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The Hepatoprotective Activity of the Traditional Multicomponent Formulation Gurgem-13 on Carbon Tetrachloride (CCL4) Induced Experimental Liver Injury in Rats

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Objectives: The purpose of present study was intended to evaluate hepatoprotective effects of traditional multicomponent formulation Gurgum13 on carbon tetrachloride (CCI4) induced liver injury model in rats. **Methods:** Chronic toxic liver injury was induced experimentally by intraperitoneal injection (IP) of 1.0 ml/kg body weight of 10% carbon tetrachloride (CCI4) three times per week for duration of three months in rats. A total of five groups of animals with 15 rats in each were used for the experiment. Group 1 was as a normal control (intact). Group 2 served as a control group received CCI4 1.0 ml/kg IP three times a week for 3 months. Group 3, 4 and 5 were as the treatment groups received CCI4 1 ml/kg IP and administered traditional multicomponent formulation Gurgum-13 as a test drug (100 mg/kg) for 3 months daily. Milk thistle (50 mg/kg) and Lilicoaguliant (LLC) (100 mg/kg) were given daily for 3 months as a reference drug respectively. The activity of Gurgum-13 formulation and other comparative drugs were investigated with the dynamic study of liver marker enzymes, AST, ALT, ALP, bilirubin, and the antioxidant and blood coagulating effects of the remedy, which were studied in three months of experimentation. Gurgum-13 is one of the Mongolian traditional multicomponent formulation used in practice for a range of diseases including liver disorders. **Results:** Carbon tetrachloride significantly decreased the liver functions as assessed through an increase in blood serum and liver marker enzymes in control groups. Gurgum-13 treatment significantly prevented hepatotoxicity of carbon tetrachloride by reducing ALT, AST, ALP marker enzymes and bilirubin in blood serum and liver tissue in the three months period of chronic hepatitis ($p<.05$) similar to reference drugs. The result of the study revealed that Gurgum-13 is significant hepatoprotective against liver injury from CCI4 induced hepatotoxicity by having antioxidant effect and decreasing lipid peroxidation in liver. **Conclusion:** Multicomponent traditional formulation Gurgum-13 demonstrated very good hepatoprotective effect against CCI4 induced liver injury in rats. Thus, Gurgum-13 can be an effective remedy for the treatment of chronic hepatitis and biliary tract disorders.

Keywords: Multicomponent formulation, Liver injury, Gurgum-13, Hepatoprotective remedy, Lilicoaguliant

Introduction

Despite the scientific advances in the field of modern hepatology, liver disorders are still increasing as a worldwide health problem. Mongolia has the highest rate of hepatocellular carcinoma (HCC)-related mortality due to its high prevalence of viral hepatitis caused by hepatitis B virus (HBV), hepatitis C virus (HCV) and hepatitis Delta virus (HDV) [1]. Health statistics of Mongolia showed that in 2017, liver cancer accounted for 38.1%, of all new recorded cancer cases and the incidence rate of liver cancer was 74.6 per 100 000 population [2]. Synthetic conventional medicines for the treatment of liver disorders are inadequate and have serious side effects. Consequently, we need to seek innovative hepatoprotective constituents by searching for alternative drugs for the treatment of liver diseases. In recent years, traditional systems of medicine have gained importance and popularity. These systems include numerous hepatoprotective components and formulations which are safe, effective, relatively inexpensive with low side effects to treat a wide variety of liver disorders.

According to the principles of Traditional Mongolian Medicine, the multi-component formulation Gurgum-13 is an important therapeutic agent in various traditional formulations used for the treatment of liver and biliary tract disorders. The clinical application of a multicomponent formulation of Gurgum-13 dates back to ancient times. As written in ancient scripture the composition cleanses the liver and blood, normalizes the function of the liver, gallbladder and biliary tract, eliminates intoxication, eliminates the "heat" of the liver, helps with general weakness and physical fatigue («nourishing» - increases blood and 7 bodily constituents) [3]. It is used traditionally to treat viral hepatitis, hepatitis of a different etiology, including chemical medications. Gurgum-13 is based on the dye from a safflower flower and consists of thirteen different components, namely flowers *Carthamus tinctorius L*., fruits of *Terminalia chebula* Retz., *Terminalia belerica Roxb*., *Gardenia jasminoides* Ellis., *Caesalpinia crista L*., Buds of *Eugenia caryophyllata* Thunb., the roots of *Aconitum Kuznezoffii* Reichenb., and *Saussurea lappa* C.B.Clarke., wood *Peterocarpus santalinus* L.F., *Cinnamomum cassia* Presl., bark, horns *Saiga tatarica Linnaeus*., Musk, *Moschus mochiferus Linnaeus*., and gallstones Bos taurus domesticus [3-4].

