Central Asian Journal of Medical Sciences
CAJMS

Original Article
Cent Asian J Med Sci. 2018 Dec;4(4):293-299. https://doi.org/10.24079/CAJMS.2018.12.007

The Protective Effect of Traditional Medicine Tagtaggunsel on Carbon Tetrachloride Induced Chronic Liver Cirrhosis in a Rat Model

Tsogtoo Bukhbayar[1], Dejidmaa Buyantogtokh[2], Chimedragchaa Chimedtseren[2], Badamsuren Dorjgotov[3], Tserendagva Dalkh[1]

[1]Department of Theory of Mongolian Medicine, School of International Mongolian Medicine, Mongolian National University of Medical Sciences, Ulaanbaatar, Mongolia; [2]Reasearch Center, Institute of Traditional Medicine and Technology, Ulaanbaatar, Mongolia; [3]Department of Gastroenterology, Shastin Central Hospital, Ulaanbaatar, Mongolia

Submitted: October 22, 2018
Revised: December 17, 2018
Accepted: December 24, 2018

Corresponding Author
Tsogt Bukhbayar, MD
Department of Theory of Mongolian Medicine, School of International Mongolian Medicine Mongolian National University of Medical Sciences, Ulaanbaatar 14210, Mongolia
Tel: +976-99125890
E-mail: b.tsogtoo2018@yahoo.com

Objective: To study the effect of traditional medicine tagtaggunsel on the development of chronic cirrhosis in rat model. **Methods:** Rats were divided into 4 groups of 18 animals each. Group I served as the normal healthy control, groups II rats were intoxicated with carbon tetrachloride subcutaneous by injection (0.1 ml/kg body weight carbon tetrachloride /olive oil, three times per week for 12 weeks), group III rats received carbon tetrachloride intraperitoneally plus tagtaggunsel orally (100 mg/kg daily) and group IV rats received carbon tetrachloride intraperitoneally plus Silymarin orally (50 mg/kg daily). The hepatoprotective potential of tagtaggunsel in rats was evaluated by measuring the enzyme levels of ALT, AST and serum iron, ferritin, hepcidin in addition to other liver biomarkers. Histopathological changes in the liver were assessed using hematoxylin and eosin, Masson-trichrome staining. **Results:** The administration of tagtaggunsel showed hepatic protection at an oral dose of 100 mg/kg. tagtaggunsel significantly reduced the elevated serum levels of liver enzymes as well as liver biomarkers in comparison to carbon tetrachloride–intoxicated group. Notably, tagtaggunsel significantly reduced the expression level of ferritin, but hepcidin significantly increased compared to their levels in carbon tetrachloride intoxicated group. These findings were confirmed with the histopathological observations, where tagtaggunsel was capable of reversing the toxic effects of carbon tetrachloride on liver cells compared to that observed in carbon tetrachloride-intoxicated rats. **Conclusion:** Our results show that tagtaggunsel has potential hepatoprotective effects at 100 mg/kg. These effects can be regarded as antioxidant properties of the extract.

Keywords: Tagtaggunsel, Carbon tetrachloride, Hepatoprotective, Silymarin, Liver fibrosis, ferritin, hepcidin.

Introduction

Liver fibrosis is the healing process of the acute and chronic liver injury response. Liver fibrosis and cirrhosis account for a significant proportion of the deaths of the world population[1-3]. As a consequence, liver cirrhosis and liver cancer can be a serious problem in Mongolia. Many researchers have been investigating this field and the natural products are considered very promising[4,5]. Therefore, the investigation of signaling pathways and identification of the potential therapeutic targets is extremely important[6]. TTG is usually used to treat human liver disease and what is called in Mongolian traditional medicine disease of "bad blood"[7,8]. It is used to relieve fever, headaches, toothaches, and bleeding. Phytochemical analysis of TTG revealed the presence of several active ingredients that include rutin, gallic acid, and insulin, in addition to other compounds and some minerals. Minerals include Ca, O, Zn, Mg, Mn and others[9-12]. The classic toxicity of carbon tetrachloride (CCl4) is to induce liver lesion and liver fibrosis[5]. Animal models using CC14 to induce chronic cirrhosis have been developed to study the effect of medicines on chronic cirrhosis[13,14]. In the present study, we aim to investigate the hepatoprotective effects of water extract of TTG against CCl4-induced liver fibrosis and its role in the alleviation of iron metabolism and restoration liver enzymes activities.

