Available online on www.ijtpr.com
International Journal of Toxicological and Pharmacological Research 2017; 9(3); 179-188

ISSN: 0975-5160

Research Article

Hepato- and Nephroprotective Effects of the Polyphenol Concentrate From Cabernet Sauvignon Grape Varieties Cultivated in Kazakhstan in the Experimental Model Of CCL_4-Induced Toxicity

Nurgozhin T[1*], Sergazy SH[1], Adilgozhina G[1,2], Gulyayev A[1], Shulgau Z[3], Abuova G[4], Tritek V[1], Saparov A[2]

[1]*PI "National Laboratory of Astana", Nazarbayev University, Astana, Kazakhstan, 010000*
[2]*Nazarbayev University School of Medicine, Astana, Kazakhstan, 010000*
[3]*National Center for Biotechnology, Astana, Kazakhstan, 010000*
[4]*South Kazakhstan state pharmaceutical academy, Shymkent, Kazakhstan*

Available Online:25th July, 2017

ABSTRACT

Objective:This study investigates the hepatoprotective effect and the antioxidant role of polyphenol concentrate in the experimental model of carbon tetrachloride (CCl_4) induced toxicity. Methods: Antioxidant activity of Cabernet Sauvignon grape polyphenol were evaluated by radical scavenging of 1,1-diphenyl-2-picryl hydrazyl radical (DPPH), 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) ($ABTS^{\cdot+}$). In addition, the effects of polyphenol concentrate on the survival of Wistar rats in the toxicity model, was also investigated. The polyphenol concentrate was administered for 5 five days prior to injection of carbon tetrachloride in a sub-lethal dose of 300 mg/kg of animal body weight in order to perform histological examinations of the liver and kidney, and detect the levels of AST, ALT and bilirubin. Results: Administration of polyphenol concentrate increased animal survival in the experimental model. Moreover, the intragastric administration of polyphenol concentrate prior to the initiation of the experimental model of toxicity, which was caused by a sub-lethal CCl4 dose, reduced morphological injuries in the liver and kidney, decreased the AST and ALT levels of the blood serum. Discussion and conclusion: Our data demonstrate that polyphenol concentrate possesses an antioxidant potential both in vitro and in vivo by reducing antioxidant stress that was caused by CCl4 administration into rats.

Keywords: Free radicals; histopathology; hepatoprotective effect; antioxidants.

INTRODUCTION

Increasing human life expectancy is one of the most important aims of modern preventive medicine[1,2,3]. Currently, there are more than 20 substances, known as geroprotectors, which demonstrate the ability to increase the lifespan of animals[4,5,6]. Based on the free-radical theory of aging, antioxidants are also considered to be geroprotectors[7,8]. According to this theory, free radicals form as a result of oxidation in the various biochemical pathways of the body, which have damaging effects on multiple macromolecules (proteins and nucleic acids), thus causing their degradation and aging. This theory not only explains the mechanism of aging, but also stimulates the search for geroprotectors among antioxidants[9]. This search is conducted among the compounds of different chemical structures, often including heterocycles[10]. Researchers have paid particular attention to screening plant polyphenols, with the greatest focus aimed at phenolic compounds of grapes and resveratrol[11,12,13]. Investigation of individual grape polyphenols or polyphenol complexes in their ability to possess anti-oxidative and anti-aging effects has been a topic of interest for the past ten years[14]. Flavonoids, whose cardioprotective, antioxidant, anti-inflammatory, anti-cancer and anti-bacterial properties were identified earlier, can be characterized as a widespread group of natural polyphenols[15]. Some studies demonstrate that polyphenols have general cytoprotective properties[16,17,18,19]. Therefore, it is extremely important to test the effects of flavonoids in the model of liver and kidney toxicity in order to understand the principles of the biological activity of grape flavonoids.

Furthermore, the selected area of grape growth determines the polyphenol composition and concentration of the individual polyphenols. Thus, it has been reported that grapes from northern breeding areas can contain a greater amount and variety of polyphenols[20,21]. To this point, Kazakhstan's location as a very northern winegrowing region can lead to interesting compositions of grape polyphenols.

METHODS

Selection and description of animals

Healthy adult Wistar male rats, aged seven to nine weeks and weighing 150±20 g were housed in the animal facility of the National Center for Biotechnology, Astana, Kazakhstan. After a one-week adaptation period, the rats

**Author for Correspondence: shynggys.sergazy@nu.edu.kz*

were randomly divided into groups (6 rats/cage) and housed in a room with controlled temperature and a 12-h light-dark cycle with unlimited access to standard food and drinking water *ad libitum.* Animal experiments were conducted according to "the rules of pre-clinical studies" (approved by the order of the Minister of Health and Social Development Republic of Kazakhstan on May 29, 2015 № 415) and approved by the Ethics Committee of Nazarbayev University (Approval no: 18 from 02.04.2015). In addition, the animal studies were approved by the Ethics Committee of the National Center for Biotechnology, Astana, Kazakhstan.

Technical information

Grape polyphenol concentrate

Cabernet Sauvignon-old French grape variety of medium-term maturity is currently located in the Almaty and Zhambyl regions of Kazakhstan. The samples were selected from the Chilean area of the Almaty region, harvest 2014 year. The concentrate polyphenols were obtained from seed ridges and grape skin, secondary wine products, by water-alcohol feedstock extraction (40% aqueous - alcoholic solution of ethyl alcohol in the ratio 1: 5), followed by concentration of the extract on a rotary evaporator to obtain a dry matter content of 25%. The total concentration of phenolic derivatives in the concentrate of grape polyphenols used in this experiment was 10000 mg/l.

Determination of phenolic components in polyphenol concentrate

An analysis of polyphenols content was conducted by HPLC using the Agilent 1290 chromatograph. The separation was performed in a gradient mode column ZORBAX RRHD SB-C18 2.1 × 100 mm, 1.8 mm. The mobile phase consisted of A: 0.1% aqueous formic acid B: acetonitrile containing formic acid at a concentration of 0.1%. Gradient was performed in the following sequence: initial eluent consisted of component A. Within five minutes, the concentration of component B increased to 10% and continued at 10% for the following five minutes. From the 10^{th} to the 25^{th} minute, concentration of component B increased to 35%. The column was then cleaned and conditioned.