According to the Pharmacopoeia of traditional medicine, raw materials used in the Mongolian traditional medicine Gurgum-13 have cooling properties and it is recommended for liver dysfunction, poisoning, inflammatory diseases of the kidneys, urine, edema, sinusitis and helminth infections [4].

However, until now, no systematic study has been carried out to reveal the pharmacological activity and hepatoprotective efficacy of the multicomponent formulation Gurgum-13 for hepatic diseases in Mongolia. Therefore, it was essential to evaluate the pharmacological hepatoprotective effect of Gurgum-13 in an experimental chronic liver damage model induced by carbon tetrachloride in rats. This effort may lead to broadening the horizon of existing remedies for liver diseases by use of a traditional formulation which provides multiple protection effects by supporting liver function through antioxidant effects and regeneration of hepatic cells.

We have undertaken this study to evaluate the hepatoprotective and pharmacological efficacy of this traditional multicomponent formulation Gurgum13 in an experimental model of liver damage induced by carbon tetrachloride (CCl4) in rats.

Materials and Methods

Preparation of Multicomponent Formulation

Traditional multi-component formulation Gurgum-13 is a uniform mixture of thirteen components, specifically flowers *Carthamus tinctorius L.,* fruits of *Terminalia chebula Retz., Terminalia belerica Roxb., Gardenia jasminoides Elli., Caesalpinia crista L.,* Buds of *Eugenia caryophyllata* Thunb., the roots of *Aconitum Kuznezoffii Reichenb.,* and *Saussurea lappa* C.B. Clarke., wood *Peterocarpus santalinus L.F., Cinnamomum cassia Presl.*, bark, horns *Saiga tatarica Linnaeus.,* Musk, *Moschus mochiferus Linnaeus.,* and gallstones Bos taurus domesticus [3-4].

Animals

Wistar albino rats with weight range of 180-220 gr, of either sex were obtained from the Institute of Traditional Medicine and Technology. All animals were kept under condition of room temperature (20 ± 2°C) and 12 h cycles of light and dark and received standard diet and water.

The rats were randomly selected and were divided into different groups with fifteen animals in each group, except

Table 1. The model of hepatotoxicity for experimental animals

Groups	n	Treatment
Normal group	10	2.5 ml distilled water was given orally for each day
Control group	15	10% CCl4, 1.0 ml/(kg·d), IP 3 times a wk 0.25% CMC, 1 ml/(kg·d), PO 5 times a wk
Milk thistle	15	10% CCl4, 1.0 ml/(kg·d), IP 3 times a wk 50 mg/kg Milk thistle was given orally each day for 3 months
Lilicoaguliant	15	10% CCl4, 1.0 ml/(kg·d), IP 3 times a wk 100 mg/kg Lilicoaguliant was given orally each day for 3 months
Gurgum 13	15	10% CCl4, 1.0 ml/(kg·d), IP 3 times a wk 100 mg/kg Gurgum-13 was given orally each day for 3 months

normal control (n=10). Experiments were carried out according to the rules of procedure with the use of experimental animals (see Table 1).

Experimental Model of Hepatotoxicity for Experimental Animals

Method: Carbon Tetrachloride induced experimental liver injury in rats. Chronic toxic liver injury was induced experimentally by intraperitoneal injection (IP) of 1.0 ml/kg body weight of 10% carbon tetrachloride (CCI4) three times per week for duration of three months in rats (method of Da Hee Jeong, Gippeum Lee, Won-Il Jeoung 2005) [5]. The animals were divided into five groups containing fifteen animals in each group. Except normal control (intact) all other groups (CCI4 control, Gurgum-13 formulation treated, reference groups 1.2) received 1.0 ml/kg CCI4 IP three times per week for duration of three months to induce hepatotoxicity. Carbon tetrachloride is a widely used hepato-toxin which has been widely used to induce hepatic injuries in animal-based pharmacological studies of liver disorders [5, 6-8].

The hepatoprotective effects of multi-component formulation Gurgum-13 was assessed over a three-month experimental period. The normal control group received distilled water daily. The treatment group animals received Gurgum-13 formulation at a oral dose of 100 mg/kg according to the acute toxicity LD 50 as a test remedy. The reference groups 1.2 received Milk thistle orally at a standard dose of 50 mg/kg, and Lilicoaguliant orally at 100 mg/kg, while the control group (CCI4) received distilled water daily. The standard reference drug Milk thistle is one of many examples of pharmacologically known natural substances that have shown a strong hepatoprotective potential due to their antioxidant, anti-inflammatory, and liver regenerative capabilities [9].

Assessment of Hepatoprotective Activity

Liver function activity was assessed by using biochemical parameters for ensuring the liver damage or efficacy of test formulation.