Materials and Methods

The study was carried out at the Laboratory of Pharmacology in Institute of Traditional Medicine and Technology of Mongolia (ITMTM) and Mongolian National University of Medical Science (MNUMS), Institute of Veterinary medicine. TTG mongolian medicine was prepared in the traditional medical factory of Institute of Traditional Medicine and Technology of Mongolia. The remedies contained in the TTG were collected and authenticated by the experts at the Department of Botany, Institute of Biology and Mongolian Academy of Sciences. Ten grams of crushed dried TTG raw material was suspended in 250 ml water and boiled until water had evaporated to 100 ml. The prepared compound was then used for pharmacological tests.

CCL4 Induced liver cirrhosis in experimental rats

The study was performed over a period of 12 weeks using 60 rats, randomized into 4 groups (group I through IV) of 15 animals each. Group I were untreated 15 normal healthy control rats received saline intraperitoneally (i.p.) 3 times weekly for the 12 weeks. Group II were rats intoxicated with CCl4 i.p. (0.1 ml/kg body weight CCl4/olive oil, 1:1 v/v, three times weekly) for 12 to induce chronic liver injury[13]. Group III were (TTG-treated) rats received the same dose of CCl4 as group II along with a daily oral dose of TTG (100 mg/kg). Group IV: (Silymarin-treated) rats received the same dose of CCl4 as group II along with a daily oral dose silymarin[14] (100 mg/kg). In groups III and IV, the treatment with TTG or silymarin was initiated 24 h after the first dose of CCl4. The rats were fed these substances daily until euthanized. Animal experiments were conducted following the guidelines for the care and use of laboratory animals of the National Institutes of Health (NIH publication No.22–27, revised 2007). The study protocol (№11/3/2016-11) was approved by members of "The Research Ethics Committee" and by the National Mongolian university of medical sciences.

Blood samples

After 4, 8, and 12 weeks 5 rats from each group were anesthetized with ketamine hydrochloride (90 mg/kg, i.p.). A 5 ml blood sample was collected from each rat by cardiac puncture. Blood samples were collected for biochemical analysis, and liver tissues were excised rapidly and prepared for histological investigation. The serum was separated by centrifugation at 3000 rpm for 15 minutes. The level of serum hepcidin, ferritin was measured by ELISA according following the kit's instructions (Shanghai MLBIO Biotechnology Co.Ltd).

Statistical analysis

Mean ± standard deviation (SD) were calculated for the observed values in each experimental group. Statistical analysis was done by one-way ANOVA followed by a priori comparison tests to compare treatment groups. Graph Pad Prism-5 software was used for statistical analysis with $p<.05$ considered statistically significant.

Results

Serum AST, ALT, ALP, Albumin levels

The serum transamineses and ALP, Albumin levels are reported in Table 1.

Table 1. Effect of TTG on serum AST, ALT, ALP and albumin levels in rats injected CCL4.

Period	Group	AST (mg/dl)	ALT (mg/dl)	ALP (u/l)	Albumin (g/L)
4 week	Healthy	111.2±10	98.8±1.7	200.5±12	45.0±1.7
	Control+CCL4	269.3±23.5**	219.8±26.3**	260.3±17.9*	39.0±3.8
	TTG+CCL4	189.0±19.4*	207.6±7.5*	215.4±10.4*	41.3±3.1
	Sylimarin+ CCL4	235.3±16.0**	213.9±21.6	207.8±21.3*	38.2±1.5
8 week	Control+CCL4	290.3±26.4**	209.3±8.8**	322.1±25.7*	32.5±2.1
	TTG+CCL4	227.0±13.7*	176.3±15.4*	292.0±10.2*	42.6±2.07
	Sylimarin+ CCL4	191.1±14.6**	118.8±7.09**	267.8±19.5*	35.3±1.6
12 week	Control+CCL4	591.0±36.6**	352.2±9.1**	807.3±31.2**	30.2±1.8
	TTG +CCL4	157.1±11.3**	141.5±11.8**	223.2±12.0**	38.4±1.05
	Sylimarin+ CCL4	158.4±7.2**	130.7±3.9**	238.1±22.4**	40.6±4.9