Flow rate was 0.3 ml / min at 30ºC (the samples were stored at 4˚C in dark glass tubes). Registration was performed by using a diode array detector at 280 nm and 325 nm. For the analysis, 3µl of solution was used. External standards used: gallic acid, a certified reference material; (-) - Epigallocatechin, analytical standard; (+) - Catechin, analytical standard; Chlorogenic acid, a primary reference standard; Caffeic acid, ≥99,0% (HPLC); (-) - Epicatechin, analytical standard; Epigallocatechin gallate, the primary reference standard; Epicatechin gallate, the primary reference standard; trans-p-coumaric acid, analytical standard; trans-Ferulic acid certified reference material; m-coumaric acid (99%); o-coumaric acid (97%); Catechin gallate, analytical standard; Quercetin, the primary reference standard; Myricetin, analytical standard; Resveratrol, an analytical standard; Piceid, ≥95% (HPLC); Morin hydrate; Quercetin dihydrate, a primary reference standard; (±) -Naringenin, analytical standard; Apigenin, analytical standard; Apigenin 7-glucoside, analytical standard; Kaempferol, analytical standard; Kaempferol 3-glucoside, analytical standard. All manufacturing standards were purchased from Sigma-Aldrich.

Myricetin, Resveratrol, Quercetin, Kaempferol and Apigenin were dissolved in 96% ethanol, while other chemicals were dissolved in a mixture of ethanol / water (50/50) to a concentration of 5 mg / ml. Afterwards, calibration mixtures with different concentrations were prepared.

1,1-dyphenyl-2-picryl hydrazyl radical scavenging activity

Radical scavenging activity of the extracts was determined using DPPH radical scavenging method. 10 µL of grape polyphenol (0.1796-17.960 mg/ml) were added to 1 mL DPPH (0.39 M) and mixed. The mixture was left to stand for 10 min in the dark at room temperature and the absorbance was measured at 515 nm by a UV-Visible spectrophotometer. Ethanol (1 mL) was used as the blank, and ascorbic acid was used as standard. The DPPH radical scavenging activity of the extracts was calculated using the following equation:

% Inhibition = $[(A_0) - A_1)/A_0)] \times 100$

where, A_0 is the absorbance of the (DPPH + ethanol) and A_1 is the absorbance of (DPPH + samples). IC_{50} value is the concentration of extracts provided 50% inhibition of DPPH radicals.

2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS.+)

Antioxidant activity of the grape extract was evaluated by using an Antioxidant Assay kit (Sigma Aldrich). Slight modifications were made in readjusting the concentration of TROLOX to mg/ul. The antioxidant concentration of the test sample was estimated by using the equation obtained from linear regression of the standard curve:

X (mg/ul) = $\frac{y(A_{405}) - Intercept}{Slope}$ *dilution factor; where

X (mg/ul) – antioxidant concentration mg/ul relative to the concentration of the Trolox standard

$y(A_{405})$ – the average absorbance of the Test Sample at 405 nm

Intercept – intercept of the y axis by the standars curve

Slope – slope of the standard curve, a negative value

Pretreatment and carbon tetrachloride (CCL_4)-induced hepatorenal toxicity

The effects of polyphenols concentrate on the survival of laboratory animals with acute toxic hepatitis was evaluated in the first series of the experiments. For this purpose, the acute toxic hepatitis was induced by a single intraperitoneal injection of 50% mineral oil solution of carbon tetrachloride at a dose of 400 mg/kg of animal body weight. The rats were divided into 2 groups: experimental and control. Five days prior to and after injection of carbon tetrachloride, the rats of the experimental group were administered with 0.5 ml of polyphenol concentrate intragastrically once daily, which is equivalent to 50 mg of phenolic compounds per 1 kg of animal body weight. Rats of the control group received 0.5 ml drinking water. The animal observation period was 2 weeks.

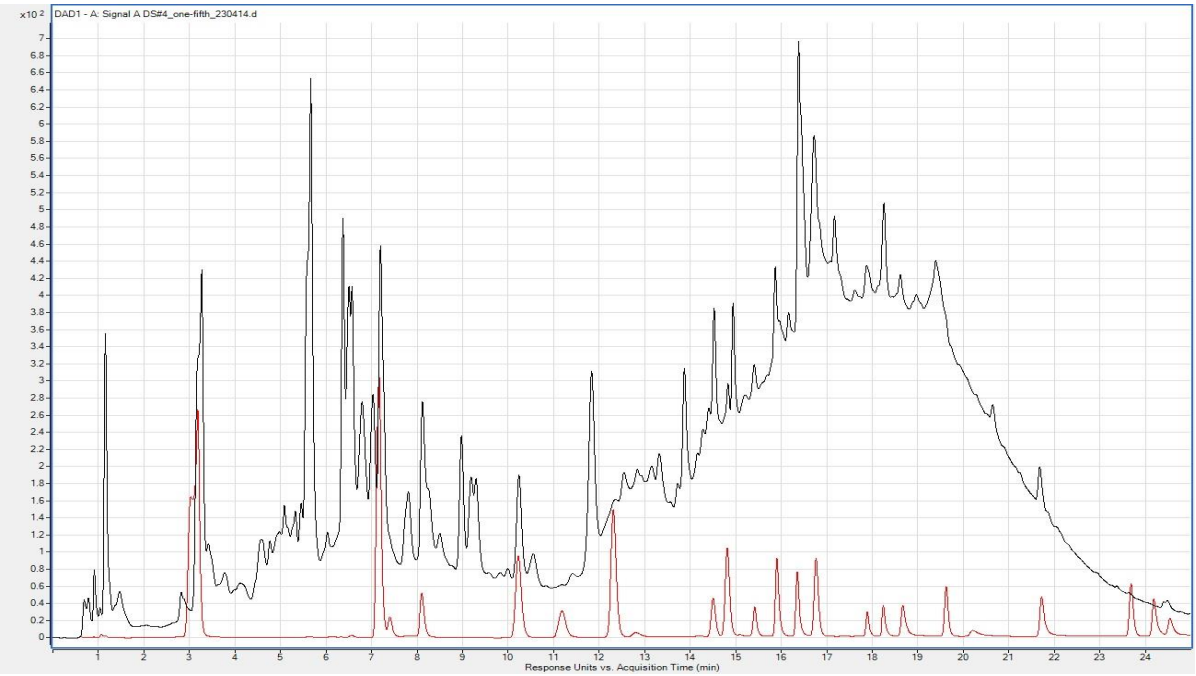

Figure 1: Chromatogram of polyphenol concentrate from Cabernet Sauvignon grape varieties cultivated in Kazakhstan (five-fold dilution).