Liver function tests: blood samples were collected to measure biochemical parameters for dynamic changes in Serum Aspartate transaminase (AST), Alanine transaminase (ALT), Serum Alkaline Phosphatase (ALP), Serum total bilirubin (TB), Serum Albumin (ALB), Lipid peroxidation marker (LPO), and Superoxide dismutase (SOD) in blood serum and in the liver tissue homogenate each month over three-month experimental period. Biochemical parameters were defined by using a fully automated biochemical analyzer. Finally, at the end of each months five rats of each group were sacrificed, liver removed and observed for morphological change and then fixed in 10% formalin for histological studies of the liver to determine the degree of hepatic change in different groups.

Assessment of Blood Coagulation

Prothrombin time (PTT), activated partial-thromboplastin time (APTT), and thrombin activity (TT) were estimated by reported standard methods as indication for blood coagulation. PTT was measured using the Quick's one-stage assay and APTT by means of modified APTT assay using an EA-containing APTT reagent. Thrombin activity (TT) was determined by PTT assay with a PK-B hem-agglutination analyzer (Zhongshan Peikang Limited Company for Medical Electronic Instruments, Zhongshan, China) [10-11].

Statistical Analysis

All data set were expressed as means ± SD. Groups of data were compared with an analysis of the Kruskal-Wallis H test. Pairwise comparisons were performed. Significant values have been adjusted by the Bonferroni correction for multiple tests. Differences between the groups were considered significant at p<.05. Statistical analysis was performed in SPSS version 23.0.

Ethical Statement

Animal experiments were carried out 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 (№ 2019.3-07, June 06, 2019) was approved by members of "The Research Ethics Committee" and by the Mongolian National University of Medical Sciences. All animals were kept in the standard condition.

Results

The Result of Acute Toxicity

In the acute toxicity study, the LD_{50} value of Gurgum-13 was 7.08 (6.2-8.0) gr/kg, which is considered as a compound of low toxicity according to the V.A Berezovskaya classification.

The Result of CCL4 Induced Experimental Model of Hepatic Injury in Experimental Rats

Carbon Tetrachloride (CCI4) induced hepatotoxicity CCl4 in a dose of 10%-1.0 ml/kg by injection intra-peritoneally IP produced significant hepatic injury in control group (CCI4 treated) when compared with normal control. Administration of CCI4 causes chronic liver damage that mimics natural liver damage causes. It mediates changes in liver function that ultimately leads to destruction of hepatocellular membrane, peroxidation of lipids and elevated levels of liver marker enzymes AST, ALT, ALP and bilirubin, causing extensive liver damage and necrosis. Accordingly, in the present study, similar substantial elevation in serum marker enzymes levels such as AST, ALT and ALP were observed in blood serum of control group in comparison to a normal control group. It shows the hepatocellular necrosis and liver damage caused by CCI4. Thus, in the case of serum AST, a nearly 2,42-fold elevation in the control group in first month (normal control 111.2±10 mg/dl, control 269.3±23.5 mg/dl), a 2.61 times elevation in second month (control 290.3±26.4 mg/dl) and a 5.3 times elevation in third month (control 591.0±36.6 mg/dl) was found respectively when compared to a normal control group. ALT level was elevated by 2.1-3.5 times during the three months period of experiment. (p<.001) (see Table 2).

The Result of Biochemical Analysis

Effect of traditional multicomponent formulation Gurgum-13 on serum ALT, AST and ALP levels.

Treatment with the traditional multicomponent formulation Gurgum-13 at a dose of 100 mg/kg for 30 days after CCl4 intoxication showed a significant decrease in the levels of serum marker enzymes indicating the protection of hepatic cells as well as the reducing of liver necrosis, improving the regeneration of liver membranes and having a hepatoprotective effect. Consequently, the elevated level of hepatic biochemical parameters AST marker enzymes decreased by 26.2%, 23.4% and 74% respectively in a three-month period of chronic toxic hepatitis, whereas ALT enzyme levels decreased by 41%; 44% and 66.4% respectively

Table 2. Effects of carbon tetrachloride (CCI4) on biochemical parameters of blood serum experimental model of chronic hepatitis

Variables	Normal Control Group (I) (n=10)	Control Groups (CCI4) (II)		
		I month (n=5)	II month (n=5)	III month (n=5)
	Mean+/-SD	Mean+/-SD	Mean+/-SD	Mean+/-SD
AST[a] (mg/dl)	111.2+/- 10	269+/-23.5**	290+/-26.4	591+/-36.6
ALT[b] (mg/dl)	98.8+/-1.7	219.8+/-26.3	209.3+/-8.8	352+/-9.1
Total bilirubin	8.5+/-0.7	17.01+/-2.1	14.5+/-0.8	10.1+/-1.0
ALP[c]	200.5+/-12.0	260.3+/-17.9	322.1+/-25.7	807.3+/-31.2
Albumin	45.0+/-1.7	39.9+/-3.8	32.5+/-2.1	30.2+/-1.8

[a]AST-Aspartate aminotransferase, [b]ALT-Alanine aminotransferase, [C]ALP-Alkaline phosphatase. Values represent Mean ± SD. (n=15 in each group). **p-value<.001 is significant, *p-value<.05 is significant (differences between indicators of normal control (intact) and control (CCI4) groups. Group I: normal control (intact); Group II: control (CCL4 treated).