Values represent Mean ± SEM (n=15), ALT-Alanine aminotransferase, AST-Aspartate aminotransferase, ALP-Alkaline phosphatase
**p<.01 (compared to CCl4 (group II)) and +++p<.001 (compared to control group I). No significant difference (p=.3 or higher) between group III (100 mg/kg TTG) and group IV (silymarin), using one-way ANOVA test followed by a priori comparisons *p<.05. TTG treatment resulted in a significant improvement in the albumin compared to CCl4 treated animals (n=15, p<.05). Silymarin-treated group and control (n=15, p<.05). Data are expressed as mean ± SEM, significance was calculated using one-way ANOVA followed by a priori comparisons.

TTG treatment restores serum liver enzymes activity

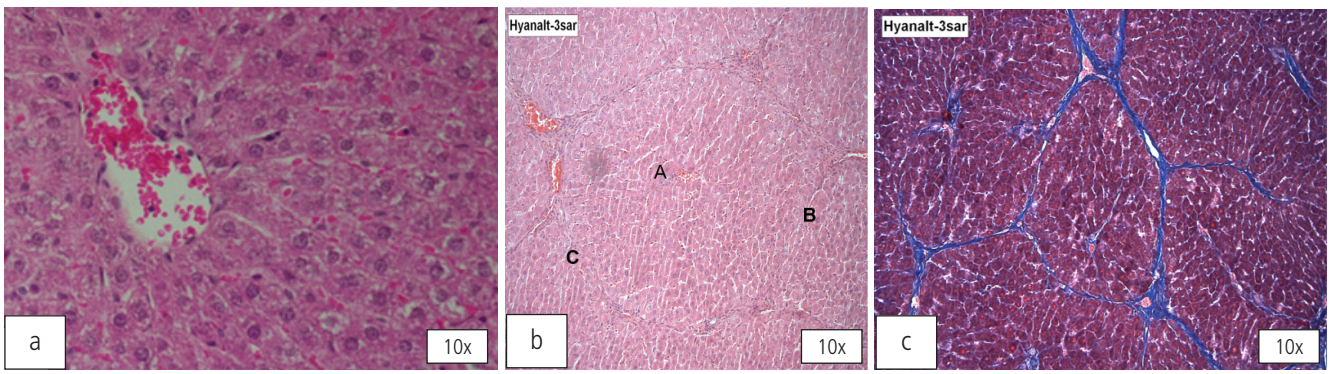
The released biomarkers of liver cell integrity; ALT, AST, ALP were investigated in the serum of the different groups. Administration of CCl4 resulted in an approximately 3.3 fold increase in the mean value of ALT levels in comparison to the healthy controls (Table 1). Interestingly, the daily treatment with 100 mg/kg TTG reversed the elevation in the levels of ALT caused by CCl4 resulting in values that were comparable to healthy control and to that produced by silymarin, noting that silymarin is well known for its hepatoprotective action[4]. Notably, this reduction in serum activity of ALT was significant (p<.001) in comparison to that observed after CCl4 treatment (Table 1). A similar tendency was also observed in the AST, ALP enzyme levels as shown in Table 1 but this was not statistically significant.

TTG treatment restores albumin levels

Figure 1 shows the change in the serum levels of albumin following the different treatments. The administration of CCl4 resulted in a significant reduction in albumin level (p<.01). Interestingly, TTG treatment was capable of preventing the decrease in serum albumin levels induced by CCl4 (p<.05).

