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Original Research Article

Hepatoprotective effect of a Tagtaggunsel on carbon-tetrachloride-induced liver fibrosis

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In Mongolia and worldwide, hepatic disease remains one of the leading causes of population mortality. The aim of the present study was to investigate the effect of a Mongolian medicine formula, Tagtaggunsel (TTG), on the treatment of liver fibrosis in rats. Rats were divided into 4 groups of 15 animals each. 1. Healthy group, 2. Control groups rats were intoxicated with CCl4 i.p injection (10%, 0.1 ml/kg body weight CCl4/olive oil, three weekly for 12 weeks), 3. Treatment group rats received CCl4 i.p. and TTG orally (100 mg/kg daily) and 4. Comparative group rats received CCl4 i.p. and Silymarin orally (100 mg/kg, daily). The hepatoprotective effect was assessed estimation of serum concentration of ALT, AST, albumin and 2 types of antioxidant markers-Lipid peroxidase, Superoxidismutase (LPO, SOD) Histopathological changes in the liver were assessed using hematoxylin and eosin staining, Masson-trichrome. Showed that TTG significantly serum level AST, ALT and ALP but Albumin increased compared to CCL4 intoxicated group. In addition TTG significantly reduced the level of LPO, but SOD significantly increased compared to CCl4 intoxicated group. These findings were confirmed with the histopathological observations, TTG had effect of anti inflammatory processes and decreased the connective tissue CCl4 on liver cells compared to that observed in CCl4-intoxicated rats. In conclusion TTG had potential hepatoprotective effects at 100 mg/kg.

Key words: Tagtaggunsel, carbon tetrachloride, hepatoprotective, silymarin, liver fibrosis, LPO, SOD

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 (Constandinou et al., 2005, Friedman et al., 2003, Seeff et al., 2001). 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 (Elpek et al., 2014, Seki et all., 2015). Therefore, the investigation of signaling pathways and identification of the potential therapeutic targets is extremely important (de Oliveira da Silva et al., 2017). Tagtaggunsel (TTG) is a traditional Mongolian medicine

prescription herb and mineral complex that containing *Calcite, Arnebia guttata. Bge, Inula Helenium, Gardenia jasminoides, Bos Taurus domesticus Gmeline*, *Odontites rubra (Baung), Schizostachoum chinense Rendle,Glycyrrhiza uralensis Fisch*. TTG is usually used to treat human liver disease and disease of blood, anti inflammatory (Zhang et al., 2013; Baavgai et al., 1990). 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 inulin, in addition to other compounds and some minerals. Minerals include calcium, oxygen, zinc, magnesium, manganese others (Tumurbaatar, 2006; Spiridon et al., 2013; Xiao et al., 2017; Jeong et al., 2005).

The classic toxicity of carbon tetrachloride (CCl4) is to induce liver lesion and liver fibrosis (Seki et al ., 2015). Animal models using CC14 to induce chronic cirrhosis have been developed to study the effect of medicines on chronic cirrhosis (Jeong et al., 2005; Wahid et al., 2016). 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 antioxidant effect and liver enzymes activities.

MATERIAL AND METHODS

Remedy materials and preparation of TTG

The plants were collected in august 2016 from the region of Khangai, Khuvsgul, Gobi-Altai in the Mongolia. Was identified by Professor E.Ganbold botanic and classification department, Ulaanbaatar university, Mongolia. A voucher specimen of the plant under the number 2016/08/27/105 was deposited in the Herbarium of Pharmacology Department, Center of research, Institute of traditional medicine and technology. The herbs were air-dried and reduced to fine powder suitable for extraction.

Animal and experimental design

The wistar rats (250 g average weight) were purchased from the animal house of research center, institute of traditonal medicine and technology, Mongolia. Sixty healthy male wistar rat of weighing between 250-280 gm were purchased from the Experimental Animal Center, Institute of Traditional Medicine and Technology of Mongolia. They were kept under controlled conditions of temperature (20±1°C) and humidity (about 50-60%), with a 12-hour light/dark cycle, and automatic ventilation 8-15 times every hour. Rats could drink ad libitum, and were fed with standard nutrient.

The study was carried out in accordance with the Health Ethics Guidelines issued by the Mongolian Ministry of Health (2018). 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.

CCL4 induced liver fibrosis model in rats

Rats were divided into 4 groups of 15 animals each. 1. Healthy group, 2. Control groups rats were intoxicated with CCl4 i.p injection (10%, 0.1 ml/kg body weight CCl4/olive oil, three weekly for 12 weeks), 3. Treatment group rats received CCl4 i.p. and TTG orally (100 mg/kg daily) and 4. Comparative group rats received CCl4 i.p. and Silymarin orally (100 mg/kg, daily) (Wahid et al., 2016). In groups III and IV, the treatment with TTG or silymarin was initiated 24 h after the first dose of CCl4. The study results were obtained by evaluating AST, ALT and ALP, albumin in serum level and histomorphological examination.