Table 1: The polyphenols composition of the concentrate derived from Kazakhstan selection of Cabernet Sauvignon grape variety.

Symbol	Phenolic compound	Content [a] (mg/L)
GA	Gallic acid	121.5 ± 1.20
Cat	(+)-Catechin	258.5 ± 1.78
ChA	Chlorogenic acid	0.68 ± 0.01
CA	trans-Caffeic acid	22.6 ± 0.68
Epi	(−)-Epicatechin	106.6 ± 3.25
pCA	trans-p-Coumaric acid	1.39 ± 0.07
FA	trans-Ferulic acid	22.4 ± 0.39
P	Polydatin	8.80 ± 0.11
K3G	Kaempferol 3-glucoside	15.3 ± 0.43
M	Myricetin	8.51 ± 0.40
R	trans-Resveratrol	1.05 ± 0.08
Q	Quercetin	18.8 ± 1.25
N	(±)-Naringenin	0.34 ± 0.01
K	Kaempferol	3.21 ± 0.04

[a] — mean of three determinations ± s.e.

Table 2: DPPH and ABTS $^{\cdot+}$ radical scavenging activity.

	DPPH inhibition IC_{50}, mg/ml	TEAC
Grape polyphenol concentrate	0.47 ± 0.03	7.98 ± 0.068
Ascorbic acid	0.55 ± 0.02	-

In the second series of experiments, the activity of transaminases and bilirubin concentration in blood serum, as well as histological examinations of liver and kidney, was evaluated. For this purpose, the acute toxic hepatitis was induced by a single intraperitoneal injection of 50% mineral oil solution of carbon tetrachloride in a dose of 300 mg/kg of animal body weight. One day after the injection of carbon tetrachloride, measured parameters were investigated. Animals were divided into two study groups: experimental and control. The rats in the experimental group were administered with 0.5ml of polyphenol concentrate intragastrically for 5 days prior to injection of carbon tetrachloride. Rats of the control group received drinking water in equivolume amount. As a negative control, the intact animals were also examined for the activity of transaminases and bilirubin concentration in blood serum. Liver and kidney tissues were taken for histological examination in order to compare the morphological changes between the control and experimental groups.

Liver function tests

Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined colorimetrically by using AST and ALT Activity assay kits, respectively (Sigma-Aldrich, St. Louis, MO, USA). The bilirubin level in the serum was determined by using a Bilirubin Assay kit (Sigma-Aldrich, St. Louis, MO, USA).

Oxidative status of blood plasma

The oxidative status in the blood plasma of rats was measured by a FRAS 4 Evolvo device (Italy) by using sets of d-ROMs Test (measures amount of reactive oxygen species in blood plasma) and PAT Test (characterizes the total antioxidant activity of blood plasma).

Histological Analysis

In the end of the observation period, the rats were sacrificed by using the CO_2 inhalation method according

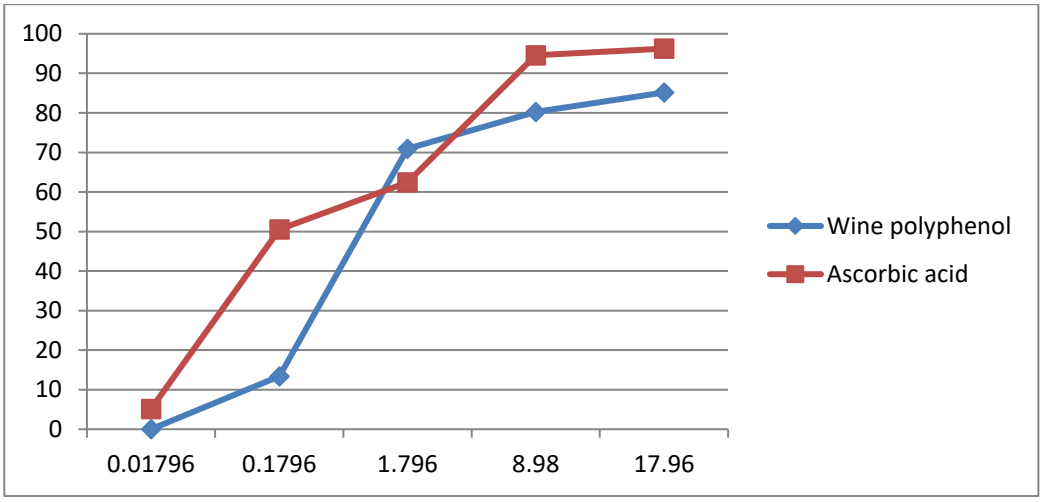

Figure 2: DPPH radical scavenging activity of the grape polyphenol concentrate and comparison to ascorbic acid.

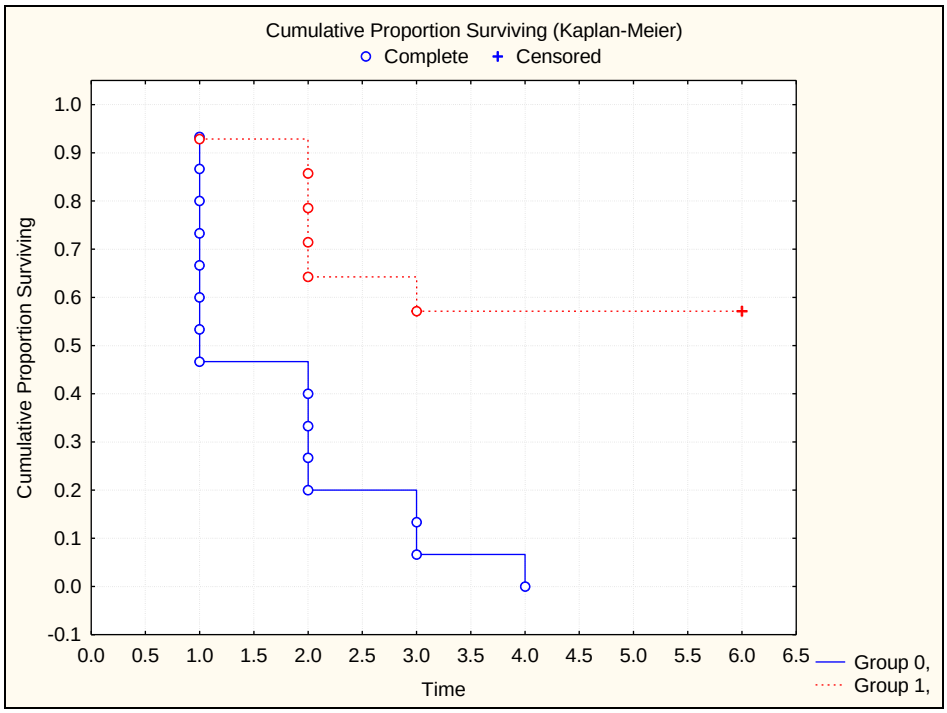

Figure 3: Increase in animal survival after administration of grape polyphenol concentrate.