(p<.001). Also, AST enzyme level decreased by 28.8%, 44.2 % in the Gurgum-13 treated group in the third month experiment when compared with first and second month, whereas ALT enzyme level decreased by 9.5 % respectively. These results were confirmed by histopathological examinations of liver sections and were comparable with the reference drug Milk thistle (see table 3).

Effect on Serum Bilirubin Level

Total bilirubin level was markedly elevated by 16-41.2% in the control group (CCI4) when compared with the normal control (p<.001) in chronic hepatitis model, induced by carbon tetrachloride (CCI4) in rats. Table 2. Treatment with multicomponent formulation Gurgum-13 significantly reduced the level of total bilirubin by 29.4%, 43%, and 12% respectively when compared with the control group (CCI4) in three-months of the experimental period as shown in Table 3. This result shows that the formulation has a good effect on biliary tract obstruction.

Effect on Serum Alkaline Phosphatase Level

Alkaline phosphatase (ALP) levels increased in the control group (CCI4) by 29%, 60.6 % in the first and second months, whereas 4 times in the third months of experiment respectively (p<.001) when compared with the normal control group (see Table 2). It illustrates the biliary tract obstruction process, which occurred during the chronic liver damage model caused by CCI4. However, in contrast with the control group, ALP in the Gurgum-13 treated group decreased by 38.2%, 6.2%, 67.1%, and in Milk thistle group by 20.2%, 17%, and 70.5% respectively during the three months. Although, in Lilicoaguliant group, it decreased by 14.3%, 16.2% and 74,1% in the three months period of experiment. In addition, ALP enzyme level decreased by 13.6% in the Gurgum-13 treated group in the third month of experiment when compared with the second month. The results of the present study confirmed the hepatoprotective effect of Gurgum-13 in CCl4-induced liver toxicity (p<.001) (see table 3).

Table 3. Effects of traditional multicomponent formulation Gurgum-13 on biochemical parameters in chronic hepatitis, induced by carbon tetrachloride (CCI4) in rats

Variables	Experimental groups			
I month	I	II	III	IV
AST[a] (mg/dl)	269.3±23	198.7±18.2*	205.0±24.8	205.0±24.8
ALT[b] (mg/dl)	219.8±26.3	129.7±4.8*	213.9±21.6	154.5±9.7
Total bilirubin	17.01±2.1	12.0±0.2*	13.6±2.2	8.9±07*
ALP[c]	260.3±17.9	207.8±21.3*	207.8±21.3*	223.0±12.0
Albumin	39.0±3.8	38.2±1.5*	38.2±1.5	37.6±3.4
II month				
AST[a] (mg/dl)	290.3±26.4	222.5±16.3*	191.1±14.6**	212.0±8.8**
ALT[b] (mg/dl)	209.3±8.8	117.2±9.2*	117.2±7.09**	169.0±5.8*
Total bilirubin	14.5±0.8	8.3±0.8*	9.9±0.7	6.6±0.5*
ALP[c]	322.1±25.7	302.8±23.5*	267.8±19.5	269.8±17.4*
Albumin	32.5±2.1	33.6±2.07	35.3±2.05*	32.9±3.1
III month				
AST[a] (mg/dl)	591.0±36.6	154.3±13.3**	158.4±7.2**	132.3±9.7**
ALT[b] (mg/dl)	352.2±9.1	118.4±8.2**	130.7±3.9**	144.3±13.0
Total bilirubin	10.1±1.0	8.9±0.9	7.3±0.5*	6.6±0.6*
ALP[c]	807.3±31.2	266.6±18.8**	238.1±22.4	209.5±16.4**
Albumin	30.2±1.8	41.0±1.2**	40.6±4.9**	44.6±2.8**

[a]AST- Aspartate aminotransferase, [b]ALT- Alanine aminotransferase, [C]ALP- Alkaline phosphatase

Values are mean ± SD, (n=15 in each group) **p-value<.001 is significant, *p-value<.05 is significant (differences between indicators of II and I groups, III and I groups, IV and I groups.

Group I: Control (CCL4 treated); Group II: Gurgum-13 (100 mg/kg) + CCl4 treated; Group III: Milk thistle (50 mg/kg) + CCl4 treated; Group IV: Lilicoaguliant (100 mg/kg) + CCl4 treated.