Blood samples

After 4, 8 and 12weeks experimental rats from each group were anesthetized with ketamine hydrochloride (90 mg/kg, intraperitoneally). A 5 ml blood sample was collected from each rat by cardiac puncture. The serum was separated by centrifugation at 3000 rpm for 15 minutes. The level of serum LPO, SOD was measured by ELISA according following the kit's instructions (Shanghai MLBIO Biotechnology Co.Ltd).

Liver specimen preparation

Rats euthanized the end experiment. Each liver specimen was dissected into 2 parts. One part was fixed and embedded in 10% paraffin for histopathological examination. The second part was homogenized for total protein extraction in 20 mM Tris, 100 mM NaCl, 1 Mm EDTA and 0.5 % Triton X-100 buffer.

Blood tests

The enzymes of hepatic damage including serum ALT, AST, ALP, albumin, were estimated according to commercial reagent following manufacturer's instructions (Shanghai MLBIO Biotechnology Co.Ltd).

Histomorphological examination

Formalin-fixed liver specimens were prepared from four randomly chosen rats per group. Specimens were dehydrated in a series of increasing ethanol concentrations then embedded in paraffin. Tissue sections (5 μm) were stained with haematoxylin and eosin (HE) and Masson-trichrome. At least three slides were prepared from each specimen and blindly examined. Histopathological scoring was achieved via an expert pathologist using Nichon microscope for detection of pathological changes. 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. The scoring system was devised in conjunction with the experienced liver hist opathologies of Kerry Thompson to

Table 1. Effect of TTG on the levels of liver enzymes compared to silymarin after CCl4 intoxication Group I Group II Group III Group IV

Period	Group	AST (mg/dl)	ALT (mg/dl)	ALP (u/l)	Albumin (g/L)
4 week	Health	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 = 10)
ALT Alanine aminotransferase, AST Aspartate aminotransferase, ALP Alkaline phosphatase, **p < 0.01 (compared to CCl4 (group II)) and +++p < 0.001 (compared to control group I). No significant difference (p = 0.3 or higher) between group III (150 mg/kg SFEE) and group IV (silymarin), using one-way ANOVA test followed by Tukey-Kramer test *p < 0.05

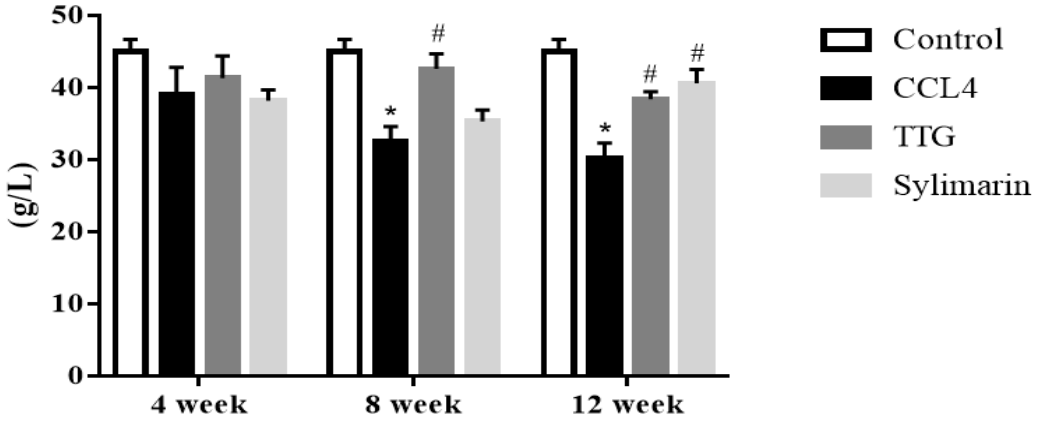

Figure 1: Effect of treatment on the serum albumin level. a. TTG treatment resulted in a significant improvement in the albumin compared to CCl4 treated animals (n=15, p < 0.05). Silymarin-treated group and control (n = 15, p <0.05). Data are expressed as mean ± SEM, significance was calculated using one-way ANOVA followed by Tukey-Kramer multiple comparisons post hoc test.

assess both qualitative and quantitative changes.

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 tukey post hoc test was performed. Graph Pad Prism-5 software was used for statistical analysis with p< 0.05 considered statistically significant.