to the approved protocol. The liver and kidney samples were fixed in 10% neutral buffered formalin (Sigma-Aldrich, St. Louis, MO, USA) for 24h and embedded in paraffin. Microtome sections of 4-5 µm thickness were prepared and stained with haematoxylin-eosin. Histopathological liver and kidney sections were examined using a computerized complex «Leica microsystems» (Sweden) with a microscope «Leica DM1000».

Statistics

Statistical analysis was performed by using «Statistica 6.0» program (StatSoft, Texas, USA). The results are presented in the form of "mean value ± SD". The normality of the distribution of performance was evaluated by using Shapiro-Wilk`s W-test criteria. Between-group differences were evaluated by using a nonparametric Mann-Whitney U-test.

RESULTS

Determination of phenolic components in polyphenol concentrate

Analysis of the polyphenol composition, which was performed using HPLC (Fig. 1), showed the presence of the following polyphenols: catechin - 258.5 mg / l; gallic acid – 121.5 mg / l; epicatechin - 106.6 mg / l; quercetin - 18.8 mg / l; myricetin - 8.51 mg / l; kaempferol 3-glucoside - 15.3 mg / l; polydatin - 8.8 mg / l; trans-caffeic acid - 22.6 mg / l; trans-ferulic acid – 22.4 mg/l; kaempferol - 3.21 mg / l; trans-p-coumaric acid - 1.39 mg / l; trans-resveratrol - 1.05 mg / l; chlorogenic acid - 0.68 mg / l; naringenin – 0.34 mg.