Effect on Serum Albumin Level

Serum albumin level decreased by 13.3-33% in the control (CCl4) group during three months of experimental period leading to structural and functional abnormalities of the liver when compared with the normal control (group I) (see table 2). Treatment with the Gurgum-13 formulation increased albumin levels by 5.8%, 32% and 27.2% within the three months period respectively ($p<.05$), preventing liver cell damage, and increasing the synthesis of albumin levels in blood serum. Serum albumin level increased by 7.3 and 22% in the Gurgum-13 treated group in the third month of experiment when compared with first and second months. So, it is reasonable to assume that Gurgum-13 is hepatoprotective. The treatment with Gurgum-13 formulation showed a reduction of raised liver marker enzymes level induced by CCL4 with parallel increase of serum albumin level, indicating the formulation could improve liver function and protect liver. Thus, multicomponent formulation Gurgum-13 has demonstrated very good hepatoprotection against the CCl4 (see Table 3).

Effect on LPO in Chronic Hepatitis, Induced by CCl4 in Rats

It has been reported that lipid peroxidation (LPO), which reduces the activity of antioxidant enzymes and generates free radicals, is the main method by which CCl4 induces liver injury.

Consequently, the CCl4 control group showed a significant increase in lipid peroxidation (LPO) level in blood serum and liver tissue homogenate as compared with a normal group. Thus, it was shown that lipid peroxidation toxic products (LPO) in blood serum intensified by 3 times in the first month (see Table 4). Further, LPO intensified by 71% in second month and 41% in the third month of experiment. ($p< .001$). This indicates that CCl4 toxin induced peroxidation in blood serum and liver tissues, leading to liver damage. However, this elevated level of serum and liver tissues LPO was markedly inhibited by the test medicine Gurgum-13 formulation and the standard reference drug Milk thistle. Hence, treatment with Gurgum-13 formulation reduced liver LPO by 14.3%, 13% and 29.1% during the three months period of experiment, whereas Milk thistle reduced liver LPO by 50.1% only in third month, while Lilicoaguliant reduced liver LPO by 14.3%-22% within 3 months. Gurgum-13 formulation also inhibited serum LPO by 34%, 17.7% and 16%, while Milk thistle reduced 26%, 38% and 6 %, and Lilicoaguliant 24%, 30% respectively within 3 months. In addition, liver LPO level decreased by 30.3%, where as serum LPO level decreased by 74.6% and 29.5% in the Gurgum 13 treated group in the third month of experiment when compared with the first and second months respectively (see Table 5).

Effect on antioxidant enzyme Superoxide Dismutase (SOD) in chronic hepatitis, induced by CCl4 in rats.

Superoxide dismutase (SOD) is the body's primary free radical scavenger, and a poweful antioxidant enzyme was found to be reduced in blood serum and liver homogenate in chronic hepatites induced by CCl4 (see Table 3). However, treatment with Gurgum-13 formulation markedly increased the SOD in liver homogenate by 4%, 12% and 40% respectively in three months period, whereas blood serum SOD increased by 23%, 14.3% and 14.6% in the three months period of experiment ($p< .05$). Moreover, SOD level in the liver homogenate increased by 20.3% in the third month of experiment, in the Gurgum-13 treated group when compared with the first month, where as serum SOD level decreased by 4.5% in the third month to compare with the first month and it was increased by 2.5% when compared with the second months, which was not significant. The multicomponent formulation Gurgum-13 produced a significant decrease in the lipid peroxidation process with associated increase in the level of superoxide dismutase

Table 4. Effect of carbon tetrachloride (CCl4) on LPO and SOD of blood serum in experimental model of chronic hepatitis

Variables	Normal Control Group (I) (n=10)	Control Groups (CCl4) (II)		
		I month (n=5)	II month (n=5)	III month (n=5)
	Mean+/-SD	Mean+/-SD	Mean+/-SD	Mean+/-SD
LPO[a] liver	707+/-16.5	1175+/-15.5	887.8+/-67.0	1435+/-35.8
LPO[a] serum	573.6+/-60	1872+/-82.9	980+/-81.2	807.0+/-24.8
SOD[b] liver	19.8+/-2.5	12.8+/-0.2	12.9+/-1.4	10.6+/-2.1
SOD[b]serum	8.4+/-1.0	7.0+/-0.8	7.5+/-0.6	7.5+/-1.0

[a]LPO - Lipid peroxidase, [b]SOD - Superoxide dismutase. Values are mean ± SD, (n=15 in each group) **p-value < .001 is significant, *p-value < .05 is significant (differences between indicators of normal control (intact) and control (CCl4) groups. Group I: normal control; Group II: control (CCL4 treated).

