5): 271-274.

Yan JZ, Chen SR (2000). Curative effects of *Leonuru Heterophyllus* on coronary heart disease. J. Shantou Univ. Med. Coll., 13(1): 20-22.

Yin J, Liao JY, Chen XQ, Guo YL, Lin P, Zhang ZW, Yu WJ (2010). *Leonurus heterophyllus* sweet extract improves hemorheology and inhibits thrombosis in rat with induced myocardial ischemia. J. Med. Plants Res., 4(9): 732-740.

Zou QZ, Bi RG, Li JM, Feng JB, Yu AM, Chan HP, Zhen MX (1989). Effect of motherwort on blood hyperviscosity. Am. J. Chin. Med., 17(1-2): 65-70.