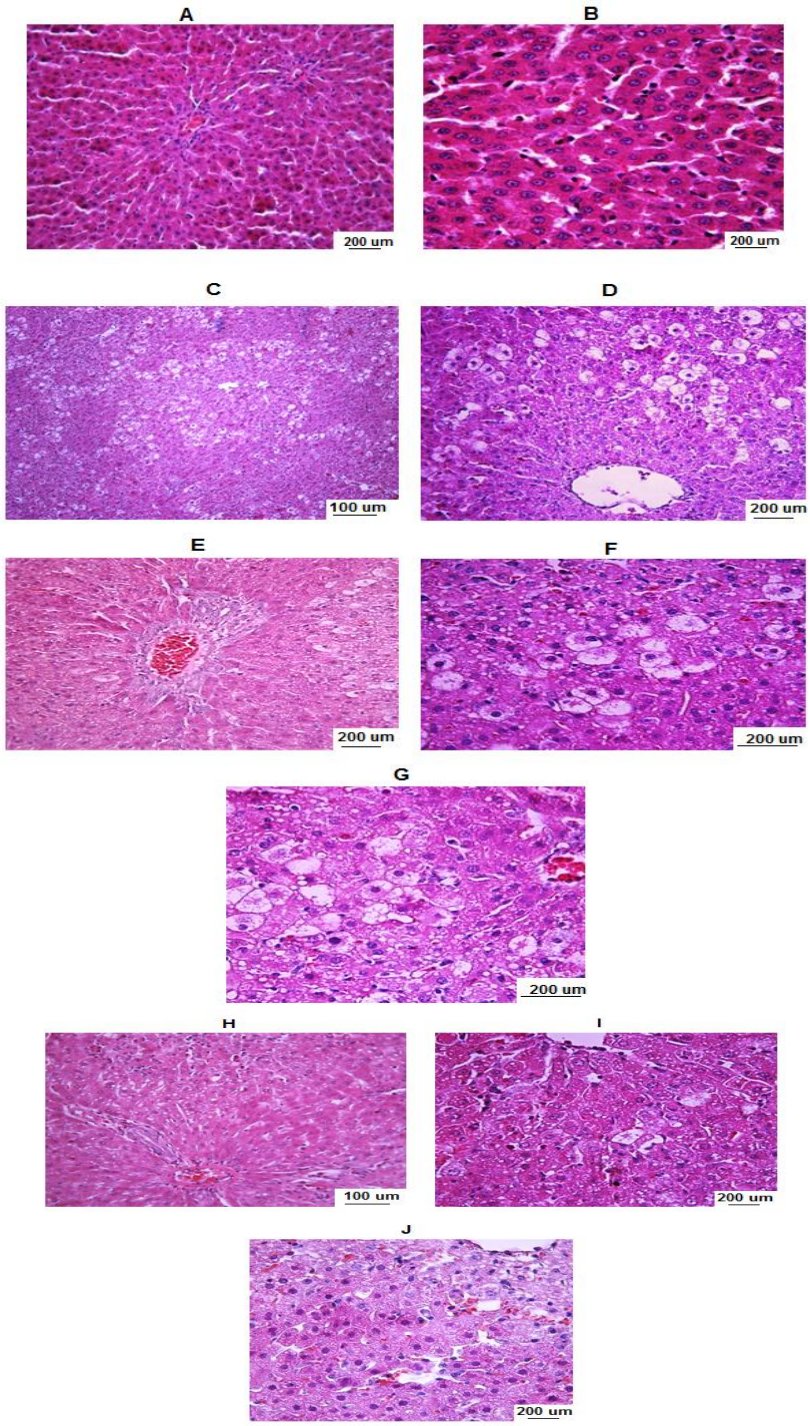

Figure 4. Liver tissue. Hematoxylin and eosin, A and B- healthy animals
Control group.C - The structure of the hepatic lobule is broken, hydropic and vacuolar degeneration of hepatocytes.100x. D - Hydropic and vacuolar degeneration of hepatocytes, mainly periportal areas of the liver lobules. Vacuolated cytoplasm, pyknotic nuclei, focal necrosis of hepatocytes, nuclei are lysed. 200x. E - Congestion periportal vessels. 200x. F- Congestion sinusoidal capillaries. Hydropic and vacuolar degeneration of hepatocytes cytoplasm vacuolated, foamy and non-homogeneous, focal necrosis of hepatocytes. 400x. G - The cytoplasm in the form of soft mesh formations, frothy, lysed nuclei. On the periphery of the hepatic lobule lymphoid infiltration, vascular congestion. 400x
Effect of the grape polyphenol concentrate.
H - Restoration of beam structure of hepatic lobules. Congestion of Central vein and sinusoidal capillaries. 100x. I - Nucleus in cells located centrally, some ballooning degeneration of hepatocytes. 400x. J - The proliferation of hepatocytes of peri-portal areas of the liver lobules. The nuclei in hepatocytes located centrally, binucleated cells. Sinusoids congested. 400x

Figure 1. Chromatogram of polyphenol concentrate from Cabernet Sauvignon grape varieties cultivated in Kazakhstan (five-fold dilution). Marked peaks correspond to the standard samples (chromatogram of a mixture of standard samples is shown with a red line). The concentrations of polyphenols are shown in table 1. Chromatographic conditions are described in the text. Detection was conducted at 280 nm.

In vitro antioxidant 1,1-dyphenyl-2-picryl hydrazyl radical scavenging activity

The DPPH radical scavenging of either extract of *Cabernet Sauvignon* or standards was determined at different concentrations. As it is evident from Fig. 2, DPPH radical scavenging activity of the extracts was increased by increasing the extract concentrations.

The IC_{50} and TEAC values of poly phenol extract and ascorbic acid standards are shown in table 2.

All animals were injected with carbon tetrachloride as described in the Materials and Methods section. Afterwards, the experimental group (blue line, n= 15) received the polyphenol extract, whereas the control group (red line, n= 15) was injected with water. Animals developed acute toxic hepatitis with substantial mortality. X-axis time reflects days after the injection. Effect of polyphenols on survival was statistically significant at $p < 0.005$ according to the Student t-test.

The present study revealed that intraperitoneal injection of carbon tetrachloride at a dose of 400 mg/kg in the control group caused the death of 53% of animals after day 1. After two days, the mortality rate reached 80%, 93% after day 3 and 100% mortality rate was observed at day 4. In the group of animals that received the polyphenol concentrate, the mortality rate was 7% one day after the administration of carbon tetrachloride, after 2 days - 36% and reached 46% at day 3. Four days after the injection of carbon tetrachloride, 54% of the animals were still alive and remained alive throughout the observation, follow-up was 2 weeks (Figure 3).

Histopathology of liver

The liver tissue from the intact group of animals exhibited normal histological structures, consisting of well-arranged liver cell cords along the central vein, well-defined structure of hepatic lobules, normal hepatic sinusoid, and round nuclei with no sign of inflammatory infiltration (Figure 4A and B).

The control group, in contrast, exhibited the development of acute hepatitis (toxic steatosis), which was characterized by significant changes in all structural components of the liver lobules. The impairment of blood circulation in the capillary of sinusoids of the liver and periportal vessels was detected. The most common structural change was the development of hydropic and vacuolar degeneration of hepatocytes in the periportal zones of hepatic lobules. Hepatocytes with hyperchromatic nuclei were located at the periphery of the hepatic lobules, as well as the presence of some large binucleated cells. The changes in the structure of the hepatic lobules were accompanied with pronounced polymorphism of hepatocytes, which were swollen and of different sizes with nuclei of various structures. There were some hepatocytes with frothy or vacuolated cytoplasm that indicates the development of hydropic and vacuolar dystrophy, and some of the hepatocytes developed atomizing fat dystrophy. The nuclei of hepatocytes were of irregular rounded forms, vacuolated and heterogeneous or with piknotic changes. The presence of binucleated cells were also observed, which were localized in the periportal zones of hepatic lobules. In some vacuolated hepatocytes nucleus pushed to the periphery, there were pockets of the center focal lobular liquefactive necrosis, which was characterized by soft mesh structures in the cytoplasm, nucleus lysed in them and were reminiscent of "desolate cells" (Figures 4 C, D, E, F, G).

Treatment with the concentrate of polyphenols reduced the destruction of the lobule structure. Nuclei in cells were located centrally; there were some isolated hepatocytes with vacuolar degeneration (Figure 4 H, I, J).

Histopathology of kidney

Histological examination of kidney tissue in the intact group of animals showed that the kidney cells presented a normal form with clear glomerular and renal tubular structures and the cavity of the Bowman's capsule was free (Figure 5 A, B). No abnormal pathology was observed.

In the kidneys of the control group, the glomeruli comes closer and vascular endothelial denudation and congestion of interstitial and glomerulural capillaries were observed. There was a visible accumulation of transudate in the cavity of the Bowman's capsule. Membranous glomerulitis was also observed; glomerular structure was disrupted, and vascular loops exposed (Figures 5 C and D). Rats pretreated with polyphenol concentrate exhibited a noticeable reduction of renal injury as demonstrated by the apparent improvement of tubular and glomerular architecture and reduction of inflammatory cells (Figure 5 E and F).

A tetrachloromethane-induced acute liver injury model was successfully established, as confirmed by elevated serum ALT and AST levels detected in the experimental and control groups, compared to the intact group (rats that were not injected with tetrachloromethane), followed by a single intraperitoneal injection of 50% mineral oil solution of carbon tetrachloride in a dose of 300 mg/kg of animal body weight.

In the second series of experiments, the intraperitoneal injection of carbon tetrachloride at a dose of 300 mg/kg in the control group dramatically increased the activity of aminotransferase and bilirubin concentration compared to the intact animals. In the experimental group of animals that received 0.5 ml of the grape polyphenol concentrate for 5 days prior to carbon tetrachloride injection, increased levels of ALT and AST in the blood serum were also observed but to a lesser degree compared to the control group. In addition, a decreasing trend in the bilirubin concentration was detected in the group of rats treated with the polyphenols concentrate compared to the control group of animals (Table 3).