Table 5. Effect of traditional multicomponent formulation Gurgum 13 on lipid peroxidase (LPO) and superoxide dismutase (SOD) in chronic hepatitis, induced by carbon tetrachloride (CCl4) in rats

Variables	Experimental groups			
I month	I	II	III	IV
LPO liver	1178±15.5	1010±78.8*	1072±12.0	1010±77.8*
LPO serum	1872.8±82.	1240.7±24.4*	981.2±12.2**	1311.4±58.9*
SOD liver	12.8±0.2	12.3±2.6*	21.2±0.4*	14.4±1.4
SOD serum	7.0±0.8	8.6±0.6*	8.5±1.9*	7.9±0.5
II month				
LPO liver	887.8±67	780.7±80.0	876.7±38.0	731.0±14.5*
LPO serum	980.5±81.2	920.0±15.0*	608.2±14.1*	535±48.0**
SOD liver	12.9±1.4	14.4±0.2*	16.5±0.1*	13.8±2.3
SOD serum	7.5±0.6	8.0±1.0*	10.3±0.6*	7.4±0.3
III month				
LPO liver	1435±35.8	1017±81.0*	716.0±22.8**	1123±16.0*
LPO serum	807.0±24.8	710.5±12.6*	760.3±48.3*	614.0±69.2*
SOD liver	10.6±2.1	14.8±0.2**	16.1±0.9*	13.8±0.2*
SOD serum	7.5±1.0	8.2±0.6*	8.5±0.2*	8.9±0.8*

[a]LPO - Lipid peroxidase, [b]SOD - Superoxide dismutase. Values are mean ± SD. (n=15 in each group) **p-value< .001 is significant, *p-value <. 05 significant. Group I: Control (CCL4 treated); Group II: Gurgum 13 (100 mg/kg) + CCl4 treated; Group III: Milk thistle (50 mg/kg) + CCl4 treated; Group IV: Lilicoaguliant (100 mg/kg) + CCl4 treated.

(SOD) against hepatotoxicity induced by CCl4 in rats compared to control group as shown in Table 5. The present study suggests that multicomponent formulation Gurgum-13 possesses a wide range of hepatoprotective effect in experimental model of liver damage. It relieves symptoms of cytolysis, liver function failure, and demonstrated antioxidant, hepatoprotective and choleretic effect, which is probably due to its antioxidant property and scavenging free radicals. The hepatoprotective activity can be connected with the bioactive compounds of the formulation.

Effect on blood coagulation disturbance and hemostasis in liver injury, induced by CCL4

The liver plays a vital role in the blood coagulation process because it is the location of synthesis of all coagulation factors and their inhibitors. So, chronic liver injury and cirrhosis induced by CCl4 is constantly connected with variable changes of blood hemostasis. In our study the basic laboratory tests of coagulation such as prothrombin time (PTT), activated partial-thromboplastin time (APTT) and thrombin time (TT) have been used to assess the risk of bleeding in experimental chronic liver injury. These measurements remain as a prognostic marker in a variety of conditions in both acute and chronic liver diseases. Results of the studies showed a significant increase of PTT values after administration of CCl4, reflecting a prolonged coagulation time. For instance, the values of PTT activities of control group were increased by 1.5 times in comparison with the normal group (control 14.7±0.3 sec, normal group 9.5±1.0 sec.) ($p<.05$). Table 6. However, treatment with Gurgum-13 reduced PTT values by 1.9-2.0 times in second and third months of experiment. We can assume that Gurgum-13 nearly returned the prolonged PTT close back to the normal control group values in experimental chronic liver injury (normal group 9.5±1.0 sec, Gurgum-13 group 9.6±0.7 sec) in the second month). PTT values decreased by 75.5% in the Gurgum-13 treated group in the third month of experiment when compared with the first month. PTT is used to evaluate the extrinsic clotting pathway. A prolonged PTT indicates a deficiency in coagulation factors V, VII and X (Azevedo APS et al 2006) [10-11].

An interesting result was in the Lilicoaguliant treatment group, where PTT reduced by 53.4 % in the second month, 32% in the third month respectively, while APTT reduced by 20 % in the second month ($p<.05$). Additionally, Gurgum-13 formulation reduced the thrombin time (TT) by 40% in the third month, while APTT values decreased by 14.2%-40% in the first two months of experiment ($p<.05$). Further, thrombin time (TT) decreased by

15.3% and 21% in the Gurgum-13 treated group, whereas APTT values increased by 23% and 84.5% in the Gurgum-13 treated group in the third month of experiment when compared with the first and second months respectively. In case of fibrinogen, it decreased by 35% in control group compared with the healthy group (control group (CCI4) 2.8±0.2 gr/l, normal control group 2.07±0.5 gr/l) because of CCI4 induced injury ($p<.05$). Treatment with Gurgum-13 formulation increased fibrinogen level by 16%-19% during the first two months, and 43% in the third month respectively when compared with CCI4 control group ($p<.05$). However, fibrinogen level decreased by 20% in the Gurgum-13 treated group in the third month of experiment when compared with the first and second months respectively. From the results of this study we propose that Gurgum-13 has a stimulatory effect on the synthesis of fibrinogen in the liver and has a protective effect against CCI4 induced coagulation disturbances in rats (see Table 6).