Serum hepcidin, ferritin levels

The serum hepcidin was measured in the healthy control, CCl4, silymarin/CCl4 and TTG/CCl4-treated groups as shown in Table 2. Observed values revealed a significant reduction in the hepatic hepcidin level in the group of rats treated with CCl4 (p=.05) when compared to healthy control rats (CCl4-treated group). Notably, TTG treatment effectively prevented the CCl4-induced



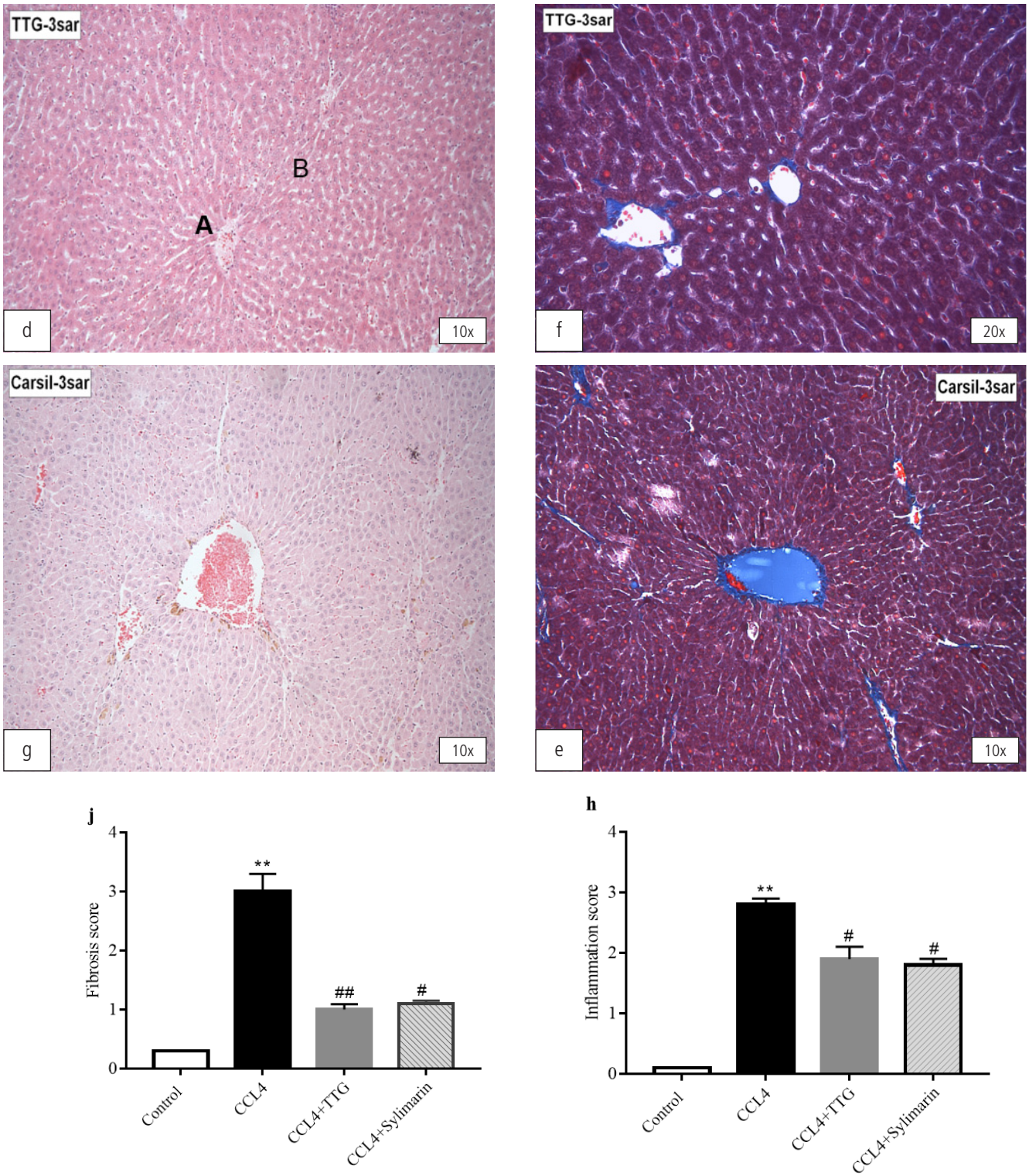


Figure1. Histological examination of liver sections from different groups.
Liver sections from healthy control show normal hepatocytes architecture (a), whereas CCl4 treatment resulted in damaged cells, shrunken nuclei, mitotic activity (arrow heads) and centrilobular congestion (b & c). TTG treatment resulted in restoration of the normal architecture and absence of congestion (d & e) in a similar way to that observed in silymarin treatment (f &g). Bars represent mean ± SEM of histopathological scoring (h) inflammation score and (j) fibrosis score. #, *: significantly different compared to CCl4-treated group or control group respectively, $p<.05$. Significance was calculated using one way test followed by a priori comparisons.