Our data indicate that injections of the polyphenols concentrate, derived from the Kazakhstan selection of

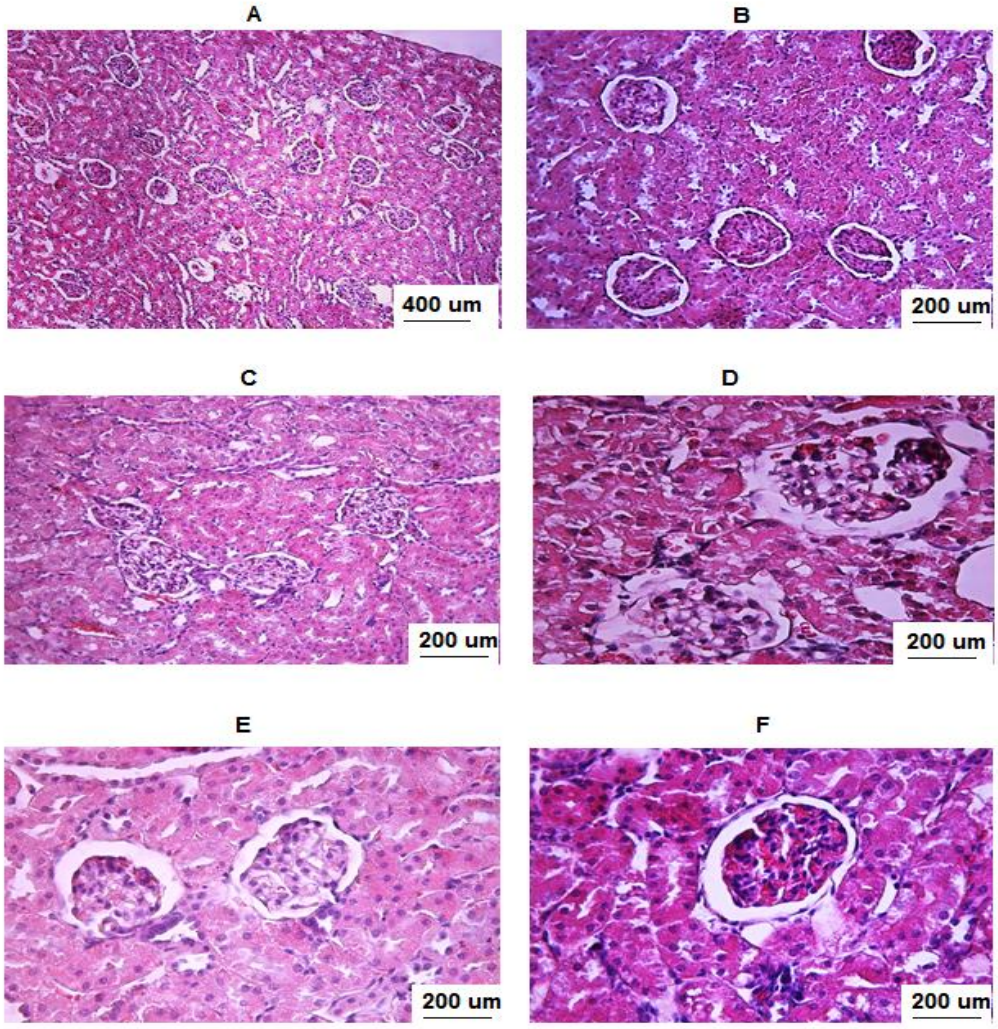

Figure 5: Kidney. Hematoxylin and eosin
A and B – Healthy animals
Control group
C -The convergence of the glomeruli, the exposure of the vascular endothelium, congestion of glomerular and interstitial capillaries.
D. Congestion glomerular capillaries, accumulation of transudate in a cavity of Bowman's capsule.
Effect of the grape polyphenol concentrate
E - Protection of renal glomerular structure. Cavity of Bowman's capsule is free.
F- Kidney. Experimental group. Glomerular capillary congestion. Tubule epithelium cube shaped with hyperchromatic nuclei.

grapes, in the experimental model of CCl4-induced acute toxicity, contributed to the preservation of the liver and kidney tissues.

One of the possible mechanisms by which polyphenol concentrate mediates their hepato- and nephroprotective properties is the enhancement of the protective function of liver and kidney cells due to the antioxidant effect of flavonoids, which are components of polyphenol concentrate. Changes in antioxidant activity in the blood plasma (PAT test) and the concentration of free radicals in the blood plasma (D-ROMs test) of rats after toxicity caused by CCL4 are shown in table 4.

As shown in Table 4, the levels of free radicals increased and antioxidant activity decreased in the group of rats that received CCl4 compared to the intact animals. However, the group of rats that received grape polyphenol concentrate demonstrated no changes in the levels of free radicals according to the D-ROMs test compared to the intact animals and showed low levels compared to the control group. Moreover, according to the PAT test, integral activity of the antioxidant system in this group of animals decreased slightly compared to the intact group of animals.

DISCUSSION

Table 3: Effect of the polyphenol concentrate on serum markers of the liver function.

Animal groups	ALT, µmol /s/l	AST, U/l	Bilirubin, µmol/l
Intact group (n=8)	0.25 ± 0.05	53 ± 3	1.0 ± 1.8
Control group (CCl_4) (n=9)	1.39 ± 0.05	291 ± 43	25.7 ± 3.3
Experimental group (CCl_4 + polyphenol concentrate) (n=9)	0.88 ± 0.09º*	202 ± 32º*	21.2 ± 3.4º

Values are the mean ± SEM. CCl_4 control group compared with the normal intact group ºp < 0.001. Experimental groups compared with CCl_4 control group *p < 0.05.

Table 4: Effects of grape polyphenol concentrate on the level of free radicals and antioxidant activity in blood plasma of rats with CCL_4 toxicity.

Test	Intact group (healthy animals) (n=8)	Control group (CCl_4) (n=9)	Experimental group (CCl_4 + polyphenol concentrate) (n=9)
D-ROMs test, CARR U (1 CARR U = 0.08mg/100ml H_2O_2)	311 ± 13	398±43º	315±37*
PAT test, U Cor (1 U Cor = 1,4 µmol/L ascorbic acid)	2636±259	1956±112º	2335±126

Values are the mean ± SEM. CCl_4 control group compared with the normal intact group ºp < 0.001. Experimental groups compared with CCl_4 control group *p < 0.05.