Discussion

Traditional multicomponent formulations are as an effective source of liver disease treatment. The multicomponent traditional formulation Gurgum-13 contains thirteen different herbal, animal and mineral compositions which contain specific therapeutically active ingredients, which are traditionally used in different liver disorders. According to the Tibetan, and Mongolian traditional medicine theory three of thirteen components of Gurgum-13, such as *Cartamus tinctorius* L., gallstones from Bos Taurus Domesticus, and *Terminalia chebula Retz.* are considered as major components, which have a general balancing effect, others are auxiliary, or assistant components having local and symptomatic effects. As distinct from the target effect of the drugs, the multicomponent formulation produces influence at all levels of the functional regulation. In that way they act according to the principle of multi-target mechanism [12-14]. More than 200 compounds have been isolated from *C.tinctorius* L. and the commonly known ones are flavonoids, phenylethanoid glycosides, coumarins, fatty acids, steroids and polysaccharides. Safflower is a very good purgative, analgesic, antipyretic and an antidote to poisoning. Flavonoid glycosides, carthamin, a flavonoid type dye and safflower yellow are the main constituents in the flower of C.tinctorius L. [15-17]. Scientific research conducted by Badamsuren D (2009) et al. [19] discovered that *C.tinctorius* L. reduced ALT, ASP, ALP, glutamine-

Table 6. Effects of traditional multicomponent formulation Gurgum 13 on blood coagulation in chronic hepatitis, induced by carbon tetrachloride (CCI4) in rats

Variables	**Experimental groups**				
I month	I	II	III	IV	V
PTT[a]	9.5±1.0	14.7±0.3*	17.9±1.2*	12.0±0.6**	12.8±1.1**
APTT[b]	11.2±1.4	19.22±1.8*	16.5±1.8*	13.7±1.7*	18.0±0.9
TT[c]	27.05±4.1	40.0±2.1*	41.4±4.8*	43.0±1.2*	43.1±0.8*
Fibrinogen	2.8±0.2	2.07±0.5*	2.4±0.3*	3.11±0.2	2.01±0.5
II month					
PTT[a]		18.9±1.2	9.6±0.7	12.1±2.5*	8.8±1.7**
APTT[b]		18.3±0.6	11.0±0.9	19.3±2.5	14.7±0.6*
TT[c]		36.5±1.0	38.6±1.2	47.6±1.0*	35.5±3.8
Fibrinogen		2.02±0.2	2.4±0.6	2.3±0.5	2.2±0.3
III month					
PTT[a]		20.6±2.5	10.2±1.4*	11.2±1.3*	14.0±1.2*
APTT[b]		16.1±0.6	20.3±2.0	16.3±0.6	17.2±0.5
TT[c]		54.1±4.5	32.7±4.8*	49.2±1.4	45.1±3.4*
Fibrinogen		1.4±0.3	2.0±0.3	2.2±0.7	2.0±0.4

[a]PTT - Prothrombin time, [b]APTT- Activated partial-thromboplastin time, [c]TT- Thrombin time. Values are mean ± SD. (n=15 in each group). **p-value< .001 is significant, *p-value< .05 significant. (differences between indicators of II and I groups, III and II groups, IV and II, V and II groups). Group I: Normal control (intact); Group II: Control (CCL4 treated); Group III: Gurgum-13 (100 mg/kg) + CCl4 treated; Group IV: Milk thistle (50 mg/kg) + CCl4 treated; Group V: Lilicoaguliant (100 mg/kg) + CCl4 treated.

pyruvate transaminase and lactate dehydrogenase enzyme levels on chronic liver injury induced by CCl4. Additionally, it reduced hepatic cytolysis and necrosis, and improved regeneration of hepatic cells [17-18]. *C.tinctorius* L. flower can act as a biological antioxidant [19]. Gallstone from Bos Taurus Domesticus (natural bezoar, gallstone) contains bilirubin, bile acids, deoxycholic acid, bile salts, cholesterol, ergosterol, fatty acids, lecithin, vitamin D and mineral elements of calcium, sodium, iron, potassium, copper, magnesium and phosphorus. In addition, it contains carotenoids, alanine, glycine, taurine, aspartic acid, arginine, leucine, methionine, a variety of amino acids. It was revealed that taurine, contained in gallstone has a significant protective effect on acute and chronic liver damage induced by carbon tetrachloride [20-21]. *Terminala chebula Retz.* is considered as a 'King of Medicine' due to its promising medicinal value in the management of various diseases and disorders such as antidiabetic, antimicrobial, antioxidant, anti-mutagenic, anti-proliferative, anti-inflammatory, cardioprotective and wound healing activity. The fruit extracts of T.chebula Retz. contain different bioactive phytochemicals such as alkaloids, glycosides, flavonoids, saponin, quinine, steroids and tannin.