Table 2. Effect of TTG on serum hepcidin and ferritin levels in rats injected CCL4.

Period	Group	Serum hepcidin (pg/ml)	Serum ferritin (pg/ml)
4 week	Healthy	15.3±1.1	53.1±5.3
	Control+CCL4	13.7±1.7	127.9±6.8
	TTG+CCL4	23.7±2.9*	69.9±3.3**
	Sylimarin+ CCL4	29.9±2.5*	58.6±4.7**
8 week	Control+CCL4	14.5±0.3	98.6±7.7*
	TTG+CCL4	18.0±1.9*	89.3±9.1*
	Sylimarin+ CCL4	15.8±0.9	86.6±2.0*
12 week	Control+CCL4	14.7±0.4	108.2±9.2*
	TTG +CCL4	20.2±0.9*	76.9±11.0*
	Sylimarin+ CCL4	19.8±0.7*	92.3±8.7*

*p=.05, **p=.001

depletion of hepcidin of liver caused by CCl4 administration. The effect obtained by TTG treatment was comparable to that observed in silymarin/CCl4-treated groups (p=.05).

Ferritin is a universal intracellular protein that stores iron and releases it in a controlled fashion. In the current study, we found that CCl4 treatment of animals resulted in a significant, 2.4 folds, increase in the serum level of ferritin (p<.01) compared to healthy control animals as expected (Table 2). To our interest, TTG treatment resulted in a significant prevention of the CCl4-induced overexpression of ferritin (p<.01) compared to the CCl4-treated group. The values observed following TTG/CCl4 treatment were comparable to those observed in healthy control as well as silymarin/CCl4-treated groups.

Histopathological findings of the TTG/CCl4-treated group

To assess the effect of the different treatment protocols on liver architecture, paraffin section prepared from the hepatic tissues of the different groups were stained with

hematoxylin/eosin and Masson/trichrome examined. Histologically, liver from rats in the healthy control group showed a normal liver lobular architecture and hepatocyte structure (Figure 1a). In contrast, CCl4 administration resulted in histopathological lesions and extensive hepatocellular damage, as represented by the presence of portal inflammation, fatty change and venous congestion (Figure 1b, c). Treating the tested animals with TTG ameliorated these histopathological changes (Figure 1d, f), producing similar effects to that achieved by the treatment with Silymarin (Figure 1g, e). TTG as well as silymarin were able to significantly decrease the signs of CCl4 –induced fibrosis (p<.05) (Figure 1h, j).

Discussion

Liver fibrosis is the excessive accumulation of extracellular matrix proteins including collagen that occurs in most types of chronic liver diseases[1,2]. Liver fibrosis is a consequence of chronic liver lesion, which can progress into liver cirrhosis even hepatocellular carcinoma[3]. In light of the limited pharmacological options available for the treatment of liver diseases, identification of effective hepatoprotective agents derived from is an urgent necessity. Therefore, it is important to evaluate plant extracts that can help in restoring liver functions. Since ancient times, natural products such as herbs have been used as a remedy for various diseases. Indeed, plant extracts usually contain variable amounts of phenolic and polyphenolic compounds, which are responsible for the antioxidant effects of these medicinal plants[15-18]. The toxicity was evaluated for 100 mg/kg dose of TTG and was assessed based on the changes in liver as well as kidney biomarkers. Interestingly, the investigated parameters revealed the safety of the whole set of doses of TTG used in the experiment compared to the healthy control group. In the present study, a rat model of cirrhosis was successfully established by the injection of CCL4. The results of our study showed TTG had significant effect on chronic cirrhosis in rats. Compared with the control group, the laboratory and light microscopy findings were

significantly different in groups receiving TTG.

The pharmacological research of TTG to date has been performed only on experimental animals and it is necessary to conduct future clinical trials to see if these results apply to humans.