CCl_4 is the most commonly used hepatotoxin for the experimental modeling of liver disease. CCl_4 undergoes microsomal oxidation, with the participation of cytochrome P-450 to form the electrophilic alkylating intermediates and free radicals that are capable of inducing lipid peroxidation and modifying biomolecules as a result of covalent bonding with them. These changes lead to the development of severe liver disease with manifestations of cytolytic syndrome, cholestasis, mesenchymal inflammation, steatosis, and hepatocellular failure[22]. Under normal physiological conditions, reactive oxygen species play a major role in promoting cell growth and differentiation, adaptation to metabolic and physiological stresses, immune response as well as protection from pathogen invasion. However, CCl_4 causes over accumulation of reactive oxygen species and induces oxidative stress. When cells are exposed to oxidative stress, they easily undergo oxidative damage that leads to a cascade of degenerative processes[23-24].

It was shown that Cabernet Sauvignon grape polyphenols possess antioxidant properties in vitro and the inhibition in IC50 DPPH test was comparable to ascorbic acid. In the ABTS+ test, the antioxidant properties of the polyphenol concentrate was higher than the antioxidant activity of trolox.

In our experiments, the protective effect of the studied grape polyphenol concentrate was more obvious in relation to transaminases. Transaminases constitute a group of enzymes that catalyze the interconversion of amino acids and α-keto acids by the transfer of amino groups. It has been reported that changes in transaminases activity is a sensitive and reliable test for the identification of the liver parenchyma and the liver membrane cells injuries in the case of a CCl_4 poisoning[25]. Our results show stabilization of the liver cell plasma membrane with the grape polyphenol concentrate after the CCl_4-induced toxicity. The data on ALT levels are also supported by the morphological changes that showed the positive dynamic in the liver and kidney structure regeneration when the grape polyphenol concentrate was used to treat the acute toxic hepatosis. The development of abnormal morphological features in the liver tissue was prevented in the rats treated by the grape polyphenol concentrate. Moreover, reparative processes were detected in the morphological structures of liver. In general, the liver tissues from the rats of the experimental and control groups were similar to each other in structure.

The treatment of rats with the polyphenol concentrate improved the structure of kidney glomeruli and demonstrated that the epithelial structure of kidney tubules was similar to that of the intact group of animals. These morphological observations indicate a cytoprotective effect of the grape polyphenol concentrate after the CCl_4-induced lesions.

Oxidative stress plays a crucial role in the pathogenesis of acute liver toxicity[26]. Oxidative stress initiates changes related to blood supply to the liver such as vascular endothelial and liver sinusoids damage with a change in the production of nitric oxide, deterioration of rheological properties of blood, and the status of the microvasculature, which exacerbates the pathological process[27]. Our data demonstrate that the grape polyphenol concentrate injected into the rats decreases the severity of the oxidative stress induced by CCl_4.

Thus, we demonstrated that administration of polyphenol concentrates into the rats prior to intoxication by CCl4 did not increase the concentration of free radicals in the blood plasma and did not significantly decrease antioxidant activity in the blood plasma. The high content of polyphenolic substances with pronounced antioxidant activity makes a significant contribution to the cytoprotection mediated by the concentrate of polyphenols that are derived from Kazakhstan selection grape varieties Cabernet Sauvignon.

There are several possible mechanisms which might explain the cytoprotective effects of the grape

polyphenols. Although the polyphenols demonstrate hepatoprotection due to their antioxidant effect, there are other effects such as immunomodulatory and anti-inflammatory[28].

Different *in vivo* studies on animals reported that carbon tetrachloride causes liver and kidney injuries[29,30] through generation of electrophilic trichloromethyl (CCl_3) radicals[31]. Similar hepatoprotective effects of different seed extracts of red grape varieties were reported not only in the model of CCl4 induced liver damage in rats[32], but also in the experiments that involed the poisoning of rats with ethanol[33,34], acetaminophen-induced liver injury[35,36], dimethylnitrosamine-induced liver damage in rats[35,36], as well as on liver steatosis[37] and in γ-irradiated rat[38]. Our results on hepatoprotective and nephroprotective effects of grape polyphenol concentrate are similar to the effects of grape seed extract, described by other researchers. These effects may be due to general cytoprotective effects of a group of polyphenols, or any individual component. Further studies should clarify the contribution of certain grape polyphenols on the cytoprotective effect and the mechanisms by which it is mediated. Thus, our data demonstrate that polyphenol concentrate, which is derived from Kazakhstan selection grape varieties Cabernet Sauvignon, possess hepatoprotective and nephroprotective properties and can serve as a promising cytoprotective agent.

CONCLUSION

The polyphenol concentrate derived from Kazakhstan selection grape varieties Cabernet Sauvignon has hepatoprotective and nephroprotective effects on CCl_4-induced tissue lesions in rats. Our results indicate that the grape polyphenols concentrate prevents or decreases CCl_4-induced liver and kidney damage due to its antioxidant effect. However, additional experiments should be performed to prove cytoprotective (hepatoprotective and nephroprotective) properties of polyphenol concentrate by using other methodological approaches.

ACKNOWLEDGMENTS

This work was supported by the grants from the Ministry of Education and Science of the Republic of Kazakhstan (0112 PK02309 and 0114 PK00491).