RESULTS

Effect of TTG on the activation liver enzymes of serum

In the group treated with TTG (treatment group), serum ALT level decreased by 29.8% in 4 weeks, % 22 in 8 weeks, % in 3.7 fold 12 weeks (Table 1) compared to the control group (p<0.001). There were statistically significantly decreased in serum AST level between the control groups. Serum ALP level was reduced by 17.2%, 9.3%, 3.6 fold in 4, 8, 12 weeks compared to the control group (p>0.05). According to Figure 1, serum albumin level in the TTG group was increased by 31.1% in 8 weeks, 27.1 % in 12 weeks respectively, compared to the control group (p<0.05).

Effect of TTG on the SOD level of serum

The hepatic tissue content of SOD was measured in the control, TTG, silymarin treated groups as shown in Figure 2a. The SOD of the control group significantly decreased from the healthy group (p < 0.05). In the treatment group

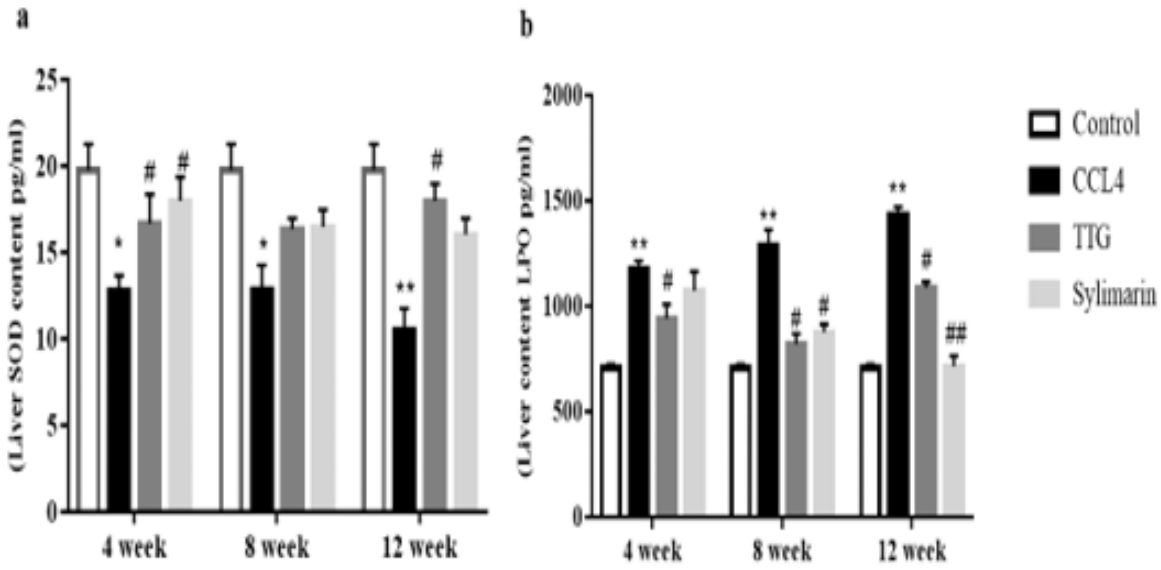

Figure 2: Effect of TTG treatment on the total hepatic SOD content and lipid peroxidadase. a. TTG treatment resulted in a significant improvement in the hepatic SOD content compared to CCl4 treated animals ($p < 0.05$). No significant difference could be detected among TTG-treated group,silymarin-treated group and control ($n = 15$, $p > 0.34$). b. TTG treatment resulted in normalization of lipid peroxidation (measured as LPO) despite CCl4 co-administration ($n = 15$, $p < 0.001$). Data are expressed as mean ± SD, significance was calculated using one-way ANOVA followed by Tukey-Kramer multiple comparisons post hoc test.

treated with the TTG the SOD level is increased by compared to the control group and similar results were found to have the same effect on the Silymarin.

Effect of TTG on the lipid peroxidation

Lipidperoxidase (LPO) of lipid peroxidation and is considered as one of its markers. In the current study, we found that CCl4 treatment of animals resulted in a significant, 2.06 folds, increase in the tissue level of LPO ($P < 0.001$) compared to healthy rats (Figure 2b). TTG treatment resulted in a significant prevention of the CCl4-induced level of LPO ($P < 0.05$) compared to the control group.

Histopathological observations of the TTG

From the histological point of view, liver from rats in the healthy control group showed a normal liver lobular architecture and hepatocyte structure (Figure 3a). In contrast, CCl4 administration resulted in histopathological lesions and extensive hepatocellular damage, as represented by the presence of portal inflammation, and venous congestion (Figure 3b, c). Treating the tested animals with TTG was capable of ameliorating these histopathological changes (Figure 3d, f), producing similar effects to that achieved by the treatment with Silymarin (Figure 3 g, e). TTG as well as silymarin were able to significantly decrease the signs of CCl4 –induced fibrosis (Figure 3 j, h).