In addition, it is important to study the TTG effects in a chronic cirrhosis model like CCL4 induced liver injury in experimental animals to determine its therapeutic benefits.

Conclusion

TTG showed potential hepatoprotective effects against chronic liver injury.

Conflict of Interest

The authors have declared no conflict of interest.

Acknowledgements

This is based on the work supported by Mongolian Foundation for Science and Technology. Any findings, conclusions and recommendations expressed in this study are those of the authors and do not necessarily reflect the views of Mongolian Foundation for Science and Technology of Mongolia.

Reference

1. Friedman SL. Liver fibrosis - from bench to bedside. J Hepatol 2003; 38: 38–53.
2. Elpek GÖ. Cellular and molecular mechanisms in the pathogenesis of liver fibrosis: An update. World J Gastroenterol 2014; 20: 7260-76.
3. Seki E, Brenner DA. Recent advancement of molecular mechanisms of liver fibrosis. J Hepatobiliary Pancreat Sci 2015; 22: 512-8.
4. Seeff LB, Lindsay KL, Bacon BR, Kresina TF, Hoofnagle JH. Complementary and Alternative Medicine in Chronic Liver Disease. Hepatology 2001; 34: 595-603.
5. Constandinou C, Henderson N, Iredale JP. Modeling liver fibrosis in rodents. Methods Mol Med 2005; 117: 237–50.
6. Silva OB, Ramos LF, Moraes KCM. Molecular interplays in hepatic stellate cells: apoptosis, senescence, and phenotype reversion as cellular connections that modulate liver fibrosis. Cell Biol Int 2017; 41: 946-59.
7. Baavgai C, Boldsaikhan B. Mongolian traditional medicine. Ulaanbaatar, Mongolia: State Publishing House; 1990. p 153-154.
8. Tumurbaatar N, Khatanbaatar Z, Tserendagva D. An introduction to Mongolian traditional medicine. Ulaanbaatar, Mongolia: Munkhiin Useg; 2006. p 25-27.
9. Zhang Z, Guo Y. Curcumin modulates cannabinoid receptors in liver fibrosis in vivo and inhibits extracellular matrix expression in hepatic stellate cells by suppressing cannabinoid receptor type-1 in vitro. Eur J Pharmacol 2013; 721: 133-40.
10. Spiridon I, Nechita CB. Antioxidant and chemical properties of Inula helenium root extracts. Cent Eur J Chem 2011; 1699-709.
11. Xiao W, Li Sh, Wang S. Chemistry and bioactivity of Gardenia jasminoides nitric oxide synthase. Korean J Physiol Pharmacol 2016; 15: 123-8.
12. Dai Sh X, Li WX, Han FF. In silico identification of anti-cancer compounds and plants from traditional Chinese medicine database. Sci Rep 2016; 6: 25462.
13. Jeong DH, Lee GP, Jeong WL, Do SH, Yang HJ, Yuan DW, et al. Alterations of mast cells and TGF-β1 on the silymarin treatment for CCl4-induced hepatic fibrosis. World J Gastroenterol 2005; 11: 1141-8-15.
14. Wahid A, Hamed AN, Eltahir HM, Abouzied MM. Hepatoprotective activity of ethanolic extract of Salix subserrata against CCl4-induced chronic hepatotoxicity in rats. BMC Complement Altern Med 2016; 16: 263-68.
15. Niki E. Lipid peroxidation: Physiological levels and dual biological effects. Free Radic Biol Med 2009; 5: 469-84-7.
16. Ohta Y, Kongo M, Sasaki E, Nishida K, Ishiguro I. Therapeutic effect of melatonin on carbon tetrachloride-induced acute

liver injury in rats. J Pineal Res 2000; 28: 119-26-30.

17. Ferreira EA, Gris EF, Felipe KB. Potent hepatoprotective effect in CCl4-induced hepatic injury in mice of phloroacetophenone from Myrcia multiflora. Libyan J Med 2010; 5: 10.3402.
18. Messner DJ, Murray KF, Kowdley KV. Mechanisms of Hepatocyte Detoxification. In: Johnson LR, Ghishan FK. Physiology of the Gastrointestinal Tract. 5th edition. Academic Press; 2012. p 1507-1527.