CONFLICT OF INTEREST

The authors declare no conflict of interest

REFERENCES

1. Ebrahim S et al. eds. *Epidemiology in Old Age*, London: BMJ Publ. Group, 1996: 436
2. Rattan S.I. Aging is not a disease: implications for intervention. *Aging Dis* 2014; 5(Suppl.3): 196–202.
3. World Health Organization. Life in the 21st century: A vision for all (Report of the Director-General) 1998; 1-14.
4. Moskalev A et al. Developing criteria for evaluation of geroprotectors as a key stage toward translation to the clinic. *Aging Cell* 2016; 15(Suppl.3): 407-415.
5. Moskalev AA, Pasyukova EG. From theories of aging to anti-aging interventions. *Front Genet* 2014; 5: 276.
6. Zs-Nagy I, Harman D, Kitani K. *Pharmacology of Aging Process: Methods of Assessment and Potential Interventions*, 1st ed. Ann. N.Y. Acad. Sci., 1994
7. Arutiunian AV, Kozina L.S. Mechanisms of free radical oxidation and its role in aging. *Adv Gerontol* 2009; 22(Suppl.1): 104–116.
8. Harman D. Free-radical theory of aging: increasing the functional life span. *Ann NY Acad Sci* 1994; 717: 1–15.
9. Wang CH et al. Oxidative stress response elicited by mitochondrial dysfunction: implication in the pathophysiology of aging. *Exp Biol Med (Maywood)* 2013; 238(Suppl.5): 450–460.
10. Panda SS et al. Xanthones as potential antioxidants. *Curr Med Chem* 2013; 20 (Suppl.36): 4481–4507.
11. Jayasena T et al. The role of polyphenols in the modulation of sirtuins. *Ageing Res Rev* 2013; 12(Suppl.4): 867–883.
12. Kasiotis KM et al. Resveratrol and related stilbenes: their anti-aging and anti-angiogenic properties. *Food Chem Toxicol* 2013; 61:112–120.
13. Marchal J et al. Resveratrol in mammals: effects on aging biomarkers, age-related diseases, and life span. *Ann N Y Acad Sci* 2013; 1290: 67–73.
14. Fraczek M et al. Resveratrol--phytophenol with wide activity. *Pol Merkur Lekarski* 2012; 32 (Suppl.188): 143-146.
15. Lin JK, Weng MS. Flavonoids as Nutraceuticals. In: Grotewold E., eds. *The Science of Favonoids.* New York: Springer Science+Business Media, Inc, 2006: 213–238.
16. Bhullar KS, Rupasinghe HP. Antioxidant and cytoprotective properties of partridgeberry polyphenols. *Food Chem* 2015; 168: 595-605.
17. Dani C et al. Intake of purple grape juice as a hepatoprotective agent in Wistar rats. *J Med Food* 2008; 11(Suppl.1): 12-32.
18. Kim Y et al. Antioxidant and cytoprotective effects of oligomeric and polymeric procyanidin fractions from defatted grape seed in PC12 cells. *J Med Food* 2012; 15(Suppl.5): 490-494.
19. Singha I, Das SK. Antioxidant potential of different grape cultivars against Fenton-like reagent-induced liver damage ex-vivo. *Indian J BiochemBiophys* 2014; 51(Suppl.5): 372-7.
20. Ky I et al. Wine by-products: phenolic characterization and antioxidant activity evaluation of grapes and grape pomaces from six different French grape varieties. *Molecules* 2014; 19(Suppl.1): 482–506.
21. Stockham K et al. Comparative studies on the antioxidant properties and polyphenolic content of wine from different growing regions and vintages, a pilot study to investigate chemical markers for climate change. *Food Chem* 2013; 40(Suppl.3): 500-506.
22. Weber LW et al. Hepatotixicity and mechanism of action of haloalkanes: carbon tetrachloride as a toxicological model. *Crit Rev Toxicol* 2013; 33(Suppl.2): 105-136

23. Hikita H et al. Activation of the Mitochondrial Apoptotic Pathway Produces Reactive Oxygen Species and Oxidative Damage in Hepatocytes That Contribute to Liver Tumorigenesis. *Cancer Prev Res (Phila)* 2015; 8(Suppl.8): 693-701.
24. Georgiev V et al. Recent Advances and Uses of Grape Flavonoids as Nutraceuticals. *Nutrients* 2014; 6(Suppl.1); 391–415.
25. Choudhary AK, Devi RS. Serum biochemical responses under oxidative stress of aspartame in Wistar albino rats Asian. *Pac J Trop Dis* 2014; 4 (Supp.1): 403–410
26. Hassan HM et al. The role of inflammatory and oxidative stress, CYP2E1 and bile acid disturbance in rat liver injury induced by isoniazid and lipopolysaccharide co-treatment. *Antimicrob Agents Chemother* 2016; 61 (Suppl.4).
27. Rajendran P et al. The Vascular Endothelium and Human Diseases. International *Journal of Biological Sciences* 2013; 9(Suppl.10): 1057–1069.
28. Sandur SK et al. Role of prooxidants and antioxidants in the anti-inflammatory and apoptotic effects of curcumin (diferuloylmethane). *Free Radic Biol Med* 2007; 43: 568–580.
29. Çetin A et al. Protective effect of hesperidin on oxidative and histological liver damage following carbon tetrachloride administration in Wistar rats. *Arch Med Sci* 2016; 12(Suppl.3): 486-493.
30. Sajid M et al. Proficiencies of Artemisia scoparia against CCl4 induced DNA damages and renal toxicity in rat. *BMC Complement Altern Med.* 2016; 16(Suppl.1): 149.
31. Recknagel RO et al. Mechanisms of carbon tetrachloride toxicity. *Pharmacol Ther* 1989; 43(Suppl.1): 139-154.
32. Deng JS et al. Hepatoprotective effect of the ethanol extract of Vitis thunbergii on carbon tetrachloride-induced acute hepatotoxicity in rats through anti-oxidative activities. *J Ethnopharmacol* 2012; 142(Suppl.3): 795-803
33. Dogan A, Celik I. Hepatoprotective and antioxidant activities of grape seeds against ethanol-induced oxidative stress in rats. *Br J Nutr* 2012; 107(Suppl.1): 45-51.
34. Bak MJ et al. Antioxidant and Hepatoprotective Effects of Procyanidins from Wild Grape (Vitis amurensis) Seeds in Ethanol-Induced Cells and Rats. *Int J Mol Sci* 2016; 17(Suppl.5).
35. Madi AA, Farouk EM. Lipid-lowering and hepatoprotective effects of Vitis vinifera dried seeds on paracetamol-induced hepatotoxicity in rats. *Nutr Res Pract* 2015; 9(Suppl.1): 37-42.
36. Matsura T et al. Mechanisms of protection by melatonin against acetaminophen-induced liver injury in mice. *J Pineal Res* 2006; 41(Suppl.3): 211-219.
37. Charradi K et al. Protective effect of grape seed and skin extract against high-fat diet-induced liver steatosis and zinc depletion in rat. *Dig Dis Sci* 2014; 59(Suppl.8): 1768-1778.
38. Ismail AF et al. Hepatoprotective effect of grape seed oil against carbon tetrachloride induced oxidative stress in liver of γ-irradiated rat. *J Photochem Photobiol B* 2016; 160: 1-10.