DISCUSSION

Liver fibrosis is the excessive accumulation of extracellular matrix proteins including collagen that occurs in most types of chronic liver diseases (Constandinou et al., 2005; Friedman, 2003). Liver fibrosis is a consequence of chronic liver lesion, which can progress into liver cirrhosis even hepatocellular carcinoma (Seeff et al., 2001). 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 (Niki et al., 2009; Ferreira et al., 2010; Messner et al., 2012; Fang et al., 2008). 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

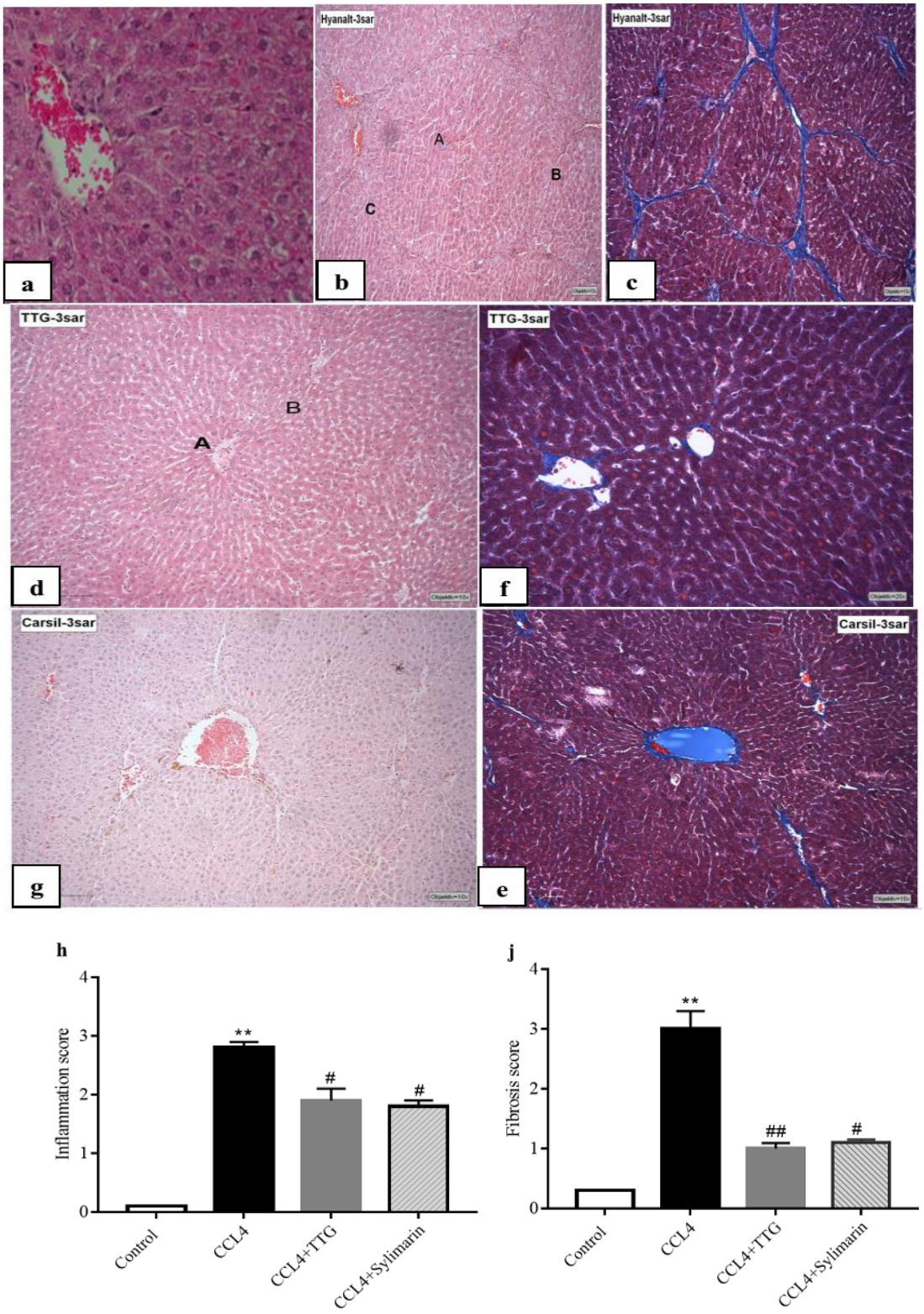

Figure 3: Histological examination of liver sections from different groups. Liver sections from healthy control show normal hepatocytes architecture (a), whereas CCl4 treatment resulted in damaged cells, shrunken nuclei, mitotic activity (arrow heads) and centrilobular congestion (b & c). TTG treatment 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, $p < 0.05$. Significance was calculated using Kruskal-Wallis test followed by Dunn's multiple comparison post hoc test.

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.

Competing interests

The authors declare that they have no competing interests.

Acknowledgements

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