Phenolic compounds from the fruit of T.chebula Retz. exhibited good antioxidant properties [22-23]. Terminalia chebula Retz. fruit represents all tastes except salt, one of the many reasons it is designated in traditional medicine as a tonic, good for health and long life. It is also can be used with any type of health imbalance [24-25]. Consequently, combined action of all the ingredients of Gurgum-13 formulation helps to normalize the liver function and can cure complex liver disorders. Administration of CCl4 causes chronic liver damage that mimics natural liver damage causes. CCl4 is widely used hepatotoxic drug for pharmacological studies [6-8]. It mediates changes in liver function that ultimately leads to destruction of hepatocellular membrane, which leads to peroxidation of lipids and elevated levels of liver marker enzymes AST, ALT, ALP and bilirubin, causing extensive liver damage and necrosis. In our study, similar substantial elevation in AST and ALT, ALP enzyme levels were observed after administration of CCl4 showing the liver damage and inflammation of liver tissues in rats [6-8, 17]. Thus, in case of serum AST, a nearly two-fold elevation in the control group was found when compared to the normal control group. Total bilirubin was markedly elevated in the control group. Treatment with Gurgum-13 resulted in a decreased in the elevated levels of enzymes, bilirubin and lipid peroxidation marker (LPO) in experiments compared with the control group. The results of biochemical analysis and hepatoprotective effects were supported by the result of histopathological study of liver.

In case of lipid peroxide, the CCl4 control group showed a significant increase in LPO level when compare to a normal control group. This indicates toxin CCl4 induced peroxidation in blood serum and liver tissues, leading to cause liver damage. Similar experiments reported by Badamsuren observed that C. tinctorius L. reduced ALT, ASP, ALP enzyme and LPO levels on chronic liver injury induced by CCl4 [17]. In our study, this elevation level of serum and liver tissues LPO was markedly inhibited by test medicine Gurgum-13 formulation, which contains C.tinctorious L. and standard drug Milk thistle. Antioxidant enzyme superoxide dismutase (SOD) was found to be reduced in liver homogenate. Treatment with Gurgum-13 formulation markedly increased the SOD in liver homogenate by 4%, 12% and 40% respectively in the three months period ($p < .05$), whereas blood serum SOD increased by 23%, 14.3% and 14.6% in the three months period of experiment ($p < .05$). Additionally Gurgum-13 formulation has a stimulatory effect on the synthesis of fibrinogen in the liver and has a protective effect against CCl4 induced coagulation disturbances in rats. Our results demonstrated that the traditional multicomponent formulation Gurgum-13 possessed a wide range of hepatoprotective effects in the experimental of liver injury model which is probably due to the bioactive substances contained in Gurgum-13 which have hepatoprotective, antioxidant properties and scavenging free radicals. Its effect was similar to those of reference drug Milk thistle. However, a limitation of our study was relatively small sample size. In addition, the pharmacological research of Gurgum-13 has been performed only on experimental animals. Consequently, it is desirable to conduct future clinical trials to see the proper hepatoprotective antioxidant effects of Gurgum-13 in reversing hepatic fibrosis in humans.

Conclusion

In conclusion, the result of the current study clearly demonstrated hepatoprotective effects of Gurgum-13 in CCl4 induced liver injury in rats. It was discovered that traditional multicomponent formulation Gurgum-13 has low toxicity and a wide range of therapeutic hepatoprotective activity. The Gurgum-13 significantly reduced the toxic effect of CCl4, in levels of liver function markers, AST, ALT, ALP, total bilirubin and increased the albumin syntsesis. Thus,

Gurgum-13 formulation was an efficient hepatoprotective agent and its activity was found to be comparable with the Milk thistle, which is used as a standard reference drug. Moreover, Gurgum-13 formulation has effectively inhibited the lipid peroxidation process, enhansing endogenous antioxidant defense due to the presense of biologically active components contained in Gurgum-13 formulation. Combined action of all the ingredients contained in Gurgum-13 helps to normalize the liver function and protects the liver cells. The beneficial effect of the above formulation is based on their free radical scavenging, choleretic, blood coagulation regulating and antioxidative actions, which provide not only hepatoprotective effect but also the regulating effect on homeostatic system of the organism according to the multicomponent formulation theory. In the present investigations, the results of biochemical analysis and hepatoprotective effects on CCI4-induced liver injury through induction of antioxidant defence and scavenging free radicals were further confirmed by histopathological observations. This study results scientifically demonstrate the pharmacological activity of Gurgum-13 and hence it will widen the horizon of traditional medicine varieties with hepatoprotective effects.

Conflict of interest

The authors state no conflict of interest

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